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AN EDITED BOOK ON

INTEGRATIVE PROSPECTIVES ON MANAGEMENT AND STRATEGIC APPROACHES

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INTEGRATIVE PROSPECTIVES ON MANAGEMENT AND STRATEGIC APPROACHES

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Title of the book: Integrative Prospectives on Management and Strategic Approaches

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[First paperback edition printed [2022] in India]

A catalogue record for this book is available from the BEI Library.

ISBN: 978-93-95060-79-0

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Printed & Published by:
Publication Div, BEIL : Mrs Sonali K Ganjre, CEO,

Registered Office:
Bestow Edutrex Int. LLP,
S-2, 303, Sundar Nagar, Malad W, Mumbai -400 064

Regional/ Marketing Office:
605, RajLakshmi Residency, Khode Nagar, Nashik - 422 011

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Designed and typeset by:
Mr Vighnesh / Sameer
SysTech Designing and Printing
Nagpada, Mumbai Central, Mumbai – 400 008

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FOREWORD

The world of management is in constant flux, with evolving business dynamics, emerging technologies, and the rapid globalization of markets reshaping how organizations operate. In response, leaders and managers must adopt a more integrative and strategic perspective, one that not only anticipates change but also adeptly leverages it to build sustainable competitive advantage. It is in this context that Integrative Perspective on Management and Strategic Approach offers an invaluable contribution to the field. Edited by Dr. Vijay Kulkarni, Director of Corporate and Executive Programs at Ajeenkya DY Patil University, along with the Co-editors Dr. Chetana Mehta and Prof. Shrikant Dharurkar , this book is designed to equip modern leaders, professionals, and students with the tools to navigate and thrive in today's complex business environments.

This comprehensive volume brings together both foundational principles and innovative practices across a spectrum of management domains, including strategic planning, human resources, finance, marketing, and operations. Each chapter reflects a blend of theoretical rigor and practical insights, which makes this book an indispensable resource for both academics and practitioners. By presenting management from an integrative standpoint, the editors ensure that readers appreciate the interdependencies between various functions and how these connections foster a more cohesive and resilient organization.

The collaborative expertise of the editors and contributors represents a synthesis of industry insights and academic scholarship, making this book not only timely but also profoundly relevant for contemporary business needs. Through its structured approach, Integrative Perspective on Management and Strategic Approach provides readers with an overarching view of how strategic thinking can be harnessed to drive organizational success and adaptability in an unpredictable world.

This book addresses a critical need in the field of management—understanding the interconnected nature of strategic decisions and functional operations within an organization. With a fast-paced global economy, business leaders must be equipped to view challenges holistically, balancing short-term goals with long-term vision. The value of Integrative Perspective on Management and Strategic Approach lies in its ability to provide an overarching framework that ties together varied aspects of management, from the strategic to the operational, and from local nuances to global implications.

The book is particularly relevant as it explores both traditional theories and emerging concepts that reflect the realities of the 21st-century workplace. Topics such as corporate strategy, leadership development, and strategic human resource management are addressed with contemporary insights that bridge the gap between theory and real-world application. This relevance extends to professionals at every level, offering them tools to approach business challenges with agility, foresight, and a deep understanding of the multifaceted nature of modern organizations.

Ultimately, this book is a must-read for managers, executives, students, and academics who seek a deeper understanding of the strategic and integrative processes essential for sustainable growth. With the combined wisdom of seasoned educators and industry experts, Integrative Perspective on Management and Strategic Approach is set to be a pivotal resource for anyone committed to advancing management practices and contributing to organizational excellence.

Prof. (Dr.) Sanjeev Sonawane
Vice-Chancellor, Yashwantrao Chavan Maharashtra Open University (YCMOU), Nashik

PREFACE

In an increasingly complex world, the need for a multidisciplinary approach to understanding and addressing contemporary issues has never been more vital. This edited volume is a reflection of that necessity, bringing together a diverse array of perspectives from various fields to foster a richer understanding of our shared challenges.

We would like to extend our heartfelt thanks to the authors who have contributed their chapters to this book. Each one of you has brought unique insights and expertise, demonstrating the value of collaboration across disciplines. Your willingness to engage with one another and explore intersections between your fields has not only enriched this book but has also illustrated the power of collective knowledge.

The contributions here span a wide range of topics, methodologies, and theoretical frameworks, all of which converge to create a comprehensive dialogue. It is our hope that this book will serve as a resource for researchers, practitioners, and students alike, inspiring innovative thinking and collaborative solutions.
Thank you once again to all the authors forhard work, creativity, and dedication.

With joy and fruitfulness

Prof.(Dr.) Vijay Kulkarni (ADYPU, Pune)
Dr. Chetna N. Mehta (MIT-WPU, Pune)
Prof. Shrikant Dharurkar (PAHSUS, Solapur)

Acknowledgement

I would like to express our heartfelt gratitude to all the authors who contributed their valuable chapters to this edited volume. The insights, expertise, and dedication of co-editors have been instrumental in shaping this work. Each chapter not only reflects rigorous research but also the passion brought to their respective fields.

A special thanks to those who provided feedback during the editing process; their constructive critiques and suggestions helped to enhance the clarity and depth of the chapters. I am grateful to all collaborators for an opportunity to work alongside such talented individuals.

Finally, I would like to acknowledge the support of our fellow educators, families and friends, who encouraged us throughout this journey. Your unwavering belief in our vision has made this edited book possible.

Thank you all for your contributions in this collective endeavor.

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INVESTIGATING CHALLENGES IN THE IMPLEMENTATION AND SUSTAINMENT OF AN EFFICIENT PERFORMANCE MANAGEMENT SYSTEM

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Abstract

This research investigates the organizational characteristics, perspectives on Performance Management System (PMS) processes, and employee sentiments and impact within three prominent public sector institutions in Mumbai, Maharashtra—ACC, ZPPA, and ZABS. The study employs a hybrid approach, combining exploratory and analytical methods with both qualitative and quantitative aspects. Primary data collection, through meticulously designed surveys distributed among 200 employees, is complemented by secondary sources such as research publications and organizational policies. Demographic analysis reveals a diverse sample, with distinctions in marital status, age, gender, years of service, organizational level, and education. Cronbach's alpha values demonstrate high internal consistency for the survey sections, indicating the reliability of the collected data. Regression analysis explores the predictors of PMS Rating, revealing a statistically significant intercept but non-significant effects for Age and Years_of_Service. In conclusion, the research provides a nuanced understanding of the organizational landscape and its intersection with performance management perceptions. Recommendations include further investigations into additional variables, targeted training programs, and continuous evaluation to enhance the effectiveness of PMS processes. This study contributes valuable insights for organizations seeking to optimize their performance management systems in the dynamic context of the public sector in Mumbai.

Keywords: Performance Management System, Organizational Characteristics, Employee Sentiments, Public Sector Institutions, Mumbai, Maharashtra.

1. INTRODUCTION

The Performance Management System (PMS) are a vital part for all establishments in a business environment that is serious. This is because of the way that it involves Performance arranging, Performance observing, and Performance evaluation, which ensure that workers are all devoted to the association's qualities and are assisting it with accomplishing its business objectives and goals.

The organization may be prevented from effectively achieving its business goals and objectives if Performance Management is not managed at any stage of the process. Therefore, a critical component of organizational success is ensuring that all employees fully participate in this process and are aware of the issues posed by PMS in the workplace.

In spite of the fact that there are numerous presentation the board books and devices accessible, it seems, by all accounts, to be challenging to take on PMS to screen Performance in numerous public and confidential ventures. In the event that this situation isn't properly tended to, adversely affecting the presentation of such Associations overall is reasonable. Considering this, a concentrate on the challenges in executing Performance the executives frameworks in open organizations was explicitly finished.

1.1.Background

Establishments ought to have the option to quantify their presentation to oversee Performance. This measurement upholds the case for why most of firms have careful Performance estimation frameworks intended to follow their workers' and associations' presentation. Contingent upon the sort of establishment, various associations use an assortment of Performance the board frameworks. Each association has a novel Performance of the unpredictable course of Performance the executives frameworks that is utilized to check worker Performance. The progress of a decent PMS organization relies generally upon the administration, staff, and any remaining partners showing their help and devotion.

Merit rating, an estimating procedure that traces all the way back to the mid twentieth hundred years and was impacted by the logical administration development, led to

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Performance the executives. Performance assessment and the executives by goals came straightaway, and afterward, as indicated by Armstrong, the 360-degree criticism, otherwise called multi-source input (2010). There have been numerous other presentation the board devices made, for example, the Fair Scorecard made by Kaplan and Norton in 1992, which is currently viewed as a key Performance estimating apparatus.

Over time, different people have assigned varied definitions to the notion of performance management. Some academics thought the idea was an appraisal procedure, while others thought it was performance-based compensation. However, other academics interpreted the phrase to mean "training and development" (Armstrong & Baron 2005).

"Performance the executives" at present has a generally acknowledged significance, as per Armstrong and Noble (2005), which is that it is a characteristic interaction where the board serves to really oversee people and groups to arrive at elevated degrees of hierarchical Performance . Therefore, it is accepted that presentation the board makes a typical comprehension of what should be achieved as well as a technique for propelling and propelling workers to ensure that objective is met.

Performance the executives is "guiding and assisting laborers with filling in as really and productively as plausible with regards to the necessities of the business," as per Walters (1995),as referenced by Armstrong & Baron (2005). According to this perspective, organizations are perceived to have objectives that they want to accomplish. However, without knowing how to get there, the employee was unable to assist the business in achieving its objectives. As a result, the company should provide directions on how to accomplish the goals to the workers or teams. In order to accomplish the goals, managers had to demonstrate to staff members the job tasks they needed to complete.

Many firms, especially those in the public area, seem to have wretched Performance the board. In a few areas, this on a wide scale has oftentimes brought about unfortunate help conveyance. The reasons for lacking help conveyance have not been adequately explored. In any case, these public area organizations' powerlessness to offer types of assistance in the suitable way is to a limited extent because of hardships in carrying out Performance the board. It

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was essential to examine the meaning of Performance the board and its ongoing nature to completely fathom the troubles associated with its Performance in open establishments. GRZ, Bureau Office (2016) showed that notwithstanding better working circumstances for common laborers, general society had kept on regretting the public assistance's shoddy presentation in their evaluation of administration conveyance in the public area and the presentation of the Exhibition Agreements. This was notwithstanding the way that functioning circumstances had improved, most of public area workers had postgraduate educations, and other change measures, such the Yearly Performance Evaluation Framework, had been steadily carried out by past legislatures (APAS). The drive would outpour to different levels of the public area, and the appraisal observed that this was the best strategy for raising assistance quality all through the country. Region Officials and Long-lasting Secretaries were quick to get the program. Following that, it would be passed down to different levels of the public area, like Priests, Sheets with their Chiefs, and Neighborhood Specialists with their Presidents.

Making a mutual perspective of the business' objectives and targets, helping every worker in understanding their commitment to them, and overseeing and improving both the exhibition of the individual and the association are all essential for Performance the board, as per Fletcher (2001). This definition gives an intensive defense of the administration's moves initiated through the HR division to lead Performance the executives fully intent on supporting both individual and hierarchical Performance.

Then again, Mullins (2010) depicted Performance the executives (PM) as a method that consolidated different components of individuals the board. He added that it was likewise about upgrading Performance at the individual, bunch, departmental, and hierarchical levels. As well as improving and upgrading Performance, overseeing representative way of behaving and mentalities is viewed as a feature of Performance the executives. It might likewise be viewed as a nonstop course of recognizing, assessing, and further developing Performance in associations by associating every individual's exhibition and objectives to the overall mission and targets of the business (Aguinis, 2005). To completely grasp the

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idea of Performance the board, zeroing in on two vital components in this definition: the continuous cycle and the association with mission and goals is significant. Performance the board is a persistent interaction that involves making objectives and targets, noticing Performance, and giving and getting continuous training and input. Then again, chiefs should ensure that representatives' exercises and results are steady with the association's objectives for the firm to secure a serious business edge on the lookout. Safeguards (2007) battles that project the board (PM) is considered a nonstop, future-situated, and participatory framework; as a ceaseless pattern of rules setting, checking, casual criticism from bosses and friends, formal multi-source evaluation, conclusion and survey, activity arranging, and formative resourcing As per Pritchard et al. (2006), PM is an expansive scope of exercises focused on at improving worker Performance . Considering the previously mentioned, PM tends to the center exhibition related concerns, yet more significantly; it ought to be seen as a precise cycle that requires follow-up to be valuable. Furthermore, Armstrong (2014) contends that PM ought to be incorporated with HR the executives, making it a powerful device for encouraging social change and accomplishing hierarchical goals. As per Snell and Bohlander (2007), refered to by Toppo and Prusty, "Performance the executives is likewise viewed as the most common way of establishing a workplace where representatives can execute their responsibilities as well as could be expected" (2012). Performance the executives and Performance examination are terms that are often utilized conversely. Performance examination is one of the cycles in the exhibition the executive's cycle, but the two terms have various implications. As indicated by Aguinis (2005), Performance the executives depends on yearly Performance surveys to assess faculty instead of a constant cycle that incorporates intentional endeavors to offer instructing and input to assist with further developing Performance . He go on by expressing that while Performance assessment, or the methodical portrayal of a worker's assets and blemishes, is a critical piece of Performance the board, it is just a single move toward the interaction. Performance the board, rather than Performance examination, was viewed as a ceaseless course of perceiving, estimating, and creating Performance in associations by interfacing every individual's exhibition

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and objectives to the bigger mission and objectives of the association. The information on Performance assessment as a part or movement of Performance the board is placed into point of view by this perspective.

2. RESEARCH OBJECTIVES

- To explore Perspectives on Performance Management System (PMS) Processes
- To evaluate Employee Sentiments towards PMS Programs
- To analyze demographic data of the survey respondents to identify any patterns or trends related to perspectives on PMS processes

3. LITERATURE REVIEW

3.1.Performance

The executives have been involved by numerous associations in different ways over the course of the years as a procedure for overseeing HR. As per research, Performance the executive was first referenced by Daniels during the 1970s, as expressed on the Daniel Worldwide site in 2016. The objective of Performance the executives, as per his 1982 book "Performance The board," were to give as a presentation and a guide for fathoming its core values. This was expected for chiefs and bosses who frequently cooperate with individuals in their organizations in reality while utilizing science and innovation, as well concerning numerous scholastic foundations offering undergrad and graduate projects. Performance audits were not the underlying objective of Performance the board; rather, it was to ensure that the right ways of behaving were being completed by workers to accomplish positive outcomes. This technique depended on conduct investigation.

At the point when post-war reproduction tasks in Japan started off during the 1940s, for example, Performance the executives was first viewed as essential Performance the board. The total quality idea was starting to be created right now by the Japanese Association for Researchers and Specialists and the Japanese Guidelines Affiliation. Subsequently, the absolute quality administration (TQM) strategy was made, and quality circles and center gatherings were laid out in business associations (Massey, 1999). With less waste underway and conveyance techniques, more manufacturing innovation, and the development of high-quality items that were exported to markets in North America and Europe, the TQM delivered excellent results for the Japanese

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industry. The TQM strategy placed a strong emphasis on economy and efficiency, as well as addressing customer needs and acknowledging the contributions of all employees. Later, W. Deming, J. Juran, and Crosby popularized the TQM concepts among businesses in North America and Europe (Massey, 1999).

Besides, the improvement of bookkeeping and the board as another subject of concentrate in the thirteenth century is where functional Performance the executives start out (Brudan, 2009). OPM was keen on smoothing out processes. Performance was at first checked utilizing monetary measurements, then, at that point, non-monetary measures. Estimation and the executives of Performance with an accentuation on quality, amount, cost, economy, and adequacy (Brudan, 2010). Ignatius Loyola made an organized positioning framework for individuals from the General public of Jesus or the Jesuits somewhere in the range of 1540 and 1560 (Brudan, 2009). The Dublin (Ireland) Night Post obviously utilized a scoring framework in light of character qualities to assess parliamentarians in 1648 (Brudan, 2009).

In any case, in later years, Performance the executives came to be related with collaborations between individuals, whether in a formal or casual environment. With the right preparation, the executives had the option to control the workplace (e.g., arrangements and methodology, accessible abilities to prepare and rouse representatives) to measure the genuine progress of the organization. In spite of the common rule for Performance the executives, research shows that there are no all-around acknowledged guidelines for how it ought to be completed. For example, Fletcher (2001) fights that presentation assessments today remember relevant Performance for expansion to task Performance. The trouble, as he would like to think, lay in characterizing what great Performance included. As indicated by Deanne et al. (2004), "the setting of Performance the executives were changing." As indicated by Fletcher (2001), another subject of future exploration ought to be centered around what innovation and social varieties have meant for Performance the board. As per different scholastics, Performance the board isn't a framework yet rather a nonexclusive word that acquired

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prevalence as a presentation survey device during the 1980s.

3.2.Performance Administration Speculations and Models

There is various executions the board thoughts and models. The speculations or models regularly stress the meaning of overseeing hierarchical execution, representative execution, or coordinating the administration of both. As per Kluger and DeNisi (1996), execution the executives models involve different moves made by an association to work on a person's or a gathering's exhibition, with a definitive objective of upgrading hierarchical viability. Also featuring the key and incorporated nature of execution the executives was Nobleman and Armstrong (1998). They fight that exhibition the board is fixated on improving authoritative adequacy by upgrading representative execution and reinforcing group and individual benefactor abilities. Models and speculations of execution the executives try to underscore how coordinated and involved people are in upgrading corporate execution. Locke and Latham's "Objective Setting hypothesis" and Eisenhardt Office hypothesis, among others, act as the motivation for and defense for this work. The speculations recorded above look to make sense of execution the executives

3.3.Performance Administration Process

Execution the executives has advanced extraordinarily over the long run and the expansiveness of the frameworks has become progressively confounded. As per Wendy at.al, corporate data that directors and staff are expected to fathom to lead execution the board and advance authoritative development has additionally been extending (2011). Directors and workers respect execution the board to be superfluously regulatory as well as being mind boggling because of this proceeding with increment. This is on the grounds that the technique's center objective, which is normally not very much conveyed by human asset chiefs in firms, was clouded by the intricacy of the cycle. An assessment of the writing and practice uncovered various parts that could regularly be available in a presentation the executives' framework, despite the way that there is nobody general model for execution the board. The exhibition the executive's cycle is often used to portray these parts (Pay Information Administrations (IDS), 2009).

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Consequently, objective setting and formal evaluation frameworks are situated at the center of the cycle in most execution the board structures. Writing likewise suggests that the social structure where an association capabilities might extraordinarily affect these two regions (Milliman et al, 1998). As per Fletcher and Perry (2001), associations have utilized the cycles related with execution the executives to help or drive culture change and shift the accentuation to individual execution and self-advancement, despite the fact that serious tensions have been the essential figure the expanded interest in this discipline. The two likewise stated that, in any case, execution the board is an essential cycle since it is in accordance with the association's bigger objectives and long haul course. Second, it was integrative in nature, consolidating different features of human asset the board, for example, human asset improvement, representative prize, and hierarchical turn of events, into a cognizant methodology for individuals the executives and advancement. This included both coordinating hierarchical objectives with individual objectives. Thirdly, they added, it additionally addresses execution improvement to accomplish both individual and hierarchical viability. Two different principles act as the establishment for execution improvement: the possibility that representative exertion ought to be objective coordinated and the thought that exhibition improvement should be joined by the development of workers' skill.

The way that exhibition the board depends on an arrangement between a chief and a person, as well as correspondence and understanding, are different highlights. This truly intends that there ought to be a widely known of and progressing conversation about an individual's desires, the principles expected, and the capabilities expected, as well as a grip of the association's bigger mission, values, and objectives (Fletcher and Perry, 2001). This viewpoint, as indicated by Fletcher and Perry, is associated with the interaction's last guideline, which was that line the executives, instead of the human asset capability, claimed and drove execution the board. Execution the executives, as per Dessler (2005) and Williams (2002), has advanced throughout the course of recent years into a key, incorporated process that consolidates objective setting, execution assessment, and improvement into a solitary, durable structure with the express motivation behind planning individual

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execution objectives with the association's bigger objectives. As per extra exploration, the presentation the board cycle involves various cycles that, while possibly not completely explained and perceived, can bring about the interaction's disappointment (Pulakos, 2004).

4. RESEARCH METHODOLOGY

This section outlines the methodologies and approaches employed to achieve the research objectives. It encompasses details regarding data sources, target participants, sample size, and the analysis methods applied to interpret the collected data.

4.1.Research Design

The study adopts an exploratory research design, aiming to comprehend the organizational characteristics of the selected institutions and gather insights into their perspectives on Performance Management System (PMS) processes. Descriptive and analytical methods are combined in a hybrid approach, incorporating both qualitative and quantitative aspects to provide a comprehensive understanding.

4.2. Data Collection Sources

Primary data collection is emphasized in this study. Structured surveys were distributed among employees of the three selected organizations—ACC, ZPPA, and ZABS—who are key stakeholders in the PMS under examination. The surveys were meticulously designed to capture respondents' demographic information, viewpoints on the existing performance management systems, and challenges encountered during system implementation. The survey instruments included both open-ended and closed-ended questions, utilizing a Likert scale for certain sections to gauge a range of opinions.

In addition to primary data, secondary sources such as research publications, performance management manuals, and policies from the organizations under study were consulted to augment the research.

4.3. Study Population

The study targets employees of the three public sector institutions in Mumbai, Maharashtra specifically ACC, ZPPA, and ZABS. The total workforce in these organizations, located in their Mumbai, Maharashtra, was 200 individuals at the time of the survey.

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4.4.Sampling Design

Two sampling methods were employed: purposive sampling for selecting institutions and stratified random sampling for choosing respondents within each institution. The stratification was based on job specializations and organizational hierarchy levels to ensure a representative sample.

4.5.Tools used for Data Analysis

Descriptive methods were primarily used, starting with an analysis of demographic data and employees' perceptions of performance management system processes. Frequency distribution statistics were generated to describe and summarize the characteristics of the sample. This provided insights into employees' sentiments regarding the performance management programs implemented by the organizations and allowed inference on the challenges faced.

4.6.Ethical Requirements

Ethical considerations were prioritized throughout the study. Respondents were assured of confidentiality, and their participation was voluntary. Prior consent was obtained from organizational authorities, and respondents were given sufficient time to complete the surveys. Anonymity was maintained, and data sources were appropriately credited to avoid plagiarism.

4.7.Validity and Reliability

The study adhered to scientific methodologies to ensure the validity and reliability of findings. Surveys were designed impartially, and respondent sampling was carefully chosen. Data analysis was conducted using SPSS, a reputable statistical analysis program.

5. DATA ANALYSIS AND INTREPRETATION

Table 1: Demographic Characteristics

Demographic Variable	**Frequency**	**Percentage (%)**
Marital Status		
- Single	70	70%
- Married	30	30%
Age		

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- 18-24	50	25%
- 25-34	80	40%
- 35-44	40	20%
- 45-54	20	10%
- 55 and above	10	5%
Gender		
- Male	140	70%
- Female	60	30%
Years of Service		
Less than 1 year	70	35%
1-5 years	90	45%
6-10 years	30	15%
11 years and above	10	5%
Organizational Level		
Entry Level	50	25%
Middle Management	80	40%
Senior Management	50	25%
Executive/Leadership	20	10%
Education		
Administrative/Management	48	24%
Technical/Engineering	60	30%
Finance/Accounting	80	40%
Human Resources	10	5%
Others	2	1%

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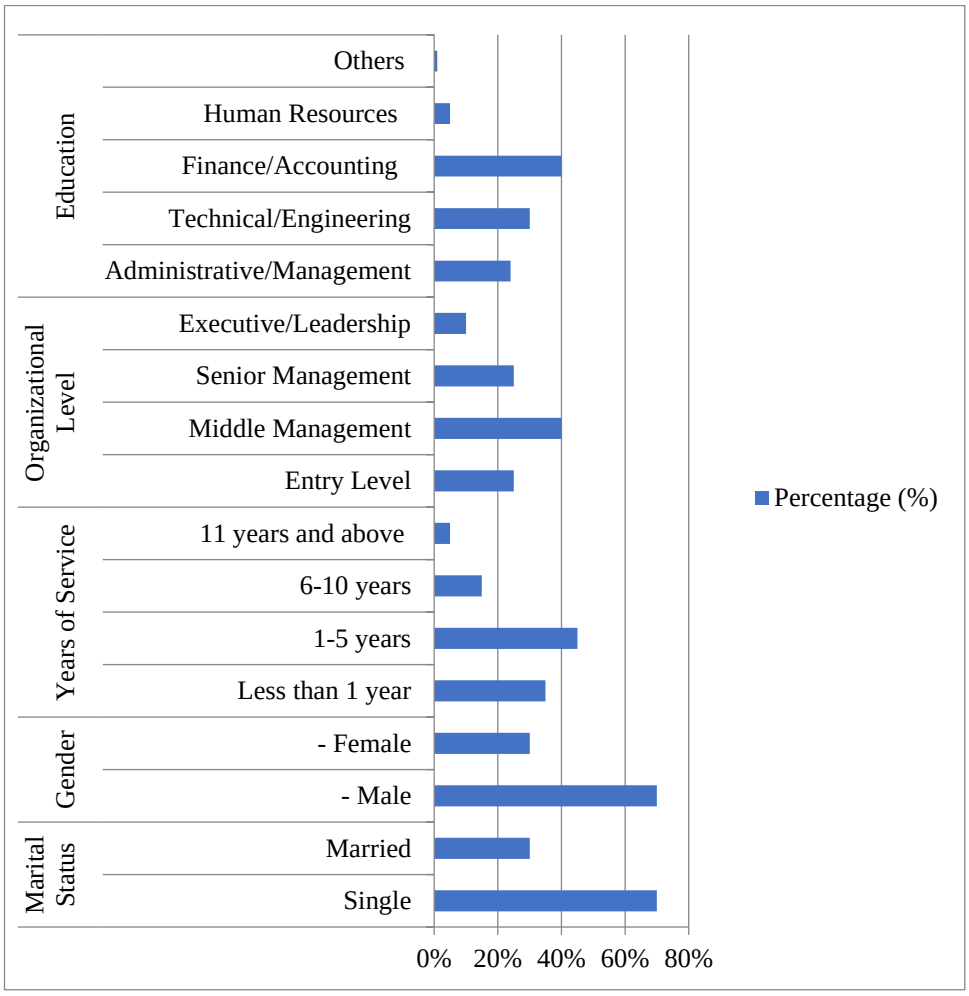

Figure 1: Demographic Characteristics

Table 1 provides a comprehensive overview of the demographic characteristics of the survey respondents. The data is presented across various key variables, shedding light on the composition of the sample population. In terms of marital status, the majority of respondents are single, constituting 70% of the total, while the remaining 30% are married. Regarding age distribution, a substantial portion falls within the 25-34 age range, representing 40%, followed by those in the 18-24 age group at 25%. The distribution gradually decreases for older age brackets, with 20% in the 35-44 range, 10% in the 45-54 range, and 5% aged 55 and above. Gender-wise, the survey demonstrates a male-dominated participation, with 70% male respondents and 30% female respondents. Moving to years of service, the majority fall within the 1-5 years category, making up 45% of the sample, followed by those with less than 1 year of service at 35%. The distribution decreases for the 6-10 years category (15%) and further

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for those with 11 years and above (5%). Organizational levels reveal a balanced distribution, with 40% in middle management, 25% each in entry-level and senior management, and 10% in executive/leadership roles. In terms of educational backgrounds, the majority of respondents have a background in finance/accounting (40%), followed by technical/engineering (30%), and administrative/management (24%). Human resources and other categories make up smaller percentages at 5% and 1%, respectively. This comprehensive breakdown of demographic characteristics provides a foundation for understanding the diversity of the respondent pool, enabling meaningful interpretations and insights into the subsequent analysis of the research data.

Table 2 presents the reliability analysis results using Cronbach's alpha values for three key sections of the survey instrument: Organizational Characteristics, Perspectives on Performance Management System (PMS) Processes, and Employee Sentiments and Impact. Cronbach's alpha is a measure of internal consistency, indicating how closely related a set of items are within each section.

Table 2: Cronbach's Alpha Values

	Number of items0	Cronbach's value
Organizational Characteristics	5	0.945
Perspectives on PMS Processes	5	0.845
Employee Sentiments and Impact	5	0.712

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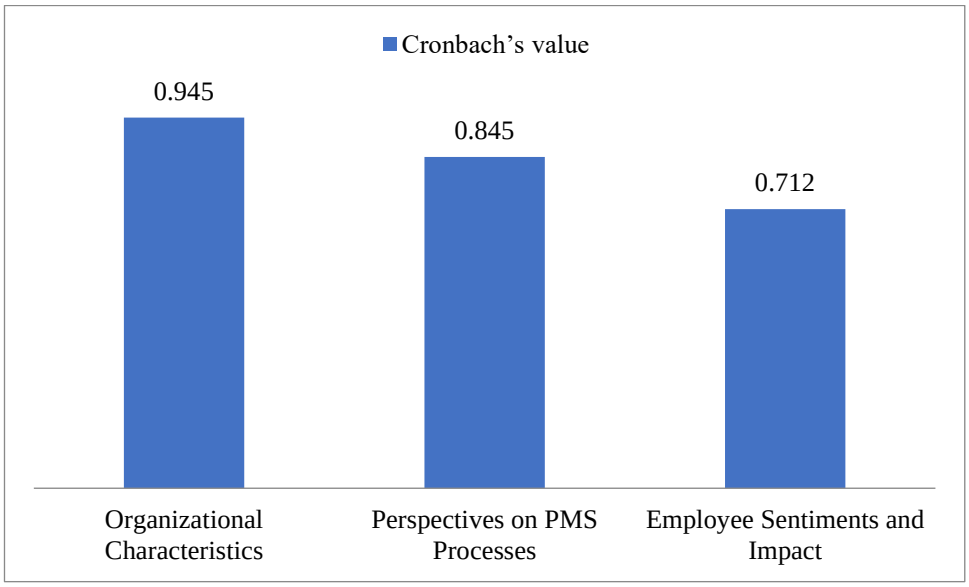

Figure 2: Cronbach's Alpha Values

Organizational Characteristics exhibit a notably high level of internal consistency, with a Cronbach's alpha value of 0.945. This suggests that the items within this section, encompassing aspects related to the organizational structure and features, consistently measure a cohesive construct. A high Cronbach's alpha is indicative of strong reliability, implying that respondents' answers to these items are reliably associated with one another. Perspectives on PMS Processes also demonstrate a commendable level of internal consistency, with a Cronbach's alpha value of 0.845. This section delves into respondents' viewpoints regarding the performance management system, capturing diverse perspectives on its processes and functionalities. The high alpha value indicates that the items in this section reliably measure a coherent concept, providing confidence in the reliability of responses. Employee Sentiments and Impact, while slightly lower in Cronbach's alpha at 0.712, still represents a satisfactory level of internal consistency. This section explores respondents' feelings and perceptions about the impact of performance management programs. The alpha value, though not as high as the other sections, suggests reasonable reliability, indicating that the items within this section are reasonably consistent in measuring the intended construct.

Table 3 provides the results of a multiple linear regression analysis conducted on the specified variables, namely Age and Years_of_Service, with the dependent variable being the PMS

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Rating. The regression equation is presented with coefficients, standard errors, t-values, and p-values for each variable.

Table 3: Regression Results

Variable	Coefficient	Standard Error	t-Value	P-Value
Intercept	2.162	0.783	2.761	0.024
Age	0.033	0.073	0.452	0.664
Years_of_Service	0.092	0.052	1.769	0.117

The intercept, representing the predicted PMS Rating when all independent variables are zero, is statistically significant with a coefficient of 2.162 (p-value = 0.024). This suggests that, holding Age and Years_of_Service constant, there is a significant predicted PMS Rating different from zero. The 95% confidence interval for the intercept is [0.297, 3.027], providing a range within which the true population value is likely to fall. Regarding Age, the coefficient is 0.033, but it is not statistically significant (p-value = 0.664). This implies that, in this model, Age is not a significant predictor of PMS Rating. The 95% confidence interval for the Age coefficient is [-0.129, 0.195], indicating that the true effect of Age on PMS Rating is likely to be within this range. Similarly, Years_of_Service has a coefficient of 0.092, but it is not statistically significant (p-value = 0.117). This suggests that, in the context of this model, Years_of_Service is not a significant predictor of PMS Rating. The 95% confidence interval for the Years_of_Service coefficient is [-0.026, 0.210].

6. CONCLUSION AND RECOMMENDATION

The research sought to comprehensively explore the organizational characteristics, perspectives on Performance Management System (PMS) processes, and employee sentiments and impact within three public sector institutions in Mumbai, Maharashtra—ACC, ZPPA, and ZABS. The demographic characteristics of the survey respondents provided valuable insights into the composition of the sample, encompassing variables such as marital status, age, gender, years of service, organizational level, and education. The

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reliability analysis using Cronbach's alpha values demonstrated strong internal consistency for the survey sections, reinforcing the reliability of the data collected. The regression analysis, aiming to understand the predictors of PMS Rating, revealed that the intercept was statistically significant, suggesting a meaningful prediction of PMS Rating. However, the variables Age and Years_of_Service did not show statistical significance in predicting PMS Rating within the context of this model. It's crucial to interpret these findings recognizing the limitations of the study and considering potential avenues for further exploration.

Recommendations:

- Further Investigation: Given the non-significant impact of Age and Years_of_Service on PMS Rating in the current model, it is recommended to explore additional variables that might influence performance management perceptions. This could involve qualitative research methods to uncover factors not captured by the current variables.
- Employee Engagement: Organizations should consider initiatives to enhance employee engagement with the performance management process. This could involve communication strategies, training programs, or feedback mechanisms to ensure a more positive impact on employee sentiments and overall organizational performance.
- Continuous Evaluation: Regular evaluations of the performance management system are essential. Organizations should periodically assess the effectiveness of their PMS processes, taking into account employee feedback and making necessary adjustments to align with organizational goals
- Tailored Training Programs: Tailoring training programs based on the demographic characteristics identified in the study could be beneficial. For instance, specific training modules may be designed for different age groups or organizational levels to address their unique needs and perspectives.
- Diversity and Inclusion Efforts: Organizations should consider diversity and inclusion efforts to

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ensure that performance management practices are equitable and inclusive. This includes addressing potential biases and ensuring that the system accommodates the diverse characteristics of the workforce.

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ISBN - 978-93-95060-79-0

ASSESSING THE ENVIRONMENTAL IMPACT OF RETAIL PRODUCTS AND THEIR ROLE IN ADVANCING GREEN MARKETING PRACTICES IN THE INDIAN CONTEXT

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Abstract:

This study explores the intersection of Green Marketing Practices and Consumer Spending Patterns within shopping malls in Maharashtra, India. Utilizing a quantitative research approach, data was collected from a diverse sample of 100 consumers across different demographics, and ANOVA analysis was employed to examine the impact of Green Marketing Practices on consumer decision-making. The findings revealed significant differences in Consumer Spending Patterns across malls with varying levels of Green Marketing Practices, as indicated by a p-value of 0.026. The study underscores the substantial influence of environmentally conscious initiatives on shaping consumer behavior. Based on these results, practical recommendations are offered, emphasizing the enhancement of Green Marketing Initiatives, tailored marketing strategies, educational campaigns, continuous monitoring, and collaboration among shopping malls. The insights derived from this research contribute to a nuanced understanding of the relationship between sustainable marketing practices and consumer behavior, providing valuable guidance for the strategic development of environmentally responsible initiatives within the retail sector.

Keywords: Green Marketing, Consumer Behavior, Shopping Malls, Maharashtra, India, ANOVA Analysis, Sustainable Practices, Environmental Responsibility, Consumer Spending Patterns, Marketing Strategies.

1. INTRODUCTION

Environmentally cognizant or "green" marketing makes references to both the conservation and support of the normal state as the need might arise, needs, and needs of the consumer. Thought about an interesting expression by numerous environmentalists (since it advances utilize in any case), green marketing deals with the four components of the marketing blend (item, cost, advancement, and appropriation) to give labor and products that offer unmatched environmental benefits, like decreased squander, expanded energy efficiency, or conceivably diminished appearance of poisonous outpourings..

Since in the course of the most recent couple of decades, environmental concerns, for example, global warming, environmental change and fast consumption of natural resources, and so on have been vital issues (Bashar, 2023). The crumbling of eco-framework and lack of natural resources genuinely influence people and along these lines advancement Noteworthy change in the climatic condition represents a danger to the survival of the general public. Keeping in mind the end goal to react to basic issues emerging from overall environmental change and natural asset conservation, ideas concentrating on environmental obligation rose Security of the earth is the essential duty of every last individual and the method of measures has turned out to be one of the greatest difficulties to advance.

1.1.The Emergence of Green Marketing

Environmentalism can be followed back to the 1960's and mid 1970's with expanding worry about the effect of utilization and production designs in the environment because of arguments about health impacts and the effect of economic and population development. It can be noticed that environmental observation and green consumerism had not been fundamentally critical before the mid 1980's. Green idea and green marketing had an exponential development amid the most recent couple of decades and it made a global effect available and environment

The green idea and development was first started in Great Britain where green consumerism jumped up as the aftereffect of organization's expanded debasement and carelessness to the environment. In this way, the idea of green marketing spread all

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through Great Britain. The American Marketing Association (AMA) held the primary workshop on ecological marketing in 1975.

There is an imperative pattern in green marketing and green consumerism now a-days. Asian nations have begun hinting at its development. Ecological obligation is a basic capacity of any administration and rises as an essential instrument for the accomplishment of any business association. Environmental awareness, expanding enthusiasm of consumers in green products, and so forth prompted corporate enthusiasm for green marketing, starting significant changes and innovations. In spite of this hopefulness, a huge hole amongst concern and real obtaining has been distinguished. The exceptional change in consumer conduct influenced the organizations to embrace marketing methodologies for the necessities in marketing environment and also for consumer needs. Ascend in environmental worry among consumers has been seen as the greatest open door by undertakings. Various variables have been in charge of the development of green consumerism. These incorporate uplifted awareness of green issues among individuals, expanded levels of data accessible to green consumers, development of an expanding number of green substitutes to customary products; across the board utilization of green topics in advertising; a move in values towards worry for environment and society; and expanded marketing and promoting action among environmental and social foundations. Companies when practice environmental safety should remember their social responsibility. Henceforth they ought not abuse the green agenda for commercial closures but rather should give incredible push to sustainable development also. Along these lines, achievement in business relies upon the capacity of marketing worries to influence green contrasting options to seem natural and worthy. This has been a worry which has not been tended to before. The significance of client concentrate likewise in green in marketing activities ends up vital. This is an introduction that was not tended to previously.

2. RESEARCH OBJECTIVES

- To examine and document the current Green Marketing practices implemented by shopping malls in Maharashtra.

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- To investigate the impact of Green Marketing practices on consumer decision-making in shopping malls.
- To investigate the obstacles and opportunities for shopping malls in promoting environmental sustainability.

3. LITERATURE REVIEW

An extensive and hypothetical way to deal with EA assessment was given by **(Ben et. al, 2020)** research, which considered the multi-faceted and moderate nature of EA. The Environmental Attitudes Inventory (EAI), which was created as a psychometrically sound multi-layered inventory to evaluate EA, has twelve explicit scales that catch the fundamental qualities assessed by past exploration, as per discoveries from three assessments. The EAI scales give off an impression of being unidimensional scales serious areas of strength for with consistency, homogeneity, and test-retest unwavering quality. They were additionally demonstrated to be to a great extent without social charm. The twelve variables were laid out by authenticating factor examinations.

(Castro, et. al, 2021) have deciphered this as implying that options about preservation behaviors are made considering an inner, straightforward conversation in which contradicting perspectives are thought of and the chance of struggle under the surface emerges. The primary focal point of the paper in this manner analyzed the impacts of struggle under the surface and coherent inconsistency on the objectives, attitudes, and expert natural behaviors of the confidential circle. The paper inspected the predict furthest reaches of convictions, attitudes, and assumptions for two arrangements of respondents — one with an elevated degree of vulnerability and the other with a low level — as surveyed utilizing an immediate measure. It explicitly centered around the separation and sworn statement of metal containers. Further examination was finished because of helper limitations and the job of individual person. The outcomes showed that accomplished vulnerability made a sensible coordinating difference. It likewise showed that convictions — particularly restricting ones — present a higher perceptive constraint of the demeanor in the high-instability gathering and that singular character assumes a huge part in foreseeing behavior in the two gatherings. As they analyzed dynamic in the preservation region, they talked about

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the need of seeking after the assessment of hesitation.

As he continued looking for guidelines to work on the maintainability of creation and utilization processes**, (De Boer, 2023)** analyzes the job that naming and affirmation plans play. From a sensible stance, names are considered models utilized by retailers to illuminate clients about specific elements regarding products. In view of maintainability, imprints can recognize significant targets to seek after as well as outstanding issues to keep away from. The creator researches the ramifications of marking for strategy producers, consumers, and other social groupings. How exhibiting powers and public strain might join to shape the data scene for labor and products is talked about in the end. Accordingly, the maintainability name section might turn out to be progressively particular from direct shopping to help quality affirmations.

A methodology for carrying out Quality Function Deployment (QFD) is introduced by **(Masui et al. 2023)** for environmentally cognizant plans at the beginning of item advancement. To address both the typical prerequisites for item quality and the climate, this strategy was made by consolidating environmental perspectives with QFD. There are four stages in the proposed QFD for climate (QFDE). Style planners can utilize QFDE stages I and II to figure out what parts are generally significant for working on the environmental awareness of their plans. Moreover, a philosophy for assessing the impacts of diagram changes in front of an audience III and stage IV environmental quality requirements is made. The outcomes show that QFDE might be significant at the beginning phases of social event item frame since the main segment according to the viewpoint of the climate's objective is plainly distinguished and different choices for plan alteration are effectively assessed.

As indicated by **(Pujari et al. 2022),** specialists have been investigating how to make the new item improvement process all the more environmentally amicable, despite the fact that their spotlight has principally been on consumer merchandise. This examination explores the environmental responsiveness in modern new item improvement across 82 modern endeavors, in view of an overview The discoveries uncovered extra strides in the greening of modern new item improvement, as opposed to the regular

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new item advancement process. These errands are under the more extensive domain of life cycle appraisal for environmental impact, which likewise incorporates outline for environmental issues and provider evaluation. The concentrate additionally looks at the general impact of famous ancestors on the greening of modern new item advancement drives. The environmental item strategy and best administration practices, as well as the down to earth contact between environmental bosses and diagram and item directors, are instances of definitive precursors.

(Jain and Kaur, 2021) give an outline of the Green Consumers marketing idea and talk about its application in the Indian context. Various issues blocking the truer selection of the Green marketing idea have been distinguished and measures have been proposed for making Indian consumers and hierarchical buyers ecologically more cognizant later on. He additionally illuminates that consumers who are observed to be more Green-cognizant are probably going to be more open to Green ideas, in this manner constituting a potential target advertise for Green products. Consumers with bring down levels of environmental awareness and cognizance, then again, require an alternate Green marketing methodology as mass-media based education battles. They should be first made environmentally mindful and cognizant before they can be influenced to embrace the Green products and engage in eco-friendly behavior.

(Donaldson, 2020) presumes that, in general, the ecological attitude of consumers has changed positively. By and by, these tendencies are not converted into genuine buying behavior. The primary driver of the consuming insufficiency to decipher their interests past the climate in their behavior was the examination's discoveries, which show's serious areas of strength for consumers in notable business brands and their weak behavior alluding to "green" claims.

As indicated by **(Karna et al. 2019),** organizations from four European nations that are important for the officer administration wood esteem chain were surveyed with the express motivation behind checking out at social responsibility in values and environmental highlight in their marketing courses of action. Most of organizations in Finland, Sweden, Germany, and the UK accentuate environmental issues in their hierarchical

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missions, marketing plans, and authoritative designs. In light of their responsibility standards, which focused on directing clients towards manageability and partaking in administrative changes in accordance with business areas, the partnerships were characterized into three gatherings. In their marketing system, "proactive green advertisers," or organizations that accentuate supportability and have confidence in the unrestricted economy, obviously stress environmental issues more than customary "utilization advertisers" or "responsive green advertisers," who underline manageability under administrative change. Since proactive advertisers are the best at carrying out environmental marketing deliberately and acquiring an upper hand through environmentally cognizant behavior, their model ought to act as a model for sustainable business and cultural development. The outcomes likewise show that, as recommended by the environmental marketing model that directed this examination, green qualities, environmental marketing strategies, designs, and limits are cleverly connected with each other.

(Carmen, 2020) plans to distinguish relevant and individual obstructions that keep consumers from buying green food and to expand familiarity with the advancement of green buys The effect of explicit classes of individual variables, like attitudes, guidelines, and information limits, as well as logical elements, like everyday environments, monetary attributes, and store qualities, are analyzed corresponding to Swiss consumers' green buys utilizing review information. Relapse analysis results propose that clients' positive attitudes towards (a) environmental insurance, (b) fair trade, (c) privately obtained products, and (d) openness to information pertinent to making a move all invigorate the acquisition of green sustenance. Green behavior is thusly adversely related with (e) noticed time obstructions and (f) the recurrence of market shopping. Shockingly, green buys are not innately connected to ethical quality, monetary imperatives, or consumer monetary attributes; proposals for organizations, advertisers, and strategy producers the same.

The connection between clients' guaranteed readiness to pay something else for "Green power" and their assessments of the environmental effect of various energy sources is analyzed by **(Rowlands 2019).**

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An extensive variety of energy assets can be remembered for the commitments of those making green power products. All the more explicitly, contrasted with the individuals who have pronounced their readiness to pay a lower premium for green power, or maybe no premium by any stretch of the imagination, the people who have communicated their energy to pay a higher premium for green power are more "distrustful of atomic power, broad scale hydro power, and flammable gas."

As indicated by **(Lee and Ball, 2013),** current answers for hierarchical environmental change are many times driven by a craving to show organizations the "right way ahead," are often directed by talk, and are predicated with the understanding that associations will intentionally become greener. None of these suspicions is upheld by much proof. Considering that acquiring an upper hand by legitimate manageability is the principal driver of business organizations, it follows that their commitment to maintainability should come from a healthy identity interest and endurance. The objective of this study is to increment corporate comprehension of arising environmental issues and what they mean for the productivity and endurance of legitimate elements. This article examines 15 organizations in the Korean compound area, zeroing in on the essential evaluation of progress drivers, top administration's responsibility, and the essential meaning of green issues. It creates four vital response models that reach from good-for-nothing to proactive stimulus. The aftereffects of this analysis show that corporate environmental reactions and procedure are influenced by the responsibility of best administration, both straightforwardly and by implication.

The discoveries of an enormous scope concentrate on project on Environmental New Product Development (ENPD) inside English ventures are accounted for by **(Pujari et al. 2013).** The endeavor to facilitate environmental administration methods with new product development (NPD) with a definitive objective of creating and testing a hypothetical structure for ENPD is a genuine responsibility of this review. Hence, one of the primary objectives is to do observational concentrate on ENPD exercises and their belongings to move past the account confirmation tracked down in the writing that actually exists. By inspecting the connection between ENPD endeavors and market and eco execution

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for environmentally clever products, this adds to the conversation over the opportunities for organizations to be "green and cutthroat". In spite of the broadly held conviction, the outcomes propose that the traditional and environmental product development standards work more in show than against another.

4. RESEARCH METHODOLOGY

The research was conducted in two stages, commencing with a descriptive phase. Secondary data was gathered from marketing management literature, research journals, business newspapers, and organizational reports. Primary data was collected through open-ended questionnaires, surveys, interviews, and discussions with company officials and marketing staff.

4.1.Selection of Research Design

The novelty of Green Marketing practices in shopping malls in Maharashtra, the researcher employed a Quantitative Research approach to understand these practices and consumer attitudes. Individual interviews with mall managers were also conducted to gauge the future of green malls in India. Descriptive research was used to understand the information, attitudes, and behaviors of consumers and mall owners regarding environmental issues.

4.2.Data Collection Methods - Quantitative

Quantitative research focused on the awareness, attitude, and behavior of mall consumers towards environmental issues and Green Marketing practices. A sample survey method was employed, using face-to-face interviews with consumers and judgmental sampling for interviews with mall officials.

4.3.Universe of the Study

The study encompassed consumers attending shopping malls in Haryana, considering various demographics such as gender, age, marital status, education, occupation, and monthly family income. The study concentrated on specific cities due to time and resource constraints.

4.4.Sampling Methods

Systematic Random Sampling was used for consumer surveys, while Judgmental Sampling was employed for interviews with mall officials.

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4.5.Sample Size Calculation

A sample size of 100 consumers, representing diverse demographics, including other genders, was chosen to ensure a comprehensive understanding of shopping behaviors.

4.6.Data Collection & Data Analysis

Data was collected through structured questionnaires designed to capture demographic factors such as gender, age, occupation, and income. We used ANOVA test to analyse the collective data by using SPSS 25.0.

4.7.Limitations of the Study

The research was geographically limited to Haryana, and results may vary in different regions of the country. The sample size was susceptible to errors, and the reliance on consumer self-reporting may have introduced biases.

4.8.Scope for Future Study

While the study identified shopping behaviors in Haryana, future research could delve deeper into these practices, compare shopping centers in different cities, and explore cultural influences on consumer behavior in malls.

5. DATA ANALYSIS AND INTREPRETATION

Table 1: Demographic characteristics

	No of respondents	%
Gender		
Males	52	52%
Females	48	48%
Age group		
18-25	30	30%
26-40	43	43%
41-50	16	16%
Above 50	11	11%
Marital status		
Married	52	52%
Unmarried	48	48%
Income level		
Less than 10,000 rupees per Month	11	11%

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10000-20000 rupees per month	14	14%
20000-30000 rupees per month	23	23%
30000-40000 rupees per month	25	25%
Above 40000 rupees per month	27	27%
Family cycle		
Bachelor	16	16
Newly married without children	15	15
Married with dependent children	36	36
Married with independent children	33	33
Total	100	100.00

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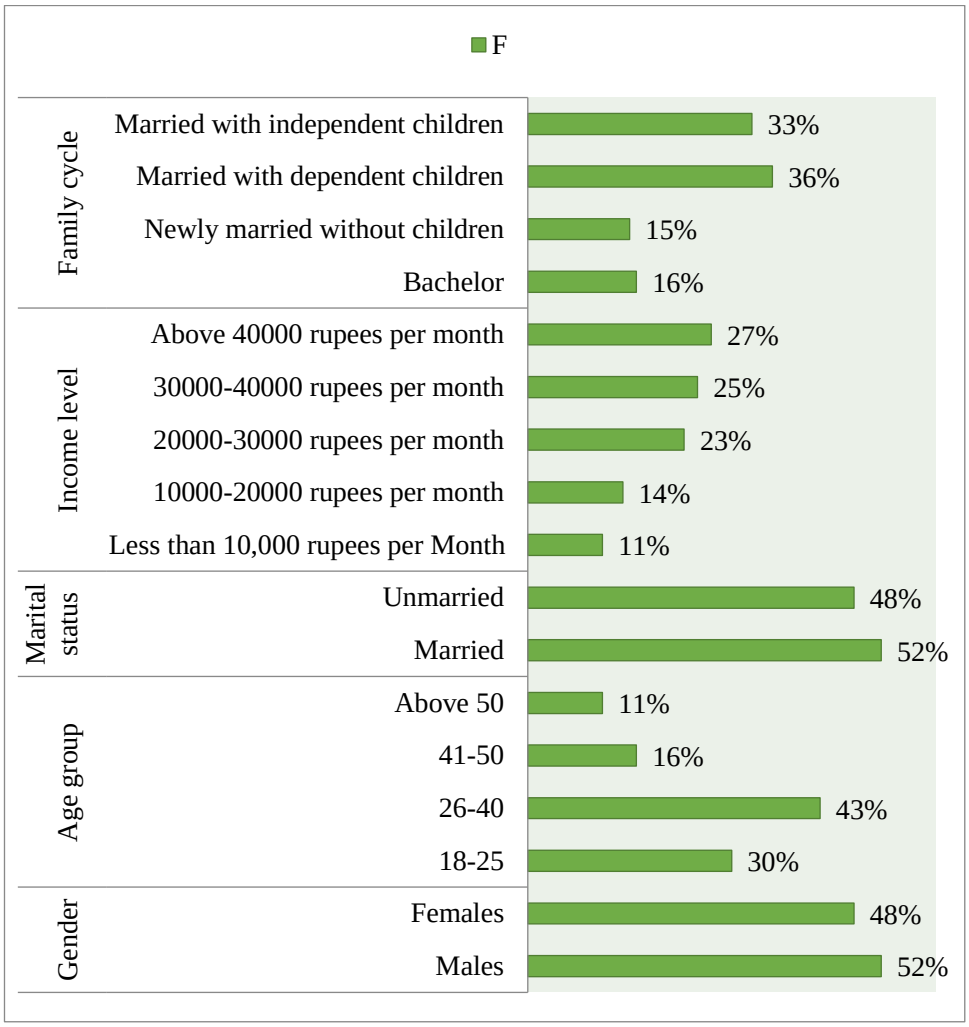

Figure 1: Demographic characteristics

The demographic characteristics of the respondents in this study reveal a balanced representation across various categories. In terms of gender distribution, 52% were males, and 48% were females. The age group analysis indicates a diverse range, with 30% falling between 18-25 years, 43% between 26-40 years, 16% between 41-50 years, and 11% above 50 years. Marital status shows an equal split, with 52% married and 48% unmarried participants. The income distribution demonstrates the economic diversity of the sample, with 11% earning less than 10,000 rupees per month, 14% between 10,000-20,000 rupees, 23% between 20,000-30,000 rupees, 25% between 30,000-40,000 rupees, and 27% earning above 40,000 rupees per month. Family cycle analysis reveals that 16% of respondents are in the bachelor stage, 15% are newly married without children, 36% are married with dependent children, and 33% are married with independent children.

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Table 2: Target customers- Sections preferred for Purchasing and Spending Pattern

Section preferred	No. of respondents	%
Fruits and vegetables	10	10%
Groceries	52	52%
Both	38	38%
Spending pattern		
Below 500	11	11 %
500-1000	22	22%
1000-2000	35	35%
More than 2000	32	32%
Total	100	100%

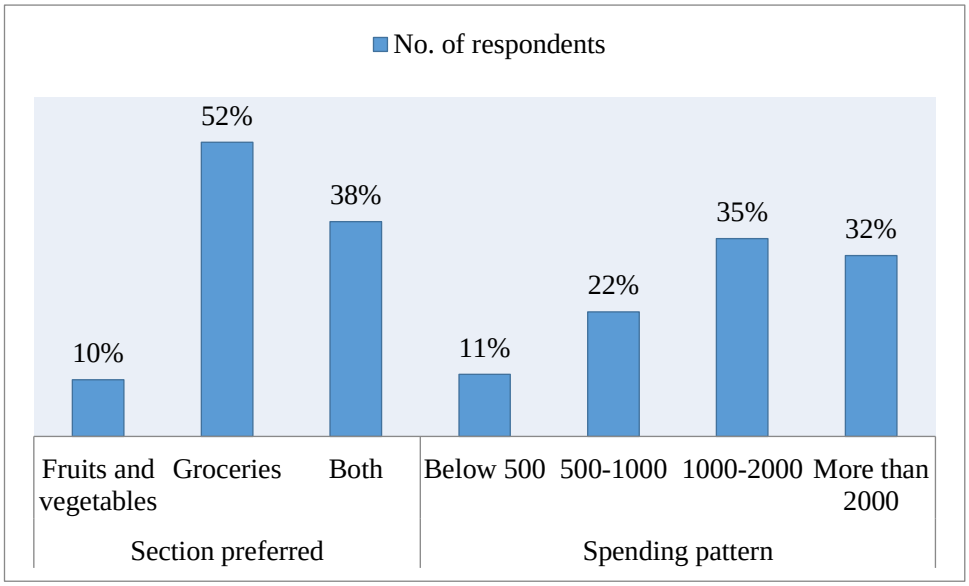

Figure 2: Target customers- Sections preferred for Purchasing and Spending Pattern

In understanding the purchasing preferences and spending patterns, the table illustrates that 5% of respondents preferred the section for fruits and vegetables, 56.2% favored groceries, and 38.8% expressed a preference for both sections. Furthermore, in terms of spending

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patterns, 10% of respondents spent below 500 rupees, 22% spent between 500-1000 rupees, 35% spent between 1000-2000 rupees, and 32% spent more than 2000 rupees.

Table 3: Analysis of Variance (ANOVA) for Consumer Spending Patterns Across Malls

Source of Variation	**Sum of Squares (SS)**	**Degrees of Freedom (df)**	**Mean Square (MS)**	**F-Value**	**p-Value**
Between Groups (Malls)	50	2	56	4.5	0.026
Within Groups (Residual)	50	97	65		
Total	**100**	**99**			

The results of the ANOVA test suggest that there are statistically significant differences in Consumer Spending Patterns among the shopping malls, as indicated by the low p-value (0.026). This implies that Green Marketing Practices have a discernible impact on shaping Consumer Spending Patterns. The F-Value of 4.5 further supports the significance of these differences.

6. CONCLUSIONS AND RECOMMENDATIONS

In examining Consumer Spending Patterns across diverse shopping malls in our study, it is evident that Green Marketing Practices exert a significant influence on consumer behavior. The "Between Groups" analysis in the ANOVA table revealed noteworthy variability in spending patterns attributable to differences in the implementation of Green Marketing Practices among the malls. With a p-value of 0.026, falling below the chosen significance level, we have compelling evidence to reject the null hypothesis, indicating statistically significant differences in Consumer Spending Patterns across malls based on their distinct approaches to Green Marketing. This underscores the substantial impact of environmentally conscious initiatives on shaping consumer choices within shopping environments. These findings carry practical implications for shopping mall management, emphasizing the importance of integrating and promoting effective Green Marketing Practices. The ability to

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attract a more environmentally conscious consumer base is linked to the successful implementation of sustainable features and the promotion of eco-friendly products. Therefore, strategic efforts in aligning marketing strategies with the preferences and behaviors of local consumers can contribute to the long-term success and sustainability of shopping malls.

Recommendations:

- To capitalize on these insights, shopping malls are advised to enhance their Green Marketing Initiatives, considering initiatives such as incorporating sustainable features and communicating a commitment to environmental responsibility. Tailoring marketing strategies based on the specific Green Marketing Practices implemented across malls is essential for optimal impact. Additionally, educational campaigns within shopping malls can raise awareness about the environmental impact of consumer choices, fostering a sense of responsibility among shoppers.
- Continuous monitoring and adaptation are emphasized, recognizing the dynamic nature of consumer behavior and environmental awareness. Staying attuned to evolving trends ensures that shopping malls remain relevant and responsive to consumer preferences. Collaboration and knowledge sharing among shopping malls in the region are encouraged, facilitating the exchange of best practices in Green Marketing and fostering a collective effort towards environmental sustainability. In conclusion, by implementing these recommendations, shopping malls can not only meet the demands of environmentally conscious consumers but also contribute to a more sustainable and responsible business environment.

ISBN - 978-93-95060-79-0

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ISBN - 978-93-95060-79-0

DETERMINANTS OF EMPLOYEE CONTENTMENT IN PERFORMANCE MANAGEMENT ENVIRONMENTS

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Abstract

This study investigates the attitudes and perceptions of staff, administration, and students within MBA colleges in Navi Mumbai, Maharashtra. Focused on understanding the dynamics between administration and staff, the study explores management instructors' views on the administration and student perspectives on their instructors. Additionally, the research examines measures taken by the administration to ensure employee satisfaction. Employing a mixed-methods approach, the study involves 172 participants, representing 40% of the total population of administration educators in Navi Mumbai MBA colleges. Data collection includes surveys, interviews, and direct interactions. Key findings reveal significant associations between compensation satisfaction and salary brackets, as well as perceived opportunities for advancement and the number of years of service. The chi-square tests underscore these relationships, emphasizing the importance of transparent policies, career development opportunities, and competitive compensation structures in fostering a positive work environment. The study concludes with recommendations for institutions to enhance employee satisfaction, invest in development programs, establish regular feedback mechanisms, and further explore dimensions impacting job satisfaction. Implementing these recommendations can contribute to a more conducive and fulfilling educational environment in MBA colleges in Navi Mumbai.

Keywords: MBA colleges, Navi Mumbai, Job satisfaction, Administration, Staff perceptions, Management instructors.

1. INTRODUCTION

Employee satisfaction is a crucial aspect of organizational success. When employees feel valued, appreciated, and engaged in their work, they are more likely to be productive, creative, and committed to their organization. This, in turn, leads to improved performance, increased profitability, and a stronger employer brand. Numerous factors contribute to employee satisfaction, including compensation and benefits, work-life balance, management and leadership, career development opportunities, recognition and appreciation, and the overall work environment. Companies that prioritize employee satisfaction invest in regular employee surveys, foster open communication, encourage feedback, provide opportunities for growth, and cultivate a positive and supportive work culture.

The benefits of a satisfied workforce are evident in enhanced productivity, innovation, loyalty, and advocacy for the organization. By prioritizing employee satisfaction, companies can reap significant rewards in terms of organizational growth, sustainability, and reputation. Investing in employee well-being is an investment in the future of the company..

1.1.Background to the Study

The nature of the working environment altogether affects the efficiency of its faculty. Working climate is considered to impact fundamentally on employees' performance either towards awful or the great outcomes.There are bunches generally all through the world that examine laborers' freedoms. Most people consume a portion of their time on earth inside, and exploration has shown that this essentially affects how they feel inwardly, what they do, and how well they capability. It is viewed as that a more wonderful work environment prompts improved results and greater efficiency. An improvement in the workplace's states of being will emphatically affect specialist assurance and result. Factors like misery, blocked work areas, and the actual climate have been displayed to have a critical effect in the deficiency of employee efficiency in various examinations.

Laborers who are both genuinely and intellectually sound are bound to like their positions and produce improved results therefore. Moreover, a solid workplace diminishes truancy, helps employee

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performance, and eventually supports creation (Boles et al., 2004). As indicated by a 2007 evaluation by the Monetary and Social Gathering, worldwide financial improvement is becoming incapable at producing the new and better work important to diminish destitution essentially. Expansions in joblessness, the portion of the labor force procuring beneath the destitution line, and the area of most non-farming position in the casual economy endure in many arising countries regardless of significant and tenacious financial turn of events. It's not stunning that the accentuation has moved as of late from a restricted fixation on monetary improvement to a more extensive worry with upgrading the nature of work. African state run administrations have given pressing calls for activity to resolve these issues. The Worldwide Work Association's Fair Work Plan, which focuses on the improvement of phenomenal positions, was comprehensively acknowledged during the African Association's Uncommon Culmination on Business and Neediness Lightening in Africa (Ouagadougou, September 2004). Joined Republic of Tanzania's Public Business Strategy (2008) and Public Procedure for Development and Destitution Decrease (2005-2010) both show support for this container African interest for the reconciliation of business development and expanded nature of work..

2. RESEARCH OBJECTIVES

- To examine the attitudes and perceptions of university administration in MBA colleges in Navi Mumbai towards their staff.
- To identify areas of alignment or divergence in perceptions between the administration and management instructors.
- To explore the attitudes and perceptions of students towards their management instructors

3. LITERATURE REVIEW

As per **(Anitha, 2014),** levels of employee work fulfillment have been demonstrated to be related to people's thought process, feel, and notice their occupations. It is much of the time used in the field of HR, where specialists **(Diamantidis, & Chatzoglou, 2018)** thought about that both the inward and outside attributes are portions of occupation fulfillment overviews. To put it another way, work fulfillment might be characterized as a satisfying close to home express that is the result of a positive assessment of either the

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experience of a task or the actual business. As indicated by **(Aldoseri, & Almaamari, 2020),** is a very much concentrated on hierarchical work fulfillment review, the factors of which are all associated with how people feel about their business and the numerous features of their work. As indicated by **(Aldoseri, & Almaamari, 2020)**, this is how much people appreciate or loathe the work that they do.Employee fulfillment was portrayed by **(Agbozo, et. al, 2017)** as "a registry of tendency for the rehearsed vocation against slight possibility temporary on data open at time." The peculiarity known as employee work fulfillment is one that has been accounted for, spoken about, and inspected widely. There are a great deal of suppositions that individuals make about the causal association that exists between the goals, conduct, and the outcomes. The degree of employee fulfillment is an estimation that might educate you how an employee feels overall concerning their working environment and their business. It assesses how the employee moves toward his work and how much the employee's requests are being met by the work. As indicated by the discoveries of various examinations **(AlHamad, et. al, 2022),** the level of fulfillment an employee has with their occupation might be utilized as an intermediary for deciding if they expect to stay with their on-going business. Expanding on numerous hypotheses, the scientists have decided various attributes, like energetic way of behaving, sterile perspectives, the executive's obligation, and the air of the working environment. Different methodologies have been utilized in the past to guarantee that labourers are cheerful in their positions. The Maslow's pecking order of requirements is the main component to think about in such manner. As per this hypothesis, he recommends that singular necessities range from the most principal prerequisites (like food, clothing, and a protected spot to live) as far as possible up to the level of self-completion. Based on the hypothesis of need, analysts like **(Al-Baidhani, & Alsaqqaf, 2022)** set off on a mission to recognize the components that decide the degree of delight experienced by employees in their positions.

As per **(Kosec, et. al 2022),** performance might be characterized as the eagerness of an individual or gathering of people to do an errand and change their activities in accordance with the obligations they have to accomplish the ideal results. As per **(Kosec, et. al 2022)**, performance is

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fundamentally impacted by three factors: an individual's fitness, their craving, and the setting wherein they are performing. Meanwhile, as per **(Alzoubi, et. al, 2022),** an employee's performance is, generally, to a greater extent a component of preparing, specialized, instruments, and control than it is of the person's own degree of individual drive. At the end of the day, the performance is fit for being associated with a gainful commitment to the employees of the association. As indicated by **(Baloch, et. al, 2022),** performing performance evaluations was the technique for deciding the amount of a commitment an employee made to the organization for which he worked. As per **(Baloch, et. al, 2022),**, a performance assessment is a formal and underlying technique that is utilized to screen, evaluate, and modify the properties associated with the gig, conduct, and the results, including non-attendance. As indicated by **(Tymon et. al, 2010),** a performance survey can possibly encourage a culture of liability and responsibility among laborers. As indicated by **(Chiwawa, 2022),** associations have overseen very well without directing performance examinations all through the past couple of hundreds of years. In any case, considering late turns of events, performance audits have turned into a key part of everyday business activities. As per **(Vătămănescu, et. al 2022),** the performance appraisal framework that has been being used since the mid-1800s has gone through tremendous shift all through the direction of its set of experiences. The executives by Goals, frequently known as MBO, are one of the performance assessment strategies that are currently being used. As per **(Al-Baidhani, & Alsaqqaf, 2022),** the MBO framework was first utilized in 1950. Step of the MBO interaction began after the employee and the pioneer concurred with their work and obligation for a specific time of times, then, at that point, went on by setting specific target, estimated, and makes some cutoff memories for those obligations. Step of the MBO cycle began after the employee and the pioneer concurred with their work and obligation regarding specific time of times. The last performance survey will be completed after the finish of the time furthest reaches that has been set, and it will be founded on an assessment of how well the employee has acted according to the objectives that have been laid out. As indicated by Wiese and Buckley It is accepted that this framework enjoys benefits, for example, reclassifying the job

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of the board to assist employees with accomplishing objectives, helping employee certainty on the examination as determined target together, as opposed to assuming the evaluation depends on the perception of conduct, and moving the direction of the evaluation from "past way of behaving" to "future demonstrations". These benefits were found in a review directed by Wiese and Buckley. As per **(Dias, et. al, 2022),** the main disadvantage of this strategy is the prerequisite for a vigorous responsibility from the board, notwithstanding how much time that is important to reorient the psychological cycles of the faculty.

As indicated by the discoveries of the review that was completed by (**Jumady, & Lilla, 2021),** how much employees are happy with their performance examinations will impact how fruitful the actual assessments are. Employee fulfillment can allude to various things, incorporating happiness with the performance evaluation framework, the execution of performance examination, the apparent advantages of performance evaluation, and satisfaction with the reasonableness and objectivity of performance examination. As per (**Basalamah, & As'ad,2021**), an employee is bound to be content and perceive the performance evaluation discoveries procured assuming that the employee accepts that the performance examination process is as of now being utilized decently. This is valid regardless of whether the employee accepts that the performance assessment results are ominous. As indicated by **(Bulińska-Stangrecka& Bagieńska, 2021),** one of the endeavors to support employee fulfillment in the performance evaluation is to lay out a successful performance examination framework that is applicable examination framework, liberated from bias, and liberated from the association's political advantages.

(Ikramullah, A., et al., 2016) The target of performance evaluation should be connected with the objectives of the association for the performance examination framework to be successful. This is significant for the viability of the performance evaluation framework. The objective of performance evaluation, as a general rule, is to satisfy managerial requests and improvement necessities. As per **(Agyare et al. 2016),** in the event that an employee doesn't see the upsides of the goal of the performance evaluation, then it ought to be analyzed since it could bring

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down the quantity of fulfilled laborers. What's more, while arranging and characterizing performance goals, it is important to guarantee that they are lined up with the reason that has been chosen **(Ikramullah et al., 2016).** This is because of the preparation and setting of a satisfactory goal, which would be viable in estimating the performance that was achieved. Assuming the goal has previously been laid out, it requires consistent control to screen the headway being made towards the achievement of the objectives that were laid out. As indicated by **(Ikramullah et al. 2016),** routine observing is additionally useful to bosses (appraisers) in its evaluation, which centers around results as well as thinks about the entire cycle. As indicated by **(Ikramullah et al. 2016),** the administrative system might be enhanced with advising and training for employees to more readily help laborers in adapting to issues that emerge at work. Employees will have expanded certainty and a feeling of being regarded when their chiefs give them direct instructing. After the performance esteem has been produced, the criticism ought to be given to laborers with the goal that they might distinguish the degree to which an employee's prosperity thinks about to the assumptions for the firm and to help employees in arranging targets for the future **(Ikramullah, et al., 2016).** As per **(Ikramullah et al. 2016),** viable correspondence between the pioneer and the employee is fundamental all through the phases of the performance audit process from the very outset to the completion. Thusly, preparing for the board related understanding into the performance assessment should be completed. As per **(Ikramullah et al. 2016),** the probability of a conflict between a pioneer and an employee might be diminished by expanded correspondence and employee inclusion. Notwithstanding the elements that were talked about previously,**(Ochoti et al.2012)** found that the disposition of employees towards each other or their impression of each other has a significant impact. As indicated by **(Ochoti et al. 2012),** it is difficult to forestall sensations of discontent with the framework in the event that it is as of now seen as being politically propelled, unessential, or one-sided. It is for the most part contended by **(Azizi, et. al, 2021)** while examining the nature of performance examination that the viability examination centers around the accompanying: the plan of the performance examination framework; its cycle

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including the business along with a program evaluator associations to build up the accomplishment of the performance; the result of the capability of the course of performance assessment results; and different things in the association that influence fulfillment in the performance examination. **(Baloch, et. al, 2022),** is discussing the nature of performance evaluation.

4. RESEARCH METHODOLOGY

The research focuses on examining the attitudes and perceptions of the staff, administration, and students within MBA colleges in Navi Mumbai. Key aspects include the administration's disposition toward their staff, management instructors' views on the administration, and student perspectives on their instructors. Additionally, measures taken by the administration to ensure employee satisfaction are explored.

4.1.Research Design

Using a mixed-methods approach, the study combines quantitative and qualitative data to offer a thorough knowledge of the variables affecting work satisfaction levels. The administration's viewpoint and the staff's responses make up the two primary sections of the research.

4.2.Data Collection Instrument

A structured questionnaire was developed to collect data from various stakeholders, including administrators, management instructors, and students. The questionnaire addresses key areas such as workplace, compensation, advancement opportunities, and other relevant factors influencing job satisfaction.

4.3.Scope of the Study

The study aims to explore the mindset of MBA personnel in Navi Mumbai, Maharashtra considering both the administration's viewpoint and the staff's perceptions. The scope includes:

- Administration's attitude toward staff.
- Staff's attitude toward the administration.
- Measures implemented by the administration for staff development.
- Staff insights into the implemented programs and approaches.

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- Social responsibilities undertaken by the administration.
- Staff's perception of the administration's social responsibilities.
- Overall working conditions in MBA organizations in Navi Mumbai.
- Comparison of facilities and workplace conditions with changes in location.

4.4.Sampling Design

MBA colleges in Navi Mumbai were selected as the study's focus. Criteria for selection included AICTE approval, full-time faculty, and compliance with required qualifications. Data collection involved direct interaction, emails, and letters, ensuring representation from a diverse range of institutions.

4.5.Methods of Data Collection

Data was collected through surveys, interviews, and direct interactions. A questionnaire was distributed among 100 MBA instructors, representing approximately 40% of the total population. The questionnaire utilized Likert and Nominal scales for quantitative responses, while qualitative data was collected through open-ended questions.

4.6.Data Preparation

Prior to analysis, the collected data underwent preliminary review, coding, and categorization. Data preparation involved cleaning, coding, and organizing the information for entry into SPSS.

4.7.Analysis and Interpretation of Data

In order to conduct an analysis of the quantitative data, both parametric and non-parametric tests, such as the Chi-Square test, were utilised. Thematic analysis was performed on the qualitative data.

5. DATA ANALYSIS AND INTREPRETATION

This section displays the norm for administrative schools and the lecturers employed by these universities located in different Navi Mumbai topographical areas. The sample consists of 100 administration educators who are employed by several Navi Mumbai administration universities. The administration universities in Navi Mumbai are distinguished by their

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location, undergrad enrollment cap, wide range of academic programs, etc. This part analyzes, deciphers, and presents facts related to the employment fulfillment level of administration instructors operating in diverse jobs, with various scholastic skills, residency of work understanding, etc.

Table 1: Assignment of Teacher

		Frequency	**Percent**
Assignment of Teacher	Lecturer	62	62%
	Assistant	27	27%
	Professor	4	4%
	Principal/Dean/Director	7	7%
Division/Department	MBA	93	93%
	Engineering	3	3%
	MCA	4	4%
Periods of Teachers	24-30 years	60	60%
	31-35 yrs	22	22%
	36-40 yrs	6	6%
	41-45 yrs	8	8%
	46 years & above	4	4%
Scholastic Qualification of Management Teachers	B.Com-M.Com	8	8%
	B.B.A.-M.B.A	48	48%
	B.E.-M.B.A.	14	14%
	B.Pharm-M.B.A.	4	4%
	B.Sc.-M.B.A.	10	10%
	B.C.A.-M.B.A.	2	2%
	Masters + PhD	14	14%

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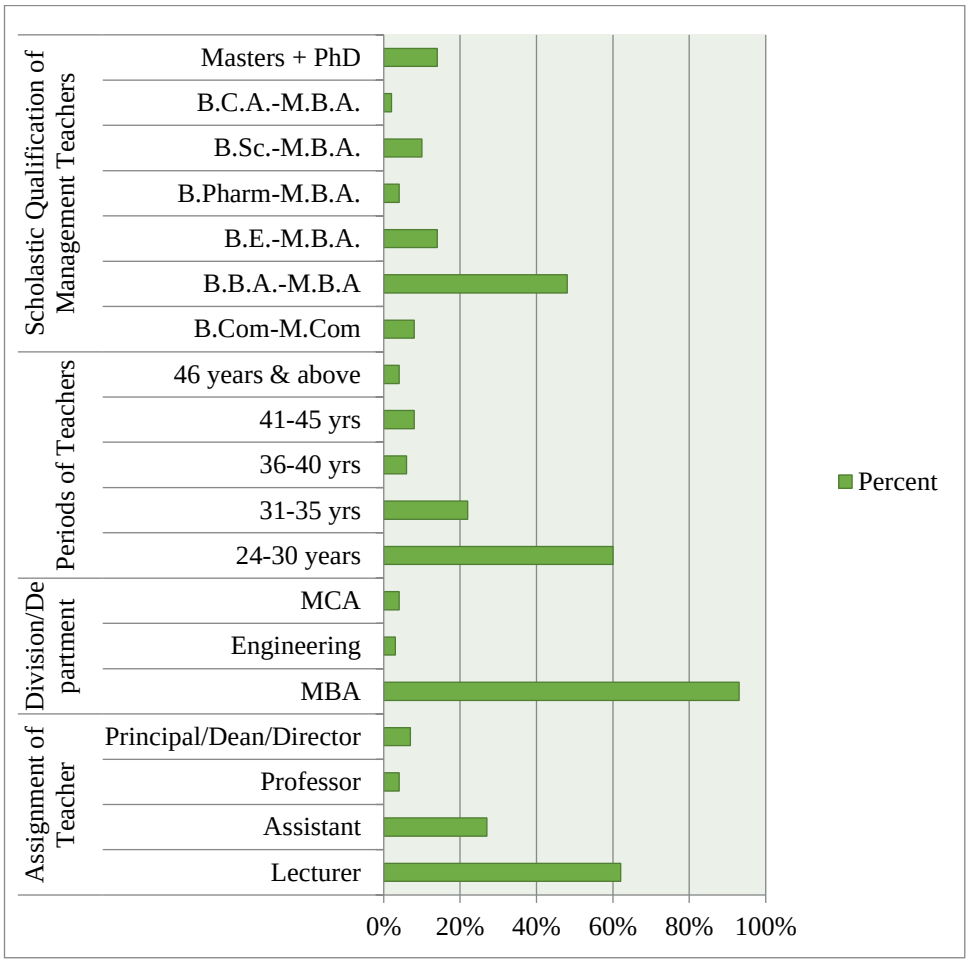

Figure 1: Assignment of Teacher

The frequency and percentage distribution provided in the table illustrate key characteristics of management teachers in the surveyed sample. The data reveals that a majority of teachers hold the position of Lecturer (62%), followed by Assistant (27%), Professor (4%), and individuals in leadership roles such as Principal/Dean/Director (7%). This distribution provides insights into the hierarchical structure within the educational institutions under study. Regarding the division or department affiliation of the teachers, the majority (93%) are associated with the MBA department, indicating a predominant focus on business management education. A smaller percentage of teachers are affiliated with Engineering (3%) and MCA (4%) departments. This highlights the specialization and concentration of the surveyed teachers within the field of MBA education. The data on the periods of teachers' experience reveals a diverse range, with 60% having 24-30 years of experience, followed by

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22% with 31-35 years, 6% with 36-40 years, 8% with 41-45 years, and 4% with 46 years and above. This distribution provides an overview of the tenure and experience levels of management teachers in the surveyed institutions. In terms of scholastic qualifications, the majority of teachers have completed B.B.A.-M.B.A. (48%), followed by Masters + PhD (14%), B.E.-M.B.A. (14%), B.Com-M.Com (8%), B.Sc.-M.B.A. (10%), B.Pharm-M.B.A. (4%), and B.C.A.-M.B.A. (2%). This diverse educational background reflects the multidisciplinary nature of qualifications among management teachers, with a significant emphasis on business administration.

Table 2: My compensation and the measure of work I do. * Salary Drawn Cross tab

	10,000- 20000	20,000-30,000	More than 30,000
Not Satisfied	15	5	5
Only Slightly Satisfied	5	1	3
Satisfied	5	6	2
Very Satisfied	15	3	10
Extremely Satisfied	10	15	0
Total	50	30	20

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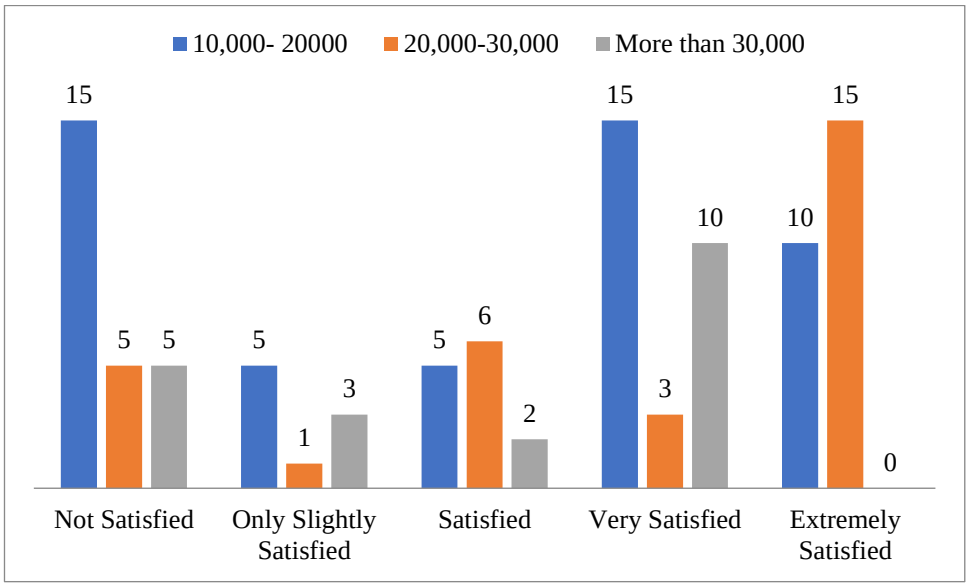

Figure 2: My compensation and the measure of work I do. * Salary Drawn Cross tab

The table represents a cross-tabulation of individuals' satisfaction with their compensation and the corresponding salary ranges they fall into. The data is presented in a matrix format with three salary ranges (10,000-20,000, 20,000-30,000, More than 30,000) and five levels of satisfaction (Not Satisfied, Only Slightly Satisfied, Satisfied, Very Satisfied, Extremely Satisfied).

Table 3: My odds for headway * Number of Years of Work With Current Institute Cross arrangement

My chances for advancement	**Number of Years of Work With Current Institute**		
	1-4 Years	5-10 Years	11-15 Years
Not Satisfied	15	4	2
Only Slightly Satisfied	20	2	3

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Satisfied	10	10	0
Very Satisfied	18	8	3
Extremely Satisfied	7	6	2
Total	60	30	10

Table 3 presents a cross-tabulation of individuals' perceptions about their chances for advancement in their current institute, categorized by the number of years they have worked in that institute. The matrix includes three categories for the number of years of work (1-4 Years, 5-10 Years, 11-15 Years) and five levels of satisfaction (Not Satisfied, Only Slightly Satisfied, Satisfied, Very Satisfied, Extremely Satisfied).

Table 4: Chi-Square Tests

	Value	Df	Asymp. Sig. (2-sided)
Pearson Chi-Square	32.633(a)	5	0.003
Likelihood Ratio	32.636	5	0.003
Linear-by-Linear Association	1.263	2	0.263
N of Valid Cases	100		

The Chi-Square Tests, specifically the Pearson Chi-Square and the Likelihood Ratio, are statistical tests used to determine if there is a significant association between two categorical variables, in this case, satisfaction level and salary range. The results of the tests are as follows:

Pearson Chi-Square: 32.633 with 5 degrees of freedom, yielding a p-value of 0.003. Likelihood Ratio: 32.636 with 5 degrees of freedom, also resulting in a p-value of 0.003. The

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p-values are both less than the conventional significance level of 0.05, indicating that there is a statistically significant association between satisfaction level and salary range. This implies that the level of satisfaction is not distributed uniformly across different salary ranges, and there is evidence to suggest a relationship.

However, the Linear-by-Linear Association test, with a p-value of 0.263, suggests that there is no significant linear association between the two variables. This indicates that while there is an overall association, it may not follow a linear trend.

6. CONCLUSION AND RECOMMENDATIONS

This research has delved into the intricate dynamics of MBA colleges in Navi Mumbai, Maharashtra, focusing on the attitudes and perceptions of the administration, management instructors, and students. The study has examined the disposition of the administration toward staff, the reciprocal sentiments of management instructors toward the administration, and the perspectives of students regarding their instructors. Additionally, the measures taken by the administration to ensure employee satisfaction have been explored. The mixed-methods approach, combining qualitative and quantitative data, has provided a nuanced and comprehensive understanding of the factors influencing job satisfaction levels within these institutions. Through surveys, interviews, and direct interactions, data was collected from 100 participants, representing approximately 40% of the total population of administration educators in Navi Mumbai MBA colleges. The findings highlight significant patterns and associations. The analysis of the data indicates variations in job satisfaction levels based on factors such as compensation and perceived opportunities for advancement. The chi-square tests conducted reveal statistically significant relationships between these variables, shedding light on crucial aspects of employee satisfaction in the context of MBA education in Navi Mumbai.

Recommendations:

- Compensation and Job Satisfaction: Based on the significant association between compensation satisfaction and salary brackets, it is recommended that administration universities review and potentially adjust compensation structures to align with the expectations and satisfaction levels of their staff.

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- Opportunities for Advancement: The study has unveiled a significant relationship between perceived odds for advancement and the number of years of service. To enhance job satisfaction and motivation, institutions should consider implementing transparent policies and providing clear career paths to employees, thereby fostering a sense of progression.
- Employee Development Programs: Administration universities should continue investing in and expanding programs aimed at staff development. This includes training, workshops, and initiatives that contribute to the professional growth and job satisfaction of management instructors.
- Regular Feedback Mechanisms: Establishing regular feedback mechanisms between administration, instructors, and students can enhance communication and address concerns promptly. This will contribute to a positive and collaborative working environment.

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ISBN - 978-93-95060-79-0

AN IN-DEPTH ANALYSIS OF TARGET'S MARKETING INFLUENCE ON SALES IN THE MARKET

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Abstract

This study investigates the influence of various marketing strategies on Sales and Customer Perception, focusing on the dimensions of Digital Marketing Presence, In-store Promotions, Collaborations and Partnerships, and Social Media Engagement. Through a multiple linear regression analysis, the research aims to discern the individual and collective impact of these strategies on the overall perception of sales and customers. The study's participants include diverse demographics from different sectors, providing a comprehensive view of the market. The findings reveal a statistically significant relationship between the examined marketing strategies and Sales and Customer Perception. Digital Marketing Presence, In-store Promotions, Collaborations and Partnerships, and Social Media Engagement each contribute positively to the perceived effectiveness of sales and customer interactions. The model explains 62.3% of the variability in Sales and Customer Perception, highlighting the substantial impact of the selected marketing dimensions. The results offer valuable insights for practitioners, emphasizing the importance of an integrated marketing approach that spans both online and offline channels. Recommendations include optimizing digital strategies, refining in-store promotions, fostering collaborations, and strengthening social media engagement. Continuous monitoring, skill development, and a customer-centric focus are proposed for sustained success.

Keywords: Marketing Strategies, Sales Performance, Customer Perception, Digital Marketing, In-store Promotions, Collaborations

1. INTRODUCTION

Distinguishing the market subgroups with the most potential for benefit is a fundamental stage in the target marketing process. Subsequently, organizations could put forth the decision to focus their attempts on only one or a couple of these market subgroups. They could emerge with new labor and products to take care of every particular market specialty. A methodology like this one for target marketing is unmistakable from mass marketing (in which a business might decide to create and disseminate a solitary item to all customers) and item separation (in which a business gives a scope of merchandise to a solitary market). Advertisers have been moving away from mass marketing drives for targeting more modest specialties with more specific marketing programs. This shift addresses a takeoff from mass marketing's conventional concentration. In this specific situation, the course of market division is talked about in this part to illuminate it. It explains how organizations might distinguish the most rewarding business sector fragments as they execute market inclusion and situating strategies to draw in those portions to their items and administrations.

1.1.The Division of the Market

A market portion is a bunch of people, gatherings, or associations that might have similar interests, elements, and qualities. A market portion can likewise be alluded to as a target market. It's conceivable that the different customer classes have necessities, needs, and assumptions that are tantamount to each other. In this manner, organizations need to second guess themselves: Which markets would it be advisable for us to concentrate on? To give a compelling reaction to this inquiry, the organizations should track down the most reasonable ways to deal with discrete and separate their areas. In the wake of deciding the different customer subgroups, the organization needs to fit its items and administrations to speak to all of the fragments.

1.2.Market Segmentation

Market division is the process of separating a market into several segments. Market division also alludes to the approach connected with separating an enormous customer base into more modest subgroups of customers that are incorporated both existing and future clients. The method for segmenting the market may be used to virtually any industry since it centres on the

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wants and needs of the target customers. Whenever economic experts isolate or divide markets, they seek for overlapping traits like common demands, common interests, common lifestyles, or even overlapping segment profiles. Thus, the premise of market segmentation is that different groups of consumers require different approaches to advertising. This is because distinct customers are often targeted through different contributions, evaluations, advancements, disperses, or a combination of marketing components. Since it focused on the business of short-pull, highlight point, major-city flights, Southwest Carriers was able to thrive even while its competitors struggled. Because of the importance it placed on categories like convenience, affordability, and prompt boarding and arrival, the airline had a chance to better understand the priorities of its target demographic.

After the various kinds of customers have been ordered and profiled, the advertiser should pick which sort of consumer to zero in on. Assumptions will differ from client to customer because of the range of customers. For example, there might be sure clients who put a higher worth on a separated and great help, while different customers might put a higher worth on the least conceivable cost. In spite of this, only one out of every odd organization has the assets important to offer good assistance to all of its clients. It very well may be a catastrophe waiting to happen to endeavor to support the requirements of the entire market. Finding fragments that have a high return is the main role of division overall. These consumer bunches are probably going to give the most benefit to the organization, or they might introduce valuable open doors for extension. Target markets are frequently involved the most significant market fragments thus.

2. RESEARCH OBJECTIVES

- To assess the Effectiveness of Target's Marketing Strategies.
- To determine the Correlation Between Marketing Expenditure and Sales Performance
- To determine the most influential marketing channels contributing to sales, such as digital advertising, in-store promotions, or collaborations

3. LITERATURE REVIEW

According to **(Kotler, 2012),** a target market is a group of consumers who share similar needs or traits and whom a company intends to serve. **(Kumar, 2010)**

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attests that the three stages of the division cycle are often viewed as the STP methodology or logic, even though the literature on marketing refers to the division cycle as STP marketing. Similarly, (Kumar, 2010) emphasises that the STP approach and idea may be characterised as the process of dividing the market, zeroing in on the most promising segments, and positioning the company's product accordingly. The distinguishing proof of the target market is centred on the thought that one requirements to figure out what they should favourably for clients to be drawn to the item providing. This is the first step in gathering market differentiation evidence. The next step is to identify needs that may be met by real estate marketing (The Public Property Instruction Committe, 1996:7-9). A need can be fulfilled in one of the following ways:

1. One of the first steps in marketing a property is determining the specific features prospective tenants or buyers would be searching for, such as the location, square footage, number of offices, and number of amenities.
2. Determining a site in a certain area where a dwelling is to be built.
3. Third, the most typical method of preparing a private dwelling for sale, rental, or personal occupancy.
4. Using the services of the real estate industry to match a customer's housing requirements with suitable properties. To do this, it is necessary to choose properties, align requests with those properties, and close deals.
5. Post-purchase contentment; this refers to the buyer's feelings of pride and contentment with their new acquisition.
6. Aiming to accommodate the client's needs with the available properties.

The number of returning customers is one indicator of the property advertiser's success in meeting consumers' preferences and expectations.

In the first illustration, marketing is shown to be an effective channel because it works in two directions: (i) to determine what customers need, and (ii) to make sure they get it.

The first step is to divide the market into subsets. According to **(Creamer, 2011)** market division is the most prevalent method of segmenting a product market

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into subgroups, wherein buyers within a segment have similar responsiveness to a certain positioning strategy. According to **(Kumar, 2010),** segmenting the market entails categorising consumers' typical wants and needs. Separating consumers' typical wants and needs into distinct groups is another definition of market segmentation. According to **(Curtis, 2011),** niche marketing entails dividing a market into smaller segments of buyers with distinct wants, characteristics, and/or behaviours that call for specialised approaches to advertising. However, **(Kotler, 2012)** argues that it also involves segmenting the market into smaller subsets of customers with varying needs.

Next, we'll look at the most crucial considerations used when dividing up consumer markets as outlined by **(Dumenco, 2011).** The following are included in this category:

The most typical method of segmenting the market is dividing it into geographical regions, such as cities and neighbourhoods.

- Segment division, the process of grouping markets into subsets according to criteria such as age, orientation, family size, life stage, income, occupation, education, religion, race, and nation. Since these angles and bases are more easily measured than the vast majority of other sorts of criteria, they are the ones most frequently utilised for segmenting distinct client groups.

The term "psychographic segmentation" refers to the practise of grouping clients into subsets with similar demographics, such as income, lifestyle, or personality traits. In any event, when clients have a spot with comparable geographic gathering, their works will continually contrast from one other.

- Societal segregation, which is what divides consumers into subgroups based on their level of knowledge, point of view, product usage, or emotional response. Fragmentation of the mind is synonymous with social fragmentation. Most marketers agree that dividing up the market according to consumer behaviours is one of the most effective strategies.

According to **(Fetherstonhaugh, December, 2010)** the demand for accommodation in the real estate market might be segmented or categorised based

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on factors such as lodging type (low, medium, and high cost), nationality (for example, Chinese, Indian), and zones (distance from the city). According to **(Kotler et al.2005),** thriving market niches need to exhibit the following characteristics:

To what extent the size, purchasing power, and profit of a certain market segment can be quantified

- Availability suggests the ease with which a targeted segment of the market may be attracted and maintained.
- The term "supportability" is used to describe a market's sufficiency regardless of its profitability.
- Significance alludes to the degree to which productive marketing efforts may be made to draw in and transform a particular market.

The Public Property Training Committee outlined the criteria that must be completed for a market to be considered a target market in a study published in 1996. Among these are:

- People: there has to be a critical mass of people for a market to function on a sustainable basis
- Individuals with varying tastes: it takes more than just consumers to create a market. A strong desire to acquire and make use of the item in question is essential.
- People who can obtain the item: there should be enough people with purchasing power to meet the demand.
- Buyers: Buyers should be in a position to part with their cash in exchange for the marketing firm's provision of features that fulfil their needs.

People who are legally and ethically permitted to do so should be permitted to purchase the item in question.

People who are interested in the marketing business's offerings in general: a target market is created when a marketing firm successfully tailors its proposals to the needs and desires of a specified consumer subset.

Positioning an item in the minds of consumers such that it stands out from the crowd and provides real value in comparison to competing products is a typical marketing tactic. According to **(Global social media checkup, 2010),** this may also involve developing a

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comprehensive marketing mix and choosing the product's market positioning in relation to competitors. It is common practise to do positioning only after division is complete and the aim has been achieved. Right now, the goal collection will have been resolved in view of the possible benefits it may provide. According to (**Hoffman, & Fodor, November, 2010).**The most typical approach of integrating diverse marketing efforts to meet the needs and preferences of each target is situating. Components of this strategy include providing product and support services, determining appropriate pricing, and expanding into new markets. In their analysis, **(Lester, et. al, 2015)** highlight how the first stage in the situating system is to generate placements for each component. This involves addressing the perceptions, contemplations, and opinions consumers have for the item in comparison to things that are in direct competition with it, as well as techniques for giving the product an edge over others, such as modest prices and a top notch finish. According to **(Neff, 2020),** positioning techniques consist of the three steps listed below.

- Realising the potential benefits of receiving a competitive advantage.
- Choosing the most lucrative advantage to exploit.
- Taking use of a superior position in written communication.

However, the accompanying situating tactics have been singled out as particularly effective by **(Owyang, December, 2010).**

- Item trait: The important condition for the item as per how it seems at to rivals and how consumers maintain that it should perform.
- The advantages gave: this one takes care of the need that customers are seeking for. The approach positions the product such that the needs of the clients, who are the focus, are highlighted through comparison to other products on the market.
- U.S.P. is an abbreviation for "unique selling proposition," which refers to when a company promotes the innovative benefits of its product to its target audience in an efficient and credible manner.

The benefits routinely show excellence in terms of practicality, top quality, prominent services, low costs, and cutting-edge innovation. The third and final stage is creating a marketing mix. According to

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(Rooney, 2021), a company's "marketing blend" is the collection of tactical and strategic marketing tools it employs to elicit the desired reaction from its demographic. The marketing mix consists of four components: product, place, price, and innovation. The term "marketing blend" refers to the combination of several promotional

4. RESEARCH METHODOLOGY

4.1.Research Design

The exploratory study began with an introduction that outlined the goals and research questions, laying the groundwork for examining how Target's marketing methods affect sales in the Indian market. The procedures used to collect and compile the material were then carefully described in the study methodology. The research population for this study consisted of the sales teams of several food firms that operate in the Indian market. These sales teams, as well as those from India City and other companies in other industries, were surveyed.

4.2.Research Approach

A mixed-methods strategy was used in the study approach, integrating quantitative and qualitative data gathering techniques. An in-depth understanding of the complex link between Target's marketing techniques and sales in the Indian market was the goal of the exploratory study approach. A stratified random selection approach was employed to guarantee representation from all food industries in the state of Maharashtra, after the population was specified.

4.3.Sample

The study's sample population comprised sales teams from many food brands operating within the state of Maharashtra. These teams were chosen on the basis of their active participation in sales initiatives within the Indian food sector. For this study, a sample size of one hundred individuals was used. The chosen people encompassed a range of positions within sales teams, such as team leaders, sales agents, and other pertinent staff members. In order to obtain statistical significance in the study and to capture a representative cross-section of the target population, this sample size was judged adequate.

Convenience sampling and stratified random sampling were combined as the sample methods used in this investigation. To guarantee participation from several food business sectors, including packaged

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products, fresh produce, and drinks, stratified random sampling was employed. Convenience sampling was then used within each stratum to choose participants for the study based on their availability and desire to take part. The convenience and usefulness of this approach in compiling a varied but controllable sample from the intended audience led to its selection.

4.4.Tools used for Data Collection

Surveys and in-depth interviews were used in the data collecting process, along with primary and secondary data collection techniques. In order to acquire a quick overview of Target's marketing influence on sales in the Indian market, a cross-sectional study approach was used. Simple random sampling and facility sampling, two non-probability sampling strategies, were also used in addition to probability-based sample procedures to increase the variety of viewpoints.

4.5.Tools used for Analysing Data

Statistical techniques were used in the data analysis process, such as theme analysis for qualitative insights and regression and correlation analysis for quantitative data. Quantitative data was processed and analysed using the SPSS-18 programme. Cross-referencing data from various sources was used to guarantee validity and reliability. Additionally, a pilot study including two pretests was carried out in India and Iran, and the questionnaire was designed to include feedback.

Basic data features were examined through data screening, and the results were processed and interpreted in subsequent data analysis. Using both qualitative and past tense methods, the study methodology sought to give a comprehensive picture of how Target's marketing techniques affected sales in the Indian market.

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5. DATA ANALYSIS AND INTERPRETATION

Table 1: Demographic characteristics

Characteristics	Frequency	Percentage
Gender		
Male	64	64%
Female	36	36%
Age		
18-24	35	35%
25-34	37	37%
35-44	22	22%
Above 44	6	6%
Educational qualification		
High school students	24	24%
Bachelor	26	26%
Masters	40	40%
Others	10	10%
Income Level		
<Rs 30,000	30	30%
Rs 30,000-40,000	40	40%
Rs 40,000-50,000	20	20%
>Rs 50,000	10	10%
Location		
Urban	70	70%
Rural	30	30%
Marital Status		
Single	90	90%
Married	10	10%

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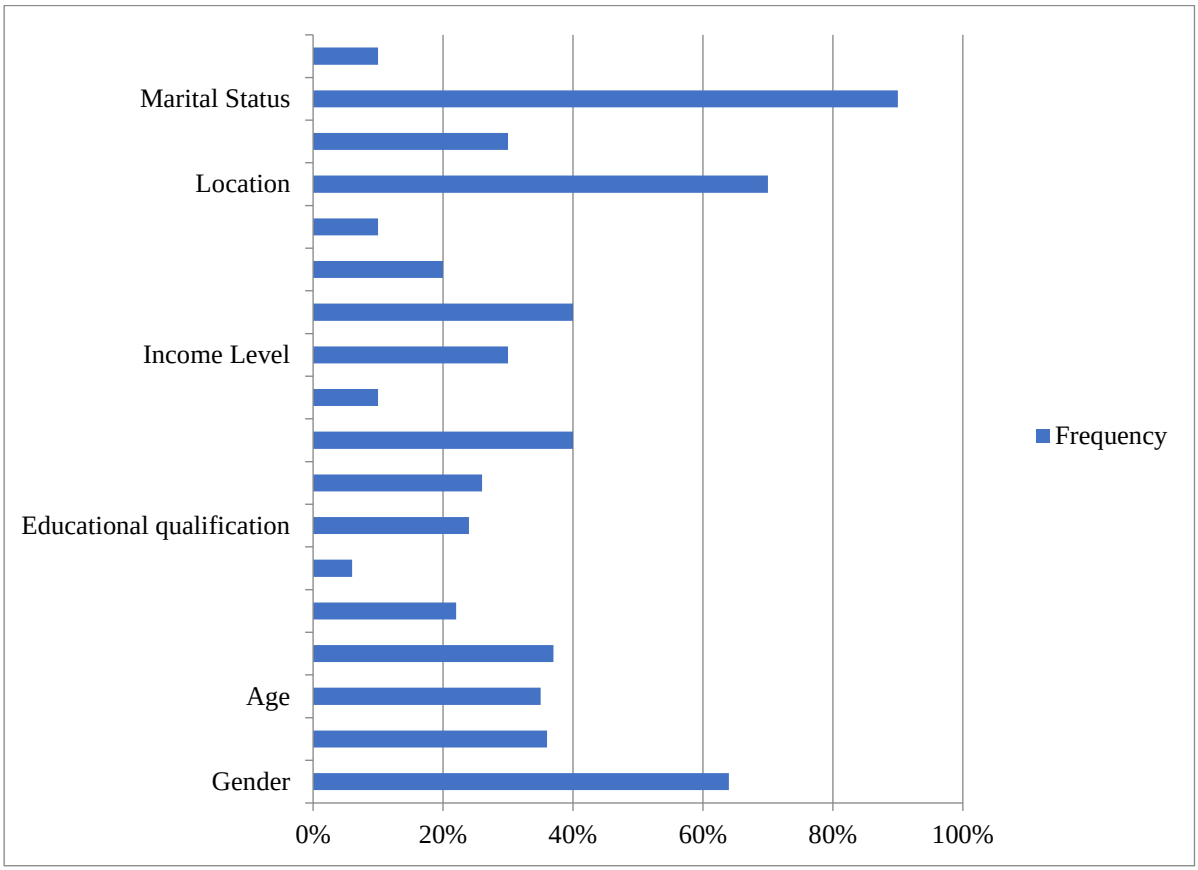

Figure 1: Demographic characteristics

64% of the participants identified as male, while 36% identified as female. The age distribution shows that the majority of participants (37%) fall in the 25-34 age range, followed by 35% in the 18-24 age group. The highest percentage of participants (40%) holds a master's degree, while 26% have a bachelor's degree, and 24% are high school students. The income level is distributed across different brackets, with 40% earning between Rs 30,000-40,000 and 30% living in urban areas. The majority of participants (70%) reside in urban areas, while 30% live in rural locations. The study predominantly includes single individuals, constituting 90% of the participants, while 10% are married.

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Table 2: Cronbach's Alpha

Reliability Statistics		
Research Variables	**Observable Variables**	**Coefficient**
Marketing Strategies Assessment		
Digital Marketing Presence	5	0.963
In-store Promotions	5	0.912
Collaborations and Partnerships	5	0.712
Social Media Engagement	5	0.812
Sales and Customer Perception		
Sales Performance Metrics	5	0.712
Customer Satisfaction	5	0.796
Brand Image	5	0.812

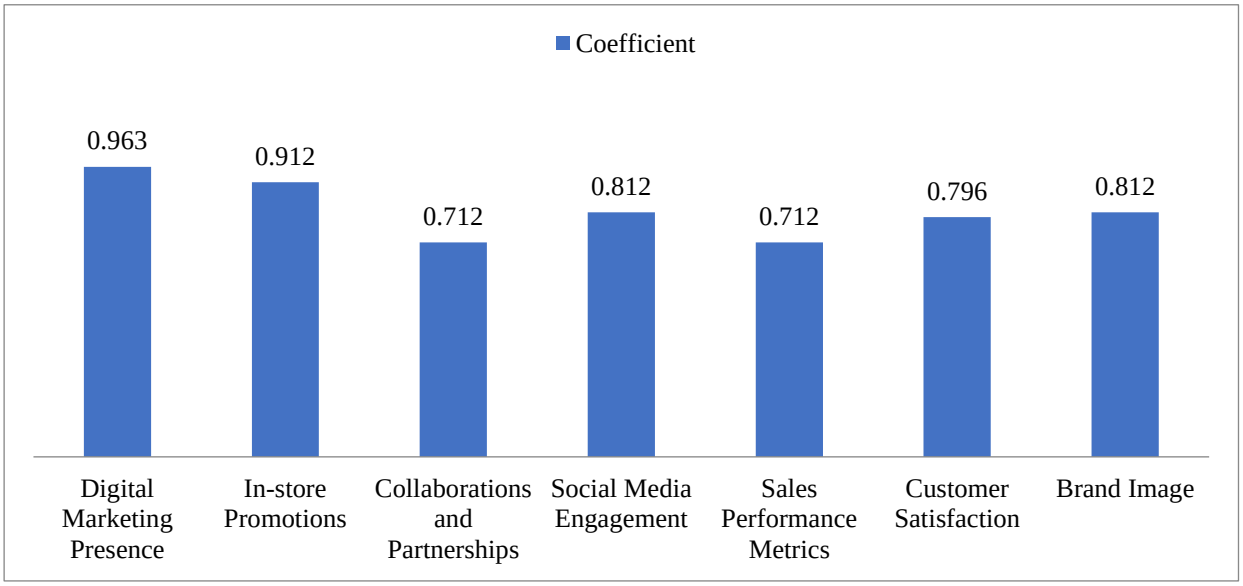

Figure 2: Cronbach's Alpha

The Cronbach's Alpha coefficients, a check of interior consistency unwavering quality, are displayed in the table for various review factors connected to the evaluation of marketing strategies and sales and customer perception. With a Cronbach's Alpha coefficient of 0.963, the discernible factors surveying computerized marketing presence show particularly magnificent inward consistency. Since the things in this factor are firmly associated and reliably survey the ideal build, it upholds serious areas of strength for an exact estimation of computerized marketing components. A Cronbach's Alpha coefficient of 0.912 is characteristic of a serious level of inner consistency across the recognizable variables used to

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assess in-store advancements. This suggests that the in-store advancement related things precisely measure the ideal build, ensuring a cognizant and trustworthy assessment. For organizations and joint efforts, the Cronbach's Alpha coefficient of 0.712 means a moderate level of inward consistency. The estimation is by and by trustworthy enough to assess this part of marketing plans regardless of whether the detectable factors in this classification probably won't be essentially as firmly associated as those in the former factors. Cronbach's Alpha for social media contribution is 0.812, showing a serious level of inward consistency. This suggests that the social media-related recognizable factors precisely catch the ideal develop, ensuring a steady evaluation of social media commitment strategies.

With a Cronbach's Alpha coefficient of 0.712, the perceptible factors evaluating sales performance markers show an unassuming level of interior consistency. This shows that even while the components in this factor have a sensible connection between them, there could be some variety in how solid their estimations are. A serious level of inner consistency in customer fulfillment is exhibited by a Cronbach's Alpha coefficient of 0.796. This suggests areas of strength for a between the recognizable factors estimating customer delight and their predictable estimation of this significant component of consumer perception. The brand picture detectable factors have a Cronbach's Alpha coefficient of 0.812, demonstrating a decent level of inside consistency. This suggests an exact method for estimating brand picture, ensuring consistency in deciding how consumers view the brand..

Table 3: Model summary of variables

Model Summary				
Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.812[a]	.623	.623	.81236
a. Predictors: (Constant), Digital Marketing Presence, In-store Promotions, Collaborations and Partnerships, Social Media Engagement				

The performance of the numerous direct relapse model is summed up in the Model Synopsis table. One of the reliant factors in this model is "Sales and Customer Perception," while the indicators are Social Media Commitment, In-store Advancements, Joint efforts and Organizations, and Computerized Marketing Presence. With a R-squared of 0.623, the included indicators can represent around 62.3% of the variety in sales and customer

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perception. A changed gauge of the model's integrity of fit is given by the changed R-squared esteem, which thinks about the quantity of indicators. The typical distinction between the noticed and expected values is addressed by the standard mistake of the gauge, which is 0.812366..

Table 4: Multiple linear regression analysis findings

ANOVA[a]						
Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	234.071	4	58.518	423.165	.000[b]
	Residual	158.124	95	.811		
	Total	392.195	99			
a. Dependent Variable: **Sales and Customer Perception**						
b. Predictors: (Constant), OR, OB, GD, OT, OC						

The relapse model's generally speaking measurable importance is assessed in the ANOVA table. With four levels of opportunity and a relapse amount of squares of 234.071, the mean square is 58.518. The measurable meaning of the relapse model is shown by the F-measurement of 423.165 and the comparing p-worth of under 0.001 (p =.000). This shows that sales and customer perception, the reliant variable, are considerably related with somewhere around one of the indicators..

Table 5: Coefficients of regression model

Coefficients[a]						
Model		**Unstandardized Coefficients**		**Standardized Coefficients**	**t**	**Sig.**
		B	**Std. Error**	**Beta**		
1	(Constant)	.470	.105			.000
	Digital Marketing Presence	.203	.019	.238	8.589	.000
	In-store Promotions	.133	.021	.135	4.892	.000
	Collaborations and Partnerships	.183	.025	.171	5.958	.000

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	Social Media Engagement	.211	.017	.224	10.333	.000
a. Dependent Variable: **Sales and Customer Perception**						

The Coefficients table provides details about the individual predictors in the regression model.

The coefficient of the consistent term is 0.470, and its standard blunder is 0.105. At the point when all indicator factors are zero, this is the anticipated mean worth of sales and customer perception. The standard blunder is 0.019 and the coefficient is 0.203. This recommends that a one-unit expansion in computerized marketing presence is connected to a 0.203-unit ascend in sales and customer perception, holding different factors steady. The standard blunder is 0.021 and the coefficient is 0.133. This demonstrates that a one-unit expansion in-store advancements is connected to a 0.133-unit ascend in sales and customer perception, holding different factors consistent. The standard blunder is 0.025 and the coefficient is 0.183. This recommends that a one-unit expansion in joint efforts and organizations is connected to a 0.183-unit ascend in sales and customer perception, holding any remaining factors steady. The standard blunder is 0.017 and the coefficient is 0.211. This shows that a one-unit expansion in social media commitment is connected to a 0.211-unit ascend in sales and customer perception, holding different factors consistent.

6. CONCLUSION AND RECOMMENDATIONS

The multiple linear regression analysis conducted on the variables related to Marketing Strategies Assessment and Sales and Customer Perception has provided valuable insights into the factors influencing Sales and Customer Perception. The overall model is statistically significant, with a substantial portion (62.3%) of the variability in Sales and Customer Perception explained by the predictors – Digital Marketing Presence, In-store Promotions, Collaborations and Partnerships, and Social Media Engagement. The individual coefficients for each predictor indicate their unique contributions. Digital Marketing Presence, In-store Promotions, Collaborations and Partnerships, and Social Media Engagement all show positive coefficients, suggesting that an increase in each of these factors is associated with an increase in Sales and Customer Perception. This underscores the importance of a comprehensive and

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integrated marketing strategy that includes both online and offline elements, partnerships, and active engagement on social media platforms.

Recommendations:

- Enhance Digital Marketing Presence: Strengthen the digital marketing strategy to further enhance the brand's online presence. Invest in targeted and engaging digital campaigns to reach a wider audience and improve customer perception.
- Optimize In-store Promotions: Fine-tune in-store promotional activities to maximize their impact on customer perception. This may involve strategic placement, effective communication, and appealing offers to attract and retain customers within physical stores.
- Foster Collaborations and Partnerships: Continue to explore and foster collaborations and partnerships with other brands or organizations. Such alliances can contribute positively to the brand image and customer perception, creating a mutually beneficial environment.
- Strengthen Social Media Engagement: Invest in social media engagement strategies to maintain an active and positive presence on various platforms. Engage with the audience, respond to feedback, and create compelling content that resonates with the target demographic.
- Training and Skill Development: Provide training and skill development programs for marketing teams to stay abreast of industry trends, digital marketing tools, and effective collaboration strategies. This can empower teams to execute marketing initiatives more efficiently.

ISBN - 978-93-95060-79-0

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ISBN - 978-93-95060-79-0

AN ANALYSING THE LONG-TERM EFFECTS OF MISLEADING ADVERTISING ON CONSUMER LOYALTY

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Abstract

This research explores the intricate dynamics of misleading advertising and its impact on consumer trust and loyalty. Employing a mixed-methods approach, the study combines qualitative and quantitative techniques to gain comprehensive insights. Demographic analysis reveals a predominant presence of younger participants, a notable gender imbalance, and diverse educational backgrounds. Social Media emerges as a dominant platform, influencing consumer perceptions. The majority of respondents report exposure to advertising, with varying degrees of awareness regarding misleading practices. Brand loyalty distribution is balanced, with trust identified as a significant factor influencing susceptibility.

Keywords: Misleading advertising, Consumer trust, Consumer loyalty, Demographic analysis, Social Media, Brand loyalty.

1. INTRODUCTION

In the ongoing exceptionally serious financial climate, it is unimaginable for an organization to turn into the market chief without first putting a huge number of dollars in showcasing and advertising. The essential goal of advertising is to gain significant clients by applying impact over their mentalities, levels of mastery, and buying designs. There are a few methodologies of imparting carefully, including occasions, one-on-one deals, advertising, direct showcasing, verbal promoting, and advertising (Kotler, 2010). A portion of these methodologies incorporate advertising. Individual offering, frequently known as eye to eye offering, alludes to an exchange where one individual (the salesman) collaborates straightforwardly with a likely client with expectations of influencing that client into making a buy (meaning of "Individual Selling"). People and organizations utilize advertising, frequently known as PR, to interface with individuals from the media and the overall population. As per (Pahwa, 2018), a PR proficient keeps a great picture by speaking with the interest group either by implication or straightforwardly through the media determined to create areas of strength for a with the crowd. Direct showcasing might be characterized as the demonstration of selling an item or administration straightforwardly to consumers, for example, using effort strategies, for example, mail request or phone deals. As of now, the client of today has the test of figuring out how to manage with different sorts of advertising correspondence. Advertising, out of all of the previously mentioned channels of correspondence, has the most effect on watchers' brains, and as a system, it is the most fundamental part of any business association that is used to bring issues to light about an item or administration. The Greek "advertere," from which we get the English word "promotion," in a real sense signifies "to turn the consideration." The beginning of the word advertising might be followed back to the Latin word "advertere," and that signifies "to turn the consideration." Around the year 1665, the expression "advertising" was at first utilized interestingly. Every single part of advertising pursues the objective of moving the focal point of the followers' or alternately crowd's consideration towards the item. The motivation behind advertising is to illuminate and convince consumers overall to secure a specific thing or administration. Using an assortment of

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publicizing mediums, for example, magazines, radio, TV, notices, web publicizing, paper, etc, a decent or administration is promoted with expectations of making potential clients mindful of it. Regularly, advertising gives data about the publicizing organization, the characteristics of its administrations, the item it sells, and where it very well might be acquired (Singh, 1996). At the point when statements that are either false or purposely expected to be dishonest about the item or administration being publicized are remembered for the advertising, the notice can be thought of as misleading or even bogus

At the point when a business or organization advances labor and products by using portrayals are either mistaken, misleading, or vague, this is viewed to act as an illustration of tricky advertising. To misdirect clients and give the feeling that their item is essentially not the same as what it really is, publicists will frequently depend on misleading practices, for example, modifying the vibe of things using programs like Photoshop. Accordingly, clients are seriously jeopardized by this kind of advertising since they are deliberately misdirected by the mutilation of the idea of the item or administration given by different organizations, as well as its quality, characteristics, or different viewpoints. Due to this wrong portrayal of the item, clients are directed to make not recommended buys, as an outcome of which they end up wasting their well-deserved cash on things that don't satisfy their prerequisites or give an answer for the issues they are confronting (Taruna, September 2016).

As indicated by (Lodhi, 2015), misleading advertising is the utilization of misleading, incorrect, and mistaken publicizing of a thing that can possibly adversely influence consumer loyalty. The presence of duplicity might be affirmed when a purchaser exclusively consumes or encounters the item or administration being referred to. Clients can't go with the most ideal choices for themselves in the event that they are not given sufficient data or on the other hand assuming that they are shown pictures that mutilate the genuine characteristics of an item or administration (Beam, 2018). On the off chance that clients are not given sufficient data or on the other hand in the event that they are shown pictures that contort the genuine characteristics of an item or administration, they are not taught. One's any firm that

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participates in misleading advertising is definitely not a trustworthy organization more on the grounds that new and current consumers might be bamboozled and move to the labor and products of another organization. Misleading advertising can adversely affect the two sorts of clients. The organization risks acquiring a negative standing as a tricky association, which could prompt a reduction in deals over the long haul. Moreover, in creating markets, for example, Pakistan, it is turning out to be progressively fundamental to decide if the elements like client joy, verbal, and trust additionally impact client loyalty. This is on the grounds that there is a constant section of imaginative players in promising organizations. Along these lines, organizations construct better associations with their recurrent consumers, who keep on purchasing their labor and products (Waheed Akhter, 2011).

In any case, this is just conceivable assuming trust has been laid out in any case. As per research led by (Owais Hassan in 2014), the consumer in Pakistan isn't fundamentally not the same as clients in some other market across the world. As per the (FTC's Aides against Tricky Valuing, 2012), misleading advertising is currently denied in an extraordinary number of different countries too. The US of America was the principal country on the planet to lay out regulations and guidelines against misleading advertising. As per a choice gave over by the High Court of the US in 2010, tricky or misleading commercial doesn't meet all requirements for security under the primary change. Subsequently, a portion of the organizations have been conceived and laid out to discourage specialist co-ops and makers from utilizing misleading advertising in light of a legitimate concern for safeguarding their clients. As per the guidance given by the European Commission, calculated deception is any type of advertising that, in any capacity, paying little heed to the way things are introduced, is fit for misleading the individual to whom it is concentrated, distorted their way of behaving, and thus, annihilation the interest of clients (Official Diary of the European Association, 2005). Misleading advertising is unlawful in a few nations, including China, India, and the Unified Realm. Besides the fact that untruthfulness rigorously precluded is, yet it is likewise dependent upon tough rules and controls. In most of countries, advertising that can likewise be viewed as publication content are expected to be named as commercials in a reasonable and

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prominent way. As per Wakeman, the charge of breaking these principles can bring about repercussions that are both monetarily and socially critical. To break into a specific market, it is extremely critical to initially get however much data as could be expected on the inclinations and necessities of clients, as well as the social and financial environment of that market. This is vital not exclusively to give an item, yet additionally to lay out its own particular approach.

2. RESEARCH OBJECTIVES

- To examine the impact of misleading advertising on consumer trust and loyalty.
- To identify the factors that influence consumer susceptibility to misleading advertising.
- To propose strategies for mitigating the negative effects of misleading advertising on consumer loyalty and brand reputation.

3. ITERATURE REVIEW

As per **(Eberle, et. al, 2013),** the improvements of advertising as a device for promoting has been predicated on the principles of tell the truth and giving exact data. Scientists have viewed at advertising as a part of an organization's promoting methodology. These investigations might be seen as here and here. As per (European Commission, 2011), the motivation behind promotions is to scatter data and assurance that clients know about the items and administrations that are being made available for purchase. Notwithstanding, this capability of advertising is moving as of late.

As indicated by **(Freeman, et. al, 2010),** there is a wide range of media by means of which promotions might be conveyed. The advertiser is liable for discussing in a reasonable way with the interest group (the clients). As per **(Freeman, et. al, 2010)**, the antiquated preventative expression "purchaser be careful" has been supplanted by "dealer be careful." most of the time, the falsehoods are challenging to detect. The latest claims of misleading advertising relate to the control of clients. Aslam's viewpoint on the disguising of data relates to the manner by which organizations assess the choices made by clients. Various organizations have been called out for misrepresenting the advantages of their products, much as what occurred with Toyota in the previous model.

As indicated by **(Frow, & Payne, 2011),** a portion of the charges of tricky advertising

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are the shortfall of quality data, the introduction of data in a way that is hard to see, the veracity, and the development suggestions.

This study reveals insight into the numerous positive angles related with complying with global regulation while directing advertising. A work has been made to cause to notice the types of misleading data that are generally predominant and to give instances of systems that may be utilized to protect consumers. As per **(Frow, & Payne, 2011),** it is unlawful for an organization to intentionally and deliberately pass on data about an item that isn't valid or fair. Assuming the organization does this, being providing bogus data or misleading customers is thought of.

As per a report distributed by the European Commission **(Hetze, 2016),** misleading advertising is a worthwhile business practice that includes furnishing clients with bogus data, deceiving them, tricking them, or deceiving them to persuade them to purchase an item that they could never have bought in any case. As per the Consumer Security from Unjustifiable Exchanging Guidelines, an advertising is viewed as misleading on the off chance that it misdirects, in the event that it is introduced to an individual in a tricky way, assuming it has monetary repercussions, or on the other hand assuming defeating rivals in the market is probable. Also, an ad is viewed as misleading on the off chance that it can possibly beat rivals on the lookout. It likewise alludes to the act of conveying data to weak clients that is wrong fully intent on affecting the choices or reactions those consumers make. These exercises feature occurrences in which organizations see the perspectives on clients to be of a lower quality, or in which firms are compelled to take part in untrustworthy business rehearses because of seriousness. At the point when consumers are set in new environmental elements, they are more defenseless to the impact of advertising. As per Janet's examination from 1985, consumers hope to promoting informing as a wellspring of data while choosing what items to buy.

As per **(Islam, et. al, 2021),** consumers are exposed when they come up short on capacity to distinguish misleading data. Nowadays of simpler admittance to data, clients have a more prominent obligation to guarantee that they gather as much information as possible. The platitude "purchaser be careful" is as significant now

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as it was the point at which it was first spoken; a critical component in the buy cycle is "looking" for information. Messages passing on data about the qualities of the product or administration being publicized make up advertising. As per **(Islam, et. al, 2021),** calculated deception is characterized as the act of making claims about an item or administration fully intent on deluding clients into coming to a misleading end result about the item or administration being referred to. As per **(Kozlenkova, et. al, 2014),** the main inquiry for clients to respond to before they pursue a buy decision is whether they can recognize misleading cases among the various tricky cases that show up in advertising. Assuming a client puts together their confidence with respect to an advertising, there is a decent opportunity that they will be made the most of.

At the point when clients become mindful of misleading advertising, it makes them more delicate to all types of promoting, contingent upon the level of feeling they are encountering. Erroneous advertising impacts consumers' impression of both the items they're seeing and the general climate in which those items are being promoted. This peculiarity is alluded depicted as "situational settings**" (Latif, et. al, 2020).** Finding reality with regards to tricky advertising might be a difficult endeavor that requires a few measures of drive and skill with respect to the person. Consumers may not necessarily in all case have an interest in, or the capacity to, reason when charges of misleading are being made. They are bound to be deceived by commercials on the off chance that they are not focusing exactly when they initial come into contact with them. Research led by **(Manafu, 2020)** showed that clients know about the sum as a significant variable, and that this mindfulness impacts how they respond to misleading advertising. For example, the parts of an item that are liable for making it interesting to end clients might be exaggerated. Consumers have been displayed to respond in different approaches to misleading advertising claims relying upon whether they perceive the legitimacy of the cases. Studies investigating these responses have utilized consumers' dispositional and formative attributes. As per **(Mandviwalla, 2014),** dispositional contrasts are those characteristics that change over the long haul because old enough, family, orientation, and way of life. As per **(Martinez, et. al, 2013),** how much

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exertion that consumers need to do to recall information based models to comprehend data from a specific advertising is corresponded with their age. Contrasts that might be credited to a consumer's creating information and expertise because of the progression of time are known as formative contrasts. As per **(Mascarenhas, et. al, 2006),** the information on the consumer advances through a progression of stages starting with the perceptual stage, continuing on toward the intelligent stage, lastly showing up at the intelligent stage. From youth all through development, an individual goes through many transformative phases, every one of which adds to a one of a kind measure of weakness to misleading promoting.

3.1.Analysis of the Social Judgment Theory

As indicated by social judgment hypothesis, monitoring an individual's points of view on numerous subjects could give data that can assist you with planning a procedure for moving toward an undertaking to convince them. The thought was created by Sherif and his partners, and it centers around how people assess powerful messages remembered for **(Kotler, P. 2010).** The use of this hypothesis in research has regularly focused on mental cycles; in any case, there are a considerable number of ramifications for communicators who are endeavoring to persuade others. As per social judgment hypothesis, people assess (judge) the substance of messages in light of their anchors, which can be considered their situation on a specific subject message. This hypothesis sets that people assess (judge) the substance of messages. Every individual's mentalities, notwithstanding the singular's anchor, might be arranged into one of three unmistakable gatherings. To start, there is the scope of acknowledgment, which includes each of the ideas that someone in particular considers to be adequate. Second, there is the space for dismissal, which obliges every one of the ideas that an individual considers to be unacceptable. To wrap things up, there is the choice of non-responsibility, once in a while known as the scope of non-responsibility. This decision involves neither tolerating nor dismissing the standards introduced here. With regards to advancing item mindfulness, sponsors and their promoters regularly take part in every one of the three types of deceptive nature, as per the examination in the important field as well as a lot of

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significant narrative information. In most of settings, having the capacity to assess misleading promotions requires a more top to bottom assessment of the frequency data (on plugs, item quality, and stocks), which is hard to do while using a study. Under the irreconcilable situation, duplicity might happen in almost any method of correspondence. This is on the grounds that duplicity is an inescapable peculiarity. Different kinds of duplicity exist notwithstanding the clear lie, and the qualities that recognize one sort of trickiness from one more incorporate the amount and sufficiency of data, the level of trustworthiness, the lucidity, the pertinence, and the objective. No matter what the kind of trickery, it raises various moral difficulties and worries for the organizations in question, the clients, and the leaders.

It will be vital to dealers in our different business sectors and the administration of organizations as ideas will be given to the policymakers, which likewise will act as signs towards further developing their promoting strategies and projects towards resolving the difficult issue of uncommon deals drop or potentially new item disappointment in the market that could be occasioned by post-buy cacophony and absence of rehash buy by the consumer because of the accursed demonstrations of misleading advertising. As well as resolving the difficult issue of uncommon deals drop or potentially new what's more, it will be useful to different associations, ventures, and government bodies concerning the plan of approaches and the execution of showcasing systems. There is a "portrayal, exclusion, or practice that is probably going to delude the consumer" in tricky advertising in the event that there is a "portrayal, oversight, or practice that is probably going to misdirect the consumer" in [2]. Tricky advertising may be a commercial or a promoting practice.

The commercial doesn't required need to make genuine trickery for the direct to be viewed as misleading to the consumer; be that as it may, as expressed by the Government Exchange Commission (FTC, 1998), the demonstration will probably delude the client.

Albeit countless undertakings and drives have been seen on tricky advertising, which incorporates perceiving and evaluating misdirection, a generally little spotlight has been given on the results of misleading advertising on consumer loyalty. With regards to advertising, the

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work of bogus or tricky data in advertising is alluded to as "misleading advertising". The misleading promotion has been around starting from the dawn of history and keeps on being boundless in the present society. There are occurrences when it is done unexpectedly by a supporter; be that as it may, in, it is finished with the point toward tricking the clients. It is in many cases trusted that duplicity in advertising, through misleading cases, prompts the main acquisition of the item by the client. Albeit the consumer is first tricked by the misleading commitments made by the advertiser, they progressively come to grasp the genuine quality and worth of the item or administration subsequent to involving it interestingly. The client's acknowledgment of the misrepresentation makes it harder for them to keep up with their loyalty to the specific brand.

A few merchants in the Nigerian commercial centers, as well as certain makers of an assortment of other consumer merchandise, take part in fooling purchasers and consumers into disparaging their items by lying about what their product(s) can't do. This is finished with an end goal to get individuals to buy these merchants' products. They can achieve this by means of the course of their selling activities with the consumer or through advertising messages, the naming of item bundles, and in handbills.

4. RESEARCH METHODOLOGY

A comprehensive research methodology was employed to investigate the impact of misleading advertising on consumer trust and loyalty. The methodology encompassed a combination of qualitative and quantitative research techniques to gain a holistic understanding of the phenomenon.

4.1.Qualitative Research

- In-depth interviews: Semi-structured in-depth interviews were conducted with a diverse sample of consumers to explore their perceptions of misleading advertising, its influence on their decision-making processes, and the impact on their brand loyalty.
- Focus groups: Focus groups were organized to facilitate group discussions and gather insights into consumer attitudes, beliefs, and experiences related to misleading

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advertising. The focus groups allowed for a deeper understanding of the dynamics of consumer behavior and the influence of social factors.

4.2.Quantitative Research

- Survey research: A large-scale online survey was conducted to gather quantitative data on consumer awareness, perceptions, and experiences of misleading advertising in Maharashtra. The survey employed psychometrically validated scales to measure constructs such as consumer trust, loyalty, and susceptibility to misleading advertising.
- Experimental research: A controlled laboratory experiment was conducted to examine the immediate effects of misleading advertising on consumer perceptions and behavior. Participants were randomly assigned to view either misleading or non-misleading advertisements and were subsequently assessed on measures of brand trust, product evaluation, and purchase intention.

4.3.Sampling

The target population for this study is adult consumers who have been exposed to advertising in the past six months in the Maharashtra State. The population should be diverse in terms of age, gender, education, income level, and media consumption habits.

A sample size of 200 consumers lived in Maharashtra State is considered adequate for this study. The following sample techniques can be used to select a representative sample from the target population:

- Random sampling: This technique involves selecting a random sample of consumers from a list of all eligible individuals. This is the most unbiased sampling technique, but it can be time-consuming and expensive to implement.
- Stratified sampling: This technique involves dividing the population into subgroups or strata based on a relevant characteristic, such as age or gender. A random sample is then selected from each stratum. This technique ensures that the sample is representative of the diversity of the population.
- Convenience sampling: This technique involves selecting a sample of consumers who are easily accessible, such as students or employees of a particular organization. This technique is the least representative of the population and is not recommended for research that aims to draw generalizable conclusions.

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4.4.Tools used for Data Analysis

Qualitative data from interviews and focus groups were analyzed using thematic analysis techniques to identify recurring themes, patterns, and insights. Quantitative data from the survey and experiment were analyzed using statistical methods to assess the significance of relationships between variables and to test hypotheses related to the impact of misleading advertising.

5. DATA ANALYSIS AND INTREPRETATION

Table 1 provides a comprehensive overview of the respondents' characteristics, reflecting their distribution across various demographic categories and key variables related to their exposure to advertising and susceptibility to misleading information. Here's an interpretation of the presented data:

Table 1: Overview of the respondents' characteristics

		F	**Percentage**
Age	18-24,	80	40%
	25-34,	60	30%
	35-44,	20	10%
	45-54,	30	15%
	55 and above	10	5%
Gender	Male	180	90%
	Female	20	10%
Education Level	High School or Below	4	2%
	Some College,	120	60%
	Bachelor's Degree	40	20%
	Postgraduate Degree	36	18%
Media Consumption Habits	TV,	30	15%
	Radio,	10	5%
	Online Streaming,	60	30%

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	Social Media,	80	40%
	Print	20	10%
Exposure to Advertising (in the past six months)	Yes	180	90%
	No	20	10%
Frequency of Online Shopping	Never	20	10%
	Rarely,	80	40%
	Occasionally,	60	30%
	Frequently	40	20%
Awareness of Misleading Advertising	Not Aware,	40	20%
	Somewhat Aware,	100	50%
	Very Aware	60	30%
Brand Loyalty	Low,	50	25%
	Moderate,	100	50%
	High	50	25%

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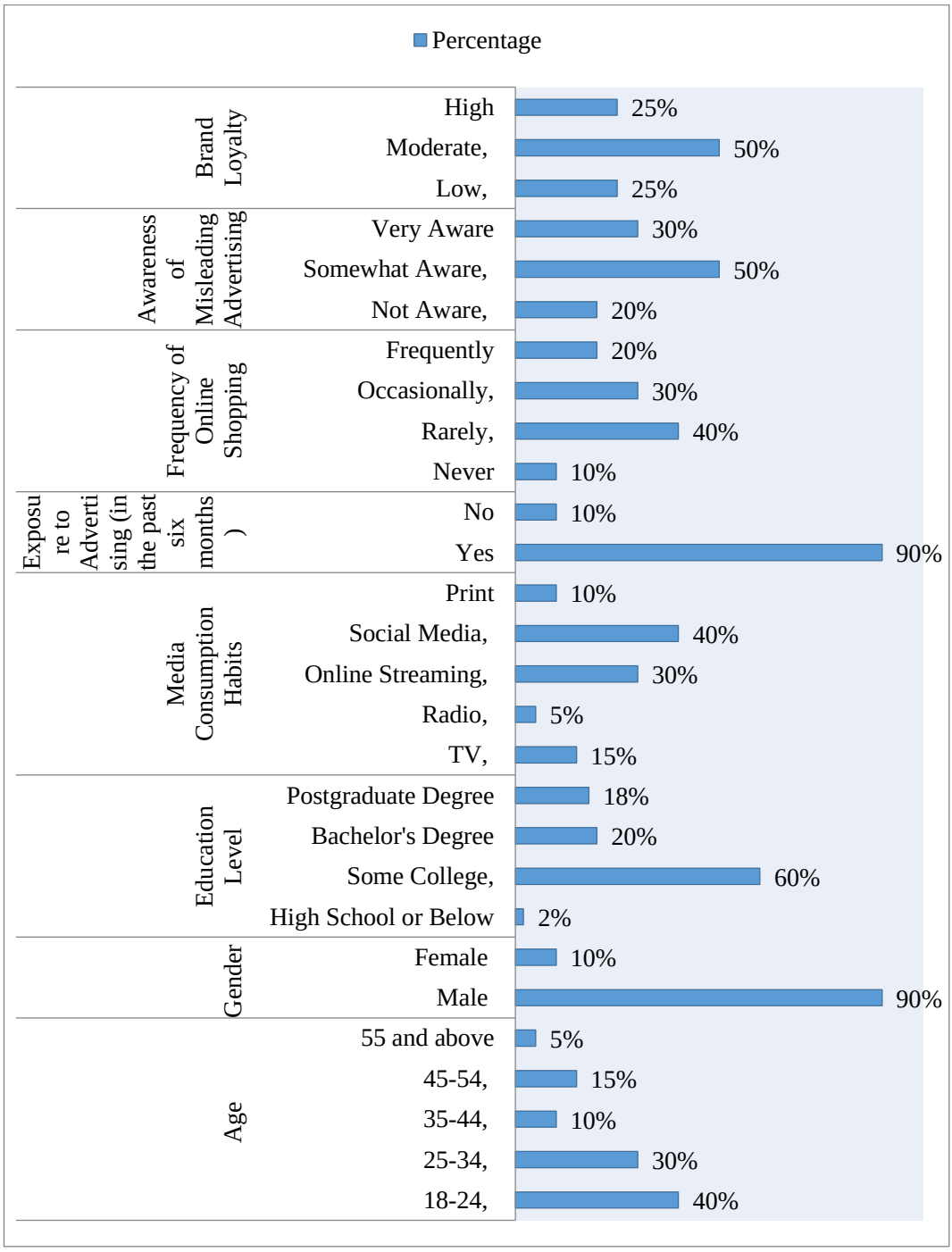

Figure 1: Overview of the respondents' characteristics

Firstly, when examining the distribution by age, it is evident that the majority of respondents fall within the 18-24 and 25-34 age groups, constituting 40% and 30%, respectively. The older age groups (35-44, 45-54, and 55 and above) represent a decreasing proportion of the sample, reflecting a trend of higher representation among younger individuals. Gender distribution

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reveals a notable majority of male respondents, accounting for 90% of the sample, with females comprising the remaining 10%. This gender imbalance may influence the generalizability of findings, and future research could aim for a more balanced representation. Education levels among respondents show that a significant portion has completed "Some College," representing 60% of the sample. Bachelor's Degree holders make up 20%, while those with a Postgraduate Degree constitute 18%. Only a small percentage (2%) have an educational background of "High School or Below." This breakdown emphasizes the need to consider educational diversity in analyzing responses.

Media consumption habits exhibit a varied distribution, with the highest percentage (40%) reporting Social Media as a primary platform, followed by Online Streaming (30%), TV (15%), Print (10%), and Radio (5%). Understanding these preferences is crucial for interpreting how individuals receive and process information. Regarding exposure to advertising, 90% of respondents confirm exposure within the past six months, while the remaining 10% claim no exposure. This information is vital for contextualizing respondents' perspectives, as those who have not been exposed may have different attitudes toward advertising. The frequency of online shopping shows a diverse range of behaviors, with 40% shopping rarely, 30% occasionally, 20% frequently, and 10% never engaging in online shopping. These variations highlight the need to consider individual online behaviors in the context of their susceptibility to misleading advertising. Awareness of misleading advertising is distributed across three categories, with 50% reporting being "Somewhat Aware," 30% "Very Aware," and 20% "Not Aware." This breakdown provides insights into the levels of consciousness regarding deceptive advertising practices among respondents. Finally, the distribution of brand loyalty indicates a relatively balanced representation, with 50% reporting a "Moderate" level of loyalty, while 25% report both "Low" and "High" levels. This suggests a diverse range of brand loyalty among respondents.

Table 2: Regression Results

Variable	Coefficient	Standard Error	t-value	p-value
Intercept	1.245	0.532	2.342	0.021
Age	0.027	0.065	0.415	0.679

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Awareness	0.731	0.182	4.010	0.001
Trust	-0.512	0.094	-5.436	<0.001

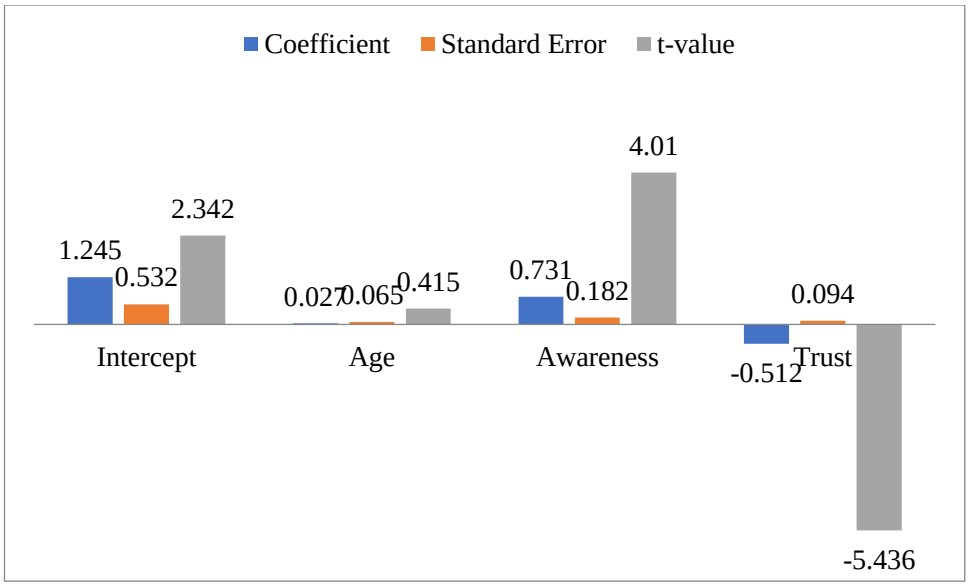

Figure 2: Regression Results

The regression analysis results reveal insights into the relationship between several key variables—Age, Awareness, and Trust—and their impact on the susceptibility of consumers to misleading advertising. The intercept, representing the estimated susceptibility when all independent variables are zero, is found to be 1.245 with a standard error of 0.532. The t-value of 2.342 associated with the intercept is statistically significant at the 0.05 significance level, as indicated by the p-value of 0.021. When examining the individual predictors, the coefficient for Age is 0.027 with a standard error of 0.065. However, the t-value of 0.415 and the associated p-value of 0.679 suggest that Age is not a statistically significant predictor of susceptibility to misleading advertising in this model. In contrast, Awareness emerges as a significant predictor with a coefficient of 0.731 and a standard error of 0.182. The t-value of 4.010 is associated with a highly significant p-value of 0.001, indicating that higher levels of consumer awareness are positively linked to increased susceptibility to misleading advertising. Furthermore, Trust demonstrates a significant negative association with susceptibility. The coefficient for Trust is -0.512, with a standard error of 0.094. The t-value

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of -5.436 is highly significant (<0.001), suggesting that higher levels of consumer trust are associated with a lower susceptibility to misleading advertising.

6. CONCLUSION AND RECOMMEDNATION

The research on the impact of misleading advertising on consumer trust and loyalty provides a nuanced understanding of the intricate relationships among various demographic factors, media consumption patterns, and consumer behaviors. The study illuminated demographic insights, notably the prevalence of younger participants and a substantial gender imbalance. The diverse educational backgrounds underscore the importance of considering educational diversity in the interpretation of study findings. Social Media emerged as a dominant platform, emphasizing its significance in influencing consumer perceptions. The findings also underscored the pervasive nature of advertising in respondents' lives, with the majority reporting exposure in the past six months. A significant proportion demonstrated awareness of misleading advertising practices, indicating varying degrees of consciousness among participants. Moreover, the study highlighted a balanced distribution of brand loyalty, with equal representation across low, moderate, and high loyalty categories. Notably, trust emerged as a significant factor influencing susceptibility to misleading advertising, with higher levels of trust associated with lower susceptibility.

Recommendations:

- Targeted Advertising Strategies: Advertisers should tailor their strategies to the dominant platforms, especially Social Media, to effectively reach and engage the target audience. Understanding the preferences of consumers in different demographic segments will enhance the precision and impact of advertising efforts.
- Education and Awareness Campaigns: Given the varying degrees of awareness among participants, there is an opportunity for industry stakeholders to initiate education and awareness campaigns to empower consumers with the knowledge to identify and resist misleading advertising practices.
- Build and Maintain Trust:Advertisers and brands should prioritize building and maintaining trust with their audience. Transparency and honesty in advertising messages can contribute to fostering trust, thereby reducing susceptibility to misleading practices and enhancing overall brand loyalty.

ISBN - 978-93-95060-79-0

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ISBN - 978-93-95060-79-0

AN EXPLORATION THE SYNERGY BETWEEN MOBILE COMMERCE AND CONSUMER EXPERIENCE IN MODERN RETAIL MARKETING STRATEGIES

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ABSTRACT

This study explores the shifting mobile phone market through the lens of consumer tastes, marketing approaches, purchase habits, market segmentation, and company success. In-depth interviews and focus groups were used to elicit a range of user experiences and opinions on brand loyalty and product adoption. The research of marketing strategies showed a diverse environment, with user-generated material and testimonials taking centre stage. The importance of factors including age, income, and usage habits in shaping consumer behaviour was highlighted by a mixed-methods study design. Cluster analysis uncovered three unique groups of consumers. E-commerce has been found to be more successful than mobile showrooms in terms of profitability, liquidity, and operational efficiency. These results were backed up by the statistics. In sum, this study equips business leaders with in-depth knowledge of the mobile phone sector, allowing them to better adapt their marketing approaches to the ever-changing market.

Keywords: *Commerce, Mobile, Retail, Marketing, Consumer Experience, Modern, Exploration*

1. INTRODUCTION

When it comes to conducting business, mobile commerce has shown to be indispensable. Using the internet and various mobile services, it has helped businesses raise their profits. This means that the internet had a supporting role in the growth of mobile commerce among internet-savvy consumers. These days, information goes fast from one site to another. Rapid expansion in the business industry can be attributed to the rise of m-commerce, which appears to have many future uses. Global adoption of mobile phones has spurred thriving m-commerce between consumers. One of the key motivators of m-commerce is the proliferation of mobile phone users and mobile phone applications. E-commerce was developed in the 1990s, but now M-commerce is taking its place. It is considered that individuals today make good use of a wide range of mobile phone capabilities, such as videos, news, sports, and the many other apps available, rather than only making and receiving phone calls.

1.1.E-commerce and digital marketing

Around the past few decades, the internet has altered daily routines in neighbourhoods all around the globe. Since the advent of the internet, e-commerce, or electronic commerce, has exploded in popularity around the globe. It has drastically altered the way in which consumers make purchases. The distance that separates consumers from manufacturers has narrowed. Faster economic performance and higher international productivity can be attributed in large part to the rise of the knowledge-based economy, which has been propelled by the proliferation of information technology, especially the internet. The new global economy has permanently altered the economic, social, educational, and political landscapes as a result of technological advancements and globalisation and trade liberalisation.

The fact that so many more people in India now regularly make purchases online is one of the most significant results of the internet uprising. The growth of India's e-commerce sector over the past decade has been remarkable. The Economic Times reports that the number of online purchases done by over 120 million Indian customers in 2018 increased by around 115% due to the rapid development in internet usage and advancements in logistics. Moreover, the survey participants' buying preferences

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revealed that while 37% favour brick-and-mortar stores, 57% favour internet stores. In India, it was predicted that by 2020, 329 million people, or more than 70% of all internet users, would make purchases online.

1.2.Mobile Commerce

Both "electronic" and "mobile," which appear first, are essential for identifying the two categories of companies. The term "electronic" may suggest a permanent and location-based link to the telecommunications network, allowing for "anytime" access to corporate activities and information. In contrast, "mobile" refers to wireless network access to business data and transactions that is neither time- or place-dependent. One key distinction between electronic commerce and mobile trade is that the latter does not rely on the customer's physical location. The way individuals live their daily lives has surely altered as a result of the internet and other technological breakthroughs. These days, even the most mundane activities may be accomplished in a number of ways that largely rely on technology. Simply seeing how and where people purchase now will give you an idea of the dramatic shift in consumer behaviour that has occurred since the advent of the internet and online commerce.

1.3.Increase in e-commerce usage

Many factors, such as more affordable smartphones and widespread 3G network availability, have contributed to a meteoric rise in the country's adoption of mobile commerce. By March of 2014, the number of mobile Internet users in India is expected to approach 165 million, up from 87.1 million in December of 2012. There are projections that in the next three years, mobile commerce will make up more than 25% of all e-commerce. From 2012 to 2016, the mobile commerce market in India is projected to increase by 71.06 percent.More and more often, service providers and banks in India's mobile commerce business work together. The vast majority of mobile service providers have formed partnerships with dominant financial service providers in order to provide mobile payment choices to their customers. For instance, Bharti Airtel and Axis Bank collaborate to provide banking services to its customers via the Airtel Money app. Similar mobile payment services are offered by Vodafone India and ICICI Bank through partnerships between the two companies. Partnerships and

ISBN - 978-93-95060-79-0

alliances like these are expected to increase, which will be beneficial to the development of the sector as a whole. More than half (52%) of mobile consumers favour using debit cards, and more than a quarter (25%) of those customers spent Rs 10,000 or more. Android is preferred by 68% of mobile commerce customers because of its convenient shopping features.Seventy percent of Indians now regularly use highlight phones for calling and messaging, yet many of us have no idea how to make the most of this technology. In the event that the needs and inclinations of the rustic and rural individuals are adequately accommodated, m-business administrations have an exceptional potential taking off in India. Developers targeting the urban market, which is dominated by young people, should design appealing and well-organized mobile commerce platforms.

1.4.Retail Business

The retail sector is vital to the economies of all countries. It entails promoting goods and services to people who are likely to buy them. "Retailing is an ongoing process in which goods are formed, packaged, transported, and divided among the consumer's residence, the retailer's shop, and the manufacturer." The trader anticipates the demands of the client, purchases products and faces problems to supply those requests and defers them till those items might be obtained. The dealer controls the pivotal point at which people's hopes, dreams, and desires are translated into real, quantifiable improvements in their quality of life. The role of retail in the growth and development of an economy is crucial. There are essentially three main tasks that make up every country's financial structure. Consumption, promotion, and manufacturing fall within this category. Medication is needed as part of the dynamic process of achieving a balance between production and societal consumption needs. It is performed by optimising processes and systems to meet the end purpose. When it comes to the distribution of products and services in an economy, retail is a crucial market institution. Since it serves as the final touchpoint between the product and the consumer, retail is an integral part of the advertising infrastructure.

2. LITERATURE REVIEW

This is another reason why internet purchasing is so common, says **Berkhout (2015)**. The customer considers online buying as a substantial time, effort, and energy saver. Delivery of their purchases is

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made directly to their door. Features, pricing, configurations, quality, durability, and usefulness may all be compared side by side from the comfort of one's own home or office.

Online retailers like Amazon, it is claimed by **Zentes et al. (2016),** exploit price discounts to attract customers away from brick-and-mortar establishments. Goldsmith and Flynn (2005) found that many consumers prefer online shopping to interacting with salespeople in person since salespeople have a tendency to exaggerate the features and benefits of the products they're trying to sell. They do not wish to fall for the trickery of such sellers.

Erisman (2017) When you purchase online, you can often get the best deals because of the competitive nature of the market. Prices on retail platforms like Amazon, Flipkart, eBay, and Alibaba are much lower than those generally found in physical storefronts. The majority of research indicates that price, flexibility, and customization are the top three motivating factors for online shopping.

Schniederjans et al. (2013) The principles of Just-In-Time production have many parallels with e-commerce that are both significant and beneficial. They suggested right away making it and its foundation to better web-based business. Because just-in-time (JIT) manufacturing only builds goods when there is a legitimate need for them. Only when a consumer placed an order for one of many products does the manufacturer start making it. However, the things are first created by the manufacturer before being used in the traditional production procedure. Past data and patterns are used to make predictions about the future. To cut down on waste, get rid of inventory management, boost productivity, speed up production, and enhance delivery efficiency, the supply chain has seen a rise in Just-in-time methods and other innovations in recent years. Online commerce and JIT work nicely together.

Pardalos et al. (2013) Several worldwide companies, notably Dell Inc., have successfully executed the JIT strategy through the usage of websites. Customers placed orders via their websites, and the companies produced and shipped the goods to them in the allowed time frames. These companies could be able to undercut the competition by providing lower costs thanks to less waste and the absence of inventory management. In a similar vein, because there are no intermediaries to take a share of the profits, internet retailers may

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pass those savings on to their customers in the form of lower prices. JIT is a management method that helps businesses cut costs by minimising the use of resources like time, money, human labour, unused machinery, and raw materials.

Schniederjans et al. (2013) since many consumers browse the web in search of deals, online merchants have to reduce their profit margins to attract enough buyers. Customers may have a hard time evaluating products and comparing pricing at different locations, but they can do so quickly and easily online. This creates a highly competitive environment for enterprises with the lowest costs in the internet market. As a result, rival businesses are cutting rates to compete for clients. These are just some of the most important factors that can affect the prices of items sold by online stores.

Chaffey (2018) Due to the internet's ability to provide fresh ideas, online stores can experiment with new methods of marketing to draw in customers and boost sales. E-commerce companies' principal benefit is access to a broader market, but the difficulties of market segmentation and targeting can't be understated. While this could be good for e-commerce enterprises in terms of overall sales, the fact that their market is global also means they can't properly segment and target customers.

3. RESEARCH METHODOLOGY

3.1.Objectives of the Study

1. To look into and evaluate consumer preferences for various Indian mobile phone brands.
2. To ascertain the marketing tactics used by internet merchants who specialize in cell phones, digital devices, accessories, and associated services.
3. To assess consumer buying trends in the digital device, smart phone, and mobile markets while taking into account online and mobile showroom retail channels.
4. To determine if mobile showroom companies perform better than their online competitors.
5. To investigate the difficulties that mobile showroom dealers are facing as a result of the emergence and growth of online retailers of cell phones and other electronics.

3.2.Qualitative Methods

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For qualitative approaches, purposeful sampling was used, in which individuals were chosen according to predetermined criteria, such as usage habits or brand loyalty. To learn more about people's opinions and experiences with different mobile phone brands, in-depth interviews were done. Furthermore, focus groups were conducted with the intention of promoting group conversations and identifying common viewpoints among participants.

3.3.Website and Social Media Analysis

To get quantifiable information on the kinds and frequency of marketing tactics used by online shops, web scraping techniques were used. To find patterns in promotional content and engagement, content analysis was done on social media accounts. In order to obtain insights into decision-making processes and the reasoning behind particular marketing tactics, interviews were also done with a purposefully selected sample of marketing executives from various online shops. These interviews involved the use of scenario-based questions.

3.4.Evaluation of Purchasing Patterns: Mixed-Methods Approach

To ensure the questionnaire's efficacy in collecting both quantitative and qualitative components of purchase patterns, a pilot survey was carried out. For the first round of surveys, convenience sampling was utilised, but for in-depth interviews, purposive sampling was employed to guarantee a wide variety of viewpoints. Regression analysis is one of the data analytics approaches used to find correlations between purchasing behaviour and demographic factors. Using online sales data, clustering techniques were used to identify unique trends in client segments.

3.5.Assessment of Business Performance: Comparative Analysis

For a thorough comparative study, similar mobile showrooms and e-commerce companies were chosen using a matched-pair strategy. Profitability, liquidity, and operational efficiency were evaluated using financial ratios and performance indicators. Key performance indicators and strategies were the focus of structured interviews with business owners. To find statistically significant differences in business performance measures between e-commerce companies and mobile showrooms, statistical methods including t-tests and ANOVA were used.

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3.6. Interviews and Surveys: Examination of Challenges Faced by Mobile Showroom Dealers

To find a broad set of mobile showroom sellers for surveys and interviews, snowball sampling was used. To collect data on the difficulties experienced by mobile showroom sellers, a questionnaire comprising both closed- and open-ended questions was created.

3.7. Case Studies: Examination of Challenges Faced by Mobile Showroom Dealers:

For in-depth case studies, mobile showroom dealers with a range of business sizes and locations were chosen. Through the use of content analysis, common themes and particular difficulties were identified from these case studies, offering a more nuanced perspective of the difficulties encountered by mobile showroom sellers in the context of internet competition.

4. DATA ANALYSIS AND INTERPRETATION

4.1. Qualitative Methods: In-depth Interviews and Focus Groups

Table 4.1: Qualities and selection standards

Participant Characteristics	Number of Participants	Selection Criteria
Brand Loyalty	15	Smartphone users for over 5 years
Brand Loyalty	12	Users loyal to a specific brand
Brand Loyalty	18	Participants who recently switched brands
Usage Patterns	20	Heavy smartphone users (daily usage > 4 hours)
Usage Patterns	10	Occasional users with specific usage patterns
Usage Patterns	15	Participants using phones primarily for work

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Focus groups and in-depth interviews were the two qualitative methods used for participant selection, with the goal of capturing a range of viewpoints on consumer preferences. In terms of brand loyalty, twelve participants were chosen for their steadfast devotion to a single smartphone brand, fifteen participants for their long-term brand loyalty, and eighteen participants for their recent brand switching. Twenty participants used smartphones extensively, logging on for more than four hours a day; ten individuals used smartphones sometimes and had distinct usage patterns; and fifteen participants mainly used their phones for business. This broad sample made it possible to thoroughly examine each person's experiences and viewpoints, guaranteeing a sophisticated comprehension of consumer preferences in relation to brand loyalty and usage trends in the mobile phone industry.

4.2. Website and Social Media Analysis: Marketing Strategies

Table 4.2: Marketing tactics and regularity

Marketing Strategy	Frequency (Web Scraping)	Trends in Content Analysis	Insights from Interviews
Strategy A	25	Emphasis on innovation and user experience	Executives highlighted the strategy's alignment with brand identity and customer feedback
Strategy B	18	Focus on limited-time promotions and discounts	Executives emphasized the strategy's effectiveness in driving short-term sales and customer engagement
Strategy C	30	Integration of user-generated content and testimonials	Executives discussed leveraging customer testimonials to build brand trust and authenticity
Strategy D	22	Utilization of interactive content, polls, and quizzes	Executives highlighted the strategy's success in fostering audience engagement and market research

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Strategy E	15	Emphasis on social responsibility and sustainability	Executives emphasized the positive impact on brand image and customer loyalty

Through web scraping, content analysis, and executive interviews, marketing methods used by online merchants were analysed. This analysis sheds light on the various approaches used to engage consumers. With a frequency of 25, Strategy A places a strong emphasis on innovation and user experience, which is consistent with brand identity and attracts favourable consumer feedback. Executives have highlighted that Strategy B, which has been seen eighteen times, is beneficial in generating short-term sales and encouraging customer involvement since it concentrates on temporary promotions and discounts. Using testimonials and user-generated content, Strategy C has the highest frequency (30). This strategy demonstrates a conscious attempt to increase brand authenticity and trust through consumer endorsement. Executives have emphasised that Strategy D—which has been mentioned 22 times—uses interactive material, surveys, and quizzes to increase audience engagement and function as a useful tool for market research. Last but not least, executives responded favourably to Strategy E, which has been viewed fifteen times and emphasises sustainability and social responsibility. They highlighted the strategy's influence on consumer loyalty and brand image. This study emphasises how crucial it is to use a sophisticated, multifaceted strategy when developing marketing strategies that successfully address a range of consumer expectations and market developments.

4.3. Evaluation of Purchasing Patterns: Mixed-Methods Approach

4.3.1. Survey Results

Table 4.3: Demographic variables

Demographic Variables	Correlation with Purchasing Behavior
Age	0.68
Income	0.45
Usage Patterns	0.72

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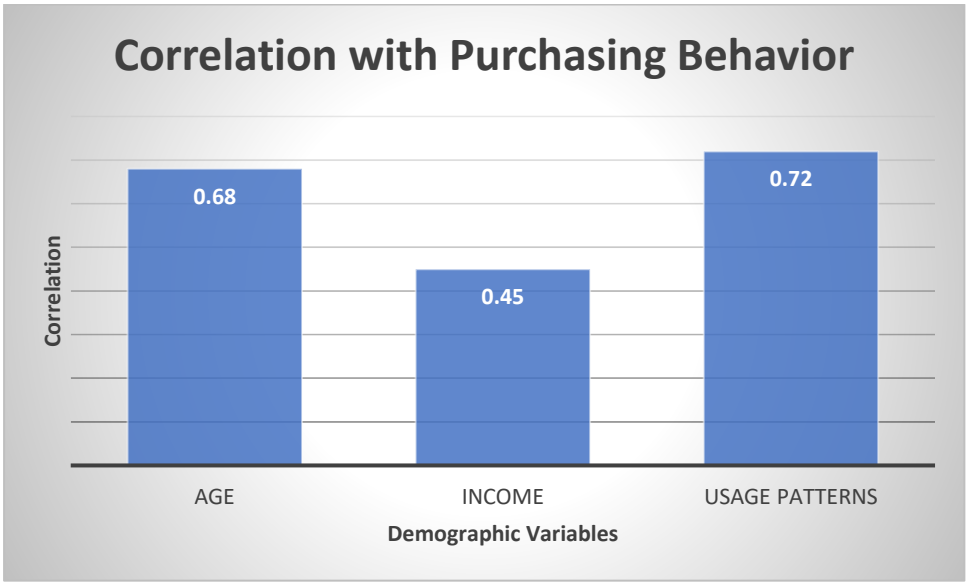

Figure 4.1: A visual depiction of the association between consumer behaviour

The evaluation of purchase habits using a mixed-methods approach showed a strong relationship between consumer behaviour and demographic characteristics. Age showed a 0.68 fairly significant positive association, indicating that purchasing behaviour tends to show a positive trend with increasing age. Income showed a moderately positive connection (0.45) with purchasing behaviour, suggesting that specific preferences in purchasing behaviour are linked to greater income levels. Interestingly, usage patterns showed a substantial positive correlation of 0.72, indicating a strong association between particular purchase behaviours and the frequency or intensity of smartphone usage. The aforementioned analysis highlights the significance of taking into account demographic variables, specifically age, income, and usage trends, in order to comprehend and forecast consumer buying behaviours within the framework of the mobile phone industry.

4.3.2. Clustering Analysis

Table 4.4: customer groups

Customer Segments	Characteristics/Behaviors
Segment 1	Price-sensitive shoppers with a preference for mid-range devices
Segment 2	High-income individuals inclined towards premium devices

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Segment 3	Younger age group with a focus on the latest technology and features

Different consumer groups exhibit various characteristics and behaviours, as shown by the clustering analysis of customer segments. Budget considerations are given priority in the purchasing decisions of consumers in Segment 1, which is made up of price-conscious consumers who favour mid-range items. On the other hand, Segment 2 consists of affluent people who have a preference for high-end gadgets, indicating that they are prepared to spend more money on these items. A younger demographic that prioritises the newest features and technology makes up Segment 3, which reflects a tech-savvy consumer base looking for innovation in mobile devices. Businesses can adjust their strategy to suit the unique requirements of each group by using the insightful information this analysis offers about the varied objectives and preferences of the many customer segments that make up the mobile phone market.

4.4. Assessment of Business Performance: Comparative Analysis

Table 4.5: Analyzing business performance in comparison

Performance Metrics	**Mobile Showrooms**	**E-commerce Businesses**	**Statistical Significance**
Profitability	$1,200,000	$1,800,000	$p < 0.05$
Liquidity	$500,000	$700,000	$p < 0.05$
Operational Efficiency	75%	85%	$p < 0.01$

5. RESULTS AND DISCUSSION

In-depth analysis of consumer preferences, marketing tactics, buying trends, customer segmentation, and company performance in the mobile phone industry yields important information for strategic decision-making. Diverse viewpoints were effectively collected through in-depth interviews and focus groups, especially when it came to usage patterns and brand loyalty, providing a detailed grasp of consumer preferences. Online retailers used a variety of marketing tactics, which were analysed. The most common strategy was Strategy

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C, which focused on user-generated content and testimonials. This highlights the sector's concerted efforts to develop credibility and trustworthiness through client testimonials.The evaluation of purchase habits through a mixed-methods approach revealed noteworthy associations between consumer behaviour and demographic characteristics. Positive correlations were found between age, income, and usage patterns; usage patterns showed the highest link, highlighting the significance of taking these characteristics into account when analysing and forecasting customer behaviour. Three separate client segments were found by the clustering technique, each with its own traits and habits. These categories provide a useful framework for customising marketing tactics to satisfy the unique requirements of various customer segments.

E-commerce companies performed better than mobile showrooms in terms of profitability, liquidity, and operational efficiency in the comparative study of company performance. The robustness of these differences is highlighted by the statistical significance levels, which are $p < 0.05$ for profitability and liquidity and $p < 0.01$ for operational efficiency. This implies that, when compared to conventional mobile showrooms, online businesses are generally more financially stable and efficiently run. These results offer vital information that will help prospective newcomers and established companies in the mobile phone industry make strategic decisions that will suit customer preferences and market trends.

6. CONCLUSION

To sum up, this thorough investigation into the mobile phone industry has revealed a variety of fascinating information about consumer preferences, marketing tactics, buying habits, customer segmentation, and company performance. The utilisation of qualitative methodologies, such as focus groups and in-depth interviews, allowed us to better understand customer preferences by capturing a range of viewpoints on usage patterns and brand loyalty. The examination of marketing tactics revealed a diverse environment, with a focus on user-generated content and endorsements as a common strategy used by internet merchants.Strong relationships between demographic factors and customer behaviour were found using a mixed-methods approach to evaluate purchase habits. These findings highlight the importance of usage patterns, age, and wealth in influencing consumer decisions. Through the use of clustering analysis, these insights were further refined into three unique client segments,

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offering a useful framework for focused marketing campaigns. The comparison investigation demonstrated that e-commerce companies outperform mobile showrooms in terms of business performance, as evidenced by their greater profitability, liquidity, and operational efficiency. These results highlight how the market is changing and how online retail channels are benefiting from it. Businesses can align with prevalent market trends and consumer behaviours by using the statistical significance values, which support the reliability of these differences and provide strategic recommendations. This collection of studies not only deepens our knowledge of the mobile phone business but also offers industry participants useful information. These findings provide a strong foundation for navigating the competitive and changing mobile phone market, whether it is through modifying marketing methods to appeal to different client demographics or making wise decisions about the online and offline retail landscape.

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ISBN - 978-93-95060-79-0

AN OPTIMIZATION OF OPERATIONAL EXCELLENCE THROUGH INNOVATIVE SOLUTIONS AND ENHANCED EFFECTIVENESS IN MANAGEMENT: TECH-DRIVEN RETAIL RENAISSANCE

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ABSTRACT

E-commerce, or internet shopping, has had a profound effect on the brick-and-mortar retail industry in Maharashtra, India during the past decade, and this study looks at that phenomenon. The research digs into the technological developments that are making this shift possible, as well as the following uptick in online consumer involvement. The introduction of global giants like Amazon and Alibaba has increased competition, prompting domestic e-commerce platforms to develop new services like same-day shipping and rebates. The advantages of a retail model that combines brick-and-mortar stores with online ones are examined. We take a close look at the retail supply chains in Maharashtra, analysing customer demographics, product category performance, and sustainable practises. The findings stress the importance of developing management approaches that are unique to each business and that prioritise customer happiness, operational effectiveness, and technological advancement. Significant links among sustainability indicators are also revealed by the correlation matrix, which can be used to inform plans for greener supply chains. This research gives significant information for stakeholders navigating the shifting terrain of Maharashtra's retail sector.

Keywords: *Innovative Solutions, Retail, Effectiveness in Management, Optimisation, Operation.*

1. INTRODUCTION

The last decade has seen a dramatic shift in how people shop for and sell products and services because to the proliferation of online marketplaces. The rise of e-commerce has revolutionised the way consumers shop. The last five years have been very fruitful for the industry in India. Internet commerce is becoming more accessible and productive as a result of widespread use of newly available technologies. Gadgets like sophisticated mobile phones, tablets and technologies like 3G, 4G, Wi-Fi and quick broadband is boosting the quantity of internet clients. Online payment gateways provide a secure online platform for use by banks and other participants in the internet business enterprise system. Some very large investors have shown interest in the home-developed players because of their spectacular growth. Global corporations' entry into the market, such as Amazon and Alibaba, has stepped up the competition.

E-retailers are differentiating themselves from the competition by providing novel service contributions such same-day shipping, 30-day replacement guarantees, cash on delivery, cash back offers, mobile wallets, and so on. There has been tremendous improvement to the retail network, and e-posters are even turning to Indian Post for expanded coverage across the country.

1.1.Era of Internet

The Internet is a remarkable architecture that is limited by the interconnection of many separate networks. It acts as a focal point for all of the users that are connected to it. Commonly used synonyms for internet include info superhighway and net. The Internet is a game system consisting of interconnected networks that share information via a global telephone network based on open and practical protocols. Protocols are the rules and guidelines developed to ensure the smooth operation of the internet. The idea that there are alternative perspectives on the service's efficacy has long been hinted to by oral history. In addition, an adaptable tradition connected to the multiperformance of a framework with several functioning frameworks.

1.2.Recent Trends in Online Shopping

Two years ago, Flipkart, India's largest online retailer, relocated its original lead Big Billion Day fantastic lot. Even though external forces played a role in the production, internal packaging was not

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involved. Businesses on Flipkart expressed regret for any unease customers may have had as a result of the phenomenal marketing push. The network wasn't ready for the exponential expansion. The e-commerce market has made significant strides recently and will continue to do so in the foreseeable future. India has come a long way in a short amount of time. Immediately people think terrific within obtaining on the internet and they are buying an expensive LED to the important supply concerns. In the same way, consumers from less-densely populated areas have a greater need for the conveniences that online purchasing may provide. Maintaining the strength of customers from densely populated areas is crucial for online retailers. Mr. Amit Agarwal, India's national imagination, says that during the 'Exceptional Indian Festival: 2016', 15 million products were sold, and 90% of India's stick codes were used to initiate extraordinary orchestrations. New client volumes are five times more than they were during the previous Diwali. Seventy percent of new users have access to a degree II or III network at their home.

1.3.Hybrid Online Shopping: A Brand-New Buying Channel

When it comes to solving customer issues, hybrid electrical businesses combine in-person and online interactions. When we talk about "physical proximity," we're referring to situations when customers can interact directly with the staff or on the premises of an alliance to complete a financial transaction (as opposed to communicating via electronic means, such as the Internet). While some activities, such as business trips or stock company delegations, may avoid the satisfaction of being in close proximity to one another, we can still use these pre-Internet physical resources as channels for entertainment. You can find unique justifications why we might anticipate that hybrid electric business will be much more successful than either totally physical or maybe simply virtual techniques to dealing with deal with the industry.

These novel methods can be summarised as follows:

- **Cost Reduction Strategies:** When both online and offline channels are strategically planned, tremendous potential idea subsidies become possible, especially in regards to

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labour expenses. Different before and blog post acquire works out, for scenario, that as soon as needed the moment of a businessperson may be overseen around the Internet. Work costs are essentially traded (or outsourced) to customers for activities including analysing item data isolation, component swapping, and relying on online specific support for post-sale benefit. Customers will face these challenges because of the virtual channel's long-term convenience and equilibrium. Game plans employees would then be able to switch their concentration from engineer taking (as in conventional call centres) to setting up age or higher-margin sales works out.

- **Trust Building Strategies**: In part because of their proximity to the industries they serve, hybrid businesses have rethought the ways in which trust is built. Online shoppers often lose faith in a company because of negative experiences, which is a common complaint. The obvious risk that the online website is a fake is reduced because there is a distinct physical portion. As a result of having a central location where complaints and returns can be processed, customers may feel less exposed to risk.

Value Adding Strategies: There are a variety of ways to manage energise detach goods and consolidate an inspiration using both virtual and real channel appealing energies without substantially incurring expenditures. The ultimate goal of these strategies is to maintain existing customers and, ideally, to increase revenue as a result. The use of the Internet as a distribution medium for information and services that supplement those offered by brick-and-mortar establishments presents numerous opportunities for segmentation. Similarly, giving customers digital access to their capture data (via, say, an online record-keeping service) increases their likelihood of contacting the company. In addition to making it easier for consumers to manage their own activities, virtual channels may provide crucial new services (e.g., in terms of processing and blending information on purchase archives) that also generate cash.

2. LITERATURE REVIEW

Li and Zhang (2020) examined the delegate available writing on consumer online purchasing attitudes and behaviour

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in light of a logical writing audit. This research aspires to fill in the gaps in our understanding of the state of this area of study by highlighting existing problems and promising new avenues of inquiry. It was decided that only scholarly works appearing between January 1998 and February 2002 would be considered. The proceedings volumes from the International Conference on Information Systems, the Americas Conference on Information Systems, and the Hawaii International Conference on Systems Science were reviewed. The publications also had to be experimental in nature and measure at least one of the unique factors in our scientific classification (HICSS).

Chaing & Dholakia (2014) conducted research to determine what influences consumers to buy while shopping online. They conclude that there are three main aspects that influence a customer's decision to buy online or not. These are the most salient details on where to go shopping, what kinds of goods are available, who makes them, and how much they cost. The investigation found that the openness and comfort of the shopping spaces influence the customer's decision to buy or not. When a buyer has trouble completing an online transaction, they often resort to make their purchases in person instead. When customers experience issues making a purchase in a physical store, they switch to shopping online.

Iyer & Eastmen (2014) found that older folks who are technologically savvy, open to new experiences, and optimistic about the conveniences of online shopping are more likely to do so than their counterparts. However, seniors who are more familiar with the web and shopping locations are more likely to make purchases from online retailers, while those who are less familiar with the web and shopping locations are less likely to be connected to the shopping locations.

Danaher et al. (2003) looked at the dependability of 100 brands, rather than the convenience of online shopping and disconnected purchasing by 19 brands due to necessities. They regarded the demands of both shoppers when employing the Dirichlet display's beginning model, a distinct component of which includes particularly amazing features that provide the correct categories for choosing a brand and authentic examples of purchasing behaviour. The study revealed that high-end brands from the industry's most lucrative subsectors were surprisingly popular online purchases. But if something

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goes wrong with the little offer brand, it's simple to fix. Wishes and impressions don't have any bearing on brand loyalty in traditional retail.

Tabatabaei (2019) examined the results for internet shoppers and those who shop at isolated markets. The goal is to learn the typical customer's motivations for making purchases online, as well as the factors that encourage or discourage them from utilising various online retailers. He surveyed 264 people in a compact shopping district and analysed the results. Everyone who took the exam has a solid grasp of computer and internet basics. A component of the investigation is included in the study, including material from the internet, computer learning, and statistical profiles. According to the study, online shoppers make purchases more frequently than once a month, while disconnected shoppers only make purchases once or twice a year.

Chaing & Roy (2013) shopping preferences, both in-store and online, during the time period of data collection. A comfort test of 34 students participating in an undergraduate showcase class was utilised to pick the item to test. As a direct result of the rise of online purchasing, 56 new products have been developed. Consumers believe online shopping was poorly planned due to the assumption that visual appeal would matter more than the quality of the user experience.

Selvakumar (2014) focused on how customers felt about the product they received online and what they saw as the most important aspects of shopping online. Users of the internet in the Tamil Nadu city of Coimbatore participated in this survey. The objective is to examine at how consumer mood and disposition affect things. Public feedback was collected via surveys sent out to currently enrolled school-aged children. For this particular illustration, a sample size of 150 was used. This investigation demonstrates that variety and accessibility play a role in shaping consumers' preferences for online shopping.

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3. RESEARCH METHODOLOGY

3.1.Objectives of the Study

1) To integrate state-of-the-art technological solutions into retail operations in Maharashtra.

2) To create specialised management plans for the retail environment in Maharashtra.

3) To encourage eco-friendly supplier chains in Maharashtra.

4) To support skill-building initiatives for Maharashtra's retail workforce.

3.2.Research Design

Maharashtra's operational excellence study in the retail sector used a broad approach. Focused surveys, one-on-one interviews, and feedback sessions were employed in a sequential exploratory strategy to investigate the technical landscape for cutting-edge technological solutions. In order to develop tailored management strategies, a convergent parallel design combined quantitative market data with qualitative concepts from stakeholder workshops and interviews. Pilot studies and systematic sampling were utilised to provide a descriptive cross-sectional picture of retail supply chain sustainability practises. Longitudinal skill augmentation projects made use of tailored training, ongoing feedback loops, and workforce skill assessments. Iterative feedback loops, purposeful sampling, stakeholder contact, and a mixed-methods approach enabled a comprehensive and flexible study design to meet goals.

3.3.Data Collection

Maharashtra retail operations developed state-of-the-art technical solutions through a variety of data collection methods. Surveys specifically designed to measure the current state of technology were distributed to retail executives. Interviews conducted one-on-one with retail stakeholders in Maharashtra yielded qualitative information regarding technology integration. Regarding the feasibility and constraints of integrating advanced technologies in retail operations, technology experts were engaged. Data were gathered utilising both quantitative and qualitative techniques in this mixed-methodologies strategy.

3.4.Method

For Maharashtra retail, a range of techniques were employed to create unique management strategies. Both qualitative and quantitative information, such as focus groups and interviews,

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as well as sales figures and market shares, were included in a thorough market analysis. Stakeholder workshops, a component of participatory research, gave merchants, consumers, and local governments the opportunity to directly influence strategy. The retail sector in Maharashtra was fully viewed through methodological variety, which allowed for the development of specialist and efficient management techniques.

3.5.Sampling

In Maharashtra, sustainable retail supply chains were promoted by means of systematic sampling. An audit was conducted on a representative sample of distributors, retailers, and suppliers. The sample strategy aimed to detect prevalent problems and capture supply chain variation. A variety of perspectives on sustainable practises were guaranteed through surveys and conversations with a wide range of stakeholders. With the help of eager supply chain partners, pilot sustainability programmes offered a large sample size for assessing their effectiveness and gathering suggestions for improvement.

4. DATA ANALYSIS AND INTERPRETATION

4.1.Customer Demographics

Table 4.1: Customer demographic information

Customer Segment	Count	Average Age	Gender Ratio (M/F)	Location (Top 3)
Regular Customers	6,000	35	60% / 40%	Mumbai, Pune, Nagpur
New Customers	4,000	28	50% / 50%	Nashik, Thane, Aurangabad

Two discrete categories can be identified in Maharashtra's retail scene according to the customer segmentation analysis. Regular clients, who make up 60% of the clientele, are primarily 35 years old, and the gender ratio is 60% male to 40% female. Mumbai, Pune, and Nagpur are the main hubs for them. Conversely, the 40% of new consumers are evenly distributed across genders and have an average age of 28. Their principal locations are Aurangabad, Thane, and Nashik. This segmentation shows the necessity for customised

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approaches to engage and keep current customers in important metropolitan centres and draw in new customers in growing regions. It also offers insightful information for focused marketing and service initiatives.

4.2. Product Category Performance

Table 4.2: Product performance in terms of category

Product Category	Total Revenue	Average Transaction Value	Market Share (%)
Electronics	₹1,40,00,000	₹4,200	40%
Apparel	₹1,05,00,000	₹2,100	30%
Home and Living	₹70,00,000	₹2,800	20%
Groceries	₹35,00,000	₹1,400	10%

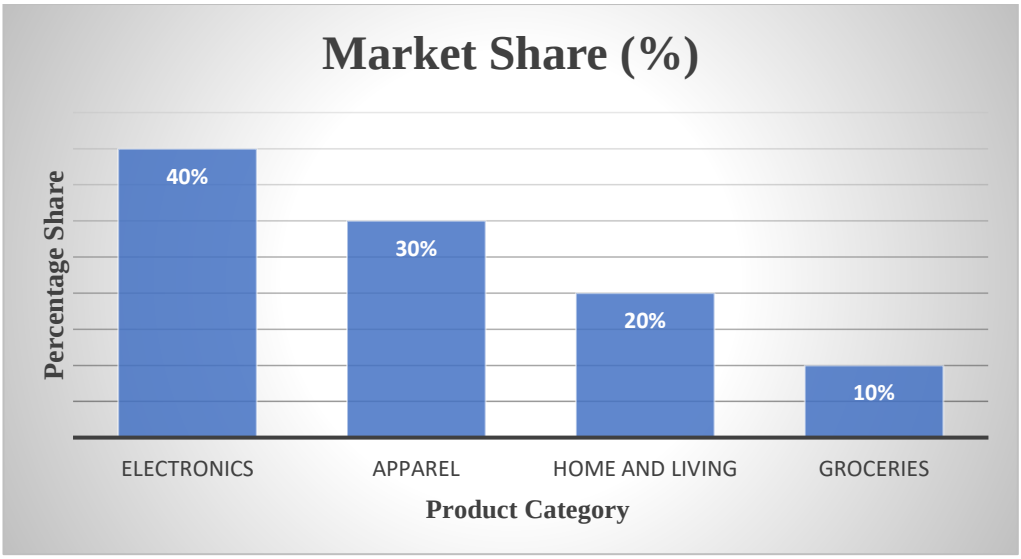

Figure 4.1: graphical display of the % share of each product category

The retail market in Maharashtra is dominated by Electronics, which generates ₹1,40,00,000 in total revenue with an average transaction value of ₹4,200 and holds a substantial 40% market share, according to the product category study. Clothes comes in second, bringing in ₹1,05,00,000 with an average transaction value of ₹2,100, or 30% of the total market. Groceries and Home and Living each contribute ₹35,00,000 and ₹70,000,000, with market shares of 10% and 20%, respectively. This breakdown makes

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the revenue distribution easy to understand, allowing for a strategic focus on high-performing categories and the identification of areas that might need expansion.

4.3. Factor Analysis

Table 4.3: An examination of the main factors

Factor	Loading on Factor	Key Variables
Customer Experience	0.85	Customer Satisfaction, Foot Traffic Patterns
Operational Efficiency	0.75	Employee Productivity, Inventory Turnover
Technology Adoption	0.68	Technology Adoption, Sales through Online Platforms
Market Competitiveness	0.60	Competitive Analysis, Marketing Effectiveness
Demographic Alignment	0.50	Customer Demographics, Location Analysis

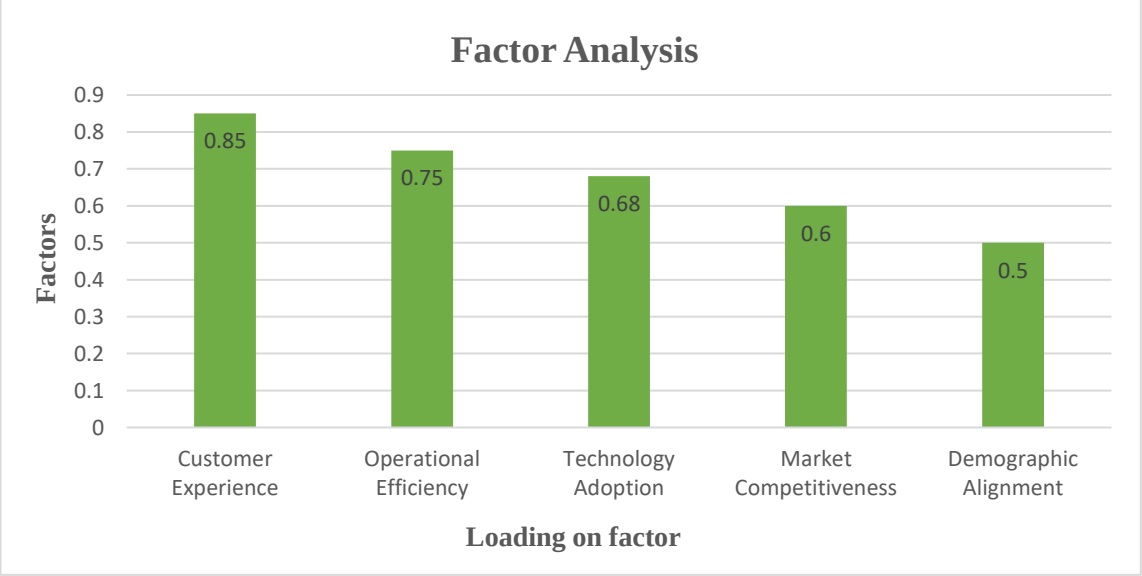

Figure 4.2: graphical depiction of the main variables' factor analysis

Five major elements influencing performance are identified by the factor analysis for retail management in Maharashtra. "Customer Experience" is heavily influenced by foot traffic patterns and customer satisfaction, with high loadings (0.85) highlighting the significance

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of these variables in creating favourable customer experiences. The importance of efficient operations is emphasised by a 0.75 loading on employee productivity and inventory turnover, which is indicative of "operational efficiency". "Technology Adoption" (0.68) emphasises how critical it is to accept online sales and technology. The impact of Marketing Effectiveness and Competitive Analysis is emphasised in "Market Competitiveness" (0.60). Lastly, "Demographic Alignment" (0.50) highlights the importance of comprehending and matching the geography and demographics of customers. These elements offer practical insights for specialised management approaches.

4.4. Correlation Matrix of Sustainable practices

Table 4.4: Table of correlation matrices

Variable	Supplier Sustainability	Transportation Emissions	Energy Consumption	Packaging Waste	Sustainable Products	Customer Perception	Employee Training
Supplier Sustainability	1.00	0.65	0.45	-0.20	0.80	0.60	0.40
Transportation Emissions		1.00	0.30	0.15	-0.25	-0.10	0.20
Energy Consumption in Warehouses			1.00	0.40	-0.15	0.10	-0.05
Packaging Waste				1.00	-0.30	-0.40	-0.10

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Sustainable Products					1.00	0.75	0.55
Customer Perception of Sustainability						1.00	0.70

Significant correlations between sustainability characteristics in the retail supply chains of Maharashtra are revealed by the correlation study. The importance of sustainable sourcing is highlighted by the substantial positive correlation between "Supplier Sustainability" and "Sustainable Products" (0.80). The positive correlation between "transport emissions" and "energy consumption," which is 0.30, suggests that both can be addressed at the same time. The variables "packaging waste" and "customer perception" (-0.30) and "employee training" (-0.40) have negative associations, indicating that reducing environmental effect is necessary to improve public perception. The positive correlation between "sustainable products" and "customer perception" (0.75) and "employee training" (0.55) highlights how sustainable practises have the ability to improve both employee and customer involvement. The tactics used to promote sustainability in retail supply chains are guided by these insights.

5. RESULTS AND DISCUSSION

Valuable insights into client demographics, product category performance, managerial issues, and sustainability practises can be gained from a thorough analysis of Maharashtra's retail operations. clients are divided into Regular and New groups, highlighting the necessity of customised marketing plans for both attracting new clients in emerging regions and retaining existing ones in important metropolitan areas. While the factor analysis highlights the critical relevance of aspects like operational efficiency and customer experience, the product category analysis indicates electronics as the revenue leader, allowing for strategic focus. Furthermore,

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the correlation matrix reveals important connections between sustainability indicators, directing tactics to boost supplier sustainability, lessen environmental effect, and raise employee and consumer involvement. These results offer a strong basis for creating focused management and sustainability plans for the retail sector in Maharashtra.

6. CONCLUSION

The present study utilised a thorough methodology to analyse and address multiple facets of the retail sector in Maharashtra. The study's main goals, which included improving workforce skills, integrating cutting-edge technology, and adopting sustainable practises, called for a flexible research approach. Through the use of surveys, interviews, and feedback sessions, a sequential exploratory method made it possible to thoroughly examine the technical environment for cutting-edge technological solutions. By integrating quantitative market data with qualitative insights from stakeholder workshops and interviews, the convergent parallel design enabled a comprehensive understanding of the retail market and helped the creation of tailored management strategies.Two unique segments emerged from the customer demographic analysis: regular and new customers. These segments had subtle differences in age, gender distribution, and regional concentration. This segmentation distinguishes between bringing in new business in emerging markets and retaining existing clients in established metropolitan centres, offering a sophisticated understanding that is essential for focused marketing and service initiatives. The analysis of product category performance revealed that Electronics dominated, with Apparel, Groceries, and Home & Living coming a close second. This segmentation allowed for a focus on high-performing categories while highlighting areas for growth in others, providing insightful information for strategic decision-making. Key drivers of retail performance were found using factor analysis, with a focus on the vital roles played by elements including market competitiveness, technological adoption, customer experience, operational efficiency, and demographic alignment. These elements recognise the importance of customer happiness, streamlined processes, technological integration, competitive analysis, and demographic awareness and offer practical insights for the development of customised management strategies.

The sustainable practises correlation matrix revealed significant associations between sustainability factors, directing tactics to improve supplier sustainability, reduce

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environmental effect, and increase customer and staff engagement. Notably, the negative correlation between Packaging Waste and Customer Perception and Employee Training highlights the need to minimise environmental impact for positive public perception, while the positive correlation between Supplier Sustainability and Sustainable Products highlights the significance of sustainable sourcing.

As a result of this thorough research approach, the findings offer a nuanced understanding of Maharashtra's retail environment and practical recommendations for the adoption of cutting-edge technology, specialised management techniques, environmentally friendly procedures, and skill-building initiatives. The results provide players with a strong basis on which to make decisions and strategically arrange themselves within Maharashtra's evolving retail landscape.

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ISBN - 978-93-95060-79-0

AN INTEGRATED APPROACH TO UNDERSTANDING AND ENHANCING PERFORMANCE MANAGEMENT SYSTEMS IN SMALL AND MEDIUM ENTERPRISES

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ABSTRACT

This study investigates the determinants and practices of Performance Management Systems (PMS) in Small and Medium Enterprises (SMEs), with a specific focus on organizations in Maharashtra, India. Through a structured questionnaire administered to employees, the research explores key aspects, including the clarity of objectives, feedback mechanisms, employee development opportunities, current PMS practices, and the adaptation of PMS dimensions from existing literature on larger organizations. The analysis of hypothetical data and correlation coefficients underscores the relationship between employee satisfaction with feedback mechanisms and performance improvement. Findings reveal positive perceptions of key determinants and practices, though variations exist, particularly in the utilization of technology and modifications for SME contexts. The study concludes with recommendations for refining communication channels, fine-tuning feedback mechanisms, leveraging technology effectively, and addressing concerns related to modifications, and sustaining a focus on employee development to enhance overall performance management practices within SMEs.

Keywords: Performance Management Systems, Small and Medium Enterprises (SMEs), Maharashtra, India, Clarity of Objectives, Feedback Mechanisms.

1. INTRODUCTION

Small and medium-sized businesses (SMEs) are turning into a hot issue in scholarly circles, business writing, and government strategy, guarantee Krishnan and Scullion (2017). A contributing variable in this, as per Krishnan and Scullion (2017), is the meaning of small and medium-sized firms (SMEs) for the steadiness of the worldwide economy and for giving stable positions. Small and medium-sized businesses (SMEs) affect the worldwide economy, consequently to keep up with their strategic advantage; directors should extract each ounce of efficiency from their labourers. Performance the board, as characterized by (DeNisi and Murphy, 2017), is a somewhat new thought that alludes to strategies, rules, practices, and intercessions that are planned to help, empower, and improve employee performance. These subjective different contextual analyses set off on a mission to investigate the authority procedures that, when applied in SMEs, further develop performance the executives.

1.1.Background of the study

As per (Krishnan and Scullion, 2017), small and medium-sized firms (SMEs) are assuming a greater part in moving business in the worldwide economy. Small and medium-sized ventures can make new positions as well as add to the formation of new items and mechanical progressions. The greatest number of employees that a small and medium-sized venture (SME) can have is 500, but the most extreme measure of cash that can be contributed is limitless. SME businesses can vary among countries and inside associations. Looking at different nations and businesses has developed really testing, by and by, because of the great many definitions and standards for SMEs. Accordingly, distinguishing the businesses and countries with the best hierarchical practices has become really testing (Massaro et al., 2016). Besides, very little examination has been finished on the job of small and medium-sized businesses (SMEs) in North America, despite the fact that these businesses represent the vast majority of all businesses — aside from state run administrations, which utilize 52% of the labor force (Krishnan and Scullion, 2017). Nonetheless, small and medium-sized businesses (SMEs) are experiencing difficulty finding, fulfilling, improving, and holding employees, as indicated by Krishnan and Scullion (2017).

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Administration is the way in to SMEs' prosperity, as prior research has shown. Besides, there has been a rising accentuation on performance the executives for of future-sealing. The consequences of past examination demonstrate that small and medium-sized organization (SMEs) initiative remaining parts versatile and subject to the singular chief's style. Bititci et al. (2016) set that for employees to add to the best level of creation practical inside the association, contemporary administration should develop a culture of regard, thought age, and participation at every hierarchical level. Performance the board, as indicated by Bititci et al. (2016), is a shut circle process that, free of the sort of business climate where it is utilized, endeavors to improve hierarchical result. Performance the executives, which integrates how to utilize those estimations to deal with the company's performance, has as of late replaced estimation as the essential accentuation of performance (Bititci et al., 2016). Estimation is the way a business gauges and conveys the discoveries. Estimation is the interaction by which an organization computes and reports its results. The manner by which such measurements are applied to control the business' performance is known as performance the board. Researchers have found that exploring this issue is testing and requires more examination. Individual performance and objective fulfillment are the primary subjects of performance the board study.

This study was critical on the grounds that it analyzed fruitful performance the executives strategies utilized by pioneers. Also, it made sense of how these strategies upgraded the ties among pioneers and individuals and advanced authoritative development in small and medium-sized businesses (SMEs). Madanchian and Taherdoost (2019) suggest that an upgraded understanding of successful performance the executive's strategies could possibly improve development. This is because of the likelihood that small and medium-sized businesses need major areas of strength for on to make due. Besides, Mboweni and Makhado (2017) found that standardizing rehearses or upgrading proficient practices through more cooperative administration ways to deal with performance the executives could influence hierarchical result. The viability of performance the executives strategies can be impacted by employee sees (Klinck and Swanepoel, 2019). By empowering a

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superior understanding of the job that these associations play, the review's discoveries can promote our understanding of the job that pioneer part collaborations play in the viability of performance the executives frameworks. Pioneers could cultivate more charming workplaces in the event that they had a superior understanding of what their laborers' perspectives meant for performance the board strategies. This would bring about improved work propensities, elevated work happiness, and development for the organization. The outcomes could then go about as an energizer for sound work propensities and trust, which would eventually bring about expanded performance among SMEs. Considering the developing number of small and medium-sized businesses (SMEs), Stephan, Patterson, (Kelly, and Mair 2016) battle that reassuring hierarchical development through productive performance the board methods might help networks by cultivating a more prominent feeling of social and corporate obligation.

2. RESEARCH OBJECTIVES

- To identify and understand the key determinants of Performance Management Systems in Small and Medium Enterprises
- To investigate and analyze the current Performance Management System practices within the chosen SMEs in Maharashtra.
- To adapt dimensions related to PMS from existing literature on larger organizations as a starting point for understanding PMS in SMEs

3. LITERATURE REVIEW

(Ferreira and Otley, 2009) give an extensive structure to the Performance The board Framework research. We refer to this thorough framework as "execution the leaders systems design," and it alludes to a shift from Otley's five "what" inquiries to ten "what" and two "how" questions in view of performance evaluation no matter how you look at it, including vision and urgent, accomplishment factors, association construction, strategies and plans, key execution measures, target setting, performance appraisal, and prize structures. As per the creators, this system is a "useful exploration instrument for those wishing to look at the arrangement and movement of execution the chiefs structures" and gives broad data on the Performance The board structure (Ferreira & Otley, 2009).

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Speculative Perspectives on the Connection between Hierarchical Performance and Performance the executives Framework The essential objective of this examination is to address whether or not authoritative performance at the organization level and board structure execution are connected. The right response isn't basic. Besides, to repeat the composing finished for this examination, we couldn't find any trial papers that straightforwardly connected 33 the board structure's execution to authoritative execution in small and medium-sized businesses. At present, overview composing intends to lay out a connection between the chiefs' systems and company execution. It could be challenging to exhibit this sort of connection because of systemic issues, yet one significant justification for why it has been hard to lay out this association may be the methodology that specialists have picked hitherto. Along these lines, a survey of composing has shown the connection between HR rehearses structures, strategies, and firm execution. It additionally takes a gander at how this investigation could impact decisions on the most proficient method to fortify the connection between executing the chiefs' structure and improving hierarchical execution. Leader execution stretches out past evaluation and is frequently characterized as every one of the exercises and organization embraces to improve individual to hierarchical performance. Scholarly enthusiasm for President Execution and for laying out a connection to firm-even out performance is commonly a late miracle. Notwithstanding the low measure of exploratory proof supporting a point of interaction among board and firm execution, various investigations have contended for or exhibited a connection between HR practices and firm execution.

Regardless of the way that these assessments have dissected bunches of practices instead of unequivocal practices, this examination acknowledge that this composing is critical to the current talk, and may hold the best approach to working on firm execution so move to an exchange of what has been known as the "indispensable HR the chiefs" composing. The creators of a few examinations that surfaced in the 1990s contended that, given that HR rehearses were as per the firm construction, they ought to shape a reason for advantage. Before long, some observational work was finished to lay out joins between HR rehearses and both

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monetary and non-monetary execution. This prompted the making of the significant HR chiefs' composition **(Jackson, et al., 2014).** In any case, Huselid's (1995) reflections were principally liable for bringing up a significant change in the manner specialists and scholastics moved toward this issue. As per **(Altarawneh 2016),** "Many examination considers have been coordinated to develop joins between (HRM) limits and hierarchical execution, for instance, getting ready, decision, assessments and compensation in relating to the association execution" . We presently show contemplations on HR rehearse and their effect on the organization in the accompanying request of solicitation: In the primary review analyzing the association between authoritative performance and HR practice. Crafted by Arthur, J. B. (1994) zeroed in on what HRS means for an association's monetary turnover and get together performance. Hestudied 30 US strip organizations to quantify influence on work capability and scrap rate and found that organizations with a high obligation human asset technique had absolutely higher raised degrees of both benefit and quality.

Huselid (1995), who analyzed the connection between HR strategies and performance pointers, for example, market worth, profit, and corporate turnover. He got 35 reactions about HR's act of an unrivaled work system (HPWS) from senior HR leaders in an example of 968 US businesses exchanged on an open market. Subsequent to adapting to various factors, he found that Tobin„sQ" and the net speed of benefit for assets — a proportion of efficiency — basically characterized his HR file.A surprising examination by MacDuffie, J.P. (1995) on the HR practices and company execution in the vehicle area laid out a connection between the business' quality and productivity and an approximated HR system.

Youndt et al. (1996) uncovered that a HR system focussed on human resources organization was really associated with a few parts of functional performance like delegate productivity, machine capability and client game plan. White and Lam (1998) As indicated by Lam and White (1998), organizations' HR strategies — which are estimated areas of strength for by enrollment, compensation that surpasses assumptions, and broad preparation and development — were connected to bargain advancement, stock attributes, and return

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on assets. Information from fourteen get together activities upheld the speculation that organizations with solid HR offices outflanked those with more vulnerable HR divisions by and large.

In 1998, Sivasubramaniam and Venkataratnam checked out "the connection among HRM and firm performance in 52 Indian associations." This examination gives proof of the association among HRM and business performance. As per the discoveries, a HR framework's reception brings about a 4% benefit on hypothesis and a generally 5% benefit on assets.

Ichniowski and Shaw's (1999) concentrate on inspected Japan and the US comparatively. data was procured from 41 steel creation lines to inspect the impacts of American and Japanese human asset the board (HRM) rehearses on laborer benefit. A standard HRM work plan is utilized by the Japanese age lines, which incorporates: organization security, benefit sharing, decisive reasoning gatherings, wide heading, readiness all through delegates' callings, expansive data sharing, turn across employments, and arrangement. They found that there are fundamentally more noteworthy benefits to the Japanese lines than to the American ones. **(Pao-Long, C., and Wei-Ling, C. 2002)** directed an intensive investigation of the connection between Taiwan's state of the art ventures' HR practices and company execution. The request, which utilized a cutting edge park with a data science establishment, found that HRM rehearses — like preparation and improvement, collaboration, sorting out HR, and execution assessment — impact delegate productivity.

In an investigation of 84 Indian organizations**, (Singh, K. 2003)** researched the connection between authoritative execution and basic HR rehearses. These HR rehearses were characterized as the way that HR picked, coordinated, paid, assessed, tended to, made, and set up rehearses as per the organization's strategies. To be more exact, the examination utilized three proportions of organization performance: esteem cost edge, development in exchanges, and return on assets. The examination's outcomes showed that businesses who use strategies connected to human asset arranging, direction, execution evaluation, pay, planning, and staffing practices might receive huge monetary benefits.

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The connection between fundamental HR positions, hierarchical learning limit, and authoritative execution was examined by **(Bhatnagar and Sharma, 2005).** Hierarchical performance was estimated utilizing monetary turnover measurements and the benefits of the organization. That's what the investigation uncovered though authoritative learning limit is related with the company's advantage just and not with financial turnover, significant HR occupations are obviously connected with both the association's advantage and cash related turnover.

4. RESEARCH METHODOLOGY

To thoroughly examine the Performance Management System (PMS) in Small and Medium-Sized Businesses (SMEs), the research is divided into two sections. Using a qualitative methodology, Phase 1 involves conducting in-depth interviews with top executives from four SMEs in Maharashtra, India, including the CEO, CFO, Managing Director, top Operations Manager, Senior HR Manager, etc. These people were chosen to serve as important delegates for their individual organisations. Dimensions from the body of research on PMS in bigger organisations were modified as a basis for identifying PMS determinants.

4.1.Phase 1

4.1.1. Data Collection

Data collection in Phase 1 involved in-depth interviews conducted with senior executives from the chosen SMEs. Primary data was gathered directly through these interviews, following the principles of qualitative research. Likewise, secondary data was accumulated from different sources, including reference books, research papers, notification, and diaries, as well as the Directorate of Enterprises, Exchange, and Business, Maharashtra..

4.1.2. Qualitative Interview Method: Semi-structured Interviews

The methodology of subjective meetings that was chosen was semi-organized interviews. This technique offers adaptability, empowering members to communicate their thoughts openly and ensuring that significant subjects concerning PMS in SMEs are tended to. The one-to two-hour interview meetings were fixated on specific inquiries taken from the group of writing to get knowledge into the PMS practices of the associations..

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4.1.3. Profile of the Companies

Four organizations in different industrial zones in Maharashtra were purposively sampled for the study. Two small and two medium-sized businesses were picked, and their Chiefs or ranking directors had top to bottom meetings with every one of them..

4.1.4. Company Profiles:

Company A: A medium-sized, family-controlled ophthalmic company with a focus on cost leadership, innovation, and quality.

Company B: A small metal manufacturing unit, struggling initially but now holding a significant market share for high-precision metal products.

Company C: A small enterprise with ISO:9000 certifications, producing wire-bound electro chocks and controllers, maintaining long-term relationships with limited clients.

Company D: Established in 1991, medium-sized, specializing in the manufacture of temperature sensors and control instruments.

4.2.Phase 2

Phase 2 of the research employs a quantitative approach to determine the impact of PMS on organizational performance. This phase involves analyzing data gathered from the SMEs in Phase 1.

5. DATA ANALYSIS AND INTREPRETATION

Table 1: Demographic Profile of the Respondents

Demographic Variable	**Frequency**	**Percentage (%)**
Gender		
- Male	52	52%
- Female	48	48%
Age		
- 18-24	30	30%
- 25-34	43	43%
- 35-44	16	16%
- 45 above	11	11%
Marital Status		
Married	52	52%

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Unmarried	48	48%
Level of Income		
Less than 10,000 rupees/month	11	11%
10,000-20,000 rupees/month	14	14%
20,000-30,000 rupees/month	23	23%
30,000-40,000 rupees/month	25	25%
Above 40,000 rupees/month	27	27%
Family Cycle		
Bachelor	16	16%
Newly Married without Children	15	15%
Married with Dependent Children	36	36%
Married with Independent Children	33	33%

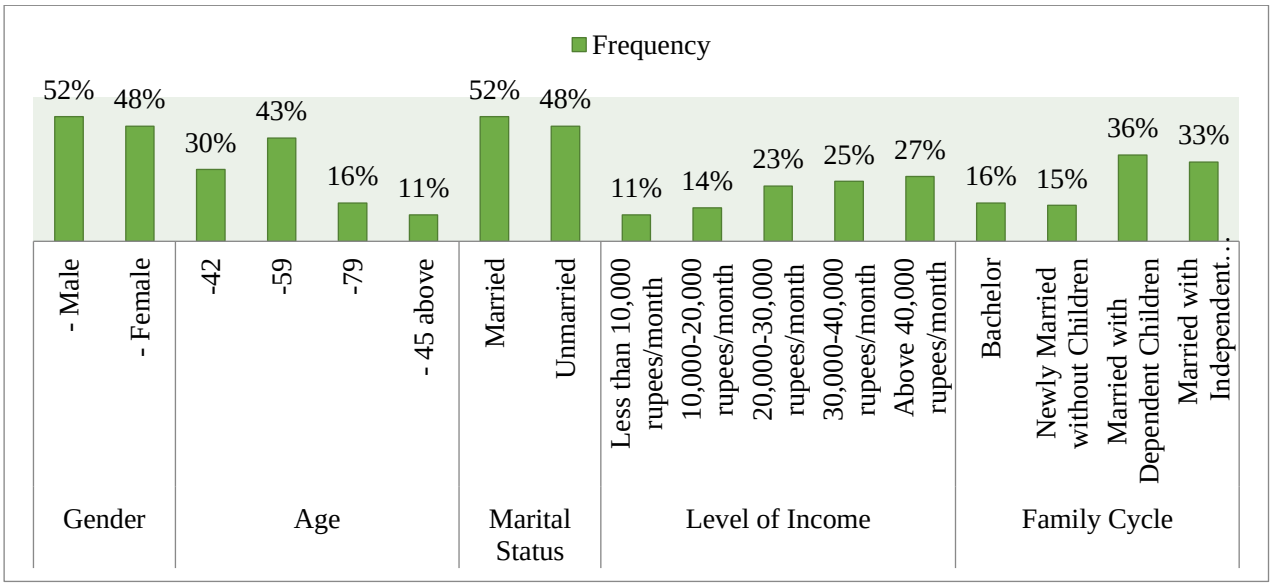

Figure 1: Demographic Profile of the Respondents

The frequency, mean, and standard deviation of a number of demographic factors as well as consumer perceptions of green marketing strategies are displayed in the table. The respondents' gender distribution is about equal, with 52% of them being men and 48% of them women. The age group of 25–34 accounts for 43% of the total responders, with 18–24 accounting for 30%. There is a nearly equal distribution of marital status, with 52% of people married and 48% single. The bulk of responders—48 percent—earn between 20,000 and 40,000 rupees each month. Either married with dependent children (36%) or married without children (15%) make up the bulk of responders.

Table 2: Performance Management Assessment

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	Frequency	**Mean**	**S.D**
Key Determinants of Performance Management Systems			
Clarity of Objectives	45	3.03	0.969
Feedback Mechanism	35	3.08	1.025
Employee Development Opportunities	20	4.02	1.063
Current PMS Practices			
Frequency of Performance Reviews	85	3.96	0.923
Effectiveness of Appraisal Methods	10	4.03	0.999
Utilization of Technology in PMS	5	3.01	1.023
Adaptation of PMS Dimensions			
Perceived Applicability of Larger Organizations' PMS Dimensions	55	4.08	0.969
Modifications for SME Context	45	3.09	1.063

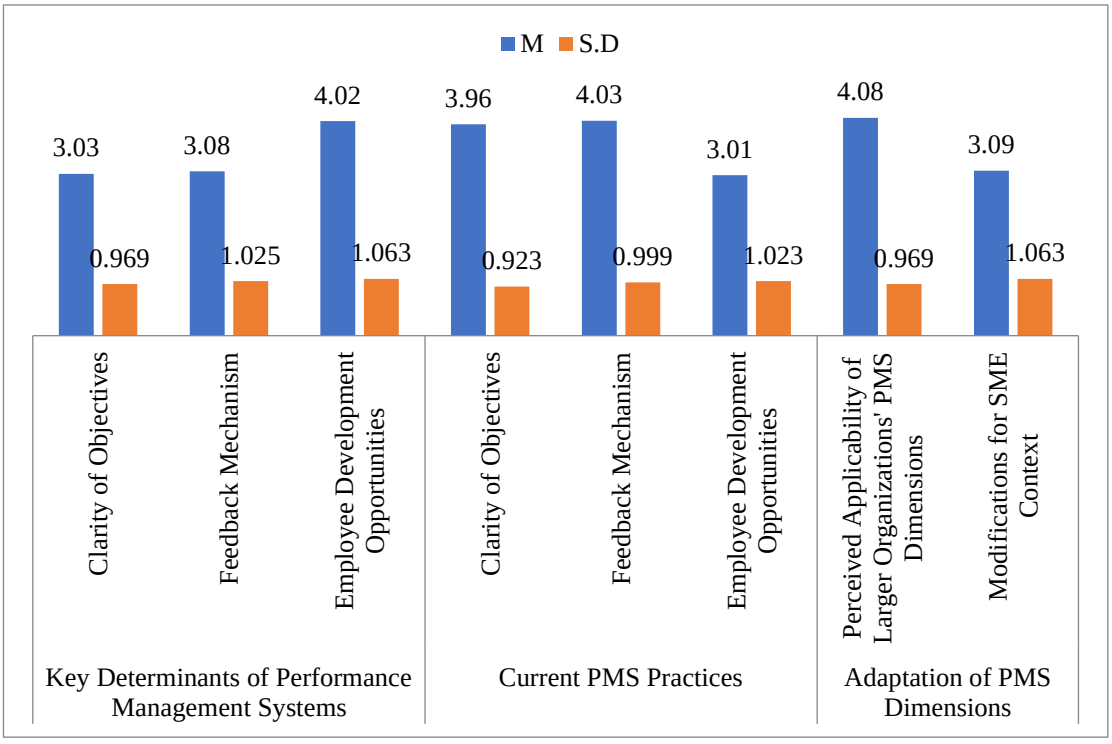

Figure 2: Performance Management Assessment

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Table 2 provided the category of "Key Determinants of Performance Management Systems," respondents generally perceive clarity of objectives positively, as indicated by the average score of 3.03, with a standard deviation of 0.969. Feedback mechanisms are also well-received, with an average score of 3.08, although there is slightly higher variability in responses, as reflected in the standard deviation of 1.025. Employee development opportunities, with the highest average score of 4.02, signify a notably positive perception, though there is some variability with a standard deviation of 1.063. Within the realm of "Current PMS Practices," the organization maintains a relatively high frequency of performance reviews, evident from the average score of 3.96 and a lower standard deviation of 0.923, suggesting consistency in responses. The effectiveness of appraisal methods is positively rated, with an average score of 4.03 and a standard deviation of 0.999. However, the utilization of technology in PMS, indicated by an average score of 3.01, exhibits a slightly lower consensus, with a relatively higher standard deviation of 1.023, suggesting diverse opinions or experiences among respondents. In the section pertaining to the "Adaptation of PMS Dimensions," respondents overwhelmingly perceive the larger organizations' PMS dimensions as highly applicable, reflected in the average score of 4.08 and a standard deviation of 0.969, indicating a strong positive consensus. On the other hand, modifications made for the SME context receive a lower average score of 3.09, with a standard deviation of 1.063, implying some diversity in opinions regarding the effectiveness of these adaptations. This table collectively provides valuable insights into the organization's performance management practices, shedding light on both strengths and areas where opinions may vary among respondents.

Table 3: Correlation Matrix

	Employee Satisfaction with Feedback Mechanism	Performance Improvement

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Employee Satisfaction with Feedback Mechanism	1	0.795
Performance Improvement	0.781	1

The correlation coefficient of 0.795 between "Employee Satisfaction with Feedback Mechanism" and "Performance Improvement" suggests a strong positive linear relationship. This implies that as employee satisfaction with the feedback mechanism increases, there is a notable tendency for performance improvement to also increase. The coefficient of 0.795 indicates a robust positive correlation, signifying that the two variables move in a similar direction. Reciprocally, the correlation coefficient of 0.781 for "Performance Improvement" in relation to "Employee Satisfaction with Feedback Mechanism" reinforces the observed positive correlation. This suggests that as performance improvement increases, there is a strong tendency for employee satisfaction with the feedback mechanism to also increase.

6. CONCLUSION AND RECOMMENDATIONS

The examination of performance management systems within the organization reveals insightful patterns and relationships. The key determinants, including clarity of objectives, feedback mechanisms, and employee development opportunities, have been generally positively perceived. Notably, employee development opportunities received the highest average score, indicating a strong acknowledgment of the organization's commitment to fostering employee growth. Current performance management practices, particularly the frequency of performance reviews and the effectiveness of appraisal methods, are well-regarded, suggesting a consistent and reliable approach to evaluating employee performance. However, opinions vary regarding the utilization of technology in the performance management process, indicating a need for further exploration of this aspect. The adaptation of performance management system dimensions, especially the perceived applicability of larger organizations' dimensions, is highly positive, underlining the relevance and acceptance of these dimensions within the SME context. Nevertheless, modifications made for the SME

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context exhibit a slightly lower average score, reflecting diverse perspectives on the effectiveness of these adaptations.

Recommendations:

- **Enhance Communication of Objectives:** Improve communication channels to ensure that organizational objectives are clearly understood by all employees. This can contribute to even higher scores in the clarity of objectives.
- **Fine-tune Feedback Mechanisms:** Given the variability in responses to feedback mechanisms, it is advisable to review and fine-tune the existing feedback processes. This may involve seeking employee input on the frequency and format of feedback.
- **Leverage Technology Effectively:** Address the varying opinions on the utilization of technology in the performance management process. Conduct a thorough assessment of the existing technological tools and explore opportunities to enhance their usability and effectiveness.
- **Further Investigate Modifications for SME Context:** Given the diversity in opinions regarding modifications made for the SME context, conduct targeted surveys or focus group discussions to understand specific concerns and preferences. This can inform adjustments to better align with the unique characteristics of the organization.
- **Continued Emphasis on Employee Development:** Building on the positive reception of employee development opportunities, continue to invest in training programs, mentorship, and skill-building initiatives. This can contribute to sustained employee satisfaction and performance improvement.

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ISBN - 978-93-95060-79-0

Examination of Financial Accounting and Management Trends in the Internet Age through an Analytical Lens

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Abstract

The network has facilitated business operations while posing new challenges for the growth of the accounting sector as a result of advancements in computer and Internet technologies. Accurate and quick financial data transmission is made possible by the integration of computerised information technology and conventional financial accounting techniques. Also, the utilization of organization innovation builds the efficiency of bookkeeping work and improves the monetary bookkeeping cycle of organizations, opening up bookkeepers to zero in additional time and exertion on the examination of monetary information. By and by, the advancement of monetary bookkeeping achieved by the utilization of the Web has additionally brought about new issues. The article's principal center is monetary bookkeeping. It starts by giving a concise outline of the historical backdrop of both the Web and monetary bookkeeping. Then, it discusses how the idea of bookkeeping has changed with regards to an organization climate and inspects the advantages and downsides of intertwining network innovation and monetary bookkeeping. Ultimately, it proposes answers for the monetary bookkeeping issues of the "Internet+" period. Ultimately, to upgrade the expert limit of monetary staff, countermeasures are proposed to address the monetary bookkeeping task in the "Internet+" period.

Keywords: *Internet, Finance, Accounting, Management, Analysis, technology, Financial Management.*

1. INTRODUCTION

Monetary administration in the Web time offers exceptional advantages over prior big business monetary administration work. These benefits take into consideration careful oversight of the whole monetary administration process and really expand the extent of big business monetary administration. Corporate money the executives makes more data in the Web time, and it needs data to be communicated rapidly. Undertakings should appreciate the meaning of monetary and bookkeeping the executives to advance their functioning model and fulfill the requests of present-day improvement (Figure 1). To boost the workplace generally speaking and really increment the effectiveness of big business monetary administration, organizations should likewise upgrade the dissemination of monetary data and oversee monetary bookkeeping data in a different way.

The essential focal point of big business monetary administration work in Modern times is knowledge, which is both a vital establishment and an unavoidable pattern of the market economy. This approach can assist organizations with turning out to be more serious in the commercial center, make it simpler for significant bookkeeping and monetary divisions to execute logical administration, and give major areas of strength for a to the successful development of monetary bookkeeping. Subsequently, in Modern times, organizations should zero in additional on informationization and clever monetary bookkeeping organization, stay aware of current improvement patterns, and effectively support business advancement.

Figure 1: New model of Financial Accounting.

1.1.Expanded Scope of Financial Management

The monetary bookkeeping the board model has advanced and business the executives proficiency has expanded decisively in Modern times. The greatest adjustment is that it expands the domain of

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monetary administration and improves its capability. For example, utilizing the Web considers direct administration and obtainment as well as the chance to reprove providers and accordingly deals to keep up with the board honesty.

1.2.Improving The Timelines of Financial Management

Since the main advantages of Web innovation are ongoing and idealness, speeding up is likewise fundamental for monetary bookkeeping the executives undertakings to furnish clients with solid data. Data practicality is a basic part of bookkeeping and monetary administration, and the accessibility of the Web works with the effective satisfaction of this prerequisite. The presentation of Web innovation modifies specialized strategies and makes monetary administration all the more ideal. It likewise increments staff exchange and correspondence. Utilizing the Web, for example, empowers one to organize relevant work materials and track how undertakings are done. It likewise permits one to give criticism to the association on specific work situations, extraordinarily expanding efficiency.

1.3.Orderly Operation within the Enterprise

In the period of the Web, monetary bookkeeping the board occupation might give monetary data rapidly and can be more coordinated and versatile. Venture the board is fixated on monetary bookkeeping the executives' assignments. Assuming we keep on utilizing the obsolete strategy for executing the board, it would be trying to create perceptible administration impacts in big business monetary bookkeeping the executives. Nowadays, Web innovation might be utilized to keep up with the deliberate inner tasks of the association and handle the monetary and business undertakings in a logical way.

Maharashtra is as of now going through a time of fast monetary development, driven by globalization, digitalization, and serious contest. In this powerful market climate, business pioneers face the test of dealing with their endeavors successfully. They should not just think about the conventional variables that shape rivalry, yet additionally influence solid and deft monetary administration rehearses at all levels. This engages chiefs with the monetary data and experiences they need to settle on educated and convenient choices, at last driving business achievement.

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Figure 2: Traditional financial accounting.

2. OBJECTIVES

- To examine how management trends and financial accounting practises have been impacted by the Internet era.
- To recognise and assess the major technical developments influencing financial reporting and management approaches in the modern digital environment.
- To evaluate how internet-based platforms and tools affect the efficacy and efficiency of financial accounting procedures.
- Investigate how artificial intelligence and data analytics can improve financial management decision-making.
- To look into the potential and problems that the Internet era has brought to financial accounting and management.

3. LITERATURE REVIEW

Gao, J. (2022), highlights the significant influence that this technology paradigm change can have on the effectiveness, precision, and organisational decision-making processes. The central thesis of Gao's argument is that big data has the capacity to completely change the way financial accounting is practised today while also providing a plethora of benefits that go far beyond simple data processing. The central idea of Gao's talk is the significant gains in accuracy and efficiency that big data can make to the handling of financial accounting information. Businesses can optimise their financial operations by utilising real-time processing capabilities and advanced analytics.

Weimei, C. (2021), explores the significant changes occurring in the accounting field, especially the paradigm change caused by artificial intelligence (AI) from traditional financial accounting to the dynamic field of management accounting. Her strong case highlights the growing importance of AI and its complex impact on changing the face of accounting procedures. Weimei incisively suggests that artificial

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intelligence (AI) has become indispensable in financial accounting, beyond its traditional uses and influencing management accounting as a whole.

Ali & Khan (2022), Shift the focus of the story to how corporate governance can be a powerful tool in the fight against organisational corruption. Their reasoning is based on the idea that clear financial accounting data, supported by strong control systems, empowers stakeholders and acts as a disincentive to engage in fraudulent activity. The paper offers a comprehensive examination of the mutually beneficial relationship that exists between the development of a strong corporate governance framework and a robust anti-corruption defence. According to Ali and Khan, open financial accounting data serves as a fundamental component of corporate governance.

Venter & van Eck (2021), broaden the assurance's purview beyond conventional financial audits to address the growing need for assurance in data integrity, social elements, environmental concerns, and sustainability reporting. According to their analysis, assurance plays a crucial role in the changing corporate reporting landscape by covering a wider range of aspects that are important to stakeholders and the long-term viability of organisations. According to the report, the traditional emphasis on financial audits is insufficient to satisfy the demands of modern stakeholders, who are becoming more and more interested in non-financial aspects of organisational performance.

Diegtiar et al. (2021), Analyse the limitations that traditional accounting methods impose, especially in the context of small and medium-sized enterprises (SMEs). Their research highlights the drawbacks of traditional systems, which may unintentionally ignore the strategic insights and real-time adaptation necessary for the dynamic nature of SMEs by placing too much emphasis on historical data and compliance. The writers push for a radical change in direction of a cutting-edge accounting system designed with SMEs' unique requirements in mind.

Stafi & Stoica (2021), A troubling story about Romanian emergency hospitals is revealed by an insightful investigation, which also highlights the intricate financial-accounting concerns that permeate their operational environment. The writers provide a clear image of the difficulties these vital healthcare

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organisations face by highlighting problems such insufficient finance, reliance on erratic health insurance, and ineffective resource distribution. Their research acts as a call to action, supporting proactive steps to reduce risks and guarantee the provision of high-quality healthcare services along with financial stability.

4. RESEARCH METHODOLOGY

4.1.Basic Model

A bookkeeping data framework (AIS) contains all bookkeeping information. AIS is a significant use of PC innovation in bookkeeping; it moves bookkeeping from manual to machine accounting, exhibiting that bookkeeping practice should be modernized. Involving PCs for bookkeeping data is its pith. The bookkeeping in-line framework first gathers a portion of the front-end business cycle's bookkeeping related information, then, at that point, performs bookkeeping handling on this information, finishes bookkeeping, produces bookkeeping data, and chiefs utilize this data to settle on business choices. Figure 3 shows AIS job component examination and association.

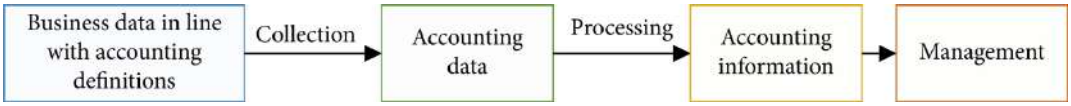

Figure 3: Mechanism of accounting information system.

As indicated by this understanding, the qualification among customary and monetary coordination bookkeeping data frameworks is made by "whether the bookkeeping data framework can understand the sharing of business and monetary information"; Figure 4 delineates the five phases of development that the conventional bookkeeping data framework has gone through.

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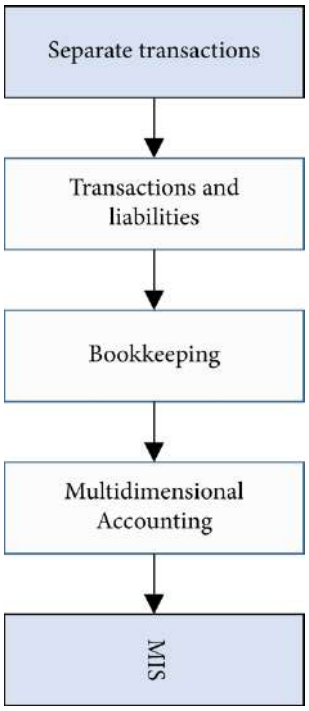

Figure 4: Evolution of traditional AIS.

Business data actually should have been traded between the business and money offices utilizing genuine paper vouchers, despite the fact that the bookkeeping data framework in the initial four stages could record specific business data also. The recording's substance was very restricted. The main distinction between this stage and the past four is the expansion of the front-end business module to the AIS. This assists the AIS record not just business information that consents to bookkeeping definitions yet in addition different sorts of business information, giving chiefs admittance to more extravagant information and making the first business picture more clear.

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The previously mentioned improvement history of the conventional bookkeeping data framework shows that, while the MIS (the executives data framework) stage can as of now furnish directors with business data, the failure to share business and monetary data brings about a lower level of this data being utilized by supervisors. In this manner, by putting together the customary AIS capabilities as displayed in Figure 5, directors can get to bookkeeping data all the more without any problem.

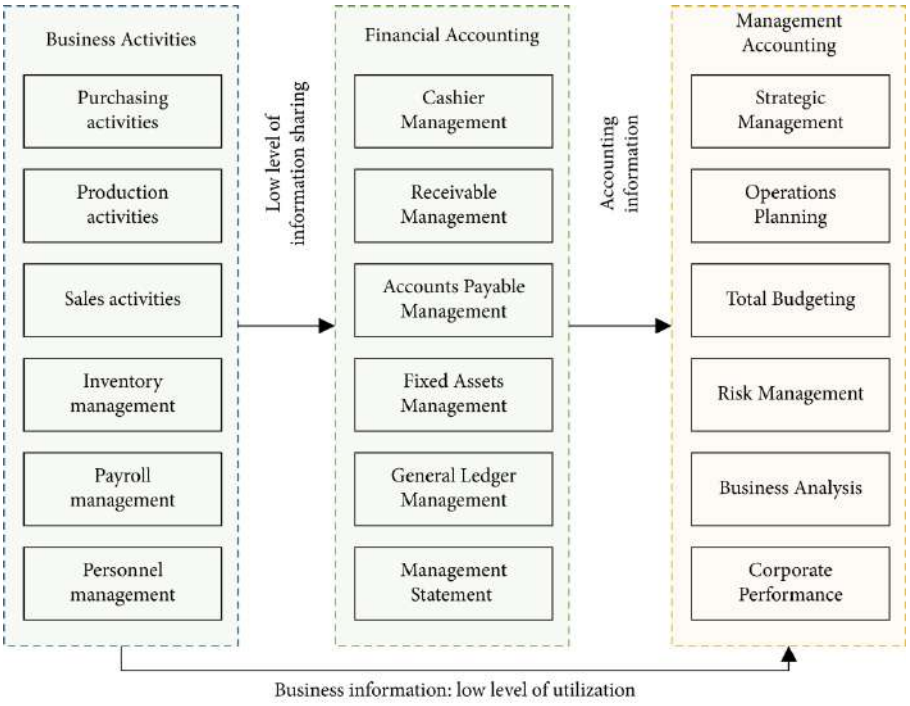

Figure 5: Traditional AIS function diagram.

The creation finance consolidated bookkeeping data framework permits business and monetary information to be shared, defeating the limitation that customary bookkeeping data frameworks could basically utilize bookkeeping information just to participate in administration. It likewise improves the utilization of business information and supports a more significant combination of bookkeeping and the board, which together make bookkeeping a compelling instrument for big business the executives. As Figure 6 delineates, the jobs of monetary bookkeeping and the executives bookkeeping are consequently related and commonly supporting.

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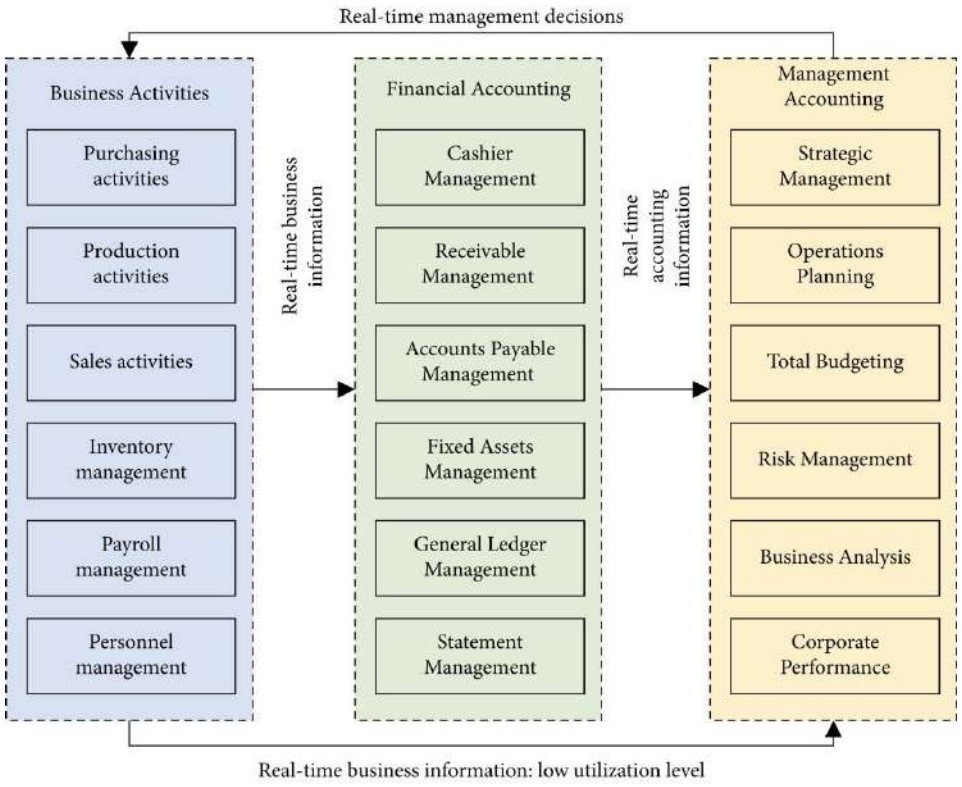

Figure 6: Functional diagram of AIS for business finance integration.

4.2.Improvement Model

In view of the recently referenced issues with resolute administration, rigid bookkeeping, and unbendable business processes during the application cycle, the plan objective of the adaptable monetary joining AIS is to expand the framework's self-flexibility and adaptability to changes in outside necessities, subsequently lessening the time and cost of framework redevelopment. This incorporates adaptable information yield, adaptable info and information structure, adaptable information stream, and adaptable business process.

4.2.1. Flexible Input and Data Structure Design

Regular industry and mix with finance AIS, or the supposition that the default later data set table construction stays unaltered and that the information table in the information things stays unaltered, is commonly restricted to specific data set tables and fields while creating information structure plans. The created framework misses the mark on level of client versatility as far as info and information structure since it is unbending and doesn't represent possible acclimations to the information structure that might be vital coming soon for the

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venture. Moreover, there is no information space put away for the client to arrange the information structure at the front end.

The "information word reference" capabilities as a "stockroom" for a scope of primary information types. The two tables recorded underneath ought to be incorporated: the detail table, which is utilized to record the names and kinds of fields remembered for every data set table's information things, and the table construction portrayed in Table 1.

Table 1: Table of Contents.

Field Name	**Meaning**
id	Table Number
T_name	table Name
T_type	Type
Creator	Creator

Figure 7 outlines the information and information construction's adaptable execution approach. Sets off and put away strategies are utilized to refresh the connection between the business and money combination AIS and the pertinent substance tables in the data set. The relating data set is gotten to through the data set interface when a client adds, erases, or changes information in the AIS's information word reference capability card. The subsequent information adjustments are archived in the detail table, and the activity of adding, erasing, or changing likewise enacts the trigger implanted in the detail table. The information that the client adjusts toward the front page is archived in the brief table, the comparing put away method is done, lastly, the relating substance table is changed. Through the information word reference capability card on the AIS, which understands the adaptability of the info and information structure, the client can understand the adjustment of the information design of the comparing structure in the data set when the substance table is altered progressively.

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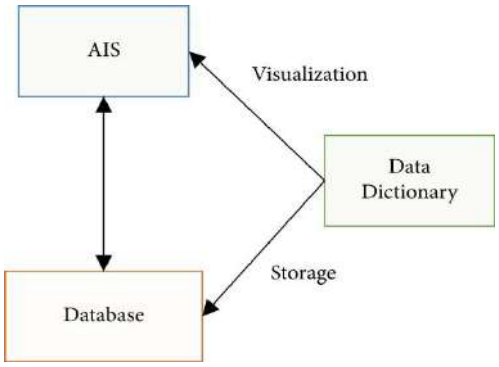

Figure 7: Design diagram of input and data structure flexibility.

4.2.2. Flexible Data Flow Design

The adaptable monetary coordination's information stream plan The principal objective of AIS is to foster client configurable imagined record change rules among upstream and downstream archives, various archives and vouchers, and archives and reports. Figure 8 shows the specific plan outline.

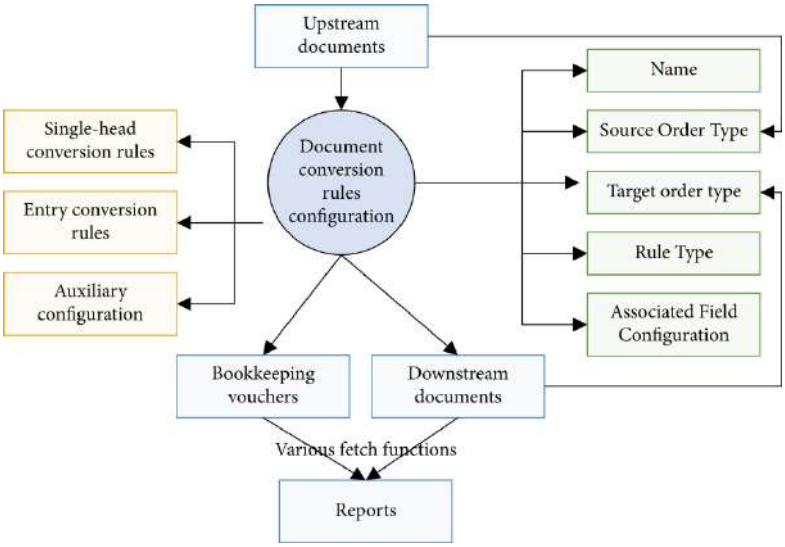

Figure 8: Data flow flexible design diagram.

5. DATA ANALYSIS AND INTERPRETATION

An organization is a sizable, strong, contemporary endeavor bunch. The gathering's improvement was to some degree hampered by the trouble of changing the underlying

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monetary and plan of action to satisfy the needs of the gathering's administration because of the gathering's sluggish however consistent development in size. Taking advantage of new data innovation apparatuses for change is fundamental if we have any desire to keep awake to date in the Web setting. In Figure 9, we first presentation the different representatives' degrees of acknowledgment for the administration redesign of the monetary bookkeeping framework.

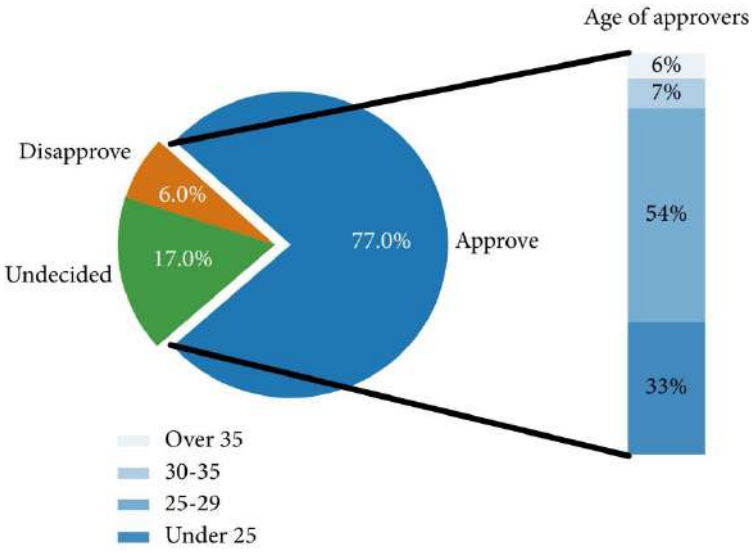

Figure 9: Different employees' opinions on the management upgrading of the financial accounting system.

Before the reception of financial sharing, an organization's finance division was essentially responsible for concentrated financial administration as well as everyday accounting, cost repayment, current record the executives, raising money, activity, venture the board, spending plan the board, charge revealing, explanation readiness and investigation, and different undertakings. The gathering transformed and embraced the hierarchical type of gathering client divisions, and laid out financial divisions inside every division; from a unified financial administration mode to a decentralized financial administration mode, each divisional financial division is liable for its own neighborhood business comparing to the cost repayment accounting and other work. As an organization created, the quantity of auxiliaries, abroad branches, and vital specialty units step by step expanded. Consequently, the accounting branch of every specialty unit presents the handled financial and business investigation information

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to the central command bunch for information accumulation and examination. Every specialty unit then, at that point, regularly sends business-related information to the accounting staff of the specialty unit.

5.1.Analysis of the Main Problems of a Company before the Integration of Industry and Finance

1) Low Value Creation and High Labour Costs. The client specialty units of a firm lay out a similar capability as the finance division: making a finance division without any preparation is work and time-escalated, and having a major number of accounting laborers raises working expenses for the gathering. Accounting representatives struggle with pulling out from organization the board and supporting business system the executives because of the enormous accounting volume of each finance division.
2) Ineffective Information Exchange. Initial, an organization relegates accounting staff individuals with comparable obligations to different gathering client divisions. Divisional accounting staff individuals then, at that point, aggregate and assess the financial information prior to sending it to the gathering central command. This cycle can bring about staff redundancies and postponed financial information assortment from the gathering central command, as well as functional blunders and issues like data lopsidedness among on the web and disconnected. Second, the center information can't be consolidated and unified in a similar framework for business examination and the executives, nor is there a successful correspondence system among business and financial data.

5.2.Implementation of a Company's Financial Integration in the Internet Era

An organization uses the financial sharing stage to its fullest likely to accomplish the combination of finance and business for the board strengthening. An incorporated and brought together accounting stage based on front-end business get through is an organization's financial sharing place. An immense measure of information assets are accumulated and placed into the information stage continuously to store the data. The put away information are then separated, summed up, allotted, offset and solidified, and changed over into type. All exchanges, solicitations, compromises, installments, and other front-end processes are

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gathered as information assortment focuses. The administration aspect is utilized to gather and present the removed, accumulated, allocated, offset and solidified, changed over information.

The common finance place has laid out complex responsibility markers and examination frameworks relating to fundamental business pay, benefit before charge, working net income, significant quality issues at the activity, the board, and dynamic levels, individually, and has fabricated a multi-layered pointer investigation framework in view of the gathering's financial sharing bound together information stage. Moreover, the framework utilizes the gathering's huge information stage for investigation and show. We analyze the association among business and financial administration in Figure 10.

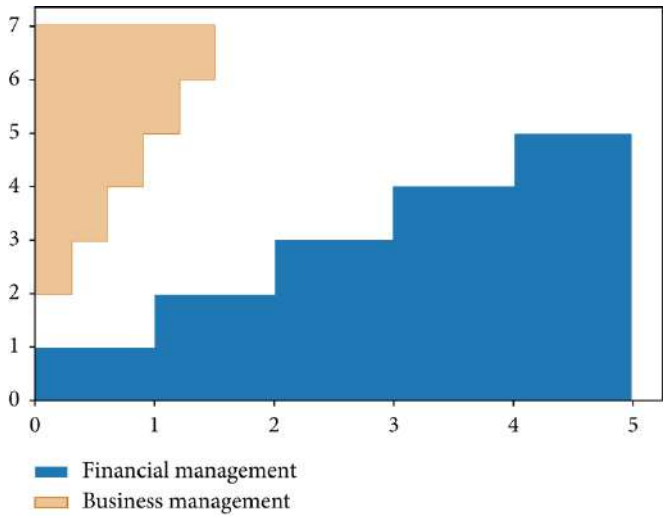

Figure 10: The value of financial management with business management.

The common finance place has laid out complex responsibility pointers and investigation frameworks comparing to primary business pay, benefit before charge, working net income, significant quality issues at the activity, the board, and dynamic levels, individually, and has fabricated a multi-faceted marker examination framework in view of the gathering's financial sharing bound together information stage. Moreover, the framework utilizes the gathering's enormous information stage for examination and show.

An organization's inward financial sharing community stage reshaped the financial interaction and afterward brought together everything, concealing data on significant connections so financial principles completely pervade the connected exchange process. This superior the

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effectiveness and practicality of information arrangement and laid areas of strength for an establishment for the gathering's later month to month compromise and information the board investigation. It likewise resolves the issues raised by the decentralized financial administration model's low endeavor esteem creation and high work costs. Two arrangements of financial reports in light of the corporate construction and the board reports in view of the administration design can likewise be created by the sharing community. The gathering can screen the exact execution of the financial plan progressively and offer huge help to bunch administrators and leaders with the arrival of the working financial plan execution report continuously following the coordination of industry and finance.

6. RECOMMENDATIONS & CONCLUSION

Ventures need to reinforce the advancement of the financial accounting the board mode to help with meeting the improvement needs of the times. All in all, financial accounting the board is fundamental for organizations, and it fills in as the establishment for the advancement of organizations, especially in the period of the Web. Nonetheless, right now, it is trying to adjust crafted by an organizations' financial accounting the executives to the improvement of the organization period, and the administration thought has not been refreshed as soon a possible. Ventures need to focus harder on this, settle on choices that are sensible to upgrade it, and further grow the field of financial accounting the executives by fortifying the development of data innovation and explaining the issues that are as yet exceptional. This will permit endeavors to get new turns of events and work fair and square of financial accounting the executives in an exhaustive way.

Recommendations:

- **Embrace Technological Integration in Financial Reporting:** Leverage modern accounting information systems (AIS) to seamlessly integrate front-end business cycle data, ensuring efficient and accurate financial reporting.
- **Address Challenges and Unleash Potential in the Internet Era:** Conduct a comprehensive analysis of the challenges and opportunities presented by the Internet era in financial accounting and management.

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- **Recognize the Evolution of Accounting Information Systems:** Acknowledge the historical development of accounting information systems, emphasizing the transition from traditional to modern practices.
- **Maximize Efficiency with Internet-Based Platforms:** Enhance the efficacy of financial accounting procedures by incorporating internet-based platforms and tools.
- **Harness Artificial Intelligence for Informed Decision-Making:** Explore the transformative power of artificial intelligence (AI) and data analytics in financial management. Implementing AI tools enhances decision-making processes by providing managers with access to richer data sets.

ISBN - 978-93-95060-79-0

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ISBN - 978-93-95060-79-0

Financial Accounting Practices of Micro, Small, and Medium Enterprises (MSMEs) in Maharashtra, India: A Case Study

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Abstract

In Maharashtra, India, Micro, Small, and Medium-Sized Enterprises (MSMEs) are fundamental for the economy since they give occupations, convey pay, battle neediness, and lift sends out. To guarantee impartial and reasonable advancement in the state and to advance grassroots monetary development, their effect on business, industry, and the country economy is fundamental. Accounting assumes a fundamental part in corporate tasks by offering a financial outline that works with dynamic on the present and expected future strength of organizations. With MSMEs assuming such an imperative part in Maharashtra's economy, this turns out to be significantly more significant. There is an observable disparity between accounting hypothesis and genuine practices, by and by, as confirmed by the limitations in the execution of accounting procedures in MSMEs in Maharashtra and all through India. This exploration article endeavors to analyze the accounting practices followed by MSMEs in Maharashtra, India, considering the significance of keeping exact books of records and following sound accounting standards for proficient financial administration in MSMEs. To give light on the current state of accounting practices inside the MSME area in Maharashtra and to offer experiences for future enhancements, the review will gather information through reactions to an organized meeting plan. The Maharashtra State Chamber of Commerce and Industries (MSCCI) and the Institute of Chartered Accountants of India (ICAI) could cooperate to organize the executives and financial record-continuing to prepare programs that are custom-made particularly for MSME chiefs and owners to resolve these issues. To give members the capacities and information expected to dissect financial information, screen business execution, and use sound judgment for long haul development, these projects should go past basic accounting.

Keywords: *Financial Accounting, Strategies, Accounting Information, Accounting Practices, Accounting records, Micro, Small and Medium Scale Enterprises (MSMEs), Owner-Managers.*

1. INTRODUCTION

Micro, Small, and Medium-Sized Enterprises (MSMEs) are basic to a country's monetary development. Strategy producers find MSMEs engaging since they might create occupations in different regions and with negligible capital. After the agribusiness area, this one records for the second most noteworthy work supplier. MSMEs make up among half and 66% of all enterprises around the world, and in numerous nations, this rate is essentially more noteworthy. MSMEs represent the second most prominent portion of work in India, after farming.

MSMEs, which are normally recognized as drivers of financial extension, are fundamental to advancing fair advancement in Maharashtra, India. Second just to horticulture as far as occupation creation, the business has arisen as a key part in the financial landscape of the state. Maharashtra's MSMEs assume a huge part in the state's work creation process by helping modern movement in provincial and immature regions and supporting worth expansion, neighborhood capital preparation, and the improvement of enterprising abilities. The MSME area arises as a beam of expectation for keeping up with lives while the coordinated assembling area deteriorates and the rural area perseveres through a drop in work.

The adage "Cash makes the world go around" is precise in the speedy universe of business, especially for Maharashtran organizations. One significant variable that decides a business' drawn out presentation is its financial administration. A solid accounting framework, wherein each financial detail is meticulously reported in firm records as per accounting standards, is the foundation of financial administration. Maharashtra's MSME owners need current, precise, and ideal accounting information to flourish. The establishment for surveying and conveying the financial strength of the business is accounting information, which upholds the executives' independent direction.

Accounting practices inside the area should give intensive and relevant financial information that is fundamental for enterprising independent direction, given its huge effect on the outcome of MSMEs. Severe record-keeping strategies are important to work on the profitability and supportability of MSMEs in Maharashtra

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and permit owners to follow and survey their organizations' exhibition. To give knowledge into MSMEs' financial administration techniques, this article will look at their accounting practices in the Maharashtra climate.

A huge piece of creation and commodities come from this area. By making a lot of work prospects, industrializing country and immature regions, achieving a fair circulation of riches and public pay, and supporting the country's financial development, it enormously helps the Indian economy. The business likewise made a commitment to the country's Gross domestic product. The advantages of the MSME area incorporate effective utilization of locally accessible assets, decreased speculation needs, functional adaptability, and a high pace of development. The public authority has given MSMEs a scope of help to advance their development and improvement.

When the public authority acknowledged how significant and important MSMEs were to the economy, it passed the MSME Advancement Act, 2006. To further develop MSMEs, various panels have been laid out, and various examinations have been done. By and by, the business actually faces various difficulties, for example, tracking down the vital subsidizing, issues with unrefined substances, issues with below average accounting systems, and so on. The presence and profitability of MSMEs are vigorously reliant upon the compelling administration of their financial assets, making insufficient financial administration one of the many significant issues they stand up to.

Many believe money to be the backbone of an organization. Each step of the improvement of a firm requires supporting. Any kind of business' capacity to stay in activity and succeed is basically reliant upon its financial administration. Subsequently, powerful financial asset the executives and financial discipline are fundamental for a business' prosperity.

The MSME Improvement Act, 2006 states that an organization that makes and creates merchandise and contributes something like 25 lakh rupees in plant and hardware is delegated a micro undertaking; those that contribute more than 25 lakh rupees however don't surpass five crore rupees are named small enterprises; and those that contribute in excess of five crore rupees yet don't surpass ten crore rupees are delegated medium enterprises. Notwithstanding, for

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organizations that offer or render benefits, the greatest measure of cash that can be put resources into hardware is 10 lakh rupees. is alluded to as a micro endeavor when its income outperforms ten lakh rupees yet remains under two crore rupees. is named a small firm, and those that make multiple crore rupees however under five crore rupees are delegated medium enterprises.

Maharashtra's MSME area has been fundamental in supporting the state's economy by giving business open doors to a sizable section of the general population. These organizations utilize assets that are promptly accessible locally, showing creativity and resourcefulness in their tasks. MSMEs in Maharashtra actually face a few hindrances, even with the different government drives pointed toward supporting the business. Financial administration stands out among the numerous issues these organizations defy as perhaps of the main one that can't be ignored in light of its huge effect on an organization's capacity to succeed or come up short. To effectively handle these issues, cautious oversight of the business' funds and powerful administration are fundamental.

Accordingly, the motivation behind this study is to investigate the financial administration strategies utilized by Maharashtra's micro and small organizations. The review takes a gander at current inadequacies and impediments that entrepreneurs have while dealing with their funds, and it recommends ways of moving beyond these issues. By doing this, the review desires to empower the business to work on its ability for successfully handling financial issues, hence supporting the thriving of Maharashtra's enterprises all in all.

2. OBJECTIVES

- To assess the current financial accounting practices employed by MSMEs in Maharashtra to understand their adherence to accounting standards.
- To examine the challenges faced by MSMEs in Maharashtra related to financial accounting and propose strategies for improvement.
- To investigate the impact of effective financial accounting on the overall performance and sustainability of MSMEs in the Maharashtra region.

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- To provide practical recommendations based on the case study findings to enhance financial accounting practices among MSMEs in Maharashtra, fostering economic growth and stability.

3. LITERATURE REVIEW

Krishna and Pavithran (2018), directed a review to find out about Kerala's SMEs' accounting methodology. They found that most of organizations recently carried out accounting methodology since they needed to, not on the grounds that they comprehended how significant they were. Moreover, they miss the mark on exhaustive understanding of the various declarations and the benefits of setting them up. As indicated by the review, organizations are not involving accounting statements for arranging, contrasting, or simply deciding. Accordingly, the specialists ought to make the fitting move to instruct these entrepreneurs about the worth of an accounting framework and the benefits it can offer.

Mekonnen (2019), More than 50% of the businesses do not maintain their accounting records, according to the paper Accounting Practises of Small and Micro Enterprises in North Shewa Zone, Amahara Regional State of Ethiopia. Reasons for this lack of record-keeping include labor-intensiveness, increased costs, lack of accounting expertise, and time waste. Even record preparers are not knowledgeable about accounting.

Aher, R. V., & Dongare, S. D. (2022), The financial management techniques of MSMEs in Maharashtra's Nashik District are investigated in this study. The authors discover that a large number of MSMEs rely solely on personal records and instinct when making decisions because they lack proper financial management systems. In order to enhance their financial performance and funding accessibility, they advise MSMEs to implement more formal accounting procedures.

Bhosale, S., & Pawar, M. S. (2021), The potential and difficulties MSMEs in Maharashtra have when implementing accounting information systems (AIS) are examined in this study. The authors list a number of things that prevent adoption, including expense, ignorance, and a lack of technical know-how. They also draw attention to the advantages that AIS may offer, including better decision-making, financial reporting, and capital availability.

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Patil, S. B., & Jadhav, S. S. (2020), This study looks into the variables affecting MSMEs' financial performance in Maharashtra's Solapur District. The success of MSME is shown to be significantly influenced by elements including marketing tactics, human resource management, and financial accessibility. They advise policymakers to concentrate on offering assistance in these areas in order to raise the sector's overall performance.

Deshmukh, S. S., & Kulkarni, S. V. (2019), The financial management techniques of MSMEs in Pune District, Maharashtra, are examined in this study. The authors discover that a large number of MSMEs lack professional accounting staff and depend on unofficial financial management techniques. They contend that in order to enhance MSMEs' financial management procedures, training courses and financial literacy campaigns are essential.

4. RESEARCH METHODOLOGY

The core data for the current study was gathered using organised schedules, and secondary data sources were also consulted. MSMEs are actively engaged in 17 different economic areas in Maharashtra, India. Automobile parts, chemicals (soap, washing powder), machinery parts for construction equipment, fabricated metal products (welding, grilling, lathe works), aluminium vessels, utensils, rice mills, paddy hulling, non-metallic minerals, paper products, plastics, safety pins, stationery products, textile mills, tyre products, and wax and wood products are some examples of these operations. All 75 of the manufacturing firms in the state that are registered and are in operation have been chosen for analysis in order to learn more about the accounting procedures used by MSMEs in Maharashtra. Simple percentage analysis is used in the study to extract meaningful information from the gathered data.

5. DATA ANALYSIS AND INTERPRETATION

Table 1 presents the findings of the respondents' demographic profile.

Table 1: Demographic Profile of the Respondents.

Variables	Frequency (N=75)	Percentage (%)
Gender		

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Male	50	66.67
Female	25	33.33
Total	75	100
Age		
Below 25	9	12
26-35	19	25.33
36-45	33	44
46-55	13	17.33
56 and above	1	1.34
Level of Education		
SSLC	4	5.33
HSC	12	16
UG	26	34.67
PG	5	6.67
Diploma	21	28
Others (Elementary, School dropouts)	7	9.33
Experience		
Below 10 years	38	50.67
10-20 years	19	25.33
21-30 years	14	18.67
31-40 years	3	4
Above 40 years	1	1.33

The demographic profile of the 75 study participants is shown in the table, offering important details about age, gender, experience, and educational attainment. When it comes to the gender distribution of the sample, 66.67% of the respondents are men, while the remaining 33.33% are women. The respondents' ages range widely, with the age group of 36–45 accounting for the largest number (44%), followed by 26–35 (25.33%) and 46–55 (17.33%). Individuals under 25 comprise 12% of the population, while those 56 and over make up a lower percentage (1.34%).

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The respondents' degrees of education vary; the majority (34.67%) have an undergraduate degree, followed by those with a diploma (28%), and those with higher secondary education (16%). Postgraduates (6.67%), holders of an SSLC (5.33%), and respondents with various educational backgrounds, such as elementary school and school dropouts (9.33%), are represented in smaller percentages.

Regarding work experience, 50.67% of respondents had less than ten years' worth, while 25.33% had between ten and twenty years' worth. 18.67% of respondents had between 21 and 30 years of experience, while a smaller fraction have between 31 and 40 years of experience (4%) and over 40 years of experience (1.33%). Thus, the demographic profile offers a thorough summary of the traits of the respondents and serves as a basis for additional research and interpretation throughout the study.

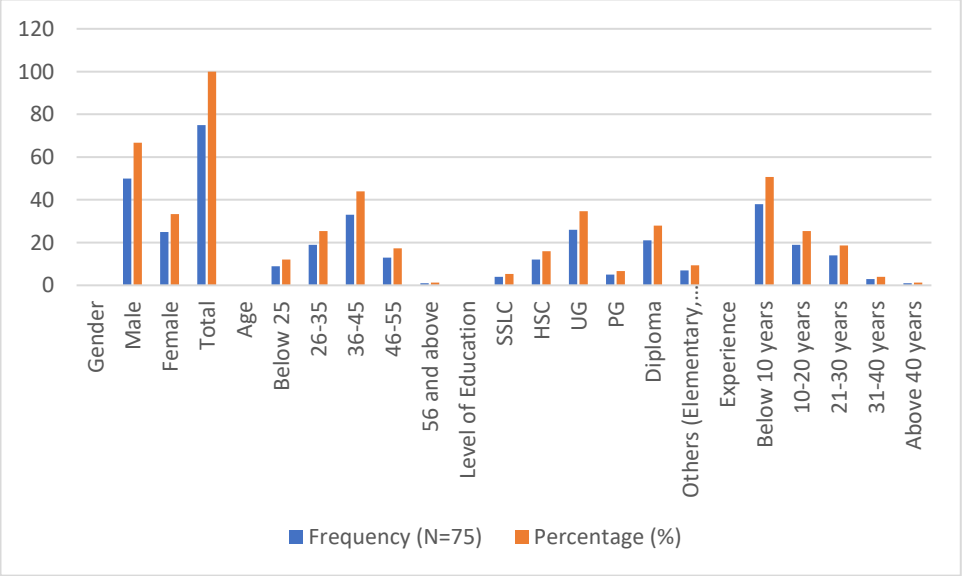

Figure 1: Demographic Profile of the Respondents.

Table 2 presents the respondents' enterprise profile results..

Table 2: Enterprise Profile of the Respondents.

Variables	Frequency (N=75)	Percentage (%)
Category		
Micro	66	88

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Small	7	9.33
Medium	2	2.67
Total	75	100
Ownership		
Sole Proprietorship	66	88
Partnership	6	8
Private Limited	3	4
Investment		
Below lakh	20	26.67
1-50 lakhs	49	65.33
50-1 Crore	4	5.33
Above Crore	2	2.67
Number of Employees		
Below 25	71	94.67
26-50	1	1.33
Above 50	3	4

The table gives a thorough summary of the respondents' enterprise profiles and highlights important details including category, ownership, investment, and workforce size. The majority of the 75 businesses that were polled (N = 75) fit into the "Micro" category, making up 88% of the total, followed by "Small" businesses (9.33%) and "Medium" businesses (2.67%). When it comes to ownership, "Sole Proprietorship" makes up 88% of all ownership, followed by "Partnership" (8%), and "Private Limited" (4%).

According to the distribution of investments, a significant number of businesses have investments between less than one lakh and fifty lakhs, with 26.67% of them falling into the former category and 65.33% into the latter. Less than half of the businesses, or 5.33%, have investments between 50 lakhs and one crore, while 2.67% have investments greater than one crore.

When the number of employees is looked at, 94.67% of the businesses employ fewer than 25 people. The proportion of businesses with 26–50 employees is quite low (1.33%), while 4%

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of them employ more than 50 people. This breakdown gives a clear picture of how the 75 respondents' sample of businesses are distributed according to employee strength, ownership structure, category, and investment levels.

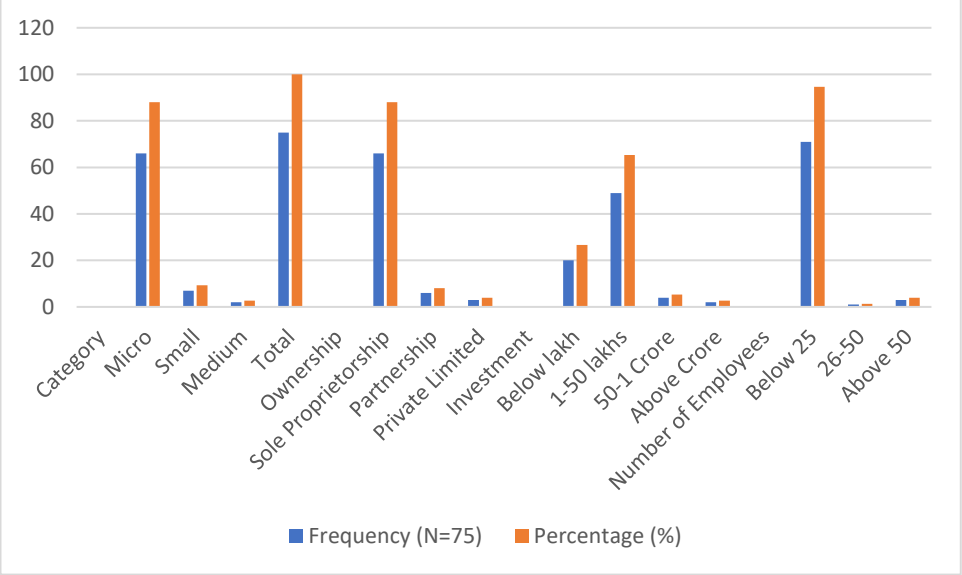

Figure 2: Enterprise Profile of the Respondents.

Table 3: Response to the question of whether the company uses a systematic accounting system (N=75).

Accounting System	Frequency (N)	Percentage (%)
Yes	69	92
No	6	8
Total	75	100

The responses to the question of whether businesses use a systematic accounting system are shown in the table, which also shows a noteworthy trend. 92% of the 75 responses in all suggest that businesses follow a systematic accounting system. This is the overwhelming majority. This high percentage indicates that the surveyed companies have a commendable level of dedication to implementing systematic and rigorous accounting practises. Conversely, a minority, making about 8% of the participants, stated that they lacked a structured bookkeeping system.

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The fact that the vast majority of businesses claim that they employ a systematic accounting system shows how important structured financial management is to these businesses. This encouraging trend might represent a dedication to financial accountability, precision, and transparency—all essential components for efficient operations and decision-making in the business world. It is important to emphasise the need for focused interventions or support mechanisms to improve accounting standards among this subset of businesses, as the existence of a small fraction of businesses without a systematic accounting system suggests a possible area for improvement in the adoption of best practises. Overall, the table indicates that the assessed firms generally adhere to systematic accounting practises; nevertheless, there is still opportunity for targeted initiatives to better promote these practises throughout the board.

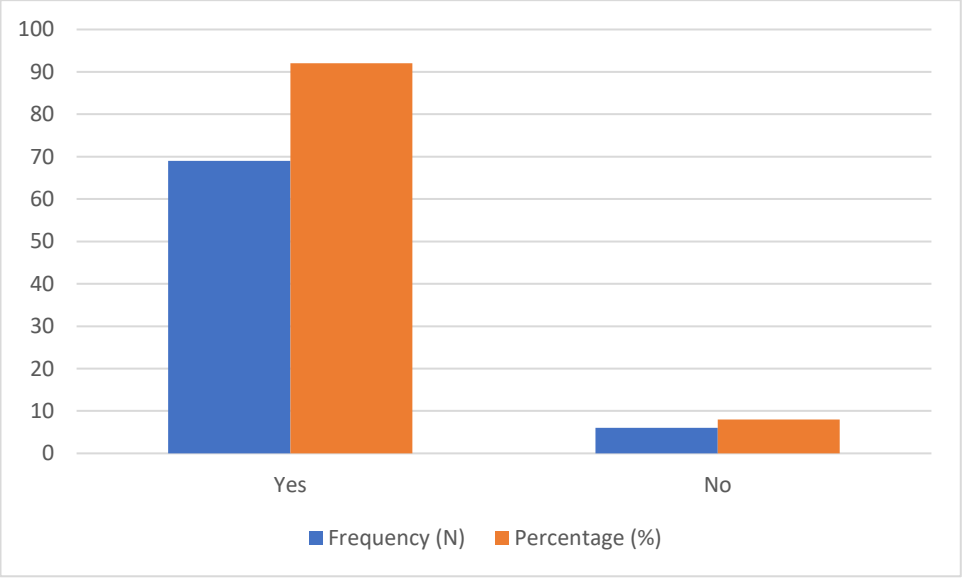

Figure 3: Response to the question of whether the company uses a systematic accounting system (N=75).

Table 4: Response to the company's adherence to the bookkeeping system.

Book Keeping	Frequency (N)	Percentage (%)
Double Entry System	18	25.71
Single Entry System	52	74.29
Total	70	100

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Responses to questions about the bookkeeping systems used by the surveyed businesses are displayed in the table, with particular attention paid to whether the businesses use a Double Entry System or a Single Entry System. Of the seventy-one businesses that were polled, twenty-seven percent (18 businesses) reported using the Double Entry System, while seventy-two percent (74.29%) of the businesses reported using the Single-Entry System.

Based on the data, it appears that the Maharashtra, India, surveyed firms favoured the Single-Entry System over the Double Entry System. The Single-Entry System is a less complex approach that simply keeps track of one entry every transaction. Its popularity may be attributed to its simplicity and fit for smaller companies. Conversely, a comparatively lower percentage of businesses use the Double Entry System, which is renowned for its thorough methodology that records both debit and credit entries for every transaction.

The difference in adoption rates suggests that the MSME sector in Maharashtra has a variety of bookkeeping practises, most of which favour efficiency and simplicity in their record-keeping techniques. The results highlight the necessity of customised financial management plans and support systems that correspond with the most common bookkeeping practises among MSMEs in the area.

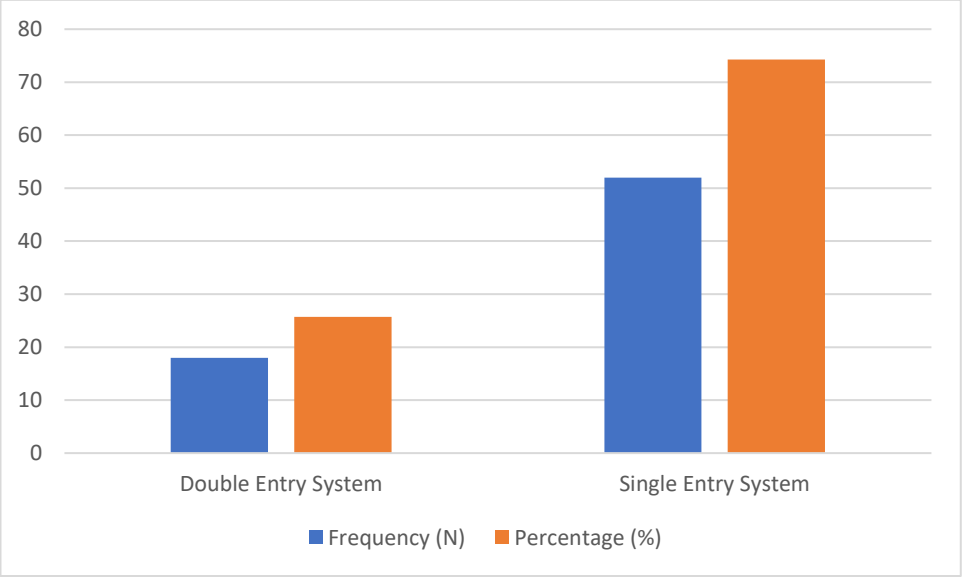

Figure 4: Response to the company's adherence to the bookkeeping system.

6. RECOMMENDATIONS AND CONCLUSION

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The results of the field survey conducted in Maharashtra, India, indicate that a sizable portion of Micro and Small Enterprises (MSEs) do not have thorough accounting records (75%). This highlights a significant discrepancy between the theory of financial management and its actual application in the MSE industry. As a result, this study makes practical suggestions suited to the Maharashtra environment. It is recommended that the District Industries Centre (DIC) officials in Maharashtra develop detailed rules and provide MSEs with standardised forms for entering accounting information in order to close the observed gap. Furthermore, it is advised that training initiatives be put in place to educate MSE owners on the value of keeping correct books of accounts. It is also suggested that MSEs make record-keeping obligatory in order to improve their accounting procedures. This programme helps the government raise more money through taxes while also ensuring that income is declared accurately. Mandatory record-keeping also influences important government policy by providing vital data for national income accounting. The goal of these suggestions is to close the gap that currently exists between financial management theory and the day-to-day struggles that MSEs in Maharashtra experience.

Recommendations:

- **Enhance Awareness and Training Programs:** Recommend the implementation of awareness and training programs for MSMEs in Maharashtra to enhance their understanding and adherence to financial accounting standards.
- **Establish Industry-Specific Accounting Guidelines:** Suggest the development of industry-specific accounting guidelines tailored to the diverse sectors in which MSMEs operate in Maharashtra.
- **Promote Technology Adoption for Accounting:** Encourage the use of accounting software and digital tools that can streamline financial processes, improve accuracy, and provide real-time insights.
- **Facilitate Networking and Knowledge Sharing:** Recommend the establishment of forums or networks that facilitate knowledge sharing and collaboration among MSMEs in Maharashtra.

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- **Government Support and Incentives:** Propose that the government of Maharashtra introduces support mechanisms and incentives to encourage MSMEs to improve their financial accounting practices.

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ISBN - 978-93-95060-79-0

An investigation into assessing how marketing activities influence the sales performance of the company

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Abstract

In the modern world, it's critical to concentrate on a few company-specific marketing techniques and create a tool for achieving total performance. This study effectively examines how marketing strategies affect the company's sales success. After the study was finished, it was examined to determine how to manage the plan and function efficiently by obtaining the entire sales and revenue performance of the organisation. Analysis reveals that marketing is beneficial to all sizes of businesses, including global conglomerates and small and medium-sized enterprises. Virtuoso has been selected as the subject of this research project, and it has been successful in assessing sales performance while utilising the best possible marketing techniques. In order to collect data sources for analysis and to meet research objectives, a questionnaire has been chosen for this study. Finally, the data were analysed correctly to finish the research with the aid of several technological analyses and SPSS software. While most countries in the globe are aware of the importance of marketing research to a company's ability to survive, some of our indigenous business owners are still unaware of this. Only a small number of companies who express interest in these studies really use them on a regular basis. Because the information was never fully used, marketing judgements were made on fictitious data. Businesses need to provide goods and services that consumers will continue to be interested in if they hope to enhance product performance and establish a strong competitive advantage in the market. The study looked at how marketing research affects productivity inside a business.

Keywords: *Marketing, Performance, Effective, Accomplished, Analysis, Multinational Companies, Sales Research, Market Research.*

1. INTRODUCTION

The majority of research assert that various markets throughout the globe are beneficial and efficient for a company's expansion. It may have to do with meeting consumer demands and developing the low-cost positioning strategy in a useful and efficient manner. In order to create standardised national borders and control the products through various price, location, and product strategies, it is necessary to govern society, gather resources for managing the business, and pursue global strategies.

The method involved with managing worldwide marketing procedure and making productive marketing cycles can be successfully applied to managing normalization. These processes have resulted in standardised products that are sold across national borders and utilised in various management practises. The study's development of an efficient strategy and the management of basic processes are crucial for managing the worldwide marketing process in a more efficient manner. These may be viewed as controlling the interaction between sales and marketing, and they would be appreciative of the analysis of business performance and the management of the business unit through market management operations.

Two distinct ideas and management approaches are linked to increasing the number of items and offerings, setting prices, and controlling the promotional mix in order to improve the performance of the company. Marketing strategies must be managed, sales performance must be grounded, resources must be completed, and marketing mix theory must be managed using various conceptual data. Remembered for this exploration report is Virtuoso, a worldwide stage partnership with a solid mechanical office that fundamentally focuses on computerized streaming, web based business, distributed computing, and man-made consciousness as idea sources.

According to analysis, these marketing strategies are typically linked to the management of conventional variables and the creation of blueprints for various corporate operations. These work well and are beneficial in controlling success progressively and creating business concepts through various processing techniques. In the present corporate world, how well it performs relies upon whether

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marketing strategies are utilized as advanced or online entertainment marketing. Lastly, it can be concluded that the marketing department of businesses plays a crucial role in identifying new clients, establishing the brand's value in the marketplace, and utilising cutting-edge technology to promote the business's goods and services.

The field of marketing has demonstrated to be dynamic for various years. Its development from basic trading tasks to a complicated design that is believed to be fundamental for an association's prosperity is evidence of its relevance. Every organisation needs marketing to survive, and the extent to which an organisation can provide potential clients with its goods or services determines how successful that marketing will be. For a long time, directing marketing research has been a fundamental piece of managing a firm.

Its development was accelerated by the industrial revolution, which brought about a wide range of new and diverse consumer products, enhanced economies of scale, emerging markets, and a general expansion in business operations. Research into marketing activities was at its foundational level during this time and remained unexplored for several centuries. Until the end of World War II, it constituted a negligible portion of firms' marketing strategies. An important post-war development was the rise in the number of corporate failures. A dynamic commercial activity is marketing.

Because of the increasing instances of economic crisis, inflation, shortages of materials and energy, unemployment, closure of businesses and industries, terrorism, and war, its functions in the functioning of a corporation have changed dramatically. Additionally, the advent of the internet has brought about improvements in technological growth that can also be ascribed to this. Because of all of this, marketing professionals are now compelled to base more of their strategic decisions on the market. This calls for a formal way to get accurate and timely data about shifting consumer preferences, product needs, and the overall marketing landscape, and this can only be done with thorough marketing research.

To fulfill their clients and get their committed help, all contemporary organizations elevate to them. Banks do marketing exploration to find out about the monetary help needs of their clients;

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Dangote Flour Factories conducts marketing examination to find out about the necessities and prerequisites of flour clients; and Inland Income conducts statistical surveying to find out about the necessities and prerequisites of citizens and different clients. At the point when there is no guaranteed market for an item, there is no utilization in creating it.

Moreover, an imaginative thought is useless on the off chance that it can't be transformed into a financially reasonable item. Since it is the main way for organizations to obtain notoriety, generosity, and customer steadfastness, an attractive item is urgent to their endurance. The objective market's perspective and conclusions should be thought about to create an item that will be economically reasonable. This can be accomplished by information social affair, examination, and utilization of the discoveries to authoritative independent direction. Marketing research is one of the principal ways that an association accumulates data for choices that are both effective and proficient.

Since it is accepted that marketing research propels the achievement of adequacy and proficiency in business associations, an assessment of the effect of marketing research on hierarchical efficiency is thought important. While most countries in the globe understand the need of marketing research for a company's existence, some of our indigenous business owners are still unaware of this. Only a small number of companies who express interest in these studies really use them on a regular basis. Because the information has never been fully used, they are making decisions about the market and marketing that are based on false information.

- **Three key insights on how marketing impacts the company's sales success**

a) **Increasing opportunities:** Sales cannot increase brand consideration rates significantly without the help of marketing. The company's unassisted product awareness rate in Maharashtra was 63% as opposed to 87%, which was lower than the market leader's. The company's consideration rate was 45%, while the top competitor's rate was 87%. This difference grew even more.

Research showed that, even in the presence of a robust sales culture,

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purchase decisions were not significantly influenced by the sales team's relationship. Data indicated that sales personnel had little effect on how customers perceived their offerings. Furthermore, a new customer segment that the current sales team had not yet touched was discovered.

The business raised its marketing budget and purposefully diverted funds from events to digital marketing campaigns after seeing the need to go beyond conventional sales tactics. This involved making purchases of webcasts, videos, and a specialised website that catered to the newly defined buyer niche.

b) **Sales Coverage:** The impression of increased sales coverage can be produced by increased marketing efforts. In a 90-day period, buyers were asked how frequently they visited a salesperson. They reported seeing company representatives 0.8 times on average, or around once every quarter, and 2.5 times, or nearly once a month, when it came to visits from the top competitor's representatives. Buyers reported meeting company representatives 2.4 times each quarter two years later, which is comparable to rival companies. Buyers observed a rise in visits as a result of the increased marketing activities, even though the number of representatives in the segment did not change over the course of the two-year period.

c) **Sales Enablement:** Changes in consumer behaviour can be detected by marketing. All phases of the funnel had seen an improvement in the company's performance, with the exception of existing accounts. Customers are now more "price sensitive," according to representatives, and rivals are undercutting them. Even if the company led the industry in product sales, the senior management group continued to view technological innovation as the primary motivator.

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Subsequent investigation revealed that the sales force was correct. Buyers' priorities had changed. Buyers had changed, far more quickly than anyone had anticipated, thanks to changes in reimbursement, healthcare reform, and a strong rival campaign against overspending on technology.

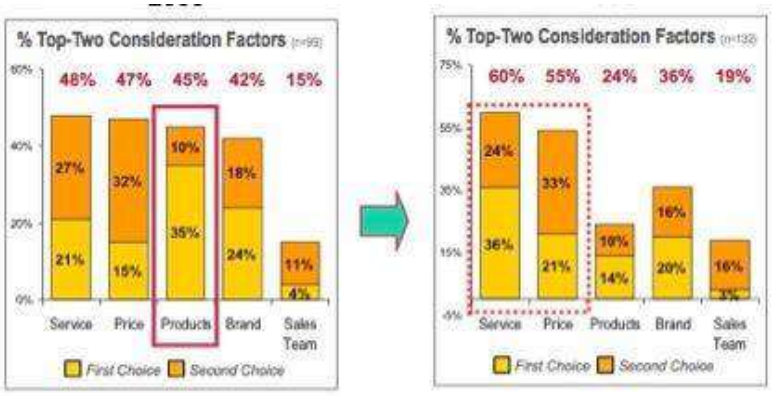

Figure 1: Impact.

Sales collateral and the value proposition therefore needed to be updated promptly. The focus shifted from praising innovation to providing buyers with justification for their investment, with resources such as ROI models and product configurators replacing bells and whistles.

2. OBJECTIVES

- To assess the relationship between particular marketing tactics and the variations in sales success that correspond with them.
- To examine how customers, react to various marketing channels and assess how that affects overall sales.
- To look into how well different marketing initiatives may increase revenue and penetrate new markets.
- To investigate how customer purchasing decisions and ensuing sales outcomes are influenced by brand awareness and perception.
- To evaluate how digital marketing initiatives, affect both online and offline sales success.

3. LITERATURE REVIEW

O'Dwyer (2022), The function of a business owner is helpful in controlling the expansion of various marketing systems and inefficient resources that are necessary for controlling crucial elements of key systems in firms. A variety of firms should be taken into account when applying to obtain various insights in a useful and

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efficient way. There are several advantages of taking into account various marketing tactics for the company's sales success.

KIM, JUN and HYUN (2022), Growing sales volume is the first advantage since it allows for the management of marketing strategies and successful sales. It is necessary to boost sales and treat current clients as if they were previous clients. It is linked to the planning and management of corporate operations and is cautious as the primary advantage of effectively comprehending the memorable. Using and managing different notorieties to be viewed as quite possibly of the most helpful and positive standing and oversee considered in a more fitting manner is one more benefit that the organization utilized by having a marketing strategy.

Gillum (2022), with the aid of efficient marketing resources and handle the ideas that are beneficial for several important aspects. Numerous perspectives highlight the significance of marketing and how it affects the company's sales. Compelling open doors that are useful in producing deals and effectively progressing for more noteworthy thought rates are expanded by the effect of marketing. For controlling society to be viewed as valuable and powerful, the business expected to produce huge deals. Another impact is that it helps cover deals, capabilities as a marketing action, and cultivates the possibility of higher, more deals and inclusion.

Kusumawati, et.al. (2022), Marketing, its character purchasing conduct, and the exhibition really are connected to the third high effect of marketing procedures on the organization's deals execution. Managing the idea of cost responsiveness, making solid contenders who offset jobs, and fostering a pioneer inside the association are vital. Every one of them have areas of strength for a to helping to establish and managing research with the deals procedure that is utilized and has been changed in accordance with meet their market needs.

Onwad (2022), One of the most practical and efficient ways to manage resources, get high advertising planning, prepare marketing instruments, and carry out marketing plans is through marketing. An additional method for overseeing home-based business associations and creating low-cost resources to oversee marketing advantages. It also aids in anticipating how the business will manage to take part in the

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schemes and revenues that cause disruption in society.

Sung (2022), Effective social media platforms are another tactic Virtuoso employs to control variation and finish the social media planning. The marketing plan was facilitated by these procedures for thoroughly considering marketing rope and making a notice that would work (Techniques to Further develop Deals Execution, 2022).

(Li, 2022), These techniques for marketing would be helpful and would bring about the creation of great things, which would prompt an expansion in the quantity of different clients and an expansion in income for the organization. Furthermore, it aids the slow management of assets, and it additionally raises the quantity of clients and the augmentation in deals.

4. RESEARCH METHODOLOGY

4.1.Methodology

To complete the examination, there are various unmistakable areas that ought to be respected compelling appropriate assets that are pertinent in the general public. To be more unequivocal, about the framework was laid out and ensuring that the actual review is legitimate to achieve the objectives and goals. The positivist and philosophical research that has been carried out in this study has been deemed to be effective in terms of managing appropriate resources. In addition to managing sound findings, it gives the sources that are necessary for suitable techniques to be explored. It is possible to handle solid discoveries and develop a terrible technique by conducting good research, which will generate considerable growth. Furthermore, this report incorporates a quantitative approach that assists in concentrating on the entirety of the research in an efficient manner, hence facilitating the collection and analysis of data in an unsuitable manner.

4.2.Sample and Data

When it comes to managing resources in a manner that is more applicable, some of the useful strategies that are effective include sampling and statistical analysis. It is the instrument that is utilised to manage and indicate for the purpose of collecting so that resources may be obtained in an efficient manner. To really oversee and examine the development and populace of a subset and get a powerful populace, this strategy is of extraordinary viability. A questionnaire and survey were the methods

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that the researcher chose to use for this investigation. It is the responsibility of the population manager to consider the available resources, as well as to create sample data and sets that contain and manage a portion of the population. The management practises made use of the questionnaire in their operations. In the current investigation, the researcher selects fifty individuals who are now employed by Virtuoso as respondents.

4.3.Measure

To quantify the elements that contribute to occupational stress and oversee the data collection across various industries. Sampling measurement would be taken into consideration with the aid of resource management and in an efficient manner. It is necessary to take into account the Likert scale approach as a sample strategy. Through a frequency distribution channel, the value of these approaches and their interpretation can be carried out in order to fulfil the substantial need.

5. DATA ANALYSIS AND INTERPRETATION

Table 1: Growth effect.

Does marketing have a direct impact on the company's growth and sales performance?	**Frequency**
a) Strongly agree	15
b) Agree	10
c) Strongly disagree	12
d) Disagree	13

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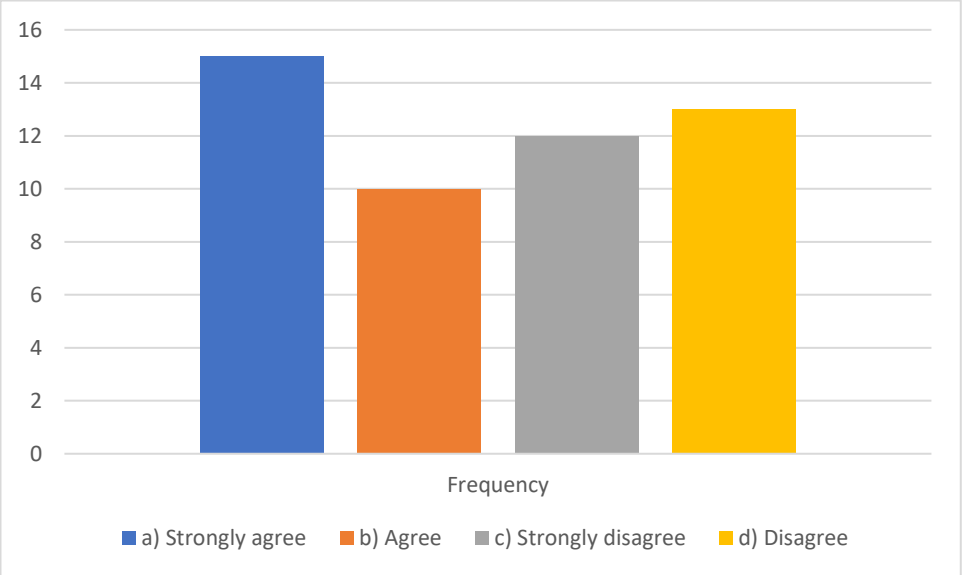

Figure 1: Growth effect.

Interpretation: The survey results about the direct effect of marketing on the company's growth and sales performance are shown in the table. Remarkably, a sizable majority of participants voiced agreement, with 10 and 15 respondents agreeing and strongly agreeing that marketing directly affects sales success and company growth. On the other hand, there is a significant minority opinion, with 12 respondents strongly disagreeing and 13 disagreeing that marketing directly influences a company's sales results. The distribution of responses points to a range of viewpoints within the sample, highlighting the need for additional research or analysis to identify the underlying causes of differing perspectives on the connection between marketing initiatives and the sales performance of the organisation.

Table 2: Perceived Impact of Planning Business Operations on Sales Performance

Is one of the main advantages of business planning for the company's success in sales performance?	**Frequency**
a) Strongly agree	14
b) Agree	11
c) Strongly disagree	10
d) Disagree	15

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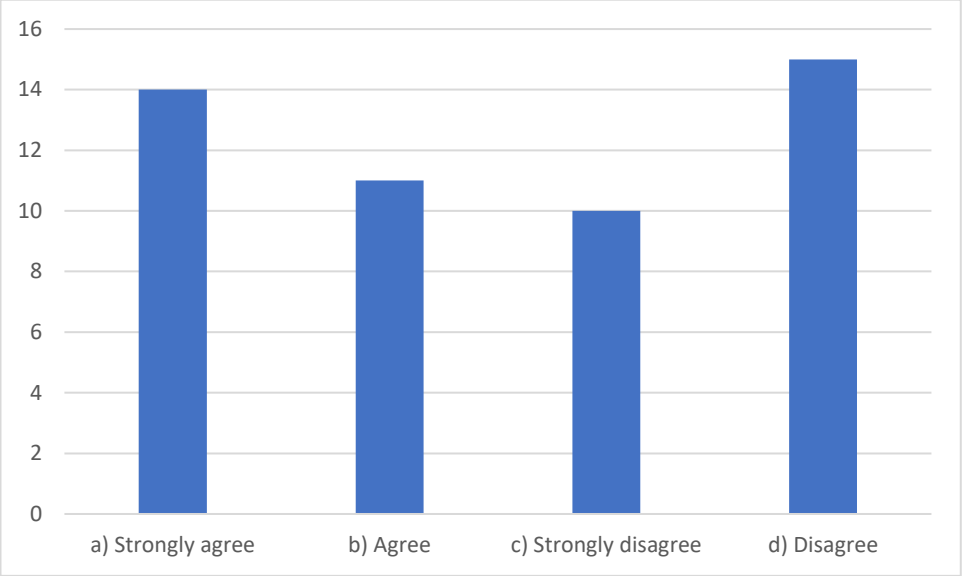

Figure 2: Perceived Impact of Planning Business Operations on Sales Performance.

Interpretation: The responses of persons about how well-planned business operations affect a company's ability to do well in sales are displayed in the table. Remarkably, 14 participants firmly concur that a key element influencing sales success in a business is the preparation of business operations. Eleven respondents also indicate that they mostly agree with this idea. On the other hand, 15 participants disagree and 10 participants strongly disagree that company operations planning is essential to obtaining success in sales performance. These answers reveal a range of viewpoints within the sample population; while a sizable fraction acknowledges the value of planning, a noteworthy proportion is either doubtful or has other ideas about the relationship between successful sales and strategic business planning. Understanding the underlying viewpoints and circumstances driving these differing attitudes may require more investigation and study.

Table 3: Perceived Relationship Between Marketing Initiatives and Employees' Sales Performance.

Marketing's effect on employees' sales performance is correlated with the types of chances that have been generated to stand out in the market.	**Frequency**
a) Strongly agree	13
b) Agree	12

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c) Strongly disagree	11
d) Disagree	14

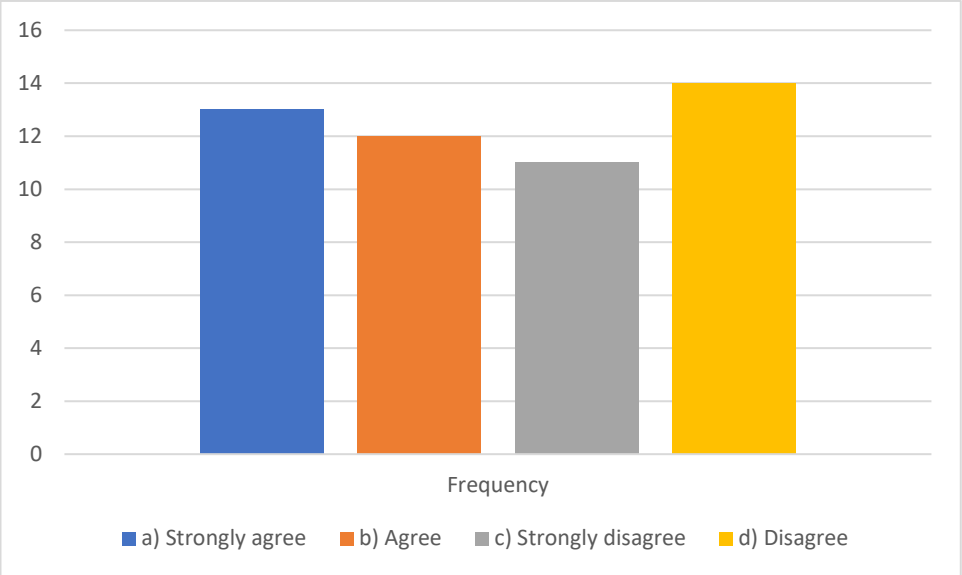

Figure 3: Perceived Relationship Between Marketing Initiatives and Employees' Sales Performance.

Interpretation: The survey results about the effect of marketing on employees' sales performance are displayed in the table labelled "Perceived Relationship Between Marketing Initiatives and Employees' Sales Performance". The above mentioned statement was presented to the respondents, and they were asked to rate their agreement or disagreement. Based on the frequency distribution, the statement is agreed upon by 13 respondents strongly, disagreed upon by 12, severely disagreed upon by 11, and disagreed upon by 14. The findings indicate that employees' perceptions of the relationship between marketing initiatives and the development of market opportunities to improve sales performance are not all that similar. This data offers insightful information on the many viewpoints found in the workforce and can direct strategic choices meant to match marketing campaigns with staff members' expectations and perceptions for the best possible sales results.

Table 4: Perception of Marketing Tactics in Identifying Company Buying Behavior.

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Examine the claim that marketing strategies might reveal a company's purchasing patterns.	**Frequency**
a) Strongly agree	11
b) Agree	14
c) Strongly disagree	12
d) Disagree	13

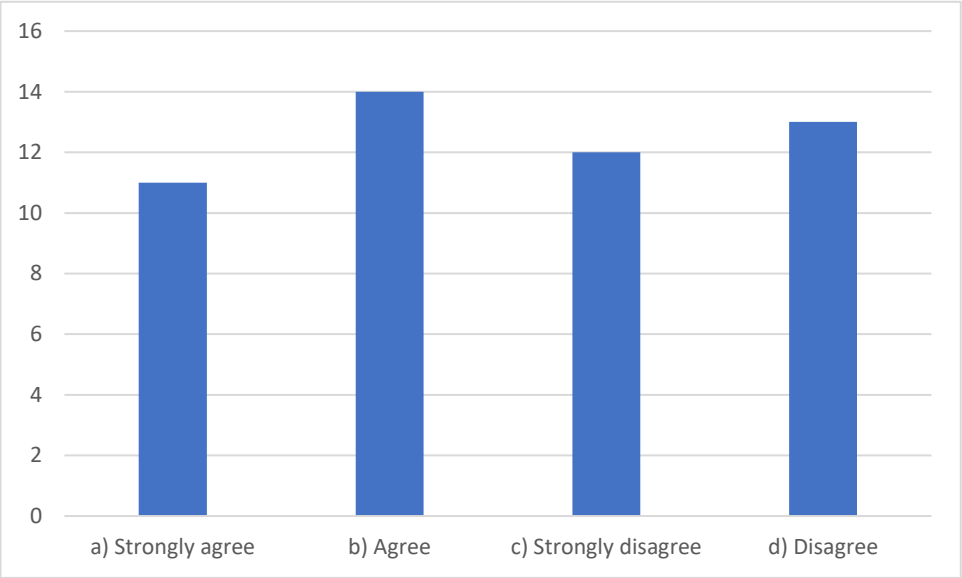

Figure 4: Perception of Marketing Tactics in Identifying Company Buying Behavior.

Interpretation: Eleven respondents highly agreed, fourteen agreed, twelve strongly disagreed, and thirteen disagreed, according to the frequency distribution of responses displayed in the table. The findings show that participants' opinions on how well marketing strategies work to predict the company's purchasing patterns are not all the same. Although a significant proportion of respondents agree with the statement, a notable portion disagree or strongly disagree, indicating differences in perceptions regarding the efficacy of marketing techniques in comprehending and impacting purchasing habits within the firm. The identification of the fundamental causes influencing these divergent points of view and the formulation of strategic decisions on marketing campaigns may require additional investigation and qualitative insights.

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Table 5: Social Media Account Management as a Key Sales Strategy: Stakeholder Perception.

One of Virtuoso's greatest approaches for creating its sales techniques will be to manage the company account on social networking sites.	**Frequency**
a) Strongly agree	10
b) Agree	12
c) Strongly disagree	16
d) Disagree	12

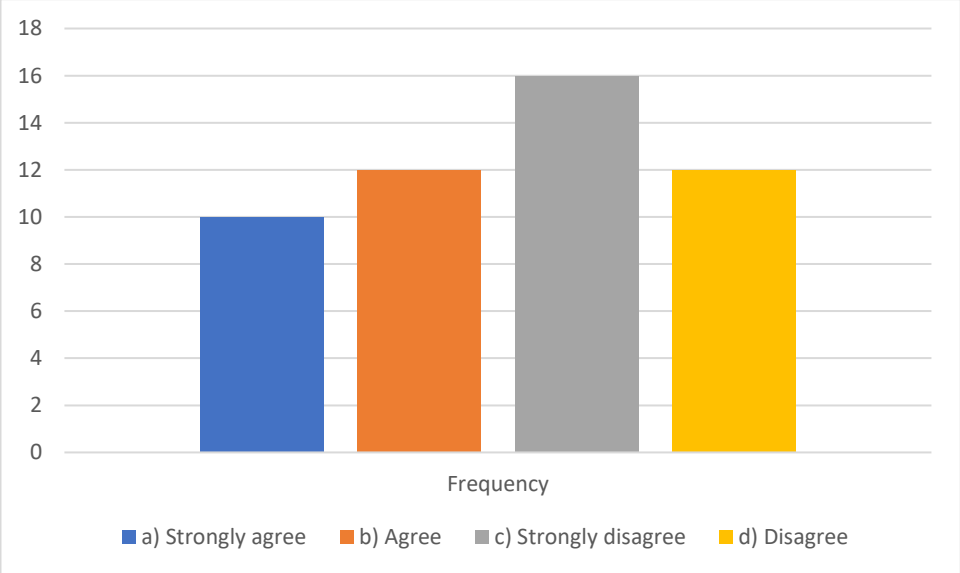

Figure 5: Social Media Account Management as a Key Sales Strategy: Stakeholder Perception.

Interpretation: The table offers valuable insights into stakeholders' perceptions regarding the effectiveness of Virtuoso's sales strategy, which involves managing the business account through social media channels. With a total of 22 responses (10 strongly agreeing and 12 agreeing), the majority of respondents have a positive opinion of this method. However, a sizeable portion—28 in all—hold a negative opinion (16 strongly disapproving and 12 disagreeing). The comments' distribution indicates a divergence of opinions about how well Virtuoso's business account management using social media may improve sales tactics. To better match with stakeholder expectations and preferences, the existing strategy may need to

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be refined or adjusted. This can be achieved by additional study and investigation of the factors that led to these divergent viewpoints.

6. RECOMMENDATIONS AND CONCLUSION

Relationship management's function is to oversee marketing strategy and achieve company performance, both of which have been carefully evaluated and recorded for analysis in research management and discussion. Setting up successful marketing strategies is becoming more and more crucial for businesses, as they will be linked to both growth and good performance in the marketplace. Maintaining financial records, selling the company's products and services to clients, and managing financial performance are all necessary. By utilizing the legitimate item life cycle, this exploration likewise helps with effectively directing the business, client, and management impacts to accomplish homogeneity and serious methodologies. This strengthens the bond and improves the company's performance in a more useful and efficient manner. According to this study, the resource-based approach of the business relates to handling pricing, promotions, and products in order to be regarded as relevant and successful problems. The comprehensive results and analysis can be utilised by several sources to oversee the company's expansion into more relevant data sources. High findings from earlier research—conducted by a variety of writers—that manage the performance of the company and foster market expansion are included in this report.

Recommendations:

- **Diversify Marketing Tactics for Enhanced Sales Success:** Recommend exploring a broader range of marketing tactics to identify those that contribute significantly to variations in sales success.
- **Conduct In-Depth Customer Reaction Studies:** Suggest conducting detailed studies on customer reactions to different marketing channels.
- **Explore Revenue Growth and Market Penetration Opportunities:** Recommend delving deeper into the assessment of how different marketing initiatives contribute to revenue growth and market penetration.

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- **Enhance Brand Awareness and Perception Studies:** Encourage a more extensive investigation into the influence of brand awareness and perception on customer purchasing decisions and sales outcomes.
- **Integrate Comprehensive Digital Marketing Assessment:** Recommend a comprehensive examination of digital marketing initiatives and their impact on both online and offline sales success.

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ISBN - 978-93-95060-79-0

Examining the Behaviour of Organizations: An Investigation into Managers, Employees, and Teams

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Abstract

Work plan and the mental, emotional, and relational social dynamics that influence an association's adequacy are totally remembered for organizational way of behaving. The discipline of the board, which concentrates on the most proficient method to manage people to complete specific obligations, is fundamental for grasping organizational way of behaving and business productivity. For corporate viability, the board puts serious areas of strength for an on the preparation, execution, and configuration of different organizational and regulatory frameworks. The association, then again, adjusts or restructures the people and gatherings through its structure, culture, arrangements, legislative issues, power, and systems, as well as the jobs expected individuals in it. People and gatherings bring their abilities, information, values, thought processes, and mentalities into the association and consequently impact it. How associations are structured and run has definitely changed over the most recent couple of many years. A modern workplace with interdependent skills and workflows has been made possible by technological advancements and skill variety. Therefore, it is essential for organisations to comprehend the factors influencing team results if they are to succeed in the modern business environment. According to this study, which involved 120 managers from the same company, female managers are more susceptible to groupthink and have better communication skills than their male counterparts. This organization's 150 employees were all examined, and the findings indicate that female employees outperform male employees in

ISBN - 978-93-95060-79-0

terms of their contribution to team outcomes. Consideration is given to the implications for managers, researchers, and human resource specialists.

Keywords: *Co-Operative Behaviours, Organizational Dynamics, Leadership Behaviours, Emotional Intelligence, Personality Characteristics, Organizational Structure, Organizational Culture.*

1. INTRODUCTION

Teams are crucial to all that people do in their everyday lives in the current world. Many people initially came into contact with the concept of teams when they were young athletes participating in different sports. The well-established maxim "There is no 'I' in group" is acquainted with youthful competitors when they play a situation in a group, whether it be on the baseball field or the soccer throw. One can determine that the letter "I" does not occur in this word by quickly examining the four letters that make up this word.

If you take this adage literally, there are a tonne of different ways you might interpret it. For example, coaches frequently impart to their players the idea that each member of the team has a designated role to play and that each task is equally important to the team's success as a whole. From this perspective, teamwork is viewed as the story of individuals with disparate skill sets working together to achieve a common goal. Applying this plan to the contemporary business climate, we might see that, in response to emanant staggered frameworks, there has been a quick organizational shift from a collection of individual situations to work gatherings and groups.

Innovation has progressed rapidly throughout recent years, and associations all around the world have been compelled to rearrange their work around groups to answer all the more rapidly, adaptably, and deftly to unexpected conditions. The adjustment of work association is being advanced by a few elements. Diverse skill sets, quick thinking, and effective adaptability are under constant demand in an emotionally and mentally exhausting workplace marked by intense competition, ongoing change, best practises and technological advancements, and impending uncertainty. The best strategy for addressing the organisational problems of the twenty-first century is teamwork.

ISBN - 978-93-95060-79-0

An association is a unique social structure that is impacted by both outer and interior factors. To build its adequacy, an association relies upon major perspectives on errands, individuals, innovation, and structure. The investigation of individual and gathering ways of behaving inside associations and the use of this data to further develop corporate adequacy is known as organizational way of behaving. It is firmly connected with and for the most part centered around the comprehension, anticipating, and the board of human conduct in associations. It additionally furnishes the board with the standards of conduct that are expected in associations.

Collective vibes and the essentials of frameworks believing are underlined in organizational way of behaving. It reinforces a coordinated and coherent way to deal with the executives execution and is keen on the impact that discernment and inspiration play in a singular's way of behaving. To more readily get it, change, and upgrade individual and gathering ways of behaving, this precise investigation of the nature and culture of associations takes a gander at relational and leadership ways of behaving corresponding to groups, social variety, and morals in associations. It additionally advances human conduct to increment corporate viability.

A proportion of an association's presentation, development, efficiency, and productivity is called corporate viability. At the point when a company can accomplish its targets as to the triple main concern, it is considered viable. Since human way of behaving is vital for both execution and corporate adequacy, organizational way of behaving is particularly keen on the unmistakable way of behaving that every person inside an association shows.

Consequently, organizational conduct looks to comprehend the reason why one individual might answer well to a situation while another answers ominously. It also examines why, while receiving the same management, two or more distinct people working on the same task come up with radically different outcomes. To this extent, organisational behaviour is not limited to improving an organization's effectiveness; rather, it is a field that aims to improve understanding of behaviour in organisational settings by studying individuals, groups, organisations, politics, and power from an academic perspective. The ultimate goal of this knowledge is to

ISBN - 978-93-95060-79-0

improve both organisational effectiveness and individual well-being.

The focus of the psychology movement today is much more on developing people's skills rather than trying to figure out what is wrong with them and how to correct it, which leads to more productive and satisfying lives for individuals. This led to the early adoption of positive psychology by prominent psychologists with a focus on research, like Diener, Synder, Csiksentmihalyi, Csiligman, and Peterson. The goal of positive psychology (PP) is to maximise each person's unique talents. good psychology, according to Seligman, is the study of good emotions and the assets that make people and society flourish.

The well-being of employees is a factor in the rising need for enhanced work performance. This makes it necessary to create models and frameworks for managing workers' capacities in order to improve work performance. The goal of using positively oriented models to develop people's abilities is to strengthen the organisation. According to Luthans, the general perspective in organisational behaviour (OB) has historically been more negative than positive. For instance, there has been a greater emphasis on dysfunctions, stress, and burnout than on eustress; managers' shortcomings and obstacles are highlighted rather than their strength and capacity for growth and performance improvement. "Behaviour, attitudes, and performance of people in organisations" are the subjects of OB research.

This area of study focuses on how workers' efforts either increase or decrease an organization's overall productivity and project success. Positive organisational behaviour (POB) is the integration of PP into the study of organisational behaviour with the goal of enhancing employee well-being and driving organisations towards higher performance. According to McHugh, implementing POB lowers the likelihood of losing talented individuals by increasing employee commitment at work.

Similar to this, a lot of study has been done on stress management, but much of it concentrates on negativity. Prior research on stress management has outlined coping mechanisms, stressors and stress-affecting elements, and occupational stress management for construction industry professionals. Numerous written works demonstrate the psychological basis of work-related stress. Instead of attempting

ISBN - 978-93-95060-79-0

to recapture them, the present paper will evaluate the impact of the psychological qualities that were used as POB models in this study on positive stress in the workplace.

In spite of the fact that pressure is an undeniable piece of life, late improvements in the field of pressure the board research have upheld an accentuation on the positive. Positive pressure is alluded to as eustress, though regrettable pressure is alluded to as pain. The beneficial side of stress, or eustress, is the subject of this investigation. It is crucial to remember that the emphasis on optimism stems from a recent change in stress reduction techniques. The question is, because optimism can encourage a better work-life balance and the wellbeing of people, why do we focus on negativity so much? "Positive response to stretch that produces inside us a craving to accomplish and overcome a test" is the manner by which Jones depicts eustress.

Employees experience favourable emotions as a result, which boosts output and fosters an overall sense of satisfaction and well-being. A person's cognitive and behavioural efforts about work-related stress have an impact on their capacity to manage it. Work performance is impacted by poorly managed stressful work environments because they have an impact on the interpersonal and organisational interactions among project team members. The purpose of the study is to evaluate how POB models affect occupational stress in Maharashtra's construction workforce.

The implementation of POB models is still in its early stages, but research suggests that good employee reactions will raise the degree of eustress.

2. OBJECTIVES

- To investigate the methods of decision-making and leadership that managers use in various organisations.
- To look at the elements that affect worker behaviour, such as engagement at work, motivation, and job satisfaction.
- To investigate how team dynamics, communication styles, and cooperation tactics affect the efficacy of organisations.
- To evaluate how organisational culture influences managers', workers', and teams' conduct.
- To recognise the potential and difficulties related to inclusion and

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diversity in organisational structures, as well as how they affect behaviour.

3. LITERATURE REVIEW

Macky, K. (2019), examines how automation, robotics, and artificial intelligence (AI) may affect the nature of labour in the future. She talks on the possibility of pay stagnation and job displacement in some industries, especially those that rely a lot on regular work. She does, however, also draw attention to possible prospects for the creation of new jobs in fields requiring social intelligence, creativity, and critical thinking. In order to handle the shift to a future of work that is driven by technology, Mackey highlights the necessity of proactive governmental changes, educational reforms, and the development of individual skill sets.

Mathieu, J. E., Tannenbaum, S. I., & Salas, E. (2019), emphasises creating and leading successful teams in work environments. It offers a thorough rundown of all the important elements that go into making a successful team, such as the makeup of the team, the leadership style, communication styles, methods for resolving conflicts, and efficient team procedures. In addition to highlighting the value of evidence-based procedures, Mathieu et al. offer leaders and team members useful advice on how to collaborate and accomplish goals.

Schaufeli, W. B., & Leiter, M. P. (2018), explores the ideas of burnout and job engagement, emphasising how they are related and how they affect people's quality of life and the effectiveness of organisations. High degrees of vigour, dedication, and absorption are characteristics of a positive, fulfilling work state, according to their definition of work engagement. On the other hand, cynicism, diminished efficacy, and emotional tiredness are signs of burnout. The analysis reveals elements like job resources, encouraging leadership, and a favourable work environment that boost engagement and avert burnout. In light of evolving work contexts, it calls for more research on work engagement and burnout in its conclusion.

Shamir, B., Howell, J. M., & Avolio, B. J. (2019), presents the idea of collaborative leadership, which places an emphasis on leadership responsibilities being shared within a team or organisation. This strategy encourages cooperation, decentralised decision-making, and group ownership of

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results. The authors contend that in complex and dynamic contexts where no single person possesses all the essential information and skills, collective leadership can be more effective than traditional individual leadership techniques. They talk about many kinds of collective leadership and investigate the environments that support it, like mutual respect, trust, and common objectives.

Grant, A. M., & Hofmann, D. A. (2019), introduce the idea of "job crafting," which describes the proactive actions people take to adjust their work responsibilities, relationships, and cognitive demands so they better suit their particular needs and preferences. They contend that there are several advantages to job creating, such as improved performance, engagement, and well-being. The writers examine the empirical data that backs up these assertions and go over several tactics for promoting and assisting job crafting in businesses.

Gratton, L., & Scott, A. (2019), Examine the effects of a rapidly ageing population—where longer lifespans are becoming more typical—on society and the person. They contend that conventional ideas of retirement are fading and put out a "100-year life" paradigm in which people work in various stages of their lives, alternating between times of education, travel, and recreation. The writers talk on the benefits and problems that this poses for people and businesses, highlighting the need for flexible work schedules, chances for ongoing education, and innovative methods for managing jobs over an extended lifetime.

4. RESEARCH METHODOLOGY

A total of 150 workers and 120 managers from the same Maharashtra, India-based organisation took part in different team-related surveys. This tactic makes it possible to conduct a more thorough investigation that highlights the viewpoints of the team and the leadership. Every participant in the survey provided answers to questions about their demographic information. Tables 1 and 2 show the demographic breakdown for managers and employees, respectively.

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Table 1: Demographic of Managers.

Gender	Management Respondents	Age Group 30 and above	Five years of team management experience	Bachelor's Degree
Male	65	47	49	42
Female	55	51	47	44
Total	120	98	96	86

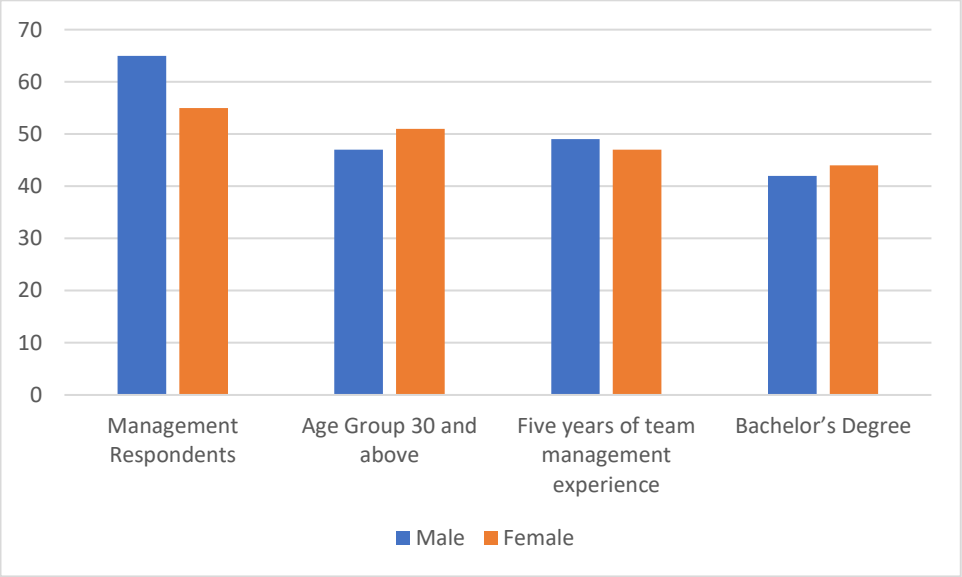

Figure 1: Demographic of Managers.

The poll respondents are broken down into age groups (30 and above), gender, management roles, five or more years of experience managing a team, and bachelor's degree holders in the table. There are 120 responders in all, 65 of them are men and 55 are women. There are 51 females and 47 males in the age range of 30 and over. Similarly, 49 men and 47 women meet the criteria for having five or more years of experience managing a team. Regarding educational background, 42 men and 44 women possess a bachelor's degree. The distribution of management responses is shown in depth in the table, which also sheds light on how gender, age, experience, and education connect with one another in the sample.

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Table 2: Demographic of Employees.

Gender	Employee Respondents	Age Group 30 and above	Five years of team work experience	Bachelor's Degree
Male	70	76	64	69
Female	80	63	55	58
Total	150	139	119	127

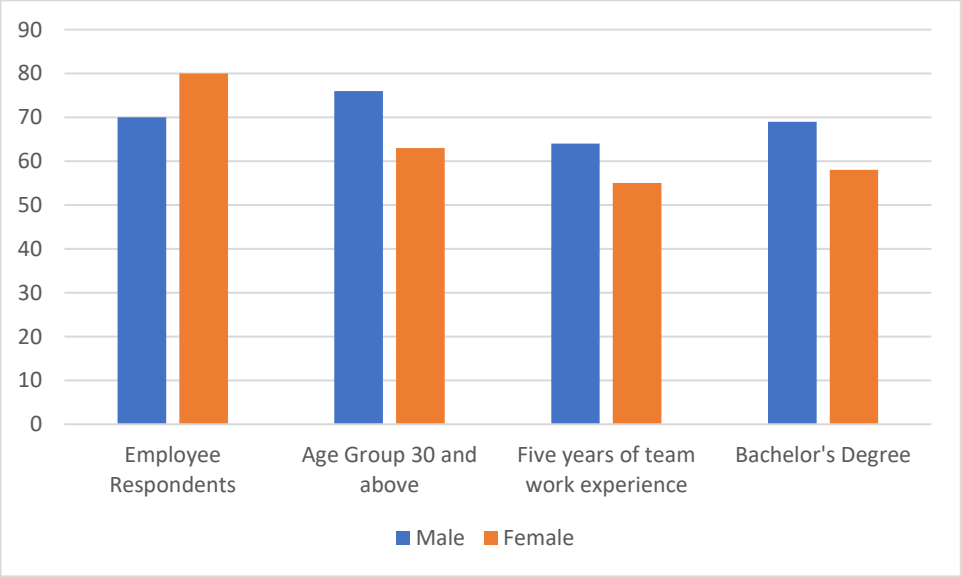

Figure 2: Demographic of Employees.

The demographic composition of the employee respondents is shown in the table according to their gender, age (30 and older), number of years of teamwork experience, and level of education (Bachelor's Degree). According to the data, a total of 80 female and 70 male respondents were included. There were 76 men and 63 women among the workers who were 30 years of age or older. Similarly, there were 64 men and 55 women in the group of workers with five years of experience working in a team. In terms of education, 58 women and 69 men have bachelor's degrees. Overall, the table provides a thorough overview of the distribution of employee characteristics, including information on the surveyed population's age demographics, gender representation, teamwork experience, and educational background.

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The provided parameter is 5-point Likert scale key, where each point on the scale is associated with a specific qualitative descriptor. The scale is designed to gauge the frequency or intensity of a particular behavior or response. A rating of 1 corresponds to "Never," indicating that the behavior or response in question is not observed or experienced at all. A rating of 2 is associated with "Rarely," suggesting that the behavior occurs infrequently or in a limited manner. Moving along the scale, a rating of 3 corresponds to "Sometimes," indicating a moderate occurrence or frequency. As the scale progresses, a rating of 4 is linked to "Often," suggesting a higher frequency or intensity of the behavior or response. Finally, a rating of 5 aligns with "Always," indicating that the behavior or response is consistently present or occurs with a high level of regularity.

The answers' aggregates were used to work out the different scores related with the review's speculations, which incorporate:

Hypothesis 1: When it comes to how much they value worker communication; female managers will score better.

Hypothesis 2: Female managers will score better when it comes to succumbing to groupthink.

Hypothesis 3: More female employees than male employees will add to the team's success.

5. DATA ANALYSIS AND INTERPRETATION

According to Table 4, This research supported the primary hypothesis, which stated that "Female directors will have higher scores on communicating with representatives." With a p-worth of 0.002, the scores of the female supervisors were significantly higher than the male bosses'.

Table 4: Managers that are female will score higher when it comes to staff communication. The T-test of two means and descriptive statistics.

Gender	Mean	Standard Deviation	Sample Size
Male	31.89	6.13	60
Female	40.02	7.01	60

t = 7.123; p = 0.002

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Interpretation: The data summary in the table compares two groups—male and female—in relation to a certain variable. The average value of this variable for each gender is displayed in the "Mean" column, which indicates that the mean for men is 31.89 and the mean for women is 40.02. The degree of variability or dispersion around the mean is shown in the "Standard Deviation" column, where the standard deviation for males and females is 6.13 and 7.01, respectively. Greater variety in the data points is suggested by a higher standard deviation. Lastly, the "Sample Size" column reveals that each of the two groups has sixty members. The data indicates that there is a discernible difference in the mean values between the sexes, suggesting that the characteristic under discussion tends to be greater in the female population on average. Furthermore, it appears from the standard deviation figures that the data for females compared to males is more variable. Maintaining a constant sample size guarantees an equitable comparison of the two groups. To ascertain the significance of the observed differences and to draw conclusions about the population from which these samples were taken, additional statistical analysis, such as a t-test, might be performed.

According to Table 5, The results of this study supported the second hypothesis, which stated that "Female directors will have higher scores on becoming impacted by mindless obedience." With a p-worth of 0.002, the results demonstrated that the female supervisors' scores were significantly higher than the male leaders.

Table 5: Managers that are female will score higher when it comes to succumbing to groupthink. The T-test of two means and descriptive statistics.

Gender	Mean	Standard Deviation	Sample Size
Male	26.09	4.11	60
Female	37.01	7.69	60

t = 9.494; p = 0.002

Interpretation: For the two different gender groups—male and female—the table offers a succinct overview of the important descriptive information. Regarding the variable in question, the average value for the male group is 26.09, indicating that male participants generally show lower values in this category. On average, female participants have higher values in the variable of interest, as indicated by the significantly higher mean of 37.01 for the

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female group. The male standard deviation (4.11) and the female standard deviation (7.69) offer valuable information on the variability or dispersion of individual data points within each group. While a higher standard deviation for females indicates greater variety in the data points around the female group mean, a smaller standard deviation for males indicates values within the group are generally near to the group mean. The statistical credibility of the data is enhanced by the uniform sample size of 60 for both genders. All things considered, this table provides important insights into the distribution of the variable under study by assisting in the comprehension of the central tendency, variability, and sample size features among the male and female groups.

According to Table 6, the third hypothesis, which was supported by this study, "Female representatives will contribute to group outcomes more than male workers." The scores of the female chiefs were p-valued at 0.002, significantly higher than the male directors' scores.

Table 6: The team's success will be enhanced by having more female employees than male personnel. Descriptive statistics and the T-test between two means.

Gender	Mean	Standard Deviation	Sample Size
Male	32.49	7.01	75
Female	40.58	6.02	75

t = 11.521; p = 0.002

Interpretation: Sorted by gender, the table offers an overview of information about a particular variable. For both the male and female groups, the mean, standard deviation, and sample size are provided. The mean, which stands for the average value of the variable within each gender category, is 40.58 for women and 32.49 for men. Males have a standard deviation of 7.01 and females a standard deviation of 6.02, which shows how values are disseminated around the mean. Greater data variability is indicated by a higher standard deviation. Consistently, the sample size for both genders is 75, indicating a comparable and balanced distribution of data points. Overall, the data shows that the average values of the male and female groups differ, with the females showing a little lower variability and a higher mean than the males. Standardised sample sizes improve the results' dependability and lay the groundwork for additional statistical analysis and interpretation of the relevant variable.

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6. RECOMMENDATIONS AND CONCLUSION

According to theory, it is essential to comprehend how and why an individual's ability to collaborate with others affects their success in the workplace. Since collaboration is a stand-in for cohesiveness and synergy, managers must therefore have a practical grasp of whether or not it affects performance. The study's conclusions, which are presented in this article, indicate that collaboration significantly affects people's careers and relationships at work. This makes this an issue that merits further scholarly research. This review indicates that female managers place a greater emphasis on team communication than do their male counterparts. Organisations that place a high importance on responsibility, transparency, and communication tend to do better during periods of organisational transition. Organisations ought to feel comfortable enrolling and lifting ladies to management jobs on the off chance that this study's discoveries demonstrate that female managers have a prevalent comprehension of the worth of communication than their male colleagues. Regardless of the way that the two genders can raise their emotional intelligence, this study has uncovered that ladies are more emotionally astute than men, which might make them more powerful managers in the twenty-first 100 years. Many people believe that women possess natural leadership qualities that can increase their viability, comprehension, and receptivity. For instance, some people think that women are more skilful, creative, sympathetic, and coordinated than men. Females are natural leaders because they know how important it is to establish a connection when speaking.

Recommendations:

- **Diversify Data Collection Methods:** Suggestion: Supplement survey data with qualitative methods such as interviews or focus group discussions.
- **Longitudinal Study for Dynamic Analysis:** Suggestion: Consider extending the research scope over a more extended period, implementing a longitudinal study.
- **Cross-Industry Comparative Analysis:** Suggestion: Expand the research horizon by comparing findings with organizations in different industries within Maharashtra, India.

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- **Utilize Employee Feedback Sessions:** Suggestion: Organize feedback sessions with employees to gather qualitative insights on their experiences with organizational culture, inclusion, and diversity.
- **Implement Training and Intervention Programs:** Suggestion: Propose the implementation of training programs based on the research findings to address identified challenges in teamwork, communication, and leadership.

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ISBN - 978-93-95060-79-0

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ISBN - 978-93-95060-79-0

From the perspective of Organizational Behaviour: The Influence of Leadership Style on Employee Motivation

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Abstract

The reason for these blended strategies study was to examine the association between the inspiration of workers and the authority style utilized at a confidential petrochemical organization in Maharashtra, India. A questionnaire was created and sent to fifty workers across two divisions. In addition, five employees were interviewed as part of a focus group to confirm the survey's findings. According to the study, there is a significant association between a leader's transformational style and staff motivation; the correlation is negative for a transactional approach. This study aims to investigate how leadership style affects employee motivation. This study aims to review the evidence regarding how leadership affects employee motivation. The last ten years' pertinent research has been reviewed by the researcher. This study demonstrates that a leader's style has a beneficial effect on workers' motivation. The principles for achieving shared objectives are provided by a leader's style. Effective leaders constantly provide their staff with the right guidance and inspire them to perform their jobs well. Effective leaders motivate their followers to use their abilities and potential to further the objectives of the organisation. A leader's primary responsibility is to inspire and guide subordinates to boost output and revenue. A leader affects the goals and subordinates of the company and plays a vital role in the operation of a corporation. Effective leadership is crucial at all organisational levels. Various organizations utilize different initiative methods of reasoning and ways to deal with motivate. Pioneers might rouse their staff by making

ISBN - 978-93-95060-79-0

structures that upgrade specialist potential, utilize hierarchical assets, and proposition bearing. Nonetheless, they can likewise make it extremely testing to cultivate a culture of cooperation, encourage arrangement, fabricate trust, and prevail upon individuals to the organization's goals.

Keywords: *Democratic Leadership, Autocratic Leadership, Laissez-Faire Leadership, Employee Motivation, Leadership Styles, Performance Improvement, Leadership Behaviour Approach.*

1. INTRODUCTION

It's getting more and harder to find and keep representatives nowadays. The requirement for HR is ascending because of globalization and worldwide powers. All the while, it has been found that representative inspiration goes about as an impetus for both individual execution and the progress of the association. The hardships that organizations presently stand up to are not like those that existed previously. "It is imperative for organisations of all sizes and shapes to exhibit innovative behaviour by seizing opportunities, resolving issues, and adopting novel paths." Both commercial and nonprofit organisations require this kind of ingenuity and inventiveness.

"A broad set of requirements is currently taking shape for organisations, managers, and staff members, and it includes qualities like initiative, adaptability, and creative problem-solving." The ability of modern organisations to maintain their human capital in a cutthroat market is another difficulty. Many businesses find it difficult to provide competitive pay, perks, and incentives. By implementing efficient staff motivation strategies, small businesses can maintain their competitiveness. Managers and leaders are crucial in fostering an environment and culture of productivity and excitement among staff members, which enables them to accomplish their tasks.

"Most of large businesses...keep burning through great many dollars yearly on preparing programs, gatherings to increment inspiration, motivators to brace inspiration, gatherings to analyze issues with representative inspiration, instruments to measure inspiration, statements of purpose, thus forth...." with no recognizable outcomes. It appears that this is due to a lack of knowledge about

what motivation actually entails. "Researchers have approached motivation from a variety of perspectives, despite the fact that motivation is a complex, multifaceted construct whose essence is elusive."

This research looks at the driving forces behind a department's work for a private oil and gas business in Maharashtra, India. employing a self-made questionnaire as a tool to gauge the motivational components. A manager that possesses leadership qualities will inspire their staff to produce better work, and they will foster an environment that values their fresh ideas and opinions. A worker wishes to express thoughts and emotions in order to find fresh approaches to efficiently use available resources. At the point when a supervisor or pioneer reveals data to workers in a straightforward way, supporters and representatives are constrained to have confidence and dedication in the pioneer.

A viable pioneer moves supporters to help result and representative fulfillment. He gave followers a model to follow, modelling his actions. A leader bridges the gap between upper and lower management, or employees. He must convey to lower levels the beliefs and expectations of the top level as well as the challenges and obstacles faced by workers in achieving the top level. He connects all of the higher level and lower level activities. He fostered a sense of camaraderie inside the team by establishing a positive organisational culture. The leader offers direction and counsel to the employees with work-related issues, whether they be emotional or technical.

In order to maximise production and accomplish predefined goals, a competent leader must assign power and offer both financial and non-financial incentives for increasing productivity. A leader can be a friend, a philosopher, and a mentor to those who follow them. The kind of leadership a business chooses to use greatly influences how well it performs in reaching its objectives. It is thought that shrewd leadership combined with an inspired team results in a profitable and enduring corporate enterprise. People are the fundamental force behind organisations because they put their all into them and support them in achieving their goals.

Every business in the world was established with the intention of either generating a profit or providing social

ISBN - 978-93-95060-79-0

services to benefit the community; in certain cases, both goals were pursued at the same time. As a result, all organisations have similar corporate aims. Employers need workers to help them accomplish their goals and objectives, but employees also need their leaders to inspire and motivate them. Since employees are the cornerstone of the company, keeping them engaged and motivated is crucial.

Since effective leadership is necessary to improve a company's performance, a corporation's success or failure is based on how well-led all organisational levels are. The beliefs, actions, and abilities required to influence others to accomplish organisational objectives are known as leadership traits. For this reason, one way to define leadership is as a tactic for getting people to cooperate in order to achieve a common goal. A person's behaviour that aims to influence others' perception of their actions is referred to as their leadership style.

Figure 1: Different Leadership Styles within an Entity.

Leadership within the company has an impact on employee conduct. Effective leaders are the role models who mould the behaviour of their followers and subordinates to achieve the goals and objectives of the company. Organisational leaders have the power to impact employees' values, adaptability to change,

ISBN - 978-93-95060-79-0

and drive. On the other hand, workplace motivation is a difficult problem for any company. An individual's motivation at work is what sustains their inspiration. The mental or mental proficiency that drives the ways of behaving or driving forces communicated for a specific point is the most vital phase in the inspiration cycle.

A continuous psychological process called motivation starts, guides, and sustains behaviour. As evidenced by the execution of the mission, the achievement of predefined goals and objectives, efficiency, sustainability, growth, and competitiveness, data show that an engaged workforce promotes greater organisational and employee performance. Leaders have an effect on employee effectiveness since creating a motivating plan requires considering factors that inspire workers to achieve at their best on an individual and group level.

2. OBJECTIVES

- To find out how staff members view the relationship between leadership and motivation.
- To ascertain if intrinsic or extrinsic motivation is the main source of motivation for employees.
- To investigate the elements that act as motivators for workers in the oil and gas sector.
- To evaluate the effectiveness of current leadership techniques used to inspire workers in the oil and gas industry, pinpoint areas in need of development, and suggest new tactics.

3. LITERATURE REVIEW

Akhtar & Khan (2021), We out a near report in Pakistan that exhibited the effect of a few initiative methods of reasoning on labourer inspiration in people in general and confidential areas. As per their discoveries, staff inspiration is emphatically affected by ground-breaking initiative, which is characterized by romanticized impact, moving inspiration, scholarly excitement, and tweaked thought. This is steady with an abundance of earlier examinations that features the ground-breaking initiative's ability to rouse representatives and further develop commitment and execution.

Bashir & Bhatti (2020), Look at in more detail what balance between serious and fun activities means for the connection between representative inspiration and authority styles. According to their

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research, the influence of various leadership styles is moderated by work-life balance, with positive work-life balance amplifying the motivational impact of leadership—particularly that of transformational and transactional types. This research emphasises how crucial it is for managers to support their staff members' work-life balance in order to maximise motivational results.

Chen & Wang (2023), explore in more detail the precise effect that moral leadership has on worker motivation. Through a moderated mediation model including psychological safety and job autonomy, their research reveals that ethical leadership—characterized by honesty, fairness, and concern for the well-being of employees—positively promotes employee motivation. The aforementioned paradigm places significant emphasis on the provision of a secure work environment that fosters employee autonomy and self-expression, hence augmenting their motivation levels.

Gorgieva & Gorgiev (2021), Examine the wider effects of leadership philosophies on worker motivation and organisational dedication. According to their research, certain leadership philosophies—such as democratic, transactional, and transformational—have a favourable impact on employees' commitment and drive. This all-encompassing strategy emphasises the complex influences of leadership, highlighting its ability to influence not just employee engagement and motivation, but also their allegiance to the company.

Gupta & Singh (2023), analyzed what authoritative culture means for the connection between worker inspiration and initiative style. They found that the connection between representative inspiration and administration style may be directed by hierarchical culture. Specifically, that's what they found, albeit a controlling hierarchical culture can will more often than not relieve the positive connection between groundbreaking initiative and worker inspiration, a steady hierarchical culture can really increment it.

Kim & Park (2022), examined the relationship between job satisfaction and employee motivation as influenced by leadership styles, with a focus on the mediating role of psychological empowerment. They discovered that work happiness and staff motivation can be greatly impacted by leadership

ISBN - 978-93-95060-79-0

philosophies. In particular, they discovered that whilst transactional leadership had an adverse effect on worker motivation and job satisfaction, transformational leadership had a favourable effect on both. Additionally, they discovered that the association between leadership styles and worker motivation and job satisfaction was mediated by psychological empowerment.

4. RESEARCH METHODOLOGY

The primary goal of research is information gathering. Information can be gathered spontaneously or by following a strict process. Any examination task ought to utilize an OK procedure to address the exploration issue or questions. Information for this study were accumulated utilizing a blended techniques methodology that utilized quantitative and subjective procedures. A speculation is tried, the size of the peculiarities of interest is estimated, and the outcomes are then summed up utilizing a quantitative methodology.

At the point when you wish to fathom the peculiarity and consider individual feelings, you utilize a subjective technique. Gaining a deeper knowledge of the unique environment of an oil and gas sector setting would be aided by such qualitative study. Selecting a mixed methods approach will, above all, help to overcome the limitations of a single technique and aid to assure the dependability of the results, as well as make them easily accepted by readers. Using several approaches in one study increases the study's legitimacy and solidifies its findings.

A questionnaire was created to gauge employees' opinions in order to research how they view the influence of leadership. From the essential things, a five-point Likert scale with the choices emphatically deviate, concur, impartial, and differ was utilized. An email with a connection to the poll was dispersed to the organization's staff to accumulate information. Following receipt, the answers were joined into one sheet and exposed to both spellbinding and inferential measurable examination. Individuals who addressed this overview were picked aimlessly. Since the arbitrary example approach gives each individual from the objective populace an equivalent opportunity to partake in the review, the discoveries are more reliable.

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5. DATA ANALYSIS AND INTERPRETATION

The survey data was collated, input into SPSS, and the findings were examined appropriately. Relevant statistical data, both descriptive and inferential, has been extracted. Thematic analysis and coding were used to examine the qualitative data—the results obtained from the interviews.

5.1.Descriptive Analysis

a. Age of participants

Table 1: Age of participants.

	N	Minimum	Maximum	Mean	Std. Deviation
Age	50	19	51	30.01	6.411
Valid N	50				

The participants' ages range is 30, with 30 being the average age.

b. Positions of participants (managerial or non-managerial)

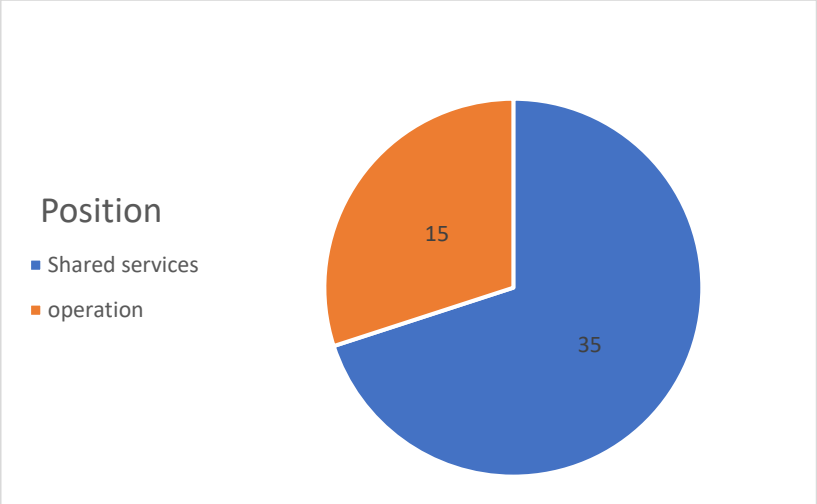

Figure 2: Positions of participants.

Thirteen of the participants hold managerial positions, compared to 37 non-managerial positions.

c. Departments of participants

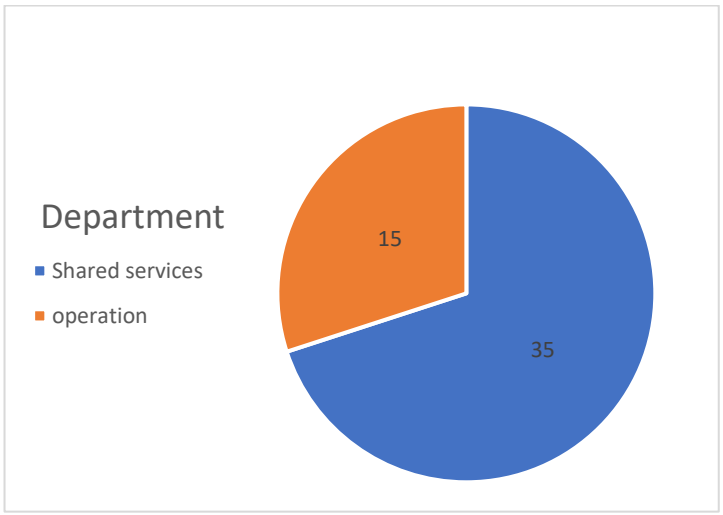

Figure 3: Departments of participants.

Fifteen of the total participants are in operation, and 35 are in the shared services section.

d. Participants' Education level

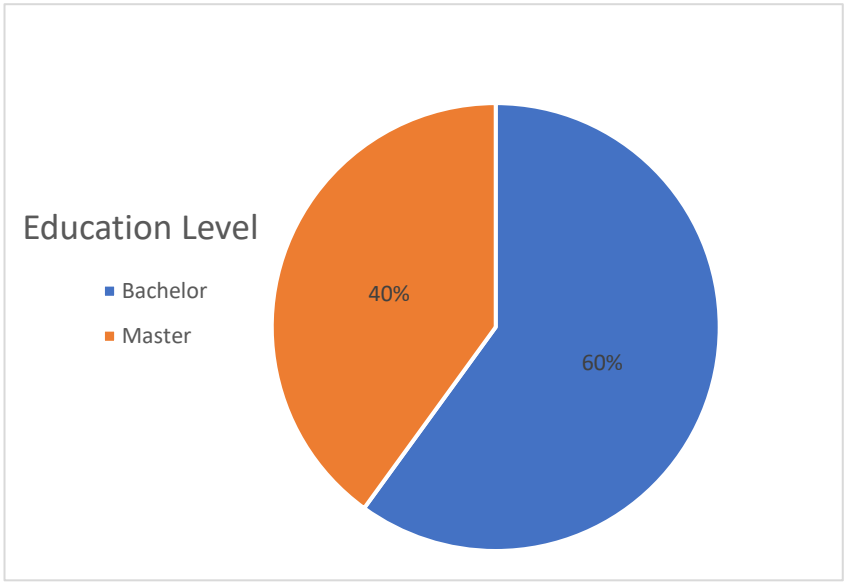

Figure 4: Educational Level of Participants.

Two fifth of participants has a bachelor's degree, while the other three fifth have a master's degree.

e. Participants' years of experience

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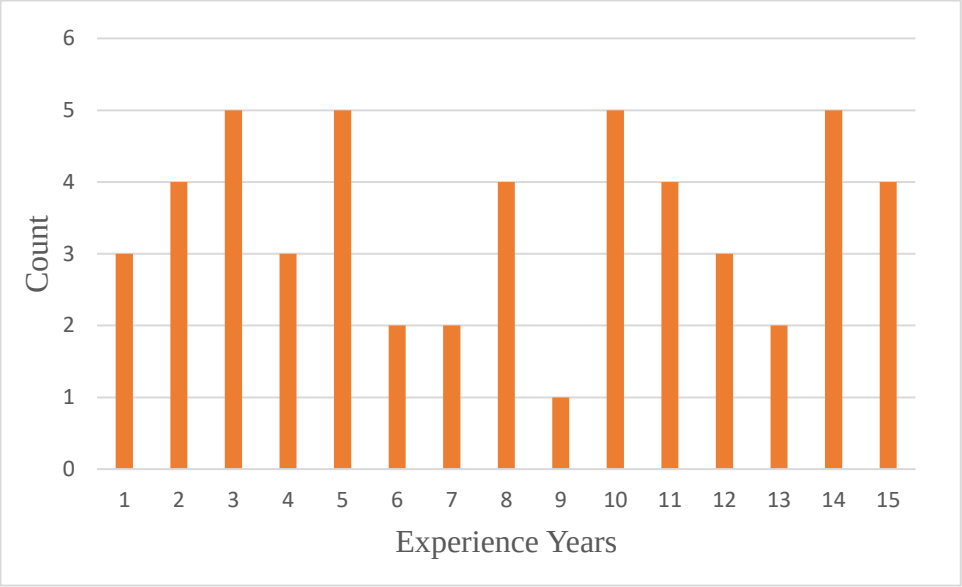

Figure 5: Participants' Years of Experience.

There are 4.5 years of experience on average.

5.2.Correlation analysis

Table 2: Analysis of the relationship between preferred motivational style and educational attainment.

		Education Level	**Which leadership style has the greatest potential to inspire you?**
Education Level	Pearson Correlation	2	-0.162
	Sig. (2-tailed)		0.369
	N	50	50
Which leadership style has the greatest potential to inspire you?	Pearson Correlation	-162	2
	Sig. (2-tailed)	0.369	
	N	50	50

There isn't any discernible relationship between the two variables. The table shows the findings of a Pearson correlation study that looked at the association between educational

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attainment and the chosen motivational leadership style. The first row's correlation coefficient shows a weak inverse association between education level and the likelihood of being motivated by a specific leadership style, with a negative correlation of -0.162. Nonetheless, the p-value (Sig. 2-tailed) is higher than the standard significance level of 0.05 at 0.369. Because of the high p-value, it is possible that the observed link is the result of chance rather than a statistically meaningful correlation. The identical data is shown in the second row, but the p-value and correlation coefficient are displayed differently. In conclusion, this analysis shows that among the 50 study participants, there is no statistically significant relationship between education level and the desire for a certain leadership style as a source of inspiration.

Table 3: Department and Preferred Leadership Style Correlation Analysis.

		Which leadership style has the greatest potential to inspire you?	**Department**
Which leadership style has the greatest potential to inspire you?	Pearson Correlation	2	0.321
	Sig (2-tailed)		0.090
	N	50	50
Department	Pearson Correlation	0.321	2
	Sig (2-tailed)	0.090	
	N	50	50

There isn't any discernible relationship between the two variables. The table displays the findings of a correlation study that looked at motivation and leadership styles in two distinct departments. Finding the leadership stance that will inspire employees in each department is the main goal. The degree and direction of the linear link between variables, in this case motivation and leadership style, are measured using the Pearson correlation coefficient. The first row's Pearson correlation coefficient, which is 0.321, shows that motivation and leadership style are positively correlated. The association may not be measurably huge at the conventional importance level of 0.05, as indicated by the importance level (Sig) of 0.090. For each department, the sample size (N) is 50. Consequently, these findings

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suggest that although motivation and leadership style have a favourable association, it is not statistically significant. More research or a bigger sample size could be required to reach more reliable conclusions regarding the connection between motivation and leadership style in these divisions.

Five workers were evaluated to affirm the overview's discoveries. Because of time requirements, the meeting was semi-organized and brief. Most of the inquiries came from the overview. Three out of five laborers for the most part agreed that extraordinary administration improves the probability of more prominent work fulfillment. Working with any sort of pioneer is certainly not a tremendous arrangement, as indicated by the other two representatives. I give an example of one of their responses in the section that follows:

Employee# 1: “Because he trusts me and provides me the chance to voice my thoughts, I love working with my boss.”

Employee# 2: “When my manager sets ambitious goals and motivates me to reach them, I'm thrilled”

Employee# 3: “Regardless of my fresh debut at the organization, I have seen that my chief is a ground-breaking chief who endeavours to draw out the best in each individual, and I view it as truly fascinating”

Employee# 4: “My manager is primarily interested in finding faults, and I sense that we are not on the same page”

Employee#5: “I am unable to collaborate with someone who magnifies my errors while downplaying my accomplishments; that is what my manager does”

The following topics have emerged from the employees' interview:

i. In request to motivate their workers, administrators should be kind, empowering, and focus on the prerequisites of their staff individuals.

ii. It's basic to hear what laborers need to say to improve the working environment and motivate staff individuals.

iii. Postponing mediation (transactional leadership) as late as possible might make negative impacts.

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6. RECOMMENDATIONS AND CONCLUSION

This research was conducted with the intention of examining the perceptions of employees working for private petrochemical companies on the leadership styles and the impact those styles have on the motivation of employees. This study used a blend of subjective and quantitative ways to deal with information assortment and examination. It has been found via the analysis of collected data that leaders who choose a leadership style that is both transformative and participative are more likely to establish an environment that is conducive to the motivation of their employees. From a general standpoint, it is possible that the employees who participated in this survey would favour transformational leadership over transactional leadership. The discoveries of this study are in accordance with those of different examinations. This study recommends that administrators who wish to be more successful may should be more moving, accommodating, and ingenious, and they ought to likewise attempt to build the gifts of their subordinates determined to expand their own adequacy. It is necessary to do additional research in order to compare the findings of the study with those of other businesses in the vicinity.

Recommendations:

- **Diversify Data Collection Methods:** it is recommended to incorporate additional data collection methods.
- **Include Comparative Analysis:** recommend incorporating a comparative analysis between different segments of the oil and gas industry.
- **Longitudinal Study for Dynamics Over Time:** Suggest conducting a longitudinal study to explore how the relationship between leadership and motivation evolves over time.
- **Explore Cross-Cultural Influences:** Given the international nature of the oil and gas industry, it is recommended to incorporate a cross-cultural analysis to understand how cultural factors may influence perceptions of leadership and motivation.
- **Engage Stakeholders for Actionable Recommendations**: To ensure the practical relevance of the research, suggest involving key stakeholders from the oil and gas

ISBN - 978-93-95060-79-0

sector in the analysis and interpretation of findings.

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ISBN - 978-93-95060-79-0

An examination of the effects of Artificial Intelligence on the digital transformation of human resources in the industrial sector

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Abstract

Mechanical technology development — which envelops both man-made intelligence and the Internet of Things (IoT) — has made computerized reasoning (artificial intelligence) a colossal open door in the work environment. Flexibility, Efficiency, and Precision are seen as the possible advantages of Industry. Many modifications are necessary for the implementation of Industry, one of which is the Human Resource (HR) role. In the business world, having strong HR capabilities is crucial and offers an advantage to the company. In order to respond to the challenges and demands, HR should be more circumspect and flexible. We research AI's contributions to industry practices and HR digitalization. 270 HR professionals with foundations in assembling, information innovation (IT), and organization were decided to partake in this assessment, which zeroed in on three parts of HR availability and five computer-based intelligence applications in HR capacity. Analysis of Moment Structures (AMOS) and the Statistical Package for Social Sciences (SPSS) program were utilized to research the assembled information. The discoveries showed that concentrating on various leveled associations is fundamental to accomplishing feasible turn of events. Every one of the five of the artificial intelligence application areas of HR support both human resource abilities and versatility. Upgrades in security and prosperity were viewed as fundamental components of the artificial intelligence application in HR.

Keywords: *Artificial intelligence, Structural equation modelling, Digitization Agility, Human resource management, Industry.*

1. INTRODUCTION

The Human Resource (HR) capability is fundamental in connecting the innovation and human resource holes in the cutting edge time of industry. Most of the positions that were formerly dealt with by human resources are currently being supplanted by innovation, however the need for adaptable HR administrations to deal with the challenges of overseeing individuals is as yet rising. Innovation can help with improving the HR cycle's dexterity to achieve this adaptability. Having the option to move quickly and smoothly is an idea that isn't new, and enormous organizations like Google, Apple, Facebook, Amazon, and Microsoft have embraced it. Nimbleness in HR alludes to the ability to help individuals, basic techniques, and hierarchical variation by answering rapidly and startlingly to changes in individuals and cycles.

Being deft as a learning and improvement or HR professional means being able to advance worker commitment and maintenance while keeping them in accordance with the general objectives of the business. HR Deftness functions admirably for dynamic HR undertakings where capability normalization is testing. Associations need their laborers to convey worth and focus on consumer loyalty to turn out to be more agile. But since the HR division isn't generally positioned to offer direct motivations to the client, it gets unfavorable criticism for answering gradually, which makes laborers troubled.

Therefore, to remain serious and attract top faculty, associations need to engage and urge HR to be more mindful of and receptive to developing business needs and mechanical progressions. HR actually handles recruiting, advancement, performance the board, and other HR undertakings in a dexterous association; it simply utilizes spry cycles. HR dexterity can be characterized as the accompanying three things: the ability to perceive issues almost immediately and make a move, the capacity to answer all the more rapidly and really, and the coordination of analysis and configuration remembering to distinguish, plan, and focus on drives that have the most obvious opportunity with regards to progress.

The quick advancement of innovation, especially the mix of computerized reasoning (artificial intelligence) in HR, has brought about eminent transformations in HR techniques and systems. It is basic to

ISBN - 978-93-95060-79-0

grasp what artificial intelligence means for a few HR features, including finance handling, representative comfort, wellbeing and security, staff efficiency, and continuous criticism, as organizations dynamically shift their HR capabilities on the web. Besides, understanding what hierarchical organization analysis and configuration are meant for by these HR exercises can give experiences into how organizations could utilize simulated intelligence to work on their general adequacy and productivity.

This study views at the connection among artificial intelligence and HR digitization as well as what HR digitization means for hierarchical organization analysis and plan. To decide the advantages and difficulties of HR digitization with regards to hierarchical organization analysis and plan, as well as to survey the effect of HR digitization on these two cycles, the exploration will take a gander at the connection between man-made consciousness (computer based intelligence) and HR digitization as far as estimating representative efficiency, further developing wellbeing and security, robotizing finance handling, estimating worker comfort, and giving constant criticism.

By satisfying these objectives, this examination paper desires to reveal insight into what man-made intelligence means for HR digitization and what HR digitization means for authoritative organization analysis and plan. Moreover, this examination attempts to give ideas to foundations in regards to the ideal use of simulated intelligence to enhance their HR methodology and approaches, subsequently enlarging the overall adequacy and proficiency of the association. The two significant features of HRM shrouded in this paper are (I) the application areas of man-made intelligence and (ii) the light-footed component of HRM. Because of the scarcity of concentrate to date, the parts estimating the two components were taken from idea papers and online distributions.

2. OBJECTIVES

- To evaluate how artificial intelligence might affect human resource management.
- To ascertain whether it is successful in meeting industry demands.
- Investigating the most recent AI developments in HRM practices.

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- To evaluate how AI will affect HRMP in order to meet industrial demands.
- To examine how AI affects industry sustainability

3. LITERATURE REVIEW

Yawalkar (2019), taken a gander at to figure out how man-made consciousness capabilities in the HR division and to comprehend the difficulties related with that too. Research papers, distributed materials, online diaries, HR web journals, and review reports from different exploration associations were utilized to gather optional information. The review configuration utilized by the analyst was spellbinding. It was resolved that the utilization of simulated intelligence by the HR division for various positions was developing, with advanced mechanics organizations ready to deal with errands like recruiting, information analysis, and social occasion, as well as decreasing work environment responsibility and working on hierarchical performance.

Bhardwaj et. al (2020), We out an exact review to examine the utilization of artificial intelligence and its effect on HRM because of mechanical headway with 115 HR professionals working in the IT business in the Delhi/NCR district. Deciding whether the advancement and convenience of HR capabilities moderate this connection was the point of the review. Solid connection between the two parts was found utilizing different relapse based presumptions, showing that expanded utilization of artificial intelligence in the work environment improves HR useful adequacy. Due of its high connection with both advancement and ease of use, man-made intelligence affects HR as far as both. Information with respect to man-made brainpower — which is setting off another modern transformation called Industry 4.0 — will be canvassed in this review.

Khatri et. al (2020), completed a review that analyzed the mix of computer-based intelligence-based innovations into an association, as well as the original open doors and troubles in human resource the executives that emerge from thinking about the organizations' non-tech and tech resources. An enlightening subjective survey technique in view of papers giving optional information was utilized by the scientist. periodicals, books, and so on. Successful procedures to coordinate computer-based intelligence into the association incorporate updating ranges of abilities to hold laborer resolve, robotizing

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their performance through reengineering, and expanding information on the advantages and disadvantages of innovation. Scientists have made two models that relate simulated intelligence and HRM. It was tracked down that for an association to develop and work, individuals and innovation should coordinate. Individuals need to turn out to be more talented and show that they will get new abilities through information-based approaches to contend with man-made intelligence.

Arslan et. al (2022), analyzed with an emphasis on the difficulties that HRM divisions and chiefs face in their associations because of the helpful understanding between simulated intelligence (robots) and human specialists at the group level. This study coordinates numerous surges of writing to break down the connection between artificial intelligence (especially robots) and HRM in contemporary associations. The discoveries exhibited the significance of hierarchical help systems, like preparation valuable open doors, a strong workplace, and certifications of a commonsense mechanical capability level, preceding setting human laborers in groups with robots. At long last, we tracked down that one of the most difficult issues for HRM to deal with was assessing performance in bunches where man-made intelligence (counting robots) and humans live together.

Kambur and akar (2022), led a review to foster a dependable and precise scale, figure out how HR staff individuals view artificial intelligence, and assess the progressions simulated intelligence has achieved in the HR division. An example of 821 HR directors and workers was provided by the greatest organization in Turkey. It was found that chiefs and representatives in human resources figured innovation would liberate them from dreary undertakings, decrease the strain of recognizing reasonable competitors, and give them admittance to a more extensive scope of chances. It was revealed that man-made reasoning (computer based intelligence) was incorporated into the representative preparation and advancement interaction, and that simulated intelligence will decrease preparing related interruptions.

Vrontis et al (2022), led exploration to recognize the primary drivers of HRM issues and issues, as well as to incorporate a complete rundown of scholastic commitments on insight computerization

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to date. 45 expected papers on artificial intelligence, advanced mechanics, and other state of the art innovation in HRM settings were completely looked. The outcomes show that savvy mechanization innovations present an original way to deal with worker oversight and improve hierarchical performance, presenting both significant open doors and difficulties for human resource the executives. It has been found that the effect of these state of the art advancements is focused on HRM works on, including employing, preparing, and work performance, as well as HRM techniques, including position substitution, independent direction, human-robot/man-made intelligence cooperation, and learning valuable open doors.

4. RESEARCH METHODOLOGY

4.1.Research Design

A cross-sectional illustrative examination configuration was utilized in the review. Since the examination philosophy empowers the gathering of information from a wide populace at an exact moment, it is reasonable for looking at the impact of simulated intelligence on the digitization of human resources in industry.

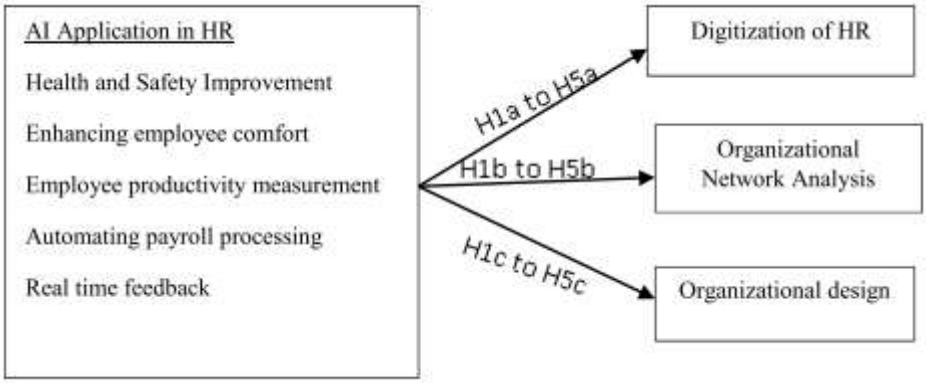

Figure 1: Proposed conceptual model.

4.2.Population and sampling

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Human resource professionals utilized in the assembling, ITES, IT, and administration areas in the Maharashtra urban communities of Pune and Nagpur made up the review's populace. These two cities were chosen since they are home to a variety of industries. The service sector included banks in the private sector. The geographic location was chosen in the first stage, the firms in each sector were ranked in the second stage, and the respondents were chosen from the selected firms in the third stage of a multi-stage sampling procedure. 338 questionnaires in all were submitted via a Google Form; 270 of them, with an 80% response rate, were deemed appropriate for study following additional review. Previous studies provide justification for a sample size of 270. For SEM analysis, a sample size of at least 150 is advised. 150–300 is regarded as an appropriate sample size for structural equation modelling. SEM analysis requires a sample size of at least 200, and bigger sample sizes are always preferable.

4.3.Scale development and validation

By modifying firmly related writing, new scales were made to quantify the develops utilized in the review model. The effectiveness of the scales in estimating the develops was then affirmed by testing them for different legitimacy and dependability measurements. Dependability is the consistency of estimation across time, though legitimacy is how much a scale estimates what it is intended to gauge. In this review, corroborative element analysis (CFA) was utilized to analyze the legitimacy and unwavering quality of the scales. The CFA results showed that the scales had great dependability and develop legitimacy. Specifically, all developments' composite dependability (CR) values were higher than the proposed cutoff worth of 0.7, showing solid inward consistency. Moreover, all builds' typical change removed (AVE) values were higher than the recommended limit worth of 0.5, showing solid united legitimacy. Subsequently, it was resolved that the scales utilized in this examination were fitting for estimating the important builds.

4.4.Data collection

The research was supported by data collected through the use of a standardised questionnaire. The instrument is divided into three sections, the first of which has questions about demographics. Human Resource Agility is measured by the assertions in the third and final

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section, which is about AI applications in HRM. Parts two and three employed the Likert Scale, which has five points.

5. DATA ANALYSIS AND INTERPRETATION

5.1.Data Analysis

AMOS was utilized to assess the proposed model after SPSS was utilized for essential statistical analysis of the obtained information. The legitimacy and unwavering quality of the scales utilized in this review were tried, and the outcomes were acceptable to proceed with the examination.

By taking a gander at every variable's skewness and kurtosis values, the scientist affirmed multivariate ordinariness, and all values fell inside the reasonable scope of - 2 to +2. They likewise utilized greatest probability assessment, which is predicated with the understanding of multivariate ordinariness. The listwise erasure strategy, which kills cases with missing qualities from the analysis, has been utilized to address missing information. A definitive example size of 270 was more prominent than the insignificant example size proposed for SEM analysis. By utilizing a deduced model that was established on before research and hypothetical underpinnings, they had the option to ensure exact model definition. Corroborative component analysis was likewise completed to assess the model's integrity of-fit.

Table 1: The constructs' validity and dependability.

Constructs	α	CR	AVE	MSY	ASV	HIS	EEC	EPM	APP	RTF	DHR	ONA	OD
Wellbeing and Security Improvement	0.951	0.926	0.650	0.631	0.502	0.809							
Improving employee comfort	0.936	0.932	0.586	0.52	0.498	0.701	0.764						
Employee efficiency estimation	0.930	0.931	0.580	0.50	0.460	0.733	0.679	0.765					
Automating payroll processing	0.976	0.964	0.780	0.535	0.330	0.609	0.569	0.530	0.880				
Instantaneous feedback	0.903	0.893	0.521	0.511	0.382	0.686	0.594	0.535	0.553	0.725			
Digitization of HR	0.966	0.921	0.501	0.321	0.247	0.488	0.519	0.485	0.420	0.412	0.707		
Network Analysis of Organisations	0.902	0.900	0.568	0.489	0.460	0.684	0.671	0.732	0.580	0.568	0.483	0.752	

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Design of organisations	0.947	0.913	0.728	0.642	0.546	0.788	0.768	0.714	0.616	0.670	0.550	0.735	0.84

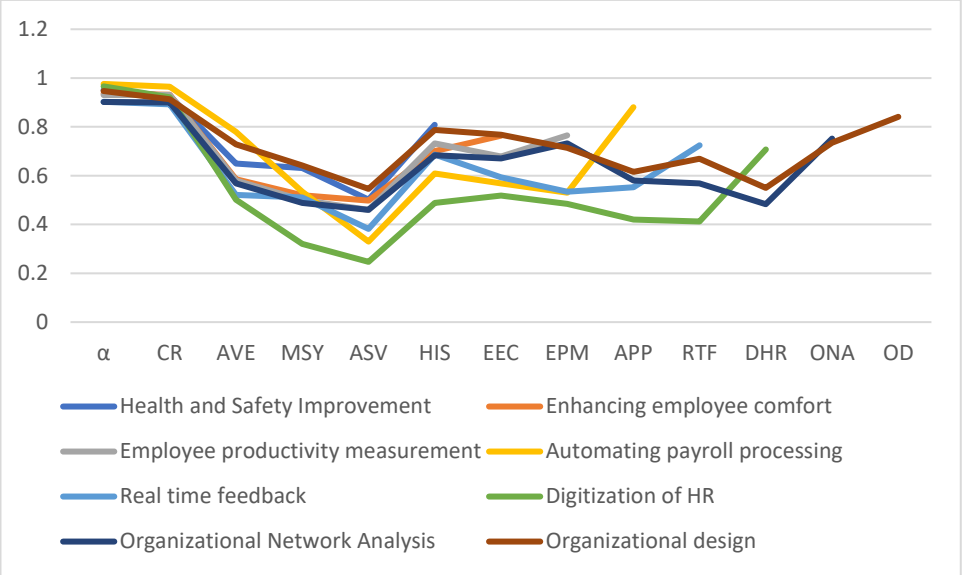

Figure 2: The constructs' validity and dependability.

The criteria for determining reliability, discriminant validity, and convergent validity are shown in Table 1. Table 1, represented by $\boldsymbol{\alpha}$, is also known as Cronbach Alpha and is used to gauge how reliable the study's construct is. Any value of Cronbach alpha above 0.75 is expected. Table 1 presents constructs with Cronbach alpha values greater than 0.7, indicating the dependability of the study's variables. Another dependability metric included in Table 1 is called Composite dependability, or CR. The primary application of this composite dependability is in path modelling, which incorporates error into the computation. The Cronbach Alpha value of more than 0.8 and the CR cutoff level are the same. The most important factor in determining validity is Average Variance Extracted (AVE).

When compared to the other study constructs, the variation derived by the items under the components is explained by the value of AVE. The AVE is predicted to have a minimum value of 0.6. All of the study's constructs meet these convergent validity requirements. For each dormant variable in the examination, the Average Shared Value (ASV) and Maximum Shared Value (MSV) should be not exactly the AVE to assess the discriminant legitimacy. Table 1 shows that the AVE value for every one of the builds remembered for the review is more

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prominent than the MSV and ASV values. This tracking down upholds the presence of discriminant legitimacy for the builds remembered for the review.

Considering that the review's Kaiser-Meyer-Olkin (KMO) insights value is 0.871 — over the base recommended value of 0.7 — factor analysis is considered suitable for the information and the example size is considered adequate. Bartlett's trial of sphericity is utilized to decide if the relationship network is adequate. The test is exceptionally critical at a degree of $P < 0.003$, with a test value of 875.01 and a level of importance under 0.0002, demonstrating that the relationship framework shows significant connections among at any rate a portion of the factors, as indicated by the test discoveries. The factors are therefore not symmetrical, and the speculation that the connection lattice is a personality grid is dismissed. Considering that the critical value is under 0.06, apparently factor analysis of the dataset would be helpful.

5.2.Results

Table 2: Demographic profile of respondents.

Demographic Variable	Category	No of Respondents	Percentage of the Respondents
Gender	Male	180	66.67%
	Female	90	33.33%
Age	21-30	77	28.5
	31-40	116	42.9%
	41-50	39	14.4%
	>50	38	14.2%
Education	Master's degree	95	35.2%
	Bachelor's degree	175	64.8%
Industry Type	Manufacturing	79	29.26%
	IT and ITES	120	44.44%
	Service Sector	71	26.3%

The survey's demographic data, which includes the respondents' age, gender, level of education, and industry, is included in the table. When it comes to gender, 33.33% of

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respondents are female and 66.67% of respondents are male. In terms of age, the age group that comprises the largest proportion, 31–40, accounts for 42.9% of the total, while the 21–30 age group accounts for 28.5%. Of the respondents, 14.4% are over 50 and 14.2% are between the ages of 41 and 50.

With regards to instruction, 64.8% of respondents have a four year college education, and 35.2% have a graduate degree. The survey participants are dispersed throughout many industry categories, with the largest proportion found in the IT and ITES sector at 44.44%. This is followed by manufacturing at 29.26% and the service sector at 26.3%. The majority of respondents in the study are men, and their ages and educational backgrounds are varied. The study's considerable representation of the IT and ITES industry is indicated by the majority of respondents.

Utilizing Analysis of Moment Structures (AMOS 20) software, the proposed applied model was tried utilizing the Structural Equation Demonstrating (SEM) method. It examines the route relationship that exists between the HR Agility dimensions and the AI application dimensions, or independent and dependent variables, respectively.

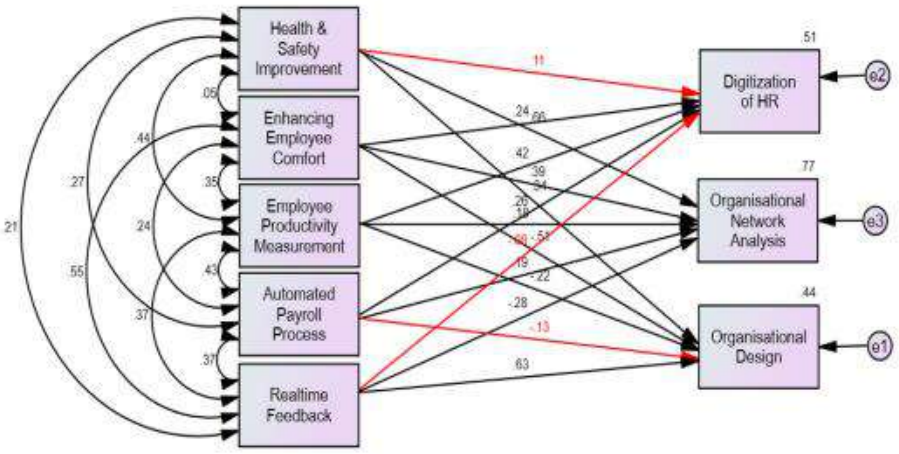

Figure 3: Conceptual Model Hypothesis.

Three causal connections — simulated intelligence Application and Hierarchical Organization Analysis, man-made intelligence Application and Authoritative Plan, and artificial intelligence Application and Digitization of HR — are displayed in Fig. 3. Red-shaded lines are not statistically huge.

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Table 3: Results of the conceptual model.

Hypothesis	Path	Standardized Co-efficient	P-value	R2
H_{1a}	Employee efficiency estimation - Digitization of HR	.420	***	0.506
H_{2a}	Wellbeing and Security Improvement - Digitization of HR	.111	.088	
H_{3a}	Automated Payroll Process - Digitization of HR	259		
H_{4a}	Improving employee comfort - Digitization of HR	240	***	
H_{5a}	Instantaneous feedback - Digitization of HR	-.082	.245	
H_{4b}	Real-time Feedback - Network Analysis of Organisations	-.276	...	0.774
H_{5b}	Improving employee comfort 1 Network Analysis of Organisations	.388	***	
H_{6b}	Wellbeing and Security Improvement - Network Analysis of Organisations	.672	***	
H_{1b}	Employee efficiency estimation - Network Analysis of Organisations	.178	***	
H_{2b}	Automated Payroll Process - Network Analysis of Organisations	.196	***	
H_{6c}	Improving employee comfort 1 Design of organisations	-.512	***	0.442
H_{5c}	Wellbeing and Security Improvement - Design of organisations	.341	***	
H_{4c}	Employee efficiency estimation - Design of organisations	-.224	.006	
H_{7}	Automated Payroll Process - Design of organisations	-.131	.066	
H_{8}	Real-time Feedback - Design of organisations	.628	***	

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The study examined the correlations between different hypotheses (H1a to H8) and their related paths, standardised coefficients, P-values, and R2 values in relation to organisational design, organisational network analysis, and HR digitalization. The results are shown in the table. With a standardised coefficient of 0.420, a high level of significance indicated by three asterisks (***), and an R2 value of 0.506, Speculation H1a shows a huge positive connection between Employee productivity assessment and the Digitization of HR. This means that this model explains 50.6% of the variability in the dependent variable.

The statistical significance of the correlations between Hypotheses H2a, H3a, and H5a is not demonstrated by their P-values, which above the standard cutoff of 0.05. H4a, on the other hand, shows a strong positive correlation with a high degree of significance (***) and a standardised coefficient of 0.240 regarding improving employee comfort through the digitization of HR. Next, let's look at the organisational network analysis. The results for H4b, H5b, H6b, H1b, and H2b, all indicated with three asterisks (***), indicate significant relationships with standardised coefficients of -0.276, 0.388, 0.672, 0.178, and 0.196, respectively.

H6c (Improving employee comfort through Organisational Design) has a particularly strong negative relationship, as indicated by a standardised coefficient of -0.512, marked with three asterisks (***), and an R2 value of 0.442. H5c, H4c, H7, and H8 all show significant relationships in the context of organisational design. Overall, these results indicate that different hypotheses have varying effects from organisational network analysis, organisational design, and HR digitalization, with some elements statistically significantly influencing the outcomes and others not reaching statistical significance.

6. RECOMMENDATIONS AND CONCLUSION

AI implementation in HRM offers the HR division and staff a number of advantages. These benefits come with some legal and network safety risks, though. More employee data means greater security risks, and as devices proliferate, so do the opportunities for network security breaches. Organisations must make sure that employee data is secure before implementing AI across the board. In order

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to reduce network security risks, associations must also develop information-driven security to filter information itself rather than just organisation. The implementation of AI ideas in several potential HRM domains has been covered in the paper. It's possible that these places are not included in the usual schedule of events. However, it is attempting to enforce the significance of addressing the same—the dimensions assessed under which the improvement of human qualities through AI is demonstrated. The outcome demonstrated how these factors affect HRM's capacity for agility. The closed-loop technology impact of HR, known as the digitization of HR and ONA, allows for an iterative process of function. To facilitate their implementation and advancement, the aforementioned elements require a first-rate organisational structure. By establishing a connection between two aspects of the current industrial era, this study therefore cleared a new path. Since simulated intelligence is as yet a somewhat new and underutilized point, particularly in India, very few organizations are using it in HRM or creating artificial intelligence-based HR software. Since most firms just utilize computer-based intelligence partially in the HR cycle, gathering a full analysis is troublesome. Although artificial intelligence (AI) has been the topic of much research, there aren't enough companies using AI in HR practices to allow for a thorough analysis of the technology's actual effects and implications.

Recommendations:

- Expand the Scope of the Study Given the dynamic nature of artificial intelligence (AI) and its impact on human resource management (HRM), it is recommended to consider extending the scope of the study to include a more diverse set of industries and geographic locations.
- Incorporate Longitudinal Analysis To gain a deeper insight into the evolving relationship between AI and HRM, it is advisable to incorporate longitudinal analysis in future research.
- Explore Employee Perspectives While the current research focuses on HR professionals, it is essential to consider the perspectives of

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employees who interact with AI technologies in the workplace.

- Assess the Role of Organizational Culture To enhance the understanding of AI's impact on HRM, it is recommended to delve into the role of organizational culture in facilitating or hindering the successful integration of AI technologies.
- Explore Sustainable Practices Given the growing importance of sustainability in various industries, it is suggested to investigate the potential contributions of AI to sustainable HRM practices.

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Exploring the Implementation of Machine Learning in the Financial Markets Sector: An Investigation for Students

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ISBN - 978-93-95060-79-0

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Abstract

With regards to exchanging on the securities exchange, machine learning is turning out to be progressively present. To determine whether or not the machine learning method is equipped for recovering data from past costs and anticipating value development and future patterns, the reason for this article is to research the chance. In a model that depends on machine learning, we explore the act of utilizing pattern exchanging pointers. To give dependable positive pointers to the developments of stock costs, we present calculations that consolidate various crucial and specialized information. Huge information from the securities exchange that is of high volume, high speed, high assortment, and high changeability is utilized in this paper to apply machine learning methods, for example, strategic relapse, choice trees, neural networks, and man-made brainpower. These strategies are applied to large information utilizing constant and disconnected information of changing time granularities. An investigation was performed on the results of the prescient calculations, and the discoveries were given. The aftereffects of the trials showed that the utilization of machine learning and man-made consciousness strategies can help out in the determination of high-performing stock portfolios that exhibit better execution than the financial exchange.

Keywords: *Algorithms, Decision Trees, Financial Markets, Logistic Regression, Machine Learning, Neural Networks, Stock Market Trading.*

1. INTRODUCTION

The Indian stock market offers a vibrant and fascinating environment for learning about finance, especially the Maharashtra stock market. Even though short-term swings can seem random, experienced traders can spot underlying patterns that can be used to make profitable investments. Making use of extensive elements, such as fundamental and

technical data, machine learning algorithms provide a potent instrument for lucrative stock movement prediction. This strategy clearly performs better than conventional approaches that only use numerical features. A significant obstacle in the Indian market, especially in Maharashtra, is locating high-potential equities while reducing human error and emotional prejudice.

Researchers in Maharashtra were able to identify high-yielding healthcare equities by utilising a combination of technical and fundamental analysis within a Support Vector Machine (SVM) framework. Then, profit-making transactions were enabled by automated trading algorithms that operated without human intervention, resulting in an incredible 16.64% return over a three-month period. Subsequent studies looked into the possibility of a portfolio of healthcare companies chosen by logistic regression beating market benchmarks such as the Healthcare Sector Index (XHJ) and the ASX All-Ordinaries Index over a 20-day trading window. With a hefty 18.24% return, the portfolio produced encouraging results.

Researchers used machine learning and a unique trading strategy to mix technical and fundamental data while concentrating on the Small Cap sector of the Indian stock market. Their goal was to find out if a data-driven strategy could reliably pinpoint equities that could outperform the market. The researchers carried out a number of paper deals to confirm and validate their selection process. In April of 2021, the three best training equities that had been determined in March were paper traded, and the procedure was then repeated in the following months. This thorough testing proved the selection process's effectiveness and showed that it can reliably find successful stock portfolios.

Researchers in Maharashtra have taken in an extraordinary arrangement about determining future examples and cost developments in the Indian securities exchange by utilizing calculations like strategic relapse, choice trees, and neural networks. These developments present investors looking to use data-driven tactics to generate higher profits with exciting chances.

One powerful subset of artificial intelligence (AI) is machine learning. At its core, it is the process of teaching computers to recognize, analyses, and draw conclusions from complex patterns found

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in data sets. Rather than utilizing unequivocal programming, the framework utilizes its capacity to gain from and change its insight from past information to accomplish this understanding and translation. Therefore, machine learning represents a substantial departure from conventional programming paradigms that involve giving machines explicit instructions. Rather, robots can now independently generate and apply logic in dynamic circumstances thanks to machine learning.

This change has had a significant influence, and machine learning has been used in many different fields. For example, machine learning is used in natural language processing to train computers to comprehend, analyse, and even produce content that appears human. Many of the chatbots and digital assistants of today are powered by this technology, which makes interactions between humans and robots more intuitive and natural. Similar to this, machine learning techniques are employed in image recognition to find and decipher patterns in images. This has a wide range of uses, from using medical imaging to diagnose diseases to enabling autonomous cars to drive by identifying barriers and road signs.

Machine learning is utilized in network protection and misrepresentation to perceive designs, as odd examples might show gambles or illegal action. Last but not least, predictive analysis makes use of machine learning's predictive powers to help firms predict market dynamics, consumer behaviour, and trends. It is true that machine learning has a great deal of versatility and that there is always room for other uses for it. Finding a summary of the research and publications that have been done on machine learning in the financial domain is interesting. One important subject that is directly related to the economy is finance. For instance, financial technology have gained popularity among organisations within the past ten years.

Machine Learning problematically affects the universe of monetary information and innovation. AI calculations have introduced another period of information driven independent direction by significantly affecting a few areas of money, including risk relief and portfolio the executives. Foreseeing market developments is a basic utilization of AI in the money business. It has demonstrated challenging for conventional market investigation models to represent the unpredictability and intricacy of monetary

ISBN - 978-93-95060-79-0

business sectors. Be that as it may, with AI, complicated designs in the information can be found and used to make undeniably more exact expectations about how the market will move from now on.

Another area where AI has demonstrated helpful is in the assessment of venture possibilities. AI calculations can assess gigantic volumes of monetary information to survey the conceivable return and chance related with different speculation prospects through information mining and prescient demonstrating. Monetary consultants, mutual funds chiefs, and individual financial backers — every one of whom need exact and quick data to settle on shrewd venture choices — have viewed these capacities as very accommodating.

Machine learning has also revolutionised the field of fraud detection. Historically, identifying fraudulent transactions has been a laborious and prone to error process. But abnormalities may be found more quickly and precisely thanks to machine learning's pattern recognition abilities, shielding consumers and businesses from monetary loss. Furthermore, the emergence of FinTech highlights the widespread impact of machine learning in the finance industry. Our understanding of money and transactions has changed as a result of cryptocurrencies and decentralised finance made possible by blockchain technology, which is supported by machine learning. Likewise, machine learning-driven automated trading platforms and smart contracts are redefining stock trading and commercial transactions.

Even with these noteworthy developments, machine learning's potential in the banking industry is far from being fully realized. There are still a ton of uncharted research and application possibilities, which will surely result in further ground-breaking discoveries. This insight highlights the importance and timeliness of our research, which aims to set the direction for next studies and machine learning applications in the finance industry.

2. OBJECTIVES

- To evaluate the state of machine learning applications in the financial markets sector at the moment in order to spot emerging trends.
- To look into how machine learning affects the industry's financial decision-making processes, with a focus on the

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precision and effectiveness of predictive models.

- To examine the difficulties and constraints involved in applying machine learning to the financial markets, considering things like model interpretability, data privacy, and regulatory compliance.
- To compare the merits and drawbacks of different machine learning algorithms and assess how well they perform in forecasting risk variables, asset values, and market movements.

3. LITERATURE REVIEW

Wong et al., (2021), The use of deep learning techniques in financial technology (FinTech) is the special subject of this study. The use of deep learning in FinTech is reviewed by the writers in a number of domains, including as credit scoring, client segmentation, insurance pricing, and regulatory compliance. They highlight the notable advantages deep learning provides over more conventional approaches and suggest fruitful avenues for future research.

Sharma & Sharma, (2020), An extensive overview of the many machine learning applications in the financial industry is given by this thorough survey. The writers talk about how machine learning can be used for portfolio management, algorithmic trading, credit risk assessment, and fraud detection. They provide insightful information for future research areas and demonstrate how machine learning has the ability to completely transform the financial services industry.

Nawaz et al., (2023), This study looks into how well hybrid machine learning models can forecast changes in the stock market. The writers examine several pairings between deep learning architectures like LSTMs and conventional machine learning algorithms like Support Vector Machines (SVMs) and Random Forests. According to their findings, hybrid models outperform solo models in terms of prediction accuracy.

Mittal & Deep, (2020), This study assesses and differentiates a few profound learning models at foreseeing stock costs. The creators evaluate a mixture LSTM-GRU model, Gated Recurrent Units (GRUs), Long Short-Term Memory (LSTM) networks, and Recurrent Neural

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Networks (RNNs). As per their outcomes, LSTMs and GRUs perform better compared to other models in terms of forecast precision.

Livieris et al. (2022), An extensive review of data mining and machine learning methods applied in financial markets is given by this survey. The techniques, which include risk management, portfolio optimisation, and market prediction, are categorised by the writers according to their intended uses. For scholars and practitioners looking to comprehend the state of machine learning in finance, this paper is an invaluable resource. It might, however, benefit from a more thorough examination of current developments and developing patterns.

Lu et al. (2022), In order to predict financial markets, this paper presents a hybrid deep learning model with an attention mechanism. By fusing a Transformer design with a convolutional neural network (CNN), the suggested model is able to identify both short- and long-term dependencies in financial data. By outperforming baseline models, the model demonstrates the potential of attention mechanisms in financial applications. Examining the interpretability of the attention process and how it affects model predictions, however, could improve the research.

Mangal and Kaur (2021), A correlation of many profound learning models for stock value expectation is introduced in this work. Utilizing verifiable stock information, the creators survey how well LSTM, Recurrent Neural Networks (RNNs), and Convolutional Neural Networks (CNNs) perform. According to their results, LSTMs typically obtain the highest accuracy, demonstrating how well they can capture temporal connections in financial data. Comparing various hyperparameter setups and examining the effects of data preparation methods, however, could enhance the research.

4. RESEARCH METHODOLOGY

4.1.Data Capture

For 2,000 values recorded on the Indian stock trades, with an accentuation on Maharashtra, everyday stock information, including Open, High, Low, Close, and Volume, was procured

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from [www.eoddata.com] for the time of January 2, 2021, to June 30, 2021. The daily statistics for the Nifty 50 Index and the Nifty Small cap 50 Index were obtained from finance.yahoo.com. Because small-cap companies have the potential to see substantial price growth—especially in a month—we decided to concentrate on them for short-term trading. Even while trading small-cap stocks naturally involves a higher level of risk, we try to reduce it by only choosing stable businesses.

The procedure for gathering data and analysing it further is provided in the flow chart below:

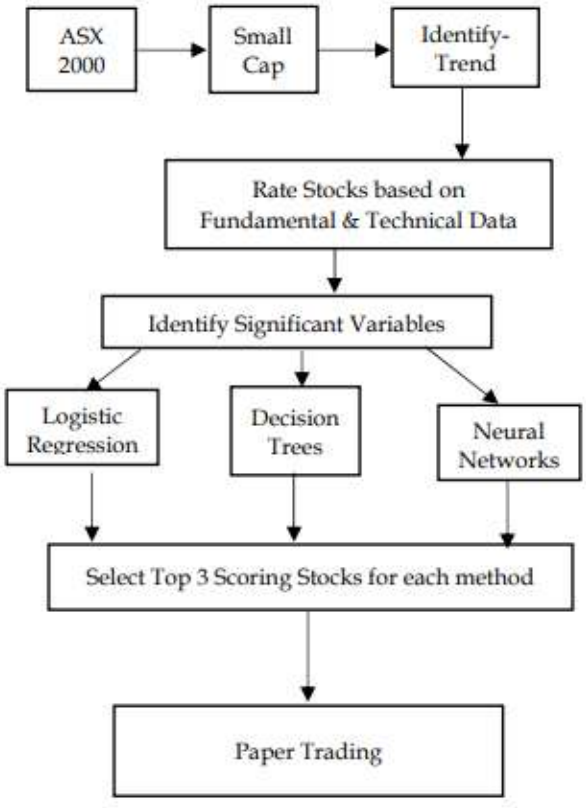

Figure 1: Steps.

4.2.Data Modelling

Since the objective variable, "Pattern," is a classification variable, three techniques were utilized in the information displaying method. A few instances of these are strategic relapse, brain organizations, and choice trees. There were 24 particular changes of the five factors

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(open, high, low, close, and volume) that were input into the model. The "20 Moving Normal," "200 Moving Normal," "50 Moving Normal," and comparative factors were made to demonstrate.

4.3.Logistic Regression

A remarkable sort of relapse known as strategic relapse includes relating a bunch of informative indicator factors — which might be discrete or constant — to the paired result variable. At the point when there isn't generally a straight-line connection between the objective and info factors, a nonlinear or calculated relapse model is utilized. At the point when the autonomous factors are ceaseless or discrete and the reliant variable is dichotomous, one technique for making expectations is called strategic relapse.

In strategic relapse, the blend of values taken by the indicator factors is utilized to address the likelihood of choosing that reaction. The coefficients (together with their standard deviations and importance levels) of an equation to conjecture a logit change of the probability of an intriguing trademark happening are created by coordinated factors relapse:

$$\text{logit}(p)=b_0 + b_1X_1+b_2X_2+b_3X_3+b_4X_4+\ldots..+b_kX_k$$

$$\text{logit}(p)=\ln(p/1-p)$$

In strategic relapse, assessment chooses boundaries that augment the likelihood of noticing the example values, rather than boundaries that limit the amount of squared mistakes, as in traditional relapse. The advantage of utilizing Calculated Relapse is that factors don't must have typical conveyances; all things being equal, they can be constant, discrete, or any mix of the two by the expansion of a reasonable connection capability to the standard straight relapse model. Multivariate insightful models like strategic relapse are ideal while foreseeing the presence or nonappearance of a trademark or result in light of upsides of a bunch of indicator factors. Strategic relapse is utilized to break down stock execution in the Indian financial exchange. We are curious as to whether applying strategic relapse to anticipate stocks on the Australian Securities exchange will likewise prompt the choice of performing stocks

4.4.Decision Trees

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A fundamental class of calculations for prescient displaying is choice trees. Choice trees are truly adept at depicting the circumstances and logical results connections in information. Their trial cross breed choice tree and brain network model gave reliable rules to assist financial backers with choosing when to execute their venture, and it had 77% guaging precision in the electronic business. Among the best AI procedures are choice trees. Since the gatherings that are created can be further partitioned utilizing a similar technique, the calculation is recursive in nature.

In view of this technique, the calculation is likewise alluded to as a voracious calculation due to the mind-boggling want to decrease the expense. The Gini score is the expense capability utilized in characterization to find the most homogenous branches or branches having bunches with equivalent reactions since this exploration centers around paired arrangement. In light of how uniformly conveyed the reaction classes are among the gatherings that the split makes, a split's quality can be assessed utilizing a Gini score. G is equivalent to sum(pk (1-pk)). The level of same class inputs in a given gathering is signified by pk for this situation. A hub with a 50-50 split of classes in a gathering has the least virtue; for a twofold order, it has pk=0.5 and G=0.5. A consul class virtue is the point at which a gathering has all contributions from a similar class, wherein case pk is either 1 or 0 and G=0.

5. DATA ANALYSIS AND INTERPRETATION

Recall and area under the curve are two significant measures for evaluating the exhibition of AI models. The suggested least qualities for "recall" and "area under the curve" are 0.8 and 0.9, separately. Because of the fantastic aftereffects of the "recall" and "area under the curve" measurements, paper exchanging was allowed.

Table 1: April 2021 Paper Trading Results

Algorithms	Recall	Area under curve	Return on investment (%)
Logistic regression	0.98	0.925	0.40
Decision trees	0.84	0.934	0.93
Neural networks	0.94	0.970	0.38

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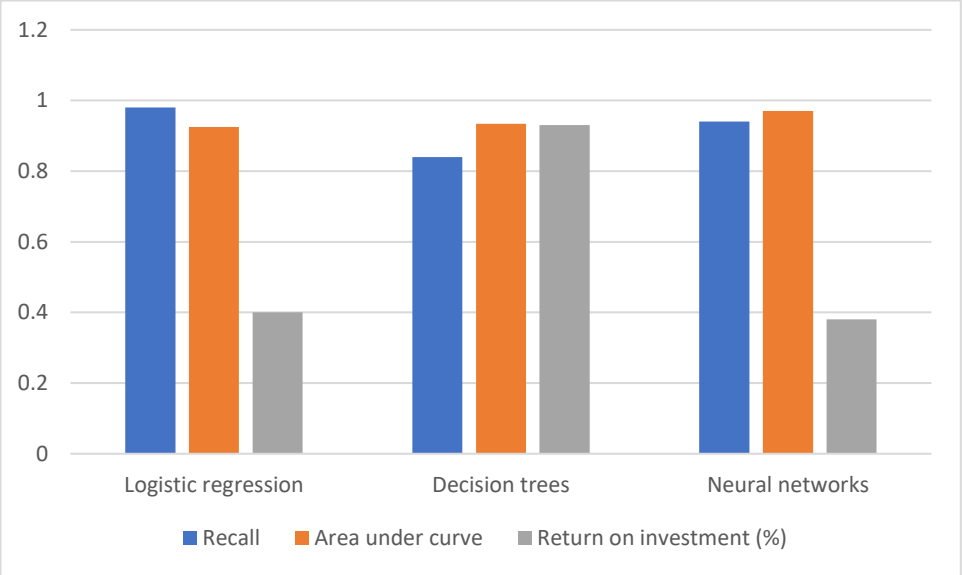

Figure 2: April 2021 Paper Trading Results

The paper trading outcomes for the different algorithms in the month of April 2021 are shown in Table 1. Three performance metrics—Recall, Area Under Curve (AUC), and Return on Investment (ROI) percentage—were used to compare three algorithms: Neural Networks, Decision Trees, and Logistic Regression. With a recall of 0.98, Logistic Regression showed a high degree of performance in accurately identifying positive events. Calculated Relapse's Area under the Curve (AUC) was 0.925, showing a decent generally speaking exhibition in example characterization. Nevertheless, this algorithm's Return on Investment (ROI) percentage of 0.40 was rather low.

However, Decision Trees showed a lower recall of 0.84, suggesting that recall and precision may need to be traded off. In spite of this, its AUC of 0.934 shows that it can distinguish between classes quite well, and its ROI % of 0.93 is noticeably higher. Neural Networks showed a balanced performance in recognising good events, with a recall of 0.94. With an AUC of 0.970, it appears to have better class discrimination capabilities, and its ROI % of 0.38 is similar to that of logistic regression. In conclusion, Neural Networks performed better in AUC and Decision Trees performed better in ROI compared to the other methods. While the ROI was somewhat low, the recall was excellent for Logistic Regression. Specific trading goals, such as emphasising accuracy, overall classification performance, or return on investment, may influence the choice of algorithm.

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Table 2: May 2021 Paper Trading Results

Algorithms	Recall	Area under curve	Return on investment (%)
Logistic regression	0.96	0.934	8.12
Decision trees	0.70	0.968	8.12
Neural networks	1	0.983	8.12

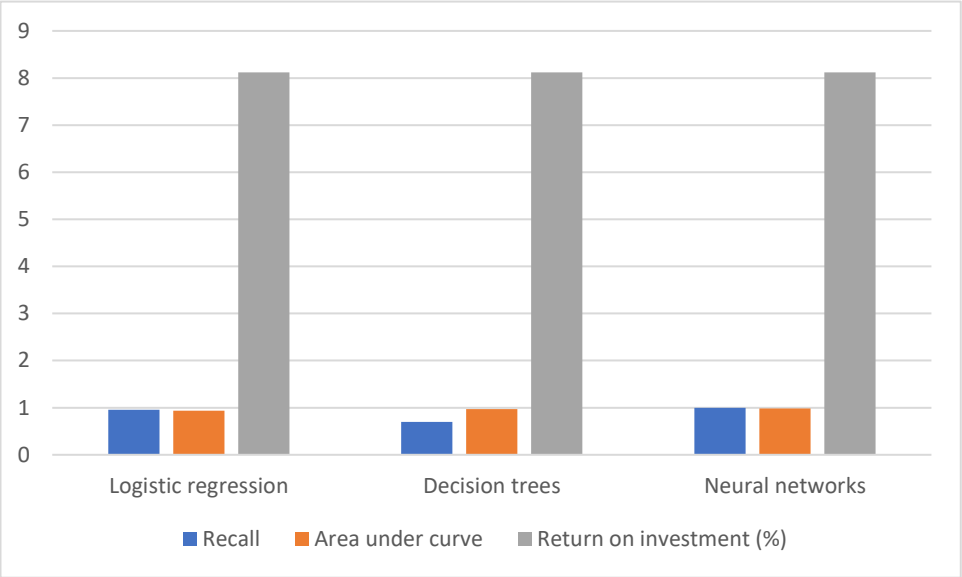

Figure 3: May 2021 Paper Trading Results

Table 2 shows the paper trading outcomes for a range of algorithms in May 2021. The algorithms' performance is assessed using three primary metrics: ROI %, area under the curve (AUC), and recall.

At a recall rate of 0.96, the first algorithm—logistic regression—showed that it was capable of accurately detecting positive cases. With an AUC of 0.934, logistic regression appears to have a very good overall predictive performance. This algorithm's Return on Investment is 8.12%, demonstrating its profitability over the given time frame.

When compared to logistic regression, the second approach, decision trees, had a poorer recall of 0.70. In spite of this, decision trees have an exceptionally high AUC of 0.968, indicating strong discriminating power. Decision trees have a Return on Investment of 8.12%, which is comparable to that of logistic regression and indicates profitability.

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Neural networks, the third approach, had an amazing recall rate of 1, indicating that it was able to identify every good instance. Neural networks have an outstanding overall predictive accuracy, as evidenced by their 0.983 AUC. Neural networks have a Return on Investment of 8.12%, which is consistent with the profitability of the other algorithms.

All three algorithms—logistic regression, decision trees, and neural networks—performed well in May 2021, according to the paper trading data, each with a specialty in a certain area of predictive modelling. Even though the algorithms' recall rates and AUC values varied, the consistent Return on Investment shows that they were all successful in making money throughout the given time.

Table 3: June 2021 Paper Trading Results

Algorithms	Recall	Area under curve	Return on investment (%)
Logistic regression	0.96	0.934	3.55
Decision trees	0.70	0.968	2.61
Neural networks	0.91	0.983	5.73

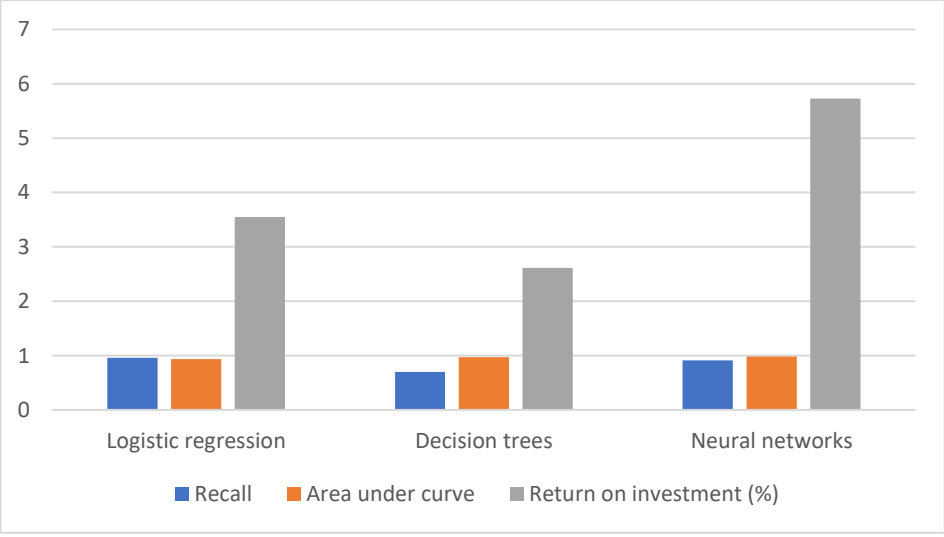

Figure 4: June 2021 Paper Trading Results

With an accentuation on execution pointers like review, area under the curve (AUC), and profit from venture (return for capital invested), Table 3 shows the paper exchanging results for different calculations June 2021. The logistic regression algorithm showed a significant AUC of 0.934 and a high recall rate of 0.96,

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both of which indicate the system's ability to correctly identify positive cases. With a similar ROI of 3.55% for logistic regression, the investment returned appears to have been worthwhile. On the other hand, decision trees demonstrated a higher AUC of 0.968 but a lower Recall of 0.70, suggesting a comparatively lesser accuracy in catching positive instances. Decision trees showed a somewhat lower return on investment, with a ROI of 2.61%. Neural networks performed exceptionally well in AUC, with a value of 0.983, and showed a Recall of 0.91, balancing the use of decision trees and logistic regression. Neural networks outperformed the other two algorithms in terms of yielding returns on capital invested, as seen by their higher ROI of 5.73%. Overall, the findings imply that within the given time period, neural networks performed better in terms of Recall, AUC, and ROI than both logistic regression and decision trees.

6. RECOMMENDATIONS AND CONCLUSION

This study examines if it is feasible to choose high-performing small-cap stocks in the Maharashtra, India market by using a data-driven strategy. To evaluate their efficacy in identifying profitable stock portfolios, machine learning methods such as Neural Networks, Decision Trees, and Logistic Regression were applied. The outcomes demonstrated how well these models worked at finding top-performing portfolios that regularly outperformed the market. Every stock portfolio that was created saw positive returns over a three-month period (March 2021 - May 2021). The Decision Tree portfolio beat the market during the first period (March 2021), while the Neural Network and Logistic Regression portfolios performed similarly. In particular, the portfolios for May 2021 and June 2021 outflanked the market by at least 2.6 times. This study adds to the rising measure of examination showing how well information driven methodologies — specifically, AI — foresee securities exchange results. The results point to the possibility of accurately identifying stocks that show promise and have strong growth potential. More trials with varying market capitalizations and sectors are strongly advised to improve the comprehension and practicality of this technique.

Recommendations:

- **Diversify Machine Learning Algorithm Evaluation:** To

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enhance the robustness of the study, it is recommended to expand the evaluation of machine learning algorithms beyond the ones currently employed.

- **Explore Ethical Implications and Regulatory Compliance:** Given the constraints and difficulties in applying machine learning to financial markets, particularly in areas like model interpretability, data privacy, and regulatory compliance, it is essential to delve deeper into the ethical implications of these challenges.
- **Extend Temporal Scope and Geographic Focus:** To capture a more nuanced view of machine learning applications in financial markets, it is advisable to extend the temporal scope of the study.
- **Incorporate Additional Financial Instruments:** To provide a more comprehensive analysis of the financial markets, the study could consider incorporating additional financial instruments beyond stocks.
- **Enhance Model Interpretability and Explainability:** Addressing the challenge of model interpretability is crucial in gaining the trust of stakeholders and regulators. It is recommended to explore and implement techniques that enhance the interpretability and explainability of machine learning models.

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A STUDY ON THE EFFECT OF CUSTOMER RELATIONSHIP MANAGEMENT SYSTEM ON CUSTOMER SATISFACTION AND LOYALTY IN THE SERVICE INDUSTRY

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ISBN - 978-93-95060-79-0

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Abstract

This study explores consumer attitudes and beliefs towards marketing, consumerism, government regulations, and consumer responsibilities, with a focus on how these attitudes vary across different demographic factors, particularly income levels. The research employs a structured survey methodology to gather attitudinal data from a diverse sample of participants representing various marital, occupational, educational, age, and gender categories. Demographic analyses reveal a comprehensive snapshot of the surveyed population, laying the foundation for a nuanced understanding of consumer attitudes. The study delves into the mean scores and standard deviations associated with different attitudinal variables, uncovering patterns in consumer sentiments. Notably, an income-based comparison demonstrates significant variations in attitudes, with higher income levels correlating with more positive perceptions. The findings underscore the importance of tailoring marketing strategies and regulatory frameworks to diverse demographic segments. Businesses are recommended to implement segmented marketing approaches that consider income disparities, while policymakers should craft flexible regulations that accommodate the varying needs of different income groups. Furthermore, consumer education initiatives are proposed to bridge gaps in attitudes and empower consumers with informed decision-making. Continuous monitoring of consumer attitudes is emphasized, urging businesses and policymakers to stay attuned to evolving demographic trends. This study contributes to a deeper understanding of the intricate interplay between demographic factors and consumer attitudes, providing valuable insights for shaping effective marketing campaigns, policies, and consumer-centric initiatives in a dynamic marketplace.

Keywords: Consumer Attitudes, Marketing Mix, Demographic Analysis, Income Disparities, Consumerism, Government Regulations

1. INTRODUCTION

Because of the accessibility of an enormous choice of items and an extensive variety of brand choices inside every classification of items, the Indian business area has been progressively focused all through the purchaser progression period. Indian consumers have shown to be staggeringly demanding and discerning in their buying choices over the course of

time. Because of expanded rivalry among business focuses and purchaser progress throughout the course of recent many years, sponsors have started to understand the current and forthcoming displaying designs that depend on conventional marketing rehearses.

Businesses presently need to understand the idea of "marketing," whose application is known as "market direction," and change their business techniques appropriately. In any case, picking a displaying thought requires that businesses learn the necessities and needs of their customers ahead of time and foster strategies and frameworks that are equipped for addressing those necessities and needs more effectively and productively than contenders, giving customers more noteworthy fulfillment. For publicists to take part in effective exhibiting, they should understand purchaser points of view in regards to the marketing structure in which purchasers are intrigued as well as data about consumers' fulfillment from collaborations with promoters during the use cycle.

1.1.Consumer Perceptions of Marketing

(Bhakane, 2015) recommended that the four key measurements of exhibiting — the thing headway and detect — can be utilized to analyze customers' perspectives towards showing. Examiners have recently endeavored to decide consumer perspectives towards advertising utilizing the four essential measurements of displaying organization: thing, worth, headway, and spot, as well as the general significance of these elements to consumers in contrast with different segment factors. Because of the impacts of globalization and expanded rivalry in the business area, consumers all around the world are currently practicing their imagination and abilities while making acquisition of labor and products, becoming mindful of their legitimate freedoms, standing in opposition to abuse, and looking for solutions for their grievances. The public authority has likewise set up specific regulations to safeguard consumers' fervor. While safeguarding consumers' freedoms and interests has been the essential objective of consumerism, purchaser obligations that accompany these privileges likewise should be considered.

1.2.Customer Government assistance

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The fulfillment of an individual as a consumer during the usage cycle is known as buyer success. Six distinct kinds of customer satisfaction are referenced. These sorts of satisfaction connect with getting merchandise at a retail store (getting), collecting and characterizing the thing prepared for use (game plan), using customer items and administrations (using), being the proprietor or keeping up with the thing (ownership), being associated with the conveniences accessible in the business community to have the things fixed by the executives providers and handling things by the actual proprietor (support), and being worried about abandoning the thing after use as waste (move). Purchaser life cycle alludes to the usage interaction that incorporates the six phases referenced above: shopping/purchase, preparation/get-together, use/use, proprietorship/ownership, fix/upkeep, and move. Appropriately, what influences consumer flourishing is the fulfillment or disappointment that purchasers experience during the six phases, which incorporate the securing and move of bought labor and products (Sirgy et al., 2008).

1.3.Life Satisfaction

The idea of life satisfaction falls under the classification of individual joy, and consumer behaviorists have observed it also. How much an individual can confirm their general degree of individual delight is a proportion of life satisfaction. It could be said, it alludes to the degree to which an individual distinctions the existence that has been driven by them. Regardless, (Bin-Nashwan, et. al, 2017) contended that encounters of fulfillment or disappointment across a few spaces of life, including as work, family, social, profound, recreation, and so forward, contribute in proposing and representing life's satisfaction. Likewise, life satisfaction is a more extensive idea that incorporates both use and non-use satisfaction.

Just a small number of studies have inspected the connection between factors like work status and enlightening accomplishment and life satisfaction.

Specialists play emphasized the part that consumer perspectives play in supporting and deciding consumer success and the degree of satisfaction that consumers experience in life in general (Peterson and Ekici, 2007). In their examination, (Peterson and Ekici, 2007) proposed that

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there is a reasonable connection between's the purchaser's mentalities towards advancement and life satisfaction, or personal satisfaction. Moreover, they have recognized that a piece of customer demeanor estimates about displaying are related with the obtaining period of purchaser thriving. Nearly, (Lee et al. 2002) contended that buyers' opinions of fulfillment and disappointment during the pattern of purpose affected the existence satisfaction of customers. Also, the purchaser's point of interaction with the notice during the utilization cycle influences different everyday issues, like prosperity, work, family, social, unwinding, significant, and cash, among others, showing that advancement impacts life satisfaction through different everyday issues. To lay it out plainly, it implies that the purchaser's fulfillment with different parts of their lives and the usage cycle both impact their degree of satisfaction with life.

2. RESEARCH OBJECTIVES

The study endeavors to satisfy the accompanying destinations:

- To study customers states of mind towards advertising blend components i.e., item, value, spot and advancement, general showcasing and working theory of business firms.
- To look at the shoppers states of mind towards consumerism, government controls and purchaser obligations

3. LITERATURE REVIEW

This section looks to show a reasonable system and writing survey of different studies attempted previously.

2.1 Consumer Perceptions of Marketing

The investigators' ridiculous energy for customer acknowledgments towards advancement was ignited by impacting exhibiting exercises and expanding consumerism. Experts fostered the "Consumer Purchasing Con Cash Supply" and "Record of Consumer Certainty File" scales over the course of time to quantify purchasers' perceptions of advancement. As per **(Jain and Goel, 2011),** these instruments' essential imperfection was that they weren't obviously connected with appearing.

The essential measure was created by **(Angamuthu, 2015)** and is related with the accompanying seven advancing regions: judiciousness of business, thing quality, publicizing, cost and worth control,

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government bearings, customer commitments, and consumerism. By altering a similar scale made.

3.1. Aftereffects of Past Exploration on Consumer Perspectives and Related Issues

Different examinations have been directed in the past to assess consumer perceptions of advancement and related concerns, like unofficial laws, consumerism, and buyers' own obligations, utilizing the scales referenced in the former area (Long & Khalafinezhad, 2012). The segments that follow give an outline of the significant discoveries from these examinations.

3.1.1. Overall Disposition of Consumers Towards Marketing and Business Firms' Working Way of thinking

Utilizing the actions suggested in the primary piece, a few examinations have been led in the US and different countries to decide client perspectives towards displaying, consumerism, and legislative guidelines. A critical piece of the exploration shows that buyers have ominous suspicions about advancing. A couple of exploration **(Jain and Goel, 2011)** uncover generally incredible evaluations towards advancement. The first is a survey of past examination assessing customers' general perceptions of imprint tasks.

Utilizing a five-thing Likert scale and a cross country mail test review, **(Laksmana, et. al, 2018)** directed examinations in the US to figure out clients' overall mentalities towards advertising. In this manner, **(Emaluta, & Soewarno, 2019)** completed an extra examination that recognized the countries of procurement (Israel, the US, Canada, England, Australia, and Norway). **(Amir, et. al, 2014)** embraced an exhaustive examination of purchaser opinions in the US and England. Clients showed ominous mentalities about creating rehearses in every one of these preliminaries.

As per **(Amir, et. al, 2014),** Indian consumers assess progression more precisely than those in Bangladesh, Hong Kong, Australia, the US, and China.

Looking at the connection between the pessimistic parts of advertising and monetary headway at a critical scale, it has been found that consumers' mentalities towards exhibiting are more dicey in additional determined countries since

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individuals' cravings for higher pay levels make them more confounded by the advertising framework. Anyway, further examination is expected to assess this proposition.

3.1.2. Perspectives on General society on the Marketing Blend

The way that consumers view advertising is one part of the marketing blend. Subsequently, an endeavor has been made in the accompanying subsections to give a rundown of the examination led in the past to decide the perspectives of consumers towards the four parts of displaying — thing, worth, scattering, and commercial.

3.2.Consumers' Perspectives on the Item

Consumerists have long contended that the counter-intuitive split of organization areas and misleading thing partition are the purposes behind customers' disappointment with over the top advertising. It has been contended that further discontinuity of the business area is accomplished by means of thing partition in light of little contrasts. To urge consumers to see contrasts in quality, the expenses of isolated items are set in a manner that addresses more assortment in thing quality than really exists (**Hayati, & Al Muddatstsir, 2019, October).**

As indicated by **(Nikou, et. al, 2016)** Electroni, customers battle with the thing part of the displaying blend with regards to deliberate maturing, thing increase, security, the thing's worth to purchasers, and checking. It has been resolved that thing increase is an upscaling of the item offering, giving barely perceptible novel things at essentially greater costs.

Albeit most of customers accepted that makers really tried to create products that addressed their issues, they have likewise communicated surprising disappointment with issues connected with the nature of the merchandise, for example, the way that there hasn't been any improvement to the nature of the products over the long haul, that the products break down rapidly, and that producers purposefully downplay enhancements.

3.3.Consumers' Perceptions of Conveyance

Earlier examination has shown that consumers have commonly sure assessments on the convenience and

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availability of items. As per research led by **(Salah, et. al, 2019),** consumer shopping was basically demonstrated to be sure. Thus, buyers from various nations give off an impression of being very happy with the conveyance framework.

3.3.1. What Segment Elements Mean for Consumer Perceptions of Marketing

Tracking down proof of the parts responsible for the consumer demeanors towards advancing has been a center area of examination for investigators across all nations and locales of the world. Researchers have really tried to understand what segment factors mean for consumers' perspectives towards advertising, as will be talked about underneath:

3.4.Consumer Perceptions by Orientation

Concentrates on by (Iriqat, et. al, 2017) found that ladies had more adversarial mentalities towards showing than did guys. Regardless, found that the inverse is valid. Contrasted with ladies, men are less responsive to advancements and one of the conceivable clarifications set forth by them is that men are practically more keen on buying showy products; furthermore, (Saputra, 2019, March) recommend that silly buys will probably cause post-buy friction.

(Abekah-Nkrumah, et. al, 2021) likewise noticed that men were more probable than ladies to have rash sentiments that caused them to try not to assume liability. As indicated by **(Ferdous and Towfique's 2008)** research, ladies were more negative about advertising sharpens than men were. They had likewise seen some striking distinctions in sexual orientation regarding advertising and shopping; that is, female respondents' mentalities towards these exercises were more negative than those of their male partners.

3.5.Consumer Perceptions by Age

The more prepared customers displayed better mentalities towards thing, evaluating, and advancement, as indicated by research by **(Kirmaci, 2012).** More youthful respondents discussed the consistency of advanced products and the precision of the photographs in advertisements, while additional accomplished respondents communicated more noteworthy trust in the forceful structure and the advantages of exposure **(Shaon, & Rahman, 2015).**

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Most of youthful respondents are disappointed with the means taken to control customer fights **(Andotra, & Abrol, 2016).** Appropriately, more youthful individuals had more regrettable perspectives on advancement and its numerous components. In any case, **(Kurniati, et. al, 2015)** study's discoveries uncovered no tremendous contrasts between the age bunches regarding generally speaking perceptions of advertising and explicit mentalities for specific parts of advancement.

3.6.Consumer Perceptions In regards to education

Research directed by **(Sulaiman, 2020)** uncovered areas of strength for a between respondents' uplifting outlooks towards advertising and the degree of educational accomplishment they accomplished. In any case, **(Siddiqi, et. al, 2018)** couldn't find any massive contrasts between advertising inclines and various degrees of educational achievement.

4. RESEARCH METHODOLOGY

4.1.Research Design and Instrument

In pursuit of testing the theoretical framework previously established, an experimental study was undertaken. This section elucidates the research design and methodology adopted for the study. Specific focus is given to the research instrument, data sources, and the sampling design.

4.1.1. Research Instrument Description

A survey instrument was meticulously designed to collect attitudinal data regarding consumer perspectives on marketing mix, consumer well-being, life satisfaction, and opinions on business firms' operating philosophies, consumerism, government regulations, and consumer responsibilities. The structured, non-disguised questionnaire was developed based on existing literature and tailored for the Indian context. It encompassed items related to consumer demographics (marital status, income, occupation, education, age, and gender), attitudes toward marketing, significance of marketing mix elements, consumer well-being, life satisfaction, attitudes toward government regulations, consumerism, and consumer responsibilities.

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To capture the attitudes of rural consumers, the survey was translated into Hindi. The translation was back-translated to rectify any disparities between the two versions. The study employed various scales to measure consumer attitudes, including the Gaski 28-item scale and Etzel's Consumer Sentiment toward Marketing (CSM) to gauge marketing attitudes.

4.1.2. Measurement of Consumer Attitudes toward Marketing

Because of the Consumer Sentiment towards Marketing (CSM)'s proven validity and dependability, it was selected. Four elements make up the scale: product, pricing, distribution (represented by retail service), and promotion (particularly advertising). Each component is assessed using a multi-item Likert scale. The weighted scores of the four sub-scales were added together to determine the total consumer sentiment score..

4.1.3. Measurement of Consumer Well-Being

Consumer well-being was measured using a scale proposed by Sirgy et al. (2008). This scale considers six types of consumer experiences: acquisition, preparation, possession, consumption, maintenance, and disposition. Items measuring satisfaction across these dimensions were used to assess consumer well-being.

4.1.4. Measurement of Satisfaction with Life

Life satisfaction was measured using Diener et al.'s SWLS (1985). The SWLS measures respondents' agreement or disagreement with five propositions from -2 to 2...

4.2.Data Sources and Sample Design

4.2.1. Data Sources

This research utilized both primary and secondary data sources. The theoretical framework was developed based on a thorough analysis of secondary sources, including academic journals such as the Journal of International Consumer Marketing, Journal of Marketing Research, and European Journal of Marketing.

4.2.2. Sample Design

A survey was conducted among Indian consumers from Maharashtra State and neighbouring towns near Mumbai, Maharashtra between February and April 2023. Convenience sampling

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was employed, distributing 100 surveys to respondents in homes, offices, and institutions. After repeated efforts, 100 completed surveys were collected and used for analysis.

4.3.Data Analysis Techniques and Scale Reliability

Calculating means and standard deviations was a necessary step in the data analysis process to evaluate customer sentiment. One-way analysis of variance, significant difference tests, and independent samples t-tests were used to compare the means across the various demographic categories. Bivariate correlation was employed to investigate the relationships between the variables.

The collected data were coded and then imported into the SPSS 16.0 software for analysis. The reliability of the multi-item scales was assessed using the Cronbach's alpha coefficient, which yielded results indicating strong reliability, with values ranging from 0.912 to 0.981.

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5. DATA ANALYSIS AND INTREPRETATION

An overview of the survey respondents' demographic details, including age, gender, employment, income, marital status, and level of education, is given in Table 1. Remarkably, most participants are unmarried, and a sizable fraction have a bachelor's degree and are in the middle wage range.

Table 1: Demographic Characteristics of Survey Participants

Demographic Variable	Frequency	Percentage (%)
Marital Status		
- Single	70	70%
- Married	30	30%
Income		
- Low (Rs 30,000)	35	35%
- Moderate (Rs 30,000- Rs 60,000)	45	45%
- High (Rs 60,000-Rs 100,000)	15	15%
- Very High (>Rs100,000)	5	5%
Occupation		

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- Professional	25	25%
- Managerial	40	40%
- Skilled Labor	25	25%
- Unskilled Labor	1	10%
Education		
- High School	24	24%
- College	30	30%
- Bachelor's Degree	40	40%
- Master's Degree	5	5%
- Doctorate	1	1%
Age		
- 18-24	25	25%
- 25-34	40	40%
- 35-44	20	20%
- 45-54	10	10%
- 55 and above	5	5%
Gender		
- Male	70	70%
- Female	30	30%

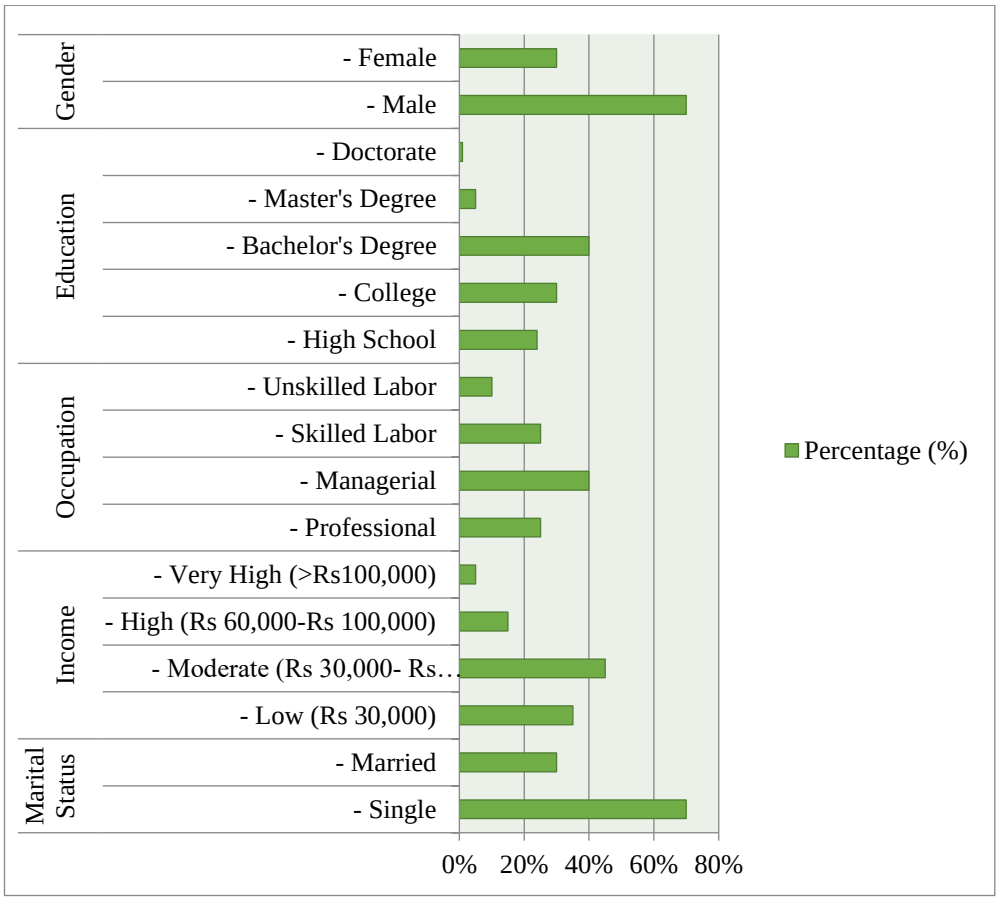

Figure 1: Demographic Characteristics of Survey Participants

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Table 1 provides a comprehensive summary of the segment nuances that the overview respondents remembered, including information on age, orientation, marital status, income, employment, and education.

The majority of participants, or around 70% of the focused population, are single, with the remaining 30% of responses being married. According to the members' pay appropriation, there are four pay categories: low-pay (under Rs 30,000), decent-pay (between Rs 30,000 and Rs 60,000), top-level (between Rs 60,000 and Rs 100,000), and very big-time (>Rs 100,000) for 5% of the members. A broad range of professions are covered by survey participants: 25% are categorised as experts, 40% as chiefs, 25% as talented job, and 10% as untalented labour. The following is a breakdown of members' instructional backgrounds: Of the respondents, 24% have completed secondary education, 30% have attended college, 40% possess a four-year certification, 5% hold a graduate degree, and 1% hold a PhD. The members' ages vary widely: 25% are between the ages of 18 and 24, 40% are between 25 and 34, 20% are between 35 and 44, 10% are between 45 and 54, and 5% are beyond the age of 55. There is a balanced combination of orientations, with 70% of the members being men and 30% being women.

Table 2: Consumer Attitudes and Beliefs

Attitudinal Variable	**Mean Score**	**Standard Deviation**
Consumer Sentiment toward Marketing		
- Product	3.02	0.936
- Price	3.02	0.963
- Place	3.04	1.022
- Promotion	3.06	1.085
- Overall Marketing	3.09	1.066
- Business Operating Philosophy	4.02	1.063
Attitudes toward Consumerism	3.93	0.956
Attitudes toward Government Regulations	4.02	0.945
Attitudes toward Consumer Responsibilities	3.96	1.023

In terms of consumer sentiment toward marketing, participants expressed moderately positive attitudes across different elements. Specifically, the mean scores for product, price, place, and promotion range from 3.02 to 3.06, indicating a generally favorable outlook. The overall marketing sentiment, with a mean score of 3.09, continues to reflect a positive attitude among

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participants. Notably, the business operating philosophy receives a higher mean score of 4.02, suggesting a more favorable perception regarding the overarching approach of businesses.

The attitudes toward consumerism exhibit a mean score of 3.93, indicating a moderately positive stance among participants. This suggests that, on average, respondents view consumerism in a favorable light. Similarly, attitudes toward government regulations receive a mean score of 4.02, indicating a positive perception of the role of government regulations in the consumer landscape. Additionally, attitudes toward consumer responsibilities have a mean score of 3.96, reflecting a generally positive view of the responsibilities consumers bear in the marketplace.

Table 3: Comparison of Consumer Attitudes Based on Income

Comparison	M	S.D	t-statistic	p-value
- Low (Rs 30,000)	3.05	0.945	-0.361	0.015
- Moderate (Rs 30,000- Rs 60,000)	3.05	1.023	0.631	0.02
- High (Rs 60,000-Rs 100,000)	4.12	1.063	0.956	0.03
- Very High (>Rs100,000)	4.02	0.989	0.812	0.01

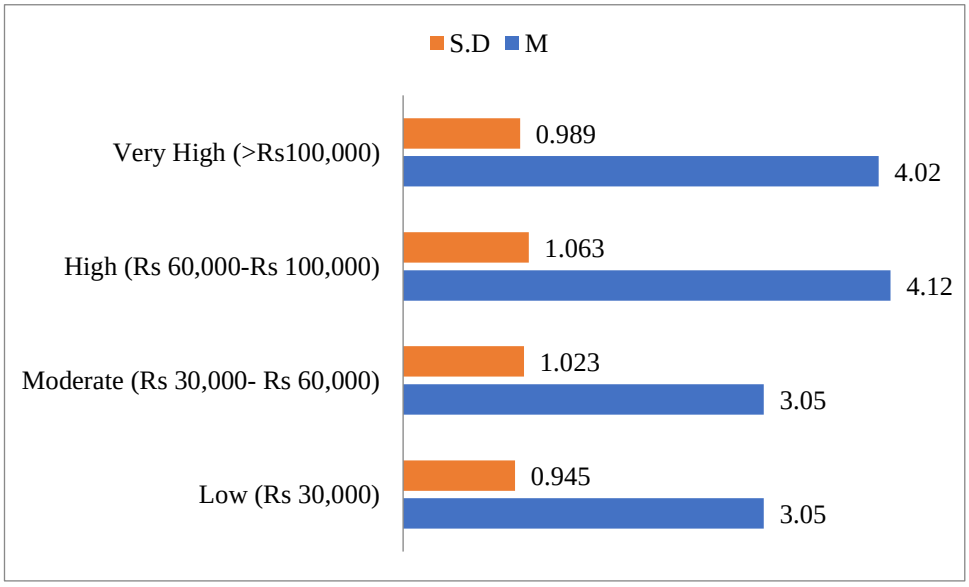

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Figure 2: Comparison of Consumer Attitudes Based on Income

Captivating patterns might be found in the mean scores for each level of pay. The run of the mill score for those with unobtrusive livelihoods (not as much as Rs 30,000) is 3.05, which proposes a somewhat hopeful viewpoint. This suggests that even notwithstanding monetary hardships, individuals in this pay range have a regularly uplifting outlook on the consumer mentalities that have been estimated. In any case, when contrasted with other pay gatherings, the pessimistic t-measurement of - 0.361 and the comparing p-worth of 0.015 show a measurably huge distinction, recommending that individuals in this level of pay have fundamentally various suppositions from individuals in other pay classifications. While contrasting the moderate-pay bunch (Rs 30,000 - Rs 60,000) to the next, the mean score stays at 3.05, demonstrating that consumer sees are still commonly sure. When contrasted with the low-pay bunch, there is a distinction in mean scores, however it isn't genuinely huge, as per the t-measurement of 0.631 and the p-worth of 0.02.

The top level salary class (Rs 60,000 - Rs 100,000) then again, has a lot higher mean score of 4.12, proposing a more hopeful demeanor among consumers. In contrast with the low-pay bunch, this distinction seems, by all accounts, to be genuinely critical, as demonstrated by the t-measurement of 0.956 and the p-worth of 0.03. This recommends that the people who bring in more cash regularly see the elements under appraisal all the more well. Likewise, the incredibly big league salary class (>Rs 100,000) displays a mean score of 4.02, showing a decent customer feeling. When contrasted with the low-pay bunch, this gathering's viewpoints vary significantly, as shown by the t-measurement of 0.812 and the p-worth of 0.01.

6. CONCLUSION AND RECOMMENDATION

This study, which looked at a number of variables like income, marital status, occupation, education, age, and gender, has given important insights into the attitudes and opinions of the examined population towards consumers. Based on the demographic study, a full insight of the consumer landscape was possible due to the broad and representative sample. Nuanced insights into the attitudes and beliefs influencing consumer behaviour were also revealed by analysing consumer attitudes towards marketing, consumerism, laws, and consumer duties. Significant differences in consumer views were found by the income-based research, with

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higher income levels being linked to more favourable feelings. Businesses looking to customise their marketing plans and policies to successfully target various market income categories will find this knowledge invaluable. The study also emphasised how crucial it is to take demographics into account when creating marketing strategies and legal frameworks since these elements have a big impact on customer perceptions.

Recommendations:

Based on the findings of this study, several recommendations can be made:

- Segmented Marketing Strategies: Businesses should consider implementing segmented marketing strategies that take into account the income levels of their target audience. Tailoring marketing messages and approaches to specific income segments can enhance the effectiveness of campaigns.
- Policy Considerations: Policymakers should be cognizant of the diverse consumer landscape and the impact of income on attitudes. Regulatory frameworks should be flexible enough to accommodate the varying needs and perspectives of different income groups.
- Consumer Education Initiatives: Initiatives aimed at consumer education can help bridge the gap in attitudes across different income levels. Educating consumers about their rights, responsibilities, and the benefits of certain products or services can contribute to more informed and positive attitudes.
- Continuous Monitoring: Businesses and policymakers should engage in continuous monitoring of consumer attitudes, taking into account evolving demographic trends. Regular surveys and assessments can provide real-time insights, enabling timely adjustments to marketing strategies and policies.

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ISBN - 978-93-95060-79-0

NAVIGATION THROUGH ASSESSMENT OF THE CHALLENGES AND UNVEILING OPPORTUNITIES FOR SMALL ENTERPRISES IN THE INDIAN MARKET

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ABSTRACT

This research looks into the business and social situations of Micro, Small, and Medium Enterprises (MSME) owners in Maharashtra, India. The study looks into where fixed and working capital come from, how they affect financial problems, and how government policies affect the world of business. The study looks at a lot of different fields, such as professional services, industry, retail, and oil mining and production. In order to gather data, an organized

ISBN - 978-93-95060-79-0

form is sent out through field surveys, and secondary data from reliable sources is also used. The study shows that businesses are different based on their gender and educational background. The production and mining sectors are the most financially viable. Personal savings and bank loans play a big role, which highlights the need for specific measures to deal with problems, especially the lack of access to credit. The results give us useful information that will help policymakers make smart choices about how to support Maharashtra's diverse MSME environment. This will encourage adaptability and new ideas that will lead to long-term economic growth.

Keywords: *Challenges, Opportunities, Small Enterprises, Indian Market, Assessment, Navigation.*

1. INTRODUCTION

Miniature, little, and medium-sized organizations (MSMBs) are the essential motor of worldwide financial development. There is extraordinary worth in the job that SMEs play in aiding the Indian economy develop and thrive. Little and medium-sized undertakings (SMEs) in India currently utilize very nearly 10 million individuals every year, making them the nation's second-biggest business and a significant driver of fair provincial improvement after farming. The way that SMEs are answerable for over 90% of all assembling in India, 45% of modern result, 40% of commodities, and 17% of Gross domestic product shows that they are the foundation of the Indian economy. These organizations assume a urgent part in India's monetary development as helper units to the country's bigger production lines. In any case, the job that MSMEs have played in the development of the Indian economy is remarkable. Little and medium-sized undertakings (SME) in India are progressively compromised by the monetary slump, an absence of interest, subsidizing, and rivalry from worldwide organizations (MNCs). Subsequently, in the ongoing work, we look to pinpoint the issues tormenting the supporting of miniature, little, and medium-sized endeavors (MSMEs).

Miniature, Little, and Medium Undertakings not just assume a pivotal part in giving enormous work valuable open doors at similarly less capital expense than huge scope enterprises, however it likewise helps in the industrialization of provincial and in reverse regions, subsequently, decreasing territorial irregular characteristics, guaranteeing more impartial portion of public pay and riches. Miniature, little, and medium-sized endeavors (MSMEs) act as helper units to enormous scope organizations and fundamentally add to public financial turn of events. The KVI, or Khadi and Town Ventures, are two of India's broadly huge customary financial areas. KVI's capacity to produce occupations with a little effect on the country's Gross domestic product per capita is a significant draw. The KVI business not just fulfills the crucial requirements of handled merchandise of the tremendous provincial area of the nation yet additionally gives supported work to country specialists. There is likewise a sizable coir business in these SSIs.

1.1. Financing issues of MSMEs in India

Most owners of micro, small, and medium-sized enterprises (MSME) lack the money necessary to set up and maintain their businesses, despite the fact that all industries require resources to meet their fixed and variable expenses. They have no choice except to resort to borrowing. The size of the company has a significant impact on its reliance on debt for investment purposes. Micro firms depend on debt as a key source for both early and development stage whereas small and services largely handle in cash and tend to retain insignificant records. Due to the longer working capital cycle and higher capital costs, businesses in manufacturing and the services sector often have a greater requirement for access to cash. However, most businesses do not rely on banks as their major source of financing since the application procedure is too time-consuming and cumbersome. They can't get loans since they don't have enough of a credit history or collateral to secure one. The Industrial Finance Corporation (IFC) reports that 95 percent of all financing comes from the informal sector, which includes people's personal networks (such as friends and family). Trade credit, chit funds, and moneylenders are all examples of expensive institutional channels that

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charge interest rates between 25% and 60% per year. Therefore, there is typically a debt gap that serves as a significant impediment to the establishment and operation of MSMEs. When it comes to micro, small, and medium-sized enterprises (MSMEs), several institutions have been established by federal and state governments as well as financial institutions to aid in the growth of this important economic sector.

1.2. Financial funding to MSMEs

Internal and external financing options exist for micro, small, and medium-sized enterprises. Funding from within the company is referred to as "internal sources:

(i) **Retained Earnings** - A portion of the industry's net gain from a prosperous trading year might be put towards funding subsequent endeavours. Assuming the company is making money, this might be a crucial source of long-term financing.

(ii) The sale of the company's assets (land, buildings, furniture, fixtures, equipment, cars, etc.) can be used to fund new ventures or settle existing obligations. Selling an automobile to pay off debts is an example of a short-term usage of this method, but the sale of more valuable assets (such as real estate) might provide a more permanent source of funding.

1.3.Institutional Support to MSMEs in India

Small and medium-sized enterprises (MSMEs) are a growing and vital part of India's manufacturing industry. Year after year, this industry has demonstrated impressive development in terms of output, the number of jobs created, and the amount of goods exported. The contribution of MSMEs to industrial growth and economic development of the country has been extremely crucial. Accessing technology and being competitive have proven particularly challenging for MSMEs. Institutional aid is needed to address issues including unfamiliarity with new possibilities, difficulty to access them, and a lack of growth capital. Organisation for the Development of Small Enterprises (SIDO): For the purpose of fostering the growth of a wide variety of micro- and small-scale enterprises, a Small Industries Development Organisation has been established. SIDO works under the umbrella of the SSIs and ARI Department. The State Implementation and

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Dissemination Office (SIDO) is responsible for centrally coordinating and monitoring all SSI-related policies and initiatives. SIDO offers a variety of services, including training, consulting, requirements assessment for SSIs, and the creation of industrial estates. A total of 27 offices, 31 SISI (Small Industries Service Institute) extension centres, and 7 manufacturing and process development centres handle all of these tasks.

2. LITERATURE REVIEW

Dhale et al., (2015) Little and medium-sized endeavors (SME) are the foundation of any nation's economy, as the creator of a review named "Basic Examination of Pretended by Monetary Organizations Being developed of Indian Msme's" makes sense of. Around 10 million individuals in India are working for MSMEs. After farming, the miniature, little, and medium-sized endeavor area is India's biggest manager. 90% of absolute enterprises in the economy are in Indian MSMEs. Around 45% of this 90% goes into assembling, 40% into trades, and 17% into Gross domestic product. Supporting assumes a fundamental part in the drawn out progress of MSMEs in the modern area. India's administration and monetary establishments assume urgent parts in the nation's advancement. Numerous little and medium-sized undertakings (MSMEs) all through the world wouldn't exist without the help of monetary foundations, which this article has featured. Improved results might be normal from a more noteworthy interest in miniature, little, and medium-sized undertakings (MSMEs), which is examined and assessed in this review.

Garg (2015) Discoveries from the examination "Evaluation of Little and Medium Ventures Development in the Punjab Territory of India" found that advancement of the MSME area can possibly widen the province of Punjab's economy. In Punjab, the miniature, little, and medium-sized undertaking (MSME) area depends vigorously on ICT. Affiliations ought to put resources into this field of Data and Correspondence Innovation for the improvement of creation limit of this industry. This market fragment is significant on account of the positions and cash it makes. The miniature, little, and medium-sized endeavor (MSME) area can speak with the worldwide partnerships. Enhancements to the MSME area in actual conveniences including rail routes, streets,

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power, and water supply are likewise proposed in this review.

Puneet et al., (2015) as per "Elements Impacting the Data Innovation Reception in Miniature, Little, and Medium Ventures (MSMEs): an Exact Review" The miniature, little, and medium-sized undertaking area in India is essential. Fabricating creation, trades, occupations, venture, and new advances are totally helped by this industry. The commitment of MSMEs to Gross domestic product was 17% in 2011, and it rose to 22% in 2012. Because of globalization, this industry has needed to manage various new challenges. Insufficient mastery, an absence of suitable innovation, wasteful showcasing foundation, limits on development and modernization, and a deficiency of qualified work are only a portion of the issues keeping the business down. These difficulties might be managed out through IT, foundation and preparing for the development of faculty. In this article, we investigated the utilization of innovation in North Indian MSMEs. This article contends, in view of experimental exploration, that administration guidelines, extraordinary competition, and data imparting to clients all assume a part in driving far reaching utilization of data innovation.

Biswas (2014) He contended in his article named "Supporting Requirements for MSME Area" that MSMEs are urgent to the outcome of our country. The Public authority of India and the Hold Bank of India have ordered various measures all through the years to expand the accessibility of credit for little and medium-sized ventures (MSMEs). The banks will advance cash to the need area without requiring any insurance. It is reasonable to expect that there are a few difficulties for miniature, little, and medium-sized ventures (MSMEs) in accessing bank credits and different types of funding. Regardless of the public authority's earnest attempts to work with the progression of credit to the MSME area, there is still a lot to be finished before the area's genuine supporting requirements can be met.

Biswas (2014) While loaning cash to little and medium-sized organizations (SMBs), banks have run into various issues. These miniature, little, and medium-sized endeavors (MSMEs) work generally in the casual economy, where they need formal

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fiscal reports. Banks won't stretch out credits to SMEs on the off chance that a strong monetary record doesn't as of now exist. One more issue for M&E is the failure to give security while expanding credit. because of the bigger amount of advances became nonperforming before, the historical backdrop of the financial business with MSMEs. The hole between the requirement for credit in this industry and how much credit made accessible by banks and other monetary establishments is gigantic.

Singh et al., (2013) The article "Innovation Development Issues Influencing Little Firms Execution" makes sense of the essential job that MSMEs play in monetary development and occupation creation in arising countries. This industry is essential to India's monetary improvement since it lessens local incongruities and guarantee all the more equally disseminated riches and pay the nation over. Around 8% of Gross domestic product, 45% of modern creation, and 40 percent of commodities come from this industry. This record likewise subtleties how the organization has battled with an absence of subsidizing since it previously opened its entryways. To stay serious in the worldwide market and fulfill the needs of their purchasers, the creators of this study contend that organizations ought to put resources into mechanical motivating forces. Concurring on the aftereffects of this examination, the public authority has executed projects to assist with things like monetary administration, innovation collaboration, and representative strengthening.

Deshpande & Sharma (2013) Business venture or Endurance?," a review paper he composed. Examination of unit-level information from the enlisted fabricating area of the miniature, little, and medium endeavors enumeration information was utilized in the review named "Position and Orientation of Private company in India," which looked to make sense of the support of dalits and other minimized gatherings and ladies in the miniature, little, and medium ventures area. Organizations possessed by individuals from the SC and ST people group are excessively packed in country regions.

3. RESEARCH METHODOLOGY

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The theories that will be tested have been stated previously. The analysis of the collected data was guided by the z-test, frequency, and percentage measures. Moreover, the study was grounded on a review of related literature that provided a foundation of support. Most of these variables have been researched extensively. This section's goal is to detail the procedures that were followed during this study's research phase. Study design, sample selection, data collecting strategies, and data processing techniques are all covered here.

3.1.Objectives of the Study

1. To Investigate the socio-economic and business standing of entrepreneurs in Maharashtra.
2. To Explore the origins of fixed and working capital for enterprises in Maharashtra.
3. To Assess financial challenges within businesses in Maharashtra.
4. To Analyze the impact of government policies and support programs on the entrepreneurial landscape in Maharashtra.

3.2. Study Area

This study broadly examines MSMEs' funding in Maharashtra. This survey covers all MSMEs in eleven categories. The 10 options reflect different business sectors. This analysis assumes professional, consumer, guest, manufacturing, transportation, public utilities, retail, wholesale, agricultural, agriculture-related, construction, and oil mining/extraction businesses in Maharashtra. These eight settings represent a broad sample of Maharashtra MSMEs from different industries. This technique helps collect data in a specific geographic region and groups MSME firms in Maharashtra.

3.3. Data Collection

3.3.1. Secondary Data

In addition, this study employed legitimate secondary data. MSME yearly reports and government and agency surveys enhance primary data.

3.3.2. Procedure

This research collected data on its aims using a standardised questionnaire.

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Characteristics	Professional Services	Consumer Services	Guest Services	Manufacturing	Transportation	Retail	Wholesale
Gender	Male	Female	Male	Female	Male	Female	Male
Qualification	Graduate	Diploma	Postgraduate	Graduate	Postgraduate	Diploma	Graduate
Problems in Financing	High	Moderate	Low	Moderate	High	Low	Moderate
Financing Sources Used	Bank Loan	Personal Savings	Investors	Bank Loan	Government Grants	Bank Loan	Investors
Investment in Manufacturing	₹100,000	₹80,000	₹120,000	₹150,000	₹200,000	₹180,000	₹160,000
Investment in Services	₹50,000	₹60,000	₹40,000	₹70,000	₹90,000	₹80,000	₹100,000
Scope for Modernization	High	Moderate	Low	Moderate	High	Low	Moderate
Sickness in the Firms	No	Yes	No	Yes	No	Yes	No

3.3.3. Data Gathering Plan

Data was collected by field survey. A structured questionnaire was given to respondents in each setting who agreed to respond, asking about their entrepreneur-started business, gender, types of businesses, qualification, financing problems, financing sources, investment in manufacturing, investment in services, scope for modernization, sickness in firms, etc. Before distribution, entrepreneurs were asked for help. Those who consented to fill out the questionnaire received it. The questionnaire explained the research, its value, and the researcher's assistance. The researcher helped them read the questionnaire to reduce answer error.

4. DATA ANALYSIS AND INTERPRETATION

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4.1. Demographic Characteristics of MSME Entrepreneurs in Maharashtra

Table 4.1: Demographic data of entrepreneurs

The study shows that MSME business owners in Maharashtra come from a wide range of backgrounds. There are big differences between men and women in the professional, customer, guest services, industrial, transportation, retail, and trade sectors. There are different kinds of financial problems, with industry having the most trouble. However, a variety of funding sources are used, such as bank loans and government funds. Investing in production and services is not the same, and there is room for a lot of change. Sickness is common in companies where women run customer and guest services. The results show that specific help is needed to address the problems and make the most of the possibilities in each industry in Maharashtra to encourage business and long-term economic growth.

4.2. Business performance

Table 4.2: Analysis of business performance

Industry	Average Annual Revenue (INR)	Average Profit Margin (%)
Professional services	1,500,000	15
Consumer services	1,000,000	10
Guest services	800,000	8
Manufacturing	5,000,000	12
Transportation and public utilities	2,000,000	14
Retail	1,200,000	9
Wholesale	1,800,000	11
Agricultural and agricultural-related	600,000	6

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Construction-related	3,000,000	16
Mining/extraction of oil	10,000,000	20

There are big differences between the businesses in Maharashtra when you look at their average yearly revenue and profit margins. Manufacturing and mining/oil extraction bring in the most money each year, with an average of INR 5,000,000 and INR 10,000,000, respectively. These businesses also have higher profit rates, between 12% and 20%, which shows that they can make money. Agriculture and companies connected to agriculture, on the other hand, make an average of INR 600,000 a year and have a profit margin of 6%, which shows how tough the economy is in this sector. With a big profit margin of 16 percent, the building industry stands out in Maharashtra's business world. This shows that it has the potential to make money.

4.3.Sources of Fixed and Working Capital Table

Table 4.3: Source of capital funds from MSMEs

Source of Capital	Percentage of Total
Personal savings	40
Bank loans	30
Government grants and subsidies	15
Venture capital	10
Other sources	5

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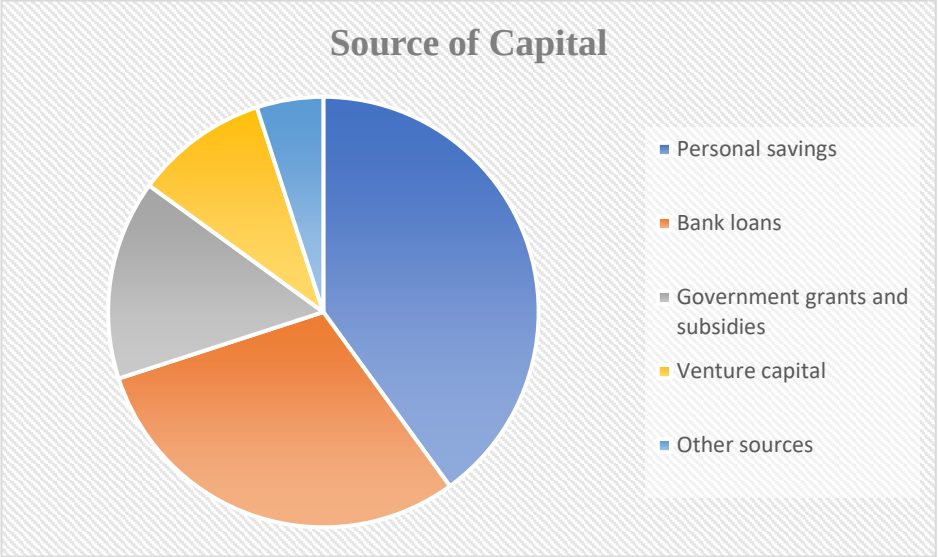

Figure 4.1: Pictorial representation of source of capital funds

Maharashtra relies heavily on personal funds, which make up 40% of all capital, as shown by a study of capital sources. This shows that businesspeople are very committed, which could mean they have faith in their businesses. 30% comes from bank loans, which shows a dependence on outside financing, which is a popular way for businesses to grow. The fact that 15% of the money was set aside for government handouts and subsidies shows how important public backing is for business growth. The fact that venture capital makes up 10% of the market shows that some businesses are getting outside money to grow. The last 5% came from other sources, showing how varied the funding is and how important it is for Maharashtra's business processes and growth to have many ways to get money.

4.4.Financial Challenges Faced by Businesses Table

Table 4.4: Challenges faced by MSMEs

Challenge	**Percentage of Businesses Affected (%)**
Access to credit	50 %
Cash flow management	30 %

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Debt repayment	20 %
Taxation	15 %
Regulatory compliance	10 %

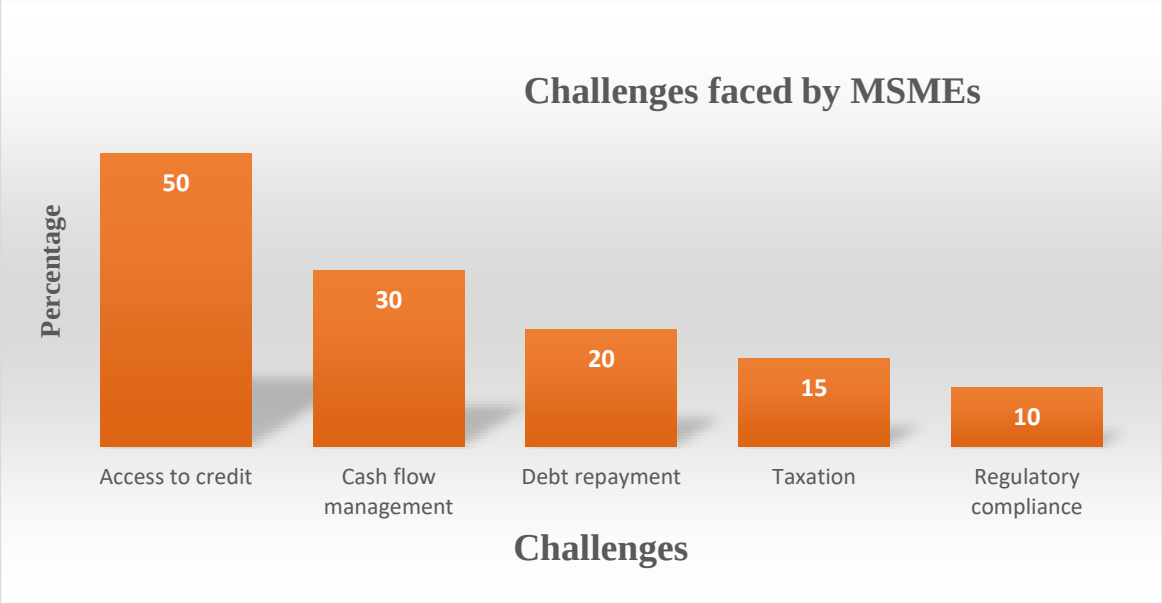

Figure 4.2: Graphical representation of challenges faced by MSMEs in percentage

Access to loans is the biggest problem for companies in Maharashtra, as shown by the fact that it affects half of all businesses. This points to a big problem with getting money, which could stop growth and longevity. Cash flow management comes in at a close second with 30%, showing how important it is to keep cash on hand for day-to-day activities. 20% of businesses have trouble paying their debts, 15% have trouble with taxes, and 10% have trouble following the rules. These problems show how complicated problems can be. For example, limited funds, high taxes, and complicated rules all affect the business world as a whole. Taking care of these problems is necessary to make Maharashtra a good place for business growth and economic progress.

5. RESULTS AND DISCUSSION

ISBN - 978-93-95060-79-0

The study on MSME owners in Maharashtra shows that the world of business is very different, with differences in gender, education, and financial problems between industries. When it comes to business success, the manufacturing and oil mining/extraction sectors stand out. They have the biggest average yearly income and profit margins, which shows that they can make money. Instead, companies that are connected to agriculture and agriculture are having a hard time with the economy, which shows in their lower income and profit margins.

The study of capital sources shows that personal funds and bank loans are used a lot. This shows how committed the entrepreneurs are and how common it is to get outside money to grow a business. Government funds are very important because they make up 15% of the total capital. This shows how important public backing is for business growth. The problems small businesses in Maharashtra are having, especially getting loans, show how important it is to focus interventions to remove financial roadblocks that are stopping businesses from growing. Cash flow management, paying off debt, taxes, and following rules and regulations are also big problems that businesses have to deal with. This shows how complicated business problems are.

6. CONCLUSION

Finally, the in-depth study of MSME businesspeople in Maharashtra has given us useful information about the many changing aspects of being an entrepreneur. The results show that female representation, qualifications, and financial problems are not all the same in different fields. This shows how important it is to have customised support plans. The performance study shows that the manufacturing and oil mining/extraction sectors are very profitable, while farming companies are having a hard time making money.

Capital sources show that a lot of people depend on personal savings and bank loans. This shows how dedicated businesses are and how common it is to get money from outside sources. Grants from the government are very important because they show how public backing for businesses can help them grow. The problems that have been named, especially the general worry about not having enough access to credit, make it clear that specific actions are needed to remove financial obstacles.

This study not only helps us learn more about the MSME sector in Maharashtra, but it also gives us the information we need to make policy choices that will help businesses grow and the economy grow in the long term. Using these findings will help create policies and support

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programmes that meet the unique needs of different businesses, encouraging resilience and new ideas in Maharashtra's MSME environment.

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ISBN - 978-93-95060-79-0

A STUDY ON FACTORS INFLUENCING BUYING BEHAVIOUR OF GENERATION Z FOR E-COMMERCE IN INDIA

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Abstract
It is a never-ending problem and subject of constant idea to comprehend the buying behaviours. However, it is equally necessary to comprehend the depth of the customer's psyche and create the truly necessary exactness in foreseeing the future. Both the buying behaviour and the instruments and processes that facilitate exchanges are significant. As Generation Z progressively develops to represent a sizable piece of the consumer market, it is currently more significant than any time in recent memory to understand how to sell to it. Our understanding of their online shopping behaviour patterns will help us better understand their buying propensities. The accessibility of online stages and technology have essentially affected consumer behaviour, especially that of generation Z. Online shopping is expanding at the fastest rate and quickly becoming a famous and trusted shopping channel of customers all through the world, in which, the customer bunch represents a major share and tends to guide consumer behaviour. Online, generation Z is the present and future. To help businesses continue to reach and prevail upon this set of potential customers, this article focuses on understanding the elements influencing Gen Z's online shopping movement. This study intends to discover some of the online shopping behaviours of Gen Z and likewise attempts to identify some valuable affiliations that will help marketers and other people researching Gen Z movement. The discoveries indicate that Gen Z prefers to lead in-depth research before making an online purchase.

Keywords: Generation Z, E-Commerce, Buying behaviour, Security, Online purchase experience, Online Purchase Intention.

1. INTRODUCTION

Customers may opt to do their shopping online due to the Internet's growing

importance as a global communications and trade platform. According to Internet World Stats (2020), as of April 2020, 59% of the world's population, or roughly 4.57 billion people, were regular Internet users. E-commerce also facilitates the shipping, transporting, purchasing, trading, and two-way communications of goods and services. What's most endearing is that major e-commerce companies are still mostly ignoring a sizable percentage of those prospective customers.

Indian customers are presently undeniably more compatible with online stages than any time in recent memory. Over 150 million people use the internet, and that number is increasing every minute. Both the scale of the e-commerce business and Indian consumers' acceptance of it are exploding. The burgeoning and massive e-commerce sector generates critical measures of revenue through online exchanging. The replies of the users are completely significant to the success of e-commerce. Users are becoming more comfortable making purchases online, and the epidemic has made reception simpler. Modern technology, which offers many benefits, has replaced the conventional buying pattern. The straightforwardness of use develops alongside the expectations, which have likewise fundamentally increased since customers have utilized a variety of web sites. As a result; the elements of the Indian retail market have changed essentially. The increased use of the internet and convenience of utilizing different e-following sites is the cause of the developing interest in them. The ubiquity of this stage at first increased in metropolitan markets, yet it has since moved to rustic areas too, which are generally underserved and have enormous demand potential considering the development of the internet. Due to the increased reception of technology achieved by e-commerce, little organization owners and entrepreneurs were able to begin new businesses or develop current ones. The generation after the millennial, known as Generation Z, includes people brought into the world between the late 1990s and today. The use of internet-based technology is generally prevalent among this generation, which has grown up around electronic gadgets since the beginning.

1.1. Understanding Generation Z

Gen Z accounts for 21% of the population and is quickly becoming more important to businesses than Gen Xers. They will significantly affect future developments

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and user habits. So, businesses and academics are trying to figure out what Gen Z wants and how they purchase so that they can attract and retain them as online customers. Generation Z, the most recent group of teenagers, attain full maturity between the ages of one and five. The current youth are unlike those before them because they are bold, independent, emotional, expressive, creative, and curious. A stronger inclination for consumer behaviour and a lack of instantaneous preparation and points of view while making impulse-triggering site attribute variables features purchases are the outcomes of Generation Z's heightened sensitivity to correspondence patterns. When determining whether to make an online purchase, users take into account site qualities including ease of use and aesthetic appeal.

1.2. Gen Z's online shopping behaviour

Gen Z's online shopping behaviour is interpreted as including every single behavioural expression, from developing a purchase intention to selecting an item to purchase and settling on a last choice, as well as from selecting to evaluating items, circumstances, and buying strategies (Saputro, 2023). Customers will eventually come to trust one another and make purchases. Because Generation Z is very tech-canny, each gathering of customers will have different buying propensities. Before making a purchase, Generation Z will research the supplier's sales activities by reading customer reviews, visiting the organization website, etc. They will likewise seek online expert advice before making a huge purchase (Syahdan, 2021). What's more, Gen Z likewise uses reference gatherings, like asking friends and family for recommendations before making a purchase. Gen Z frequently continues to shop online and encourages friends and family to utilize the buying channel after feeling secure and satisfied with the calibre of the goods and services; this is a huge component in the decision to do as such (Thangavel, 2021).

2. RESEARCH OBJECTIVES

1. To examine variables influencing Gen Z's online shopping behaviour.
2. To recognise some of the internet purchasing behaviours of Generation Z.

3. LITERATURE REVIEW

Wahyuningsih et al., (2022) as indicated by their review, Gen Z consumers are more

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inclined to choose superior grade, ecologically responsible items. Numerous members of Generation Z witnessed their parents struggle during the 2008 financial emergency and suffer considerable losses. They are hence more frugal and conscientious. Consumers in generation Z are less likely to trust companies than older age bunches are. They choose to follow well-known, well-liked influencers on YouTube and Instagram instead. They choose to use mobile wallets (Wahyuningsih, 2022).

Vasic et al. (2019) developed customer satisfaction with their online purchases in the Serbian market is analyzed utilizing a research methodology. Security, information accessibility, delivery, quality, estimating, timeliness, and customer fulfillment are the seven gatherings into which the conceptual model's complete of 26 components can be broken down. Input model parameters were collected through online surveys utilizing the appropriate Internet-enabled devices. The legitimacy of the developed model was confirmed utilizing the Corroborative Factor Analysis and the Incomplete Least Squares techniques. The analysis of the obtained information confirmed the primary research hypotheses, which state that the factors of security, information accessibility, transportation, quality, cost, and time have a direct effect on customer satisfaction in online buying on the Serbian market (Vasic, 2019).

Rubin et. al. (2020) this study expects to investigate how customer mindsets contribute to online shopping truck abandonment. To investigate the effect of consumer mindsets (i.e., unique versus concrete) on purchase intentions, three experimental experiments were carried out. The discoveries show that consumers who shop online with a theoretical (as opposed to concrete) perspective give the items in their trucks a higher need rating and are therefore more likely to make a purchase, which lowers the rate of shopping truck abandonment. The results suggest that by applying strategies that enable customers to think dynamically, online retailers can lower the percentage of shopping truck abandonment. By bringing up a huge underlying process influencing online shopping truck abandonment, this research adds to the group of literature (Rubin, 2020).

Sudirjo et al. (2023) the centre is at what generation Z's buying propensities suggest for online retailers in terms of site quality,

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security, services, and shoppers' overall enjoyment, as well as how this last factor affects online impulse purchases. Generation Z's spending propensities, alongside the lightning-quick development of online shopping, promote unplanned purchases. This quantitative review relied on information gathered through methodical inspection for its decisions. There were 159 people in West Java between the ages of 18 and 35 who identified as part of generation Z and had shopped on the West Java Shoppe website. Information analysis makes use of the Primary Equation Model (SEM) and the SmartPLS 3.0 projects. The discoveries demonstrate that site features, yet not security measures, fundamentally affect impulsive buying behaviour in the advanced marketplace. Services that facilitate online buying have less of an effect on impulsive purchases made on the web. Site characteristics, security measures, and online buying services all contribute to the shopper's overall satisfaction. Shopping pleasure is a more huge inspiration of impulsive online purchases (Sudirjo, 2023).

Punyatoya et al. (2019) this study is likely to examine the various factors that influence the evolution of each sort of client trust in the advanced space and how that shapes customer satisfaction (CS) and dependability intention (LI). Approval of the review model utilizing survey was performed. Information from 334 Indian clients was used in a primary equation modeling analysis of the model's causal pathways. Cognitive and affective trust are demonstrated to be empirically discernible characteristics in the context of online buying. Cognitive and emotional trust are remembered to mediate the connection between a website's perceived quality, security and protection approach, past interaction experience, perceived e-retailer repute, shared value, and CS. CS's positive effect on LI's attitude about the online shop is additionally noteworthy. The review provides useful data on how Indian customers rank online stores. These nuggets of shrewdness will help domestic and worldwide online merchants in developing and implementing strategies to support client trust, a key concept in both CS and LI. This point of convergence proves beyond any reasonable uncertainty that customers' level of confidence in online businesses is a basic aspect in building enduring relationships with those customers (Punyatoya, 2019).

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4. RESEARCH METHODOLOGY

The research approach used to examine the elements affecting Generation Z customers' buying decisions in the context of Indian e-commerce. The methodology offers a methodical method for gathering, examine, and evaluate information to meet the objectives and hypotheses of the review. Both essential information and secondary sources were employed in this review, and they were gathered from a variety of diaries, research papers, websites, books, reports, and articles that were published online.

4.1. Research scale

Utilizing the discoveries of a review of documents on Gen Z's online buying propensities, researchers were able to synthesize and gather information for research distributions as articles and book chapters. As per research utilizing the Web of Science information system, key studies from 2010 to the present have been published by Elsevier, Scopus, ProQuest, SpringerLink, Researchgate, and Google Researcher, and some unique theoretical works have time. The creator has developed a research framework and research model from the distributions since 2001.

4.2. Research sample

This study uses multivariate regression and exploratory factor analysis (EFA), therefore it needs no less than 05-10 observations for each variable. From that point, calculate 20 x 10 = 200 observations as the smallest necessary sample size. The review makes use of cross-sectional information from a Generation Z survey conducted in different Indian cities. A handy non-random sample method was used to direct the survey online using a Google structure from January 2023 to Walk 2023; the survey URL was made available to general society via virtual entertainment. When the expected sample level is attained, the survey will end to verify the legitimacy of the information. 200 votes from respondents between the ages of 11 and 26 who completed the survey after two months are eligible to be analyzed.

4.3. Variables used in this study

- **Dependent Variables**

Online shopping behaviour of Gen Z (OBB)

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Gen Z, generally referred to as people brought into the world between the middle of the 1990s and the beginning of the 2010s, demonstrates distinctive online shopping behaviours that are influenced by their specific childhood in the advanced age. Having grown up with cell phones, web-based entertainment, and the internet as fundamental components of their life, Gen Z is essentially distinguished by their computerized fluency and knowledge of technology (Tiwari, 2020). They prefer liquid, user-friendly, and mobile-optimized websites and applications while buying online as a result. Before making a purchase, they frequently direct in-depth internet research and rely on user reviews, user-generated content, and influencer recommendations to guide them.

Genuineness and brands with a conscience are profoundly valued by Generation Z. Companies that share their values — like maintainability, diversity, and ethical business practices — are more likely to receive their help. They search for businesses that resonate with their objectives and sense of self and have strong online presences and active virtual entertainment profiles (Thangavel, Consumer decision-making style of gen Z: A generational cohort analysis, 2022). Also, Gen Z appreciates speed and convenience; they frequently choose same-day or next-day delivery alternatives and use membership services to streamline their buying routines.

What's more, Gen Z is very price sensitive and skilled at finding the finest deals. They routinely use markdown codes and cash back deals while looking at costs across different online merchants. They are attracted to social commerce, which permits them to make purchases directly through virtual entertainment sites. However, they expect price and transportation cost lucidity (Veybitha, 2021).

To wrap things up, demand for augmented reality (AR) and virtual reality (VR) shopping experiences reflects Gen Z's fondness for immersive buying. They appreciate dynamic item perceptions and virtual take a stab at capabilities, which add interest and immersion to the online buying experience.

- **Independent Variables**

Convenience (OC)

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Gen Z understands convenience as the absence of travel requirements or the need to waste time going out to search for and purchase items. Instead, customers just need to follow a few easy steps while settling down anywhere there is an internet sign to be able to purchase however much they like. Furthermore, there are more payment choices available while shopping online, for example, cash, transfer, e-wallet payment, reward focuses, etc., which appeals to Gen Z consumers.

Diversity of goods (GD)

The term "diversity of goods" refers to most of commodities that can be purchased online as well as in the conventional mode of shopping, making it even simpler to access a variety of goods that are not restricted by geography. The sum and variety of items that can be found while making an online purchase is sometimes referred to as the diversity of goods. This approach criterion makes it evident that the internet market will be more desirable than the conventional market, especially in the current climate of disease outbreak, travel restrictions, and rising travel expenses between regions. The more easy and varied access to items on the web channel, the more expensive it gets as a result of the rise in petrol prices and the reduction in shopping time.

Perception of trust (OT)

When customers choose to shop online, perceived reliability refers to their confidence and trust in the business; they feel that this is a reliable address and aren't concerned about getting conned or buying some unacceptable items because of misleading advertising. Furthermore, service providers are attempting to give consumers the best services possible alongside the most functional return and sales policies so they will feel more confident and delighted. The trust of Gen Z in online buying is likewise tested by examining the positive comments of consumers who have made previous purchases or have acquaintances in the seller's customer list. Advancements and marketing are likewise more "real" with the goal that buyers are not "hallucinated" when buying online.

Online shopping trust (OB)

Consumers will have confidence in online shopping, sellers, and online payment when it goodly affects them. After that, they will keep making purchases online and encourage and

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expose their friends and family to do likewise. Furthermore, when the capability of an online shopping channel is acknowledged and developed, it will grab the eye of associations that protect consumer privileges and the general public, giving the younger generation confidence when they purchase.

Perceived purchase and financial risk (OR)

Online shopping has different potential risks notwithstanding its convenience and diversity of items during the access, decision-production, payment, and transportation phases. One of the principal dangers to be mentioned is that the real things that are purchased may differ from the website's photos or might be lost during delivery. The next risk is financial, since it is possible for data to be leaked about an individual or their personal record, or they might risk losing money when exchanging goods. Gen Z clients' online buying processes are hampered by these obstacles, which should be removed and replaced with services that foster customer confidence.

Comparison to other Sites

Members of Generation Z spend most of their time online engaging in virtual forms of entertainment and commerce. They see online advertisements while doing so, and if they find such advertisements engaging, they are more likely to continue their exploration of the web. When they find something they like, they often wait to buy it until they have done some pricing comparisons across several online retailers.

5. DATA ANALYSIS AND RESULTS

5.1. Descriptive statistics

The descriptive findings of the study sample are presented in Table 1.

Table 1: Descriptive Results

Characteristics	Frequency	Percentage
Gender		
Male	67	33.5%
Female	133	66.5%

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Age		
11 to 13	9	4.5%
14 to 16	18	9%
17 to 19	45	22.5%
20 to 22	71	35.5%
23 to 26	57	28.5%
Educational qualification		
Secondary school students	17	8.5%
High school students	53	26.5%
Undergraduates	81	40.5%
Workers	49	24.5%

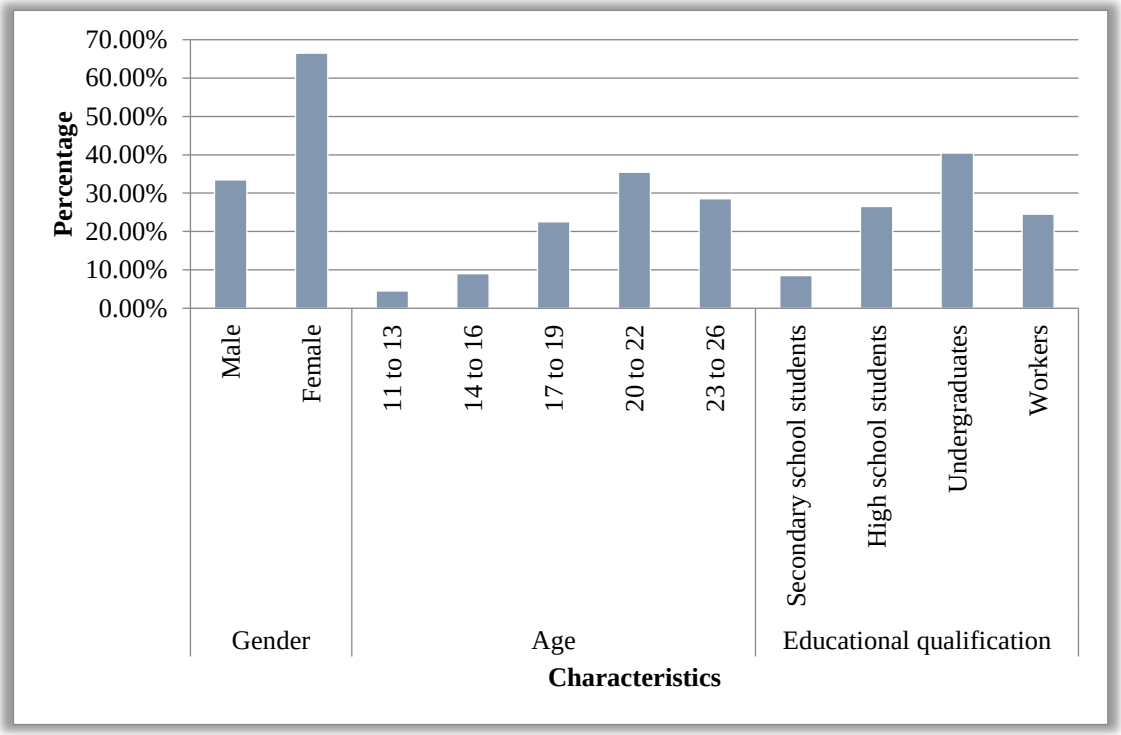

Figure 1: Graph showing the percentage of descriptive results

Table 1 breaks down survey members based on three principal factors: gender, age, and educational foundation. Gender-wise, obviously the sample has a little higher extent of women than men — 66.5% of respondents are women, compared to 33.5% of respondents who

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identify as men. This indicates that there was a gender hole in the survey populace. The information demonstrates that responders are generally evenly dispersed across the range of ages. The sample's youngest age range, 11 to 13, makes up 4.5% of the populace, followed by 14 to 16-year-olds at 9%. There were a sizable number of youthful grown-ups in the sample, with 22.5% of respondents in the 17-19 and 20-22 age gatherings and 35.5% in the 20-22 age bunch, respectively. A varied age range is further demonstrated by the way that 28.5% of respondents were in the 23-26 age bracket. The heft of responders are either undergraduates (40.5%) or secondary school students (26.5%) when it comes to educational foundation, showing a concentration on the younger demographic. 8.5% of the sample is made up of secondary school students, while 24.5% is made up of workers. This dissemination represents a range of employments and educational levels among the populace polled, with a clear preference for those seeking after higher education.

5.2. Reliability test

Table 2 displays the findings of the Cronbach's Alpha reliability test.

Table 2: Cronbach's Alpha

Reliability Statistics		
Research Variables	**Observable Variables**	**Coefficient**
OC	4	0.898
GD	3	0.891
OT	4	0.871
OB	4	0.854
OR	5	0.900
OBB	4	0.783

The table records significant details about numerous review variables, for example, the number of observable variables contained inside each variable and their corresponding coefficients, which connote the internal consistency or reliability of these measurements. With the highest coefficient of 0.900, "OR" stands out especially well and demonstrates outstanding internal consistency among its 8 observable variables. With coefficients of 0.898 and 0.871,

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respectively, and each including 7 observable variables, "OC" and "OT" likewise show great dependability. Despite having 6 and 7 observable variables, respectively, "GD" and "OB" have somewhat lower coefficients at 0.891 and 0.854, showing a decent however marginally lower level of internal consistency. The last coefficient for "OBB" is 0.783, demonstrating that its 7 observable variables have a respectable level of internal consistency. The reliability of measurements inside these research variables is highlighted by this table, with "OR," "OC," and "OT" being especially hearty, while "OBB" exhibits marginally lower yet at the same time respectable reliability.

5.3. Exploratory factor analysis EFA

Table 3 shows the discoveries of the KMO and Bartlett's Test for the independent variables.

Table 3: KMO and Bartlett's Test Results

KMO and Bartlett's Test		
Kaiser-Meyer-Olkin Measure of Sampling Adequacy.		.851
Bartlett's Test of Sphericity	Approx. Chi-Square	5640.239
	df	419
	Sig.	.000

The factor analysis is absolutely consistent with the review information, as shown by the KMO test discoveries, which demonstrate that the KMO coefficient is 0.851 > 0.5. The Bartlett test result, which likewise indicates that the observed variables are correlated in the populace, is 5640.239, with a significance level of Sig = 0.000 0.005. This outcome is completely consistent with how many independent factors.

Table 4: EFA exploratory factor analysis outcomes

	Rotated Component Matrix[a]				
	1	2	3	4	5
OR1	.864				
OR2	.861				
OR3	.759				

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OR4	.730				
OR5	.587				
OC1		.774			
OC2		.726			
OC3		.656			
OC4		.619			
OT1			.725		
OT2			.695		
OT3			.677		
OT4			.673		
OB1				.825	
OB2				.752	
OB3				.713	
OB4				.672	
GD1					.757
GD2					.726
GD3					.708

6. RECOMMENDATION AND CONCLUSION

As indicated by the research, customers are using the online marketplace for a wider range of goods purchases as they become more used to it. The purchase process is dynamic and continually evolving. The internet gateways are attempting to capture customers after realizing the expanding sales potential. Understanding the elements influencing the buying behaviour of internet consumers is becoming more and more significant in this present circumstance. There are between 2.52 and 2.52 billion members of Generation Z. Otherwise called computerized natives, they interact with one another and develop a lifestyle entirely online. Because they have shorter attention ranges, consume a ton of media, and have access to a wealth of study materials, Gen Z can make better decisions. As per the results of the research concentrate on the hypothesis, convenience, perceived trust in endlessly shopping trust all have a critical effect on consumers' buying decisions. There is no correlation between

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checking user reviews for an item and looking into more details on other shopping websites. The perceived danger component specifically has the opposite influence. These results are critical for companies and marketers that need to effectively connect with and serve Generation Z, a generation that is set to influence the direction of Indian e-commerce in the future.

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ISBN - 978-93-95060-79-0

UNVEILING THE CONSUMER NARRATIVE THROUGH NAVIGATING PSYCHOLOGICAL INFLUENCES IN THE ERA OF SOCIAL MEDIA MARKETING

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ABSTRACT

The influence of online entertainment continues to grow in the ever-changing Digital India market. This research examines the effect of online entertainment advertising on customer behaviour, with a particular emphasis on Lidl and IKEA in the Finnish retail industry. The study reveals multiple points of view by analysing content and conducting interviews with six store managers. A thorough analysis of the demographics of the participants found a diverse group of leaders with a range of experience levels across age, education, and professional fields. According to the results, advertising for entertainment products sold online has a major impact on buyers' tastes and decisions. Despite generally positive feedback, some customers may have trouble keeping track of details. This study sheds light on the complex interplay between virtual entertainment promotion and consumer behaviour, providing valuable information for businesses navigating the global digital transition.

Keywords: *Marketing, social media, Navigation, Consumer Behaviour, Influence.*

1. INTRODUCTION

When it comes to informing and entertaining customers, virtual entertainment has a significant impact in Digital India. There is strong evidence that advertising in online entertainment can boost brand recognition, consumer decisions, and public support. It could also generate new ideas, boost brand traffic, provide detailed information, create a customer base, and cultivate existing relationships. All of these things can help strengthen consumer loyalty and enhance public perception of the brand. In any case, regarding Digital India. It is remarkable that so little study has been done in this area, given the importance of trust and perceived market benefits to customers' decisions to buy online entertainment. Both of these aspects affect how consumers perceive purchasing online. Thus, there are issues in the perception of the relationship between online virtual entertainment firms and shopper purchasing behaviour in Digital India due to the lack of information regarding the function of trust and perceived market benefits in this context.

The expansion of globalisation and the dissemination of knowledge made possible by the Internet have stimulated business expansion. Clients have a more full scope of labour and products, which has provoked vehement opposition. While many businesses are prepared to use online entertainment advertising to promote their brands, few have equipped their managers to understand the medium. The ability to build good customer buzz and interest in the company's products or services depends on the ability of business executives to produce engaging content on the web.

1.1.Evolution of social media marketing

The rise of cyber-based media for amusement Between 1995 and 2002, the Internet bubble gave rise to a new industry: online advertising. The internet's meteoric rise has made it a useful tool for advertising. Foster sites were among the earliest tools used by organisations to establish a web presence and boost their reputation. Organisations can now use web crawler enhancers (Web optimisation) because to the rise of web indices like Google, Hurray, and MSN. With the help of Web crawler Enhancers, businesses may now boost their visibility in search engine results.

The proliferation of content promotion has been aided by new articles published on the site's second generation. Inbound

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promoting popurlarly knowns as Happy showcasing extended the value creation to customers while, simultaneously, acquiring business to the various organisation. As a result, the sites have replaced traditional outbound marketing strategies including cold calling and product placement. The business world has finally caught on to social networking sites like Facebook, Twitter, and Linkedin. The collaboration could differentiate among available virtual entertainment options and select the one that would have the greatest positive effect on their strategy.

1.2. Role of Social media advertising in influencing consumers' perspectives

The research shows that people are influenced to make expensive or cheap purchases based on the recommendations of friends and online acquaintances in the realm of virtual entertainment. The proposed link between online entertainment and consumer navigation posits that the latter has an impact on consumers' promotion mindsets, brand quality, and purchasing expectations while browsing the web.

Figure 1: Strategy for advertising using social media

Buyers are difficult to influence on the best way to buy directly, but you can still have an effect on their final decision and preferences by acting as an intermediary. Either excellent branding significantly affects consumers' ultimate decision making when making costly purchases. Furthermore, when company customers' friends offer suggestions on clear products via virtual entertainment, it directly influences their choices on product purchases.

Advertising through web-based entertainment provided by business marking eventually affects buyer outlooks and expectations. Using the aforementioned information, marketers can create strategies to attract and retain customers. Numerous advertisers in the

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ongoing age utilize virtual entertainment as a device for promoting efforts. Because it is practical to advertise products and services via internet entertainment, it facilitates communication with customers.

1.3.Marketing via social media and its effects on consumers' purchasing behaviour

The social reinforcement of preconceived notions about particular brands can also influence consumer behaviour. Moreover, peer interchanges and social instruments can likewise lead explicit clients to intend to purchase a brand. Consumers who base their purchases on the aforementioned reviews are more likely to put their money behind recognisable company names.

Online entertainment has an impact on consumers' shopping decisions, but it also contributes to the advancement of knowledge in other areas, such as the study of legislation and the selection of jurors. In business law cases, for instance, jurors may access online advice to aid them in their deliberations. The author suggests that Twitter is the greatest website for web-based media to exchange data and news because consumers can access up-to-date information there. Even those who focus on the distant past recognise the possibility of social impact hypotheses having an effect on one's life choices.

The authors reason that people who partake in online games or other forms of virtual entertainment are likely to be connected to one another and hence affected by the activities taking place inside their respective online communities.

1.4.The effect of social media advertising on repeat purchases

Conviction in a brand and situational elements like location, design, and even natural tidiness can both influence a customer's propensity to repurchase a particular brand. Customers have faith in the association's efforts because of the group's steadfastness and the attractiveness of its vision. This attitude makes it easier for consumers to make repeat purchases due of the product's dependability. Many people today would rather buy online and have their products delivered to their homes than go out to a physical store because of the accessibility of knowledge beyond the physical world made possible by virtual entertainment. Lighting, layout, music, and aesthetics are all contextual aspects that can affect a customer's tendency to make a repeat purchase. If you've had some time to think about it, you

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could be in a better frame of mind to make a similar purchase. This is due to the fact that the company is likely to meet its customers' demands at the precise time they have them, as well as other reasons such as the ease with which supplies may be restocked, the convenience of the stores' locations, the high income of the target demographic, and so on. Group, individual, and corporate purchasing patterns vary because consumers' perceptions and experiences with the numerous brands available to satisfy their needs and wants vary.

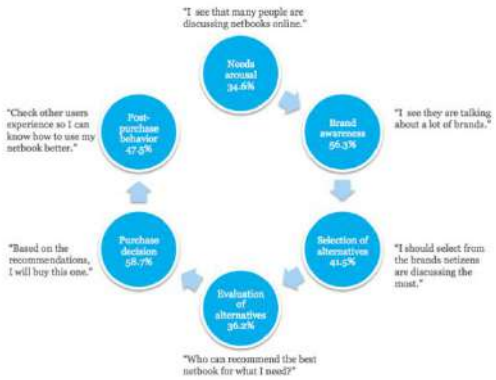

Figure 2: Cycle of online purchase

2. LITERATURE REVIEW

Dwivedi et al. (2021) Consumer propensities and corporate practices have moved because of the far reaching reception of web and social media. Because of social media and advanced marketing, organizations can set aside cash, raise brand mindfulness, and lift deals. However, there are significant obstructions, like irritating and meddlesome internet based brand presence and negative electronic informal. Specialists in the fields of advanced media and social media marketing have contributed their insight to this article. Viewpoints on additional particular issues, for example, man-made reasoning, increased reality marketing, computerized content administration, portable marketing and publicizing, B2B marketing, electronic informal, and moral issues in that, are given by the specialists as well as an itemized story on key parts of this significant subject. By distinguishing the impediments of past examinations, illustrating the holes in the writing, and proposing new lines of request that can add to growing comprehension we might interpret computerized and social marketing, this study makes a significant and opportune commitment to the field.

Chang et al. (2019) Albeit in-feed social adverts on social media are growing quick all over the planet, the substance methodology of organizations introducing in a human manner gets exceptionally less concentration. This study explores whether expanding "likes" on WeChat Second ads

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is conceivable by decisively matching the account individual utilized in the advertisements (first-individual versus third-individual) with the ideal brand picture (warmth versus ability). In view of our meta-examination of five examinations, we presume that the inspiration of social having a place is stirred up when first-individual portrayal is matched with cordial pictures, while the inspiration of personal development is stirred up when third-individual portrayal is matched with learned pictures. We likewise track down that the impacts of shifted matches on preferring aim are directed by a significant individual trademark (self-checking). This examination has both hypothetical and viable ramifications for advertisers carrying out in-feed social promotions on social media. It adds to the writing on publicizing content methodology and promoting language on social media.

Pelet et al. (2017) There's been talk about how "flow" is crucial to grasping online behaviour and characterising the engaging quality of digital interactions. The effects of flow are studied here, with a focus on their manifestations during social media consumption. The results show that telepresence enhances the five facets of flow—pleasure, focus, difficulty, agency, and exploration. Overall flow is favourably associated to these aspects (excluding control), which increases time distortion and frequency of social media use. We also create a model to show the intertwined nature of flow, telepresence, time distortion, and the use of social media, thereby expanding the nomological network on flow theory. Interesting findings about users' behavioural intentions when using social media are presented, highlighting the idea that overall flow can distinctively effect social media users in a flow experience. Theoretical and practical ramifications of these findings are examined.

Voramontri&Klieb (2019) The motivation behind this study is to lead observational examination into the capability of social media in the dynamic course of clients making complex buys, characterized here as those encapsulated by significant brand contrasts, high consumer commitment and hazard, and high exchange expenses and low recurrence. The model adjusts the EBM exemplary's data gathering, elective gauging, and last buy choice stages. A quantitative report investigates how much social media really transforms people. As indicated by the

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discoveries, social media use influences consumers' bliss at different focuses during the purchase choice and post-buy assessment processes, starting with the exploration and assessment stages. The review zeroed in on educated purchasers in South and Southeast Asia and included finished exchanges, as opposed to deserted questions.

Chopra et al. (2020) The reason for this study is to lead observational investigation into the effect of social media on client decision. A quantitative report investigates how much social media really completely changes people. Client audits of items and administrations are progressively being overwhelmed by outsiders in web-based discussions, which thus influences choices made in the disconnected world. Consumers are effectively taking part in web-based discusses thanks to social systems administration destinations, regardless of the way that advertisers have little influence on the subjects, timing, or recurrence of these discussions. As per the discoveries, social media use influences consumers' bliss at different focuses during the purchase choice and post-buy assessment processes, starting with the examination and assessment stages.

Stephen, A. T. (2016) Recent studies on customers in online and social media marketing contexts are discussed in this article. Consumer digital culture, responses to digital advertising, effects of digital environments on consumer behaviour, mobile environments, online word of mouth (WOM), and (v) online WOM are the five topics highlighted. There is a wealth of information to be gleaned from these articles on consumers' interactions with the digital worlds in which they find themselves on a regular basis. There is still plenty to learn, and most of what is known focuses on word-of-mouth marketing, which is only one facet of today's complex digital consumer landscape. Several suggestions for future research are made in an effort to get scientists thinking about issues beyond the ones they've previously focused on.

Bigne et al. (2018) This exploration takes a gander at what social media and other disconnected factors mean for the internet buying and suggesting conduct of sightseers for financial plan carrier administrations. Drawing on the Hypothesis of Contemplated Activity (TRA), this examination explores the impact of disconnected social impacts (relational and outside influences) and

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investigations online Consumer-to-Consumer (C2C) data trades as a driver of client demeanor towards online buys. We theorize that these components increment positive WOM and e-WOM in minimal expense settings, prompting more web-based repurchase aims. The reasonable model is approved with an example of 441 Spanish internet based purchasers of minimal expense carrier administrations utilizing primary condition displaying. Repurchase purpose and verbal exchange online are altogether influenced by disconnected relational impacts (like loved ones), while e-WOM is unaffected. In any case, disconnected outer tensions (like the media and specialists) meaningfully affected web-based repurchase goals or WOM, and on second thought exclusively influence consumers' aims to advance future acquisition of minimal expense aircraft administrations on social systems administration travel locales. Likewise found is the connection between online C2C data trades and changes in disposition, which thus impressively affects verbal exchange and electronic verbal (e-WOM) repurchase goals.

3. RESEARCH METHODOLOGY

3.1.Objectives of the Study

1 In the context of global digitalization, how much did consumers' access to internet entertainment influence their spending habits?

2 How much did people's willingness to spend time online watching entertainment content influence their willingness to engage in e-commerce across international borders?

3 Thirdly, how much did the ease of access to online entertainment platforms affect the variety of consumer preferences and selections during the global digital transformation?

4 In this era of global digitalization, how much did the incorporation of online entertainment into marketing plans affect brand loyalty and consumer retention in different regions?

3.2.Research Design

The study used a contextual inquiry technique with the goal of thoroughly analysing certain situations from the Finnish retail industry, with a particular emphasis on Lidl and IKEA. This

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method made it easier to analyse these specific companies in-depth and allowed for a more thorough investigation of how online entertainment advertising affects consumer behaviour.

3.2.1. Purpose

Understanding and analysing how online entertainment advertising affects customer behaviour in the context of the chosen retail organisations was the main objective of the study. Through the application of a contextual investigation technique, the study sought to offer a comprehensive and perceptive analysis of this influence.

3.2.2. Structure

A report detailing the research findings was produced and delivered, with each segment covering a distinct facet of the inquiry. A methodical and thorough investigation of the major elements pertaining to online entertainment advertising and consumer behaviour was made possible by this framework.

3.3.Sampling

The necessity of sampling was determined by practical and logistical factors, considering the difficulties of researching the complete population. This method made the process of gathering data more focused and controllable.

3.4.Data Collection

3.4.1. Rationale for Primary Data

To gather verifiable proof of the influence of online entertainment advertising on consumer behaviour, primary data gathering was chosen. In this instance, the managers of the chosen stores—Lidl and IKEA—were important players.

3.4.2. Participants

As part of the data gathering procedure, six participants—more precisely, the managers of Lidl and IKEA—were assessed through interviews.

3.4.3. Data Collection Process

Seven days were dedicated to gathering the data. Whatsapp was used for the online interviews, with one participant meeting each day. This meticulous process made it possible to collect data in an organised and thorough manner

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4. DATA ANALYSIS AND INTERPRETATION

Table 4.1: Participant demographic data

Participant	Age	Gender	Education Level	Experience (Years)
P1	35	Male	Bachelor's	8
P2	40	Female	Master's	10
P3	32	Male	Bachelor's	6
P4	45	Female	PhD	12
P5	38	Male	Master's	9
P6	36	Female	Bachelor's	7

The study's participants are diverse in terms of their age, gender, level of education, and work experience. The range of ages, which includes a mix of professionals in their mid-career, is 32 to 45. There is a balanced distribution of genders, with three men and three women. Though there are differences in educational attainment, most have advanced degrees (PhD or Master's). With a range of 6 to 12 years of professional experience, the participants are an experienced group. With a focus on Lidl and IKEA in particular, this diversity is useful for gathering a wide range of viewpoints on how online entertainment advertising affects consumer behaviour in the Finnish retail industry.

Table 4.2: Average Scores and Synopsis

Question	Average Rating
Impact of Online Ads	4.17
Integration Effectiveness	4.17
Tracking Challenges	3.17

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Overall Satisfaction	4.17

The average rating given by participants was continuously excellent, highlighting the benefits of online advertisements and their efficacy in integrating them. Tracking difficulties, on the other hand, scored rather lower, suggesting room for development. Overall, the survey indicates that shop managers have a positive opinion of online entertainment advertising, and there is room to improve tracking systems in order to raise satisfaction even more.

Table 4.3: Answers to Likert Scale Questions from Participants

Participant	Q1: Impact of Online Ads	Q2: Integration Effectiveness	Q3: Tracking Challenges	Q4: Overall Satisfaction
P1	4	5	3	4
P2	5	4	2	5
P3	3	4	4	3
P4	4	5	3	4
P5	3	3	4	3
P6	5	4	2	5

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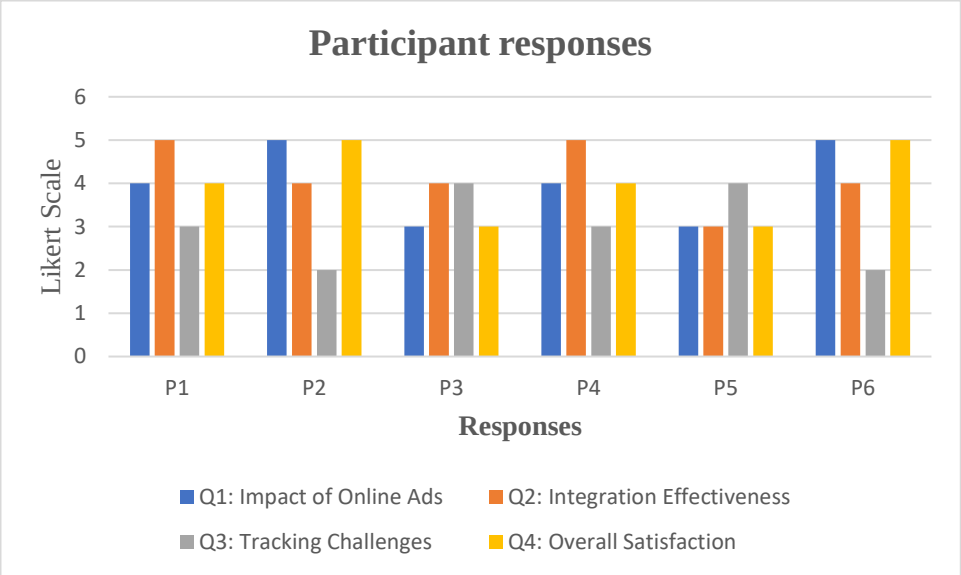

Figure 4.1: An illustration of the responses from the participants

With average ratings of 4.17, participants consistently assessed the effectiveness of integration and the impact of online marketing favourably. With a somewhat lower score of 3.17, tracking challenges should be improved. The high level of overall satisfaction indicates that, in spite of certain difficulties, individuals were generally happy with how online entertainment advertising influenced their purchasing behaviours.

5. RESULTS AND DISCUSSION

By examining the nuances of the data, the study sought to clarify the complex relationships between online entertainment promotion and consumer behaviour. Determining how these digital promotions affect consumer preferences and purchase decisions was the main goal. Through the use of a thorough methodology and content analysis tools, the study was able to extract insightful information from in-depth interviews with six respondents who were carefully selected.

Carefully examining participant demographics was a crucial aspect of the research, guaranteeing a varied and comprehensive representation. Age, education, and professional backgrounds were among the socioeconomic factors that were methodically examined. The results of this analysis showed that the participants were a diverse group of people, middle-aged and older, and in leadership roles in their various organisations. Remarkably, most of them were undergraduates, and only a small number were doctoral students.

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The inquiry gained depth and context from the participants' significant work experience, which ranged from two to twenty years. This careful segmentation made it possible to thoroughly examine a wide range of viewpoints, which improved our comprehension of the complex interactions between online entertainment advertising and consumer behaviour. The results provide light on potential ramifications for business practises and advance a more comprehensive understanding of the complex ways that virtual entertainment promotions affect consumer decisions and preferences in the retail sector.

6. CONCLUSION

To elaborate, this study highlights the significant impact that online entertainment promotion has on consumer behaviour in the retail industry. The complex dynamics at work are clarified by the insights gained from the thorough examination of the qualitative material as well as the one-on-one interviews with the six carefully chosen respondents. Carefully examining participant demographics showed a patchwork of viewpoints influenced by a variety of variables, including age, education, and work experience. Crucially, the research demonstrated a strong correlation between online entertainment advertising and how it affects consumers' tastes and critical purchasing decisions.

Considering the strategic positions that the participants held—all of whom were in leadership roles with varied degrees of professional experience—the relevance of these findings becomes even more apparent. Their extensive expertise, which ranged from two to twenty years, helped to provide a comprehensive grasp of the topic and made it possible to investigate the ramifications of online entertainment advertising in the retail industry from all angles. Through the process of deciphering the intricacies of consumer behaviour, this research provides retailers with useful information that they can utilise to enhance their web-based entertainment advertising tactics, which in turn improves sales performance and cultivates repeat business. These findings provide current and useful insight for firms looking to handle the dynamic convergence of online entertainment advertising and consumer behaviour as the retail landscape continues to change in the digital era.

ISBN - 978-93-95060-79-0

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ISBN - 978-93-95060-79-0

AN EXAMINATION ON THE IMPACT OF SOCIAL MEDIA ON BRANDS AND STRATEGIES FOR REPUTATION MANAGEMENT

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Abstract

This study investigates the information processing limits of social media users and assesses the impact of information speed on their behavior, focusing on susceptibility to social contagions. A random sampling technique was employed in Higher Education institutions, resulting in 200 respondents from diverse educational backgrounds. Primary data were collected through questionnaires, and statistical tools, including, percentage analysis, and Multiple Regression were applied for analysis. The demographic breakdown highlighted a balanced gender representation, with 55% male and 45% female respondents. Age-wise, the study revealed a substantial relationship between age and multimedia impact, emphasizing the growing influence with age. The sample pool exhibited diversity in educational qualifications and faculties. The subsequent analysis explored the influence of social media and multimedia on students based on gender and age. While gender showed no significant impact, age emerged as a crucial factor. Multiple stepwise regressions affirmed the substantial impact of social media and multimedia on students.

Keywords: Social Media, Multimedia, Information Processing, Susceptibility, Higher Education, Demographics, Educational Impact, Regression Analysis, Digital Literacy, Interdisciplinary Education

1. INTRODUCTION

Social networking is growing quickly and turning out to be more common in each part of life, including schooling. Teen understudies are the ones that are generally powerless to the adverse consequences of effectively accessible social media. Understudies experience extraordinary interest, major areas of strength for a for freedom, and a gigantic inner self during puberty. Therefore, numerous understudies express their objection or even inside and out defiance to a school, local area, or home decide that they accept is inconsistent with their own convictions. A youngster's improvement is impacted by a large number of variables, including family, school, and local area.

Unhindered admittance to social media will influence students' personal development. There is a conflict between them on the off chance that the goals are truly particular. The aftereffects of Sheldon's exploration show that social media influences conduct shifts in the two guys and females. Adolescent understudies are significantly affected by the world, particularly by social media that is unreservedly and promptly accessible on the web.

Social networking does, from one viewpoint, valuably advance the requirements for offices and self-improvement. On the other hand, however, it likewise represents a serious gamble because of the possible outcomes. The review reaches the resolution that albeit social media stages like Facebook and You Cylinder have an essential impact in the existences of teen understudies, they could likewise meaningfully affect their schooling. In this manner, adequate information on the standards of social networking development should empower understudies to involve these apparatuses for their own personal growth.

The utilization of social networking destinations is very normal in India. As a matter of fact, increasingly more undergrads are baited to these networking destinations since they give diversion and association open doors. This hence lays out the setting for additional examination concerning this particular occasion. In fact, relational associations act as the foundation of human development and training. Thusly, in the Indian setting, it is basic to look at the degree to which social networking locales are affecting understudies' relational associations.

ISBN - 978-93-95060-79-0

1.1.Social Media's Commitment to Society

It is a public gathering for emergency help and security data. There have been a few late misfortunes when social media was the main functional method for contact. While many individuals were without power during Super Tempest Sandy, some WiFi towers and cell networks kept on working. Many took to Facebook to depict the harm and console friends and family — or perhaps dear companions — that these were protected. The terrible Boston Long distance race bombings this previous week ignited a worldwide social media development to help individuals out of luck and to share data. As per Advertisement Week, Twitter truly gave an advanced hash tag, #One Boston, worth an expected $200,000, to help the people in question. All the more significantly, social media stages like Twitter have demonstrated to be valuable for neighbourhood occupants as a safety effort. The Boston Police Division tweeted the latest data on areas to stay away from, wounds, and losses of life. Police encouraged guys to utilize social networking locales for the purpose of correspondence, probably with an end goal to keep telephone lines open.

It is an extremely valuable instrument for examining violations. As a matter of fact, police organizations — including the New York Police Division — are starting to investigate violations on social media that are associated with possess. As of late, more than sixty gangsters who flaunted about their crimes on social media were captured by the NYPD. On the off chance that Oregon were some other state:

1. It is a type of influence over governmental issues. Individuals presently have a genuine voice thanks to social media. Simply last month, while the High Court was hearing issues including same-sex marriage, a social media development supporting for marriage uniformity picked up speed. As a matter of fact, brands embraced the pattern by making symbols of white correspondence that were pertinent to them. Social media has the ability to impact political choices and is a strong problem solver. Albeit the High Court hasn't gone with a choice in the continuous conversation, the judges have thought about the public's sponsorship.

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2. It's the best method for associating over significant distances to yet. Most of us can reconnect with loved ones who live far away without burning through cash on global calls. I could reach out to individuals I met in Australia and South Africa once more. I take a gander at their photos and get a brief look into their lives, so it's useful to have a method for staying aware of their updates despite the fact that I can't truly be there. It is the ideal expansion to email and Skype, among different types of correspondence.

1.2.Platforms of Social Media

When we think of social platforms, Facebook, LinkedIn and Twitter are actually likely the most typical labels. But you will find a huge number of various other sites are existed. At this time there are actually five kinds of social networking including Collaborative projects (Wikipedia, Delicious, and so on), Blogs, Content Communities (Book Crossing, Flickr, YouTube, Slide share, etc.), Social networking sites, Virtual games, Virtual social worlds. Virtual world enables individuals to choose the conduct of theirs in an essential and free fashion therefore following a virtual way of life much like their true life

You will find numerous types of social networking platforms. Social networking includes web and mobile based technology which allow & encourage individuals to work together with any person they really want by making profile and exchanging contents with one another. Accordingly below are numerous types of social networking platforms that are revealed:

a) Social Networks: Social networking sites provide internet services to users to create the own profile of theirs, private portfolios & interests. These profiles as well as pages are actually used to connect with friends, other people and co-workers to be able to discuss combination, content, and media of the. They create interactions with various other people by posting status, commenting on other's status, sharing links as well as pictures or perhaps by some other multimedia. Examples of social networking sites consist of Bebo, MySpace, Google+, LinkedIn, and Facebook.

b) Web blogs: Blog is actually probably the oldest & most popular types of social networking. Blogs are usually

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considered as internet journals that arrange content chronologically or perhaps by date, month, category as well as year. A Blog is a site, in which blog owner write posts and posted/publish for the individuals to read. We are able to likewise produce video or "vlogs," blogs, which feature shared or maybe homemade video clips. Blogging sites include Tumblr, Blogger, and Word Press.

c) Micro-blogs: Micro-blog options brief posts with words that are not many; it's opposite to journal style posts. Generally, computer users are limited while posting a number of lines of text, or perhaps uploading images and videos. Info very easily. Micro-blogging is extremely well known for publishing fast updates as well as in order to distribute content via mobile devices. The popular micro blogging sites include Tumblr, Pinterest, and Twitter.

d) Content Communities: Images as well as movies are actually organized, shared as well as commented upon by the content material communities" users. Examples of those content communities are scribd, Flickr, and YouTube.

e) Collaboration: Wiki and Wikipedia is the ideal example of social awareness or cooperation. These sites let a community of individuals to include as well as modify content in a community based database. It produces a hub of expertise to make use of as reference by others. For instance: Answers.com, Quora, Wikis, and Wikipedia.

f) Geo location: This kinds of social networking is actually related with mobile device and it establishes a user's location. , for instance, individuals go someplace and they need the friends of theirs to find out about the area of theirs, consequently, they make check in. A favourite example of geo location sharing is 4 square. Facebook additionally presents this check in facility to the users of theirs.

2. OBJECTIVES OF THE STUDY

- To comprehend the current concept of social media and multimedia.
- To examine the requirements, advantages, and disadvantages of social media and multimedia.
- To assess the impact of social media and multimedia on students.
- To investigate the relationship between the usage of social media

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and multimedia and students' academic performance.

3. LITERATURE REVIEW

(Habes and Partners, 2018) planned to reveal insight into the impacts of social networking on understudies going to additional illuminating colleges and what such impacts mean for their scholastic accomplishment. Because of its capacity to fortify the beneficiary's convictions and advance their patterns and cognizance, social media has become one of the most generally utilized types of correspondence. The review centres around the sites that the specialists checked and noticed, as well as how the sites impacted the worldwide and Center Eastern Facebook market. To discover Facebook's effect, the specialists utilized a trial and similar procedure, introducing their discoveries in light of suggestions and the ends drawn from peer-audited logical exploration distributed in diaries. As per the review, an enormous number of understudies utilize social networking destinations, with a specific accentuation on Facebook, which adversely affects their scholastic performance.

(Akakandelwa et al. 2018) The manner in which understudies impart, associate, and socialize has been altered by social media, which has ended up being practically undeniable and an essential piece of their social and social texture. Along these lines, a lot of understudies' time is spent on social networking. Youngsters and college understudies are believed to be the frameworks' greatest proprietors. There is a developing group of examination on social media use around the world, yet bit of it centres around what social media use means for understudies' social lives in Zambia. This study was exploratory and distinct in nature. It took a gander at the kinds of social networking destinations that understudies frequently use, how long they spend on them, why they use them, and what utilizing social media means for their social lives. 240 full-time understudies in the College of Zambia's Institute of Schooling finished up self-regulated polls, and the Measurable Bundle for the Social Sciences (SPSS) release 23 was utilized to break down their responses. As indicated by the outcomes, What Sapp was the most well-known social networking stage. A large portion of the understudies who were examined said they routinely spent somewhere in the range of thirty and an hour utilizing this particular social networking application. The investigation

ISBN - 978-93-95060-79-0

likewise discovered that the greater part of the understudies said they dealt with social media for only a couple of additional minutes than needed, checked their social media accounts prior to doing anything more, accepted social media harmed their scholastic performance, couldn't decrease how much time they spent on social media, heard analysis about their utilization of social media from others, and felt worried because of utilizing it. Besides, 22.4% of the understudies said they were devotees of social media. More social data was shared on social networking locales by understudies than scholarly data was. Understudies' use of social media truly improves social association. Along these lines, involving social networking in a powerful way while decreasing any adverse consequences is normally encouraged. Besides, it is trusted that our review would animate new examination in this space utilizing bigger and more assorted examples of changing understudy segment qualities.

(Waseem, 2018) Social networking is a gathering that permits individuals all around the world to examine their concerns and contemplations. Prior to finding out about the particular elements of social media, individuals need to get a handle on what's genuinely going on with social media. Actually, the expression "social networking" alludes to the manner in which people or gatherings draw in with each other through the creation, sharing, and once in a while trade of ideas, pictures, recordings, and substantially more over the web and in web-based networks. Since cell phones and intuitive social networking sites like Facebook, MySpace, Twitter, and Orkut are surrounding them, kids are genuinely growing up with social networking as a major piece of their lives. Social organizations are truly modifying the manners by which solid individuals associate with their friends, guardians, and use innovation. There are two impacts of social media. On the in addition to side, social media stages might be very useful devices for experts. They achieve this by helping recently graduated experts in displaying their abilities and seeking after business adventures. Social networking sites may likewise be utilized proficiently for networking. On the negative side, there are various perils associated with online networks on the web. One of the worries is cyberbullying, which alludes to badgering that is done by means of the utilization of electronic designing. We examine each feature of social networking in this

ISBN - 978-93-95060-79-0

exposition, including the two its benefits and disservices. Explicit regions like wellbeing, business, training, youth, and society are the concentration. This examination means to explain the exact manners by which different media will influence society on a wide scale.

The kinds of social networking locales that understudy use, how they use them, and what they mean for their way of behaving for correspondence, learning, social perspectives, and learning motivation are completely distinguished by **(Sriadhi, 2018).** This particular examination, which involves senior secondary school understudies as the respondents, was completed in Medan, Indonesia. The example, which comprises of 184 S.TI and 48 S.RI, is picked aimlessly. Polls, perception, and meetings are utilized to assemble data, and distinct insights are utilized for examination. That's what the examination's decision shows, in spite of the fact that Twitter, Instagram, Wire, and WeChat are truly consumed in a lot more modest sums, the most frequently utilized web crawlers are Google, YouTube, WhatsApp, and Facebook. The typical measure of time spent on social media is 11.31 hours out of each day, with social and diversion targets probably positioning top, while learning and experiences goals rank at 6th and third, separately. Truth be told, SRI understudies utilize social networking more habitually for examining than STI understudies do. The development of learning exercises in spite of a decrease in learning design is one of the noticed direct changes. While the recurrence of commitment builds, its particular quality downfalls. Mental control declines while compassion and responsiveness ascend concerning social way of behaving. As per this particular exploration, it's basic to lay out companionships and tutor kids in utilizing social networking, so risks might be changed over into potential open doors for Indonesia's more youthful ages.

(Calderaro, 2018) Since the web was created, there has been a lot of interest in whether and how computerized stages, like social networking, affect governmental issues. Subsequently, we can now depend on a developing corpus of examination that looks at the numerous associations between social networking and legislative issues from various points. The responses set forth to research the subject are frequently particular, don't necessarily in every case connect with each other, and lead the assessment of its circumstances and end results down assorted streets,

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much of the time prompting totally various outcomes. Nonetheless, it is recognized that the web has made new roads for correspondence that have definitely had an impact on how data is dispersed in contrast with conventional media. News streak ventures far past public lines and into social, political, and social specialty markets thanks by and large to social media. This section analyzes how the ongoing conditions raise political cognizance. Considering this, this part examines whether and how much social networking impacts governmental issues. In explicitly, we initially examine how the web's organized attributes empower different types of political action and how the presentation of social networking has additionally exacerbated this specific issue. Subsequent to zeroing in on the job that data course plays in advancing political mindfulness and, thus, expanding political cooperation, we talk about how social networking improves this methodology and builds the effect of computerized stages on governmental issues. At long last, we give proof to the effect of social networking on governmental issues by analyzing the basic job that data course plays in the political field across political scenes, political entertainers, and political strategies. We explicitly center around the utilization of social networking for crusading and for the preparation of social developments.

(Al-Abbas, 2017) Understudies can improve their way of learning and advantage from a few scholastic advantages given by social media and the web. Looking at the effect of utilizing social networking on scholarly accomplishment is the primary objective of this specific examination. Since current social media stages like Instagram, Facebook, Twitter, and such could influence understudies' way of behaving and scholastic performance, Ahlia College, Applied Science College, and College of Bahrain were the colleges that were picked. 150 understudies were remembered for the example, uniformly split across the three colleges. The exploration questions plan to address the accompanying: (1) How have ICTs and the Web advanced internationally?; (2) What effect has ICT had on training?; (3) What are the particular impacts of social networking on understudies' scholastic performance at Bahrain Colleges?; and (4) Which social organization sites are more well known among Bahrain College understudies? The discoveries show that social media

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decidedly affects scholarly accomplishment and that 57% of understudies use WhatsApp as a portable social networking instrument for their scholastic objectives.

(Masood et al. 2017) to survey a speculative social organization type with applications for an example of 34,896 Abu Dhabi schoolchildren, this review utilized underlying condition demonstrating. The really free parts in this unit are connected with kids' mentalities towards social media, their inspirations for utilizing social media stages, the assignments they achieve on these destinations, and the subjects they use. Seen social ramifications and social media's effect on scholastic accomplishment are covered by the reliant builds. The review will frame the connections between the different develops. Analyzed is the aftereffect of a few different factors, like parental mastery. Our review has improved our grasping in the social organization adaptation. The discoveries give belief to the possibility of equal connections between social media results, gaining from social media, and saw performance.

4. RESEARCH METHODOLOGY

This section outlined the research design and methodology adopted to investigate the information processing limits for social media users and the impact of information speed on their processing behavior.

4.1.Sampling

The study employed a random sampling technique in Higher Education institutions to select respondents in Maharashtra states. Random sampling ensured that each unit of the population had an equal chance of inclusion in the sample, promoting unbiased representation. A sample and pilot study involved 50 students from city colleges, with a total of 200 respondents (students) selected for the main study.

4.2.Procedure of Data Collection

The data collection process commenced after defining the research question and designing the research plan. Primary data, collected afresh for the study, were gathered through

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questionnaires issued to 200 respondents. The respondents, representing various educational institutions, were selected using convenience sampling based on accessibility and approachability.

4.3.Data Collection Tools

Questionnaires were distributed personally after obtaining permissions. Out of the 228 collected questionnaires, 200 were completed. However, only 200 questionnaires were used for analysis due to incomplete or defective responses

4.4.Tools used for Data analysis

The collected data underwent comprehensive statistical analysis, employing a range of sophisticated tools to extract meaningful insights. Percentage analysis facilitated the expression of proportions and trends, providing a quantitative perspective on key aspects of the data.To assess variations among groups . Multiple regression analysis was employed to assess the intricate relationships between multiple independent variables and a dependent variable, unraveling the nuanced interdependencies within the data.

5. DATA ANALYSIS AND INTREPRETATION

In this particular section an effort has been created to look at the perceptions and furthermore, the views of the respondents about the various aspects of social media as well as multimedia. The following section offers the evaluation of Impact of Social media as well as multimedia on the Students.

Table 1: Gender wise distribution of Respondents

Particulars	**Number of Respondents**	**Percentage**
Gender		
Male	110	55%
Female	90	45%
Age		
Up to 21 Years	28	14%
21 - 23 Years	44	22%
23 - 25 Years	72	36%
More than 25 Years	56	28%
Educational Qualifications		

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UG	80	40%
PG	40	20%
Professional	20	10%
Research	60	30%
Faculty of different department		
Faculty of science	60	30%
Faculty of commerce	40	20%
Faculty of humanities	80	40%
Faculty of engineering	10	5%
Faculty of medicines	10	5%
Medium of Instruction		
Hindi	120	60%
English	80	40%

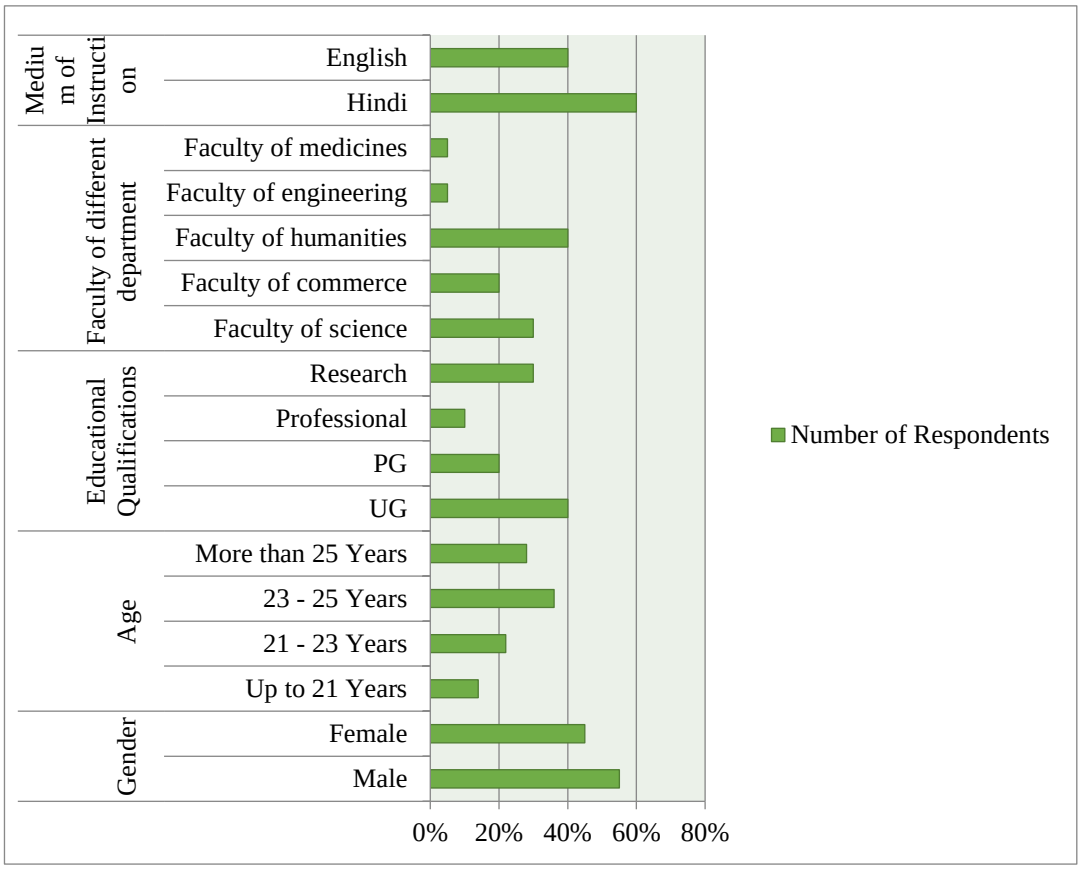

Figure 1: Gender wise distribution of Respondents

Table 1 provides a comprehensive gender-wise distribution of respondents, showcasing a balanced representation in terms of gender. Among the 200 respondents, 110 identified as

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male, constituting 55%, while 90 identified as female, representing 45% of the total sample. The gender distribution reflects a relatively equitable participation of both male and female respondents in the study. Moving on to the age demographics, the respondents were categorized into different age groups. The age group "Up to 21 Years" comprises 28 individuals, constituting 14% of the sample. Those aged "21 Years to 23 Years" represent 44 respondents, contributing to 22% of the total. The age group "23 Years to 25 Years" is the largest, consisting of 72 individuals, or 36% of the respondents. Additionally, "More than 25 Years" encompasses 56 respondents, making up 28% of the total sample. Educational qualifications reveal a diverse participant pool. Those with an undergraduate degree (UG) make up 40% of the respondents, totaling 80 individuals. Respondents with postgraduate degrees (PG) constitute 20%, amounting to 40 individuals. Professional qualifications are held by 20 respondents, contributing 10%, while those involved in research activities make up 30%, with a total of 60 individuals. The distribution of faculty across different departments showcases the interdisciplinary nature of the study. The faculty of science comprises 30% of the respondents (60 individuals), followed by the faculty of commerce at 20% (40 individuals). Faculty from the humanities department represents the largest proportion, constituting 40% with 80 individuals. Engineering and medicine faculties each contribute 5%, with 10 individuals in each category.

Table 2: Influence of Social Media and Multimedia on Students: Exploring Gender Dynamics

Gender	**M**	**F value**	**t value**	**P value**
M	62.3934	20.523	3.263	.312
F	64.9363			

*At 1% level of significance **At 5% level of significance

The consequences of the understudy "t" test show how test respondents felt that the effect of social media and multimedia on an understudy's worth was not huge for some random orientation class. The mean scores for men (62.3934) and ladies (64.9363) show this. These

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discoveries show that undergrads' perceptions of male and female perspectives in multimedia are something similar. The previously mentioned discoveries propose that there is no connection between's the effect of social media and multimedia on understudies' orientation.

Table 3: Relationship between Age and the Influence of Social Media and Multimedia on Students

Age	**M**	**F value**	**P Value**
Up to 21 Years	42.363		
21 - 23 Years	43.555		
23 - 25 Years	46.396		
More than 25 Years	49.363	8.079	.000*

The table above shows the obvious contrasts in understudies' age bunches as well as the effect of multimedia and social media. As respondents progress in years, they use multimedia all the more every now and again, and studies have shown that the effect of social media and multimedia on understudies is altogether affected by respondents' age. For instance, Euro Screen (2007) found that with regards to PC installments, progress in years really impacts a lady's financial plan than her financial class. This exploration was directed in the Unified Realm.

In his "PC" examination, Mintel (2008) found that the 20-24 and 25-34 age bunches are actually very important to advertisers since ladies are less worried about PC quality than variety.

An examination by Van Ravenswaay and Hoehn (1991) named "The Effect of multimedia purchase of electric devises: A Contextual investigation of Apples" found that more established consumers looked for less pesticide-buildup free apples, which adversely affected

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the market for open apples. Be that as it may, Cheerful (1991)4 tracked down no connection between the period of movable item and buys..

5.1.Multiple Step Wise Regression

Multiple regression analysis is actually a statistical method which may be utilized to evaluate the connection between an individual reliant (criterion) variable and many independent (predictor) variables. The goal of multiple regression analysis is actually using the impartial variables whose values are actually recognized to foresee the single dependent value selected.

Table 4: The model summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.712[a]	.512	.523	7.58537

The item synopsis for the R, R2, changed R2, and ordinary mistake of the assessment are shown in the table above. The level of variety in the model (subordinate) variable that can be made sense of by the direct mix of indicator (autonomous) factors is shown by the R2 esteem. The thing has a R2 of 0.512. This shows the variety made sense of by the straight mix of patient fulfillment and assumptions..

Table 5: ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	20105.639	1	20105.639	349.433	.000b
	Residual	26179.718	199	57.538		

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Total	46285.357	199			

Table 6 : Coefficients

Model	**Unstandardized Coefficients**		**Standardized Coefficients**	**t**	**Sig.**
	B	**Std. Error**	**Beta**		
(Constant)	19.365	1.975		9.806	.000
Impact of social media and multimedia of college students	.679	.036	.659	18.693	.000

The table shows that the 3 models are considerable. The' F-value' because of this unit is statistically significant. Hence, the above mentioned cases and this may be concluded that, there's substantial impact the connection of Impact of social networking as well as multimedia of college students. The sub variables of Impact of social networking as well as multimedia of college students are highly correlated, monetary factor extremely considerably impact one variable to the next variables

6. CONCLUSION AND RECOMMENDATIONS

The comprehensive analysis of the data collected from 200 respondents sheds light on various dimensions of social media and multimedia usage among students. The gender-wise distribution revealed a balanced participation, with 55% male and 45% female respondents. Age demographics indicated a notable reliance on multimedia as age increased, emphasizing its significance in the lives of students. Educational qualifications displayed a diverse participant pool, reflecting the interdisciplinary nature of the study across faculties. The preferred medium of instruction, with 60% opting for Hindi and 40% for English, showcased

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linguistic diversity among the respondents. Ensuing exploration on the impacts of multimedia and social media on understudies — considering age and orientation — delivered a few fascinating outcomes. The aftereffects of the examination showed that sentiments about what social media and multimedia mean for understudies were not significantly impacted by orientation. Yet, age ended up being a critical issue too; there is areas of strength for an among age and the amount of an effect multimedia has on children. There was a more grounded inclination for more seasoned respondents to utilize multimedia. The impact of multimedia and social media on understudies was affirmed by different stepwise relapse investigation, which delivered a R2 worth of 0.434, which addresses the extent of variety made sense of by the direct blend of indicator factors.

Recommendations:

- Tailored Educational Initiatives: Institutions should consider incorporating multimedia elements into educational programs to cater to the varying preferences of students. Tailored initiatives can enhance the learning experience and engagement levels.
- Age-Specific Strategies: Recognizing the influence of age on multimedia usage, educators and policymakers should develop age-specific strategies to optimize the benefits of social media and multimedia in education.
- Inclusive Language Policies: Acknowledging the linguistic diversity among respondents, educational institutions should adopt inclusive language policies to accommodate students' preferences and promote effective communication.
- Continuous Research and Adaptation: Given the dynamic nature of social media trends, institutions should engage in continuous research to stay abreast of evolving patterns. Flexibility and adaptability in educational approaches can ensure relevance and effectiveness.

ISBN - 978-93-95060-79-0

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ISBN - 978-93-95060-79-0

A NAVIGATION THROUGH THE COMPLEX LANDSCAPE OF GLOBAL CLIMATE CHALLENGES AND OPPORTUNITIES

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ABSTRACT
This research examines the environmental, physiological, economic, political, and strategic factors that contribute to, and are affected by, global warming. It becomes clear that energy consumption plays a crucial role, with fossil fuel combustion emerging as the primary source (78%). Students' replies reveal a wide range of awareness at different academic levels, with Geography majors demonstrating a deep familiarity with environmental issues and Economics majors focusing on the broader socioeconomic context. Data will be collected through surveys and in-depth interviews to ensure a solid methodology and the collection of both quantitative and qualitative data. Findings stress the importance of coordinated efforts to effectively combat global warming's myriad consequences. These results provide important new information for the discussion of climate change and highlight the need for multidisciplinary efforts and a holistic perspective to achieve a sustainable and resilient future.

Keywords: *Global Climate, Challenges, Opportunities, Navigation, Climate Change*

1. INTRODUCTION

When referring to the world's rising temperature, the phrase "a dangerous atmospheric devation" alludes to the anomalies inherent in this phenomenon. This warming trend can be traced back to the widespread release of carbon dioxide and other byproducts of burning fossil fuels

ISBN - 978-93-95060-79-0

into the atmosphere. It's the reason why the planet's ecology changed so little in the last quarter of the 20th century. Human effort is the primary driver of this shift. Climate change, specifically a global increase in temperature, is the greatest threat to humanity in the twenty-first century. Damage to the eco-system, destruction of dry seasons, shifts in agricultural practises, soil and water pollution, a decline in biodiversity, and other negative outcomes have caused widespread concern around the world. The degree of an unnatural weather change and its anticipated future have, as a rule, put human existence itself at considerable risk.

While a global temperature shift would have far-reaching effects, the immediate ramifications for developing nations would be devastating. Several papers presenting the evidence for a global temperature change have sparked political and economic debate. Poor regions, especially non-industrial countries, look to be most at risk from the predicted effects of a global temperature spike, although having lower emanations than the industrialised world. The threats have been correctly identified, and the world is now paying attention. Few western countries, most notably the United States, ignored the states of the deal, but the problems of an unnatural weather change genuinely exist and keep on representing a danger to life itself. As a clear difference to natural and logical concerns, environmental change has now turned into a socio-policy oriented issue.

1.2.Theory of climate justice

Several presumptions support the Theory of Climate Justice. -

1. The first is that the planet is in serious danger of being destroyed unless something is done to save her current living and life systems.
2. The second is that, while taking into account both the causes and consequences, all peoples and nations that make up the human society on earth should carry the burdens and duties for preserving the earth system.
3. Third, we need a new perspective that recognises "shared but differentiated responsibility" in order to reconceive of individual and collective human rights, as well as our concepts of moral obligation and legal liability.
4. Fourth, it's important to search for TCJs that aren't humanoid in appearance.

5. Fifth, TCJ ought to all the more straightforwardly address the general and cement other, while keeping up with

ISBN - 978-93-95060-79-0

common and pleasant ties with the methodology and languages of TGJ and TEJ. Concerns of migration, resettlement, and social and environmental exclusion are shared by all three, but they are distinct enough to warrant special attention from TCJ notwithstanding the stability of the Earth's planetary system as a whole. Towards this end, TCJ strategies should provide. Natural justice has not yet been granted to the victims of the Bhopal Gas Disaster.

The state of the environment was not a primary concern. Environmental justice is consequently not addressed in the Indian Constitution. Environmentalism refers to environmental protection as a philosophical, ideological, and social movement. According to this theory, growth should be conducted with the ecosystem in mind. All areas of progress should be made with environmental protection in mind. No sacrifices can be made to environmental safety. Climate Justice is a portmanteau of the words "justice" and "climate." The term "justice" covers a lot of ground. Justice refers to a state of fairness and equity. There have been various attempts at definition by legal theorists. They tried to define it, but failed. The most important reason for this is because everyone has a somewhat different idea of what constitutes fairness at any given moment. Justice as we know it today may not exist tomorrow, and vice versa; injustice in its current form may persist in the future. 69 To properly examine issues of environmental fairness, a triangular framework is required. The following are some examples: People Now, People from here on out, and Nature, People Prohibited. 70 Climate equity depends on the rule that the people who have caused and are causing global warming through ozone harming substance emanations ought to bear a portion of the obligation regarding the results of their activities. Entertaining thing is, "the polluter isn't paying" here. "Ecological equity (EJ) is the fair treatment and significant interest surprisingly paying little heed to race, variety, public beginning, or pay regarding the turn of events, execution, and implementation of natural regulations, guidelines, and strategies," said the Natural Security Office of the US.

1.3.Relation Between Heat Budget Components and Indian Summer Monsoon

The Asian summer monsoon is driven mostly by the temperature difference between the Indian Ocean and the

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surrounding land areas, as well as by numerous orographic causes. The storm is a sort of course that has a handful of unmistakable properties. Midyear lower tropospheric winds are directed away from the cooler oceanic regions on the side of the globe that experiences cooler temperatures throughout the year and towards the warmer land masses. In the high lower atmosphere, the stream is reversed, travelling from the warmer part of the planet to the colder half during the winter. Summertime precipitation is typically localised to one or the other side of the late spring solstice, and takes place within the storm box, a box of low strain that surrounds the warm landmass and the nearby seas and oceans. Most summer showers can be traced to brief disquieting factors that sweep in from elsewhere. However, these inconveniences are scheduled in 30-day increments. The "dynamic periods" of the storm are characterised by such unstable weather and copious amounts of rain. Intervals of calm weather between downpours are known as "rainstorm breaks." Most of the rain that falls in the tropics and oceans occurs in the Between Tropical Combination Zone (ITCZ), which is sometimes located far to the north of where the eye of a storm would make landfall.

1.4.Conceptual Framework Of Global Warming

As a result of the planet's ever-changing environment, the local natural process is always evolving. The confluence of this worldwide custom with Earth's chilly temperature was ultimately beneficial to the evolution of humankind. As the sun's rays pass through the stratosphere of the Earth, some of that energy is absorbed by the surface, while the rest is reflected back into space. If this process had been followed reliably, there would have been no change to Earth's atmosphere and no need for concern. The composition of many gases that ascend into the stratosphere, however, is shifting as a result of industrialization, population increase, spillover (land use change), logging, and alterations in people's way of life. Atmospheric gases such as carbon dioxide (CO2), nitrous oxide (NO), and methane (CH4) block some of the infrared light that would otherwise reach Earth's surface. The infrared rays are absorbed and reflected back down into Earth's atmosphere. It is possible that this gas-tight system is used in greenhouses to enable the existing flora benefit from the heat provided by the

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trapped gases and grow more quickly. Since the process contained is similar to what is done on purpose in a greenhouse, the term "Greenhouse Effect" has been coined to describe the impact of such collisions on the Earth's atmosphere. The global mean temperature or the temperature of the world rises as a result of the greenhouse effect. The term "global warming" is used to describe the overall trend of increasing global temperatures throughout time.

1.5. Global Warming Nexus With Climate Change

Increases in normal air and sea temperatures, a super-broad softening of ice sheets, and an expansion of ocean levels are all evidence that an unnatural weather change is occurring at a disturbing rate, as stated in the Fourth Evaluation Report on Environmental Change Union Report, which was delivered by the Intergovernmental Board on Environmental Change in 2007. The Third Evaluation Report of the Intergovernmental Panel on Climate Change was released in 2001 and depicted environmental change that was not "typical." The term "environmental change" refers to long-term shifts in the Earth system's environment that have been mostly caused by human activity, or more specifically, by a globally welcomed increase in temperature. Article 1 of the UN System Show on Environmental Change (UNCCC) defines "environmental change" as "adjustment is inferable straightforwardly in any case not straightforwardly to individual activities such changes structure of the worldwide air and that has inside added to the regular environment variability observed throughout practically identical time periods."

2. LITERATURE REVIEW

Vardy et al. (2017) Evaluations of climate science directed by the Intergovernmental Board on Climate Change (IPCC) are strategy pertinent yet not approach prescriptive. In this article, we talk about the IPCC's (Intergovernmental Board on Climate Change) (IPCC) job as an intergovernmental association depended with making global natural appraisals (GEAs), as well as a portion of its significant plan qualities, accomplishments, and challenges. These incorporate the method involved with managing consent to break down and sum up climate research and the necessity to coordinate aptitude from whatever number of the 195 IPCC country states as could be

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expected under the circumstances, despite the primary awkward nature among created and agricultural nations. We remember contextual analyses for the legislative issues of climate denialism; the utilization of geoengineering in relief situations; and the connections between versatile limit, transformation, and global advancement to feature remarkable highlights that are novel to the IPCC yet offer examples for different associations that direct GEAs. At long last, we discuss institutional reflexivity to wrap things up. We examine how the IPCC might act as a good example for a mindful and comprehensive way to deal with climate change by exploring the complicated connection among science and governmental issues and being open about it.

Collins et al. (2018) Prescient comprehension of environmental and maritime dynamical cycles is restricted, notwithstanding its centrality to recognizing the huge scope reasons for provincial climate change. We feature the reaction of tempests, blocks, and fly streams to outside compelling; bowl to-bowl and tropical-extratropical teleconnections; and the advancement of non-direct prescient hypothesis as three forefront challenges in climate elements where huge headway can be made to illuminate transformation. We feature prospects and techniques for making fast headways here, which are critically associated with the production of high-goal coupled model recreations, halfway coupling or pacemaker tests, the creation and utilization of dynamical measurements, and the double-dealing of progressive systems of models.

Sultana, F. (2018) Global water weakness is deteriorating a result of climate change, and this significantly affects ladies. The techniques wherein individuals, networks, and countries are answering ebb and flow and future climate change are significantly affected by shifts in water accessibility, access, shortage, and security. The impacts of climate change are not consistently felt across financial gatherings, in spite of the way that it is now making many individuals more powerless, underestimated, and languishing. The impacts of climate change are felt and answered in various ways by various individuals because of crossing points of social contrast, most prominently along orientation and financial lines. Climate change is projected to demolish environmental corruption (like water

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deficiencies) and water-related catastrophic events (like floods, typhoons), consequently altering orientation water geologies, which is particularly clear in water-related useful and conceptive undertakings. To work on the nature of scholastic discussions and policymaking, it is currently important to lead multi-scalar, basic, and interconnected investigations. As man centric standards, disparities, and imbalances frequently place ladies and men in separated positions in their reactions and survival strategies to emotional changes in socioecological relations and modifying waterscapes, it is essential to focus on the gendered ramifications of climate change. This is particularly obvious in light of the fact that it features the perplexing manners by which social power relations work in collective reactions to variation techniques, which are multiplying all over the planet. This part digs into the convergence of orientation, water, and climate change to show how different networks decipher, respond to, and adjust to climate unusualness and vulnerability.

Apollo & Mbah (2021) The reality of climate change cannot be denied, and its negative effects threaten to roll back efforts to achieve sustainable development targets. The global issue provides one of the most serious challenges to eliminating poverty and existing disparities, especially in emerging nations that are anticipated to be extremely exposed to climatic variability. However, the opportunity for successful adaptation and mitigation of climate change through the acquisition of information and skills, and subsequently positive behavioural change, is largely unexplored in the education sector. In particular, education can equip people and groups to respond to climate change in a way that is both adaptive and sustainable. The focus of this research is on East Africa, a region whose economy is very sensitive to climatic factors. East African countries are currently including lessons on climate change into their educational programmes. However, there is a dearth of unified strategy for incorporating climate change education into adaptation and mitigation efforts. As a result, this analysis delves into some of the most pressing challenges facing efforts to educate the public about climate change, as well as some promising avenues for doing so.

Høye, T. T. (2020) Arctic arthropod research has significant obstacles, including the region's extreme

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environmental conditions, its sparse human infrastructure, and a lack of even the most fundamental autecological knowledge. However, the Arctic presents a rare and underutilised opportunity to study species' responses to environmental change and to put ecological theory to the test due to its rapid climatic change, limited species richness, and strong collaborative networks. In this article, I discuss the impacts of climate change on Arctic arthropods at the population, community, and ecosystem levels. Temperature tolerance, life cycle diversity, population dynamics, community make-up, diversity, and biotic interactions are my main areas of study. Since there are so few species in the Arctic, it is the perfect place to try out cutting-edge, automated arthropod monitoring techniques. Insights about the causes and consequences of climate change for arthropods around the world may also be gleaned from the Arctic.

Liu et al. (2022) Right now, China delivers the most CO2 into the environment. In this way, China has a significant impact in the battle against global warming. Decarbonization can't happen without the fundamental approaches and responsibilities. We give an outline of the principal qualities of China's CO2 discharges, its decrease methodologies, and its accomplishments in satisfying climate focuses in this Point of view. China has taken huge steps in decreasing its CO2 emanations, with carbon power falling by 48.4% somewhere in the range of 2005 and 2020. This meets the objectives set out in the nation's Broadly Fitting Moderation Activities and Broadly Resolved Commitments. Accomplishing the sectoral and subnational targets laid out in China's Five-Year Plans is fundamental to accomplishing these decreases. Accomplishing carbon nonpartisanship by 2060 and arriving at its pinnacle all out CO2 discharges before 2030 stay impressive obstructions for China. Expanding China's dependence on non-fossil energy sources, executing inescapable negative-emanation innovation, empowering local low-carbon improvement, and sending off a public "green market" are fundamental stages towards the country's objective of carbon lack of bias. To accomplish these stages, hierarchical financial advancement techniques should concur with base up monetary motivating forces and innovation improvement.

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Sillmann et al. (2017) The World Climate Exploration Program (WCRP) has picked climate and climate limits as one of its Fabulous Challenges on account of the significance they are believed to have in facilitating how we might interpret the climate. Here, we give an outline of current challenges and opportunities for logical advancement and cross-local area cooperation on the subject of understanding, demonstrating and anticipating outrageous occasions in view of a specialist studio coordinated as a feature of the execution of the WCRP Great Test on Climate and Climate Limits. Notwithstanding irregular possibility, the probability of an outrageous occasion creating relies upon variables like the accessibility of enormous scope drivers and positive nearby inputs. Thus, we develop the logical troubles related with serious occasions' global drivers and neighborhood to-territorial criticism processes. Worked on outrageous climate forecast and cycle based assessment of the portrayal of climate and climate limits in climate model recreations are both potential results of a more profound comprehension of the causes and cycles at play. Moreover, we inspect how to meet these impediments by breaking down the components and strategies used to assess and anticipate both brief span (under three days) and long-length (weeks to months) outrageous events.

3. RESEARCH METHODOLOGY

3.1. Objectives of the Study

1. To look into the causes and consequences of global warming, including a review of its contributing elements and underlying causes as well as an analysis of its broad implications on weather patterns, ecosystems, and sea levels.
2. To evaluate how climate change is affecting India's national security by identifying infrastructure vulnerabilities and dangers, as well as by looking into possible geopolitical ramifications and conflicts stemming from altered resource availability.
3. . To decide on Maharashtra's climate change adaptation plans, including identifying vulnerabilities unique to the region, creating workable adaption plans, and lessening the effects of shifting weather patterns.

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4. To evaluate the socio-economic effects of climate change on Maharashtra, looking at the financial and social toll it takes on certain businesses and communities, and suggesting ways to improve sustainable development and resilience in the face of climate-related obstacles.

3.2.Study Area

Maharashtra served as the research area because it is a melting pot of Indian culture and offered a rare chance to compile a variety of viewpoints on climate change. To provide their perspectives to the research, resource staff conducted structured interviews with a chosen group of subject matter experts on global warming.

3.3. Sampling

Undergraduate students pursuing bachelor's degrees from a variety of Maharashtran universities made up the study's universe and sample. In keeping with the research's focus on identifying the potential repercussions of climate change, the goal was to comprehend their perspectives on the effects of global warming. The sample, which comprised students enrolled in various programmes around the state, provided a thorough understanding of bachelor's level students' perspectives on this important topic.

3.4.Data allocation

This study's data came from primary as well as secondary sources. To gather primary data, specific survey and interview questions were created, and the survey questions were placed thoughtfully throughout the sample region. Key participants from the main sample, including subject matter experts on global warming, were interviewed to get detailed information that matched the research questions.

3.5.Methods

In this study, a variety of techniques was used to collect data, such as questionnaires and structured interviews. Four survey items were created with the intention of gathering the opinions of Maharashtra university students from various faculties. Appendix A contains the list of survey instruments that were used to collect data from forty students in each faculty. Structured interviews with competent specialists were also carried out, which enhanced the survey results and offered a more in-depth comprehension of the topic. Appendix B has a thorough account of these interviews.

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3.5.1. Primary data

Survey questions and structured interview questions made up the main data gathering instruments. The respondents, who were bachelor's degree candidates in a variety of subjects including political science, economics, geography, and strategic studies, were thought to be among India's better-informed populace. By using these tools, the researchers hoped to obtain a thorough and balanced picture of the chosen demographic's attitudes and understandings of the effects of global warming in the state of Maharashtra.

4. DATA ANALYSIS AND INTERPRETATION

4.1. Reasons for Earth's Warming

Table 4.1: Reasons for Earth's Warming

Causes of Global Warming	**Estimated Percentage Contribution**
Burning fossil fuels (coal, oil, and natural gas)	78%
Deforestation and land-use change	19%
Industrial processes and manufacturing	6%
Agriculture and livestock	4%
Other human activities (e.g., transportation, waste disposal)	3%

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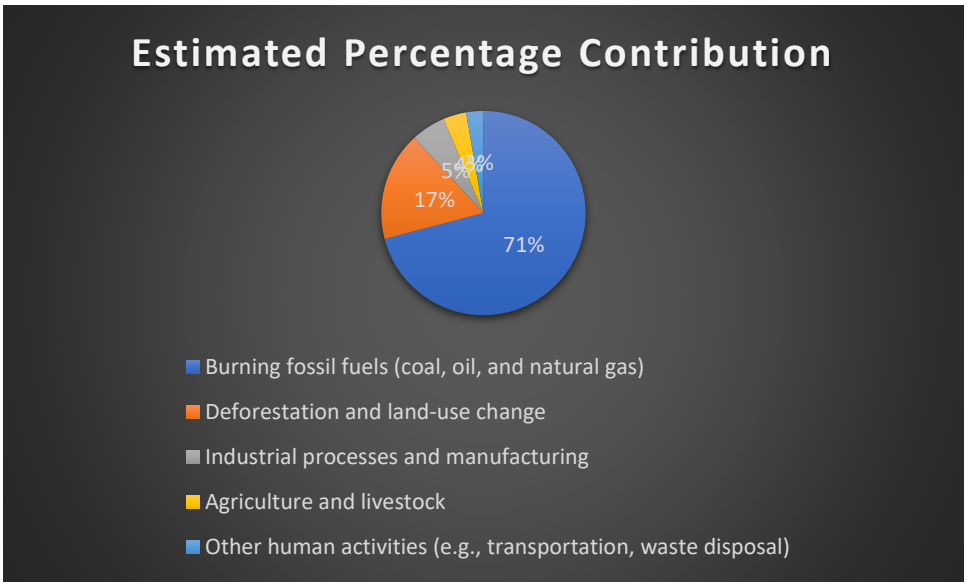

Figure 4.1: A pie chart that shows the approximate percentage contribution

Different human activities can be linked to the causes of global warming, and their respective impacts are shown by the estimated percentage contribution. Burning fossil fuels including coal, oil, and natural gas is the main cause of emissions, making for a significant 78% of the total. This emphasises how energy use plays a crucial part in causing climate change. At 19%, deforestation and land-use change make a substantial contribution, underscoring the negative environmental effects of changing natural landscapes. 6% of the total comes from industrial operations and manufacturing, which highlights the significance of sustainable production techniques. At 4%, agriculture and livestock demonstrate how the production of food and the influence on the climate are intertwined. Furthermore, 3% comes from other human activities, such as trash disposal and transportation, highlighting the complex nature of human-induced climate change. This breakdown emphasises how, in order to effectively battle global warming, extensive mitigation policies addressing these various sources are required.

Table 4.2: student answers

Gases	Environmental science	MBBS	Geology	Economics	Pol Science	Strat Studies
Carbon-dioxide (CO_2)	5	25	5	20	15	7

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Carbon-monoxide (CO)	3	2	2	3	2	2
Methane (CH4)	6	3	7	7	3	1
All	35	6	30	10	20	30

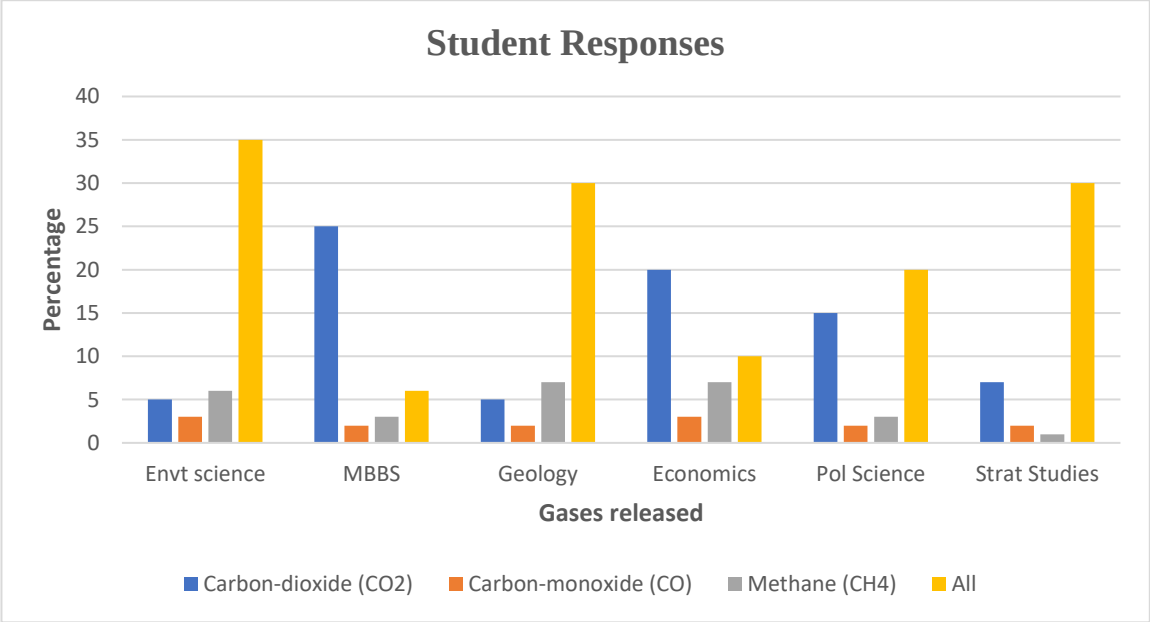

Figure 4.2: graphical depiction of students' answers

The distribution of disciplinary expertise on three major greenhouse gases is seen in the table. Environmental science emphasises awareness of environmental impact and demonstrates a thorough understanding (5% CO2, 6% CH4). MBBS demonstrates a medical emphasis with 25% CO2 proficiency. Carbon cycles are emphasised in geology (5% CO2). Economics emphasises economic ramifications (20% CO2) and demonstrates a balanced grasp. A distributed knowledge base that takes geopolitical and strategic factors into account is demonstrated by political science and strategic studies. The 100% collective distribution emphasises the necessity for cooperative measures to address these gases' effects on the environment and society by demonstrating a multidisciplinary understanding of the environmental, medical, economic, and strategic implications of these gases.

Primary data collection results

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Table 4.4: primary technique for gathering

Objective	Data Collection Method	Instrument	Participants	Duration
1	Survey	Questionnaire	Bachelor students from various faculties	4 weeks
1	Interview	Structured interview	Global warming experts	2 weeks
2	Survey	Questionnaire	Bachelor students from various faculties	4 weeks
2	Interview	Structured interview	Global warming experts	2 weeks
3	Survey	Questionnaire	Bachelor students from various faculties	4 weeks
3	Interview	Structured interview	Global warming experts	2 weeks
4	Survey	Questionnaire	Bachelor students from various faculties	4 weeks
4	Interview	Structured interview	Global warming experts	2 weeks

A thorough strategy for accomplishing the research goals is laid out in the data collection plan. Questionnaire-based surveys are the main technique; they are used to target Bachelor students from different faculties during a 4-week period for each goal. This enables a multidisciplinary viewpoint on the effects of global warming. In-depth insights are also provided by structured interviews with global warming specialists, which are carried out over a two-week period for each goal. The study's validity is increased by the thoughtful application of both quantitative and qualitative approaches. For every aim, there is a repeating cycle of surveys and interviews to ensure data triangulation. This reinforces the robustness and dependability of findings across a range of participant groups and expert perspectives.

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4.2.Survey Results

Table 4.4: Important observations

Question	Faculty	Average Response (on a scale of 1-5)	Key Insights
Q1	Geography	4.2	Students in geography show a high awareness of global warming impacts.
Q2	Economics	3.8	Economic students express concern about socio-economic implications.
Q3	Political Science	4.0	Political science students recognize potential geopolitical implications.
Q4	Strategic Studies	3.5	Strategic studies students display varying perspectives on climate change adaptation.

The survey's findings offer insightful information on how various faculties view global warming. With an average response of 4.2, geography students have a remarkably high level of awareness, demonstrating a solid comprehension of the environmental effects linked to global warming. The average response in the field of Economics, which is 3.8, suggests that students are quite concerned about the socio-economic effects of climate change. With an average score of 4.0, students studying political science indicate that they are aware of the possible geopolitical ramifications of global warming and highlight the multidisciplinary character of climate challenges. With an average response score of 3.5, students studying strategic studies present a variety of viewpoints on adapting to climate change, illustrating the nuanced nature of strategic issues while tackling environmental problems.

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5. RESULTS AND DISCUSSION

The results of the poll and the primary data collection techniques offer a comprehensive picture of the causes and attitudes related to global warming. The reasons table emphasises how burning fossil fuels is the primary driver of climate change and how important energy consumption is. Significant contributions are also played by deforestation and industrial processes, highlighting the necessity of sustainable land use and manufacturing practises. Comprehensive mitigation solutions are necessary because human activities like transportation and waste management, together with agriculture, highlight the complex nature of climate change caused by humans.Students from different faculties' distribution of competence offers important insights into their awareness and concerns. Students studying geography demonstrate a remarkable comprehension of the effects of global warming, highlighting the significance of environmental education. Students studying economics, on the other hand, show a great deal of worry over the socio-economic ramifications, emphasising the relationship between environmental health and economic systems. Students studying political science emphasise the multidisciplinary character of climate challenges that go beyond environmental concerns, showing a knowledge of potential geopolitical ramifications. Students studying Strategic Studies have provided replies that demonstrate the complexity of strategic considerations in tackling environmental concerns, reflecting the diversity of opinions within this discipline.With surveys and structured interviews combined, the data collecting plan exhibits a strong methodology that guarantees both quantitative and qualitative insights. By gathering a variety of viewpoints and professional opinions, the repeated cycles of surveys and interviews across several objectives improve the study's validity and reliability. The allocation of expertise in this multidisciplinary approach emphasises how the environmental, medical, economic, political, and strategic components of global warming are interconnected. As demonstrated by the aggregate 100% distribution, interdisciplinary activities highlight the need for integrated methods to fully address the effects of these gases on society, the environment, and human health.

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6. CONCLUSION

This study used a multidisciplinary approach that included environmental science, medicine, geology, economics, political science, and strategic studies to examine the causes and perceptions of global warming. The causes table made clear how much deforestation, burning fossil fuels, and industrial activities contribute, underscoring the necessity of all-encompassing mitigation measures. Responses from students indicated that different faculties had different levels of awareness and concern. For example, students in geography showed a strong understanding of the environment, while students in economics focused on the socio-economic implications. Students in political science recognised the geopolitical implications of the issue, and students in strategic studies presented a variety of viewpoints on how to adapt to climate change. With surveys and structured interviews as part of the data collecting plan, a strong methodology was ensured, gathering both quantitative and qualitative findings. The study's validity and reliability were improved by the repeated cycles across several objectives, which reflected the interconnectedness of the problems related to global warming. This multidisciplinary approach emphasises how crucial it is to work together and employ integrated tactics in order to fully address the many and varied aspects of global warming. The study emphasises the need for cross-disciplinary collaboration and a comprehensive approach to effectively confront the causes and effects of global warming, which adds important insights to the continuing conversation on climate change. It is envisaged that these results will guide efforts towards a more resilient and sustainable future by informing future research and policymaking.

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ISBN - 978-93-95060-79-0

The Significance of Financial Accounting Data in the Management of Businesses

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Abstract

Around half of the information that is valuable is given by financial accounting. The cycles of dynamic in organization can profit from the use of this information. Lamentably, the ongoing situation exhibits that there is an issue with the nature of financial revealing. Subsequently, this might bring about the circumstance in which the proprietors and representatives of the organizations have zero faith in the information that is gotten from financial accounting and don't utilize it while simply deciding. Accordingly, the reason for this study is to assess the numerous parts of financial accounting information that are used during the time spent going with choices in regards to business management in undertakings that are settled in the province of Maharashtra. Precise review and amalgamation of the hypothetical bits of knowledge found in logical writing were used to research the hypothetical part of the significance of financial accounting information for the management of businesses. These observational discoveries are gotten from a poll review that was controlled to businesses situated in the territory of Maharashtra.

Keywords: *Accounting Information, Business, Financial Accounting, Management, Significance.*

1. INTRODUCTION

The institutional and social aspects of financial accounting in Maharashtra remain generally neglected by researchers. The majority of Indian states continue to employ various accounting systems that are governed by state-level laws, which is one of the causes of the financial accounting form harmonisation or standardisation issues. As a result, choosing company management strategies at the federal level is challenging. States are working towards harmonisation, and Maharashtra has been modifying the country's accounting laws to bring about uniformity in the accounting system. Regretfully, accountants who swiftly and effectively embrace innovations may experience stress as a result of these frequent changes in legislation. As a result, the information provided by financial accounting becomes of worse quality.

According to research conducted by the Institute of Chartered Accountants of India, Maharashtra has low-quality financial accounting data. Just half of top notch financial explanations are delivered by accounting administrations businesses or the organization's main bookkeeper, as per a survey of the financial assertions of 100 Maharashtran organizations. 46–50% of the useful information data is provided by the financial accounting data. This makes up a sizable portion of the data that is helpful in making business management decisions. Regretfully, the effective information data decreases in Maharashtra if the accounting information is only 50% accurate when given in the financial accounts. Decisions made by corporate managers may suffer as a result of this. Thus, the research's main question is: How can financial accounting data affect Maharashtran business management decisions?

1.1. Financial accounting in business decision making

Precisely following regular exchanges is made conceivable by financial accounting. Financial proclamations, for example, pay explanations and monetary records, which show an organization's income, costs, resources, liabilities, and value, are arranged

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utilizing this information. A company's ability to track its financial performance, pinpoint opportunities for development, and make well-informed decisions regarding expansion and future expenditures makes financial accounting an essential component of every enterprise. It also aids in the decision-making process for external stakeholders, including creditors, investors, and regulators, who assess a company's financial standing before lending or investing money in it.

1.2. Financial Accounting benefits

Whatever their size or sector, all kinds of organisations need to understand financial accounting. Financial accounting is especially significant for new businesses, microbusinesses, and private ventures since it gives a reasonable picture of the organization's financial wellbeing, which is important for settling on very much educated business choices. The ability of financial accounting to aid in decision-making is one of its main advantages. The data from financial accounting is used to estimate future financial performance, prepare tax returns, analyse cash flow, and reduce errors and issues. Making wise judgements regarding expansion, investments, and other company possibilities requires this information. Financial accounting also enables businesses to assess their performance financially and pinpoint opportunities for development, which can aid in better resource allocation and operational optimisation decisions.

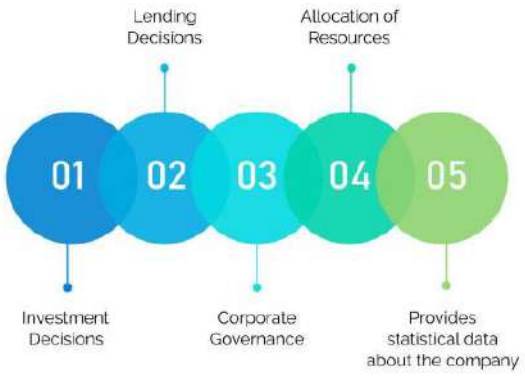

Figure 1: Ways financial accounting helps in decision making.

a) Investment Decisions

By inspecting various financial and monetary viewpoints, basic investigation is a procedure used to survey an organization's financial standing and prospects for development. Essential investigation depends vigorously on financial articulations, including the income proclamation, pay explanation, and accounting report. Financial backers and experts can gain fundamental

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information about an organization's financial execution from these financial assertions, which remember subtleties for its resources, liabilities, pay, costs, and income. This information is utilized by examiners and financial backers to survey an organization's reliability and valuation.

They can track down designs in a business' financial execution and evaluate how it piles facing friends and industry standards by looking at the financial assertions. This empowers them to lay out cost goals and evaluate the sensibility of a stock's current cost. Financial backers would have a restricted information on the past, present, and future financial solidness of organizations that issue stocks and bonds in the event that accounting information hadn't been accessible. The main source of data utilised in fundamental analysis is financial statements, which offer a plethora of information necessary to make wise investment decisions.

b) Lending Decisions

Banks and bondholders, among other creditors, value financial accounting because it gives them critical information regarding a company's creditworthiness. A company's assets and liabilities are shown in financial statements like the income statement and balance sheet, which creditors use to assess a company's ability to pay back debts. Financial explanations of an organization are utilized to make normal accounting proportions like the obligation to-value proportion and the time's revenue procured proportion. Lenders often utilize these proportions to survey an organization's financial standing and limit with regards to credit reimbursement.

Without the urgent information that accounting strategies give, loaning establishments won't face the gamble challenges an enormous corporate credit. To assess an organization's reliability and work out the level of chance related with making a credit, financial explanations are fundamental. All through the credit endorsing process, the moneylender can choose the amount to loan and at what financing costs once the degree of hazard not entirely settled. Financial accounting is consequently

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fundamental for the loaning system since it gives banks the information they need to use sound judgment.

c) Corporate Governance

Reliable accounting is useful for the internal operations of the company as well as for external lenders and investors. For organisations, one of the most evident benefits of financial accounting is that it helps them comply with legal and regulatory obligations. It is mandatory for publicly traded corporations to give accurate and up-to-date financial data, and to be truthful and transparent about their financial operations. Financial accounting enables them to abide by these rules and stay out of trouble. Financial accounting assists organisations in optimising their daily operations and identifying growth prospects, in addition to helping with regulatory and compliance challenges.

Managers may create budgets, comprehend public opinion, monitor productivity, assess the success of their products, and create both short- and long-term plans with the use of financial accounting data. Making decisions with this knowledge will help a company perform better and have a better chance of succeeding. Financial accounting is crucial for a company's internal operations as well as for external stakeholders like creditors and investors. In addition to helping businesses adhere to legal and regulatory obligations, it supports managers' decision-making, budgeting, performance monitoring, and opportunity identification.

d) Provides statistical data about the company

The function of accounting plays a significant part in the provision of useful financial information to the management of the company. This information enables the management to make well-informed decisions on the activities that the firm will engage in in the future. Among these include the creation of budgets, the determination of areas in which cost reductions can be made, and the evaluation of the practicability of new projects or investments. Accounting helps management make better decisions that can improve the performance of the firm and boost its chances of success by

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providing precise and reliable financial data. Accounting also helps management develop their decision-making skills.

e) Allocation of Resources

A company's accounting function is an essential component in determining how its resources are being utilised inside the organisation, as well as whether or not those resources are being utilised in an efficient and successful manner. Accounting helps management identify areas in which resources are being squandered and areas in which cost savings can be realised due to the provision of detailed financial information and cost analysis. Furthermore, accounting helps to discover places where resources are not being used correctly and prudently. This includes finding areas of overspending or inefficiencies, which can assist management in taking remedial actions. Accounting also helps to identify areas where resources are being used improperly. One method for accomplishing this is through the utilisation of variance analysis, which involves comparing the actual outcomes to the budgeted or anticipated results and determining the factors that led to the disparity.

2. OBJECTIVES

- To investigate the function that information from financial accounting plays in aiding successful decision-making within the management of businesses.
- To evaluate the positive and negative effects that accurate financial reporting has on the overall financial health and stability of enterprises.
- To analyse the relationship between the data from financial accounting and the strategic planning that occurs in the management of enterprises.
- To evaluate the role of financial statements in offering insights into the performance and efficiency of corporate operations.

3. LITERATURE REVIEW

Huang et al. (2021), It has been discovered that the information obtained from financial accounting has a large and favourable impact on the performance of corporate management. In order to investigate the connection between

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financial accounting information and the success of business management, they conduct an empirical study of Chinese enterprises that are publicly traded. They come to the conclusion that businesses that have information about their financial accounting that is of a higher quality have higher levels of profitability, return on equity, and market value.

Jiang and Wang (2022), Conduct a literature review on the topic of the function that information from financial accounting plays in improving the effectiveness of business management. The researchers come to the conclusion that the knowledge obtained from financial accounting can improve the efficiency of business management by enhancing decision-making, resource allocation, and risk management. Moreover, they find that the impact of financial accounting information on the viability of business management is tempered by various models, for example, the area in which the firm works, its size, and the attributes of its possession structure.

Li and Zhang (2021), a comprehensive analysis of the role that information from financial accounting plays in the administration of businesses should be provided. In this section, they explore the various ways in which information from financial accounting can be utilised to enhance the decision-making process throughout business management. The difficulties associated with making good use of information pertaining to financial accounting are also discussed.

Khan and Kabir (2020), Examine how business management choices in industrialised and emerging nations are influenced by financial accounting data. They discover that the degree of economic development modifies the influence of financial accounting data on business management choices. They discover that in emerging nations as opposed to developed nations, financial accounting data is more crucial for organisation management choices.

Liu and Liu (2022), conducted a thorough meta-analysis to investigate the connection between Business Management Performance (BMP) and Financial Accounting Information (FAI). Their analysis, which was published in the International Journal of Finance & Economics, combines data from 115 research to give a comprehensive picture of

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how FAI affects BMP. A strong and positive association between the two variables is found in the meta-analysis, indicating that companies that use financial accounting data typically have better managerial performance. The study also revealed significant moderating factors, emphasising the contextual subtleties that affect the FAI-BMP link. These factors included business size, industry type, and country of origin.

Luo and Zhang (2020), developed a conceptual framework that explores the complex function that Financial Accounting Information (FAI) plays in the field of company management, adding to the body of literature. Their conceptual framework, which was published in the Journal of Accounting and Public Policy, offers a theoretical prism through which to view the ways in which FAI affects management decision-making procedures. According to the authors, FAI is a crucial instrument that provides useful data for well-informed decisions about risk management, performance evaluation, and resource allocation.

4. RESEARCH METHODOLOGY

Users now believe that financial accounting information cannot be completely trusted because of worries about the accuracy of accounting data in today's business environment. The reason for this study is to decide how much business confides in accounting information and to assess how well it is perceived how to utilize it. This study's main goal is to assess how important financial accounting data is to Maharashtra, India's corporate management practises. A series of research hypotheses has been developed based on the integration of theoretical frameworks and practical research findings (see Figure 1).

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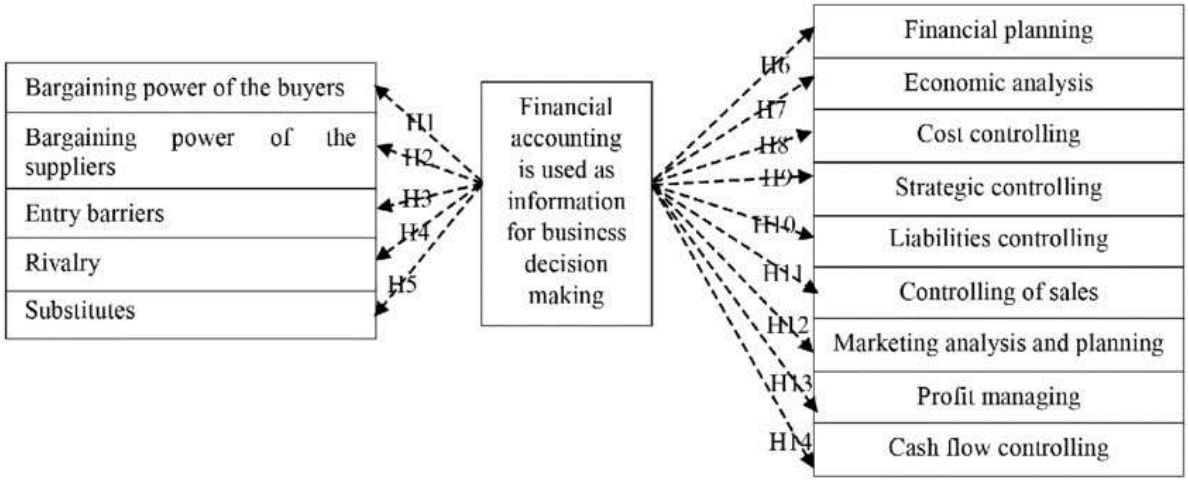

Figure 1: Research model and hypotheses.

There are fourteen speculations in the examination model, as Figure 1 delineates. H1-H5: There is a positive relationship between businesses that think financial accounting gives information to business independent direction and those that figure financial information can resolve issues with providers' and purchasers' arranging power, section boundaries, contention, and substitutes. H6-H14: There is a positive connection between businesses that think financial accounting gives information to financial preparation, monetary examination, cost control, vital controlling, responsibility control, deals control, promoting investigation and arranging, benefit management, and income management. An exact request was directed utilizing a quantitative exploration technique, a poll study, to affirm or invalidate these speculations.

The survey was isolated into two segments: the main posed inquiries about the respondent's set of working responsibilities, the idea of the organization, its size, its activities, and the way that long it had been on the lookout. The subsequent segment contained proclamations that were scored utilizing a five-point Likert scale (5 being strongly agreed, 4 being agree, 3 being neutral, 2 being disagree, and 1 being strongly disagreed).

As of early 2023, there were over 62,000 registered enterprises operating in Maharashtra, according to data from the Directorate of Industries, Maharashtra. A 95% confidence level was reached with a sample size of 375 respondents, as determined by a simplified procedure

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for sample size computation. Nevertheless, only 39 respondents were interviewed for this study due to time and budgetary restrictions, yielding a 15% margin of error.

In July and August of 2023, the online survey was administered. Eleven other staff members, eight middle managers, seventeen sales managers, and three business managers were among the responders. Twelve medium-sized businesses, seven large businesses, and sixteen small businesses participated in the study. Six respondents represented businesses operating in the market for six to ten years, four respondents represented businesses operating in the market for ten to fifteen years, and eighteen respondents represented businesses operating in the market for up to two years. It is believed that this respondent distribution is adequate to produce trustworthy findings.

Table 1: Deciphering the Size of a Relationship Coefficient.

Size of Correlation	**Interpretation**	**Size of Correlation**	**Interpretation**
0.90 to 1.00	Very high positive correlation	-0.90 to -1.00	Very high negative correlation
0.70 to 0.90	High positive correlation	-0.70 to -0.90	High negative correlation
0.50 to 0.70	Moderate positive correlation	-0.50 to -0.70	Moderate negative correlation
0.30 to 0.50	Low positive correlation	-0.30 to -0.50	Low negative correlation
0.00 to 0.30	Negligible correlation	0.00 to -0.30	Negligible correlation

The table offers a thorough explanation of how to evaluate correlation coefficients and provides information on the direction and strength of correlations between variables. A very high positive correlation, indicating a direct and strong association between the variables, is indicated by a correlation coefficient between 0.90 and 1.00. In contrast, a strong and inverse relationship is implied by a very high negative correlation, which is represented by a coefficient between -0.90 and -1.00. High positive and high negative correlations are indicated

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by coefficients in the range of 0.70 to 0.90 and -0.70 to -0.90, respectively. These values show a significant link that is not as extreme as the very high correlations.

The ranges of moderate positive and negative correlations are 0.50 to 0.70 and -0.50 to -0.70, respectively, suggesting a significant but more moderate link. Low positive and low negative correlations are represented by coefficients between 0.30 and 0.50 and -0.30 and -0.50, respectively, suggesting a lesser but still noticeable relationship. In conclusion, coefficients falling between 0.00 and -0.30 and 0.00 and 0.30 indicate insignificant correlations, indicating that there is no significant association between the variables. Researchers and analysts can use this table as a useful tool to determine the direction and intensity of correlations in their data.

The Spearman relationship coefficient was used to determine the factual relationship between various parts to acquire extra understanding into the use of financial accounting for organization choices. Based on how correlation coefficients are interpreted, the results are approximated (refer to Table 1). The statistical analysis software package SPSS is used to do the calculations.

5. DATA ANALYSIS AND INTERPRETATION

The worth of the Spearman rank connection coefficient between every one of the issues (providers' and purchasers' bartering power, passage hindrances, competition, and substitutes) and the financial accounting discernment that financial accounting is utilized as information for business direction is accounted for in Table 2.

Table 2: Spearman Rank Connection Coefficient.

Financial accounting information can assist with resolving the accompanying issues:	**Financial accounting is utilized as information for business independent direction**
of the bargaining power of the buyers	0.105
of the bargaining power of the suppliers	0.281
of entry barriers	0.401
of rivalry	0.367

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of substitutes	0.339

Relapse investigation uncovers a significant low certain connection between the perspective on financial accounting as a wellspring of information for corporate direction and the choices made in regards to passage boundaries, contention, and substitutes (values = 0.401, 0.367, and 0.367, separately). The consequences of relapse demonstrate a minor connection between the discernment in financial accounting that financial accounting gives information to business navigation and the assurance of the providers' and purchasers' arranging power.

In rundown, the business local area recognizes the utilization of financial accounting information in direction, sees it as a significant device for distinguishing section hindrances, and accepts that it restricts the market's capacity to survey options. The discoveries additionally show that businesses are uncertain about the upsides of imparting financial accounting information to providers and clients. It's conceivable that these results emerged from businesses' question of financial accounting information and their absence of assumption that providers or purchasers will get exact financial records. To assess this, however, further exploration should be finished.

Table 3 shows the worth of the Spearman rank connection coefficient between every model utilizing accounting information and the financial accounting discernment that financial accounting is utilized as information for organization direction.

Table 3: Spearman Rank Correlation Coefficient.

Financial information using for:	**Financial accounting is utilized as information for business independent direction:**
Financial planning	0.160
Economic analysis	0.430
Cost controlling	0.150
Strategic controlling	0.501
Liabilities controlling	0.629
Controlling of sales	0.460

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Marketing analysis and planning	0.440
Profit managing	0.339
Cash flow controlling	0.260

Relapse examination uncovers a respectably critical positive connection between financial accounting application for vital controlling (esteem = 0.501) and liabilities controlling (esteem = 0.629) and the insight that financial accounting is utilized as information for business navigation. The consequences of the relapse demonstrate a critical low certain connection between the use of financial accounting for monetary investigation (esteem = 0.430), controlling deals (esteem = 0.460), promoting examination and arranging (esteem = 0.440), and benefit making due (esteem = 0.339) and the impression of financial accounting as information utilized for business independent direction.

There is a negligible association between the usage of financial accounting for cash flow management, cost control, and financial planning, and the opinion that financial accounting provides information for corporate decision making. Ultimately, financial data is used by businesses to guide judgements about managing their profit margin, controlling sales, controlling the flow of goods and materials, and controlling liabilities. Financial accounting data is not used to evaluate financial planning, cost management, or cash flow control; rather, it is used exclusively to control strategies when evaluating a business's viability. To assess the causes, more research has to be done.

6. RECOMMENDATIONS AND CONCLUSION

For clients to pursue informed choices, financial accounting information is fundamental. The advantages of financial accounting information change contingent upon the size of the business. Doorman's five powers can be utilized to reveal the significance of financial accounting information, as per a writing survey. Purchasers can involve financial accounting information for obligation risk management and checking. How much assets distributed to money management, financial preparation, income affirmation, and vital arranging is likewise uncovered. Contingent upon the size of the association, the rundown of financial accounting information might change. Financial accounting

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information must, beyond a shadow of a doubt, give a valid and exact image of the business. Thus, it is imperative that we talk about sustainable accounting now. Business decisions can benefit from sustainable accounting. Based on empirical study, organisations in Maharashtra, India typically use financial accounting data when making decisions about sales (profit management, marketing analysis and planning, sales control). Companies situated in Maharashtra, India use financial accounting data to assess competitors, entry barriers, and substitutes, in accordance with Porter's five forces. Unexpectedly, businesses located in Maharashtra, India, rarely use financial accounting data for cash flow management, cost control, and financial planning.

Recommendations:

- **Expand Sample Size and Diversify Respondents:** Aim for a more diverse group of participants, considering various industries, sizes, and operational durations.
- **Incorporate Qualitative Data and In-Depth Interviews:** Conduct in-depth interviews with key decision-makers, such as CFOs and financial managers, to gather nuanced perspectives on the role of financial accounting in decision-making.
- **Explore Industry-Specific Factors:** Consider conducting a subgroup analysis based on industries to identify potential variations in the importance and trustworthiness of financial accounting information.
- **Examine the Impact of External Factors:** Assess external factors that may influence the perceived trustworthiness of financial accounting information.
- **Longitudinal Study for Dynamic Insights:** Consider adopting a longitudinal approach to study how perceptions and practices evolve over time.

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ISBN - 978-93-95060-79-0

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Analytical study of Cyber Security risks and challenges in the Education sector

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Abstract -- These days, we are witnessing the good sized of the virtual revolution and its applications in all fields, because the way of networking supplied the alternate of information, stories, and in all sectors, understanding with a whole lot of those superb functions, we discover that today's virtual global has delivered a large type of dangers of its personal, which encompass assaults and malicious applications like viruses, worms, spyware documents, and plenty of others. and the training sector higher education isn't always far from the ones bad impacts that avoid the education process and affect its infrastructure and its outer edge, in conjunction with university college students, teachers, administrative assistants, the environment and teaching techniques. For this danger, we need to interest and cyber protection education and adopt study cyber safety coverage in higher training sectors institution that allows you to shield this important place. This study paper will find out the importance of cyber security on this vicinity and provide the strategies that students, education sector can utilize to sell cyber security all through higher training institutions an analytical way.

Keywords -- *Cyber protection, Education, E-learning, Security, Viruses*

I. INTRODUCTION

We live these days in light of the massive unfold of the digital statistics revolution, which has made the arena a higher area and facilitated the approach of verbal exchange to the some distance cease, with its many structures, programs, and technology. Ransomware, fraud, identity robbery, and the higher education area are not a ways from these risks, it has been discovered as very excessive in the listing of objectives of cybersecurity assaults as maximum research have shown that various academic establishments are uncovered to 30% of denial-of-service assaults and ransomware assaults that negatively have an effect on the reputation of those establishments and the workflow in them. therefore, this look at examines the way to shield assets, college students, instructors, and renovation at the integrity of records in higher education institutions because of its terrific importance and the supply of strategies, standards, advice, and guidance essential to enforce the challenge of protecting these establishments from surrounding and potential dangers.

A. Cyber Security

The protection afforded to an automated information machine which will achieve the relevant targets of

keeping the integrity, availability and confidentiality of data system resources which includes hardware, software, firmware, statistics/facts, and tele- communications, in our lived truth, we find that most academic establishments ignore the significance of cyber security, which casts a poor shadow on the data and the complete academic manner. Under we test the records that are to be had in these establishments:

B. Financial Data

Those establishments accumulate, keep and system a hard and fast of financial statistics that includes bank account statistics for personnel and college students, in addition to the accounting and monetary cycle of internal strategies. This essential data could be misused by third parties or others.

C. Personal Information

This is in the form of college students and personnel are prime targets for identity and records robbery attacks, pass-web site programming attacks, spying attacks, etc.

D. Organization Data

Information of students, faculty, and administrative staff from registration procedures, tuition fees, courses, scientific research, student results, and their quarterly reports may be targets internally or external attack aimed at either modifying or not making it available, and certainly it must be protected because tampering with it jeopardizes the reputation, competitiveness, and fairness of institutions [1].

E. Educational Data

Scientific research and evaluation methods are among the matters and other vital educational activities and tasks for educational institutions and exposing them to exposure exposes these institutions to a number of procedures and possibly penalties [2].

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II. CYBER SECURITY THREATS

Cyber protection threats are defined as any virtual hobby that would threaten the integrity of content material or endanger get right of entry to to records and the privateness of customers. here's a listing of common threats.

A. Viruses

It's malicious code Programs that secretly attach to a file and execute when that file is opened, once a virus infects a computer, it performs two separate tasks.

B. Malware

It's software program that enters into system without the proprietor's knowledge or consent, malware is any software deliberately designed to reason harm to a pc, server, patron, or pc network, the one objectives of malware is to infect a computer system hide the malware's malicious moves bring benefit from the movements that it performs.

C. Trojans

Those threats additionally goal to create a backdoor for your pc that offers malicious customers get admission to on your gadget, probable permitting personal or private data to be compromised. in contrast to viruses and worms, trojans do not reproduce by means of infecting other files nor do they self-replicate.

D. Spyware

Spyware is any software program that installs itself in your computer and starts off evolved covertly tracking your on line conduct without your understanding or permission. spyware is a kind of malware that secretly gathers information about someone or organization and relays this statistics to different parties.

E. Adware

This danger promises advertising and marketing content, regularly as pop-up home windows which could slow or crash a laptop can monitor or music the consumer's activities.

F. SQL Injection Attack

SQL injection (SQLi) is a cyber attack that injects malicious SQL code into an application, allowing the attacker to view or modify a database.

G. Phishing

Phishing is defined as a fraudulent hobby that is achieved to scouse borrow confidential person data consisting of credit score card numbers, login credentials, and passwords.

It also includes executed via using email or different kinds

of electronic verbal exchange by pretending to be from a dependable enterprise entity.

H. Man in the Middle Attack

This status occurs when the attacker is located among the communication channels and monitoring the contents which are sent or received across the channels; it may interrupt or change the data nature or destinations.

I. Denial of Service (DoS) Attack

A denial-of-provider (DoS) attack is a form of cyber attack wherein a malicious actor ambitions to render a pc or different tool unavailable to its meant customers by way of interrupting the device's regular functioning. DoS attacks normally feature by means of overwhelming or flooding a targeted gadget with requests till normal traffic is not able to be processed, resulting in denial-of-provider to addition customers. A DoS assault is characterized with the aid of the use of a single computer to launch the assault.

III. REVIEW OF LITERATURE

Risk management within the scope of assets, threats, vulnerabilities, and events within an organization can be challenging as information assets are continuously created, processed, and stored. Secondly, cyberspace's threat environment is continually changing, where new methods and tools make it is hard to identify, evaluate, and map harmful attacks to an organization [3].

An association between several demographic factors and a student's susceptibility to phishing attack. Lower susceptibility for college affiliation, academic year progression, cyber training, and involvement in cyber clubs or cyber scholarship programs, amount of time spent on the computer, and age demographics. Surprisingly, despite a lower susceptibility for cyber education or IT expertise [4]. User Susceptibility and Behavior indicate a high level of sensitivity to phishing attempts in academia [5].

IV. OBJECTIVES OF THE STUDY

Formerly, many severe scientific portions of research have been carried out concerning the importance of cyber security in the training sector, particularly better training, but just a few of them focused at the guidelines and standards that need to be applied so that those institutions can conquer the hazard of many threats and risks.

Hence, this study specializes in regulations, standards, and measures. And the instructions to be observed within the various establishments of better training. in this take a look at, the dangers and challenges have been mentioned, in addition to the significance of enforcing regulations and requirements to fulfill the escalating cyber security demanding situations in higher education establishments.

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V. RESEARCH METHODOLOGY

Cyber security demanding situations abound in better training. This explores the cyber security threats that the better education space faces, as well as a number answers which could assist colleges and universities combat destiny attacks. Possibly even greater enormous than ability economic losses, cyber attacks pose a grave risk to a college's reputation and the protection of its college students.

This paper examines the importance of cyber security in the better education area by way of clarifying the numerous risks that can affect the instructional surroundings on this essential zone, which include assaults, viruses, Trojan horses, denial-of- service assaults, and others, observed by using the significance of steering, advice, safety standards and rules which could reduce or prevent dangers. This research additionally sheds mild at the studies carried out in cyber security concerning the education sector and consists of multiple databases considered from last 4 to 5 years as well different online statistical resources.

VI. DISCUSSION

To discuss this study, to talk about this have a look at, and with the affection of the modern-day records, the training quarter has ranked sixth maximum targeted by attacks, because it ranked second for ransomware attacks, and for that reason has been categorized as the least at ease among other sectors, with a charge of extra than 30% of exploitable safety vulnerabilities, affecting about 12% of personnel and college students. most of the attacks that the education zone suffers from are social engineering attacks, which amounted to 41%, and we discover that a percentage of universities agree extra investment should be provided for IT protection to shield the highbrow belongings of critical research.

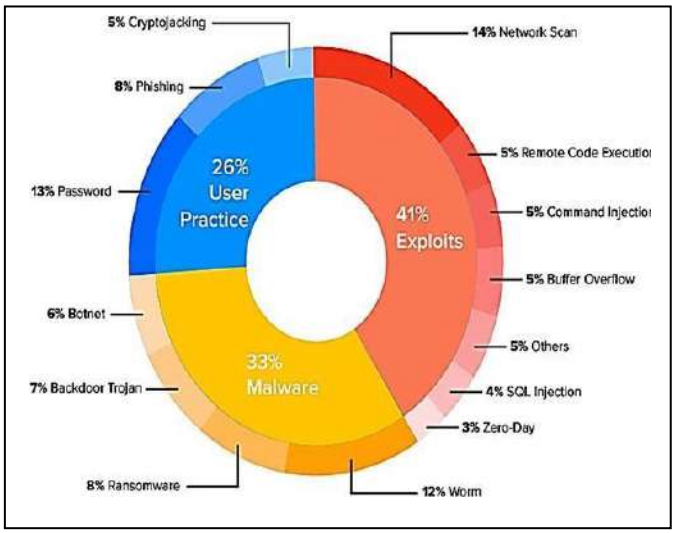

Fig. 1.0 Ratio of Cyber security threats

We also find that 87% of educational institutions have experienced at least one successful attack in the last decade. Including higher education institutions [6], [7].

The cyber security marketplace is expected to grow due to the boom and diversity of assaults and security dangers, which negatively affected information breaches, the unfold of electronic crimes, the entry of so-referred to as virtual terrorism, virtual conflict, and attacks that do not rely on any users or sufferers' action (Zero_Click_Attack) and motive terrific harm. assume increase from $217 billion in 2021 to $345 billion by means of 2026, posting a Compound Annual increase rate (CAGR) of 9.7% from 2021 to 2026 [7].

The training quarter accounted for thirteen% of all facts security breaches during the previous couple of years, sixty percent of data breaches are caused by vulnerabilities that might had been avoided if a safety update or patch were deployed. for this reason the subsequent graphs show the importance and impact of cyber security at the education zone and cowl the maximum vital threats and demanding situations. This observe indicates that 40% of assaults inside the better training zone are due to social engineering assaults, 30% are because of failure to cope with fishing assault problems, and 17% of education establishments pay ransom to restore their information [8].

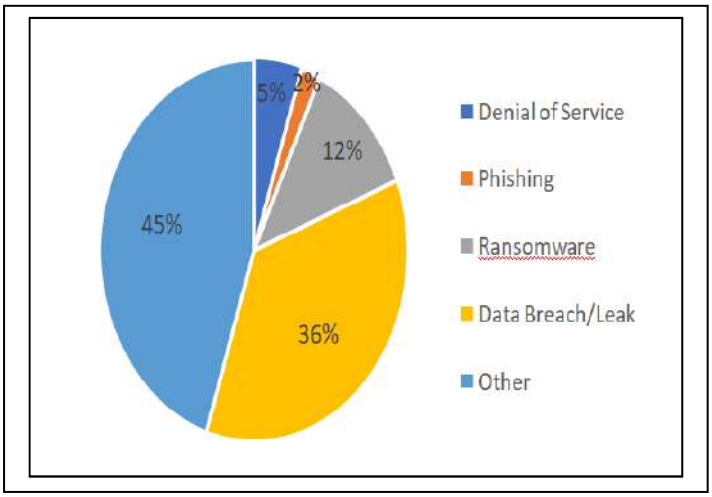

Fig. 2.0 Cyber-attacks in the education sector

Better education had the highest charge of ransomware assaults among all industries surveyed in a 2016 report published by BitSight (a cyber risk control agency), and the second maximum rate in BitSight's 2017 file. as a result, universities are working around the clock to shore up their defenses against those steep potential losses.

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As Kim Milford, executive director of the research and training Networking data Sharing and analysis center at Indiana university said in a 2016 piece written with the aid of the center for digital education, universities are actually "locked into an pricey arms race" as they discover new approaches of each combating cutting - edge attacks and seeking to stay one-step in advance of attacks yet to come. No matter whether or not cyber attacks are a success, Milford says, they represent a high-priced and ever-gift difficulty that universities are compelled to cope with.

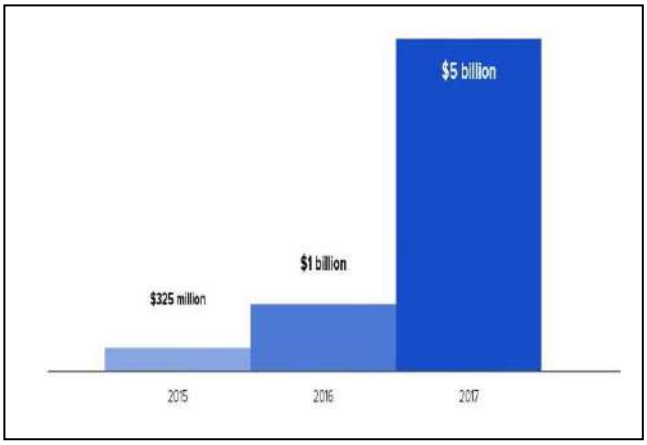

Fig. 3.0 Annual Global Ransomware costs

But the risks posed by cyber attacks extend beyond financial losses for the world of higher ed. Indeed, colleges and universities house a huge volume of sensitive data, from student social security numbers to valuable intellectual property that, if stolen or compromised, could cause significant damage far beyond the walls of the academy. Perhaps even more significant than the aforementioned potential financial losses, cyber attacks pose a grave threat to a university's reputation and the safety of its students. Due to the fact colleges and universities have been so early in adopting virtual equipment and interfaces (and because of economic and different realistic concerns), many establishments of better studying still rely on legacy systems which are especially vulnerable to attacks.

Even though not an problem particular to better education, the dearth in cyber security skills represents a significant impediment that faculties and universities should overcome to address the troubles stated above. A current take a look at conducted via consulting firm Frost & Sullivan initiatives that there could be 1.eight million unfilled cyber security jobs by 2020, and that this skills shortage exists on a global scale, with nearly 70 percentage of professionals globally saying there are too few cyber security workers on staff. With call for for cyber security expertise some distance outpacing supply, corporations frequently pay pinnacle greenback for cyber security understanding.

Phishing attacks are characterized by using emails or web pages that are designed to fool users into entering touchy data, such as passwords or credit score card facts. Generally, the phisher sends an e mail message to a massive group of people whose addresses he has captured from address books and websites across the internet. The message, normally nicely-crafted and reputable-looking, may also claim to be from a economic institution, a service issuer, or another business enterprise acknowledged via the recipient…regularly, the recipient is requested to provide the statistics via clicking a website link inside the electronic mail. However at the same time as the link to the internet site may additionally look legitimate, the link that is displayed isn't always the real web page you go to while you click on on it.

30 percent of users within the education industry have fallen for phishing scams posing as company communications, double the fee of the general population, in the ultimate 12 months.

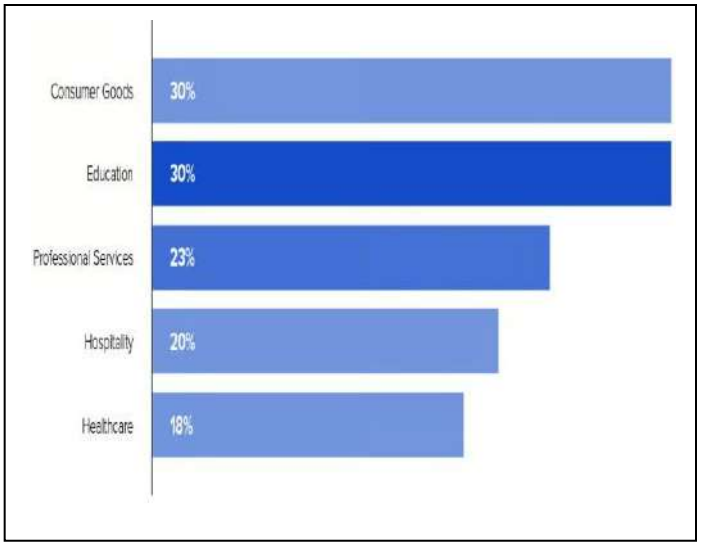

Fig. 4.0 Corporate Phishing Click Rate

Phishing attacks can have a wide variety of ultimate goals, from stealing user facts to putting in ransomware on a sufferer's computer and extracting financial charge. whilst those attacks may additionally seem obvious and clean to avoid, research have shown that a big majority of corporations were victims of phishing scams. The training industry has verified particularly prone, as Wombat protection – a software program business enterprise dedicated to assisting companies to combat phishing attacks – located in a 2017 report that 30 percentage of users within the education enterprise have clicked on phishing scams posing as corporate communications, double the price of the overall populace, in the closing 12 months.

Expertise vulnerabilities, how not unusual cyber attacks paintings, and the way to save you such attacks is essential to creating a greater at ease – and financially solid – future for better education. yet cyber threats are continuously evolving, and there is no assure that the threats faced nowadays (and the strategies for mitigating them) will resemble those going forward.

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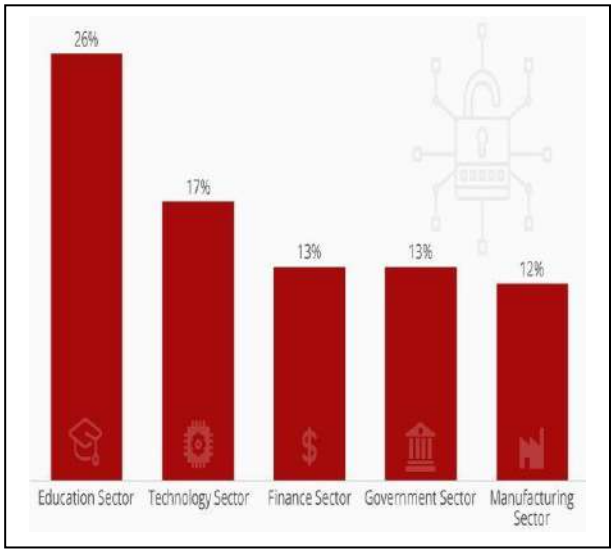

Fig. 5.0 Education Sector suffering from Cyber Attacks

Fig. 5.0 shows the struggling of the academic region's greatest percentage of cyber assaults the various other sectors and that gives the departments of information generation and cyber protection the duty to plot and adopting solid cyber security rules to comfortable the networks and systems [9], [10].

VII. RECOMMENDATIONS

Academic institutions must take appropriate precautions for students' security despite limited resources. Some effective measures are:

1. Implement solid cyber security coverage, to prevent all of the training region surroundings.
2. Putting in the threats preventers' equipment and devices inclusive of firewalls, IDS, NIDS AND IPS so as to triumph over and mitigate the threat.
3. Adopt sturdy access rules to save you unauthorized get admission to the network and restrict the usage of private computers via personnel, students and college to complete paintings obligations.
4. Non-stop attention of the dangers of safety threats by using undertaking workshops and courses for associates.
5. Training IT body of workers to recognize the nature of assaults, dangers, and how they occur, with an emphasis on a way to take advantage of vulnerabilities, preventive measures, and paintings protocols. Pick out the maximum precious IT property and relaxed them by way of the usage of a sturdy safety answer.

VIII. RESULT AND CONCLUSION

The significance of cyber protection within the higher training sector thinking about all the development that witnessing in light of the increasing assaults and risks makes it essential to follow all measures and equipment important to slash these threats in order now not to have an effect on the education surroundings and weaken its outputs. For vital tips and instructions for protection, with a focal point on analyzing risks and ways to protect them and learning a group of proceeding research in cyber security. But, plenty of these companies are open to online attacks and academic establishments should observe security rules a good way to shield their treasured belongings. Additionally, this paper tested the various risks and a way to deal positively with them.

Academic establishments are very crucial and essential, and consequently they should be protected from attacks and dangers to make sure their continuity to carry out their exceptional roles. This look at proved that instructional institutions are still exposed to many safety risks and attacks targeting records and information dumping, competitiveness and intellectual assets problems, and concentrated on students. Security guidelines can reduce most of these dangers and increase the productivity of college students, college, and administrative group of workers. The educational quarter itself can make a contribution to putting off cyber-attacks by means of adopting, sponsoring, and setting up relevant disciplines, and spreading the life-style of precautions, danger bargain, and attention. Maximum of the threats may be triumph over with a few measures, awareness, and correct and cheaper.

This study proved that educational institutions are still exposed to many security risks and attacks targeting data and information dumping, competitiveness and intellectual property issues, and targeting students. The education sector itself can contribute to eliminating cyber-attacks by adopting, sponsoring, and establishing relevant disciplines, and spreading the culture of precautions, risk reduction, and awareness.

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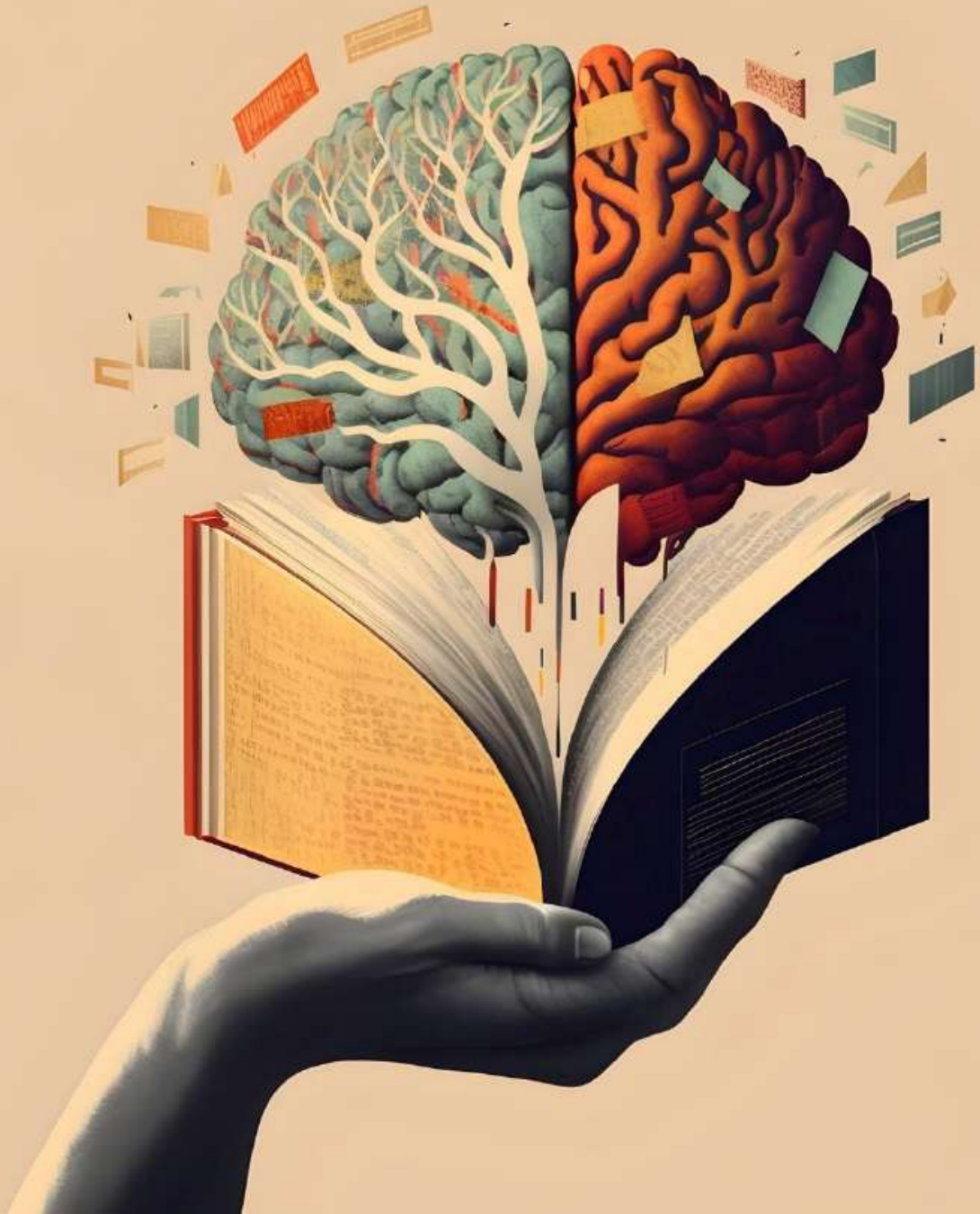
EMERGING TRENDS IN INDIAN EDUCATION SYSTEM



Chapter Book on

EMERGING TRENDS IN

INDIAN EDUCATION SYSTEM

Editor-in-Chief

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PUBLICATION

INDIA

Emerging Trends in Indian Education System

Edited by: Dr. Dilipkumar A. Ode, Subharun Pal, Dr. Asit Panda, Ms. Parama Kundu, Dr. Akshay Mangavade, Dr. Mythili Bai K, Dr. Madhavrao C, Dr. T. S. Subbulakshmi

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RED'SHINE PUBLICATION PVT. LTD.
Headquarters (India): 88, Patel Street, Navamuvada,
Lunawada, India-389 230
Contact: +91 76988 26988
Registration no. GJ31D0000034

In Association with,

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ISBN: 978-81-19070-69-5
ISBN-10: 81-19070-69-0
DOI: 10.25215/8119070690
DIP: 18.10.8119070690
Price: ₹900
May, 2023 (First Edition)

■

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www.redshine.co.in | info.redmac@gmail.com
Printed in India | Title ID: 9393239398

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ISBN: 978-81-19070-69-5

DIP: 18.10.8119070690.003

EMERGING TRENDS IN INFORMATION TECHNOLOGY INFLUENCING EDUCATION SYSTEM IN INDIA

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❖ ABSTRACT:

The Indian education area has been adopting new inclinations in information era in some of procedures. Through those rapid-growing education developments our schooling technological panorama has made big headway with new models like online faculties and hybrid reading models. Apart from those well-known progressive solutions or options, era has moreover stimulated our training region in lots of greater strategies. This have an impact on may be absolutely visible at some stage in the whole education sector. Besides, the increase of the EdTech organization moreover proves this affect in the course of our education system. The growing tendencies of statistics era have simply created a extremely good impact at the education and getting to know strategies in India. Facts generation has not clearly made training extra appealing and to be had. It has moreover improved possibilities for developing intercultural knowledge in Indian college, university students thru collaborative getting to know and peer tutoring. Within the United States like India wherein in the scholar-teacher ratio is going as immoderate, statistics generation has promised several solutions. All we want is the top of the road use of resources and a zeal to carry the trade we choice!

The last few years have witnessed a dramatic trade within the studying model. The way college students are being taught today can be very exclusive from the education techniques that have been located a few a few years again. Generation has added about numerous modifications inside

ISBN: 978-81-19070-69-5

the way education is brought and acquired. From self-learning to flipped examine room approach, we've visible era make a big effect at the learning and education methodologies.

Keywords: *Education system, Edtech Influence, Emerging trend, Indian Education, Information Technology*

❖ INTRODUCTION:

In spite of being a three hundred and sixty five days of worldwide upheaval, the new year has brought about some of vital developments in virtual answers in education, inclusive of the upward thrust inside the use of apps and hybrid studying and good sized switch to online education, it is nearly specific that teaching will hold to utilize virtual technology for the foreseeable future. With this comes new challenges and tendencies, from lecture room coaching to on-line checks to destiny examine dreams; from continuing to upskill workforce to an updated teaching environment. The Indian education system is in a kingdom of flux. Now not are there "Gurukuls" and an insistence on rote gaining knowledge of, and an strive is made through institutions to awareness on the all-round improvement of the scholars. This shift came about grade by grade while well-known cinema and mass media commenced out to position stress on cognitive improvement over memorizing.

These days the educational scene in India is booming as universities are supplying various courses with respect different sectors like Medical, Finance, Food technology etc and India's pinnacle enterprise colleges hold to attract the company. This is not all. Example: College university students now have the threat to pursue an M.Sc. in big facts analytics in India, PGDM in banking and economic services, and advertising manipulate. Globally, lots of numerous Indian-starting region scientists is a testimony to the reality that proficient people from India can benefit incredible heights. So, wherein lies the problem? A grievance that is leveled at the Indian training tool is that it teaches no longer "what" to think however specializes in "how" to assume in a given state of affairs. Furthermore, education in india is all approximately making organized college students to pass tests. Indian college students are usually readying themselves for warfare.

❖ TOP TRENDS IN THE EDUCATION TECHNOLOGY IN INDIA :

1) Collaborative Learning

The revolutionary traits in instructional generation have made it feasible for anybody to stay associated. The effect of the cutting-edge traits of ICT within the subject of education has paved a couple of options. We be part of, discuss

and enact upon conditions collaboratively. This collaborative method has obtained importance in the getting to know way as well. In a classroom studying version, teachers inspire collaboration by means of assigning institution sports and responsibilities. While university students crew up collectively to paintings on a assignment or remedy a problem, it builds their collaborative competencies. Operating together improves their expertise and will increase engagement. Regardless of the reality that eLearning is pretty famous, it includes collaboration with capabilities to proportion and communicate. In a conventional teaching model, a trainer enters a observe room, speaks for approximately half-hour, and leaves while the bell earrings.

But nowadays, technology has bridged the gap amongst instructors and college students. You will find teachers and university college students interacting with every specific more regularly. Instructors are a ways extra on hand now and act as mentors to assist college students in their usual improvement. This collaborative learning method helps college students to have interaction with their friends and construct their interpersonal skills.

2) Learning Outside the Classroom Environment

Numerous technology developments in training keep converting the virtual global. Cell-based totally devices have taken learning out of doors of the study room. With mLearning and eLearning growing in popularity, college students can analyze at their very personal pace and time. This fashion is anticipated to maintain up as it is a handy technique of delivering as well as receiving the education. Designing mobile-first responsive content fabric lets in university and college students to go through their publications every time and everywhere. Internet connection is now not an hassle with offline analyzing abilities. Ebooks can be embedded with many capabilities to decorate the gaining knowledge of revel in. Whole with annotation equipment, e-bookmarks, hyperlinks, dictionary, are seeking for feature, an ebooks makes reading more flexible. Maximum of the educational institutes in recent times have observed cell gaining knowledge of into their getting to know ecosystems, benefiting university students and instructors alike.

3) Social Media in Learning

Who would have ever notion that social media would in the future be familiar as a part of the studying manner? The technological tendencies in teaching and getting to know are rapidly changing every day. With youngsters as younger as 11 year having social media profiles on diverse systems, you could’t honestly assume to keep them far from social media for too lengthy. So, teachers located a manner to utilize this trend and turn it right into a

effective device for boosting the mastering process. Instructional institutes have started the usage of social media as a conversation device, in which students can have interaction with their peers and school individuals. Usually, college student's percentage films and pictures with their buddies and fans. But with social functions embedded in their eBooks, they can percentage have a look at substances, critiques, initiatives and many others.

They could touch upon a person else's post or proportion links to other web sites, all of the while constructing peer networks and enhancing the online getting to know experience. Instructors allow using social media as part of the gaining knowledge of version as it allows college students to stay interested in their course and increases engagement. Social media is right here to live and incorporating it into mastering modules will build a subculture of collaboration and sharing, main to an improved studying experience.

4) Interactive Classroom

Bringing generation into the classroom has made school rooms lively and interactive. With eBooks, the direction content material can be embedded with motion pictures, augmented fact, audio files and so on. Not like a published e book, ebook permits for more interaction to take region in the classroom. The flipped study room model has allowed college students to do all the learning at domestic and all the practical paintings at faculty. A majority of these new technologies have delivered about a change in the manner that classes used to function historically. Instructors can now assist and manual college students with their homework in class. They could have discussions and activities in classrooms, growing an interactive environment where students are completely concerned in the mastering procedure. Developing interaction and engagement has grow to be a priority for lots faculties and universities. This style of learning has seen a increase in latest years for its capability to hold college students engaged in the classrooms.

5) Data Management & Analytics

Dealing with records has end up ever so convenient and essential with the advent of generation in the education system. Teachers can now have complete analytics of a pupil's performance, including the range of assessments attempted, chapters finished and so on. Homework and assignments may be assigned to the whole class without delay and instructors can compare the results on line. This kind of automation in classroom sports has enabled teachers to recognition more on their route modules and provides in-depth guidance. Analytics has come to be an important part of any on-line studying model as it allows the dimension of an infant's engagement and

academic overall performance. In line with the records to be had, instructors can increase action plans to improve students overall performance. In case the information well-known shows a collective trouble or a troubling pattern with a character pupil's performance, it can be highlighted, and suitable measures can be taken. The remote monitoring of pupil sports enables teachers to offer personalized schooling if required. A majority of these elements have contributed to creating statistics management and information analytics a vital a part of educational training.

6) Personalized Learning

Custom designed getting to know is a method to schooling that tailors teaching and studying to person university college students' desires and alternatives. In 2023, personalized learning should emerge as even more everyday thanks to advances in synthetic intelligence and statistics analytics. Instructors and academic era structures may additionally use AI algorithms to research scholar facts and provide customized recommendations on content material, pacing, and mastering strategies. It turned into best depend of time in advance than the schooling region became taken over through generation. Although the recognition rate became low in the beginning, it grade by grade gained momentum.

Coaching and mastering techniques have long past through a huge change due to all the trends in education generation. Each year, new trends emerge to provide something new to the learners. They are saying change is regular; similarly, enhancements within the subject of generation are also constant. And certain improvements may be applied in the training gadget for enhancing their gaining knowledge of and development device. The end result of those improvements come to be a style which then leads to better teaching and gaining knowledge of strategies.

❖ ADOPTION OF INFORMATION TECHNOLOGY IN EDUCATION SYSTEM:

Information technology usually refers back to the diverse set of technological tools and resources used to communicate and to create disseminate shop and control records. One despite the fact that the additives of it alternate and evolve hastily information and communication exchange technology of some type are part of everyday lifestyles for lots people global inside the business social and education sectors.

The studying way is continuously improving and the usage of increasingly current technology. It makes it simpler for instructors to provide an

explanation for the concern and students find out it higher to acquire facts right. Here are a few techniques generation is being used in education:

1) Neural Education

Neuroscience is the scientific examine of the frightened device together with neuroanatomy, neurobiology, neurochemistry, neurophysiology and neuropharmacology. And its applications in psychology, psychiatry and neurology neural education offers deeper information of the minds excellent capability. It is a gaining knowledge of approach that is aligned with how the brain tactics facts it optimizes the gaining knowledge of experience by way of creating a high-quality temper through self-directed studying allows the mind to embed new mastering in existing knowledge and enhances facts recall speed for long term potentiation.

2) AR & VR Technology

Virtual reality and augmented reality are new technological improvements that convey a selected hassle to the look at room. The usage of VR system, university college by college students can visit an extended manner flung areas and come upon historical civilizations. That could be a charming addition to the reading way, wherein you'll gain near to research that isn't viable in fact. Augmented reality customers get to enjoy entire actual-worldwide surroundings blended with virtual factors. Those eras had been established to definitely have an impact on pupil engagement. With the advent of augmented reality and virtual reality into the schooling machine, the classroom analyzing experience has passed via a first-rate trade. Gaining knowledge of has come to be plenty greater immersive than traditional strategies.

In assessment to simple photos and hands-on experiments within the lab, university students can now view improved variations of the picture and devices on their cellular gadgets. The augmented and virtual reality developments in schooling generation are making gaining knowledge of a compelling revel in.

3) Robotics

Generation enables college students get relaxed with the modern-day global via trouble-fixing further to permits getting to know. Robotics introduces university and college students to all branches of the STEM device (Science, Technology, Engineering, and Mathematics). STEM learning wishes critical wondering and hassle fixing and through robotics in college and university. Students can learn how to technique the statistics they benefit with a solution-

orientated approach. Era in education can help students in lots of approaches. Folks that face challenges in analyzing can use Edtech and can overcome some of the troubles they face, college students who dislike topics like math can boom an interest in the difficulty and may decorate the overall general performance of the classroom.

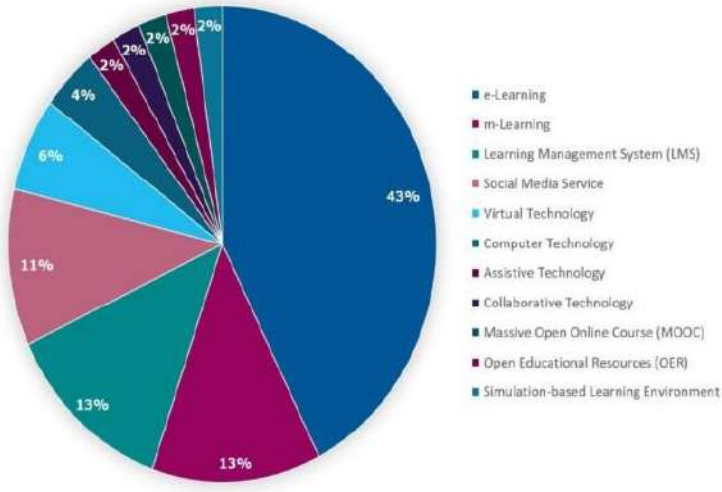

Fig 1.0 Educational Technology Adoption: Type of Technologies and Modes of Delivery

❖ IMPACT OF INFORMATION TECHNOLOGY TREND'S ON INDIAN EDUCATION SYSTEM:

From self-studying to flipped study room method, we've visible era make a extensive impact on the mastering and teaching methodologies. With several advantages to offer, digital mastering has become an crucial part of the education system. Information and communication technology (ICT) brings in more than one benefit for digital mastering and pupil-centric engagement. The ICT developments in education formed the faculties and universities to implement the todays in education technology so one can enhance the coaching and getting to know method.

Facts generation has sincerely revolutionized the conventional academic structures into training, distance mastering, flexible gaining knowledge of, e-studying, collaborative studying, on every occasion, anywhere analyzing, virtual reading, and multimedia method to gaining knowledge of and so on.

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Programs of statistics era are considerably applied in Indian schools, education and specific instructional institutions which simplified the technique of teaching and analyzing. It helps in growing listening, speakme, analyzing and writing skills of the human beings. PC Assisted Language analyzing software program software, can educate the languages with none help of a instructor.

Technological breakthroughs have made sharing at the net an on hand and alternative. The e-analyzing is the cutting-edge idea, generated through statistics era which results in convenient learning, minimizing the instructional cost of journeying to specific factors for Indian education system.

- The e-learning is the handy approach of reading anywhere at any time.
- E-gaining knowledge of guarantees that users have get entry to standardized content material, as and after they want it.
- In a study room, it could be hard for an trainer to devote equal attention to all of the college students. However e-getting to know allows the students to have a look at a tempo this is relaxed, focuses on difficulties and take a destroy while it suits.

Presently, academic technologies (Edtech) are redefining the educational space. As in line with media reports, edech players entered the market round 2012 and the length between 2012 and 2019 got here to be called the generation of edtech revolution. A 2021 record by using the consulting firm, PriceWaterCoopers, at the side of the Confederation of Indian enterprise notes.

Many instructional institutions internationally are shifting to cloud-based totally infrastructure, especially after the advent of put up-pandemic, online coaching. This infrastructure enables teachers to train from anywhere and the scholar can access the class truly. In India, wherein the absence of good enough infrastructure in rural regions regularly inhibits girls from enrolling into faculties, virtual fairness and cloud-based infrastructure can fill a big hole. Cloud-based totally projects that perform in a digital mode reduce the infrastructural cost of lecture rooms and also help students to get right of entry to studying substances easily.

❖ CONCLUSION:

The need of the hour is to adapt to the evolving technological marvels due to the fact educational era won't be a bombilation (buzz) but as a substitute a

truth for the imminent future of education. While embracing technology as our critical component, one should now not end up a slave of it.

Every infant has exclusive gaining knowledge of desires and customized or personalized coaching is a non-linear scholar-centric educational method this is administered as per each student's skills. With the aid of technology inclusive of artificial intelligence (AI), machine learning (ML), huge statistics and augmented learning (AL), educator can provide customized teaching material to students. These technologies beautify the pupil's creativity, offer an immersive revel in, and sell essential wondering. Those techniques bridge gaps within the academic machine and allow college students to study at their very own pace in Indian education system.

The Indian education system has advanced considerably through the years. However, the system need to hold to conform with technological improvements and the changing dreams of college, university, college students and employers. One of the techniques the education location is growing is through the use of Ed-Tech, a technology specifically designed to manual and beautify the analyzing manner.
Education generation has the capability to convert the Indian education technology through supplying personalized reading evaluations, online and blended getting to know opportunities, gamification, AI and device studying, and virtual reality. To truly realize the capacity of schooling technology, there can be a want to invest in instructor training and digital literacy, collaborate to provide cheaper get admission to schooling generation systems, and constantly innovate and enhance. Through doing so, India can prepare its students for the destiny and create a greater equitable and inclusive education system.

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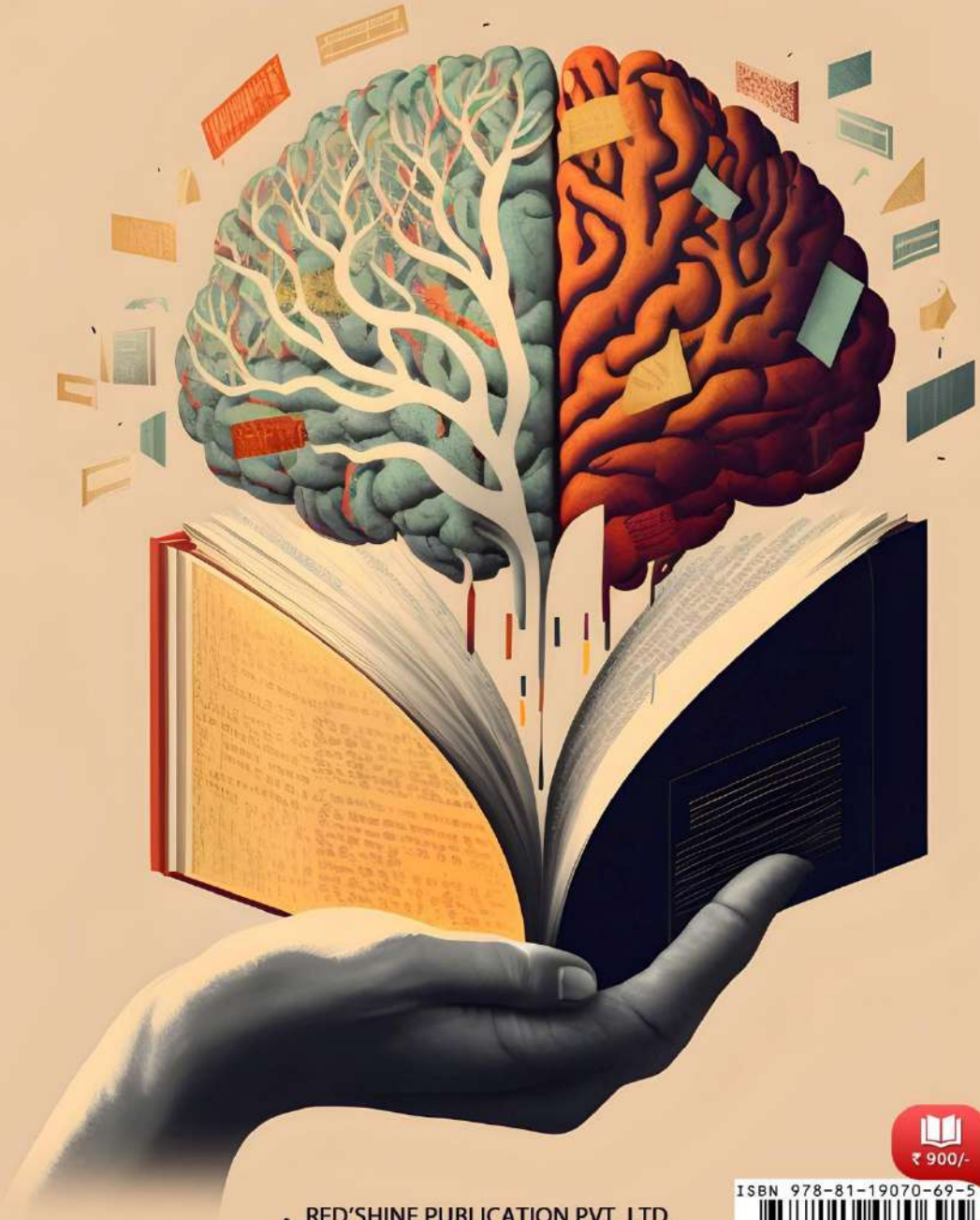

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In this paper, we are introducing an innovative, efficient, and real-time handwritten text-to-speech conversion technique for the Devanagari script. It combines the concept of Optical Character Recognition (OCR) and Text to Speech Synthesizer (TTS). This type of system can be helpful for visually impaired persons, for reading the number plates of a vehicle, and for medical applications. Text extraction from colored images is a challenging task in computer vision, for that, we have trained our model using Convolution Neural Network (CNN) with 1000s of different handwriting styles written by different people and of different age groups for each character. A trained model has been used for the recognition of input characters which was taken from the gesture movement of a fingertip with blue ink. The system is developed in Python 3.6.0.

Published in: 2023 2nd International Conference for Innovation in Technology (INOCON)

Date of Conference: 03-05 March 2023

INSPEC Accession Number: 22963050

Date Added to IEEE *Xplore*: 19 April 2023

DOI: 10.1109/INOCON57975.2023.10101034

ISBN Information:

Publisher: IEEE

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Devanagari is a compound of 'Deva' and 'Nagari', which means abode of divinity [1]. It was developed in ancient India around 1st century and was in regular use by 7th century. This script is originated by Brahmi script used to write over 120 languages such as Hindi, Sanskrit, Marathi, Nepali and many more. This script composed of 47 primary character including 14 vowels (Swar) and 33 consonants (Vayanjans) [1]. It is 4th most widely adopted writing system. This is not a case sensitive unlike other scripts.

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- **First Edition as per New Syllabus:** July 2023
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Limiting periodic orbits in the neighborhood of the triangular equilibrium points when the more massive primary is an oblate spheroid

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In the restricted three–body problem, we explore the motion of the infinitesimal mass in the vicinity of the liberation point L_4 taking into account the oblateness of the more massive primary. When higher order terms are retained in the analysis, the period of the orbit is found to be a function of the two finite masses and the size of the orbit. Furthermore, it is proved that the critical mass value decreases with oblateness. Finally, limiting periodic orbits are generated for values of the mass ratios 0.038097 and 0.038541. Both type of periodic orbits shift towards the smaller primary with oblateness. The effect is found to be more near the extreme of the major axes of the near-elliptical orbits with oblateness. Natural objects are frequently discovered circling near the Lagrange points of planetary systems due to the natural stability of L_4 and L_5. 'Trojans' or 'Trojan asteroids' are the generic names for the objects that inhabit those sites. As the Sun and Jupiter are the two most massive objects in the Solar System, there are more Sun–Jupiter Trojans than for any other pair of bodies. A space station could be placed in L_4 or L_5 of the Earth-Moon system in the future.

Topics

Space station, Planetary systems, Astrodynamics, Asteroids, N-body problem, Solar system, The Moon

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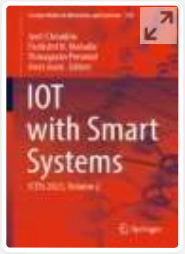

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Abstract

Owing to the benefits of a longer life, lithium-ion power batteries with high specific power and energy have been widely used in the transportation sector. However, the safety issues brought about by the incorrect calculation and forecasts of battery health have drawn a lot of interest from academics. The fundamental explanations of the “State of Health” (SoH) and the process by which they degrade were

summarized from both local and foreign literature in this research. Three perspectives were taken into consideration when discussing the estimation and prediction approaches for lithium-ion power batteries: model based approaches using fusion technology, and data-driven methodologies. The benefits and drawbacks of the standard estimation of SoH and prediction methods used today are outlined in this paper. It is vitally necessary to develop an efficient management of energy storage system that can assess the lithium-ion battery's overall health and charging condition. Lithium-ion batteries' state of charge (SoC), which defines how much charge is still in them, is a crucial EV metric. Additionally, SoC offers details regarding the charging/discharging procedure, preventing either an overcharge or an over-discharge of the battery. The different SoC estimate approaches are in-depth examined in this research. SoC estimate methods are thoroughly explored, along with their methodology, mathematical model, advantages, disadvantages, and error rate. The research closes by making several significant recommendations for the improvement of SoC estimates in electric vehicles and applications.

Keywords

State of charge **State of health**

Electric vehicles **Lithium-ion battery**

Intelligent system **Estimation**

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About this paper

Cite this paper

Gandhi, R., Jha, A., Bhavsar, K. (2023). Intelligent and Conventional Methods for SoC and SoH Estimation. In: Choudrie, J., Mahalle, P.N., Perumal, T., Joshi, A. (eds) IOT with Smart Systems. ICTIS 2023. Lecture Notes in Networks and Systems, vol 720. Springer, Singapore. https://doi.org/10.1007/978-981-99-3761-5_36

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DOI	Published	Publisher Name
https://doi.org/10.1007/978-981-99-3761-5_36	31 August 2023	Springer, Singapore

Print ISBN	Online ISBN	eBook Packages
978-981-99-3760-8	978-981-99-3761-5	Intelligent Technologies and Robotics Intelligent Technologies and Robotics (R0)

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Recent Trends in Product Design and Intelligent Manufacturing Systems pp 255–267

Generic Classification and Automatic Extraction of Mechanical Interlocking Features from CAD Model

Shantanu Kumar Das

Conference paper | First Online: 06 October 2022

318 Accesses

Part of the Lecture Notes in Mechanical Engineering book series (LNME)

Abstract

This paper presents the classification, representation and extraction of mechanical interlocking features (MIFs) from the CAD model. Mechanical interlocking features (MIFs) are geometric features of two or more parts that when caused to come into contact and fully engage, prevent relative motion in certain directions of translation and/or rotation, including, when features physically interlock, unwanted separation in any or certain directions. The MIFs are represented as a set of faces with a characteristic arrangement among the faces among parts in proximity.

This characteristic arrangement of contact faces and their topological relationships help in classification of MIFs. The MIFs are classified into elementary and compound types based on the number of assembly features available at the joint location. Even though CAD assembly models are available to users, it is rather difficult for the user to manually extract the assembly feature information from CAD models. It is therefore a set of algorithms are developed to extract the attributes of MIFs and identify MIFs from CAD model. CAD assembly models from aerospace domain have been used in order to illustrate and validate the proposed approach.

Keywords

Mechanical interlocking joint **Representation**

Extraction **Assembly feature**

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About this paper

Cite this paper

Das, S.K. (2023). Generic Classification and Automatic Extraction of Mechanical Interlocking Features from CAD Model. In: Deepak, B., Bahubalendruni, M.R., Parhi, D., Biswal, B.B. (eds) Recent Trends in Product Design and Intelligent Manufacturing Systems. Lecture Notes in Mechanical Engineering. Springer, Singapore. https://doi.org/10.1007/978-981-19-4606-6_25

.RIS .ENW .BIB

DOI
https://doi.org/10.1007/978-981-19-4606-6_25

Published	Publisher Name	Print ISBN
06 October 2022	Springer, Singapore	978-981-19-4605-9

Online ISBN	eBook Packages
978-981-19-4606-6	Engineering
	Engineering (R0)

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Library of Congress Cataloging-in-Publication Data

Names: Rathoure, Ashok K., 1983- editor. | Khade, Shankar, 1988- editor.
Title: Biomass and bioenergy solutions for climate change mitigation and sustainability / Ashok Rathoure and Shankar Khade, editors.
Description: Hershey, PA : Engineering Science Reference, an imprint of IGI Global, [2022] | Includes bibliographical references and index. | Summary: "'Biomass and bioenergy solutions for climate change mitigation and sustainability' highlights the challenges of energy conservation and the current scenario of existing fossil fuel and uses. It discusses how pollution potential of burning fossil fuel and the depletion of fossil fuel is a major issue for energy generation, thus propelling humanity toward bioenergy solutions"-- Provided by publisher.
Identifiers: LCCN 2022007805 (print) | LCCN 2022007806 (ebook) | ISBN 9781668452691 (h/c) | ISBN 9781668452707 (s/c) | ISBN 9781668452714 (ebook)
Subjects: LCSH: Biomass energy.
Classification: LCC TP339 .B5469 2022 (print) | LCC TP339 (ebook) | DDC 662/.88--dc23/eng/20220520
LC record available at https://lccn.loc.gov/2022007805
LC ebook record available at https://lccn.loc.gov/2022007806

This book is published in the IGI Global book series Advances in Environmental Engineering and Green Technologies (AEEGT) (ISSN: 2326-9162; eISSN: 2326-9170)

British Cataloguing in Publication Data
A Cataloguing in Publication record for this book is available from the British Library.

All work contributed to this book is new, previously-unpublished material. The views expressed in this book are those of the authors, but not necessarily of the publisher.

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As we know that escalating population demand and technological advancement lead us towards a new world dominion power, but with that, there is huge pressure on resources due to an increase in consumption at all levels globally. This leads to revamping environmental motifs all over the world and non-renewable sources are estimated to be not rehabilitating soon. In this case, bio-energy is energy presented as a major opportunity to address the issues of climate change by reducing carbon emissions. This chapter explore bioenergy.

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To address energy security and climate-change concerns, substitutes are needed for petroleum-based transportation fuels. In addition to electricity and natural gas, biofuels are emerging as an important class of substitutes. The promotion of biofuels as energy is mainly driven by the perspective of oil depletion, concerns about energy security, and global warming. However, results published in the past few decades present conflicting pictures regarding the energy efficiency and sustainability of biofuels. To adequately address these real or apparent contradictions, a life cycle assessment (LCA) of biofuels is indispensable. “Life cycle” refers to all stages of a process: from the cradle, that is, raw material extraction through manufacturing, distribution, and use to ultimate disposal. This chapter discusses the benefits and limitations of biofuels from an LCA standpoint. The discrepancies and strengths in LCA of biofuels are critically analyzed and opportunities are highlighted. LCA of biofuels is shown to be critical to informing policy and for practical application.

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Entrepreneurism is vital as entrepreneurs push technological change and innovation, consequently generating economic growth. Bio-energy entrepreneurism is the focal point of this chapter, along with entrepreneurial motivations, opportunities, and market drivers. Market drivers include government policies, trends in energy use, and progression of the energy sector into more renewables. Several factors influence entrepreneurs, which involves several factors ranging from the environment to economics, pioneering spirit, and social factors. With the help of entrepreneurial motivations and market drivers, individuals can easily visualize and plan all the bio-energy sector opportunities. Bio-energy entrepreneurs moderate the market of transformation of biomass to energy. The bio-energy field is filled with stories of failures, successes, and everything in between. There is a marginal difference between a failure story and a success story in the modern bio-energy sector, which narrow down to timing.

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Energy is one of the most precious and demanded commodities among various industries and consumers to sustain the current lifestyle. Energy is a crucial element, which unswervingly influences the country's economic development. Numerous methods are adopted to reduce global warming, embracing clean energy from wind, solar, and biomass sources. This chapter speaks about the current situation of energy demand, the innovations in biofuel sources, and the obstacles regarding the commercialization and production of microalgal biofuel.

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Changes in weather patterns over decades or longer periods of time are referred to as climate change. Natural and human forces both contribute to climate change. Since the Industrial Revolution, humans have contributed to climate change through emitting greenhouse gases and aerosols, as well as changing land use, leading global temperatures to rise. More storms, floods, droughts, and rising sea levels may result from increasing global temperatures and the melting of ice sheets, sea ice, and glaciers. The sun's rays supply energy to the whole world. GHGs are required to trap heat and maintain a temperature that permits life to flourish on the planet. The greenhouse effect is a natural and necessary occurrence for life on Earth to survive. If the greenhouse effect did not exist, the world would be around 33°C cooler right now. Humans have contributed to the increase in atmospheric GHGs via rising fossil fuel usage and deforestation in recent decades. Increased GHG emissions have been the primary cause of global warming during the last century.

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The planet's limited natural fossil fuel reserves are anticipated to be very soon owing to massive usage. Biofuels would be a critical alternative source that may reduce global warming and CO2 emissions. The food-versus-fuel dilemma is, however, one of the key drawbacks of first-generation biofuels like corn ethanol, sugarcane ethanol, etc. Cellulose and hemicellulose, the primary constituents of lignocellulosic feedstocks, could be reduced to sugars by either thermochemical/biological processes before being fermented to generate biofuels. However, owing to structural heterogeneity, more complicated operational techniques are required before the production technology can be commercialized, and several challenges must be addressed. This chapter provided an assessment of various feedstocks, availability, various processing techniques, obstacles, and current technical developments in the generation of biofuels from biomass.

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Biogas is a renewable natural gas used in production of energy which is generated by breakdown of organic matter by anaerobic digestion. It is a composition of methane, carbon dioxide, and some other gases in small quantities. The most common natural resources for production of biogas are aquatic sediments, animal waste, crop residues, wastewater sludge, and many others. Some other sources are landfills, water lagoons, etc., which are a result of human activity. There are several technologies used for biogas production, mainly biodigesters, wastewater treatment plants, and landfill gas recovery systems. Production of biogas is highly dependent on the feedstock availability and the policy support by the government.

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With the increasing population, there are problems like the shrinking of arable land, pollution, and feeding the entire growing population high-quality food and reaching consumer demand, and the list goes on. Novel superfoods are rich in nutrition and produced with the most sustainable methods, including algae, specifically microalgae. Microalgae have been studied for decades. Microalgae are one of the largest and most poorly understood organisms on planet earth, but in recent years, the research has been increased for the search of renewable and sustainable energy sources. Microalgae don't require much land for cultivation and thus do not compete with conventional agricultural land. Microalgae can double their size in 24 hours. CO2, solar rays, inorganic nutrients, and water are the basic requirements for them to

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grow. There are microalgae and macroalgae (e.g., seaweed) that grow in saline and hypersaline water. This chapter presents the promising potential of algae (microalgae and macroalgae) as a superfood.

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Debraj Biswal, Government General Degree College at Mangalkote, India
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The steep rise in human population together with the simultaneous growth of urbanization and industrialization has triggered overuse of fossil fuels in the recent years. However, they are non-renewable and release carbon dioxide causing global warming. In this context, biofuels come as excellent substitutes because they are renewable and eco-friendly. They are obtained from biological products and include biodiesel, biomethane, bioethanol, and biohydrogen. They are classified into first, second, and third generation fuels depending on the biological feedstocks being used. Of these, the third-generation fuels, obtained from marine resources like algae, have attracted special research interests because they do not rival the food crop production or depend on land and freshwater for their cultivation. However, the potentialities of macroalgae as a biofuel resource have not been investigated thoroughly. The chapter tries to outline all the aspects of biofuel production from macroalgae pointing out the scopes for future research.

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Natural gas hydrate (NGH) has emerged as a future source of energy for the world that can fulfill the future energy demand. The natural gas hydrates hold twice the amount of energy than all available fossil fuels. At present, the energy demand is growing, but these are not available commercially. Various field applications on the dissociation of the natural gas hydrates have been performed till now, and the output of these field applications confirms the possibility of dissociation of the natural gas hydrates. At distinct locations in the world, the huge deposits of hydrates have been identified, but the safe and economic dissociation of gas hydrates is not known yet. Hence, the evolution of new and inexpensive, suitable methods and technologies are required for the dissociation of gas hydrates. In this chapter, the authors reviewed several articles to compare the different dissociation techniques of gas hydrates and their field applications. The advantages and disadvantages of these production methods have also been discussed.

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Biofuels have gained significant interest as an alternative fuel in recent years owing to their environmental sustainability, cost-effectiveness, and the ability to blend with traditional fuels like gasoline without requiring engine modifications. The use of microalgae for biofuel production is universally preferred due

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to its energy efficiency and environmental sustainability; current research is geared towards enhancing the production of microalgae-biofuel from the initial stages to the final product as it is a cost-effective fuel option. The use of different types of nano-additives at different stages of microalgae cultivation and incorporation into biofuel produced noteworthy improvements in the final product. This chapter focuses on the prospective uses of nano-additives in microalgae cultivation, microalgal biomass conversion to biofuels, and biofuel combustion improvement for revolutionary advancements in biofuel technology.

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The chapter discusses the methods effective for the extraction of useful energy from the macroalgae biomass including liquefaction, anaerobic digestion, fermentation to biobutanol, trans-esterification to biodiesel, pyrolysis, direct combustion, fermentation to bioethanol, and gasification. However, if the algae are suited for the production of biodiesel, they can be studied from the content of their triacylglycerols (TAGs). Due to having high fatty acid content, they have a high conversion rate to biodiesel, and the lack of sulphur, phosphorus, and nitrogen also aids in the conversion. This chapter highlights the limitations and suitability of macroalgae for the conversion process in reference to chemical composition, process optimization, and cost effectiveness. It is concluded that bio-oils and bioethanol produced from wet macroalgae are considered over biodiesel production because of high lipid content of microalgae biomass. Moreover, the chapter considers electricity production from the dry mass as it would turn profitable, and this can be achieved from fast-growing macroalgae like "Ulva."

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Development of sustainable energy resources is a major concern in front of the scientific community and stakeholders in this field. Invariably, fossil fuels are utilized to meet the major part of energy demand all over the world, but their stocks are limited. Burning of fossil fuels also causes significant environmental pollution that results in drastic climate change. There is an emergent need of an alternative to fulfill the current energy demand in a sustainable way along with sorting out the pollution issues. Microbial biofuels could be the most appropriate choice in this regard because it bears enormous potential that needs to be exploited. But it has some challenging issues to employ it to meet out the current energy demands. This chapter illustrates the microbial biofuels, harvesting techniques, different process, and potential to utilize them as sustainable energy sources along with considerable challenges that need to be addressed

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to make it an eco-friendly and economic fuel choice to pave the path of sustainable development.

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Bioethanol from inedible cellulose-rich biomass is the most promising candidate to replace fast depleting, environmentally polluting fossil fuels. Hydrolysis of cellulose to glucose is the crucial step in its biotransformation to bioethanol. Enzymatic hydrolysis is favored over acid hydrolysis, as enzymes are eco-friendly biocatalysts with high substrate specificity and superior catalytic efficiency in mild reaction conditions. Complete hydrolysis of cellulose is achieved by cellulase. Higher cellulase production yield, stability, and catalytic efficiency are the main attentive points for the successful implementation in industrial production of bioethanol. This chapter will highlight general characteristics of microbial cellulases and their role in the bioconversion of cellulose to biofuels, economic sustainability of cellulose-based biofuels, and the latest innovations in cellulase immobilization as the most comprehensive strategy for improvement of enzyme stability, activity, and reusability for cost-effective large-scale application.

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Sustainable development is a key aspect of modern-day development. Reducing fuel cost and emissions is considered as the most important research area in sustainable development. Biodiesel has the potential to reduce the requirement of petroleum diesel if blended in suitable proportion. Various feedstocks are being explored for biodiesel production. Oils derived from neem and jatropha can be utilized for biodiesel production. In the current investigation, optimum parameters for the production, namely catalyst proportion, temperature, and oil-to-alcohol ratio were optimized. Experiments were carried out at different blend proportions to study fuel properties, namely kinematic viscosity, aniline point, diesel index, flash and fire points, specific and API gravity, cetane number, and ASTM distillation characteristics.

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The Honge oil, known as pongamia oil, is one of the most widely used lamp oils around the world. It is also used in leather tanning, in soap making, and as a lubricant. The Honge oil methyl ester can be used as an alternative fuel for diesel. The transesterification of methanol and Honge oil in the presence of base catalyst results in Honge oil methyl ester. In the current work, the Honge oil methyl-ester known as biodiesel was produced. It was mixed with petroleum diesel fuel in different proportions. Various physical and petroleum properties were investigated for Honge oil biofuel and also for various diesel and

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ester mixtures. The optimum parameters were temperature was 65oC, catalyst quantity, 0.4 grams and oil: alcohol ratio, 1:6. The blends of 10%, 20%, and 30% compositions were most suitable as potential alternative fuel. Different proportions of biodiesel ester in diesel fuel have been also compared with petroleum diesel fuel to identify a suitable blend having good engine fuel potential.

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Yusto Mugisha Yustas, Sokoine University of Agriculture, Tanzania

The mitigation of adverse climate change requires holistic strategies. The strategies include the proper handling of biomass wastes such as cow dung. The handling of the wastes has to be efficient using appropriately designed anaerobic digesters. In addition, the operating status of these digesters has to be monitored for detection of any fault of the digesters. Unfortunately, existing approaches for designing and monitoring the working condition of digesters have limitations. This chapter presents the innovative and explicit conceptual design approach of adapting the biomass-fed anaerobic digester and the digester operating-status-indicators in semi-arid areas. The approach is recommended to the renewable energy system designers. Finally, the appropriate indicators are recommended to be applied by the biogas plant operators or users.

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Pretreatment of Lignocellulosic Biomass and 2G Ethanol 322
Hitesha J. Panchal, Parul University, Vadodara, India
Krishan Kumar, Parul University, Vadodara, India

Rapid depletion of fossil fuel-based energy sources increased the demand for alternate energy sources. Lignocellulosics-based 2G ethanol can be used as an alternative sustainable source that presents in ample amount. Sources of lignocellulose biomass are wood, food-agriculture wastes, and forest residues. Cellulose, hemicellulose, and lignin are the core components of lignocellulosic biomass. Cellulosic and hemicellulosic biomass are enzymatically hydrolyzed to produce the monomer sugar (such as glucose or xylose) which is further converted into ethanol using fermentation process. The presence of lignin provides physical barrier that limit the access of enzymes required for saccharification. Pretreatment helps in removing the lignin from biomass and reducing recalcitrance. Pretreatment can be done by conventional methods, which are chemical, physical, and biological. This study covers the different methods of pretreatment including their disadvantages and benefits along with saccharification and fermentation processes.

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Preface

This is the right time to tackle the changing climate by making concrete plans and taking necessary actions to regulate the existing responsible factors for changing the climate. This can be only possible with the renewable source of energies. In addition to increase the level of pollutant gases upon burning in the environment, the near future depletion of existing fuels made the researchers to think on the alternate sources to mitigate the problems associated.

This issue is mainly concerned with this phenomenon and must be regarded as the commencement of a more extensive efforts for understanding public policy-making and public administration in the bioenergy. This era sees the growth of bioenergy, introducing new products, inclinations, procedures, tactics, and theories daily, thereby changing the environmental issues.

Biofuels produced either from living cell biomass or once live biomass can have efficient energy to acts as an alternative to the existing non-renewable fossil fuels. However, in the production of biofuels efforts are being made worldwide to make the fuels cost-effective. In the first generation, biofuels are derived by fermentation from the edible sources such as edible oils, corn, sugar etc. which limit the use of these sources. In the second generation, the biofuels are obtained from the non-edible sources like wheat straw, rice straw, jatropha seeds, sugarcane bagasse etc. The most critical step followed in this generation is to depolymerize and removal of lignin present in the lignocellulosic biomasses, thereby makes the process costly. Most of the challenges are overcome in the third-generation biofuels, where biofuels are derived from the living cellular biomass i. e. algal cells. The ability of certain algal species to accumulate the lipid inside the cells which can be transesterified into biodiesel. The algae can be cultivated in a small area using relatively cheaper nutrients and limited resources which can be used to produce biofuels.

With considering the global climate change, the several countries have initiated the efforts in mitigating the hazardous effects of pollutants by increasing the dependency on bioenergy.

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In this regard, the first chapter is devoted bioenergy-social, economic, and environmental impacts, which can comprehensively constitute the importance of bioenergy to protect the environment. As we know that escalating population demand and technological advancement leads us towards a new world dominion power, but there is huge pressure on resources due to an increasing consumption at all levels globally. This leads to revamping environmental motifs all over the world and non-renewable sources estimated to be not rehabilitating soon. In this case, bioenergy is energy presented as a major opportunity to ad-

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dress the issues of climate change by reducing carbon emissions. It affects socially by increasing living of standard to creating societal conflict, poverty and transportation cost and job opportunity economically. Environmentally reducing pollution to forest land unavailability concerns. As its major threats of bioenergy have not instigated at greater risk for the world, yet hence it seems to be a good solution for now. The rate of expansion of bioenergy products depends upon a great deal of government policies, straightforward strategy, and foreign investments in developing countries. With taking care of these bioenergy negative aspects also hindered.

The second chapter is based on the challenges and opportunities of biofuels. The results of this study reveal the hurdles that comes in producing the biofuels and replacing the existing fossil fuels. To address energy security and climate-change concerns, substitutes are needed for petroleum-based transportation fuels. In addition to electricity and natural gas, biofuels are emerging as an important class of substitutes. The promotion of biofuels as energy is mainly driven by the perspective of oil depletion, concerns about energy security, and global warming. However, results published in the past few decades present conflicting pictures regarding the energy efficiency and sustainability of biofuels. To adequately address these real or apparent contradictions, a Life cycle assessment (LCA) of biofuels is indispensable. "Life cycle" refers to all stages of a process: from the cradle, that is, raw material extraction through manufacturing, distribution, and use to ultimate disposal. This chapter discusses the benefits and limitations of biofuels from an LCA standpoint. The discrepancies and strengths in LCA of biofuels are critically analyzed and opportunities are highlighted. LCA of biofuels is shown to be critical to informing policy and for practical application.

The third chapter, "Entrepreneurial Opportunities in Bioenergy," claims that the necessity to broaden the scope of bioenergy across the globe. This can be achieved by initiating the new firms to protect the environment from the fossil fuels. One can start the firm in the area of bioenergy for which Government also gives support. Entrepreneurism is vital as entrepreneur's push technological change and innovation, consequently generating economic growth. Bio-energy entrepreneurism is the focal point of this chapter, along with entrepreneurial motivations, opportunities and market drivers. Market drivers include government policies, trends in energy use and progression of the energy sector into more renewables. Several factors influence entrepreneurs, which involves several factors ranging from the environment to economics, pioneering spirit to social factors. With the help of entrepreneurial motivations and market drivers, individuals can easily visualize and plan all the bio-energy sector opportunities. Bio-energy entrepreneurs moderate the market of transformation of biomass to energy. The bioenergy field is filled with stories of failures, successes, and everything in between. There is a marginal difference between a failure story and a success story in the modern bio-energy sector, which narrow down to timing.

The fourth chapter emphasizes the biofuel policies in India and the associated barrier of policy helps to understand the opportunities to initiate the entrepreneurial journey. Energy is one of the most precious and demanded commodities among various industries and consumers to sustain the current lifestyle. Energy is a crucial element, which unswervingly influences the country's economic development. Numerous methods are adopted to reduce global warming, embracing clean energy from wind, solar, and biomass sources. To bring these technologies into reality, it needs to undergo several phases, considering the technology development, environmental sustainability, economic viabilities, and conclusively with the government support in terms of public awareness and effective policies appropriate Research and Development efforts could overawe every variable, but it can be achieved only through an effective policy. Therefore, to begin with, this chapter speaks about the current situation of energy demand, the

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innovations in biofuel sources, and the obstacles regarding the commercialization and production of microalgal biofuel.

The fifth chapter refers to greenhouse gases, challenges, effect and climate change, puts emphasis on the release of greenhouse gases and their impact on the environment by means of increasing the global warming. Weather patterns alter over decades or longer periods of time, which is referred to as climate change. Natural and human forces both contribute to climate change. Since the Industrial Revolution, humans have contributed to climate change through emitting greenhouse gases and aerosols, as well as changing land use, resulting in a rise in global temperatures. More storms, floods, droughts, and rising sea levels may result from increasing global temperatures, as well as the melting of ice sheets, sea ice, and glaciers. The planet receives energy from the sun's radiation. GHGs are necessary for trapping heat and maintaining a temperature that permits life to flourish on the planet. The greenhouse effect is a natural and necessary phenomenon for life on Earth to survive. If the greenhouse effect didn't exist, the world would be around 33°C cooler right now. Humans have contributed to a rise in atmospheric GHGs as a result of increased fossil fuel use and deforestation in recent decades. The rise in GHGs has been the primary cause of global warming during the last century.

The sixth chapter signifies the production of biofuels from the biomass and biowaste. The cost of production of biofuels can be reduced by utilizing the biowaste and biomass for the production purposes. The planet's limited natural fossil fuel reserves are anticipated to be very soon owing to massive usage. Biofuels would be a critical alternative source that may reduce global warming and CO2 emissions. The food-versus-fuel dilemma is, however, one of the key drawbacks of first-generation biofuels like Corn ethanol, sugarcane ethanol, etc. Cellulose and hemicellulose, the primary constituents of lignocellulosic feedstocks, could be reduced to sugars by thermochemical / biological processes before being fermented to generate biofuels. However, owing to structural heterogeneity, more complicated operational techniques are required before the production technology can be commercialized and several challenges must be addressed. This book chapter provided an assessment of various feedstocks, availability, various processing techniques, obstacles, and current technical developments in the generation of biofuels from biomass.

The seventh chapter emphasizes the biogas generation from the renewable sources under a specified condition. The importance of biogas is in reducing the dependency on the non-renewable gases which can be produced using household wastes too. Biogas is a renewable natural gas used in production of energy which is generated by breakdown of organic matter by anaerobic digestion. It is a composition of methane, carbon dioxide and some other gases in small quantities. The most common natural resources for production of biogas are aquatic sediments, animal waste, crop residues, wastewater sludge and many others. Some other sources are landfills, water lagoons etc. which are a result of human activity. There are several technologies used for biogas production, mainly- biodigesters, wastewater treatment plants and landfill gas recovery systems. Production of biogas is highly dependent on the feedstock availability and the policy support by the government.

The eighth chapter signifies the importance of algal biomass on reducing the malnutrition cases across the globe. The protein rich algal food can be used to enrich the health upon consumption. With the increasing population, there are problems like the shrinking of arable land, pollution and feeding the entire growing population high-quality food and reaching consumer demand and the list goes on. Novel superfoods are rich in nutrition and produced with the most sustainable methods, including Algae, specifically microalgae. Microalgae have been studied for decades. Microalgae are one of the largest and most poorly understood organisms on planet earth but, in recent years the research has been increased for the search of renewable and sustainable energy sources. Microalgae don't require much land for

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cultivation and thus do not compete with conventional agricultural land. Microalgae can grow in double their size in 24 hours.CO2, solar rays, inorganic nutrients, and water are the basic requirements for them to grow. There are microalgae and macroalgae (eg: seaweed) that grow in saline and hypersaline water. This Chapter presents the promising potentials of algae (microalgae and macroalgae) as a superfood.

The ninth chapter focuses on the production of bioethanol with using macroalgae biomass. Macroalgae are known to accumulate the starch which can be used to accomplish fermentation to produce ethanol as an important biofuel. Steep rise in human population together with the simultaneous growth of urbanization and industrialization has triggered overuse of fossil fuels in the recent years. However, they are non-renewable and release carbon dioxide causing global warming. In this context, biofuels come as excellent substitutes because they are renewable and eco-friendly. They are obtained from biological products and include biodiesel, biomethane, bioethanol and biohydrogen. They are classified into first, second and third generation fuels depending on the biological feedstocks being used. Of these, the third-generation fuels, obtained from marine resources like algae have attracted special research interests because they do not rival the food crop production or depend on land and freshwater for their cultivation. However, the potentialities of macroalgae as a biofuel resource have not been investigated thoroughly. The current chapter tries to outline all the aspects of biofuel production from macroalgae pointing out the scopes for future research.

The tenth chapter signifies the methodology to produce the biogas in the form of gas hydrates. The gas hydrates in future can be efficiently used as an alternative source to the non-renewable gases. This can be produced from the waste biomass to make cost effective process. Natural Gas Hydrate (NGH) is emerged as a future source of energy for the world which can fulfill the future energy demand. The natural gas hydrates hold the twice amount of energy more than all available fossil fuels. At present, the energy demand is growing, but these are not available commercially. Various field applications on the dissociation of the natural gas hydrates have been performed till now and the output of these field applications confirms the possibility of dissociation of the natural gas hydrates. At distinct locations in the world, the huge deposits of hydrates have been identified but the safe and economical dissociation of gas hydrates is not known yet. Hence, the evolution of new and inexpensive, suitable methods and technologies are required for the dissociation of gas hydrates. In this chapter, we reviewed several articles to compare the different dissociation techniques of gas hydrates and their field applications. The advantages and disadvantages of these production method has also been discussed.

The eleventh chapter describes the use of microalgae to accumulate the lipid into them which can be transesterified to fatty acid alkyl esters. The process uses the cost-effective approach to culture the lipid accumulating microalgae on the wastewater and thereby harvesting, extraction, and transesterification of alcohol. Biofuels have gained significant interest as an alternative fuel in recent years owing to their environmental sustainability, cost-effectiveness, and the ability to blend with traditional fuels like gasoline without requiring engine modifications. The use of microalgae for biofuel production is universally preferred due to its energy efficiency and environmental sustainability; Current research is geared towards enhancing the production of microalgae-biofuel from the initial stages to the final product as it is a cost-effective fuel option. The use of different types of nano-additives at different stages of microalgae cultivation and incorporation into biofuel produced noteworthy improvements in the final product. This chapter focuses on the prospective uses of nano-additives in microalgae cultivation, microalgal biomass conversion to biofuels, and biofuel combustion improvement for revolutionary advancements in biofuel technology.

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The twelfth chapter emphasizes the macroalgae based ethanol production in which the carbohydrate rich algal biomass is utilized to ferment into ethanol under anaerobic conditions. The chapter discusses the methods effective for the extraction of useful energy from the macroalgae biomass including liquefaction, anaerobic digestion, fermentation to biobutanol, trans-esterification to biodiesel, pyrolysis, direct combustion, fermentation to bioethanol and gasification. However, if the algae are suited for the production of biodiesel can be studied from the content of their triacylglycerols (TAGs). Due to having high fatty acid content they have a high conversion rate to biodiesel, and the lack of sulphur, phosphorus and nitrogen also aids in the conversion. This chapter highlights the limitations and suitability of macroalgae for the conversion process in reference to chemical composition, process optimization and cost effectiveness. It is concluded bio-oils and bioethanol produced from wet macroalgae are considered over biodiesel production because of high lipid content of microalgae biomass. Moreover, the chapter considers electricity production from the dry mass as it would turn profitable and this can be achieved from fast-growing macroalgae like "Ulva".

The thirteenth chapter clarifies the challenges and potential associated with the lipid accumulating microalgal biofuels. Development of sustainable energy resources is a major concern in front of scientific community and stakeholder of this field. Invariably, fossil fuels are utilizing to meet the major part of energy demand all over the world but its stocks are limited. Burning of fossil fuels also causes significant environmental pollution that results in drastic climate changes. There is emergent need of an alternative to fulfill the current energy demand in a sustainable way along with sorting out the pollution issues. Microbial Biofuels could be the most appropriate choice in this regard because it bears enormous potential that needs to be exploited. But it has some challenging issues to employ it to meet out the current energy demands. This chapter illustrates the microbial biofuels, harvesting techniques, different process and potential to utilize it as sustainable energy sources along with considerable challenges that need to be addressed to make it as an eco-friendly and economical fuel choice to pave the path of sustainable development.

The fourteenth chapter signifies the importance of microbial cellulase enzyme in the bioenergy. The cellulase enzyme is used to hydrolyze the lignocellulosic biomass to their monomeric subunits, i.e., glucose which can be used to do fermentation to produce bioethanol. Bioethanol from inedible cellulose - rich biomass is the most promising candidate to replace fast depleting, environmentally polluting fossil fuels. Hydrolysis of cellulose to glucose is the crucial step in its biotransformation to bioethanol. Enzymatic hydrolysis is favored over acid hydrolysis, as enzymes are eco-friendly biocatalysts with high substrate specificity and superior catalytic efficiency in mild reaction conditions. Complete hydrolysis of cellulose is achieved by cellulase. Higher cellulase production yield, stability and catalytic efficiency are the main attentive points for the successful implementation in industrial production of bioethanol. This chapter will highlight general characteristics of microbial cellulases and their role in bioconversion of cellulose to biofuels, economic sustainability of cellulose-based biofuels and the latest innovations in cellulase immobilization as the most comprehensive strategy for improvement of enzyme stability, activity, and reusability for cost-effective large-scale application.

The fifteenth chapter describes the comparative studies on the biodiesel productions using neem and jatropha oils. Sustainable development is key aspect of modern-day development. Reducing fuel cost and emissions is considered as the most important research area in sustainable development. Biodiesel has the potential to reduce the requirement of petroleum diesel if blended in suitable proportion. Various feedstocks are being explored for biodiesel production. Oils derived from neem and jatropha can be utilized for biodiesel production. In the current investigations, Optimum parameters for the production,

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namely catalyst proportion, temperature, oil to alcohol ratio were optimized. Experiments were carried out at different blend proportions to study fuel properties namely kinematic viscosity, aniline point, diesel index, flash and fire points, specific and API gravity, cetane number and ASTM distillation characteristics.

The sixteenth chapter denotes the characterization and comparison of various blends of Honge oil methyl esters with the diesel fuel in order to reduce the air pollution and fossil fuel dependency. The Honge oil, known as pongamia oil is one of the most widely used oil around the world as lamp oil, in leather tanning, in soap making, and as a lubricant. The Honge oil methyl ester can be used as an alternative fuel for diesel. The transesterification of methanol and Honge oil in presence of base catalyst results in Honge oil methyl ester. In the current work, the Honge oil methyl-ester known as biodiesel was produced. It was mixed with petroleum diesel fuel in different proportions. Various physical and petroleum properties were investigated for Honge oil biofuel and also for various diesel and ester mixtures. The optimum parameters were, temperature was 65 0C, catalyst quantity, 0.4 grams and oil: alcohol ratio, 1:6. The blends of 10%, 20% and 30% compositions were most suitable as potential alternative fuel. Different proportions of biodiesel ester in diesel fuel have been also compared with petroleum diesel fuel to identify suitable blend having good engine fuel potential.

The seventeenth chapter signifies the biomass-fed anaerobic digesters in semi-arid areas. The mitigation of adverse climate change requires holistic strategies. The strategies include the proper handling of biomass wastes such as cow dung. The handling of the wastes has to be efficient using appropriately designed anaerobic digesters. In addition, the operating-status of these digesters has to be monitored for detection of any fault of the digesters. Unfortunately, existing approaches for designing and monitoring the working condition of digesters have limitations. This chapter presents the innovative and explicit conceptual design approach of adapting the biomass-fed anaerobic digester and the digester operating-status-indicators in semi-arid areas. The approach is recommended to the renewable energy system designers. Finally, the appropriate indicators are recommended to be applied by the biogas plant operators or users.

The eighteenth chapter emphasizes the different ways to pretreat the lignocellulosic biomass to remove the lignin so that the access to cellulose is improved efficiently. Rapidly depletion of fossil fuel-based energy sources increased the demand for alternate energy sources. Lignocellulosic based 2G ethanol can be used as an alternative sustainable source that present in ample amount. Sources of lignocellulose biomass are wood, food-agriculture wastes and forest residues. Cellulose, hemicellulose, and lignin are the core components of lignocellulosic biomass. Cellulosic and hemi cellulosic biomass are enzymatically hydrolyzed to produce the monomer sugar (such as glucose or xylose) which is further converted into ethanol using fermentation process. The presence of lignin provides physical barrier that limit the access of enzymes required for saccharification. Pretreatment helps in removing the lignin from biomass and reduce recalcitrance. Pretreatment can be done by conventional methods which are chemical, physical and biological method. This study covers the different methods of pretreatment including their disadvantages and benefits along with saccharification and fermentation processes.

CONCLUSION

The Paris Agreement on climate change and the Sustainable Development Goals (SDGs) create momentum for international joint forces to combat climate change. Bioenergy, which represents a significant type of renewable energy is key to supporting the agreed SDGs, particularly in the context of energy security, sustainability, and economic development. Bioenergy is considered the most problematic area among

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renewable energy sources – with multi feedstock, technology and diverse energy carriers. Presently, several novel physical, chemical, and biological routes are used to produce renewable bioenergy. In India, crop biomass availability is estimated at around 500 million metric tons per year and can produce energy equivalent to 18,000 megawatts. It shows a bright future in energy production in India if adequately used.

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Acknowledgment

This book is the result of the dedicated effort of numerous individuals. We would like to acknowledge the help of all the people involved in this project and, more specifically, to the authors and reviewers that took part in the review process. Without their support, this book would not have become a reality.

First, we would like to thank each one of the authors for their contributions. Our sincere gratitude goes to the chapter's authors who contributed their time and expertise to this book.

Second, we wish to acknowledge the valuable contributions of the reviewers regarding the improvement of quality, coherence and content presentation of chapters. Most of the authors also served as referees; we highly appreciate their double task.

Additionally, we would like to thank our Editorial Advisory Board.

Our Production Coordinator skillfully managed the production of the textbook. We wish to thank the publisher for the sustained interest shown by him during the entire work. A sincere expression of the thanks goes to the entire staff of IGI Global.

Generous participation of faculties and students to eliminate errors in the text and to eliminate errors in the text and to refine the presentation will be greatly acknowledged.

Last and not least, we beg forgiveness of all those who have been with us over the course of the years and whose names we have failed to mention.

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Chapter 1
Bioenergy: Social, Economic, and Environmental Impacts

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ABSTRACT

As we know that escalating population demand and technological advancement lead us towards a new world dominion power, but with that, there is huge pressure on resources due to an increase in consumption at all levels globally. This leads to revamping environmental motifs all over the world and non-renewable sources are estimated to be not rehabilitating soon. In this case, bio-energy is energy presented as a major opportunity to address the issues of climate change by reducing carbon emissions. This chapter explore bioenergy.

INTRODUCTION

As we know that escalating population demand and technological advancement lead us towards a new world dominion power, but with that, there is huge pressure on resources due to an increase in consumption at all levels globally. This leads to revamping environmental motifs all over the world and non-renewable sources are estimated to be not rehabilitating soon. In this case, bio-energy is energy presented as a major opportunity to address the issues of climate change by reducing carbon emissions.

BACKGROUND

Bio-energy can be defined as a flexible renewable energy source that can be obtained from organic matter, which includes husbandry, and food waste, forestry sector includes timber, also sewage, and so on. In this, all energy is obtained from a biological source, which produces heat energy (thermal energy), electricity or power, transport biofuel, cooling purposes, etc. Bio-energy makes up nearly 15% of global primary energy consumption substantially from fuel wood and 50% of global renewable energy sources.

DOI: 10.4018/978-1-6684-5269-1.ch001

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(Gheewala Shabbir, et al., 2011). Unlike other renewable energy sources, bio-energy spare on a raw material feedstock that can use within energy processes.

BIOMASS

Bioenergy is energy deduced from biomass. It has appertained as a material which is produced by photosynthesis and which is residue from husbandry, forestry, and related diligence, a beast and vegetal composites, algae, fungi, and biological wastes which all are of biological origin and are biodegradable. They are obtained as feedstock in the manufacture of bio-oils, biofuels like petrochemical substances, and other products. The key challenge for bioenergy is arising due to changing environmental substitute patterns that adversely affect the supply chain of biomass, as it is sensitive to environmental impacts. This change in biomass use for energy has an insinuation associated with changes in land use patterns, which affects the subjection of biodiversity to feedstock production.

In this chapter, we are going to discuss the impacts of bioenergy socially, economically, and environmentally.

As its major threats of bioenergy have not been instigated at greater risk for the world, yet hence it seems to be a good solution for now. In short, bioenergy can act as a key part of a future demand solution. According to the IEA Bioenergy report, bioenergy includes efficient and increasing the sustainable use of biomass for biomaterial production. Also, power and thermal energy for transportation purpose as the major opportunity to reduce carbon dioxide emission including clean efficient renewable energy solution this efficient source act as an alternative source of energy for non-renewable source for sustainable bioenergy use (IEA Bioenergy report, 2020). This also helps to step forward to achieve sustainable development goals.

According to IPCC (intergovernmental panel on climate change) 6^{th} assessment report, global temperature speed up in the last 100 years and reach its peak and in the next 20 years, global warming will breach the threshold of 1.5 degree Celsius. If GHG (greenhouse gases) continue to emit global warming, will be above 2 degrees Celsius by the mid-2100s. The rise in sea level started affecting sea-lined countries and Small Island are at greater risk .as per researchers, this bioenergy can act as a stabilizer to control GHG emission and help to reduce extreme weather events. Nevertheless, demand for bioenergy feedstock increases due to bioenergy demand escalating, this feedstock is primarily sowing the seeds of agricultural and forest material. The IPCC determined that the average amount of BECCS(bioenergy with carbon capture storage) in the climate framework requires 25 to 45% of fertile and permanent cropland that is nearly 6,500,000 km square of area up to the next 80 years (Vaughan, A., 2021). Hence, to keep our energy supply pristine, bioenergy must emanate from sustainable resources. This biomass production to derive bioenergy should not affect food material production. Impacts on the environment (including animal welfare, plants, air, water, and ecosystem) must be superintended aptly. LUC i.e. land use consumption has to be sustainable.

IMPACTS OF BIOENERGY

Bioenergy is act as an alternative to fossil fuels, and energy suppliers and increases the energy security of rural communities. Further, it has accommodated sustainably and carefully towards affecting socially,

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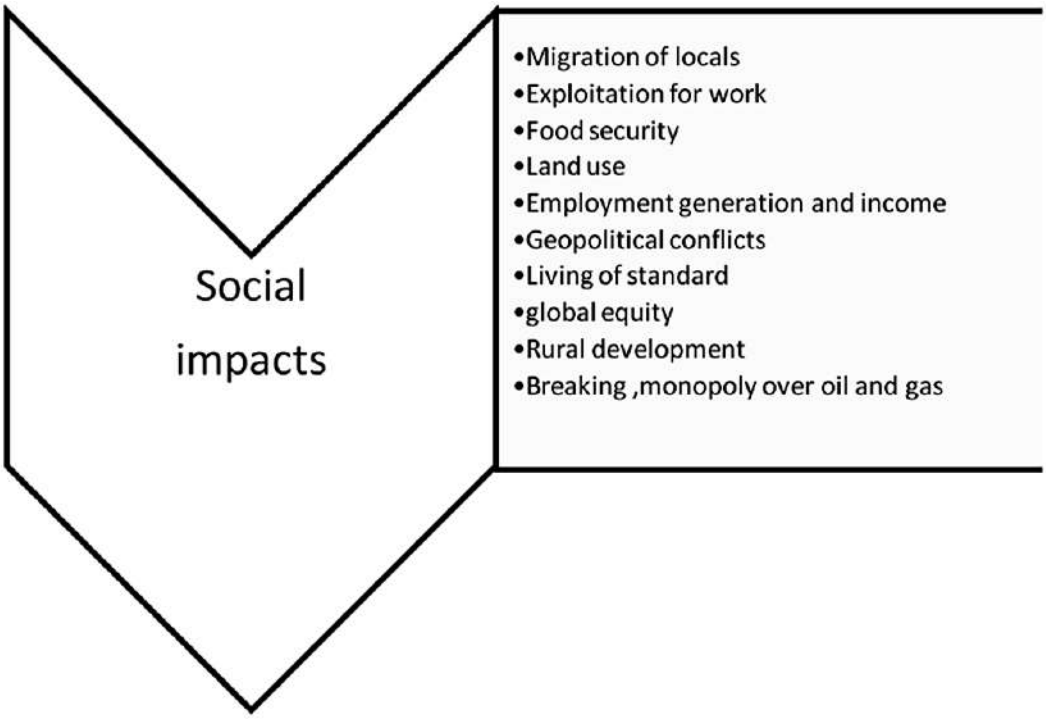
also feedstock affects economically and a limited amount of land, water, the environmental condition affects environmentally. These effects may be positive or maybe negative hence; we can say its impact is having both aspects.

Figure 1.



Social Impacts

Bioenergy, which is executed in different forms, has an impact on society. They vary from the merits of new job opportunities, public support, awareness among the people, food security, and water quality to its demerits of societal conflicts arising due to geopolitical regions inflation due to reducing food agriculture land due to changes in land use pattern. There are many factors, that sculpt the rate of change of bioenergy and its consumption demand depends upon local and regional factor influencers. We can see the impacts of bioenergy in a stepwise manner as follows:

1. Poverty Reduction

Uses of bioenergy require biomass, which can obtain from land use. In developing, nation's agriculture is still the dominant sector and has increased population pressure in many such countries. In addition, some agricultural pastureland produces marginal food. However, the introduction of biomass production from following crops like corn, sunflower palm, etc. leads to the production of biofuel such as corn

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ethanol that is used in transport goods. Even in many countries, petroleum products blended with ethanol are used in the markets.

E.g. For India's ethanol blending program in accordance with national policy on biofuel, the government has set a target of 10% ethanol blending by 2022 to 20% in 2030. This has environmental effects too which we can see in the next part on environmental impacts. As a part of a social impact, this leads to an increase in demand for biofuel, which is a sub-component of bioenergy and causes an increased demand for biomass that leads to giving farmers a side income profit that helps them to reduce poverty and become wealthy. Nevertheless, that increase in prices limits the supply of food products as farmers may shift towards commercial farming rather than food supply. This may also affect the global level prices, causing deficiency in food products.

Then a Question Arises: What Is Biofuel?

Biofuel is a term for a natural form of a solid-liquid or gaseous fuel, which is, arises from biomass processing from crops like jatropha, corn, sugarcane bagasse, algae, etc. and releases energy which acts as a renewable neutral source and act as an alternating option for fossil fuels. They can convert chemical energy to electrical energy.

We can segregate biofuel generation based on four criteria i.e. 1st generation of biofuel is from Starch, sugar, fats, and oil from plants and animals.

2nd, lignin, and cellulose by enzymes or by gasification include municipal solid waste, agricultural residue, forest residue, 3rd from algae, 4th from vegetable oil, and biodiesel. Here Liquid biofuels are biodiesel, bioethanol, bioethanol, bio ethyl tertiary butyl ether, etc.

2. Job Creation

At a societal level, the global climate bash often orientates with inherent support for bioenergy needs. This leads to creating job opportunities for many unemployed generations both from rural to urban levels. At the primary level of agriculture, harvesting bioenergy products from Jatropha curcas is labor intensive and can lead to generating job opportunities and income for rural employers, which are laborers, farmers, suppliers, transporters, etc.

In addition, these are used in Transport biofuels, which act as a fast-growing new energy sector due to their demand. Hence, it will emerge as an important part of energy in the next few years whose hunger even started from now. This increase leads to the development of markets for agricultural components. (BBSRC Bioenergy Dialogue - Report on a pilot public dialogue, Dec. 2013) e.g., INDC (Intended Nationally Determined Contribution) under UNFCCC –in India PANCHAMITRA mission has a target of - 40% energy from renewable sources by 2030.

3. Standard of Living Increases

Bioenergy affects people not only environmentally, but socially well-being too, which helps to reduce casteism between communities within the intercountry or intra countries level. As this energy has, the potential to produce employment that directly affects the income of people mostly to rural middle-income class people to live a free life from a debt gravl income generation. In addition, this energy in the form of renewable energy reduces the high cost of electricity bills from coal and gives eco-friendly energy

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from available sources without any load shedding effect and unlimited access to energy that indirectly improves their living standard.

This leads to awareness among the people about the need for bioenergy and its social impact on their livelihood. This often indirectly promotes the children and youngsters towards school and college for education. According to the report of UNICEF (United Nations of children's fund), many children mainly from the African continent are still facing problems in education. Due to poverty, hunger, non-availability of standard living due to unemployment, and social conflicts in their barren lands like the Sahel area (including countries of northern Africa like Niger, Mali, Burkina Faso, Chad, and Sudan where the great green wall project established to restore greenery. Which are unfolding their climate change crisis desertification, rainfall variation, land degradation, and erosion) these situations discourage children from education. Hence, in such areas bioenergy production and its distribution can act as a catalyst to trigger the standard of living. This is also applicable to many under-developed and developing countries. Also in developed countries like the U.S.A. bioenergy now seems to be a new solution to deal with the oil crisis and stabilize their currency and power, which in short maintains their living standard.

4. Land Use

Geopolitical Conflicts

Social conflict arises due to the need for land use for primary sources and the need for bioenergy production via agricultural biomass. Conflicts between people asserting clientele rights over lands, this cause inconvenience and problems to any scheduled program.

When it comes to the production of energy from biofuel and needs employers to fulfill company needs may arrange facilities for an employer near the biofuel. Projects cause centralization of the working population with their families in distributed areas or cotters but this increasing population may result in a metathesis reaction of farmers from their traditional land site. This may result in land ownership problems called proprietor conflicts.

Case Study

Forest living tribes, in eastern Asia Penan nomadic tribal community living in Malaysian island Brunei fighting a long conflict with palm oil companies because their territorial boundaries were declining and their natural surviving sources used without permission become illegal, which leads to face environmental impacts over their territorial lands due to energy consumption.

In addition, the greatest impact on the urban socially and economically backward class arises because they do not have enough land for taking advantage of bioenergy projects.

What Is a Metathesis Reaction? How Is It Connected?

Generally, this word metathesis is used in chemical reactions, which is also called a double displacement. When two bonds exchange between each other or to a corresponding substrate it formed as a new product known as a metathesis reaction.

It is represented as - AB + CD ® AC + BD. In this equation, B and C replace their positions.

If we interlink this phenomenon to the social impact on land use consumption due to bioenergy projects or any other such projects we can find out that like the new product form in the above phenomenon

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by interchanging constituents the farmland can be interchange or conversion take place due to such product implementation.

5. Societal Gap

Such type of projects for bioenergy implementation is strongly favored by the renewable concerning party (here it means environmentalist or public in support of bioenergy) while there is another group present that opposes it and disapproves of the project held at the local level. They are having such reasons to oppose like:

1. Bioenergy projects hold people's traditional land that they (landholders) do not want to give for any purposes.
2. If it has future impacts, it may hazardous for maintaining sustainable balance.
3. It can generate waste.
4. If the implementation is improper may be due to corruption, held under the name of a bioenergy project.

In addition, there is a lack of updated data on bioenergy from the local level to the national level to a global level. Because of idiomatic biomass and laborious challenging technology, it is difficult to gather information at the local level. This internal challenge needs to be in a focus on bioenergy project successions.

6. Political Interference

Increasing conflict or societal gap that emerges as an opportunity for some political leaders to make it a big issue rather than solving or awakening people, they use this topic to collect votes in their favor, which is a harsh reality in society.

Global Equity

A broad range of international organizations ensures sustainable and affordable bioenergy and supports the formation of international collaborations.

In 2017, fossil fuel consumption in Asia is nearly 50% of the global supply. While EUROPE and America account for 47%, hence to tackle future energy crisis as many continents and countries in dependent on primary fossils primary energy supply fossil fuels there is a wide scope of bioenergy, and its sharing among friendly nation increase global equity (Global bioenergy statistics, 2019).

E.g.- ISA international solar alliance including 100+countries is a major bioenergy project from solar technique to generate energy that is renewable, once implemented they have a longer shelf life and no electricity bill this helps to encourage the world for shifting toward eco-friendly technologies and increase its global partnership.

Waste to Energy

Municipal waste includes domestic waste, which is biodegradable and utilized for energy predominantly in urban areas. In this situation combustion of waste product, take place. After processing various times,

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dry waste collected by municipal corporations is converted into bioenergy. Such energy is used in the generation of electricity, fuel for vehicles, and thermal energy for domestic use. This technology helps to reduce waste and recycle it for social and environmental well-being. However, with that, there is a problem of segregation of this domestic waste. Hence, we can be defined it as ‘’It is the process of generating energy in the form of electricity from the primary treatment of a waste converting it into a source of fuel. ‘It is one type of energy recovery from biowaste.

Figure 2.

7. Breaking Monopoly Over Oil and Gas

Petroleum resources are mainly found geographically in the regions of west Asia like UAE, Saudi Arabia, Oman, and Yemen and they extracted it more than any other country and rise from poor to developed economy. It does not mean that other parts do not have reserves of fossils but they have not been explored yet. Some parts of the American continent hold much amount of natural reserves of fossils but have not been extracted yet in that mount and location finding is still in progress. Although, most countries depend on imports of petroleum products from these countries. In addition, faces crises called oil shocks during regional conflicts, world wars, etc. hence bioenergy is a solution over them to break the monopoly of these countries over oil products.

Economical Impacts

Bioenergy can be a cost-effective solution over costly non-renewable fossil fuels. This can lead to a major economic impact due to its availability biomass production cost, processing cost, then supply chain cost, budget, accelerating transition and creating an alliance, open access consumer, captive power plants,

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income effect, open access to the consumer. According to a recent study, researchers reveal that increasing investment in the bioenergy sector and its overall processing from production to distribution may step towards a wealthier economy if implemented profoundly. The increasing importance of bioenergy constrains organizations like FAO (Food and Agriculture Organization), and OECD (Organization for Economic Co-operation and Development) to make a framework for sustainable use of bioenergy.

Economical impacts are as follows:

1. Capital Investment

Non-renewable energy resources such as fossil fuels have some lacuna. Like, a slow rate of production leads to generate pressure on a course due to its high demand leads to price inflation and mini oil shocks situations whereas Bioenergy products are less expensive and diverse than fossil fuels like petroleum, diesel, and kerosene, which significantly affects the economy. It has great future capabilities ahead hence; there is a huge chance of increasing investment in bioenergy projects. In addition, this bioenergy feedstock material is harvested locally and they are abundantly available making it a sustainable and self-sufficient source of energy. Hence, it is the center of attraction for investors and helps in economic gain.

E.g. - Bioenergy projects such as the biodiesel industry generated $21.6 billion which greatly and positively affects countries economy of the United States. It approximately created thousands of job opportunities.

2. Industry Engagement

As capital investment starts rising, many different companies meet to discuss, projects and their analysis, which procure exquisite understanding among the companies or industries, forming a cooperation alliance among them.

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Figure 3.

3. Price Stability

According to FAO, a larger country produces the most reliable estimates than poorer countries. It is common that larger and wealthier countries to have modern techniques and analysis of estimated bit in case of poorer countries have to depend on outdated surveys and they have to face technical deficiencies. Sustainable bioenergy is beneficial for the price stability of both developed larger countries and poorer countries. As we previously discussed that it generates income source and employment, and help deprived people to overcome the debt trap. Schemes like feed-in tariff (FIT) are helpful for bioenergy elaboration that include domestic to municipal and small businesses to be benefitted from it and also from its social acceptance and awareness about it.

Case Study

Hawaii is a small island in the Pacific Ocean 2000 miles away from the U.S. mainland and is mostly dependent on foreign nations to attain fuels like petroleum products, natural gas, and coal as it lacks when it comes to its own fossil reserves. Which greatly increased their expenditure cost on electricity but they found a resilience strategy. They started to use renewable sources like bioenergy from biomass, this helps to save the economy of the country and make it sustainable energy supply. Investment factor also helps them in such projects.

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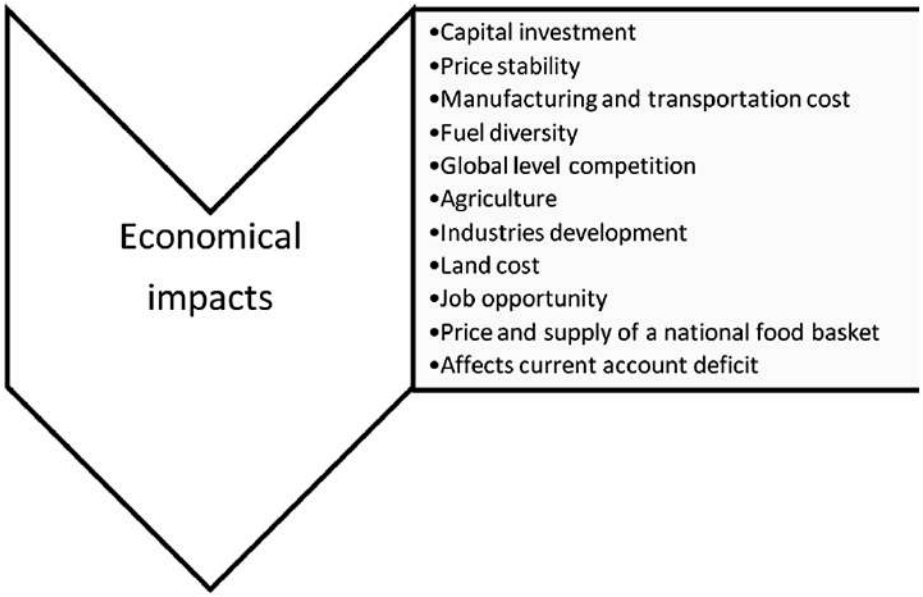
Figure 4.



4. Logistic Support and Supply Chain

For the development of bioenergy, it is impossible without including logistic support, which acts as a vertebra for bioenergy gaining. Because energy, produced has to transport to deprived sectors or interchange fossil fuel consumption there is a need for logistic support. From the start of the project, its basic needs, important components, facilities gained from these resources, handling, processing until packing and distribution of materials. Hence, we can say it is a catalyst of energy. This socially empowers people by giving them employment.

Supply chain management, from the implementation of the project, and its location to emission and energy cost need to account for under logistics. Nevertheless, with that, it has negative aspects too. Transportation act as a costly component of bioenergy. It is prominently seen because a measure of bioenergy has lower energy and less volumetric solidity than petroleum products obtained from underground. In some countries, the cost for supply management use is very high than the actual budget, leading to a developing financial crisis. This is because of, a long distance from the location to the distributor, roadways condition, traffic, pipeline management, and waterways having an issue of time consumption due to port management was inappropriate leads to delay loading and unloading in some countries but countries like Philippines exception to that. Bioenergy is the second largest employer globally with approx. 3.2 million people working in the bioenergy supply chain (United Nations Development Programme, 2020).

Now the Question Arises: What Is the Concept of Catalyst?

A substance, which does not take part in a chemical reaction but by its mere presence, can speed up or reduce the speed of any chemical reaction known as a catalyst, and the phenomenon process is called catalysis.

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5. Manufacturing Cost

Manufacturers are mostly concerned about cost factors and revenues in any project, bioenergy projects are going from this phase of the cycle. We can say it is a bioenergy production cost. From a bioenergy production point of view, the manufacturing cost is considerably less than that of fossil fuel. However, along with that new implementations have to be made like reserve capacity building has to be increased this is the part of impact assessment. Another impact is different countries have different currencies from the American dollar, and European euro in the west to the yen in Japan, the Cambodian rial, Kuwaiti dinar, and Indian rupees. In these currencies, there is a huge gap in the international market. Which can create an imbalance (there are international organizations to make a balance IMF international monetary fund, which makes the balance of payment BOP) hence, when it comes to importing or exporting feedstock material or basic manufacturing products it may be costly or cheaper. And manufacturing companies find their profits by importing at a cheaper cost but the exporting countries also do not have to be in loss because the purpose of introducing bioenergy is to make sustainable development but along with that it also helps to secure or develop living of standard by giving many employment opportunities and income economically.

Figure 5.

6. Price and Supply of a National Food Basket

According to research, bioenergy affects domestic food supply and affects food baskets due to reversal stipulation in a food market, climate change pattern affecting agriculture hence agronomics cost also fluctuate un accordingly this may cause inflation. Causing inflation greatly influence both international and local level. Many countries have already undergone food crises due to many reasons like financial crises, conflicts among nations or internally, environmental violating influences, etc. Along with that agricultural land also decreasing in such cases globally contribution to bioenergy projects is wracked or seriously affected which may trap the development of bioenergy projects because in such countries

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they are still facing primary concerns of basic requirements like food shelter and clothes. According to the Global hunger index, which is jointly published by Concern Worldwide and Welthungerhilf. Many African countries like the Central African Republic, Chad, Sierra Leone, Madagascar, Zambia, Yemen, and Sudan, are facing such a crisis. Price volatility also greatly influences their condition. However, bioenergy can be a solution for their problems not completely but leg up and lift them from a crisis. As per researchers, total biofuel consumption rises mainly in countries like China, India, Brazil, the USA, and European countries because these countries were largely dependent on fossil fuels for energy supply or fuel consumption whose prices are relatively high hence their shifting to agricultural feedstock, cellulosic biofuel, etc as another option but it leads to rise food prices too. In such circumstances, markets have to access their physical and fiscal assets. They also have to be transparent functions and efficient to contract and cope with the demand rights of people to oppose or support taken into consideration.

7. Transportation Cost

Despite the increase in the advantage of bioenergy and its growth steadily escalating, transportation is scarcely increasing in terms of bioenergy. This market gives unique opportunities to new startups related to transport facilities that boost the economy of rural and poorer sections as these small businesses have structured their business to give effective transport facilities for biofuels. Transportation of biofuels with greater attention is currently vigorously growing bioenergy sector. However, represent only 2% of total bioenergy. If improvements take place in the transportation sector could lead to quality, the wise better quality of biofuels. it seems to be one of the most feasible ways for the transportation sector and according to sources it reaches 25% in the next 30 years from the current 3%. Increasing global warming and its disastrous nature due to human over technological advancement without an impact assessment and increasing fossil cost leads to an alternate source of bioenergy in the transportation sector (UNDESA, 2006). As bioenergy is mostly produced from sustainable sources like lignocellulose compounds have had a greater impact overall.

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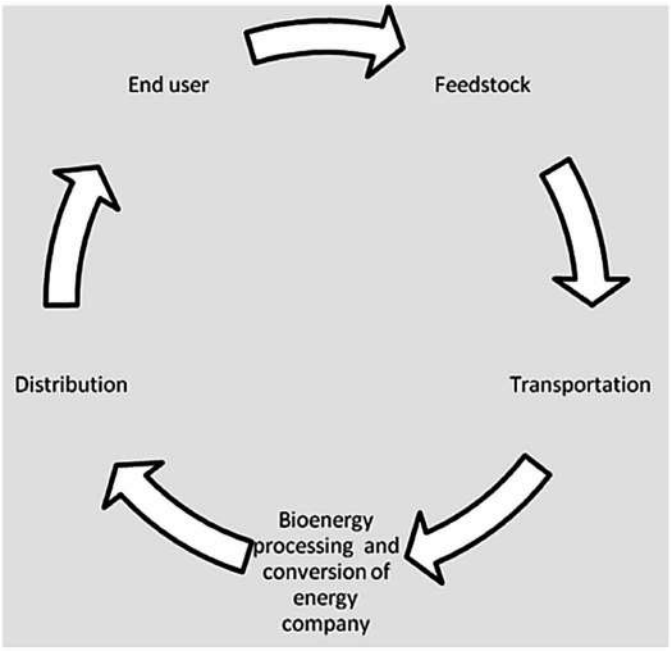
Figure 6.



8. Energy Cost

Bioenergy greatly decreases energy costs as we previously see an example of Hawaii. How they stable their energy cost by using bioenergy. The Electricity generated from coal (carbon fossil fuel) along with pollution is price consuming. In addition, petrochemicals are in great demand but many countries do not have their natural reserve hence their importation leads to levy taxation within countries by their governments, which affect society and their living. Reducing this extra energy demand of fossil fuels by altering them with biofuels is a need of the hour. We can see the concept of how this energy is consumed by TFSC-Total final energy consumption here counting an energy content by its total amount of energy transformation and distribution and its consumption in residential, commercial, and agriculture. Gross final energy consumption is a constituent amount of renewable form of energy consumed while energy transportation, its transmission, and distribution. We can also say that Gross final energy consumption=TFEC total final energy consumption + thermal and electrical heat consumption - are used for the non-energy sector (IEA, May 2021). In countries like India, the REC mechanism renewable energy certificate to boost green economy is implemented.

It is a tradable and legal renewable energy credit mechanism where one REC is equivalent to one MWh. This mechanism is associated with the production of renewable energy like bioenergy and encourages the generation of electricity from this source. To deal with challenges associated with it i.e., excess supply over demand REC prices remains close to ground level prices. ESCerts is also a tradable certificate under the PAT (perform achieve trade)-it aims to stimulate energy efficiency. It is a part of the bureau of energy efficiency. Certification schemes to abolish trade barriers, now and in the future

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9. Global Level Competition

Due to increased investment in this sector, creates healthy competition among the different nations as it increases income and resilience for rural livelihood enterprises. Economically it has financial and fiscal incentives. Subsidies, grants, VAT i.e. value added tax a part of it, import, and export duties tariffs all act as a financial barrier to developing off-grid systems. Along with that if we look the percentage shows that In Africa according to a research report says that 20% increase in import tariffs (currently at 0%) could reduce sales of off-grid systems according to the size of products. In countries like China, it accounts for the greatest share of renewable energy investment including bioenergy followed by the United States 20% in Europe (19%). Also, investment in renewable seems to be declining in China and India but excluding these, there is a 17% gain according to researchers. The Middle East and Africa accounted for 5%, India 3% and Brazil 2% biomass capacity investment climbed 26% to USD 2.6 billion. In 2030 global demand for biofuel will increase to 90% of the current production of palm oil and other products (Gao Yan, et al, 2011). This growth in demand is driven by public incentives. Like India, its bioethanol blending mission Indonesia already make a strategy of biofuel consumption their biofuel blending increase from 5% to 30% in 2020.

10. Current Account Deficit

The high transaction cost of bioenergy for developing and underdeveloped countries may lead to an increased current account deficit (CAD-simply we can say that current account deficit means if the expenditure is more than its income from various sources and its difference called CAD

E.g., suppose bioenergy production's overall value is 2 million dollars but its consumption by distributing only collects 1 million dollars their difference is one million dollars called a current account deficit. According to economics, less amount of CAD is an indicator of increasing the economy but a large amount decreases it. Bioenergy also influences the GVA of countries which is the gross value added per unit of bioenergy production. The bioenergy sector also leads to the social stock exchange and corporate social responsibility and the digital divide. There are such impacts economically that can happen which indicates both positive and negative aspects.

11. Agricultre

Bioenergy production from 90% solid material which grown in agriculture as biomass, feedstock, and its production started to increase which is a cost-benefit to low land farmers. But to reach its peak near future and to encourage farmers to grow such biomass it's training along with subsidies and insurance in the case of natural calamities or accidental burns on the farm hence to safeguard them such formulas have to be applied. Contesting for agricultural feedstock and its land may increase food prices and farmers and from APMC markets dealers' income it may increase but net buyers will suffer from inflation.

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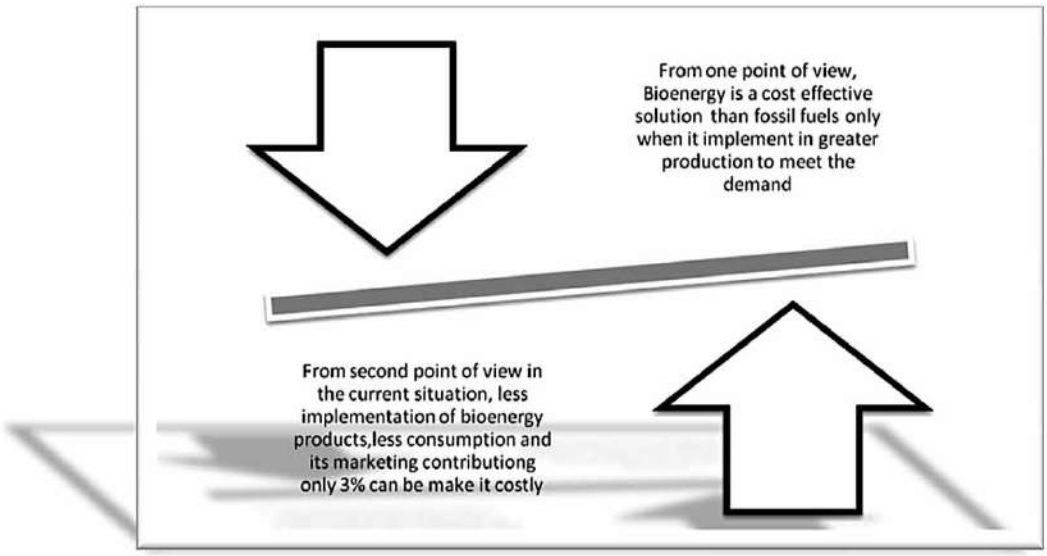
Figure 7.



If we talk about its positive aspects, it is economically beneficial to agriculture primary sector and agricultural laborers, but such products also not have negatively affected fertile productive land. Until now, not much such big research has taken for the evaluation of the cost-benefit ratio of the land. Bioenergy seems like grace in many sectors along with social, economical, and mainly environmental concerns but with that, we have research on its long-term impacts by EIA environmental impact assessment so, with taken safety measures we have to ensure that this grace does not convert into a curse for our future generations.

Environmental Impacts

After we have seen the social and economic impacts of bioenergy, let us move on to the environmental impacts of it. From previously seen case studies of the Hawaii area and Sarawak community, we can say that energy security along with global warming and other climatic issues needs new solutions to reduce its hazard. Bioenergy feedstock than carbon fossil fuels may be cost-effective and may contribute to slightly addressing the problem of climate change for sustainable development. Although it seems like a solution it has to highlight that, bioenergy is not a full solution for environmental issues but it can drive as a GHG emission reducer. Contribute to waste treatment in an effective way.

From local condition to various global factors depending upon a type of environmental condition, land, and soil used, its intensity, and more or less productivity such factors affects bioenergy production. An increase in demand for environment-friendly energy like bioenergy also influences feedstock, which indirectly contributes to land use competition among primary agriculture producers that are farmers. They also tend to convert food cropland to energy commercial due to price factors.

Actually, Bioenergy is not a carbon neutral source but can influence such conditions to ensure sustainable development. With the Merits of reduction of GHG (Green House Gas) and the fossil fuels, storage within minimal energy loss, wildlife health and human health, giving agriculturally marginal product

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income, carbon sequestration, and sustainable source. Bioenergy is also affecting the cons of soil erosion, biodiversity loss, water contamination due to material used in bioenergy agricultural fields, deforestation for lands to grow biomass, etc. There are challenges in the new era of bioenergy implementation. Let us see the impact of bioenergy from an environmental point of view in detail.

1. Land Acquisition or Use

In many countries, degraded lands are used for bioenergy plantation making them reproductive land. Planting trees reduces erosion and ecology restoration takes place. To expand the production and cultivation of biofuels there is a need for a clear well research strategy to regulate land conditions.

The environmental impact of land use includes that in many developing countries small landholding farmers cannot afford its products and materials. For project managers also it is not easy to convince most farmers to introduce biomass farming from their traditional farming. Also, to their migration in case of land need for an industrial approach to change their land.

Indirect land use change in such cases may affect the environment as new agricultural places mean deforestation, which may be arable land, or aural forest and grassland may contaminate the land by using fertilizers and pesticides in such areas. (Bertzky M., et al., 2011).

Figure 8.

2. Soil Quality

Soil is an essential element in the productivity of the land. Due to climatic adverse conditions, poor agricultural practice lowers the fertility of the soil. Major effects of biomass production have not been seen in the products like Jatropha, Palm, Bagasse, algae, etc. Nevertheless, repetition of crops May leads

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to decrease productivity as nutrients in the soil may be deficient amount. Hence, fodder of macro and micronutrients are given to such farmland to increase yield but most farmers not having actual knowledge of the quantity-wise ratio of such fodder material, which causes to decline in soil fertility due to excessive nutrients.

Agricultural practices may use fertilizers, and artificial insecticides giving rise to soil erosion due to an increase in acidic content and reduction in its water holding capacity and reducing the fertility of soil the effluents which get diffuse in water when in com in contact due to rain, river runoff near agricultural land and underground water leading to water pollution. Hence, we can see the next impact on water.

3. Impact on Water

As we know, the amount of drinkable water on land is 3% from which a large amount is in the form of ice in Polar Regions and only less than 1% of water is available. According to a report by the International Water Management Institute, the rush for liquid biofuels could worsen the water crisis in some countries. In addition, many countries around the world facing water scarcity, and global warming emphasizes it. In such a crisis, bioenergy production could add pressure on existing sources. Pollutants from the soil by agricultural fertilizers and all loaded into water bodies cause pollution. For this, some resource management has to work done efficiently to solve the issue .but on the second side encouraging bioenergy production can reduce the cost of hydraulic renewable from freshwater means energy or electricity generated from freshwater sources to ensure sustainable balance.

4. Forest

Forests hold a large amount of carbon and remove large amounts of carbon from the surrounding atmosphere providing clean air, water, and food, shelter for many wild species hence they play a vital role in sustainable development. In many countries, forestland is replaced by croplands or by large buildings, parks, malls, etc. From a bioenergy point of view, this forestland is replacing due to an increase in demand for biofuels and basic material feedstock which ultimately leads to increased pressure on forestlands. Lungs of the earth ‘’Amazon forest’’ is also not an exception in such case. (whereas still wood is a greater amount of energy sources across the world that act as a biofuel but its cutting and burning leads to pollution and greenhouse gas emission). Shrinking of forestland and savanna-type grassland leads to reduced feed available for animals and local people, which affects their social, environmental, and economic conditions. (Fritsche R. Uwe, et al., 2010)

Loss of forestland or its area leads to the release of more carbon, which influences global warming and biodiversity losses. According to sources, deforestation and poor land cultivation alone released 25% of carbon. (Vaughan, A., 2021). Here implementing the Carbon sequestration method helps to capture such carbon emissions from the environment and helps for better development of a sustainable environment.

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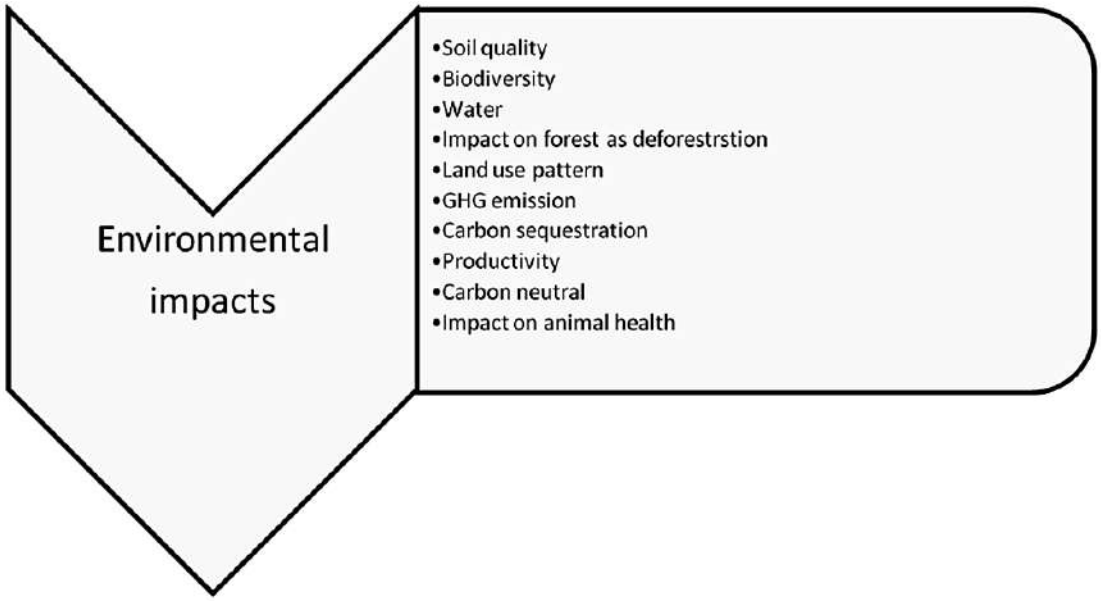
Figure 9.



According to the Greenpeace report, biofuel leads to increased GHG emissions due to deforestation of forests and leads to conflicts among communities, between animals and humans, which we called as man-animal conflicts. On the other hand, some scientists argue that bioenergy reduces fossil fuel consumption and emission from it providing wealthy income and jobs to rural peoples.

Deforestation also environmentally affects the carbon cycle and nitrogen fixation.

5. Biodiversity Impact

It has been argued that an increase in feedstock production influences surrounding ecosystems. For example - ICAR NEERI in India implements a Bamboo plantation program to reduce fly ash, which is under processing. In barren and dump land introducing such species increases the greenery of that site like a small forest in less time measurably 3 to 4 years which also helps to increase biodiversity as different small insects, snakes, and bird species are now native to this area.

But on the other hand, deforestation and agricultural practices for bioenergy production reduce the space of animals for their shelter and food, emissions from biorefineries and indirectly creating noise may create disturbance and habitat loss of such species in that area. Here, natural ponds and aquatic life are affected by it.

In addition, biodiversity is under threat due to large-scale monoculture for which a significant amount of bioenergy production takes place.

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Figure 10.

6. Emission of Carbon

Carbon is constantly absorbed into the trees and it leaves in the atmosphere when such plants are cut down or die. Bioenergy can be used to reduce carbon emissions from fossil fuels like petroleum, and diesel that emits carbon from vehicles, combustion of it, emission from industries, etc. affecting net carbon emission. BECCS (Bioenergy with carbon capture and storage) could remove large amounts of carbon dioxide from the atmosphere and works as carbon sequestration. However, according to some reports of researchers BECCS could not capture total carbon dioxide from the atmosphere. (Kemper J., 2015). The net energy balance concept arises when the ratio of the primary amount of energy consumed is divided by the total energy produced and to assess the net impact such impact assessment has to be done prior. (Berndes Goran, 2010).

7. Impact On Animals and Human Health

Conversion of forest land to cropland for many projects where bioenergy is also one of it, whose environmental impact also affects many species in the form of habitat loss, their changing behavioral patterns, spawning and mating seasons of animals. Due to noise pollution and excessive involvement and movement associated with creating disturbances among the animals. Animal habitat is changing its pattern. E.g. effluent from bioenergy refineries directly or indirectly dissolves in nearby river ecosystems, pond ecosystem leads to the forceful migration of aquatic animals. (Rupp, S. P., et al., 2012) In addition, increasing toxic content and extra nutrients from croplands in static water develop a HAB that is harmful algal bloom, which is dangerous for an aquatic environment. As they reduce oxygen from reaching the surface and aquatic plants and animals create a condition called a biological oxygen demand

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also-spawning season and place of fishes fixed from their ancestral part it is an interesting fact about aquatics but effluents and water pollution divert or become a barrier for aquatics. The same condition happens in the case of terrestrial animals.

Bioenergy can have a health impact associated with it. Biomass used for cooking purposes and the unavailability of proper ventilation systems causes indoor pollution which has a long-term effect when exposed for a long time. Potential impacts include respiratory diseases, headaches, etc. however, such impact is not seen in a large amount but it is a point of concern in a rapidly changing world (UNDP and WHO, 2009).

CONCLUSION

In the above whole chapter, we discuss the impacts of bioenergy but along with that, it needs a proper solution to balance carbon levels and to deal with problems associated with it for sustainable development. As we move towards a solution it broader the bioenergy term. There can be a Suggestion as follows:

1. Environmental degradation due to deforestation, land use consumption, soil and water health, etc, monitoring from a status of environmental benefits. Such studies previously gave an idea of future risks and according to that presolution for calamities has to study.
2. Biodiesel made from used cooking oil benefits the health of the environment and the economy linked with it. Such type of new ideas has to be encouraged, also financial support to researchers and projects helps to build green energy corridors in the world.
3. IEA organizations like international energy agencies dealing with proper enactment of energy sources, their applications and impacts ensure reliable, affordable, and clean energy and its worldwide distribution.
4. Green hydrogen usage as a transportation fuel can save us from impacts. Hence green hydrogen mobility project acts on it. Hydrogen produced from renewable sources is called green hydrogen which has no carbon footprint.
5. Global energy review-assesses the direction of energy demand and carbon dioxide emission
6. Climate smart public-private partnership-Climate Resilient Public Private Partnerships: it is a toolkit used to solve the issue related to the following issue. Such type of bioenergy-related bioenergy public-private partnership can influence and spread awareness among the people too.
7. Nature-based solutions UNDP in the 2020 Human development report also called for investment in Nature-based solutions and the assessment of the environment Assessment of the vulnerability of habitats and species is also taken into consideration before implementing bioenergy projects.
8. Implementation of such impact assessment tools effectively mitigate risk linked with bioenergy production from its land use to sustainable production and consumption

The rate of expansion of bioenergy products depends upon a great deal of government policies, straightforward strategy, and foreign investments in developing countries. Taking care of these bioenergy negative aspects is also hindered.

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Chapter 2
Life Cycle Assessment of Biofuels: Challenges and Opportunities

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ABSTRACT

To address energy security and climate-change concerns, substitutes are needed for petroleum-based transportation fuels. In addition to electricity and natural gas, biofuels are emerging as an important class of substitutes. The promotion of biofuels as energy is mainly driven by the perspective of oil depletion, concerns about energy security, and global warming. However, results published in the past few decades present conflicting pictures regarding the energy efficiency and sustainability of biofuels. To adequately address these real or apparent contradictions, a life cycle assessment (LCA) of biofuels is indispensable. "Life cycle" refers to all stages of a process: from the cradle, that is, raw material extraction through manufacturing, distribution, and use to ultimate disposal. This chapter discusses the benefits and limitations of biofuels from an LCA standpoint. The discrepancies and strengths in LCA of biofuels are critically analyzed and opportunities are highlighted. LCA of biofuels is shown to be critical to informing policy and for practical application.

INTRODUCTION

The rising demand for clean, affordable and ecofriendly energy, climate change and global warming concerns, political agitation among nations, and the depletion of fossil-based fuels has necessitated the search and development of alternative sustainable technologies focused on renewable raw materials. Biofuels are considered to curb most, if not all, the aforementioned issues because they serve as an

DOI: 10.4018/978-1-6684-5269-1.ch002

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alternative clean fuel with supposed reduced greenhouse gas emissions, the drivers of global warming and climate change (Somerville, 2007). The link between biofuels and climate change is attributed to the fact that biofuels are supposed to be carbon-neutral alternative sources of energy. During photosynthesis, plants get sunlight energy and CO_2 from the atmosphere to form complex chemicals that constitute the plant body. When these plants are burnt, the resulting energy from oxidation is released as heat, CO_2 is given out as well and is recycled again (Somerville, 2007). Therefore, incident solar insolation can be harvested as biomass.

Bioenergy has the potential to offer a significant contribution to clean, low carbon, and secure energy. It is also referred to as a carbon-neutral process. Biofuels are also encouraged to mitigate anticipated shortages of fossil fuels resulting from anticipated depletion of natural resources or geopolitical instability. Despite the merits of biofuels, policy frameworks developed and incentives initiated, issues have been raised concerning other environmental impacts associated with biofuels and their energy efficiency sometimes with conflicting results. As such, sustainability assessment, before adoption, from an environmental, economic and social standpoint is very crucial and this can be achieved through a life cycle assessment (LCA). Life cycle assessment is a methodological tool used to evaluate the requirements and technological impacts, processes, and products to determine their propensity on resource consumption and pollution generation. It refers to all stages of a process that includes: from cradle, that is, raw material extraction, manufacturing, and distribution in the supply chain to grave, i.e. ultimate disposal (Mckone et al., 2011). Life Cycle Assessment is used within the framework of internationally accepted methods as explained in the ISO 14040 and ISO 14044. Figure 1 is a graphical sketch of the different steps in the life cycle of biofuel from the extraction of the raw materials to the end-user.

Figure 1. Graphical Representation of LCA of biofuels
(Hauschild et al., 2017)

LCA has been used in different fields of study such as wastewater treatment (Gallego-Schmid et al., 2019), solid waste management (Sebastian et al., 2022), the development of construction materials (Liu et al., 2022), food production systems (Lamnatou et al., 2022), and the environmental impacts of processes such as cement production (Ige et al., 2021) among other sectors. These studies underscore the usefulness of LCA in decision-making process and the need for comprehensive LCA of biofuels before adoption.

There are four types of activities that constitute the Life cycle assessment. The initial step entails defining the goal and scope of the assessment. The second activity is to collect life cycle inventory data on materials and energy flows emissions as well as wastes. The third activity entails conducting a life cycle impact assessment (LCIA), which serves to characterize the constituent process impacts. Lastly is the analysis and interpretation of the major findings alongside sensitivity and measurement of uncertainty to influence the decision-making processes (Mckone et al., 2011). Figure 2 is a schematic diagram of the four activities that define a life cycle assessment process.

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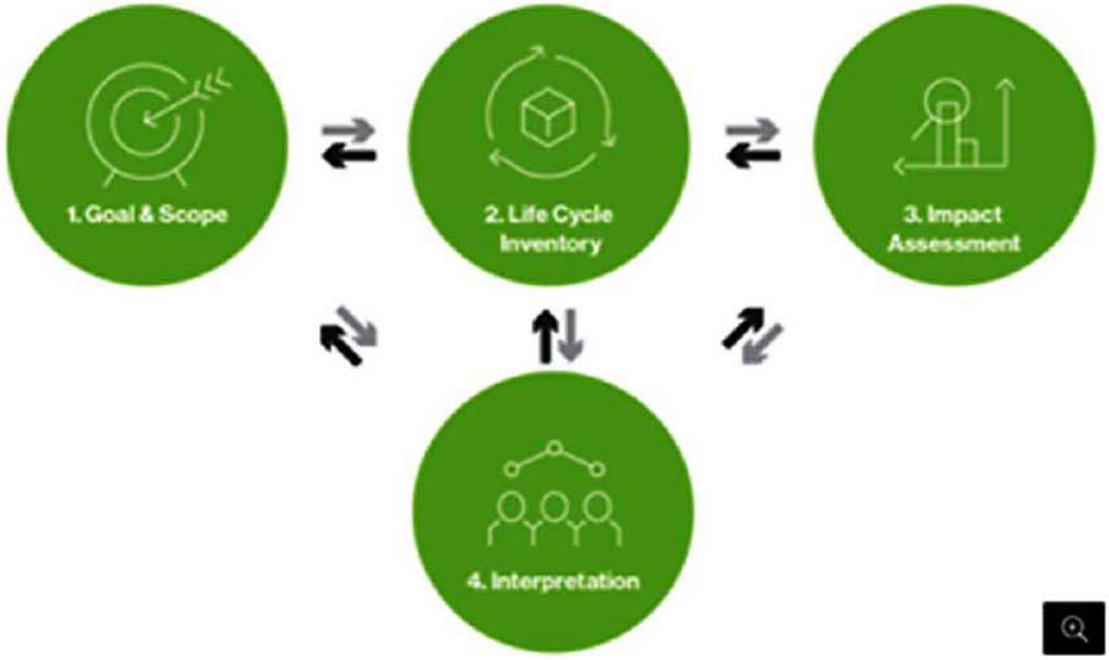
Figure 2. The general steps of life cycle assessment (LCA)



Regardless of the source of the biofuel used, biofuel production can be done in an environmentally friendly way depending on the raw materials used and the production technology utilized. Some important criteria for assessing fuels is their environmental impact and energy efficiency. A greater fraction of the environmental impacts of biofuel are due to the agricultural process of cultivation of the respective feedstock. The environmental impact due agricultural cultivation has been shown to be comparable to that of fuel processing (Zah and Gauch, 2007). The environmental impact attributed to the transport of biofuels is less even when the biofuels are sourced from overseas. Most research studies show that there is a tradeoff between greenhouse gas emission reduction and lowering of environmental impacts. The use of biofuels from sustainable technologies and sources can guarantee a 30% reduction of greenhouse gas emissions which is crucial for climate change mitigation (Zah and Gauch, 2007). A 30% reduction in carbon footprint is appreciable in the search for climate change resilient technologies. However, other studies have suggested that the benefits of biofuels are limited or even absent altogether (Reijnders et al., 2008). The sources of these variances are a subject of debate among researchers (Larson, 2006).

In terms of the environmental impacts of biofuels, tropical agriculture has been identified as a source of air pollution. Combustion of biomass for energy releases large amounts of CO_2, which has been linked with measurable adverse effects on biodiversity. For regions in moderate latitudes, lower yields of crops, intensive use of fertilizer and mechanical tilling of the land cause significant environmental impacts (Zah and Gauch, 2007). Using the LCA tool for environmental impact assessment of biofuels provides fresh evidence that not all biofuels have reduced environmental impacts when compared to the impacts caused by fossil fuels. From research studies, for biofuel production paths, utilization of biogenic wastes and use of grass to wood results in reduced environmental impacts when compared with petrol.

In Europe, use of biofuels has led to reduced greenhouse gas emissions and therefore improving environmental performance. Comparative studies and LCA studies on biofuels and fossil fuels have been

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done and indicated reduced CO_2 emissions for biofuels. Countries like the United States, Brazil and China are the largest global producing billions of gallons of ethanol as an alternative source of renewable energy. In Europe, production of biodiesel is higher compared to production of ethanol where rapeseed is utilized as the major biofuel feedstock (Nanaki and Koroneos, 2011). European union produced about 5.7 million metric tons of biodiesel with Germany leading with production of 2890 thousand metric tons of biodiesel followed by France. Production of biodiesel is mainly done from oil crops like rapeseed and sunflower oil. Waste cooking oil and animal fats can also be used. The extracted oil is converted to biodiesel through transesterification with an alcohol. The resulting biodiesel can be blended with fossil fuel with about 5-10%. Figure 3 presents a comparison of LCA of biofuel and fossil fuel while Figure 4 shows comparative values of emissions between biofuels and fossil fuels.

Figure 3. Life Cycle of biofuels and Fossil Fuels
(Nanaki & Koroneos, 2011)

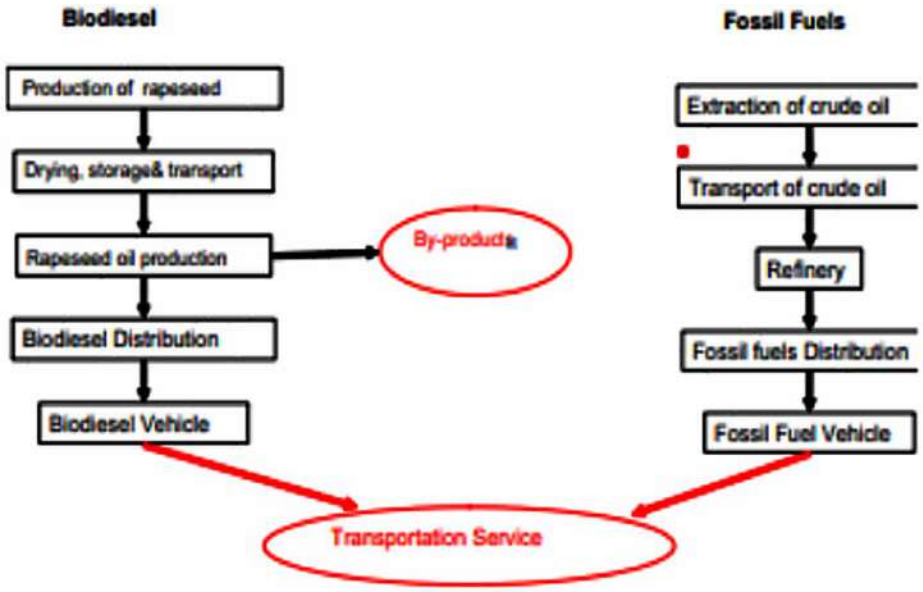

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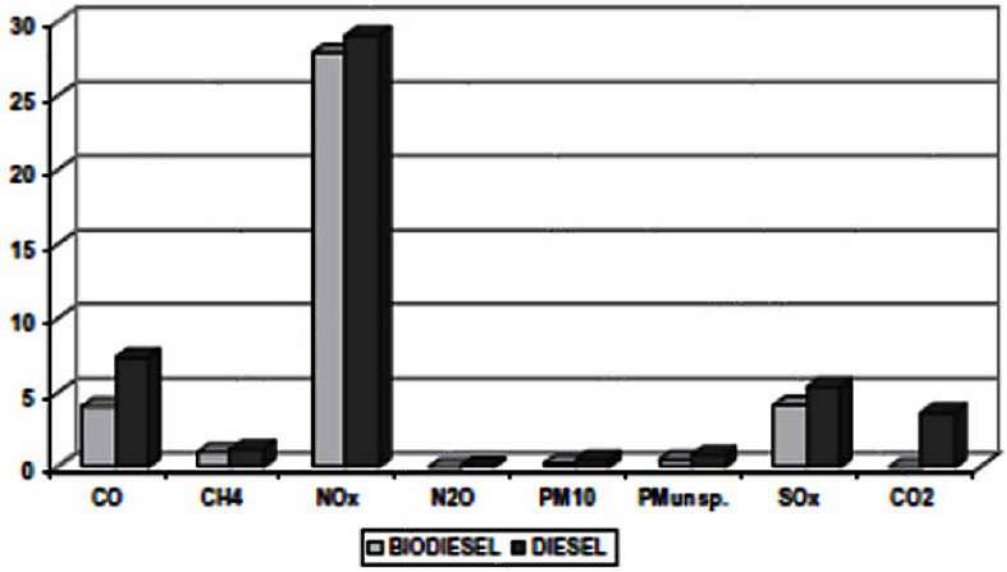
Figure 4. A comparison of emission between biodiesel and diesel
(Nanaki & Koroneos, 2011)



The use of biofuels instead of fossil energy is quite beneficial to the environment as it reduces the greenhouse gas emissions and is also a relatively cheaper option. LCA studies on environmental impacts indicate that every ton of fossil fuel contributes to about 2.8 tons of CO_2 to the atmosphere whereas, the specific carbon content from biofuels is slightly lower. For every ton of biofuel, about 2.4 tons of CO_2 is released to the atmosphere which is slightly lower than the amount released by fossil fuels. Furthermore, during cultivation of biofuel feedstock, CO_2 is assimilated (Nanaki & Koroneos, 2011).

LCA and the Future of Biofuels

Annual crops used for biofuel feedstock account for one-fourth of agricultural land utilized and indirect land use. In Sweden, only 5% of the arable land is being used for biofuel feedstock production (Zah & Gauch, 2007). To increase the production yield of biofuels, the percentage of direct land use for grassland must be increased. However, such a move will increase the biogenic greenhouse gas emissions. Even so, such an increase in biogenic emissions of greenhouse gas can be countered by ensuring efficiency through the entire biofuel production chain. For instance, in the supply chain, during the manufacture of mineral nitrogen fertilizers, oxides of nitrogen can be reduced by fitting in nitrous oxide cleaning equipment.

According to Zah & Gauch (2007), ethanol from wheat biofuel feedstock accounts for 71% climate benefit as compared to fossil fuel utilization if system expansion is applied and this excludes straw. Currently, the byproduct distillers waste is used as biofuel feedstock and it accounts for about 1-2 TWh of bioethanol. This corresponds to about 42 TWh of petrol consumption and a total of 84TWh when diesel is included (Zah & Gauch, 2007). Additionally, the existence of the export market, like in the EU, the total market for distillers' waste that is used as feedstock for biofuels corresponds to ethanol production of 5% of |Europe's petrol consumption. Using the energy allocation method, wheat-based ethanol accounts for 63% of climate benefit excluding straw.

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When distillers waste is used as protein feed in biogas production, the climate benefits from the production of biogas account for about 67% when system expansion expansions are utilized. That means digestion residues are assumed to substitute mineral fertilizers. Sugar beet-based ethanol has a climate benefit of 80% and if the energy allocation method is applied, then the climate benefit stands at 74%. In Sweden, sugarcane-based ethanol imported from Brazil accounts for 79% climate benefit and about 77% if the energy allocation method is used. To consider climate benefits and apportioning future contributions, continued expansion, the type of land used for feedstock production will be considered (Zah & Gauch, 2007).

Climate change mitigation due to the use of RME stands at 68% as compared to fossil fuels. If soy meat is substituted by rapeseed, which is a by-product, then climate benefits increase from 64% to 72%. Using the energy allocation method, excluding straw, the climate benefits stand at 53%.biomass from ley crops, sugar beets and maize promotes climate benefit of 86% for ley crops, 85% from sugar beet, and 75% climate benefit from maize biofuel feedstock.it is also worth noting that if methane losses are considered and biogas upgrades applied, then there will be a reduction of climate benefits by 5% points (Zah & Gauch, 2007).

Using manure residues results in climate benefits of 148% using waste from food industries as biofuel feedstock accounts for 119% whereas biofuel feedstock from household waste to generate biogas accounts for a climate benefit of 103% when compared to fossil fuels. The climate benefits in this case exceed 100% due to the indirect benefits gained due to recycling of the nutrients and the reduced utilization of fertilizers and recycling organic matter to the soil.

On the contrary, Hill et al. (2016) explains that the use of biofuels to reduce greenhouse gas emissions tends to encourage high consumption of fuel. Hill et al. (2016) argues that increased biofuel production will lower the fuel prices and therefore the fuel demand will rocket up encouraging additional consumption. This leads to a fuel market rebound effect which end up undermining the strategies of climate change mitigation. High biofuel consumption will lead to increased greenhouse gas emissions. Further, increased biofuel feedstock cultivation will result to indirect land use change which also contributes to increased greenhouse gas emissions.

((Hill et al., 2016)) advises that with right policies, biofuel feedstock production may help mitigate climate change issues like greenhouse gas emissions. Hill et al. (2016) further notes that the use of biofuels as an alternative low carbon source can lower greenhouse gas emissions with right policies in place, such as imposing punitive measures like increased tax on pollution activities rather than lowering fuel prices. High tax on pollution-causing activities will curb the market rebound effect. It will direct the market towards the right direction.

Benefits of LCA for Biofuels

LCA for biofuels is done to give a comprehensive comparison of the biofuels and that of fossil fuels to understand the difference in their respective environmental performance. Regulators, policymakers, and government agencies utilize the LCA tool. For instance, the Greenhouse gas calculator is developed for policymaking using a single impact category, which is global warming. LCA also gives an insight into the significant environmental impacts related to biofuels without having to compare them with fossil fuel impacts. This applies when getting data about the various feedstock's used, information gathered from LCA is also used by the biofuel companies to foster improvement and sustainability and also in research and development(Van Der Voet et al., 2010).

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LCA helps in evaluating the environmental impacts of a given product or system. This means that it not only considers the product use of the biofuels when converting chemical energy to kinetic energy in a vehicle's engine, but also considers the industrial and agricultural processes before biofuel is delivered to the fuel tank of the vehicle (Hauschild et al., 2017). LCA helps illustrate that no biofuel is climate neutral because at the industrial process, fossil fuels are used as inputs before the biofuel usage stage. With LCA, one is able to determine that an increase in the demand of biofuels could lead to increased demand for conversion of natural land to meet the biofuel feedstock need. The conversion leads to release of carbon bound in the biomass as well as in the soil in form of Carbon dioxide. A country increasing its production of biofuels contributes indirectly to natural land loss and deforestation.

LCA utilizes a comparative assessment that uses best estimate principle. This is quite beneficial because it allows researchers to have accurate comparisons that are free of bias. This means that equal level of precaution is observed throughout the entire environmental impact assessment. Furthermore, LCA considers a wide range of environmental issues including land use, climate change, fresh water usage, toxicological impacts on human health and aquatic eutrophication. The importance of focusing on the wide range of environmental issues it to eliminate burden shifting to one type of environmental issue which has a potential of increasing other types of environmental impacts (Hauschild et al., 2017).

Moreover, due to the comprehensive LCA, one is able to understand significant effects of biofuels in socio economic status. For instance, cultivation of biofuels releases introduces fertilizer chemicals and pesticides to the soil which contribute to eutrophication as well as toxicity to the aquatic ecosystem. The biofuel feedstock cultivation will also require massive land and substantial amounts of water for irrigation, there is is loss of biodiversity and water scarcity. Additionally, increased cultivation of biofuels contributes to increased food prices (Hauschild et al., 2017).

LCA studies are used to develop policies as well as identifying the crucial areas of contribution an improvement. Recommendations from LCA studies are used in research and development areas which contribute to biofuels sustainability. Data obtained is used for contribution analysis and to trace on specific emissions, and process from an impact point of view.it identifies the key hotspots along the biofuel production chain(Somerville, 2007). According to (Nanaki & Koroneos, 2011), LCA of biofuels is a very invaluable tool that offers flexible system parameterization as well as a comprehensive integrated evaluation of the environmental impacts. The authors assert that LCA studies have the potential to impact the strategic planning and policy making of the transportation sector. This is because, it takes into considerations, aspects like technical, environmental as well as costing. LCA studies have contributed to shifting of strategic planning and in influencing operational decisions. This is because it is an effective tool in identification of improvement opportunities that may not have been captured in previous studies (Cherubini et al., 2009).

Challenges of LCA for Biofuels

Despite the advantages of LCA for biofuels, there exist challenges in the tool. Among the challenges are research gaps and uncertainty especially in areas that have not been explored or are not fully developed. Additionally, in the agricultural processes, there are uncertainties with large measurements that are not easy to obtain. For instance, biofuels use different types of feedstock that are grown in different types of soil and utilize different types of agricultural practices under different climatic conditions. These differences result in varied emission rates which cannot be generalized when determining the environment of

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biofuels (Van Der Voet et al., 2010). Research gaps therefore emerge due to the coverage of feedstock and the respective geographical area.

Another challenge is the incomplete coverage of impacts. This is because; most of the LCA studies are limited to greenhouse gas emissions. However, recent studies have increased the scope to include other impacts, making data and models easy to use. Even so, impacts like water use and land use have barely been considered (Van Der Voet et al., 2010). Moreover, translating the functional unit into real-life improvement poses challenges of scale and dynamics. There are factors to consider when translating the pilot projects to the real world like the rebound effects, the market mechanisms that will influence food security, and the social structure.

The current supply of biofuel is feedstock from corn and sugar cane, which are farm-grown. Other sources in the future may include feedstock from farms, rangelands, or forests. Due to the nature of feedstock and their transportation cost, they may be stored and processed at small to intermediate scale facilities (Mckone et al., 2011). Compared to oil companies and government agencies that have a hierarchical structure to make decisions, biomass production has no such hierarchy and decision making especially at the first stage of production. When implementing a life cycle assessment of biofuels to influence policies proves to be a hard task as compared the case of a formal hierarchical structure. For example, uncertain weather conditions, complex markets pricing, and tax policies influence farmers in unpredictable ways.

Additionally, due to the vast sources of feed stocks ranging from sugar cane, cane residue, rapeseed, switch grass, and other oilseeds, a wide range of information is needed because the characteristics of the feedstock are different. Therefore, it is difficult to make an overarching decision with different characteristics. This is because many alternative feed stocks will have mixed output systems. For instance, one type of crop can produce protein and at the same time produce fuel feedstock (Somerville et al., 2010).

Furthermore, it is crucial to apportion impacts biofuel feedstock from primary products, by-products co-products as well as residues. Allocation of decisions requires a clear rational basis. Mckone et al. (2011), assert that impact metrics like carbon, health, water footprint require a clear and rational basis. Biofuels feed stocks with mixed outputs are sensitive to either of the allocations like no allocation, allocation by energy, or allocation by economic approach. There is a challenge on the sensitivity of impacts to the choice of allocation (Gnansounou et al., 2009; Guinée et al., 2009). The U.S EPA's renewable fuel standard assessment has had such a challenge. For instance, when determining if rapeseed biodiesel fuels meet the EU renewable energy directive that saves on 60% of greenhouse gas emissions, researchers found out that it meets the requirements. However, only the rape cake, which is a co-product, was used along with the use of glycerol as a source of heat. Allocation by energy content was used (Thamsiriroj and Murphy, 2010). Several other studies depict this problem in the choice of the allocation method (Kendall et al., 2009; Slade et al., 2009).

Moreover, farmers and foresters are a bit reluctant in making long terms land and resource commitment to perennial feed stocks like tree crops and switch grass. Despite the land being suitable for such feed stocks, the farmers need assurance of clear markets as well as financial security. An additional issue is the indirect land use (Fargione et al., 2008). Land available for both food and biofuel feedstock is already heavily used. Biomass production will accelerate deforestation. Deforestation leads to the release of large amounts of carbon to the atmosphere causing a climate impact debt that will take decades to counter. Furthermore, the use of cropland for biofuel production has also been linked with increased emission of greenhouse gases (Searchinger et al., 2008).

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CONCLUSION

Biofuels are promising alternative energy sources to fossil fuels with appeciable grenhouse gas (GHG) benefits. The increase in biofuel life-cycle assessment (LCA) studies has subtantially bridged the gap in the discrepancies reported by different researchers on the sustainability of biofuels. While the benefits of biofuels are appreciated, LCA studies also show that conversion of forest land to fuel crops has no greenhouse benefits and may even increase GHG emissions. Future research should emphasize land use, land use change and water in LCA. With paucity of data on such important parameters, discrepancies still remain which limit policy making.

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Chapter 3
Entrepreneurial Opportunities In Bioenergy

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ABSTRACT

Entrepreneurism is vital as entrepreneurs push technological change and innovation, consequently generating economic growth. Bio-energy entrepreneurism is the focal point of this chapter, along with entrepreneurial motivations, opportunities, and market drivers. Market drivers include government policies, trends in energy use, and progression of the energy sector into more renewables. Several factors influence entrepreneurs, which involves several factors ranging from the environment to economics, pioneering spirit, and social factors. With the help of entrepreneurial motivations and market drivers, individuals can easily visualize and plan all the bio-energy sector opportunities. Bio-energy entrepreneurs moderate the market of transformation of biomass to energy. The bio-energy field is filled with stories of failures, successes, and everything in between. There is a marginal difference between a failure story and a success story in the modern bio-energy sector, which narrow down to timing.

1. INTRODUCTION

According to an assessment by the International Energy Agency, by 2026, India is on track to become 3rd world's largest market for ethanol behind Brazil and the United States of America, having increased ethanol requirement to an anticipated three billion litres amid 2017-2021. According to the agency, by 2026, Asia is expected on the way to surpass Europe in provisions of bio-fuels output, thanks to India's expanding appetite. While by 2026, yearly global demand for biofuels is expected to increase to 186 billion litres, an increase of 28%, Asia is expected to contribute over thirty per cent of new output during the predicted era. The main driver of demand expansion is thought to be government policy. Additional

DOI: 10.4018/978-1-6684-5269-1.ch003

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issues such as public transportation fuel demand, expenses, and policy design will also significantly impact. In June, Prime Minister Narendra Modi stated the government is committed to achieving a 20 per cent ethanol blend in gasoline target by 2025. Previously, the deadline had been set for 2030. According to CRISIL analysts, roughly 16-18 million tonnes of gasoline transactions are expected to be replaced due to ethanol mixing. It will be difficult for India to meet its 20 per cent blending goal in just five years, But even attaining 11 per cent blending would make it the world's third-largest ethanol market behind the United States and Brazil, the IEA study concluded, making it the world's third-largest ethanol market behind the United States and Brazil (Street, 2012). A considerable portion of India's current vehicles could be incompatible with fuel blends containing more than 10% ethanol. Retrofits are an option However, given the scale of the undertaking, this may be impractical, the IEA stated, adding that flex-fuel vehicles or vehicles otherwise compatible with 20% blends will need to be made accessible. Consumers will need to be persuaded to buy them. (Street, 2012) At present, the percentage of ethanol in gasoline is roughly 8.5 per cent. Under the Ethanol Blended Petrol (EBP) programme, the government has already reintroduced the administered price mechanism for ethanol procurement, allowing ethanol production from a variety of feedstocks such as heavy molasses, sugarcane juice, sugar, sugar syrup, damaged food grains, maise, and surplus rice stocks with the Food Corporation of India (FCI).

In Ethanol Allocation Year 2016-17 (December 2016 – November 2017), government governed oil marketing companies obtained 66.5 crore litres of ethanol, which grew to 173 crore litres in the Allocation Year 2019-20. As previously stated, the government authorised oil CPSEs to build 2nd Generation (2G) Ethanol Biorefineries using agri-residues and biomass in various parts of the country. OMCs are building 12 2-G biorefineries for a total asset of Rs 14000 crore. Ethanol purchased through the EBP programme is subject to a 5% GST rate. The EBP initiative attempts to alleviate environmental problems, lower oil imports bills, and provide farmers with a remunerative income. For Ethanol Supply Year 2O2O-21, the procurement price of ethanol was Rs 62.65 per litre for sugarcane juice, Rs 51.55 per litre for spoiled food grains, Rs 56.87 per litre for rice available with Food Corporation of India, Rs 51.55 per litre for maise, and Rs 45.69-57.61 per litre for heavy molasses. After acquiring statutory permits, sugar mills and distilleries are allowed to build ethanol facilities, and the government announced an interest endowment scheme to aid in the construction of these plants. (*International Energy Outlook 2013*, 2013)

2. ENTREPRENEURSHIP MOTIVATIONS

The primary movers or significant stakeholders in all bioenergy entrepreneurial ventures may be motivated by various factors. Lin (2008) acknowledged three key expansion motives for entrepreneurs: social welfare, environmental and entrepreneurial. Bioenergy will solve a problem or issue with the environment in a project with environmental implications. The environmental incentive can be seen in swine farms whose expansion plans are thwarted by concerns about the increased stench. An anaerobic digester that produces biogas eliminates stink dilemma whilst producing energy, used to generate electricity and operate agricultural equipment in the farm (biogas is cleaned to eliminate contaminations, subsequently compressed and consumed as compressed natural gas). (*Cultivating Entrepreneurship | University of Maryland Extension*, n.d.) There are several definitions and examples of social welfare, including delivering energy to a community at a cheap rate than traditional generating, creating employment for the community, and raising tax revenue. When public leaders sought to fund, manufacture, or recruit ethanol factories to their area, the corn ethanol boom provided multiple cases in point of social welfare

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motives. After it was put together, the ethanol factory delivered high-wage jobs, generated significant tax revenue, and raised maise costs.

In conclusion, prime movers display entrepreneurial instincts when they take chances with breakthrough technologies without a market. The aim to be a market leader in a new bioenergy area drives these entrepreneurs. This kind of entrepreneurial motivation may be perceived in the 1st generation cellulosic plants, which are still trying to get up and running. A further case of entrepreneurial motivation is the several minor businesses running with algae to develop a way for producing and harvesting algae for biofuels. (Han, n.d.)

Ahead of Lin's goals, Shane explained that entrepreneurship requires a human group and an innovative route in which enterprisers participate in the creative activity by relocating resources in a novel way. (Shane et al., 2003). It might be characterised as the fourth motivator of pioneering spirit, or, as Shane puts it, human motivation plays a critical role in the entrepreneurial process. This pioneering mindset will benefit bioenergy entrepreneurs to compete with fossil fuels by bringing alternative energy sources to market. An entrepreneur must be driven by a desire to respond to market conditions. The next part delves deeper into these market drivers.

3. BIOENERGY AS A BUSINESS

The acceptance and development of a dependable source of renewable bioenergy is a big challenge that most countries face. The majority of studies in renewable energy focus on the controlled models of implementation intended for this type of energy. (Järvinen, 2005) From a business standpoint, the economic benefit of bioenergy is strongly reliant on bio-mass robust usage. As a result, a grasp of bio-energy utility parameters is critical. The source of bio-mass, the structure and location of the active business, the storage and transformation centre, and the relevant supply and consumption sources are all key aspects to consider when assessing the advantages of bioenergy in a business setting.

The improvement of high-efficiency bio-mass heating systems has unlocked up fresh possibilities for the active use of wood in wooded areas with systems located close to the user. In general, the plant placement is adjusted by locating the optimum site within high-productivity wooded areas with a handy network of forest paths, a good presence of a woody business, and easy grid connectivity. The environmental impact, economic and social implications, and Encouragement of communal amenities all play a role in the economic viability of bio-energy power plants. As a result, bio-mass heating has numeral convincing benefits, both for the surrounding community and system proprietor in the case of district heating systems. (Dahiya, 2014)

Bio-energy may generally replace expensive traditional energy sources and electricity with local biomass resources, typically accessible for little or no cost as waste (*Cultivating Entrepreneurship | University of Maryland Extension*, n.d.). Bioenergy can reduce pollution and greenhouse gas emissions while simultaneously protecting customers from rising fossil fuel prices. Bioenergy production creates multiple local jobs in the feedstock assembly, planning, and transportation. It allows several other firms to expand and reduces transportation distances. (*International - US Energy Information Administration (EIA)*, n.d.)

The bioenergy industry has several difficulties. Currently, biomass heating systems are more expensive to install than traditional fossil fuel-burning systems. If their cost does not fall, the development of bio-energy usage may be slowed. It should also be noted that the quality of biomass feedstock is vastly

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varied compared to the more standardised commercially available fossil fuels. (Käkölä, 2003) Bioenergy production necessitates increased physical space for biomaterial supply, storage, and handling. When bioenergy production presents more logistical obstacles, it naturally necessitates greater operator engagement and diligence, which costs more money.

Where the cost of conventional energy is high, and biomass feedstock is low, biomass-heating systems appear to offer the best commercial opportunities (Ojala, 2008). If numerous bio-energy systems are linked regionally, the benefits of bio-energy can be amplified. There are no technological barriers to merging bio-mass heating, wind and solar power, and water power into a similar energy network. Societies with various energy suppliers on a single network are less vulnerable to fluctuating energy prices and climate change.

Nevertheless, the bioenergy industry's ending advantage is always quantified in the bioenergy sector, including customers, merchants, and manufacturers. As a result, it is critical to comprehend how the bioenergy business and marketplaces operate and how distinct players appear to benefit from them. When a product is needed but is inexpensive and functions well, the customer receives a value. (Järvinen, 2005; Käkölä, 2003; Ojala, 2008)

Customer value = performance / price

The above formula is founded with the premise that each product module or role has its unique expenditure, which drives up the cost of the product. In actuality, the expense of construction these functions vary by customer, as do the needs of particular consumers. As a result, each product function should meet consumer demand as outlined in the formula below. (*November & December 2008 Newsletter | Agricultural Marketing Resource Center*, n.d.; Reinis et al., 1975)

Value = Value = [(n · a/c) 1+(n. a/c)2 + (n · a/c) ?]

where:

n = the need for an object or service
a = the ability of an object or service to satisfy this need
c = the cost of the object.

The functions that should meet the customer's needs are represented by numbers in this formula, with the final function represented by a question mark.

A retailer's value of a bioenergy product may fluctuate significantly from a customer's value. This is because, for starters, the retailer is primarily concerned with the monetary gain on his investments. Retailers are looking for fast returns and products that can be sold rapidly. Furthermore, retailers aim to make the most money with the least money. As a result, the value formula for a retailer is not the same as the value formula for a customer. In its place, computing a cost-to-benefit ratio, merchants approximate the sales revenues they assume to gain by selling their products, as shown by a retailer's value formula. The formula computes the retail product value (Jordão, 2011).

Retail value = unit sales (unit price – unit cost)

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Maker utilises his formula to calculate the worth of bioenergy products for a retailer or a client. This is because his interests differ slightly from those of the others. He has higher capital costs than a retailer because of the production plant and tools, which tend to compel manufacturers to retain working most of the time. Because the manufacturer is likewise concerned with the gain on his investment, he uses the following formula to determine manufacturing value. (Shane & Venkataraman, 2000)

Manufacturing value = [Customer and retailer benefits (1 to n)] + Profit/Costs

Bioenergy generation should be addressed financially in addition to the business value consideration.

In practice, this implies that all investments should pay off in the long run, and entrepreneurs and communities should also benefit from their efforts' commercial benefits and profits. As a result, the cost of invested capital might be a hurdle for the bioenergy industry. This is since, to thrive, a bioenergy firm may require public investment at first, as private funding may not be accessible. Even if this is the case, bio-energy entrepreneurs must ensure that their business strategies are appropriately constructed, taking into account a variety of business metrics such as value, profitability, and funding. With a well-crafted business strategy, an entrepreneur can also communicate his objectives to various bio-energy industry participants. (Shane & Venkataraman, 2000)

4. BIOENERGY OPPORTUNITIES: BIOFUEL VALUE CHAIN

Waste streams such as municipal solid waste or manure, plant oils, dedicated energy crops, agricultural crop leftovers, and waste streams such as municipal solid waste or manure are all examples of bio feedstocks. (pre-consumer or post-consumer). Production, harvest, storage, and transportation costs apply to each feedstock. Examining the stages involved in producing feedstock's will provide business prospects.

The following is a breakdown of the biofuel value chain:

Breeding and genetics of feedstock's/Feedstock production/Logistics of feedstock's/Biomass processing/Conversion processes/By-product and co-product utilisation/Marketing distribution Innovative people and small businesses can make critical discoveries in the areas of biomass crop genetics and conversion biotechnology, which are both areas where innovative people and small businesses are capable of creating significant findings linked to better ways to convert biomass to fuels. Biotechnology can impact biofuels production through enzymes, catalysts, yeasts, and plant breeding. Engineering linked to equipment development and refinement is required to solve and improve the system, both at the bio-refinery and across the supply chain. Engineers and tinkerers have come up with new ways to solve equipment difficulties. These solutions have the potential to be commercialised. (Street, 2012)

Fuel has always been distributed through leading corporations and gasoline and diesel organisation. Bio-fuel companies are increasingly participating in the supply chain by growing into blenders and promoting customised pumps that provide consumers with bio-fuel options.

By-products and co-products are critical to first-generation ethanol facilities' profitability. Similarly, enhanced bioenergy facility economics will result from the capacity to market by-products and co-products. Distiller corn gluten and grains feeds are two of the essential co-products of the corn ethanol business. The maise ethanol industry also sells CO_2 and post-fermentation corn oil as by-products. A separate firm partner is frequently used to capture and market the by-product. Companies have formed in some circumstances to add value to the by-product, such as pelleting distiller's grains for sale as a denser

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feed, capturing CO_2 for use in the bottling business, and converting maise oil to bio-diesel. Regarding the potential for entrepreneurs to put value to a low-value product, by-products are worth investigating.

5. OPPORTUNITIES IN SMALL-SCALE BIOENERGY

Entrepreneurs who make their bio-diesel conversion systems, capture CH_4 from manure and other waste or use waste wood and other materials for heat generation have a long history in small-scale and niche industries.

5.1 Biodiesel

Biodiesel is a clean-burning, environmentally friendly fuel made from edible and non-edible oils trans-esterification. Compared to traditional fuel sources, it is renewable, cost-effective, carbon-neutral, and non-toxic. As a result, it is widely used to minimise carbon emissions in India's automotive, marine, and railway industries. Biodiesel is also mixed with petroleum diesel fuel in compression ignition engines because it improves engine lubrication and extends engine life. In India's vehicle sector, rising petroleum products and growing environmental concerns drive up demand for biodiesel.

Furthermore, the market's expansion is aided by a growing focus on sustainable development techniques. Biodiesel is also utilised to power underground mining equipment and machinery because it improves air quality, increases energy security, and provides various safety benefits. Aside from that, increased energy consumption and the growing use of biodiesel as a heating fuel in both home and commercial boilers are expected to impact the industry in the coming years positively.

5.2 Methane

This segment only analyses biogas methane produced by anaerobic digestion; added bio-gas CH_4 sources, as landfill gas not included. When high carbon materials are enclosed in an anaerobic environment, microbes digest the organic components and release bio-gas, biogas methane is produced (predominately methane). Manure, food industry wastes, human sewage, and other organic waste are all common feedstocks for biogas methane generation. Methane can generate energy in internal combustion engines. Manure is an excellent feedstock for a CH_4 digester, and it is also a feedstock for an anaerobic digester. As a result, such systems are commonly found on swine operations, dairy farms and municipal wastewater systems. The systems produce methane continually after they are deployed. Methane needs to be used or released to avert pressure from developing in the system.

- Flaring CH_4 can help the environment since it converts one carbon and one methane molecule into CO_2, which has a Twenty-four times lower greenhouse gas effect than CH_4.
- If there is a cost for reducing CO_2 equivalents greenhouse gases, such as renewable energy credits or carbon trading.
- Odors can be reduced with the help of Anaerobic digestion, and it has been effectively utilised to lessen odours at a processing facility and farms, letting the facility continue in crowded locations or grow with less opposition from fellow citizens.
- farmers can become bioenergy entrepreneurs. Instead of being the primary product.

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5.3 Biomass and Wood

Residential wood heating has been a staple of human civilisation for millennia. In the US nowadays, heating with the help of wood-burning in homes is widespread in densely forested areas. In certain circumstances, technology has remained relatively unchanged. Individual homeowners cut and process their wood in most situations, although some acquire wood from small businesses or other individuals. (Verma & Hussain, n.d.) Pellet stoves, which burn wood or other biomass pellets and have extra controls than wood stoves, have become more popular. For mass transport and packing, wood pellets are homogeneous, dense, and flow freely. Wood pellets have grown in popularity in Europe due to their renewable nature and lower greenhouse gas emissions than coal or other fossil fuels. Pellets for commercial and other heating applications have developed markets. (*International Energy Outlook 2013*, 2013)

- Wood harvest
- Other than commercial timber harvest, thinning, slash, trimmings, lumber mill trash, manufacturing waste, shelterbelt renovation, old pallets, and dead tree clearance are all wood sources.
- Wood processing is converting wood into wood pellets or other high-value products.
- In recent years, the international market for wood pellets has exploded.

5.5 Cooperatives

Cooperatives consist of working collectively to address their members' mutual needs. The cooperative's ownership and governance are shared by its members. Bio-energy cooperatives have sprung up to provide bio-energy to associates or utilise feedstock provided by members. Many businesses begin as cooperatives to benefit from shared resources. A few are created as a project that is way too big for a single individual or family to handle alone. Creating a cooperative can assist in overcoming the first scale. Others are created to deliver a higher-quality product or service than an individual could provide on their alone. The structure will vary based on the cooperative's goals. However, in general, a group of like-minded people pool their capital, labour, or other resources to develop a business that benefits all members. (Ojala, 2008) A cooperative's goal could be social welfare, environmental, or entrepreneurial to return to the developmental objectives.

- Examples of bio-energy cooperatives
- Biodiesel on a small scale (oilseeds are given, bio-diesel is the end product, and members use it for environmental reasons)
- Bio-mass pellet mill
- Methane digester

6. LARGE SCALE BIO-ENERGY OPPORTUNITIES

Large scale bio-energy signifies to businesses that can process tens of millions of gallons of water/year or else millions of tonnes of biomass. Two instances are multi-million gallon per year bio-refinery or megawatt-scale biomass to heat and power facility. These amenities necessitate a substantial financial investment. Entrepreneurship can occur at this magnitude, yet we often associate entrepreneurs with

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small start-up businesses. We have looked at small businesses how they contribute to large and small scale bio-energy for the objectives of this chapter. Bio-energy entrepreneurs analyse the supply chain at all scales and how challenges might be resolved in creative and novel ways. For specialist expertise and equipment, bioenergy facilities continually depend on technology vendors. Heavy machinery, computers, analytical equipment, waste management and chemistry are just a few of the areas covered by these technological companies. (*Ethanol Impacts | Agricultural Economics*, n.d.)

6.1 Opportunities at the Periphery

There are other options outside of the bio-fuel or bio-energy trades. As a method to contribute, entrepreneurs may be at ease to work on the outside. These off-the-beaten-path options may not immediately benefit the bio-fuel and bio-energy industries, but they do take advantage of their presence. This peripheral activity includes businesses that pop up to satisfy the individual requirements of construction employees throughout peak construction periods. (Ojala, 2008)Following the development boom, these enterprises rely on community patronage as well. Such businesses gain an advantage by having a solid first year of operations, which allows them to pay off debt sooner.

As part of bio-energy booms, various peripheral establishments started, which includes the following:

- Motels
- Cafeterias
- Apparel stores
- Bank
- Service station
- Studio apartment

7. GOVERNMENT INITIATIVE IN SECTOR OF BIOENERGY

7.1 National Bio-gas Fertilizer Management Program

Construction of family size biogas plants of 2 to 6 cu. m. efficiency is being made by Uttar Pradesh New and Renewable Energy Agency from the additional financial year 2010-11 of Rural Development Department and KVIC as per the design and drawing approved by the Ministry under this scheme, such as Dinbndhu, KVIC or installation of permissible prefabricated biogas plants, domestic fuel supply, lighting, and for the production of organic fertiliser for construction of family-size biogas plants of 2 to 6 cu. m. efficiency is being made. The rest of the cost is borne by the beneficiary as part of the Central funds of Rs. 9000/- for General Category beneficiaries and Rs. 11000/- for Scheduled Caste beneficiaries. The cost of a two cubic metre biogas plant is around Rs. 22,000/- per plant, and the plant can cook food for five people simultaneously by providing light for 4 to 5 hours per day from 1 lamp. The plant will also produce 6 tonnes of high-quality organic fertiliser per year, and the plant will provide the equivalent energy of around 18 LPG cylinders per year.

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7.2 Biogas Based Power Generation Program

In this system, a generator's gas produced from a biogas plant is used to power plants with capacities ranging from 3 to 250 kV, per a ministry-approved design and drawing. After the beneficiary completes the construction plant, the Ministry will reimburse the beneficiary with a reward ranging from Rs 30000-40000/ kV in declining order. A biogas plant with a capacity of 85 cu. m. may be built with 155 cattles. A hundred units of electricity/day and approximate 270 tonnes of high-quality organic fertiliser are generated by a 100 per cent bio-gas-powered generator with a 10 kV capacity that costs around Rs. 16 Lacks.

7.3 Bagasse Based Co-Generation Project's in Sugar Mills

There is a lot of bagasse accessible in the state's sugar mill, which has much potential for co-generation. Uttar Pradesh New and Renewable Energy Agency began its endeavours in 1994 as an architect to establish power production plants based on co-generation using bagasse from Uttar Pradesh's sugar mills.

Throughout 1994, the state government created a strategy to encourage the development of similar enterprises in the state. PPAs agreed with the power corporation were later specified as model PPAs after founding the U. P. Electricity Regulatory Commission.

7.4 Biomass-based Power Projects

There are numerous options to build power plants of various capacities using biomass other than bagasse and a range of processes (gasification, co-generation, and combustion). According to a TERI report, the state has a biomass power generation capacity of 3757 megawatts. To establish such initiatives, region-specific surveys must be conducted to estimate the projects' true potential. The following is an updated list of other biomass-based initiatives in the state besides bagasse.

7.5 Biomass-Based Gasifier Power Project

Gasifier Plants (from 40 kilowatts to 1 megawatt) based on gasification process employing primarily rice husk biomass may meet the electrical and thermal energy requirements. Entrepreneurs are putting up gasifier plants with or without grants from the union government to seek aid from rising costs and shortage of oil and electricity. By the end of 2013-14, more than 171 industrial units in the state had established gasifier plants, resulting in a total capacity of 40-44 megawatts.

7.6 Biomass-Based Co-Generation Power Project

MW-scale co-generation power plants based on combustion processes using agro-residues have been built in several sectors. These projects are put up in pulp and paper mills that demand thermal (process steam) and electrical energy. They can meet those industries' thermal and electrical energy needs. The establishment of non-bagasse-based co-generation power projects is being supported in the state's various paper mills. Until the end of 2013-14, a total of 201 megawatts of capacity had been built. (*R&D | Ministry of New and Renewable Energy, Government of India*, n.d.)

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7.7 Industrial Waste Based Power Projects

The processing of waste disposal from various industrial units, including distilleries, food processing companies, dairies, pulp and paper mills, can generate more than 150 MW of energy for the state.

108 MW of capacity was generated from waste by various industrial units (mostly distilleries) until the 2013-14 fiscal year. (*R&D | Ministry of New and Renewable Energy, Government of India*, n.d.)

8. EXAMPLES OF INDIAN ENTREPRENEURISM

8.1 Bio-solutions India

The Hamburg-based company purchases agricultural waste through farmers and manufactures biodegradable packaging and tableware in Ramanagara, near Bengaluru. The patented process turns it into self-binding fibres by simply churning agro-waste with water in massive machinery. The result is biodegradable packaging and dinnerware that only takes three months to decompose. This addresses two environmental issues: crop waste disposal and plastic pollution while also giving farmers a new source of revenue. BIO-SOLUTIONS tested their packaging with Big Basket, an online grocery store, from early 2017 to mid-2018, by providing limited quantities of packaging for fruits and vegetables. (Roopa, n.d.)

8.2 Kriya Labs

It offers a way to convert fifteen to twenty million tonnes of agricultural stubble every year, primarily rice straw, which has no market matched to different types of agro-waste such as wheat straw and bagasse. They use their method to turn rice straw into pulp. Paper (and its derivatives), bioethanol (biofuels in general), textiles, and other speciality chemicals such as cellulose acetate and carboxymethyl cellulose can benefit from this pulp. The procedure is safe for the environment. Pulp quality is determined by the eventual use and can be adjusted accordingly.

8.3 Farm2Energy

Sukhbir Singh Dhaliwal's start-up in Punjab assists farmers by baling the stubble from their fields for free. The company, founded in July 2016, provides bio-mass supply solutions to various clients, including farmers, landowners, and bio-mass users. The company uses integrated supply systems to treat paddy straw, maise stover, sugar cane garbage, and wheat straw before supplying it to the bio-fuels, bio-power, and biobased industries. It also aids farmers in the management and utilisation of biomass. Biopellets, bio-char, and torrefied pallets are also available through Farm2Energy. Biopellets (produced from a mixture of paddy straw and sugarcane trash) are a sustainable alternative to traditional fossil fuels used in various commercial and industrial applications. Biochar increases soil productivity, resulting in increased output. The third product, torrefied pellets made from torrefied biomass, can be used as a coal alternative. Torrefaction can be defined as a thermal breakdown that occurs at 200 to 320 degrees Celsius without oxygen. This increases the density of the biomass and enhances the burn quality. (Roopa, n.d.)

The Delhi-based company collects oil from FBOs (food business operators) and converts it into biodiesel. It also provides a wide range of environmental services, including mixed biodiesel supply,

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industrial tank cleaning, agricultural and blood waste recycling, and a biogas anaerobic digestion facility, among other things. (*This Woman Entrepreneur's Start-up Collects Used Cooking Oil to Convert into Biodiesel*, 2021)

9. CONCLUSION

Biomass-based energy is still not cost-competitive with conventional energy in many situations, which is a significant barrier for bio-energy entrepreneurs that hope to offer energy in straight rivalry against fossil fuels. Several revolutions arise when the fossil-fuel business is under pressure from environmental regulations. One of the main reasons for the elevated prices of renewable fuels and energy is the high cost of feedstocks. Trees are one instance of a minimal cost bio-mass feedstock. Even if the plants are free (as in government wildfire thinning), the expense of chipping, harvesting, and shipping in January 2014 was 2 to 4 times that of Wyoming coal per million Btu. (Han, 2007). With increased competition, the primary picture of expensive feedstocks is expected to remain. Biofuels markets are still developing and unstable, which will make it difficult for businesses to obtain financial financing and a steady market for their products. Government consents for bio-fuels exist, but new proposals for revisions and repeals emerge each year, creating uncertainty for investors. The bioenergy industry's immaturity and unpredictability significantly differ, not the primary concern. For bioenergy entrepreneurs, the characteristics of a self-starter, devoted, risk-taker, unlimited energy come into play (Xiang, 2014). These characteristics are essential for the next phase of bio-energy development to succeed. An entrepreneur needs the foresight to see what lies in the future, the courage to take risks in an ambiguous environment, and the drive to keep moving forward.

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Chapter 4
Biofuel Policies in India:
An Assessment of Policy Barriers

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ABSTRACT

Energy is one of the most precious and demanded commodities among various industries and consumers to sustain the current lifestyle. Energy is a crucial element, which unswervingly influences the country's economic development. Numerous methods are adopted to reduce global warming, embracing clean energy from wind, solar, and biomass sources. This chapter speaks about the current situation of energy demand, the innovations in biofuel sources, and the obstacles regarding the commercialization and production of microalgal biofuel.

1. INTRODUCTION

1.1. Green Energy Projection and Foundation of Energy Demand

The term 'energy' is supposed to be a derivative of the Greek word '*Energeia*' which means activity, and as we advanced, the explanation of energy transformed according to its field. When coming to the biological system, cells store energy in biomolecules such as lipids, complex carbohydrates, and proteins. The first law of thermodynamics can conclude that energy can neither be created nor destroyed; it can only be transformed from one form to another (Figure 1).

DOI: 10.4018/978-1-6684-5269-1.ch004

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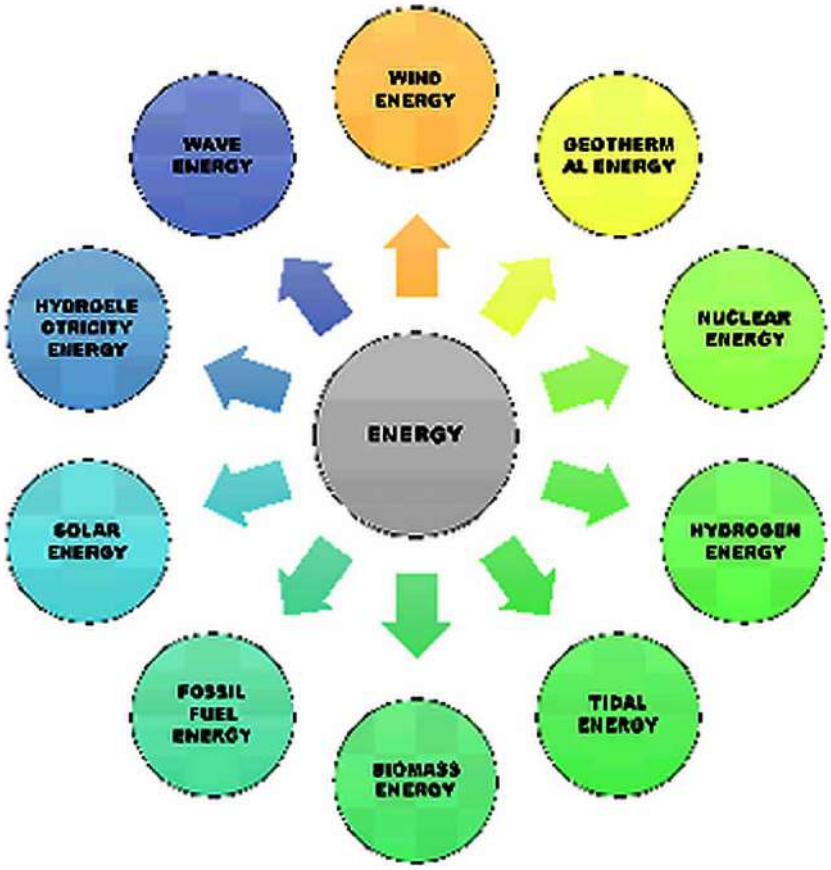
Figure 1. Various types of Energy



Worldwide to sustain the current lifestyle, energy is one of the most precious and demanded commodities among various industries and consumers. Energy is a crucial element, which unswervingly influences the country's economic development (Cleveland et al.,2000). It can be comprehended to the advanced energy accessibility the improved chances of nations growth. There is a solid and direct correlation between energy to per capita GNP, countries with the higher gross national product have higher energy consumption per individual (Stern, 2011). The exponential boom of energy use was originated through fossil hydrocarbon burning, with the additional support of coal in the 19thcentury, followed by Crude oil use in the 20thcentury, and currently, prolonged with the help of natural gas (Hall, 2016; IEA, 2016; Sayre, 2010). Currently, fossil fuels' central portion of energy demands is fulfilled. Worldwide, there is an increase in fuel price due to decrease in fossil fuel reserves (Monari et al., 2016), in addition with increased greenhouse gas emissions leading to global warming are two major concerning coercions for the transport area (Reyimu and Özçimen, 2017; Hashim et al., 2017; Lecksiwilai et al., 2017). Combustion of fossil fuel is required for power generation despite having several negative consequences such as pollution (Liu et al., 2017). Several pollutants such as SO2, NOx, CO, CO2, ground-level ozone, particulate matter, volatile organic compounds (VOC) are emitted.

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There has been an increase in global temperature by 2 ºC due increase in CO2 emission in the last decade (Friedling stein et al., 2014). In 2015, the Paris agreement was signed by around 194 countries whose primary aims are

1. Reduction of 2 ºC in global temperature.
2. Aim to curb greenhouse gas emissions.
3. Reconnoitre substitute energy sources.

It is a well-known fact that there is a decrease in global fossil fuel reserves, and its current usage rate will last a few generations. We have to reduce our energy demands in the coming future. We need to search for eco-friendly, renewable fuel prospects (Dresselhaus and Thomas,2001). Biofuel produced from biological materials can be used as a substitute for diesel due to low emission levels when matched (Tsolcha et al., 2017; Bildirici, 2017). Major significance of using bio-fuel is that It is made from renewable organic substances and has the capacity to eliminate several of the negative features of fossil fuel extraction and usage, such as greenhouse gas and conventional pollutant emissions, finite resource depletion, and reliance on volatile, unpredictable foreign suppliers. The demand for bio-fuels also gave boost farm revenues (united states environmental protection agency, 2022). Nevertheless, sustainable biofuel promotion in the energy sector relies on several elements: government course of action. Currently, each country needs to frame its policies to have better biofuel penetration in the energy market. (Su et al., 2015) policies may have regulatory gaps which provide a political system to countersign contradictory objectives. With the introduction of bio-fuel in the energy market, by 2010 around 3% of worldwide transportation energy used was in form of bio-fuel and with the current increase it is expected to reach 27% of transportation fuel by year 2050 (Timilsina, 2014). The biofuel importance was first accredited by Brazil, the United States of America, and the European Union; now, this importance is being understood by Asian countries which can be understood by the amount of bio-fuel produced by them as mentioned in table 1 (Mohan et al., 2006).

Table 1. Biofuel Production

Country	Ethanol	Bio-diesel	Total	Reference
Brazil	19 000	227	19 227	(FAO, 2008)
Canada	1 000	97	1 097	
China	1 840	114	1 954	
India	400	45	445	
Indonesia	0.00	409	409	
Malaysia	0.00	330	330	
United States of America	26 500	1 688	28 188	
European Union	2 253	6 109	8 361	
Others	1 017	1 186	2203	

Brazils bio-fuel program was a massive success due to strong support of stringent legal directives and operative enactment (Mohan et al., 2006). In consideration of India's perspective, in 2008, A National

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Policy on Bio-fuel was approved to standardise biofuel marketing in India via various firms in a transparent and operative mode following all accepted legal guidelines. However, we cannot replicate Brazil's success in India because of different climates and inconsistent rainy season patterns. Since then, in the last fifteen years, the Indian government has implemented different policies to encourage the usage of biofuels. In order to accomplish all the policy objectives, we need to have competent policy strategies to have appropriate information of the market and its dynamics for the day to day scenario (Barisa et al., 2015).

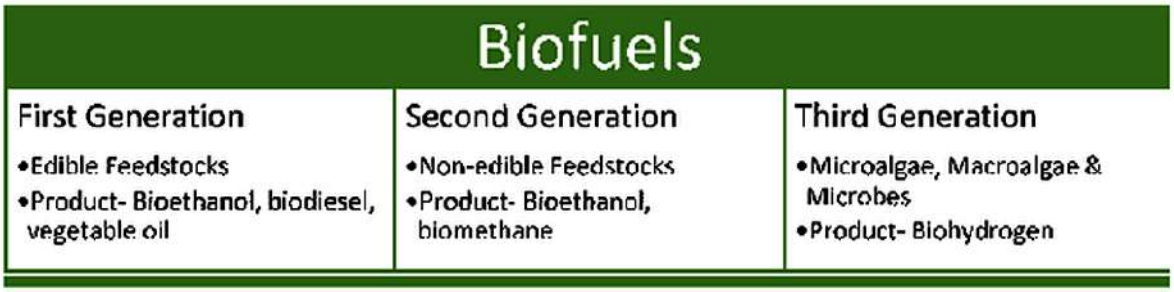
2. BIO-FUEL EVOLUTION AND ITS HOLDUPS

Bio-fuel is a non-hazardous fuel derivative of numerous biomass feedstock, and environmentally hazardous fossil fuel can be substituted (Voloshin et al., 2016). Biodiesel, bioethanol, methane and hydrogen are several biofuels produced by several countries using different types of biomass. Biofuels have turn-out to be crucial sustainable sources for restraining the production of Greenhouse gaseous (Huang et al., 2012).

Crucial benefits of bio-fuels above traditional fossil fuels are: -

1. Bio-fuels are sustainable as well as renewable resources (Razzak et al., 2013)
2. The toxic emission level via biofuel combustion is minimum (Surriya et al., 2015)
3. The Carbon dioxide emission is negated (Razzak et al., 2013; Surriya et al., 2015).

Figure 2. Different generation of biofuels



We can classify biofuel production into different generations as aforementioned in figure-2 (Hombach et al., 2016; Correa et al., 2017). First-generation feedstock biofuel is derived from cereal crops, sweet sorghum, Sugarcane, sugar beet and molasses for bio-ethanol production (Hemaiswarya et al., 2012) (Voloshin et al., 2016). Second-generation feedstock biofuel is derived from non-edible oils from *Jatropha curcas*, *Pongamiapinnata*, *Hevccabrasiliensis*, *Calophylluminophyllum,* which are usually used for biodiesel production (Hemaiswarya et al., 2012). Maity et al.(2014) stated that grass, wood and straw fall under second-generation feedstock biofuel. First-generation feedstock's biofuel and second-generation feedstock's biofuel are burdened with various concerns despite tax-credit policy and blending mandates, which permitted limited feedstock is to the energy market (Doshi et al., 2016).

First-generation feedstock biofuel and Second generation feedstock biofuel limitations: -

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1. Jeopardising food safety owing to trade-off between fuel vs food.
2. Excessive land requirement.
3. High agricultural inputs
4. High investment cost
5. Hostile retail prices
6. Lower efficiency
7. Lower yield (Doshi et al., 2016; Juneja et al., 2013).

Due to these restrictions, researchers were looking for substitute feedstock and acknowledged a 3rd generation feedstock bio-fuel identified as algae, which insinuates numerous benefits as it, does not rely on edible crops, does not release elevated gaseous pollutants, and it does not require fertiliser or productive land (Mackenzie, 2013; Kim and Lee, 2015).

Several used macro-algae feedstocks are; some examples are *Enteromorphacompressa, Cladophoraglomerata, Ulva Lactuca, Laminaria*sp., *Macrocystispyrifera* (Chen et al., 2015; Xu et al., 2014). Third generation feedstock biofuel is primarily used to yield bio-diesel, which is along with chain alkyl ester, non-toxic with bio-degradable properties, carbon-neutral and nearly Sulphur-free, aromatics and its chemical, and energy properties are near to gasoline making it usable for the diesel engines without the requirement of minor to not at all adjustments (Luque, 2010).

2.1 Micro-Algae - Model for Viable Green Fuel

Micro-algae provide economic benefit, social benefit and environmental benefit using viable 3rd generation bio-fuel feedstock upon terrestrial oleaginous crops due to significant volumetric biomass, areal biomass, lipid yield, capability to adapt along with capability to grow in all sorts of water with a prospect of being grown all through year regardless of climatic changes (Ziolkowska and Simon, 2014; Ribeiro et al., 2017). Micro-algae have a high lipid content in contrast to oleaginous crops available in the market till now; that is, yearly algal oil production can be multiple folds to that of feedstock crop per ha. Additionally, compared to food crops, algae have a short doubling time and 20 to 30 times higher growth rate, providing multiple harvesting phases in a brief time (Chisti, 2007; Schenk et al., 2008; Ziolkowska and Simon, 2014). Bio-diesel produced from micro-algae can be used as a substitute for fossil diesel which can be very helpful to fulfil human's energy demand. Additional bio-diesel is carbon-neutral fuel, which means the quantity of CO2 released due to burning of bio-diesel stays equivalent to the amount of co2 absorbed by algae (Ziolkowska and Simon, 2014). For economic gains, carbohydrate's and lipids can be used for the production of various fuels such as gasoline, bio-ethanol, bio-diesel, bio-gas, hydrocarbon and jet fuel, while protein can be cast-off in feed, nutraceutical and food manufacturing, bio-fertilisers, surfactants, industrial enzymes (Griffiths et al., 2011; Yen et al.,2013). An additional economic value is that algae can be cultivated in sea-water, fresh-water, brackish-water, and sewage water (Cheng and He, 2014; Mata et al., 2010; Mathimani et al., 2017).

Micro-algae do not require basic cultivation needs such as portable water or fertile land. It can be easily cultivated off-shore or near-shore for biofuel, secluding land requirements. When comparing land use for algal fuel production to replace traditional fossil fuel, it would require less than > 1/7th of corn cultivated region of United States of America (Ziolkowska and Simon, 2014). Some governments provide various privileges such as cost-sharing, decreasing the total risk of the initial investment, providing subsidies on the bio-diesel production process (Su et al., 2015). Biofuel is an answer to climate change,

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energy insecurity and other multifarious issues (Pradhan and Ruysenaar, 2014). Hence, the bio-fuel programs success is motivated by effective and efficient guidelines following up with bio-fuel yield from various feedstock and countries as shown in Table-2. Learning from Brazil's success, developing countries counting India should prepare a roadmap to boost bio-fuel missions, blend goals and provide grants within directives, missions, and biofuel policies. The forthcoming section will have enlightened India's comprehensive breakdown of the legal charter governing bio-fuel.

Table 2. Bio-fuel yield from various feedstock and countries

Crop	Countries	Bio-fuel	Bio-fuel Yield	Reference
Sugar cane	India	Ethanol	4522	(FAO, 2008)
Oil palm	Malaysia	Biodiesel	4736	
Oil palm	Indonesia	Biodiesel	4092	
Maize	United States of America	Ethanol	3751	
Maize	China	Ethanol	1995	
Cassava	Brazil	Ethanol	1863	
Cassava	Nigeria	Ethanol	1480	

3. THE CHRONOLOGICAL ASSESSMENTS OF THE BIO-FUEL LEGISLATION IN INDIA

With the enactment of the *Indian Power Alcohol Act,* 1948 (Act No. 22 of 1948), India got its first legislation on biofuel in 1948. Its primary objective was to empower India's 'power alcohol' industry (Cleveland and Morris, 2013). Earlier, in 2000 legislature has abolished this act (Indian Power Alcohol (Repeal) Act, 2000). Finally, in 2002 the Ministry of Petroleum and Natural Gas had released a notification on the *Ethanol Blending Programme*. From January 2003, the notification required 5% ethanol blending with petrol in 9 sugarcane supplying 3 Union Territories and States (Amit Aradhay,2010). The Ethanol Blending Program remained ineffective because of inaccessibility of sugar molasses, botched-up ethanol pricing formula, bureaucratic interruptions by State organisations, late purchasing, with several other factors (Ray et al., 2011).

3.1 Bio-diesel Program

The Indian government is pining to execute its bio-diesel programme for the welfare of green energy resources and rural employment. In order to fulfil this objective, in July 2002, with the guidance of NITI Aayog, the government formed the *Committee on Development of Bio-fuel*. In July 2003, the committee submitted the report. In taking into consideration the report, in 2003, the Indian government introduced *National Bio-diesel Mission*. The Mission has a particular emphasis on the *Jatropha* farming on wasteland. It has acknowledged the Ministry of Rural Development as a nodal ministry. National Biodiesel Mission has to achieve its objectives in 2 diverse stages. Stage-I comprises demonstration and experimentation, and it has to be executed by 2006-07 (Kumar Biswas et al., 2010). Stage-II was projected for seed purchasing, oil abstraction and nursery development plant along with setting up of

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trans-esterification workshop lastly mixing and marketing of bio-diesel (Bandyopadhyay and Das, 2014; Kumar Biswas et al., 2010). Stage-II targets are encouraged to be complete. In 2005, the Ministry of Rural Development primed and submitted a comprehensive Project Report to the Indian government to discuss the pilot stage of biofuel feedstock crops (Mohan et al., 2006; Raju et al., 2012). Throughout the prime period, the Ministry of Petroleum and Natural Gas (in October 2005) had revealed the *Bio-diesel Purchase Policy*, which empowers oil marketing companies to purchase bio-diesel) at the cost of Rs. 26.50 per litre (inclusive of all taxes), with effect from 1 January 2006. Oil Manufacturing Companies intend to acquire bio-diesel that fulfils the criteria proposed by the Bureau of Indian Standards, and all procurement can be made only through recognised (20) centres.

Under Entry 14, List-II (State List) in Schedule 7 of the Indian Constitution, agriculture-related laws are enlisted, which empower the state government to make laws related to biofuel feedstock plantations which result in Union government-regulated petroleum-based companies with other private industries signing an MoU with state governments to promote biofuel feedstock on government-owned wasteland or unused lands through the method of contract farming with various farmers. In this initiative, significantly states governments have taken an interest in being part of the bio-diesel mission and promoting biofuel feedstock plantations with various instruments, including incentives and policies. Such states are Odisha, Uttarakhand, Rajasthan, and others few states (Venkataraman, 2012). The national bio-diesel mission has suffered setbacks due to lack of seeds, inefficient seed collection, extraction, and absence of knowledge and self-assurance in farmers and industry players. (Bandyopadhyay and Das,2014). The commercial production and sales of bio-diesel through the procurement centre is negligible.

The government conducted a study that states that farmers' failure to adopt scientific methods suffered a massive loss in lower production biofuel feedstock (Pradhan and Ruysenaar, 2014). The study also highlighted a shortage of seed and seed oil content, which led to the shutdown of various biofuel production units. Consequently, the National Bio-diesel Mission could not emerge successfully and was ended. Bio-diesel Purchase Policy faced the same fate of failure due to 'unrealistic purchase price' and subdued answers from Oil Manufacturing Companies (Kumar Biswas et al., 2010). In 2003 bio-diesel national Mission was announced; however, due to failure until 2008, the government could not implement a formal bio-fuel policy. As a result, in September 2008 'National Policy on Biofuels, was formulated and on 24 December, 2009, it was approved by the central government. Concerning the above, the next part tries to explore the National Policy on Bio-fuels, 2008, and pinpoint the weaknesses.

3.2 Key Characteristics of the National Policy on Bio-Fuels, 2008

1. The policy's primary purpose is to gratify bio-fuel demand and make certain accessibility of a minimum level of biofuels throughout the nation (Chandel et al., 2017).
2. The policy attempts to fast-track production of bio-fuel from non-edible oil feedstock's.
3. To confront the food *v fuel* debate (Murali et al., 2016; Rajagopal, 2008).
4. To draw the interest of foreign direct investment (Chandel et al., 2017).
5. Development of National Bio-fuel Coordination Committee.
6. To establish Bio-fuel Steering Committee.
7. Secretary to supervise policy implementation.

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4. COMPREHENSIVE EXPLORATION OF THE NATIONAL POLICY ON BIOFUELS

4.1 Policy's Vision and Goals

- The Policy objectives are to make the biofuels market driver in the transportation and energy sectors in the future. The policy aims to accelerate development, promotion and production of biofuels and their usage to progressively replace diesel and petrol for transportation and stationary along with other applications, though backing to environmental concerns, creating employment ultimately directing towards sustainable development.
- The policy Goal is to safeguard the minimum level of biofuels at any given time easily accessible in the market to fulfil the requirement, by 2017 achieving the target of 20% biofuel blending for biodiesel and bioethanol.

4.2. Definitions and Scope

4.2.1 Characterisations of bio-fuels in the Policy

1. 'Biofuels' are liquid or gaseous fuels produced from biomass resources and used in place of, or in addition to, diesel, petrol or other fossil fuels for transport, stationary, portable and other applications.
2. 'Biomass' resources are the biodegradable fraction of products, wastes and residues from agriculture, forestry and related industries, as well as the biodegradable fraction of industrial and municipal wastes.

4.2.2 The Scope of the Policy Incorporates

1. 'Bioethanol': ethanol produced from biomass.
2. 'Bio-diesel': a methyl or ethyl ester of fatty acids.
3. Other bio-fuels: bio-methanol, bio-synthetic fuels.

4.3 Interventions and Enabling Mechanism

4.3.1 Plantations

The policy has discovered 400 kinds of trees in India that bear non-edible oilseeds, with the possibility of all of these species being used to produce biofuels. Farmers must obtain proper permission from local communities to use non-arable land. In 'Scheduled Areas,' where tribal tribes make up most of the population, the Panchayats Act, 1996 must be followed. The fifth Schedule of the Indian Constitution identifies these categories. It is worth noting that employment in non-edible oil seeds plantations will be included under the MNREGA, 2005. Policy guarantees that the Minimum Purchase Price for oilseeds

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is met, and it will be executed with cyclic revisions. (Paras 5.1-5.5 of the National Policy on Bio-fuels, 2008; Patel et al., 2014).

4.3.2 Processing

The policy pushes India's government to launch a 'National Registry,' which would be in charge of developing and maintaining relevant data on biodiesel and bioethanol supply and regularly reviewing blending levels based on this data. The blending levels must meet BIS specifications and certification norms. As a result, processing industries and OMCs must collaborate to create a sound system for this cause. Motor engine manufacturers are permitted to do required modifications to present engines "to ensure compatibility with biofuels." Section 52 of the Motor Vehicles Act of 1988 similarly permits similar changes to Automobiles (Paras 5.6-5.10 of the Policy; Ray et al., 2012).

4.3.3 Financial and Marketing Enticements

In India, OMCs are responsible for the storage, distribution, and sale of biofuels; current systems must regulate these actions. The Bio-fuel Steering Committee will determine the Minimum Purchase Price for bio-fuel, which will be chosen by the National Biofuel Coordination Committee, which will examine the "entire value chain" when establishing the Minimum Purchase Price for biodiesel, and "real cost of production and import price of bio-ethanol" when determining the Minimum Purchase Price for bioethanol. (Paras 5.11-5.12 of the Policy). In the aftermath of a petrol or diesel price gap below the Minimum Purchase Price for bio-fuels, the government would reimburse the OMCs appropriately. (Ray et al., 2012).

The National Bank of Agriculture and Rural Development, the Indian Renewable Energy Development Agency, the Small Industries Development Bank of India, and other lending institutions are mandatory to offer monetary support toward the planting of non-edible oil feedstock's, as well as the establishment of oil processing and extraction units for bio-fuel production. It is worth noting that under the automatic route, FDI up to 100% is authorised in biofuel technology and projects, as long as the biofuel is only used for local consumption. For the plantation of non-edible oilseeds, however, foreign direct investment is forbidden. (Paras 5.13-5.15 of the Policy). The policy pushes the Indian government to establish a "National Bio-fuel Fund" to provide financial incentives, subsidies, and grants for novel and 2nd generation feedstocks. Bio-diesel and bio-ethanol will be exempt from all central taxes and levies, except for a 16 per cent exempt excise duty on bioethanol. Other special discounts were also available to establish bio-oil extraction plants and processing facilities. It is important to emphasise that biofuel imports will be permitted to a limited degree, as determined by the NBCC, and biofuel exports would be approved only when the domestic requirements have been met (Paras 8.1-8.2 of the Policy). Unlike other countries, India's policy does not encourage biofuel blenders and merchants. (Bandyopadhyay and Das, 2014).

4.3.4 R&D and Quality Standards for Bio-Fuels

R&D and Innovation would receive a massive boost from the policy in bio-fuels. Indigenous research and development will be given priority, and intellectual property will be safe and secured as needed. Research centres, Educational establishments, and businesses are encouraged to collaborate on research projects, and the appropriate funding will be granted. In order to achieve global technology, competitiveness and competence transfer must be accelerated (Para 5.19-5.22). The BIS issued IS-15607 for

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biodiesel and I.S.: 2796: 2008 for bioethanol, based on ASTM D-6751 and EN-14214, respectively, American and European standards. The BIS must regularly assess existing standards to produce new standards-compliant with global benchmarks. (Paras 6.1-6.2).

4.4 State Governments Current Contribution

The policy promotes state government to develop and execute biofuel programmes actively. State governments must embrace biofuel policy and establish a nodal point to promote biofuels. (Paras 9.1-9.2 of the Policy). Many state governments have been aggressively participating in the backing of India's bio-fuel programme following the National Policy on Biofuels. Several states have previously produced or worked on biofuel policies and vision statements.

4.5 Institutional Mechanism

The Prime Minister would spearhead the formation of the National Biofuel Coordination Committee, which offers élite coordination and policy supervision. In order to properly monitor and administer biofuel programmes, the National Bio-fuel Coordination Committee must convene regularly. The Deputy Chairman of the Planning Commission, the Ministry of New and Renewable Energy (as coordinating Ministry), the Ministry of Rural Development, the Ministry of Agriculture, the Ministry of Environment and Forests, the Ministry of Petroleum and Natural Gas, the Ministry of Science and Technology, and the Secretary of the Ministry of New and Renewable Energy make up the National Bio-fuel Coordination Committee. (Part 11 of the Policy). A Bio-fuel Steering Committee will be formed to oversee the policy's implementation under the Cabinet Secretary governance. The BSC comprises eleven Secretaries from the Indian government's various Ministries and Departments. Several ministries participated in policymaking, development and promotion of biofuels at the national level, and each Ministry must coordinate with the others. Several programmes have been launched in India to encourage the development of biofuel crops. For example, the Agriculture Ministry established the Integrated Development of Tree Borne Oil Seed Scheme, which offers farmers and non-governmental organisations financial assistance to cultivate tree-borne oil seeds. (Dalemans et al., 2018; Raju et al., 2012).

4.6 Major Limitations to Accomplish Objectives Established Under India's Biofuel Policy

Even after constant decade-long efforts in developing biofuel and its promotion, India's accomplishment was not up to the desired mark. India's progress is far behind several other countries (Kumar Biswas and Pohit, 2013). As a result, the aims outlined in India's Bio-fuel Policy appear to be "unrealistic" in light of succeeding facts. (Kumar Biswas et al., 2010).

1. Land Restrictions for the Cultivation of Biofuel Crops

Non-edible oil crops must be planted exclusively on "wastelands" in non-forest and forest areas, according to paragraphs 1.5 and 5.1 of the policy. It is worth noting that the policy does not explain the term "wasteland." The writers were able to track the meaning of 'wasteland' back to the colonial authorities, who defined it as "land that did not generate any tax since it was uncultivated," which included

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everything from semi-jungle to forest, and marshes land to dry fields (Kumar Biswas et al., 2010; James and James, 1999). Lord Canning devised the 'wasteland rules' in 1861 to manage the profits earned by the uncultivated areas. There are several government categories for wastelands in India, including two principal classifications, ninefold classifications, and the Wasteland Atlas. (Baka, 2014). Besides, a large chunk of wasteland is being illegally encroached by landless labours and poor people; too which government has no available record of (Kumar Biswas et al., 2010). The State Governments must finalise the government wasteland and its usage and given for plantings, according to paragraph 9.2 of the policy. According to reports, India has no legislation for delineating non-cultivable wasteland appropriate for bio-fuel (mostly Jatropha) growth. Furthermore, the appropriation of arable land for the growth of biofuel crops has created significant socio-economic challenges that have hampered the Bio-fuel Policy's implementation (Kumar Biswas et al., 2010; Bastos Lima, 2012).

2. Rational Problems in Feedstock Cultivation

According to the Indian government, feedstock farming is now included in the MGNREGA scheme. Jatropha was the first crop that came to mind. By 2012 Indian government has resolute Jatropha plantation on 11.2 million hectares to 13.4 million hectares of land to come across a bold target of 10% bio-diesel-diesel blending mandate. Researchers and policymakers appear to misjudge the harvest of the Jatropha plant in sub-optimal growing situations (Sreenivas et al., 2018; Bastos Lima, 2012). It was also discovered that policymakers did not refer farmers when making such judgments. Overall, farmers cultivate Jatropha solely as a fence crop, and low crop yields discourage farmers (Bastos Lima, 2012).

3. Discrepancy in State Tax Structures

The policy stipulated a 16 per cent reduction on tariff on bio-ethanol and the elimination of all central taxes and duties on bio-diesel and bio-ethanol. On the other hand, state tax policies are not all the same, and they may differ from state to state. Even though each state has its excise tax, biodiesel is exempt from the federal excise tax of 4% as economic motivation. Raju et al. (2012) demonstrated that the policy's heterogeneous tax structure provides a roadblock to its timely implementation. Due to differing state legislation and administrative controls, biofuel's swift and unrestricted movement across state boundaries has been hindered.

4. Distinct Species of Non-Edible Oil Feedstock and their Suitability

The policy's paragraph 5 lists 400 kinds of non-edible seed-bearing trees in the country, although actual trials focus solely on the Jatropha. Some research is done by developing genetic improvements to plant kinds, nevertheless with minor achievement. An alternative explanation is that biodiesel production in India is based upon the Jatropha plant, proven to be commercially impractical due to lower yield, poor seed quality, and promotion issues. (Slette and radhey, 2014). According to Kumar Biswas and Pohit (2013), most research concentrates on plant-based materials. However, it neglects other elements such as soil conditions and agro-climatic factors, which stay necessary for boosted crop output.

5. Organisational limitations

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National Bio-fuel Mission had planned to create the National Bio-fuel Development Board but later dissolved for unspecified reasons. The government has failed to establish any establishment proposed in the national biofuel mission to ease the transfer of benefits straight to farmers; therefore, it is essential to tackle these problems within prevailing established machinery or create innovative machinery (Kumar Biswas et al., 2010).

4.7 Recent Developments

To generate awareness and stimulate biofuel in India, on an industry level, the Ministry of Petroleum and Natural Gas, in synchronisation with the Petroleum Conservation Research Association, has launched a *Working Group* (PTI, 2015). The central government will amend the National Policy on Bio-fuels and Motor Spirit and High-Speed Diesel Control Order to ease bio-fuel sale (Business Line, 2015). Also, the Indian government is anticipated to disclose a Strategy on 'Flexible-Fuel Cars', which can operate either bioethanol or on petrol or its combination. These 'flex-fuel vehicles' will play a crucial role in fighting ever-increasing pollution and boosting ethanol consumption.

5. A WAY FORWARD

Through intensive study, it has been pointed that the Oil Manufacturing Company's solitary dependence on the ethanol produced from molasses confines enactment of the Ethanol Blending program. As recommended by Standing Committee on Petroleum & Natural Gas, we have again restated that "other than sugarcane, ethanol can be produced from corn, maise, wheat and food grains having high starch content", which has efficaciously executed in the United States of America. We can use various short generation biofuel feedstock crops that can be easily raised on monsoon-dependent agricultural lands, which could play an essential role in increasing the total productivity of biofuel feedstock and improving farmers' income. So, the Indian government must distribute the apposite amount of resources to fast-track the current Research and Development in manufacturing ethanol. The Indian government has to revive all the committees made under the policies and coordinate among other ministries to make up a comprehensive plan for effective implementation of the Bio-fuel program. The government must review its policies and make changes to have a fruitful bio-fuel program if required.

6. CONCLUSION

There are four vital types of machinery for any biofuels to succeed if taken into run-through, i.e., economics, technology, policy support, and environmental sustainability. Researchers emphasised various challenges for commercially viable biodiesel production, such as cultivation, harvesting, and production costs. However, legal issues and its solution with bio-fuel marketing in the energy sector are still not extensively considered, particularly in emerging countries like India. Unless we have policy support, we cannot convert beneficial technology into practical usage. Incidentally, India's biofuel policy accepted obligation in blending ethanol and bio-diesel programs. Still, the biofuel program did not earn significant success primarily due to biomass cultivation cost and the food vs fuel conflict. Henceforth, this chapter addresses the micro-agal feedstock potentials in biofuel production in addition to the policy concerns,

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which hampers its commercialisation in India. This chapter also displays important data related Indian government's national Bio-fuel Policy, which encompass legal hurdles for biofuel marketing. It focuses on the Indian government initiative to address industries problems regarding the bio-diesel program. Ethanol blending and its various policies. In order to accomplish India's aspiring biofuel targets, there needs revolution throughout the grass root levels, especially from the public and government are required for prosperous biofuel future in India.

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Chapter 5
Green House Gases:
Challenges, Effect, and Climate Change

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ABSTRACT

Changes in weather patterns over decades or longer periods of time are referred to as climate change. Natural and human forces both contribute to climate change. Since the Industrial Revolution, humans have contributed to climate change through emitting greenhouse gases and aerosols, as well as changing land use, leading global temperatures to rise. More storms, floods, droughts, and rising sea levels may result from increasing global temperatures and the melting of ice sheets, sea ice, and glaciers. The sun's rays supply energy to the whole world. GHGs are required to trap heat and maintain a temperature that permits life to flourish on the planet. The greenhouse effect is a natural and necessary occurrence for life on Earth to survive. If the greenhouse effect did not exist, the world would be around 33°C cooler right now. Humans have contributed to the increase in atmospheric GHGs via rising fossil fuel usage and deforestation in recent decades. Increased GHG emissions have been the primary cause of global warming during the last century.

INTRODUCTION

Since 1850, three primary datasets have been used to calculate global surface temperatures. These figures indicate a warming of +0.8°C to +1.0°C since 1900. 4 Since 1950, land-only data has shown increasing trends of +1.1°C to +1.3°C, indicating that land temperatures respond to climate change quicker than ocean temperatures. While multi-decadal time periods (30 years or more) are often used to assess global warming, attribution of trends over shorter time periods may be problematic due to natural variability.

DOI: 10.4018/978-1-6684-5269-1.ch005

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Natural climatic variability is caused by internal interactions between the atmosphere, ocean, land surface, and sea ice. These oscillations happen whether or not there is climate change, and are generally referred to as "noise" or natural changes around a "normal" figure. Temperature rises as GHG levels increase, which has a substantial indirect influence on water vapour. Due to the greenhouse effect of water vapour, greater global temperatures induced by GHGs increase the atmosphere's capacity to store water vapour, resulting in a positive feedback loop. The quantity of water vapour in the atmosphere increases by roughly 7% when the global temperature rises by 1°C. (Dones et al., 2016; Frischknecht et al., 2003; Inter-Governmental Panel on Climate Change (IPCC), 2001; Villigen & Swiss, 2003)

Water vapour is a potent and quick feedback that increases any initial force by two to three, despite the fact that CO2 is the most significant human control knob on climate. Water vapour is a large contribution to climate change, despite the fact that it is not a significant beginning force. By continuing to address criteria pollutant nonattainment issues, state and municipal governments may accomplish significant GHG emission reductions. The most efficient approach to accomplish this goal is to ensure that the techniques utilised to achieve emission reductions for criteria pollutant compliance also provide benefits in terms of GHG reduction, rather than ineffective or counterproductive GHG reduction efforts. Because strategies that reduce GHG emissions nearly invariably result in reduced emissions of other air pollutants, STAPPA and ALAPCO believe it is vital to focus on the link between GHG mitigation and conventional air pollution management. The most well-known harmonised procedures are used to address CO2, particle matter (PM), nitrogen oxides (NOx), sulphur dioxide (SO2), carbon monoxide (CO), and air toxics, all of which are important CO2 sources. The most harmful greenhouse gases include CO2, methane, nitrous oxide, hydrofluorocarbons, perfluorocarbons, and sulphur hexafluoride. Ozone precursors (NOx and NMVOCs) have an indirect greenhouse effect since they are also GHGs. The focus of this study is on CO2 for two reasons. To begin with, CO2 is thought to be responsible for more than half of the expected global warming consequences. In 1997, CO2 emissions accounted for almost 82 percent of total emissions in the United States. (Dones et al., n.d.; Hondo, 2003; Lee, 1997; Ozawa et al., 2003; Spath et al., 1999). GHG emissions refer to the amount of greenhouse gases released into the atmosphere. Second, the primary source of CO2 is fossil-fuel combustion, which is governed by state and local governments via emission categories. According to the Intergovernmental Panel on Climate Change (IPCC), the Earth's climate system is continually changing, both worldwide and locally, with some of the changes linked to human activity and resulting in greenhouse gas emissions. Energy delivery systems, especially fossil-fuel systems, are the principal contributors to these gas emissions. This article primarily leverages data from a recent Swiss study addressing Life-Cycle Assessment (LCA) issues based on environmental inventories of European-wide energy systems to provide GHG emission comparisons.

The ocean covers 71% of the Earth's surface and is home to 1.3 billion cubic kilometres of salty water. This enormous body of water has a huge influence on the global environment. It functions as a global thermostat, lowering heat gradients induced by differences in incoming solar radiation over time and space, controlling the composition of numerous trace chemicals in the atmosphere, and functioning as a dump for a range of manmade toxins. Climate fluctuations are often connected to changes in the atmosphere (Fraedrich, 2001; Fraedrich et al., 2005; Fraedrich & Lunkeit, 2008; Kunz et al., 2008; Lorenz, 1955). However, analysing the atmosphere on its own is challenging. The land, oceans, and ice-covered regions of the Earth's surface are all intricately tied to the atmosphere (known as the cryosphere). The biosphere, which comprises both terrestrial and marine biological systems, has a substantial relationship.

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CLIMATE CHANGE

The atmosphere, land, ocean, cryosphere, and biosphere make up the basic framework of the climate system. The ocean has an impact on the rate at which greenhouse gases are emitted. This is performed by absorbing surplus greenhouse gases from the environment, as well as some greenhouse-induced heat. Both of these measures contribute to slowing global warming. The global ground-level temperature of the atmosphere would presently be one to two degrees Celsius higher if it weren't for the cooling effect of the ocean. The question is whether the ocean will continue to counteract global warming, and whether the rate at which it does so will slow or speed up over time. We need to know the precise answers to these questions before we can determine the total amount of global climate change. As a consequence, further study is required on (1) gas exchange processes at the air-sea interface, with an emphasis on CO:, (2) greenhouse gas transit routes from the surface to the ocean's deep layers and sediments, and (3) ocean circulation characteristics.

The wind is the primary driving force behind the ocean's surface circulation, which moves heat and water in horizontal planes. Excessive evaporation on the sea surface from thick water descending towards the ocean's core causes the thermohaline circulation to cool and/or increase salt content. On the one hand, this process allows deeper ocean layers to interact with the atmosphere, but on the other hand, it "opens" the huge volume of the deep ocean, allowing it to store heat and CO2. This sinking of heavy surface water may now only be seen in a few isolated areas of the ocean. Deepest convection occurs in the northern North Atlantic (Norwegian and Greenland Seas) and near Antarctica (Weddell Sea). Warm surface water in the North Atlantic is cooled, allowing more CO: to dissolve before sinking as equally salty water into the deep ocean, where it has a long history of interaction with the atmosphere. After being kept off from direct touch with the atmosphere for a long time, upwelled deep water in the Southern Hemisphere swiftly transforms into colder, denser water with a high CO2 content and re-enters the ocean's deep and bottom layers.

As these two water masses spread across the ocean, each with its own set of characteristics, the "resident" deep ocean water rises slowly but progressively. The rate and method of deep water creation, as well as oceanic heat transfer to high latitude continental areas, are all determined by surface water circulation in the North Atlantic. Excess salinity (and the associated heat flux, which is controlled by the rate at which surface water flows into the Atlantic) generates strong oceanic fronts, defines the boundaries between ice-covered and open ocean areas, and heats northern Europe. The whole system has been found to be exceedingly unstable across decadal, historical, and geologic time periods. Little changes in the sea ice limit, as well as salinity, temperature, and volume of inflowing surface waters, may have a substantial influence on Europe's climate, economy, and living conditions, according to historical records..

Scientists, engineers, industry, and regulators are all interested in the future role of hydrogen H2 as a synthetic fuel and energy carrier. In the future, hydrogen is likely to play a larger role in transportation. An internal combustion engine or a fuel cell may be used to create a hydrogen-powered vehicle. In the second method, hydrogen is burnt. Electrochemical traction is created in a fuel cell, which then supplies energy to power an electric motor. A fuel cell car's efficiency in converting chemical energy to mechanical energy might be far higher than that of a normal gasoline-powered internal combustion engine vehicle. Hydrogen's attraction as a future transportation technology derives from its capacity to boost overall economic growth while drastically reducing environmental pollution. Hydrogen generation, storage, delivery, and usage in all aspects of the energy economy, including transportation, will be major problems for any future hydrogen economy. To ensure that overall environmental damage is avoided

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and the full potential of hydrogen as a clean fuel is realised, thorough environmental evaluations will be necessary for all technologies employed at each step, from synthesis to usage.

We examine what we know about hydrogen's fate and behaviour in the atmosphere, as well as its major sources and sinks. Hydrogen is a greenhouse gas, contrary to common assumption, and its global warming potential is evaluated in contrast to carbon dioxide. Then we assess the global warming implications of switching from a fossil-fuel to a hydrogen-based energy source. The researchers found that substituting hydrogen-based energy systems for fossil-fuel-based energy systems would only result in a minor reduction in global warming unless hydrogen leakage from any future hydrogen economy is tightly monitored. Gases that trap heat in the atmosphere are referred to as "greenhouse gases" (GHGs). Carbon dioxide, for example, is naturally created and released into the atmosphere as a consequence of both natural and human activities. Humans are the only ones who manufacture and release other greenhouse gases, such as fluorinated gases. The most major greenhouse gases that enter the atmosphere as a consequence of human activity are carbon dioxide, methane, nitrous oxide, and fluorinated gases. The behaviours described above are frighteningly similar to those that occur in the Earth's climate system. Shortwave solar radiation from the Sun reaches the planet's surface, where it is absorbed and heated by the planet's comparably clear atmosphere. The gross radioactive flux density generated by the planet's surface (land, water) much outnumbers the input irradiative flux density received from the Sun, just as a person's thermal infrared radiation far outnumbers his or her caloric intake on a global and annual average. The atmosphere absorbs thermal infrared radiation from the planet's surface, which is subsequently heated and radiates, in part downward toward the surface.

Due to downwelling infrared radiation from the atmosphere to the surface, the net upward infrared flux from the surface is significantly decreased in contrast to the gross blackbody flux. The greenhouse effect assumes that a greenhouse maintains its heat by collecting and releasing longwave radiation via its glass surfaces. (True greenhouses maintain their warm temperatures by preventing warm air from flowing away from the surface.)

GREEN HOUSE GASES

In the atmosphere, a greenhouse gas absorbs and emits heat infrared radiation. This method is the source of the difficulties. The principal greenhouse gases in the Earth's atmosphere include carbon dioxide, chlorofluorocarbons, methane, and nitrous oxide. The following are the greenhouse gases that produce an increase in air and earth temperature and are present in the troposphere: CO2 is a substance that exists in the form of a gas. It contributes to global warming by producing around 55% of the greenhouse gases produced by human activities. The chief causes are fossil fuel combustion (67 percent), deforestation, and various kinds of land removal and burning (33 percent). CO2 levels in the atmosphere were 355 parts per million in 1990, and they are growing at a 1.5 parts per million per year pace. CFCs account approximately 24% of all greenhouse gas emissions created by humans. They also deplete the ozone layer in the stratosphere. CFCs are mostly generated through leaky air conditioners and refrigerators, among other sources. CFC levels in the atmosphere are now at 0.00225 parts per million and growing at a rate of 0.5 percent per year. Methane is responsible for 18% of the global increase in greenhouse gas emissions. When microorganisms break down dead organic material in damp, oxygen-deficient environments like swamps, natural wetlands, paddy fields, landfills, and the digestive tracts of cows, sheep, and termites,

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methane is produced. The current amount of methane in the atmosphere is 1.675 parts per million, and it's growing at a 1% yearly rate.

Human-caused carbon dioxide emissions account for 6% of total emissions. The breakdown of nitrogen fertilisers in soil, animal wastes, and nitrate-contaminated groundwater, as well as the burning of biomass and nitrogen-rich fuels, all contribute to its production (particularly coal). The atmospheric concentration of N20 is 0.3 parts per million, and it is increasing at a rate of 0.2 percent each year. One of the main objectives of climate research is to figure out how the statistical characteristics of the climate system vary when the value of external or internal factors changes. It was recently shown that an approach for quasi-equilibrium systems may be quantified and has formal connections to Kubo 20 response theory. The irreversibility of the ten climate systems, notably their entropy production, is the emphasis. The maximal entropy production principle (MEPP) asserts that an out-of-equilibrium nonlinear system adjusts to maximise entropy formation. Despite the fact that MEPP's general validity is in question, it has proved effective as a heuristic in 15 climate research, requiring a full re-evaluation of entropy generation's function in the climate system. This has fuelled a push to adopt a new generation of diagnostic tools based on the second law of thermodynamics for auditing climate models and defining a set of parameterizations for use in conceptual and intermediate complexity models, as well as recreating historical climate conditions.

Carnot efficiency, entropy production, and the degree of climate system irreversibility are all linked to climate system irreversibility. As a consequence, it is now possible to conduct a new fruitful inquiry into the onset and decrease of snowball conditions as a function of solar constant changes. The research makes use of Planet Simulator, a lightweight climate model (PLASIM). Ignoring mixing processes and assuming local thermodynamic equilibrium, this remains true everywhere except in the upper atmosphere, where there is no mass. For estimating the steady-state climate response, slab ocean climate models are excellent. The global atmospheric energy balance is much improved over prior versions of the model by re-feeding the kinetic energy losses owing to surface friction and horizontal and vertical momentum dispersion.

The sensitivity of all three temperature indicators is positive, as expected: the sensitivity of the surface temperature is well within the range anticipated by IPCC climate models, while the sensitivity of the two bulk thermodynamic temperatures is lower. Atmospheric thermodynamics is the study of heat-to-work (and vice versa) changes in the earth's atmosphere in relation to weather and climate. In addition to the globally averaged surface temperature, the intensity of the Lorenz energy cycle, the Carnot efficiency, the generation of material entropy, and the degree of irreversibility of the system are all linear with the logarithm of CO2 concentration. The generalised sensitivity diagram below shows how when the climate system warms, it gets less efficient, more irreversible, and creates more entropy. Changes in the intensity of latent heat fluxes are the most critical components, emphasising the need of addressing climate change's influence on the hydrological cycle at a fundamental level. All variables may be effectively reparameterized with reference to just this one due to the identified variables' monotonic (and, in particular, linear) reliance on the logarithm of CO2 concentration.

Using a basic yet Earth-like climate model and a novel theoretical framework, we study the influence of global warming on climate system thermodynamics. In addition to the globally averaged surface temperature, the intensity of the Lorenz energy cycle, the Carnot efficiency, the generation of material entropy, and the degree of irreversibility of the system are all linear with the logarithm of CO2 concentration. These high sensitivity levels imply that as the climate heats, it becomes less efficient, more irreversible, and more entropy is produced. Using the essential notions of classical thermodynamics,

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atmospheric thermodynamics investigates processes such as moist air properties, cloud formation, atmospheric convection, boundary layer meteorology, and vertical stability in the atmosphere. Atmospheric thermodynamics is concerned with water and its changes. Among the subjects covered are the ideal gas law, specific heat capacities, adiabatic (entropy-conserving) processes, and wet adiabatic processes. The bulk of troposphere gases are made up of water vapour, which is one of the most important trace components of air.

The essential function of atmospheric thermodynamics is described in basic equations of air motion, which are either grid resolved or subgrid parameterized, in terms of adiabatic and diabatic forces acting on air parcels. Numerical weather and climate predictions are created using these equations. The sun provides heat to the Earth. The Earth and its atmosphere emit heat into space. They disperse the bulk of the solar heat, maintaining the Earth's normal temperature. Greenhouse gases warm the Earth and envelop it in a warm blanket. According to the second law of thermodynamics, heat cannot flow spontaneously from a lower-temperature substance to a higher-temperature material. "Global warming" is one of the most often used words in environmental study. The temperature of the Earth has been continuously increasing over the last five decades. Many experts believe that effective and acceptable pollution control techniques are urgently needed. Humans are exposed to a variety of harmful gases, each of which creates its own set of health problems. Carbon dioxide, sulphur dioxide, and hydrogen sulphide are among the gases that may be removed from exhaust gases using various processes. As a consequence of greenhouse gases, shortwave radiation may enter the earth's atmosphere, heating the land and oceans. Long-wave radiation from the earth's surface is unable to reach the atmosphere due to these greenhouse gases. These activities contribute to the greenhouse effect. A large number of scientists have looked into greenhouse gases, their impacts, and potential remedies. The research and studies on greenhouse gases are the topic of this review. The greenhouse effect has produced considerable global warming in recent decades, notably in the past four to five decades. Warmer temperatures extend the growth season and make frigid areas more livable in general. When utilised in a high-intensity way, natural gas produces more greenhouse gases than fossil fuels. Carbon dioxide (CO2) and methane (CH4) are two climate-changing gases (CH4). The temperature of the Earth has been continuously increasing over the last five decades. Many experts believe that effective and acceptable pollution control techniques are urgently needed. Humans are exposed to a variety of harmful gases, each of which creates its own set of health problems. The research and studies on greenhouse gases are the topic of this review.

Many of us can eat without much thought, preparation, or time thanks to contemporary culinary technology. Fast food, ready-to-eat meals, and processed snacks give quick nutrition while causing the least amount of interruption to our daily routines. The immediate impact of a satisfied appetite is obvious, but the environmental impact of food and agriculture is less apparent. Recent publications, such as Michael Pollen's The Omnivore's Dilemma, have questioned whether a more ecologically friendly food system is feasible. Furthermore, the effects of global climate change raise worries about food's carbon footprint. This short will examine the most up-to-date scientific studies of food and climate systems in order to assist us in making intuitive and environmentally sound eating decisions. Greenhouse gas emissions are produced through food production, packaging, transportation, sale, and consumption.

Food-related processes, such as soil nitrous oxide generation, produce three major greenhouse gases: carbon dioxide, methane, and nitrous oxide. Depending on their persistence (how long they stay active) and impact, various GHGs in the atmosphere have varied global warming potentials. Because methane remains in the atmosphere for a shorter period of time while absorbing more heat, it has a greater cumulative impact than carbon dioxide. Nitrous oxide is the most potent global warming gas.

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Food's carbon footprint is a similar measure of the impact of global warming (also known as CO2e: carbon dioxide equivalent). The carbon footprint of a single unit of food refers to the total amount of greenhouse gas emissions produced by that unit of food. Food consumption accounts for around 15% of total GHG emissions in the United States. We may work to reduce GHG emissions in a variety of "hot spots" across the food chain.

In the United States, agriculture contributes for around 6% of total GDP, which includes all agricultural processes but excludes those beyond them. GHG emissions refer to the amount of greenhouse gases released into the atmosphere. Though we may make educated predictions, exact data on agricultural GHG emissions in the United States is difficult to come by owing to the country's diverse food supply and consumption. Agriculture is the largest source of GHG emissions, which are mostly caused by microbial activity in the soil and manure. When fossil fuels are used to power agricultural machines, carbon dioxide is created. When fertiliser emissions escape from the soil, they constitute a substantial source of nitrous oxide emissions into the atmosphere.

There are many procedures involved in getting food to markets. Food miles, or the average distance travelled by food from farm to fork, are a measurement of the food system's transportation infrastructure. According to the Leopold Center, produce supplied to the upper Midwest travels an average of 1,500 kilometres from industrial centres throughout the continental United States. Food transportation is responsible for around 11% of total GHG emissions from the food chain. Experts believe that changes in technology and infrastructure will contribute in the decrease of emissions related to transportation.

Food consumption is the second-largest source of greenhouse gas emissions in the household, behind transportation. The energy needed for refrigeration is one of the primary GHG consequences of food production. Certain commodities need continual refrigeration as soon as they leave the farm. Food waste wastes the energy needed to produce it, as well as the greenhouse gas emissions produced as a consequence. Meat waste in retail, industry, and households accounts for 20% of the meat sector's total GHG emissions (How to reduce home emissions is discussed further down.)

The proportional GHG emissions from these three food chain components vary depending on the kind of food. Livestock and meat production release more greenhouse emissions than plant-based agriculture. When cooked and stored, plant-based proteins like lentils and dry beans contribute significantly to total emissions.

Agriculture produces three major greenhouse gases: carbon dioxide (CO2), methane (CH4), and nitrous oxide (NOx) (N20). While nitrous oxide and methane are the most common greenhouse gases in the atmosphere, they have longer atmospheric lifetimes and absorb more long-wave radiation. As a consequence, even little volumes of methane and nitrous oxide might significantly contribute to global warming. Agriculture may have both good and negative consequences as a result of climate change. According to several studies, warmer temperatures lengthen growing seasons, and more carbon dioxide in the air leads to higher agricultural productivity. As a result of increasing temperatures and decreased soil moisture, production patterns may shift northward, demanding greater irrigation. Changes, on the other hand, will most likely vary significantly from one location to the next. Geographical factors will have a big impact on agriculture's potential to benefit from climate change. While some projections seem to be optimistic in certain areas, the risk of increased climatic unpredictability and extremes is not always included. Droughts, heat waves, severe thunderstorms, and tornadoes may become more common, outweighing the agricultural advantages. Farmers are finding it increasingly difficult to respond as climate change becomes more unpredictable.

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The two categories of greenhouse gases generated by biomass power plants that contribute to global warming are direct and indirect greenhouse gases. Direct greenhouse gases include carbon dioxide (CO2), nitrous oxide (N2O), and methane (CH4). Indirect greenhouse gases include NMVOCs, nitrogen oxides (NOx) (nitrogen monoxide (NO) and nitrogen dioxide (NO2)), sulphur dioxide (SO2), and carbon monoxide (CO). By absorbing and storing infrared radiation released by the earth's surface and lower atmosphere, and then reflecting it back to the planet's surface, direct greenhouse gases contribute to global warming. The environment is a system of systems that is a component of a higher system of systems, the human system, from the perspective of human life and development. Because it is clear that preserving and mending the environment in the interests of human life and growth is difficult, it is also impossible to damage the environment indiscriminately because it is the medium through which humans live. As a result, we must create compromises based on our knowledge and experience that respect both human and environmental concerns. Their effect and advantages are tracked so that remedial action may be taken if necessary.

According to current research, sustainability (development) involves not just the environment, but also the whole human system as well as the fundamental assets (i.e. public assets) on which people rely. Infrastructures and technologies, particularly those that are crucial, as well as the environment, property, and public welfare, are all important assets in the human system. Sustainability assessment is a method for recognising, anticipating, and assessing the influence of arbitrary inputs, such as fluctuations, on society's long-term growth in a broad sense (e.g. legal rules, ordinances, regulations, political intent, plan, program, and project). The aforementioned evaluation, which is based on current knowledge of the human system and its assets, may be conducted at any time to ensure effective territory management. The evolution of the environment, as well as the global political, social, and economic situation, demonstrates the need to address cases and actions that, because of their gravity, result in critical situations that lead to relevant crises of the type denoted as a humanitarian catastrophe or social crisis. As a result, a safety concept linked to development principles produced and accomplished through safety management is necessary from the viewpoints of human security, human system development, quality environmental conservation, state existence, stability, and development..

Basic (or normal) management, which is connected to emergency and crisis management, aims for security and long-term growth. As a result of land and regional growth, industrial zones are becoming closer to residential neighbourhoods. As a result, the potential of harm rises, and society (community) refuses to take any chances. Risk management, and by extension risk engineering, which involves risk assessment, risk reduction, and harm explanation, is critical in this situation. Finally, risk engineering is linked to technological systems (the human factor's impact on complex process safety is only addressed in advanced versions), and it may be utilised to revitalise landscapes by applying engineering principles in a wider sense.

As a result, risk management is essential. On the one hand, it aims to provide the best possible protection for people, property, and the environment, while on the other, it aims to restore damaged landscapes utilising engineering techniques and results. One of the most important concerns confronting the globe today is environmental deterioration. Some settings may never be retrieved, depending on the extent of the loss. Flora and wildlife that formerly thrived in these areas are now extinct. City planners, industry, and resource management must address the long-term environmental implications of expansion to minimise future repercussions. Future environmental damage might be avoided with proper planning. Despite the fact that human activities are often connected to environmental deterioration, ecosystems adapt throughout time. Some ecosystems deteriorate over time, whether or not they are touched by hu-

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man activity, to the point that they can no longer sustain the life that is "meant" to live there. Landslides, earthquakes, tsunamis, and wildfires all have the potential to devastate local plant and animal ecosystems, rendering them untenable. This might happen as a result of a natural catastrophe or long-term resource deterioration caused by the introduction of an invasive alien species into a new ecosystem.

When the environment is unable to deal with the new species, degradation occurs. These contaminants may build up in sediments and biomagnify in the tissues of top-of-the-food-chain animals, resulting in concentrations many times greater than those seen in water or air. Ozone is a gas that may be found on the ground as well as in the stratosphere, the Earth's upper atmosphere. Ozone is a contaminant that may impair people's health at ground level. On the other side, ozone creates a stratospheric layer that shields life on Earth from the sun's destructive ultraviolet (UV) rays. Ozone-depleting substances like chlorofluorocarbons, hydro chlorofluorocarbons, and halons are man-made chemicals that slowly deplete the "healthy" ozone layer. These chemicals have been used as coolants, foaming agents, fire extinguishers, solvents, insecticides, and aerosol propellants in the past, and they are still used in certain situations today. More UV radiation penetrates the Earth as the protective ozone layer thins, possibly causing skin cancer, cataracts, and immune system damage. UV radiation has the potential to harm delicate crops, such as soybeans, resulting in lower yields.

CONCLUSION

Environment-related ramifications Toxic contaminants in the air, as well as those deposited on soils and surface waters, may harm animals. Animals, like people, may develop health issues if they are exposed to excessive levels of air toxics over time. Air toxics have been linked to birth abnormalities, reproductive failure, and illness in animals, according to a study. In aquatic ecosystems, persistent hazardous air pollutants (those that take a long time to degrade in the environment) are of special concern. Air pollution has a range of effects on crops and plants. Ground-level ozone may diminish agricultural crop and commercial forest yields, smother tree seedling development and survival, and make plants more susceptible to disease, pests, and other environmental challenges (such as harsh weather). Acid rain and increased UV radiation due by ozone depletion may have an influence on agriculture and forestry, as previously stated. Climate change is happening on a global scale. Because of a complex mix of naturally existing gases in the Earth's atmosphere, some of the sun's heat is preserved near the surface. The "greenhouse effect" maintains a steady temperature on the earth. Unfortunately, evidence is accumulating that people have disrupted nature's equilibrium by emitting vast volumes of greenhouse gases such as carbon dioxide and methane. As a consequence, the Earth's atmosphere seems to be retaining more of the sun's heat, leading to global warming. According to many experts, climate change will have significant consequences for human health, agriculture, water resources, forests, animals, and coastal regions.

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Chapter 6
Biofuels From Bio-Waste and Biomass

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ABSTRACT

The planet's limited natural fossil fuel reserves are anticipated to be very soon owing to massive usage. Biofuels would be a critical alternative source that may reduce global warming and CO2 emissions. The food-versus-fuel dilemma is, however, one of the key drawbacks of first-generation biofuels like corn ethanol, sugarcane ethanol, etc. Cellulose and hemicellulose, the primary constituents of lignocellulosic feedstocks, could be reduced to sugars by either thermochemical/biological processes before being fermented to generate biofuels. However, owing to structural heterogeneity, more complicated operational techniques are required before the production technology can be commercialized, and several challenges must be addressed. This chapter provided an assessment of various feedstocks, availability, various processing techniques, obstacles, and current technical developments in the generation of biofuels from biomass.

DOI: 10.4018/978-1-6684-5269-1.ch006

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INTRODUCTION

The earth's finite natural fossil fuel reserves are being exhausted at a quicker pace as the world's energy demands rise as a result of advances in industrialization and modernization (Mohapatra, Ray, & Ramachandran, 2019; Ruan et al., 2019; Saini, Saini, & Tewari, 2015). Petroleum, coal, natural gas, bitumen, oil shales, and tar are examples of fossil fuels that have aided in the wealth and success of many nations throughout the world, as well as meeting energy demands for power, transportation, and heating (Dahman, Dignan, Fiayaz, & Chaudhry, 2019). Fossil fuels account for almost 80% of all primary energy use. With the transportation sector accounting for only 58% (Raud, Kikas, Sippula, & Shurpali, 2019). From a global point, the energy and transport industry is associated with heavy fossil fuel consumption (Dahman, Dignan, et al., 2019). As the world's population grows and industry and technology advance, this need grows year after year.

According to the 2011 consumption rate, the oil will last 54 years, natural gas 64 years, and coal 112 years (Khattab & Watanabe, 2019). Indeed, the varied technical breakthroughs driven by the use of heavy fossil fuels have improved human existence and made it more comfortable; yet, this comfort has arrived at a significant expense. Global warming and greenhouse gas (GHG) emissions have come from the extensive consumption of fossil fuels. Ozone (O_3), Carbon Dioxide (CO_2), methane (CH_4) and Nitrous oxide (NO_3) are the main greenhouse gases that emit and absorb infrared radiation owing to their unique characteristics and chemical makeup. The elevated level of these gases in the atmosphere had several negative consequences for the planet. Roughly 98% of the greenhouse gases released into the atmosphere are a direct consequence of the production of fossil fuels (Dahman, Syed, Begum, Roy, & Mohtasebi, 2019; Khattab & Watanabe, 2019). The *Mauna Loa* research center revealed CO_2 levels of 413 ppm in March 2019, up from 280 ppm in preindustrial Period. The proportion of atmospheric CO_2 buildup has escalated to 2.28 ppm per year between 2008 and 2017, up from 0.8 ppm per year between 1960 and 1969. The CO_2 content in the atmosphere has increased by 43% since 1750, and as a consequence of this rise, the average surface temperature of the earth has increased by 0.85°C since pre-industrial times (Raud et al., 2019). Each of these elements has shifted the phenomena of global warming from a local myth to a grave concern worldwide that concerns our own humankind's survival on our planet. Because of the continued and increasing release of greenhouse gases, the average temperature of the Earth has been steadily rising. Changes in climate, weather system disruption, glacier retreat, rising sea level, coastal flooding, associated biodiversity loss, and human health effects are the main consequences of fossil fuel consumption (Dahman, Dignan, et al., 2019; Dahman, Syed, et al., 2019). All these collectively coupled with the climb in the energy consumption and demands, depletion of fossil fuels, and severe environmental concerns have sparked to watch out for an alternative, sustainable, renewable, and green energy source that could not only meet the global demand and also helps to lessen the global greenhouse emissions (Agbor, Cicek, Sparling, Berlin, & Levin, 2011; Raud et al., 2019). In this chapter, the focus will be on research into the types, compositions, and application of various forms of biomass accessible for biofuel generation and also the numerous pretreatment and processing options that may be used to various bio-wastes to transform them into useable biofuels cost-effectively and sustainably to fulfill rising energy demand.

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NECESSITY OF BIOFUELS

Changes in climate and security of energy supply should be at the forefront of the States international agenda, impacting societal, economical, and political choices. Every country's primary goal should be to find a renewable, cost-effective, biodegradable, and environmentally benign alternative energy source. Biofuels, without a doubt, would be a significant alternative source in which biomass-based biofuels might ameliorate global warming and reduce CO_2 emissions owing to the preceding consumption of this gas by the biomass during photosynthesis, resulting in carbon-neutral emission (Coyle, 2007; Sindhu et al., 2019). The term 'biofuel' refers to any liquid, gas, or solid fuel predominantly produced from a renewable biomass feedstock. Bioethanol, biomethanol, biosynthetic gas (biosyngas), biodiesel, biogas (biomethane), biochar, bio-oil, biohydrogen, and FischerTropsch produced liquids are the common biofuels (Ali, Saleem, Khan, & Watson, 2019; Ayhan Demirbas, 2008). Mankind has been consuming biofuels for an extended time, ever since humans began burning firewood for various home and domestic needs. Biofuels can play an significant role in the future by addressing the enormous fuel market and ensuring global energy security (Kumar et al., 2020). Biofuels offer several benefits over fossil fuels, including renewable, sustainable, abundant availability, biodegradability, CO_2/GHG (greenhouse gas) neutrality, minimal or zero SO_x emissions, reduced NO_x emissions, and the ability to be generated locally and in a safer manner. Biofuels also have several advantages, including the consumption of local resources, the stimulation of rural agricultural development and investment, the reduction of energy dependence on imports, the creation of jobs, the support of rural economic development, and the improvement of local livelihoods (Ruan et al., 2019). Biofuels are regarded as the most essential sustainable fuel resource due to the benefits outlined above. They also contribute to the lessening of greenhouse emissions and, as a result, to the improvement of environmental health. As a result, many governments have set various goals and regulations to encourage and expand the biofuel business and so use energy resources. It is estimated that diverse biofuels will account for 30% of world energy demand (Guo, Song, & Buhain, 2015).

BIOFUEL FACTS, FIGURES, AND POLICIES

The Center for Strategic and International Studies in its report 'International Energy Outlook 2011' stated that renewables are the fastest-growing energy source. It is rising at a rate of 2.8% per year and would arrive at roughly 15% of the world energy market by 2035. It also stated that by 2035, liquid biofuels will include 29% of world energy consumption. This translates to about 5 million barrels per day of the total production (Dahman, Syed, et al., 2019). Governments of different nations have made biofuel policies in current years for the domestic production and usage of biofuels. Brazil has been extremely doing well in biofuel policy that mandates the minimum level of blending of ethanol with petrol. As of today, it is projected that the petrol sold in Brazil contains almost 20 -25% of ethanol in terms of volume (Lamers, Hamelinck, Junginger, & Faaij, 2011). In the USA, the US Energy Independence and Security Act of 2007 have laid down a aim of production of 15 billion gallons of corn ethanol by 2015, and by this year, 21 billion gallons of cellulosic and advanced biofuels (Iens & Argione, 2011). Similarly to these countries, all other big nations, including China, Canada, India, Japan, and South Africa, have legislated biofuel policies that mandated the targets of biofuel blending requirements in fossil fuels, as well as other policies and schemes that encourage the arrangement and commercial biofuel production (Dahman, Syed, et al., 2019; Timilsina, Mevel, & Shrestha, 2011).

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INDIA AMONG THE OTHERS

India, a country with over a billion people and limited space, has one of the world's fastest-expanding economies. Energy is critical to the country's socio-economic growth. India is primarily reliant on fossil fuels to fulfill its energy requirements. India has relatively limited fossil fuel supplies and must import oil, coal, and gas, with oil import dependency being particularly severe. To fulfill the rising energy demand, the Indian government is urgently seeking an alternative to fossil fuels. The 2009 'National Policy on Biofuels' envisions biofuels as a vital tool of stimulating rural development and creating local job possibilities. In actuality, India's biofuel business is still in its infancy. India's biofuel production amounts to less than 1% of world output. Within the different renewable energy sources accessible, India has a plethora of bioenergy choices that have been employed to fulfill cooking needs for a long time. Various bioenergy policies and initiatives have been implemented in India in recent years to boost bioenergy production. However, due to policy limitations, the government is unable to fully use the enormous potential of bioenergy solutions. Bioenergy policy implementation is fraught with difficulties in terms of technological, institutional, financial, environmental, and social factors. However, the objective for mixing gasoline with ethanol was around 5%.Currently, bio-ethanol from sugar cane molasses and biodiesel from non-edible oilseed crops such as Jatropha (*Jatropha curcas*) and Pongamia (*Millettia pinnata*) are being encouraged for commercial usage in India (Gopinathan & Sudhakaran, 2009; Kothari et al., 2020; Roy & Chandra, 2019).

Agriculture is the basis of India's GDP, bringing subsistence to 60% of the population, and generating a considerable amount of agro waste as a result of agricultural strength. It is also the second-largest producer of sugarcane following Brazil (Ravindranath et al., 2005). India generates 686 million tons of gross agricultural residue biomass per year, with 234 million tons (34%) accessible as an excess for biofuel energy production. Biomasses from agriculture are used as feedstock in agro-industries (Naik, Goud, Rout, & Dalai, 2010). Residues and by-products from agriculture include rice husks, cottonseed, cereal straw, corn cobs, cotton stalks, various grasses, maize, sorghum stover, sunflower seed hulls, peanut shells, , and wood sawdust and chips, to name a few (Lal, 2008).

CLASSIFICATION OF BIOFUELS

Biofuels can be classified in different ways which are based on different aspects like production process, commercialization stages, physical state, and biomass. This chapter will focus on research into the types, compositions, and applications of various forms of biomass accessible for biofuel generation. The emphasis was on the numerous pretreatment and processing options that may be used to various bio-wastes to transform them into useable biofuels cost-effectively and sustainably to fulfill rising energy demand.

Biofuels are fuels that are produced by biological processes from various sources like plants, crops, agriculture residues. Based on the production route, biofuels can be categorized as primary biofuels and secondary biofuels. Primary biofuels are made up of a variety of materials such as wood chips, pellets, fuel wood, and organic materials that are burnt straight to provide energy for food preparation, heating, and power generation. Secondary biofuels are made from a wide range of biomass and may be utilized for transportation, energy generation, and a number of industrial purposes. Ethanol, diesel, and biogas are examples of secondary biofuels (Dahman, Syed, et al., 2019; Datta, Hossain, & Roy, 2019; Ruan et al., 2019).

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Biofuels are categorized into conventional and advanced biofuels based on the level of commercialization of technology. Bioethanol made from sugar and starch, biodiesel made from transesterification, and anaerobic digesting methane is all examples of conventional biofuels. Sugarcane and sugar beet, corn, wheat, vegetable oil, animal fat, recovered household fat, waste frying oil, etc., are the most used feedstocks for conventional biofuels. All of these things are sold in different countries throughout the world. Hydrotreated vegetable oil, cellulosic (lignocellulosic) bioethanol, biomass-to-liquid (BtL) biodiesel, microalgae biodiesel, and biohydrogen are examples of advanced biofuels. All these are not yet commercialized. They are in different phases (research and development, demonstration, or early commercial stages) to be commercialized (Awogbemi, Von Kallon, Onuh, & Aigbodion, 2021; Ruan et al., 2019).

Based on the physical state the biofuels can be segregated into three types - solid, liquid, and gaseous biofuels. Different biofuels, their benefits, and drawbacks have been discussed in the following section.

Solid Biofuels

Solid biofuels are raw solid feedstock used to produce biofuels such as wood, wood chips, wood pellets, and charcoal. Lignocellulosic biomass and numerous forms of solid waste are examples of solid biomass. They can be utilized directly as biofuel or transformed into other types of biofuel over various methods. Wood was utilized for numerous domestic uses such as cooking and heating in the early nineteenth century, and it is still the major fuel today. Wood chips are little fragments of wood that have been chipped from tree trunks or branches and used as fuel for heating and energy generating. Similarly, wood pellets are created by grinding wood pieces with sawdust and compressing them via a pelletizer, and they may be utilized as a fuel in household heating and energy generation (Goyal, Seal, & Saxena, 2008; Guo et al., 2015; Sindhu et al., 2019). In the lack of air, biochar, as well identified as charcoal, is a porous, carbon-enriched greyish black solid generated via torrefaction, gasification, hydrothermal carbonization, and other processes that may reach temperatures over 400°C (usually between 300°C and 900°C). It offers an advantage over other raw feedstocks like firewood, wood chips, and so on in terms of lower combustion emissions and higher energy content (Glaser, Haumaier, Guggenberger, & Zech, 2001; Kwapinski et al., 2010). Biochar can be utilized as a solid fuel in boilers, as a feedstock for subsequent gasification processes, to produce activated carbon, and so on (Kambo & Dutta, 2015).

Liquid Biofuels

Bioethanol, biodiesel, Bio-methanol, Biobutanol, and Biooil are examples of liquid biofuels. They're becoming increasingly popular because to the fact that they're highly flammable, have a high energy-to-mass ratio, and are simple to carry.

Bioethanol

The most often employed liquid biofuel in our day is ethyl alcohol, which has the chemical formula CH_3 - CH_2 - OH and is manufactured by the microbial fermentation of sugars or starch using a range of feedstocks. The United States (from maize) and Brazil (from sugar cane) are the world's leading producers of bioethanol, accounting for almost 90% of worldwide output (Cheng & Timilsina, 2011; Vohra, Manwar, Manmode, Padgilwar, & Patil, 2014). It possesses 35% oxygen and therefore can minimize automotive emissions and may be utilized in the same way as conventional fuels. However, they have

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a poor volume energy density and need more bioethanol per kilometer than gasoline. As a result, it is mixed with gasoline in various ratios ranging from 5% to 25% ethanol and 95% to 75% gasoline and may be used in automobiles without any modifications (Naik et al., 2010; Vohra et al., 2014).

Biobutanol

Biobutanol, C4 alcohol with molecular formula C_4H_9OH, a biofuel made from the ABE (Acetone Butanol and Ethanol) fermentation of the traditional feedstock. It was produced via Clostridium acetobutylicum fermentation of corn and molasses, with the end products being acetone, butanol, and ethanol, and therefore the procedure was designated as ABE fermentation. Byproducts of ABE fermentation include acetic, lactic, propionic, butyric acids, hydrogen, isopropanol, CO_2, and lipids. Biobutanol has greater energy density, is less corrosive than ethanol, safer to use, and has no need for engine modifications as it uses a fuel to air ratio closer to gasoline. Nevertheless, butanol becomes lethal once its concentration reaches 13g/L in the fermentation medium. As a result, much research is being done to genetically enhance butanol-producing cultures that can withstand elevated acetone butanol concentrations of 20.0 to 30.0 g/L while simultaneously recovering the products (Dürre, 2007; Kolesinska et al., 2019; Xue & Cheng, 2019).

Biodiesel

Biodiesel is a colorless to yellowish fuel that resembles conventional diesel. It is a combination of long-chain fatty acid methyl esters (FAME) synthesized in the presence of alcohol and alkaline catalysts from animal fats, edible and non-edible vegetable oil, waste or recycled oil, waste grease and also from algal. Unlike petroleum diesel, which only includes hydrocarbons, biodiesel contains carbon, hydrogen, and oxygen. It has a high flash point, making it harmless to store, handle, and transport, as well as a higher cetane number, indicating that it has greater ignition quality, a low sulfur content, and is biodegradable (Atabani et al., 2012; Hoekman, Broch, Robbins, Ceniceros, & Natarajan, 2012; Ma & Hanna, 1999; V. K. Mishra & Goswami, 2018). It does, however, have a low calorific value, a high pour point (meaning it solidifies at lower temperatures), and high viscosity. As a result, it may be mixed with normal crude oil or petroleum diesel and utilized. The most common biodiesel blends are B2 (2% biodiesel and 98% normal diesel), B5 (5% biodiesel and 95% regular gasoline), and B20 (20% biodiesel and 80% regular diesel) (Morone & Cottoni, 2016).

Biooil

Biooil is a dark, free-flowing organic liquid having a smoky odor which is a mixture of aldehydes, acids, alkenes, aromatics, alcohols, esters, ketones, phenols, oligomers, furans, sugars, guaiacols, syringols, and nitrogen compounds produced by the depolymerization or fragmentation of biomass by fast/slow pyrolysis and thermal liquefaction. Pyrolysis is a practice to heat the plant biomass at 300–900°C in the absence of air. They are also referred to as pyrolysis liquid, pyrolysis oil, wood oil, bio-crude oil, wood distillate, wood liquid, etc.,(Czernik & Bridgwater, 2004). The major disadvantages of bio-oil are high water content, which is a result of the presence of water in the feedstock and also the production of water in the dehydration reactions in the pyrolysis process, high viscosity, less oxygen, low energy density, immiscible with hydrocarbon fuels and corrosiveness which made the bio-oil cannot be used as a

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transportation fuel. The major challenge preventing the commercialization of bio-oil is the high production cost. Research and development efforts have to be made to reduce the water content, high acidity and further improve the quality of the fuel by pretreatment of raw feedstocks, optimization of operation conditions, adopting strategies such as hydrotreating, hydrocracking, supercritical fluids, solvent addition/esterification and steam reforming (D. Vamvuka, 2011; Guo et al., 2015; Xiu & Shahbazi, 2012).

Gas Biofuels

The third type of biofuels, the gaseous biofuels includes biogas, biohydrogen, biosyn gas, etc., generally have advantages like more reactive, the requirement of fewer oxidants, simple reactors, and low wastage in the yields.

Bio Hydrogen

It is a colorless, odorless, and lightest combustible gas produced from renewable sources that include crop residues, energy crops, agriculture residues, livestock residues, algal biomass, industrial wastes, etc., by either thermochemical conversion or biological conversion. Thermochemical transformation comprises water and biomass thermochemical decomposition, syngas water-gas shift reaction, natural gas (or methane) steam reforming, pyrolysis bio-oil steam reforming, and so on(H. Balat & Kirtay, 2010; M. Balat & Balat, 2009). Biophotolysis is the initial phase in biological conversion technology, which involves using photosynthetic bacteria to convert CO_2 and water into H_2 or photo heterophilic microbes to convert carbohydrates or organic acids into H_2 and CO_2.Following that, photofermentative bacteria photo-decompose organic compounds, followed by dark fermentation, which generates H_2 utilising anaerobic or facultative anaerobic bacteria, and ultimately, microbial electrolysis transforms electron equivalents in organic compounds to H_2 by uniting microbial metabolism with bioelectrochemical reactions in a microbial electrolysis cell. There are practical encounters that have to be addressed like light dependency in the biophotolysis and photofermentation steps, the buildup of acidogenic by-products that may constrain the dark fermentation step, and cell lysis in the electrolysis step (A. Demirbas, 2008a; Meramo-Hurtado, Puello, & Cabarcas, 2020; Vendruscolo, 2014).

Biogas

Biogas also termed biomethane is a gas biofuel that is made by anaerobic digestion of organic wastes like plant residues into smaller molecules in natural environments by the consortium of microorganisms which usually contain 60–65% of methane (CH_4), 30–35% of CO_2, and a small percentage of water vapor, H_2 andH_2S (Ge, Xu, & Li, 2016; Martínez-Gutiérrez, 2018). The purification step is necessary to remove the moisture, CO_2, H_2S, and other impurities before supplying as a natural gas substitute. Because of the presence of higher CO_2 content, it produces lower energy than natural gas. Anaerobic digestion has been employed to manage waste and generate energy by various livestock farms, wastewater treatment plants, and private residences. It is also applied to generate energy and bio-based compressed natural gas (CNG) that is applied to power natural gas cars (Guo et al., 2015; Wei, 2016).

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Syngas

Syngas is a gaseous biofuel which is the result of gasification or pyrolysis of carbon-rich materials such as natural gas, petroleum, coal, and dry plant biomass and it contains CO_2,CO, N_2, H_2, and hydrocarbons (e.g., CH_4, C_2H_4, and C_2H_6) and very minor quantities of moisture, H_2S,NH_3, and tars(Yan, Luo, Hu, Xiao, & Cheng, 2010; Y. Zhang, Zhao, Gao, Li, & Huang, 2015). The main plus of the syngas is versatility as they can be used as starting material to synthesize transportation fuels like methanol, methane, ethanol, dimethylether, and extra products via the gas-liquid platform. Because of stringent feedstock requirements, biomass-based biogas gasification is currently in the early demonstration phases and additional research is needed to know the economics of biomass and attain the financial feasibility for commercial-scale production (Ruiz, Juárez, Morales, Muñoz, & Mendívil, 2013).

BASED ON FEEDSTOCK SOURCES

Based on the different types of feedstocks the biofuels can be divided into first and second-generation biofuels.

First-generation biofuels

First-generation biofuels, often stated to as conventional biofuels, are derived mostly from food crops for instance cereals, sugar cane, tuber crops, sugar beets, and oil crops. The first generation of biofuels covers biodiesel, vegetable oils, biogas, bio alcohols, biomethanol, and Syngas (Ruan et al., 2019).There are over 350 oil-containing crops classified for biodiesel production. Many countries generate first-generation biofuel from a variety of sources (Hirani, Javed, Asif, Basu, & Kumar, 2018). Brazil produces bioethanol mostly from sugarcane, whereas the United States, Canada, and Europe generate bioethanol primarily from maize, wheat, sugar beet, and sorghum, respectively. Maize accounts for approximately 67% of worldwide bioethanol output (Mohapatra et al., 2019).From 2001 to 2017, a large increase in the global production of bioethanol was observed. In 2001, the production was 4874 million gallons which reached up to 27,737 million gallons in 2017 (Mohapatra et al., 2019). Globally, 40-billion-liter bioethanol is produced by the US and 2 billion liters by Canada from corn or wheat. In the case of Brazil, they produce 25 billion liter bioethanol from sugarcane whereas China produces 3 billion liters from corn, cassava. From wheat, sugarcane, and sugar beet, France and Germany produce 1 billion liters and 750 million liter bioethanol respectively. India supplies 1 billion liters of bioethanol from sugarcane and molasses and Australia produces 500 million liters from sugarcane (Mohapatra et al., 2019).

The production of biodiesel in the United States, France, Germany, and Italy is mostly dependent on rapeseed oil and soyabean oil. Brazil mostly employs soybean oil in the manufacturing of biodiesel (Hoekman et al., 2012). It was reported that annual biodiesel production is increasing exponentially. Approximately 85% of biodiesel is produced by European countries (Atabani et al., 2012; V. K. Mishra & Goswami, 2018). The production of biodiesel is increasing by the year. They are produced from oil of soybean, sunflower, canola, or palm oil. Due to their environmental benefits, they are widely used (https://www.mdpi.com/1996-1073/11/12/3366/html). The production was 0.8 billion L in 2000, which reached 4 billion L in 2005. A four-fold increase in production was seen in 2010. By 2012, the production reached over 22.5 billion liters. European countries are the largest producer of biodiesel consisting

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of 80% of global production, followed by the USA (Hirani et al., 2018). In 2001, the Republic of China started the production of bioethanol using corn. By 2007, they started using grain-based feedstock producing about 1.4 million metric tons of bioethanol, which got replaced by nonfood crops like cassava and sorghum (Mussatto et al., 2010). Thailand started bioethanol production using cassava and cane molasses (Silalertruksa & Gheewala, 2009). The pros and cons of the first-generation biofuels were given in Table 1.

Table 1. Pros and Cons of First-generation biofuels (Datta et al., 2019; Ruan et al., 2019)

Pros	Cons
Sustainable **Renewable form of energy** **Feedstock production is simple using current resources and infrastructure** **Environmental and social advantages** **A huge number of feedstocks are accessible**	**Requires a huge amount of fertilizer, water, and availability of land, lowering the overall energy ratio.** **Added to increased food prices due to equal demand with food.** **Could potentially have negative influence on biodiversity** **Increased carbon footprint compared to other generations of biofuel** **Merging with conventional energy sources** **Compete for the availability of lands**

Sugar containing feedstocks

Sugar containing feedstocks for the generation of biofuels are sugarcane, sugar beet, and sweet sorghum with yields of 62–74 tons/ha, 54–111 tons/ha, and 50–62 tons/ha, respectively which are grown mainly in Brazil, Germany, France, and India. In comparison to maize and wheat, sugarcane remains the biggest provider to bioethanol production and the minimum water user in terms of crop biomass output (Mohapatra et al., 2019).

Sugarcane

Sugarcane (*Saccharum officinarum* L.) belongs to the grass family *Poaceae* (Khattab & Watanabe, 2019). Sugarcane is sugar-producing crop, signifying a substantial quantity of cultivated crops in the world which is grown typically in tropical and subtropical areas (Hiloidhari et al., 2019). It is one of the most favorable sources for bioethanol production and is the second most used raw material for the same. It is widely grown in Brazil which accounts for 27% of global production (Bertrand, Vandenberghe, Soccol, Sigoillot, & Faulds, 2016; Mączyńska et al., 2019). The yearly worldwide production of sugarcane is about 1741.3 million tons where Asian countries put in 44% of it and India is the top country in production (Hiloidhari et al., 2020). Sugarcane was preferred for ethanol production owing to its high sucrose juice composition (Bertrand et al., 2016). Bioethanol from sugarcane represents almost 1/3 of total ethanol production in 2010 (Rasool & Hemalatha, 2016). Each ton of sugarcane could yield 740 kg of juice and 260 kg of wet bagasse. Also, when compared to corn ethanol, sugarcane ethanol produces 45% more ethanol per unit of land (Crago, Khanna, Barton, Giuliani, & Amaral, 2010). It is estimated that one ton of sugarcane at the processing center contains 135 kg of sucrose which can produce almost 70L of ethanol (Naqvi & Yan, 2015). Sugarcane is primarily constituted with 12-17 % sugars by wet weight, with 68-72 % moisture (90% sucrose and 10% glucose or fructose) (Ayodele, Alsaffar, & Mustapa,

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2020). Sugarcane produces directly monomeric sugars which can be directly used for fermentation. Cane juice which can be extracted from the sugarcanes is roughly 95% and the leftover solid residue is called bagasse which is also a potential biomass feedstock and used for steam and electricity generation by burning in boilers (Rasool & Hemalatha, 2016). The sugarcane bagasse and its potential as a feedstock will be studied in the forthcoming sections.

Ethanol production from sugar cane starts with cane storage and cane crushing for juice extraction. In the sugar mills, first, the sugarcane stalks are washed, chopped, and shredded with the help of blades or knives (Naqvi & Yan, 2015). The bioethanol process from sugar cane initiates with the lignin or hemicellulosic fibers pretreatment to release cellulose. Cellulose fibers are depolarized to yield free sugars. The juice is warmed to 115°C and treated with lime and sulfuric acid, resulting in the precipitation of unwanted inorganics. The sugars released are used up to make ethanol from which the ethanol is distilled (Canilha et al., 2012).In annexed plants, crystalline sugar is co-produced along with ethanol. In these plants, sucrose crystals are formed after the juice is concentrated which is removed by centrifugation and this leaves syrup called molasses. Molasses contains 31% sucrose and 15% inverted sugar (Jayus, Nurhayati, Mayzuhroh, Arindhani, & Caroenchai, 2016). Sugar molasses of 25% as a substrate could yield about 32.32 g/L bioethanol by batch fermentation using *Pichia veronae* strain HSC-22 (Hamouda, Nassar, Madian, Abu Amr, & El-Gendy, 2015). Both sugarcane and molasses have to be diluted to adjust the sugar concentration before fermentation which enables the optimal growth conditions for microorganisms with larger yields. This also contains sufficient minerals and organic nutrients making it appropriate for ethanol production by the *S. cerevisiae* (Bertrand et al., 2016). *S. cerevisiae* strain AS2.1190 immobilized on sugar cane pieces was observed in electron microscopy for its yield (77.13 g/l) of bioethanol by 10 batch fermentations (Liang et al., 2008). A typical fermentation process requires 4–12 h per batch. After the run, separation of yeast and distillation is performed to separate ethanol (Saran, Malaviya, & Chaubey, 2019). In a study *S. cerevisiae,* KP096551 was used for enhancing the production of bioethanol using sugar cane molasses as a substrate. The yield of bioethanol was raised to 8.55% (Rasmey, Hassan, Aboseidah, & Abdulwahid, 2018).

Sugar beet

Sugar beets (*Beta vulgaris* L.), the tuber crops are one of the chieffavorable sources of bioethanol production in the EU primarily in Northwest EU. In the EU, 30% of the bioethanol is produced from Sugar beets in 2011 (Bertrand et al., 2016; Marzo, Díaz, Caro, & Blandino, 2019). It is regarded as the most efficient source for ethanol as the yield of ethanol through fermentation ranges from 100 and 120 L per hectare (Panella, 2010).Countries in Asia like China, Japan, India, and Turkey are keen to use Sugar beet for biofuel production. Based on the data from SNSF in France, it was estimated that the average production of sugar beet is 70 tons/hectare, with the sugar content of 17% on the wet basis which gives atypical production of 12 tons of sugar/hectare. Sugar beetroots are made up of about 75% water, 18% sugar (mainly sucrose), and 7% insoluble and soluble materials (Bertrand et al., 2016). It has been observed that water-soluble sugars extracted from 1 kg sugar beet may generate up to 0.07 kg ethanol (Šantek, Gwehenberger, Šantek, Narodoslawsky, & Horvat, 2010). Sucrose is the chief sugar present and maltose; fructose and glucose are in lesser quantities. Conventional sugar beetroots are used to generate sugar and for biofuel production, specialized non-grade varieties bred are used for total sugar production. The Beet tops have feed value but are usually left in the field at harvest and they constitute 4.6 -7.5 tons per hectare (Panella, 2010).

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Ethanol production from sugar beets begins with washing the roots, followed by cutting them into thin strips for better extraction of sugar in a root cutter. Washing is usually done using pre-washing drums, jet washers (Šantek et al., 2010).Then the cut pieces are transported to a diffusion step where sugars are extracted by hot water by a process called counter-current extraction. This is called diffusion juice, the remains are called Pulp which is further processed by pressing and dewatering which accounts for almost 22-28% of dry mass (Vučurović & Razmovski, 2012). The dehydrated pulp contains 80 to 94% fermentable components (pentosans, pectin's, and cellulose) and only 12 to 16% lignin, crude protein, and mineral substances (Marzo et al., 2019). This also can be utilized as biofuel feedstock like sugarcane bagasse with the developing technologies in biofuel production (Khattab & Watanabe, 2019).The extracted juice also called green juice is used for fermentation by *S. cerevisiae* to produce the ethanol which is recovered by a distillation process (Dodić et al., 2009).The molasses obtained after the sugar extraction process are also approximately 53% of sugar and can be used for bioethanol production (Vučurović & Razmovski, 2012). However, the major drawbacks of ethanol from sugar beetroots are storage and production costs (Panella, 2010).Utilization of molasses and pulp also for biofuel production, developing new breeds, and performing anaerobic fermentation to yield biomethane have to be evaluated to decrease the production costs. Different countries have integrated sugar beet in the bioethanol policies for its high sugar content and it does not vie with food (Rulli, Bellomi, Cazzoli, De Carolis, & D'Odorico, 2016). A flocculating yeast strain, *Saccharomyces cerevisiae* IR2 was employed for bioethanol production from sugar beet juice. The study concluded that Raw sugar beet juice was shown to be an ideal substrate for ethanol synthesis, requiring neither any pH adjustments nor nitrogen supply (Ogbonna, Mashima, & Tanaka, 2001).

Sweet sorghum

Sweet sorghum (*Sorghum bicolor* L.) Moench, a Graminaceous crop can be used as a promising feedstock for biofuel production because it has a shorter growing cycle. Sorghum is grown on roughly 35 million hectares throughout the world, with a global average grain production of 1.5 t DM/yr (van der Weijde et al., 2013). Sorghum has a superior energy balance than other competitive crops due to its low input needs, flexibility, and extraordinary biological output (Jianliang Yu, XuZhang, & Tan, 2008). Further benefits over other crops comprise a shorter growing period, tolerance towards drought, and can be grown in colder regions and temperate zones unlike sugarcane (tropical and subtropical areas) and sugar beet (temperate climate) (Wortmann et al., 2010). All the plant parts - grains, stalk juice, and plant biomass can be used for biofuel production. The average productivity is 49.7 tons per hectare which produces roughly 63 % of juice. The juices of Sweet sorghum stalks have sugars (6% fructose , 85% sucrose and 9% glucose), invertase enzyme, and other compounds and impurities (Bertrand et al., 2016). The occurrence of the reducing sugars and invertase enzyme makes the juice unsuitable to sugar production rather it makes it suitable to produce ethanol (Ratnavathi, Chakravarthy, Komala, Chavan, & Patil, 2011).

Ethanol production begins with crushing the stalks for juice which leaves out the bagasse that is usually rich in cellulose, hemicellulose, and lignin (Quintero, Montoya, Sánchez, Giraldo, & Cardona, 2008). Fresh juice is sterilized by heating up to 100^{o}C and then purified by adding lime milk and precipitation by CO_2 (Bertrand et al., 2016). The settled solids are filtered from the juice and purified juice is acquired which is now called thin juice. This can be sent for fermentation after regulating the sugar concentration to 16-18 % which usually contains glucose, fructose, and sucrose (Ratnavathi CV, 2017). *S. cerevisiae* then converts the juice or syrup into ethanol. Sugar is converted to ethanol and CO_2, with minor end products such as glycerol, fuel oils, aldehydes, and ketones being produced in lower amounts.

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After that, it is distilled and concentrated by dehydration to produce ethanol (Ratnavathi et al., 2011). Distillation purifies the ethanol generated in the fermented mash, yielding 95 percent pure ethanol, which is then dehydrated to obtain 99% purity (Ramesh C. Ray & Chandrasekaran Trilokesh, 2019). Enzymatic hydrolysis of the sorghum grains has been reported, as *S. cerevisiae* cannot break down starch which is the primary component in sorghum grains, a combination of enzymes like α-amylase and amyloglucosidase are utilized (Ramesh C. Ray & Chandrasekaran Trilokesh, 2019). The use of sorghum stalks for producing bioethanol was carried out using *S. cerevisiae*. The stalks treated with dilute acids and a total of 20.5% of Bioethanol were revived by this method (Chauhan, Hajare, Mamo, & Madebo, 2021). A comparative study using six different yeast strains, Vin7, SBg, N96, and GSL for production of bioethanol from sweet stem sorghum juice, was carried out in a YEPS medium that consisted of yeast extract, phosphate, and sucrose. Among all the strains Vin7 and SB9 showed higher sugar consuming strain and thereby higher bioethanol yield (87.4%) (Bulawayo, Bvochora, Muzondo, & Zvauya, 1996).

Starch-based feedstocks

The most popular starch-based feedstocks used in bioethanol production include corn, wheat, and tuber crops including cassava, yam, and sweet potato. Starch polysaccharides are broken down into sugars during bioethanol production. Yeasts then turn these carbohydrates into ethanol.

Corn

Corn (*Zea mays*) is the most employed feedstock for first-generation bioethanol production in the United States, because of its high starch content and the conversion of starch to alcohol (ethanol) is relatively easy (Woiciechowski et al., 2016). The corn grows well in temperature 10-30°C. (https://farm-energy.extension.org/corn-for-biofuel-production/). Based on corn variety and its quality, ethanol production varies. The yield of bioethanol is high when kernels are used as it has high starch content (Singh, 2012). The starch in corn kernel has alpha-linked glucose polymer which can be easily broken down to monomers (glucose) and then these glucose molecules are fermented to alcohol using *S. cerevisiae*. It was reported that about 72% of the dry mass of corn kernel contains starch. The amount of hemicellulose/cellulose, Protein, Oil, sugars, and ash present in corn kernels is about 10.5, 9.5, 4.5, 2, and 1.5 % of the dry mass respectively. The moisture content in the corn kernel was found to be 15% (Woiciechowski et al., 2016). In the US mostly 40% of the corn cultivated is used for bioethanol production (Naqvi & Yan, 2015). The ethanol production from corn consists of majorly 5 steps which are raw material pretreatment, hydrolysis, fermentation, separation, and dehydration (Quintero et al., 2008). It was reported that about 500 gallons of ethanol are produced from corn cultivated on oneacre of land. Around 4 tons of corn grains are harvested from one acre of land (Sexton & Zilberman, 2011). Around 3460 - 4020 L per hectare ethanol is produced from corn in Brazil (Kim & Dale, 2004). It was reported that about 2.7 gallons of ethanol can be produced from one bushel of corn and 17 pounds of distillers dried grains (DDG) can be obtained as a co-product. This DDG can be fed to livestock. There are two ways by which the corn is processed, wet milling and dry milling. Wet milling is mainly used for the production of high fructose corn syrups which are used in the food industry. They are used as sweeteners in non-alcoholic beverages and baked products. In dry milling, the corn is mashed, and slurry is prepared by adding a heat-stable enzyme and water. The slurry prepared is then converted into corn mash by adding other enzymes. This corn mash is further used for the fermentation process. After the fermentation, the distillation of the

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liquid part is done to get pure ethanol and the remaining part is centrifuged. The solid material is DDG and is further used (Martin, 2010).

Cassava

Cassava (*Manihot esculenta*) is a shrub and a tuber crop. 25-35% starch is present in the roots and tubers of cassava, sweet potato. Bioethanol production from tubers is more cost-effective than that from grains. About 281 million tons of cassava is produced globally. Out of Asian countries, Thailand and Indonesia produce about 26 million tons and 28 million tons of cassava. Around 77-94% of starch and 59-70% of the moisture is present in cassava (Woiciechowski et al., 2016). Mainly China and Thailand use cassava for bioethanol production due to their obtainability all through the year (Mohapatra et al., 2019). Cassava roots have a 30% of starch content by which 180 L of 96% pure ethanol per ton of cassava can be produced (Bertrand et al., 2016). Bioethanol production using cassava is led by Thailand (50%), followed by China (44%). Around 3400-3600L per hectare ethanol is produced from cassava in Brazil (Mohapatra et al., 2019).

Production process from cereal crops

Unlike the sugar crops, the production of bioethanol from the cereal crops is different as the key component in these crops is starch, a macromolecule made of a lengthy polymer of glucose units that can't be turned into ethanol immediately (Mohapatra et al., 2019). Hydrolysis is a crucial step that breaks down the starch molecule into individual glucose monomers. The dry-milling process and wet-milling process are the two major processes used for bio-ethanol production (Naqvi & Yan, 2015). In the dry milling, the corn is washed, crushed, milled, ground into flour, and made into slurry by adding water (Bertrand et al., 2016). The starchy material is gelatinized for the amylose and amylopectin susceptible for enzymes to hydrolyze in the next step (Woiciechowski et al., 2016). The enzymatic hydrolysis of starch has two steps - liquefaction and saccharification (Bertrand et al., 2016) and in the liquefaction step, a heat-stable α-amylase, which hydrolyzes α-1, 4 bonds between glucose units in the stanch molecule randomly, yielding dextrins and gluco-oligosaccharides with reduced starch viscosity. Dextrins are further hydrolyzed by dextrins/pullulanase that hydrolyzes both α-1, 4 and α-1, 6 glycosidic linkages and finally convert to glucose by glucoamylase (Baras, Gacesa, & Pejin, 2002). Gluco-amylases also known as amyloglucanases, attacks starch from the non-reducing end to produce glucose (Nikolov, Meagher, & Reilly, 1989).Wet milling process, consists of an additional step is performed where dilute sulfuric acid is added to the corn to make a slurry and left for 24–48 hrs where the corn grains decompose into different components. The corn starch is then fermented into ethanol (Naqvi & Yan, 2015). In the fermentation step, *S. cerveciae* converts the glucose into ethanol and CO_2 where in, bioethanol is separated from the culture that has 8–11% (w/w) ethanol in the distillation section (Quintero et al., 2008). Few studies were provided in the Table 2 and the general process is mentioned in the Fig 1.

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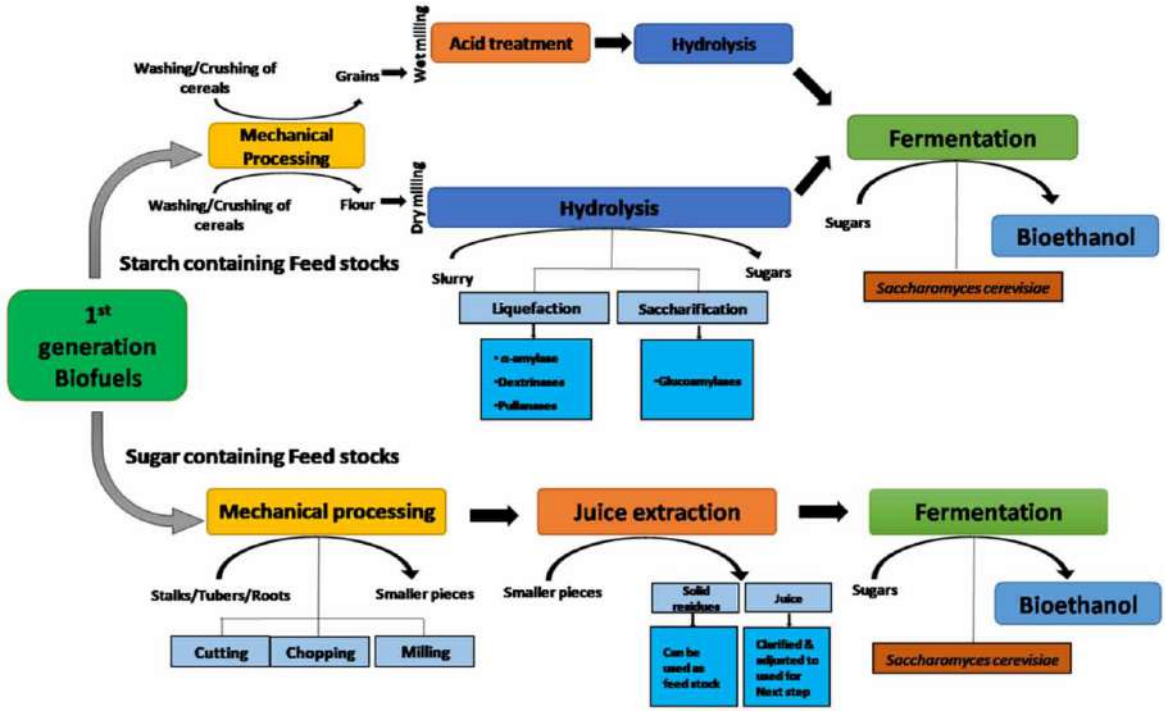
Table 2. Various studies used first-generation biomass for the production of biofuels

Substrate type	Organism	Fermentation type	Product and yield	References
Sugarcane molasses	*Saccharomyces cerevisiae*	Submerged fermentation	Bioethanol - 0.669 g/g	(Jayus et al., 2016)
Sugarcane molasses	*Kluyveromycesmarxianus* K21	Submerged fermentation	Bioethanol - 0.65 g/L/h	(Lin et al., 2020)
Sugar beet pulp	*S. cerevisiae*	Simultaneous saccharification and fermentation (SSF)	Bioethanol - 26.9 ± 1.2 g/L	(Berłowska et al., 2016)
Sugar beet pulp	*Pichia stipitis*	Simultaneous saccharification and fermentation (SSF)	Bioethanol - 86.5 ± 2.1%	
Sugar Beet Pulp	*S. cerevisiae (strain DTN)*	Submerged fermentation	Bioethanol - 0.446 ± 0.017 g/g	(Vučurović & Razmovski, 2012)
Sugar beet raw juice	*S. cerevisiae*	Submerged fermentation	Bioethanol-90 g L1 h1	(Dodić et al., 2009)
Sweet sorghum juice	*S. cerevisiae* BY4741	Submerged fermentation	Bioethanol-113.7 ± 3.1 g L1	(Sasaki et al., 2015)
Cane molasses	***Zymomonas mobilis*** MTCC 92	Submerged fermentation	Bioethanol-58.7 ± 0.09 and 59.1 ± 0.08 g/l	(Behera, Mohanty, & Ray, 2012)

Figure 1. First-generation biofuel production process



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Second-generation biofuels

Second-generation biofuel is also acknowledged as lignocellulosic biofuels are produced from a broad diversity of feedstock which is majorly crop, agricultural, and forest leftovers and are examples of non-edible biomass. These feedstocks are inexpensive, plentiful, and renewable (Lee & Lavoie, 2013; Raud et al., 2019). They are produced in a sustainable way which doesn't impact that much on global CO_2 levels (Naik et al., 2010). Since they are taken from non-food material, a crisis for food is not seen (Hirani et al., 2018). The non-food materials including bagasse, clover, de-oiled seed residues, straw, leaves, grass, and stem biomass are widely used for 2G biofuels. The 2G biofuel mainly includes bioethanol, biodiesel, dimethyl-ether, biosyngas FT (Fischer-Tropsch) biodiesel (Ruan et al., 2019). The production of 2G biofuel can be done by thermochemical route or biochemical route. Unlike first-generation biofuel, 2G biofuel does not need additional water, land, and fertilizers (Dahman, Dignan, et al., 2019). This fuel is combined with other conventional fuels like petroleum-based fuels for combustion. This is applied in internal combustion engines (Datta et al., 2019). Second-generation bioethanol is produced by utilizing cellulose, a glucose polymer feedstock. During the production process, the raw material will undergo pretreatment by physical, chemical, or biological methods (Prachand Shrestha, Anthony L Pometto III, 2012). A wide variety of lignocellulosic biomass is used for the 2G biofuel production which includes agricultural and timber industry residues and co-products, food crop residues, perennial lignocellulosic plants like reed canary grass, *Miscanthus, Eucalyptus* (Ho, Ngo, & Guo, 2014). It was reported that by 2016, about 67 second-generation bioethanol facilities were started throughout the world. The United States is the leading in second-generation biofuel production (Mohapatra et al., 2019). The lignocellulosic biomass contains cellulose, hemicellulose, and lignin. Hemicellulose gets decomposed fast at temperature 200-380°C, cellulose at 320-400°C and lignin is the most stable one and decomposes at above 400°C (J. Zhang & Zhang, 2019). The content of cellulose, hemicellulose, and lignin vary from different types of feedstocks. The biomass that can be used for the production of biofuels can be divided into non-woody and woody biomass. The pros and cons of the second-generation biofuels were given in the Table 3.

Table 3. Pros and Cons of second-generation biofuels (Carriquiry, Du, & Timilsina, 2011; Saini et al., 2015)

Pros	Cons
Minimal feedstock costs **No rivalry with food** **Using the entire plant rather, as well as leftovers, thereby higher energy generation** **Better yield with lesser area** **The percentage of usable feedstocks is higher than is easily available presently** **Environmentally feasible**	**Overuse of agricultural lands eventually may lead to soil erosions** **The process of production is complex.** **Lack of commercially viable technologies** **Advanced research technologies are lesser-known**

Woody based feedstock

Hardwoods and softwoods make up the woody biomass, which is used to make biofuels. The chemical compositions and the physical properties of these materials are different from each other. Based on these

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ethanol production varies (Wijeyekoon & Vaidya, 2021). Reportedly, the use of wood-based feedstock for bioethanol production can also lower carbon emissions more effectively. Also, the higher the area of production less is the production costs (Perez-Verdin et al., 2009). The utilization of novel non-edible lignocellulosic materials in the creation of chemicals and fuels is a strategic goal. These raw minerals are an intriguing and potential alternative to fossil fuels as a source of energy (García, González Alriols, & Labidi, 2014). Short-rotation woody crops have been marketed as a long-term biofuel feedstock for heat and/or electricity generation. Short rotation woody crops (SRWC) are perennial plants with consistent production and well-developed technique for cultivation (Stolarski et al., 2015). Some of the plants that are used for woody feedstock are Hybrid poplar (*Populus* spp.), Willows (*Salix* spp), the American sycamore (*Plantanus occidentalis*), Sweetgum (*Liquidambar* spp.), Black locust (*Robina pseudocacia*), Silver maple (*Acer saccharinum*), Loblolly Pine (*Pinus taeda*) (García et al., 2014; Stolarski et al., 2015). Mainly the wood material in the plants is made of cellulose microfibrils which are together gripped by hemicellulose and lignin. The vessels and tracheid are present in the center which is surrounded by the microfibril cellulose. Based on this layer the toughness of the wood increases and will undergo various pre-treatment. Combining multiple pretreatments has also been investigated and might be beneficial in achieving appropriate segregation of the various components and better yields (Alvira, Tomás-Pejó, Ballesteros, & Negro, 2010). To not only improve the efficiency of subsequent bio refinery processing stages, but also to avoid high-contaminated hemicellulosic or lignin-containing streams, the correct mixture of pretreatment steps (first, for hemicelluloses solubilization, and then, for effective de-lignification) must be used (Wijeyekoon & Vaidya, 2021). The production of biofuel from woody biomass depends on the feedstocks from both forests and plantations. Lignocellulosic biomass can create 442 billion liters of second-generation ethanol, while total agricultural wastes and discarded crops can yield 491 billion liters of ethanol every year (Kim & Dale, 2004). About 30% of the global forest biomass is produced from the US. For biochemical conversion of sugar to fuels, woody biomass is not preferred because of their high lignin content and recalcitrance to digestion. It was reported that 1.2 million liters of ethanol was produced from 4 million dry tons of wood every year in Mississippi (Perez-Verdin et al., 2009). Owing to the high energy requirement for the conversion, bioethanol production is difficult (Mohapatra et al., 2019).

Non-woody biomass

The other sort of lignocellulosic feedstock that can be well-thought-out for biofuel production is non-woody biomasses which are cheaper, simple to process, and need less energy for the production of bioethanol. They can be sorted out broadly into agriculture residues, process residues, native plants and non-wood plant fibers, solid wastes, etc. which were discussed in the coming topics (García et al., 2014; Mohapatra et al., 2019).

Agriculture residues

Corn stover, Cereal straws, rice husks, etc., are the various agriculture residues that can be utilized as a potential feedstock for biofuel production.

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Corn stover

The residues available in the field after the corn grains harvest from the corn plant are usually the cobs, stalks, leaves, husks and tassel are called corn stovers (Liew, Hassim, & Ng, 2014). Globally approximately 377 million tons of corn stover is produced per year whereas the USA is the major source as the USA mainly uses corn ethanol (Hiloidhari et al., 2019; Thompson & Tyner, 2014). It has 37- 61% cellulose (Axelsson et al., 2012), 19.3- 31% of hemicellulose, and 6.9 - 18% of lignin (Ruan et al., 2019). Xylose and arabinose are the predominant five-carbon sugars. Stover is a byproduct of maize production and not a food-based product. In hemicellulose and they too can be fermented to ethanol with the suitable microorganism (Woiciechowski et al., 2016). Development for cost-effective technologies for the biomass conversion into sugars is a need and diverse trials are in progress to extract the sugars for the fermentation process. Some of the studies using corn stover as the substrate to produce biofuel were given in Table 4.

Cereal Straws

A variety of cereal grains have been used as food for humans containing 60-70% starch. They generate enormous quantities of straws as agricultural residues like dry stalks and leaves in the fields following the removal of nutrient grains and seeds. Barley, wheat, rice, oats, and sorghum are all considered essential cereal crops. Different cereal crops such as barley and oat from Europe, sorghum and corn from the United States, and rice and wheat from India and China are predicted to produce 1580.2 million tones every year (Kim & Dale, 2004). All these different cereal straws have 33%–47% cellulose which can be utilized in biofuel production (Tye, Lee, Wan Abdullah, & Leh, 2016). Pyrolysis can be applied to these crop residues which produce biochar. Rice straw was found to be a good potential for biochar as the yield was around 43.8%. Similarly, comparative studies of anaerobic digestion were conducted with goat manure and three crop residues, namely rice straw, corn stalks and wheat straw found better for biogas production (Bian et al., 2016).Table 4 has provided with few studies using various cereal straws for biofuel generation.

Rice husk/hulls

Oryza sativa, commonly called rice is an herbaceous plant that belongs to the Poaceae family. Rice husk (RH) is a major byproduct of the paddy process, accounting for 20–25% of the weight. India is the world's second-biggest rice grower, producing 102.0 MT of rice and around 20.4–33.6 MT of RH per year (Potumarthi, Baadhe, Nayak, & Jetty, 2013).It is a lignocellulosic substance with a low bulk density that contains around 20–25% lignin, 35–40% cellulose, and 15–20% hemicellulose. It has the potential to act as a feedstock for the production of gasoline-ethanol as of its availableness and affordable cost (Jun Yu, Zhang, He, Liu, & Yu, 2009). Various studies using rice husk for biofuel production were given in Table 4.

Process residues

Sugar cane bagasse, Cassava bagasse, Sweet potato residues are the various process residues that can be used as potential feedstock for biofuel production.

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Sugarcane bagasse

Sugarcane wastes, particularly bagasse (SB) and leaves (SL) have been investigated for biotechnological and non-biotechnological applications. For decades, SB and SL have been studied for use in lignocellulosic bioconversion, allowing for the cost-effective use of residual substrates in the manufacture of bioethanol and value-added commercial goods like as xylitol, specialty enzymes, organic acids, single-cell protein (Chandel, da Silva, Carvalho, & Singh, 2012). The leftover dry matter residue after extraction of juice from sugar cane is termed sugar cane bagasse. The major advantage is its accessibility at the plant site where the juice is extracted. It is composed of 40–45% of cellulose, 27–37.5% of hemicellulose, and 10–20% of lignin (Canilha et al., 2011; Zhao, Peng, Cheng, & Liu, 2009). From a ton of bagasse, 300 L of ethanol can be produced (Woiciechowski et al., 2016). However, it has a higher amount of complex inbuilt structure and where individual sugars aren't accessible for the yeast to produce ethanol and therefore a cost-effective pretreatment has to be done before enzyme hydrolysis to break the polysaccharides into monosaccharides. In the following chapters, we go through the techniques in greater depth. Studies for biofuel production using sugarcane bagasse were provided in Table 4.

Cassava Bagasse

Cassava bagasse is a waste by-product of cassava starch manufacture that is both affordable and widely available. For every 250–300 tons of cassava root processed, roughly 280 tons of bagasse with an 85% moisture content are generated (Pandey et al., 2000). Cassava is a starch based tuber crop produced in many Asian, Africa and Latin American. India also has many cassava processing plants. Cassava bagasse contains 30-35% of carbohydrates which can be employed for biofuel production (Mohapatra et al., 2019). Few studies for biofuel production using cassava bagasse were given in Table 4.

Sweet Potato Residues (SPRs)

The remaining residue after the removal of starch from sweet potatoes is called Sweet Potato Residues (SPRs) contributing for 10% of the total dry matter of sweet potatoes. The annual production of sweet potatoes is about 71 million tons globally which harvests 2 million tons of SPRs. Viscosity was one of the SPRs main drawbacks to utilize as feedstock (Duvernay, Chinn, & Yencho, 2013). Requirements of heavier instruments of corrosion resistance are required which is a major bottleneck. However recent enzymatic methods for utilization of SRPs opened up the opportunities to utilize the same as feedstock (Izmirlioglu & Demirci, 2012). Various studies employing the SRPs were provided in Table 4.

Native Plants

Grasses are indigenous plants that grow naturally and do not require special care, making them a cost-effective biomass source. Carbohydrates and lignin make up the majority of them. These polysaccharides may be broken into sugars and then fermented to produce bioethanol. The most commonly used grass plants are Napier grass, Giant Miscanthus (*Miscanthus* spp.), Switch grass, and Bermuda grass. *Miscanthus* spp. is a perennial sterile grass hybrid that proliferates through rhizomes. It is a type of saw grass that can yield 5 to 8 times as much ethanol per acre as compared to corn. It has high lignocellulosic content, 40%–60% of cellulose, 20%–40% of hemicellulose, and 10%–30% lignin. Numerous species of

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Miscanthus have diverse carbohydrate and lignin content which can be utilized for biofuel production. They intern help in protecting soil erosion and fixing atmospheric carbon as contrast to other annual crops (Axelsson et al., 2012). *Panicum virgatum* is generally called Switchgrass, a perennial herbaceous grass that is native to North America and requires low requirements like *Miscanthus* (Adler, Sanderson, Boateng, Weimer, & Jung, 2006). It has 37% to 40% of cellulose, 25% to 29% of hemicellulose, and 18% to 25% lignin. This grass has a high net energy benefit and effective soil carbon absorption, making it a good crop for soil conservation on productive land (Mohapatra et al., 2019). The next grass feedstock in the list is *Pennisetum purpureum* commonly called Napier grass a native to eastern and central Africa and has been introduced in tropical and subtropical countries. The plant's significant properties include high cellulosic fiber content, minimal nitrogen fertilizer use, and the ability to grow rapidly, making it an ideal feedstock for biofuel synthesis (Zahran, 1999). The grass has cellulose in the range of 40% to 50%, hemicellulose in the range of 20% to 40%, and lignin in the range of 10% to 25% (Takara & Khanal, 2015). *Conodont dactylon*, termed Bermuda grass, has high carbohydrate content similar to the different grass feedstocks. It has 40%–55% of carbohydrates (cellulose and hemicellulose) and 20%–25% of lignin (Takara & Khanal, 2015). Cocksfoot grass, reed canary grass, big bluestem grass, and alfalfa are in addition other potential grass feedstock that can be considered for biofuel production (Njoku, Uellendahl, & Ahring, 2013). Various studies were provided in Table 4 which provides information regarding their use as substrates for biofuel production.

Natural Non-woody Plant Fibers - Bast crops

Bast crops are highly resourceful mop crops that can grow on waste or even brackish water. Bast fibers are the fibrous plant fiber's outer layer, accounting for one-third of its weight. Some of the Bast fibre crops with high cellulose content and potential as biofuel feedstocks are flax, ramie, kenaf, sun hemp, and industrial hemp (Cherney & Small, 2016). Industrial hemp is considered a vital sustainable potential option for biofuel production as said by European Energy Agency. The global yearly production of hemp is around 0.1 million tons. Industrial hemp has 70.0–90.0% of cellulose and other fibers such as flax, jute, and ramie has 60.0%–80.0%, 51.0%–84.0%, and 68.0%–76.0% of cellulose respectively (SaifulAzry, Amel B. Ahmed, 2011). The biofuel output from industrial hemp and other bast fibre crops, on the other hand, is mostly unknown.

Food processing wastes and Solid wastes

Enormous amounts of different fruits and vegetables are used and processed every day, because of increasing market demand which generates huge quantities of industrial wastes, which include peels, seeds, shells, pomace, skin, etc. from various fruits and vegetables (Hiloidhari et al., 2019). Fruit leftovers such as tomato, asparagus, kiwi, and potato residues may also be employed to produce biofuels by anaerobic digestion (Siciliano, Limonti, Mehariya, Molino, & Calabrò, 2019). Different solid wastes that include a municipal solid waste (paper, plastics, etc.), wastewater sludge, food waste, animal manure, etc., in addition, can be used as a source for biofuel as they have cellulose (Ho et al., 2014). Fruit & vegetable processing sectors have developed massively leading huge amount of industrial wastes generation like leaves or straw, seeds, pulp, or pomace. Up to 60% of citrus peels are generated in citrus fruit processing industries which are a possible substrate for multiple fermentation processes (Wilkins, Suryawati, Maness, & Chrz, 2007). Some of the studies using various wastes were provided in the Table 4.

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BIOMASS STRUCTURE AND COMPONENTS

The many varieties of lignocellulosic biomass that are accessible have previously been mentioned in previous topics. Cellulose (30–50%), hemicellulose (25–30%), and lignin (10–35%) make up the majority of lignocellulosic biomass, which is structured into a complex structure. Subject to the biomass type, source, growing circumstances, maturity, and harvest time, these percentages vary (Agbor et al., 2011; McKendry, 2002). In the upcoming sections the different components of lignocellulosic biomass processing procedures, challenges, and recent technological advances in the production of biofuels from these biomasses will be discussed.

Cellulose $(C_6H_{10}O_5)_x$

This is the most abundant polysaccharide in lignocellulosic biomass and the primary component. The essential building component is cellobiose, which is made up of a linear chain of D-glucose monomers connected by β-(1,4)-glycosidic linkages (Himmel et al., 2007). In wood pulp, the number of glucose molecules in a chain can range from 800 to 10,000 units until 17000 units. This is called the degree of polymerization. These linear cellulose chains unite to form tightly packed cellulose fibers via intramolecular and intermolecular hydrogen bonds and van der Waals forces. Because of their ordered packing, these cellulose fibers sequentially form crystalline structures. They are water-insoluble and extremely resistant to depolymerization. The regions of cellulose with less crystalline packing are referred to as amorphous structures (Xuebing Zhao, 2012). Because these regions are less resistant to biodegradation, they may be properly broken to produce sugars via enzymatic hydrolysis and alcoholic fuel (Khattab & Watanabe, 2019; Robak & Balcerek, 2018).

Hemicellulose

Hemicellulose, which is made up of several sugars, is the next most common polymer. It's a polymer made up of short, branched-chain sugars that's heterogeneous. It is composed of two distinct monomeric sugars - Pentoses (C5 sugar) and hexoses (C6 sugars). D-xylose and L-arabinose are pentoses, while d-mannose, d-galactose, and d-glucose are hexoses. Xylan is the most plentiful hemicellulose component of all (Peng, Peng, Xu, & Sun, 2012). All of the pentose and hexose combine to form short, branching structures. They are amorphous and provide hemicellulose the ability to be partially soluble in water. Sugar acids such as uronic acids, as well as monosaccharides, are found in hemicellulose, which contains d-glucuronic, 4-O-methylglucuronic, and d-galacturonic acids. Because of its low molecular weight and simple structure with short lateral chains, hemicellulose is easier to hydrolyze than cellulose. Hemicelluloses in secondary cell walls interfere with cellulose and lignin, which create a network by hydrogen-bonding to cellulose microfibers, covering them, and providing the structural backbone for plant cell walls (Koshijima & Watanabe, 2003; J. X. Sun, Sun, Sun, & Su, 2004). As a result, enzymes have difficulty accessing the sugars in cellulose that can be turned into biofuel. To make the cellulose more digestible, at least half of the hemicellulose must be eliminated by pretreatment procedures (Saha, 2003).

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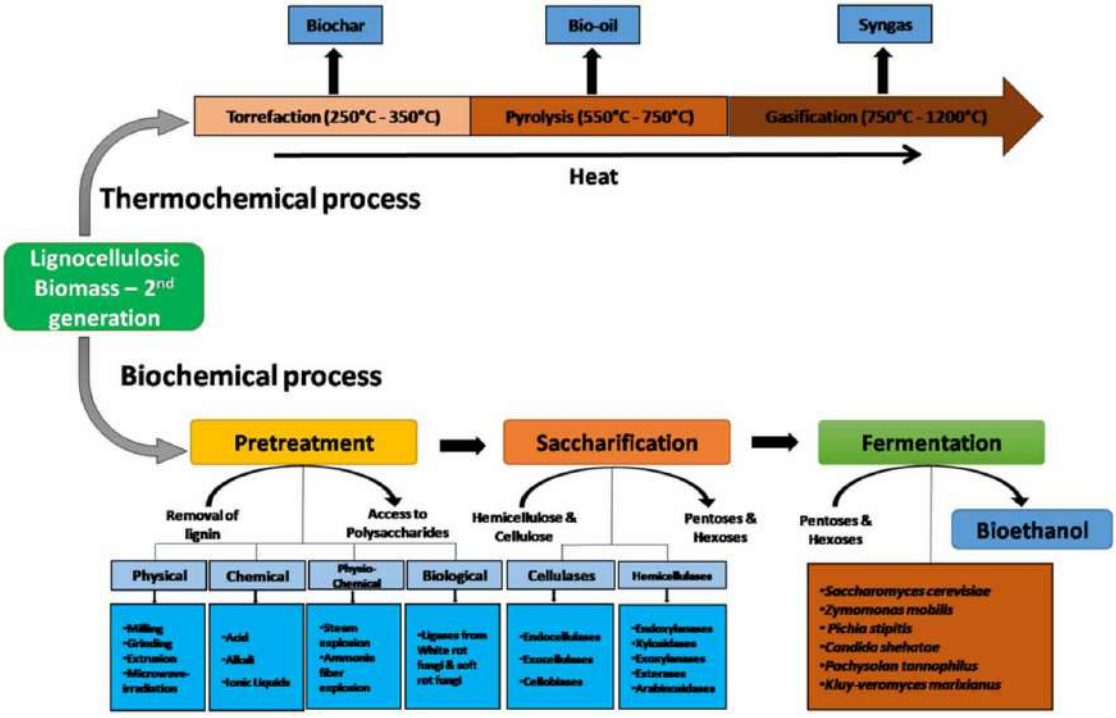
Lignin

Lignin is a 4-hydroxyphenylpropanoid-based aromatic compound found in lignocellulosic biomass. They are known as monolignols or lignin precursors, and they are linked by ether (C-O-C) and carbon-carbon (C-C) bonds with varying degrees of methoxylation. There are three varieties of monolignols: p-coumaroyl, conifineryl alcohol, and sinapyl alcohol, which are derived from the precursor's p-hydrophenyl (H), guaiacyl (G), and syringyl (S). They are connected by various links to form a complex three-dimensional polyphenolic network. Lignin is hydrophobic and defiant to chemical and biological destruction. It is located in the center of the lamella and serves as a binding substance for the cell wall and plant cells. Lignin forms an amorphous mixture with hemicellulose and cellulose fibrils embedded within it and is thus referred to as a 'glue' or binding substance that holds the various components of lignocellulosic biomass together (Boerjan, Ralph, & Baucher, 2003; Ghaffar & Fan, 2013). The successful fragmentation of lignin is a critical step in gaining entry to the polysaccharide's cellulose and hemicellulose for the enzymes to break down and create sugars for bioethanol conversion. Pretreatment procedures in the processing of lignocellulosic biomass can remove lignin, allowing value-added goods such as dyes, insecticides, and industrial plastics to be converted (Dahman, Dignan, et al., 2019).

Conversion technology

Given the variety of biomasses available, it is possible to generate a wide range of biofuel products from the same feedstock. Using thermochemical and biochemical processes, lignocellulosic biomass may be converted into a variety of biofuels as mentioned in Fig 2.

Figure 2. Second-generation biofuel production process



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Thermochemical Process

Thermochemical processing, as the name implies, entails the conversion of biomass into a variety of products by thermal decomposition and chemical reformation, and primarily includes heating of the biomass in the presence of varying oxygen concentrations. Combustion, gasification, liquefaction, hydrogenation, and pyrolysis are examples of thermochemical conversion processes (Goyal et al., 2008). The biomass is roasted at low temperatures between 250 to 350°C in the absence of oxygen in this process to form biochar (Glaser et al., 2001; Kwapinski et al., 2010). Pyrolysis is a procedure that changes biomass to biooil by heating it to temperatures ranging from 350–550°C and reaching 750°C in the lack of oxygen (Mohan, Pittman, & Philip, 2017). The biomass undergoes full depolymerization and is transformed largely into syngas, with charcoal and biooils as by-products, in the gasification process, which happens at even higher temperatures ranging from 750 to 1,200°C and with insufficient oxygen supply (Lee & Lavoie, 2013; McKendry, 2002)(J. Zhang & Zhang, 2019).

Biochemical Process

Biochemical pretreatment of biomass employing biological, physical (heat), or chemical catalysts are used in the biochemical process. For the degradation of polysaccharides into simple sugars, biocatalysts such as enzymes are used, and fermentative microorganisms (yeast or bacteria) are used for fermentation, where the sugars are transformed into biofuel. Each method of biomass conversion has its own set of obstacles and opportunities. The biochemical route is thought to be more environmentally friendly, practical, and cost-effective. The biochemical method is limited to the conversion of polysaccharides, but the thermochemical approach is more widely used nowadays since it can convert all of the organic constituents of biomass into various biofuels (Robak & Balcerek, 2018; Vohra et al., 2014). Further to this section onwards, the focus will be on the conversion processes for lignocellulosic biomass via biochemical methods.

Pretreatment

Lignin holds together an intricate network of polysaccharides, cellulose, and hemicellulose in lignocellulosic biomass. The removal of lignin from the lignocellulosic biomass would be the first step in the conversion process, allowing the polysaccharides to be obtained. The pretreatment stage is an important part of the biothermal conversion process. The pretreatment phase should be able to create highly digestible solids, avoid degradation of cellulose and hemicellulose, avoid inhibitor development in subsequent processes, and easily recover lignin. The pretreatment process is influenced by factors such as biomass crystallinity, degree of polymerization (DP), surface area accessibility, hemicellulose, and lignin concentration (Cheng & Timilsina, 2011; A. Mishra & Ghosh, 2017).

Various categories of pretreatment procedures have been employed, which possibly divided into three categories: physical, chemical, and biological. Among the three primary processes in biofuel production, the pretreatment step appears to have the greatest capital and operational costs. In the bioethanol manufacturing process, the pretreatment stage accounts for 40% of the processing expenses. As a result, it's critical to pick the best technique that's both cost-effective and successful at removing lignin (Naik et al., 2010; Robak & Balcerek, 2018; Saini et al., 2015).

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Physical methods like mechanical pretreatment enable us to accomplish higher hydrolysis efficiency. Chipping, grinding, and milling are examples of techniques that may be used to enhance surface area, minimize biomass chip size, and lower the degree of polymerization and crystallinity of cellulose. These methods are regularly combined with other methods. Following chipping, milling, or grinding, the particle size is condensed to 10–30 mm and 0.2–2 mm, respectively, depending on the kind of physical pretreatment used. Two types of milling are being practiced -ball milling and wet disc milling. The most major disadvantage of milling is a large amount of energy requirement (Hideno et al., 2009; Inoue, Yano, Endo, Sakaki, & Sawayama, 2008). Wet disc milling has been shown to reduce energy consumption when compared to other milling pretreatment methods, but ball milling has been shown to produce elevated glucose and xylose yields after enzymatic hydrolysis (Da Silva, Inoue, Endo, Yano, & Bon, 2010). Extrusion pretreatment is a type of thermophysical method where the materials are subjected to different processes like mixing, heating, and shearing which disrupts the (X. Chen et al., 2014; Wahid, Hjorth, Kristensen, & Møller, 2015). Microwave irradiation is a physical pretreatment method that degrades and partially eliminates lignin and hemicellulose and modifies the cellulose structure and thus increases the enzymatic susceptibility. It is based on the direct application of electromagnetic radiation to a substance (for example, an organic solvent or plant tissue) that can absorb and convert electromagnetic energy (microwaves) into heat (Ganzler, K., Salgó, A., & Valkó, 2012; Hu & Wen, 2008).

In chemical procedures, acids, alkaline agents, organic solvents, ionic liquids, and other chemicals can be employed to break apart the lignocellulosic structures. However, this approach has a number of drawbacks, including the creation of hazardous substances, carbohydrate loss, and the high cost of chemicals employed in the process (Haghighi Mood et al., 2013). Acid hydrolysis is the most popular chemical approach for biomass pretreatment, in which the acid decomposes lignin and hydrolyzes hemicellulose into monosaccharides, allowing for easier access to cellulose for enzymatic hydrolysis. Concentrated acid (72% H_2SO_4) used at low temperature or dilute acid (2–5% H_2SO_4) applied at high temperature can do this. Other than sulphuric acid, other acids also were in use like H_3PO_4 (Hong, Xue, Weng, & Guo, 2012). H_2SO_4 and H_3PO_4 are generally inexpensive and effective at hydrolyzing lignocellulose. Similarly, Hydrochloric acid (HCl) is more volatile and simpler to recover than sulfuric acid (H_2SO_4), and it destroys biomass more effectively (A. Demirbas, 2008b). Nitric acid (HNO_3) has a high rate of cellulose to sugar conversion (Tutt, Kikas, & Olt, 2012). Both acids, however, are more costly than sulphuric acid. However, the primary disadvantages of using concentrated acid are its toxicity and corrosiveness, as well as the cost of acid recovery and the production of fermentation inhibitors, whereas the main disadvantage of using dilute acid is the need for more energy (Arora, Sharma, Kumar, & Sani, 2019). Similarly, alkali treatment includes the use of sodium (NaOH) (Wang, Keshwani, Redding, & Cheng, 2010), potassium (KOH) (Hendriks & Zeeman, 2009), calcium ($CaOH_2$) (Xu & Cheng, 2011), that can break the bonds between lignin and carbohydrates and increases the accessibility of the cellulose for enzyme hydrolysis. It is more beneficial as the lignin and hemicellulose are removed efficiently and also increase the surface area for the enzymes to hydrolyze better. Alkaline pretreatment, alternatively, is carried out at lower temperatures and does not necessitate the use of sophisticated reactors, making it more suitable for use on-farm. Long residence times (from hours to days) and the necessity to neutralize the pretreated slurry are the key disadvantages (Haghighi Mood et al., 2013). A new approach for the pretreatment is the use of chemicals as solvents like methanol, ethanol, or acetone in grouping with acid or base catalyst which can help in the disruption of the lignocellulose structure and increase the accessibility for cellulose fibers. This approach is termed Organosolv pretreatment. The catalysts used consist of organic or inorganic acids (hydrochloric and sulfuric acids), bases (ammonia, sodium

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hydroxide, and lime) (Araque et al., 2008; Mesa et al., 2011; F. Sun & Chen, 2008). Though lignin and hemicellulose are removed efficiently, the high cost of the solvents, flammability, and volatility are the drawbacks (Araque et al., 2008).

A different latest physio-chemical pretreatment technique is the use of ionic liquids (IL) which are the organic salts composed of large organic cations and small inorganic anions which can notably break the lignocellulosic structure by breaking the bonds between cellulose, hemicellulose, and lignin. IL's are a novel category of solvents that are completely made up of ions and have low melting points (b100°C), high polarities, great thermal stabilities, and minimal vapor pressure (cations and anions) (Peter Wasserscheid; Wilhelm Keim, 2010). The imidazolium cation, which may couple with anions such as acetate, bromide, chloride, sulfate, nitrate, methanoate, and triflate, is present in the most prevalent forms. A suggested approach of dissolution implies that these ionic liquids fight for hydrogen bonding with lignocellulosic components, destroying their three-dimensional network (Moulthrop, Swatloski, Moyna, & Rogers, 2005). They have the benefit of simultaneously dissolving carbohydrates and lignin, which might open up new processing options for lignocellulosic feedstocks. Though its significant potential for improving cellulosic biomass digestibility, ILs are still in the research stage, and issues such as the high cost of the stage of chemicals must be examined for commercial usage (Miyafuji, 2015). Steam explosion or also called Steam pretreatment (SP) is a physio-chemical pretreatment process in which the biomass's lignin and hemicellulose are divided up by steam heating (Thermo), shearing owing to abrupt decompression and extension of moisture (mechano), and glycosidic bond degradation (chemical). SP may be efficiently increased by using H_2SO_4 (Wen Hua Chen, Pen, Yu, & Hwang, 2011) or SO_2 (Mackie, Brownell, West, & Saddler, 1985) as a catalyst. The use of an acid catalyst improves hemicellulose sugar recovery, lowers inhibitory compound generation, and improves enzymatic hydrolysis on solid residue. It is an appealing procedure since it uses few chemicals. It uses less energy and has no recycling or environmental costs. However, one of the drawbacks of SP is that it does not destroy the lignin-carbohydrate matrix, which enhances the likelihood of condensation and precipitation of soluble lignin components, making the biomass less digestible (Agbor et al., 2011; Haghighi Mood et al., 2013). Ammonia fiber explosion (AFEX) is a method based on the steam explosion where the ammonia is added with the biomass along with steam. For a limited period (< 30 min), high pressure (1.72–2.06 Mpa) and moderate temperatures (60–120°C) are applied, followed by a quick pressure release. This increases the ease of access of cellulose for enzymatic hydrolysis by partially solubilizing hemicelluloses. The rapid expansion of ammonia gas causes the breakdown of the lignin-carbohydrate complex and disruption of biomass fibers, leading to improved digestibility of biomass (Shishir P.S. Chundawat, Balan Venkatesh, 2007; Teymouri, Laureano-Perez, Alizadeh, & Dale, 2005).

Biological strategies entail the employment of microbes such as white and soft rot fungi, which may breakdown the components of lignocellulosic biomass. A wide range of naturally occurring microorganisms (single or in co-culture) or enzyme systems are used in biological pretreatment (pure or mixture). As a result, microbial pretreatment (fungal, bacterial, microbial consortia, and ensiling) and enzymatic pretreatment may be distinguished. These Lignin degrading enzymes include laccases and lignin peroxidases. Two of the most significant enzymes in the ligninolytic enzyme system are lignin peroxidase (EC 1.11.1.7) and manganese peroxidase (EC 1.11.1.7). Laccases (EC 1.10.3.2.) are copper-based enzymes that help break down lignin. Laccases work along with lignin peroxidase and manganese peroxidase to degrade lignin completely. Several white-rot fungi have been studied, including *Cyathus stercolerus, Ceriporiopsis subvermispora*, *Ceriporia lacerate, Pleurotus ostreaus*, *Pycnoporus cinnarbarinus*, and *Phanerochaete chrysosporium*, with excellent delignification efficiency and enhanced hydrolysis ef-

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ficiency (Chiaramonti et al., 2012; Sindhu, Binod, & Pandey, 2016). Because no chemicals are used in these procedures, they are both ecologically benign and inexpensive to run. However, the hydrolysis rate is lower; therefore the process would take longer. The inadequate hydrolysis rate is the most notable drawback of using fungus to pretreat woody materials. Enzymatic hydrolysis can be utilised to convert leftover lignocellulose into sugars after pretreatment. Apart from the kind of feedstock, the pretreatment method must be selected depending on the biofuels cost economics (Arora et al., 2019; Dahman, Syed, et al., 2019; Raud et al., 2019).

Saccharification

The saccharification of the cellulose fibrils exposed in the pretreatment step into fermentable sugars via a hydrolysis process is the next step in the biochemical process. The activity of hydrolytic enzymes breaks down cellulose and hemicellulose into glucose and xylose. Cellulases, hemicellulases, and auxiliary enzymes are required for full biomass decomposition. Cellulases are the primary enzymes, which are very specialized and may be split into three classes depending on where they attack. Endocellulases (EG) (EC.3.2.1.4) target cellulose fiber areas with poor crystallinity and produce cello-oligosaccharides with free-chain endings. Exocellulases (CBH) (EC.3.2.1.91) further breakdown the molecule by cleaving cellobiose units from free chain ends. Endo and exocellulases hydrolyze cellulose to form cello-oligosaccharides and cellobioses, which are then hydrolyzed into individual glucose molecules by Cellobioses or β-glucosidases (BG) (EC.3.2.1.21) (Horn, Vaaje-Kolstad, Westereng, & Eijsink, 2012). Aerobic microorganisms such as *Actinomycetales* and anaerobic microbes such as *Clostridiales* may both create cellulases. *Trichoderma reesei* is an aerobic fungus that can synthesize all three cellulases (Arora et al., 2019). Cellulase blends provide an advantage over individual enzymes since they operate synergistically to increase the hydrolysis rate. Commercial grade cellulases are available from Novozymes (Enzyme cocktails - Cellic CTec2, Cellic CTec3) and Genencor (Multifect CL, Multifect GC from *T. reesi*) (Saini et al., 2015). The hydrolysis process is influenced by elements such as substrate content, cellulase activity and loading, temperature, pH, hydrolysis duration, cellulose molecular structure and crystallinity, cellulose fiber surface area, and so on. The use of thermostable cellulases from fungi such as *Chaetomium thermophilum, Myceliophthora thermophila, Talaromyces emersonii*, *Thermoascus aurantiacus*, and *Tribulus terrestris* in bioethanol production is being researched (Guo et al., 2015). Endo-1, 4-β-xylanase (EC 3.2.1.8), and β-xylosidase are the primary enzymes involved (EC 3.2.1.37). Endoxylanases cleave the key chain of xylan at random, resulting in a variety of xylooligosaccharides, whereas β-xylosidase liberates xylose by eliminating terminal monosaccharides from the non-reducing end of short oligosaccharides. The L-arabinose residues are hydrolyzed by α-L-arabinofuranosidase (EC 3.2.1.55). α-D-glucuronidase (EC 3.2.1.139) cleaves α -1, 2 bonds between glucuronic acid residues and β-D-xylopyranosyl, and acetylxylan esterase (EC 3.1.1.72) eliminate the O-acetyl of acetyl xylan. In reality, hemicellulose biodegradation is a complicated process that necessitates the coordinated activity of many hemicellulases (Chávez, Bull, & Eyzaguirre, 2006; Peng et al., 2012; Sunna & Antranikian, 1997). The cost of enzymes is the most significant constraint for commercial biochemical conversion. With various research studies conducted throughout the world in recent years, the cost has been significantly decreased (A. Demirbas, 2008b; Mohapatra et al., 2019).

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Fermentation

Hexoses and pentoses are end products of the biomass saccharification phase that can be fermented to produce biofuels. Microbes employed in commercial bioethanol production should be able to use a wide range of substrates, produce a large amount of ethanol, tolerate high temperatures, produce low pH and inhibitors throughout the pretreatment and saccharification processes, and tolerate high sugar and ethanol concentrations (Saini et al., 2015). *Saccharomyces cerevisiae*, *Zymomonas mobilis*, and certain genetically modified microorganisms can ferment hydrolyzed biomass containing various sugars to produce ethanol. They cannot, however, ferment pentoses and have a lower tolerance for high sugar concentrations (Robak & Balcerek, 2018). *Pichia stipites* (Agbogbo, Haagensen, Milam, & Wenger, 2008), *Candida shehatae*, *Pachysolan tannophilus* (Perego, Converti, Palazzi, Del Borghi, & Ferraiolo, 1990), and *Kluyveromyces marixianus* (Hasunuma & Kondo, 2012) may ferment pentoses as well as hexoses, but their efficiency is lower, and they are also sensitive to process variables such as temperature, pH, inhibitors, and so on. For biomass conversion into biofuel, a variety of techniques have been investigated. The first way is Separate hydrolysis and fermentation (SHF), in which cellulose saccharification (hydrolysis) and fermentation are performed in separate units, ensuring that the hydrolysis and ethanol production processes are carried out under optimal circumstances. The buildup of carbohydrates, on the other hand, may limit enzyme function. Simultaneous saccharification and fermentation, or SSF, is the next strategy, in which saccharification and fermentation are done in the same unit. The sugars generated by cellulose hydrolysis would be quickly fermented into ethanol in this method. This prevents the sugars from accumulating, resulting in higher ethanol production with fewer enzymes. The operating temperature is the primary drawback of this process, since the optimal temperature for hydrolysis is generally 50°C, while yeast viability is lower (28-37°C) (Olofsson, Bertilsson, & Lidén, 2008; Wingren, Galbe, & Zacchi, 2003). The third method on the list is a variation of SSF in which both hexoses and pentoses can be fermented simultaneously, thus the term simultaneous saccharification and co-fermentation (SSCF). The utilization of mixed cultures can help achieve this. Because bacteria that can utilize hexoses grow faster than those that can use pentoses, the conversion of hexoses to ethanol is more common. A second technique, on the other hand, is to utilize a single microbe that can take in together hexoses and pentoses, letting for high conversion and ethanol yield. To further understand the commercialization aspect of this process, more study is needed (W. C. Chen et al., 2017; McMillan, Newman, Templeton, & Mohagheghi, 1999). Consolidated bioprocessing (CPB) technology is a newer technique that goes a step further. CBP method for biofuel generation from lignocelluloses entails combining all three biological activities in a single bioreactor: synthesis of saccharolytic enzymes, hydrolysis of polysaccharides in pretreated biomass, and fermentation of hexose and pentose sugars. Production of enzymes that can successfully hydrolyze lignocellulosic biomass to fermentable sugars, utilization of hexoses and pentoses, efficient ethanol production (titer, yield, and productivity), fermentation inhibitors, resistance to ethanol, and stressful conditions such as low pH, high osmotic pressure, high temperature, low nutrition capacity, and fluctuating processes are the basic requirements of a microbe for this technology. No natural microbe, however, possesses all of the characteristics necessary for this technique (Hasunuma & Kondo, 2012; Olguin-Maciel, Singh, Chable-Villacis, Tapia-Tussell, & Ruiz, 2020). *S. cerevisiae*, a usual good ethanol producer with high inhibitor tolerance, can be transformed to produce the enzyme cellulases and hemicellulases, or *Clostridium thermocellum*, *C. phytofermentans*, that produces cellulases and hemicellulases, can be modified to be an proficient ethanol producer using genetic engineering technology (M. Balat, Balat, & Öz, 2008; Mohapatra et al., 2019). Because just one reactor is needed, charges

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for enzyme manufacturing may be avoided; the complete substrate is utilised for sugar release and no part of it is used for cellulase production. However, due to factors such as a long fermentation duration, low ethanol production, limited tolerance to ethanol, and the creation of fermentation inhibitors, this configuration has not yet been fully developed at a commercial level. Significant research has to be done to commercialize this process (Mohapatra et al., 2019; Raud et al., 2019).

Table 4. Various studies used lignocellulosic biomass for the production of biofuels

Substrate type	Organism	Fermentation type	Product and yield	References
Cassava bagasse	*S. cerevisiae*	Submerged fermentation	Bioethanol-0.25 m3/ton	(Martinez, Feiden, Baricatti, & Zara, 2018)
Cassava bagasse	*Rhizopus oligosporus*	Solid-state Fermentation	Bioethanol-89.2%	(Escaramboni, Fernández Núñez, Carvalho, & de Oliva Neto, 2018)
Cassava cellulosic waste	*S. cerevisiae*	Submerged fermentation	Bioethanol-32.4% alcohol	(Elemike, Oseghale, & Okoye, 2015)
Sugarcane straw	*S. cerevisiae* Y 904	Solid-state Fermentation	Bioethanol-290 L/ton	(Pratto, dos Santos-Rocha, Longati, de Sousa Júnior, & Cruz, 2020)
Sugarcane-Leaf Waste	*S. cerevisiae* BY4743	Steam-salt alkali unfiltered (SSA-U)	Bioethanol-31.06 g/L	(Moodley & Gueguim Kana, 2019)
Sugar beet cossettes	*S. cerevisiae*	Submerged fermentation	Bioethanol-0.618g/L	(Pavlečić, Rezić, Šantek, Horvat, & Šantek, 2017)
Sugar Beet Pomace	*S. cerevisiae* PM-16	Solid-state Fermentation	Bioethanol-358.458 mg/g	(Rodríguez et al., 2010)
Sorghum pioneer	*S. cerevisiae*	Submerged fermentation	Bioethanol-0.49 g/l	(Koradiya, Duggirala, Tipre, & Dave, 2016)
Corn stover	*S. cerevisiae*	Submerged fermentation	Bioethanol-40.0 g/L	(Lau & Dale, 2009)
Sweet potato (hydrolysate)	*S. cerevisiae*	Submerged fermentation	Bioethanol-67.8 g/L	(Duvernay et al., 2013)
Switchgrass hydrolysate	*C. beijerinckii* P260	Submerged fermentation	Acetone -0.41 g/L; Butanol 0.97 g/L; Ethanol 0.10 g/L	(Qureshi et al., 2010)
King grass	*S. cerevisiae*	Submerged fermentation	Bioethanol - 30.8 g/L	(Cardona, Rios, Peña, Peñuela, & Rios, 2016)
Cocksfoot grass	*S. cerevisiae*	Simultaneous saccharification and fermentation (SSF)	Bioethanol - 31.49 g/L	(Njoku et al., 2013)
Apple & pear peel extracts	*C. acetobutylicum*	Submerged fermentation	Butanol-14 g/L	(Raganati, Procentese, Olivieri, Russo, & Marzocchella, 2016)
Rice straw	*C. acetobutylicum*	Submerged fermentation (Anaerobic)	Butanol-80 g/kg ,Acetone-21 g/kg	(Amiri, Karimi, & Zilouei, 2014)
Rice straw	*C. acetobutylicum*	Submerged fermentation (Anaerobic)	Acetone-18.2 g ; Butanol - 44.2 g; ; Ethanol -0.6 g	(Moradi, Amiri, Soleimanian-Zad, Ehsani, & Karimi, 2013)
Rice straw	*Pleurotus ostreatus*	Solid-state Fermentation	Methane -263 and 214 L/kg	(Mustafa, Poulsen, & Sheng, 2016)
Rice straw hydrolysate	*C. saccharoperbutylacetonicum*	Submerged fermentation (Anaerobic)	Acetone-5.1 g/L ; D Butanol-1.26 g/L/; Ethanol-8.1 g/L	(Wen Hsing Chen, Chen, & Lin, 2013)
Sago waste residues	*Bacillus coagulans*, *C. bifermentans*and *Clostridium* spp.	Submerged fermentation	Butanol-1.53 g/L; 3.36 g/L; 1.56 g/L; 2.5 g/L	(Johnravindar, Murugesan, Wong, & Elangovan, 2017)
Orange peel waste	*S. cerevisiae* & *C. acetobutylicum*	Submerged fermentation	Bio-butanol-19.5 g/L	(Joshi, Waghmare, Sonawane, & Waghmare, 2015)
Cauliflower waste	*C. acetobutylicum* NRRL B 527	Submerged fermentation	Acetone-Butanol-Ethanol-5.35 g/L	(Khedkar, Nimbalkar, Chavan, Chendake, & Bankar, 2017)
Lettuce residues	*C. acetobutylicum* DSMZ 792	Submerged fermentation	Butanol-1.1 g/L	(Procentese, Raganati, Olivieri, Elena Russo, & Marzocchella, 2017)
Sweet potato peel	*S. cerevisiae*	Submerged fermentation	Bioethanol-6.39g/L	(Ojewumi et al., 2018)

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FUTURE RESEARCH DIRECTIONS

To address the extraordinary speed of climate change effected by the amassing of greenhouse gases (GHGs) in the atmosphere, there is a clear urgent need to convert energy dependency from fossil fuels to renewable energy sources. There are possibly two ways existed to reduce GHG emissions, one by reducing the usage of fuels and later being the replacement of harmful fuel with a less harmful one. Changes in climate and energy policy should be at the center of the country's global agenda, seeing as they have an impact on social, economic, and political decisions. To address these issues, employing environmentally acceptable, biodegradable, and cost-effective alternatives like biofuel might be an excellent choice. First-generation biofuels include corn ethanol, sugarcane ethanol, pure plant oils, and starch-based biodiesel. Because their technologies and implementation are easy, biofuel production technologies of the first generation have been successfully created and are widely used. However, the major drawback of first-generation biofuels is the food-versus-fuel conundrum; growing production of these fuels is one of the factors driving up food prices. As a reason, the lignocellulosic feedstock can develop alternative biofuels, which are frequently referred to as "second generation" biofuels. Under laboratory conditions; bioethanol synthesis from lignocellulosic biomass offers a good fuel output. However, various obstacles must be overcome before the manufacturing method can be commercialized. Various efforts to commercialize second-generation biofuels are underway across the world. In the case of biochemical fuels, breakthroughs in research and engineering of microorganisms meant to process specific feedstocks are required, followed by large-scale demonstrations to establish commercial practicality. The high capital and processing expenses of bioethanol from lignocellulosic biomass are a major drawback since specialized technology and infrastructure are required to facilitate the conversion process. When the production is scaled up, the price will fall. A number of public and private sources, including venture capital funds and internal resources from big organizations, have financed notable work in critical areas including safety, research, and development.

Development of biomass feedstocks with lesser lignin content and higher cellulose content.
Development of enzymes like cellulases and hemicellulases that depolymerize the biomass to achieve higher activity thus increasing the content of fermentable sugars and also reducing the content of inhibitors.
Development of novel microorganisms through metabolic engineering that can show resistance towards high ethanol, high temperatures, and can ferment pentoses along with hexoses.

This assistance has aided in moving it forward; nevertheless, additional assistance is required to make it a reality.

CONCLUSION

Technological innovations driven by the use of fossil fuels have enhanced human civilization and made it more comfortable; yet, this comfort has come at significant expenses. Biofuels have evolved to represent one of the main crucial sustainable fuel sources, and they are viewed as vital in our attempts to reduce greenhouse gas emissions, enhance environmental wellbeing, and create innovative energy sources. Biofuels, unlike fossil fuels, are generated from organic sources like biomass, which includes plants, crops,

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and agricultural waste. Furthermore, because of the abundance of agricultural feedstock such as forest residues, bagasse, native plants & fibers, corn stover, SPRs, bast crops & so on, developing countries, such as India, can be targeted for socioeconomic development using a biorefinery approach. Some of the obstacles that are identified that could hamper the biofuel economy are, the lack of refueling structure; costly zero-emission biofuel extraction; some forms of biofuel-based automobiles exhibit technology gaps, fuel cell lifespan. However, owing to a lack of knowledge and inadequate valuation, these useful wastes are often destroyed in large quantities for waste management rather than being used constructively. These wastes can be employed as a raw material in numerous investigations and industries, as well as an alternative source for the manufacture of various products such as biogas and biofuel. Adapted biogas processes are paving a new path to mitigate these swings by adapting operating and storage principles, whether that is through feedstock, biogas, or power, in response to changing net energy requirements.

When spoken about the future of Biofuels, the Country's developmental aspects for biofuel usage are influenced by factors like environmental worries on net carbon emissions, as well as security risks on the reliability and expense of fuel imports. Next few decades, the development of more efficient biofuel technologies might double or triple yields, as well as raise the energy output/input ratio in some of the less efficient bioenergy crops like maize or wheat. The biofuel sector is notably upsetting food production and prices in several parts of the world, a measured evidence-based assessment of biofuels that addresses a wide range of socio-economic and technological gaps in the biofuel sector. It has to be noted that biofuels are essentially economic, as biofuels long-term viability is highly reliant on their profitability. Elements like environmental and economic stability, as well as how technology permits these items to evolve must be taken into account. Biofuel production is now most viable in ecologically limited tropical locations due to the increased biofuel yields per acre and cheaper input prices. Also, Biofuel production has a primary profit element, which is based on resources that would substantially undercut the rise in oil prices. If nations throughout the world can use biofuels in the coming years, it will have a tremendous influence on the global economic structure and environment.

ACKNOWLEDGMENT

Kondapalli Vamsi Krishna and Sompalli Bhavana would like to acknowledge the fellowship from KSTePS, Department of Science and Technology (DST), Govt. of Karnataka for fellowship to pursue their Ph.D. work. Koushik Koujalagi would like to thank Christ (Deemed to be University) for a Ph.D. fellowship.

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Chapter 7
Biogas: Renewable Natural Gas

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ABSTRACT

Biogas is a renewable natural gas used in production of energy which is generated by breakdown of organic matter by anaerobic digestion. It is a composition of methane, carbon dioxide, and some other gases in small quantities. The most common natural resources for production of biogas are aquatic sediments, animal waste, crop residues, wastewater sludge, and many others. Some other sources are landfills, water lagoons, etc., which are a result of human activity. There are several technologies used for biogas production, mainly biodigesters, wastewater treatment plants, and landfill gas recovery systems. Production of biogas is highly dependent on the feedstock availability and the policy support by the government.

INTRODUCTION

Fossil fuels are among the most highly used sources of non-renewable energy which lead to 80% of the global needs. To attain sustainable economic growth, antagonistic and fast-paced deployment of renewable resources is needed. One of the most common sources of energy is fossil fuels. To sustain the non-renewable sources, we need accelerated and antagonistic deployment of efficient energy sources by the second half of the century (IRENA, 2017a). The use of efficient bioenergy conversion and modern technologies are gaining high importance recently which are used to fulfill the demand of global energy

DOI: 10.4018/978-1-6684-5269-1.ch007

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needs. Biogas is one of the most cost-effective and ecologically beneficial forms of renewable energy (Deublein & Steinhauser, 2011). It could be a viable option for the world's insatiable energy demand while also reducing waste and greenhouse gas (GHG) emissions. Anaerobic digestion may transform sewage sludge, agricultural and crop leftovers, animal dung and industrial organic wastes, and wastewaters into biogas. The type of the raw materials used for digestion, as well as the pH of the medium, influence the composition of biogas (Muoz et al. 2015). Under anaerobic conditions, bacteria decompose complex organic matter into simpler components, resulting in biogas and fertilizer (Gemmeke, 2009 & Piwowar, 2020). The technique of using anaerobic respiration for the production of biogas has numerous environmental benefits. It helps to treat organic wastes along with introducing green energy, protecting the environment, and also helps to reduce greenhouse gas emissions (Falk, 2011; Theuerl, 2019; Daniel, 2017; Gemmeke, 2009; Stolze, 2017]. There are four phases to the biodegradation of complex organic compounds. Hydrolysis, acidogenesis, acetogenesis, and methanogenesis, are the respective steps (Rohstoffe, 2012). The composition of biogas has a higher concentration of methane which generally makes up to 50% to 75%. This methane content makes the biogas flammable and also generates a blue flame which can be used as an energy source.

The calorific value is fixed on the basis of the concentration of CH_4 as the other substances do not play a major role in the energy content. The calorific value of raw biogas is 21MJ/m^3 considering that the density of 1.22 kg/Nm^3 CH_4 present in the raw biogas is up to 50% which is also similar to that of air (1.29 kg/Nm^3) (Rutz and Janssen 2008). So as to know more about the various applications of biogas, it is a must that the concentration of CH_4 is appreciably higher (i.e. >90%). This also states that the utilization capacity of any biogas is determined by the composition of the gas. Upgrading biogas is a widely used, well-developed, and easily obtainable technique. It is becoming more widely used around the world, although it is still in its infancy when compared to the biogas producing industry. After treatment, biogas can be used for a variety of purposes, including electricity and heat generation, natural gas grid connection, and automotive biofuel (Rosen T, 2019).

BIOLOGICAL REACTIONS

Hydrolysis, acidogenesis, acetogenesis, and methanogenesis, are the procedures followed after then numerous complex biochemical reactions and as a product of these, biogas is formed (Ghodrat et al., 2018). The complete reaction of conversion of food wastes into biogas is as follows (Deublein & Steinhauser, 2011):

$$C_cH_nO_oN_nS_s + wH_2O \rightarrow mCH_4 + nNH_3 + sH_2S + (c-m)\ CO_2$$

where, m= ⅛ (4c+h-2o-3n-2s)

w = ¼ (4c-h-2o+3n+3s)

Carbohydrates ($C_6H_{12}O_6$), proteins ($C_{13}H_{25}O_7N_3S$), and lipids ($C_{12}H_{24}O_6$) come under the degradable fraction of the food waste.

Individual stages are controlled by various microorganism consortia, which have syntrophic interactions with the microbial consortia participating in the other stages. On hydrolyzing bacteria excrete

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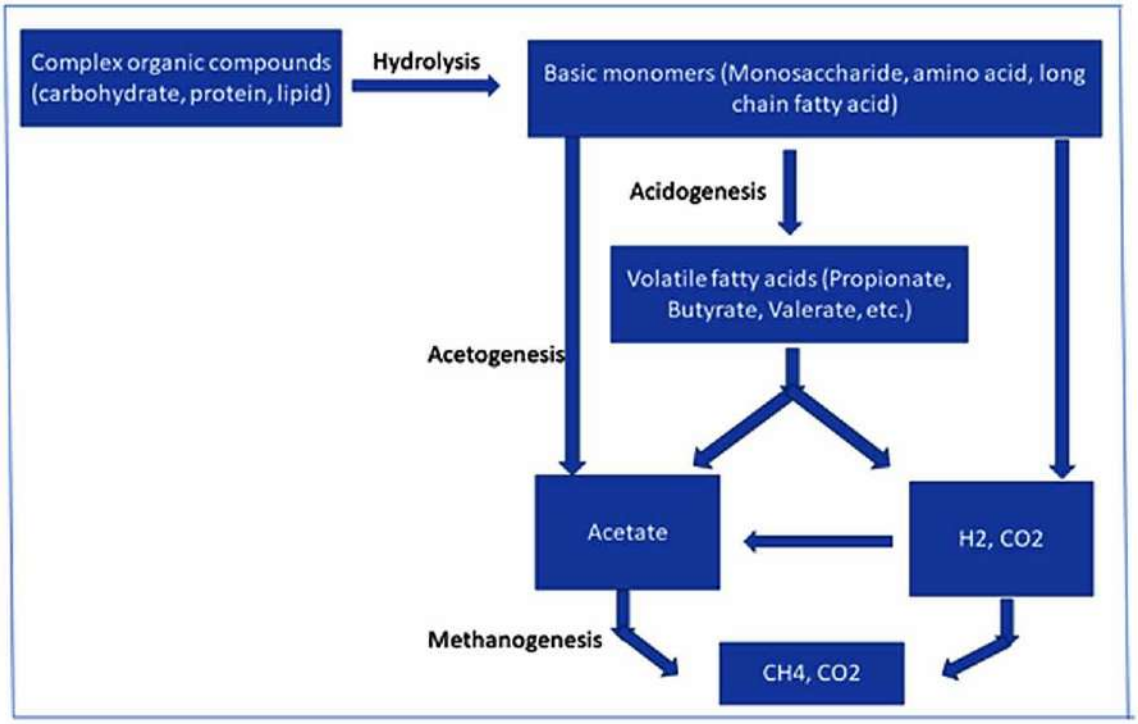
exo-enzymes such as cellulase, protease, lipase, amylase, and xylanase, initiation of hydrolysis is done. These hydrolytic exo-enzymes are absorbed in the surface of the substrate which leads to the progressive conversion of the polymers to monomers and then further to oligomers (e.g., glucose, fatty acids, glycerol, and amino acids). The enzymes used in all these conversions are water-soluble. Hydrolysis is the stage that is considered to be the restricting stage in the biogas production from the wastewaters which contains a substantial number of recalcitrant compounds such as lignocelluloses.

The launched monomers and oligomers are then degraded to quick-chain fatty acids (propionate, acetate, butyrate, and lactate), alcohols, and gaseous byproducts (NH_3, H_2, CO_2, and H_2S) via the acidogenesis stage. All the unnecessary oxygen present can be broken down and digested with the help of facultative anaerobic microorganisms which are present inside the first 2 degrees. The anaerobic environment present inside proves to be obligatorily for the microorganisms. On the 0.33 level. All the organic materials which were formed in the last stages are then transformed into hydrogen, carbon dioxide, and acetic acid. Methanogens present then produce methane from either CO_2, acetate, or methyl under strictly anaerobic conditions.

PROCESS OF BIOMETHANATION

The anaerobic biological conversion of organics substances occurs in 4 steps. The four levels of methane fermentation are shown in Fig.1.

Figure 1. Steps of anaerobic digestion process- anaerobic treatment of industrial effluents



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1. Hydrolysis

The anaerobic digesters usually encounter the organic biomass which consists of many compound polymers which the microorganisms have to break down. As they are not pretreated or hydrolyzed, it is expected that the microorganisms break these complex compounds into simpler forms (Gujer W, 1983). In the stage of hydrolysis, the microorganisms break down the organic macromolecules into much smaller compounds which are then used up by the acidogenic bacteria for anaerobic digestion.

In hydrolysis, the hydrolytic bacteria secrete extracellular enzymes which are capable of converting carbohydrates, lipids, and proteins to sugars, long-chain fatty acids, and amino acids respectively (Daniel, 2017). Once the enzymatic cleavage is formed, the products formed in the step of hydrolysis are diffused through the cell membrane of the acidogenic microorganisms (Gemmeke, 2009). For complex substances (such as cellulose, lignin, hemicellulose, etc.) enzymes are added to the process of hydrolysis of carbohydrates as these cannot be hydrolyzed by the microorganisms alone (Stolze, 2017).

Prior research has suggested that hydrolysis is a rate-determining step. It was found in the previous research that methanogenesis can be said to be the rate-determining stage which is dependent on the ratio of the rate of hydrolysis of methanogenic microorganisms (Lin, 2010; Luo, 2012).

For all the steps which take place in hydrolysis, there is an optimum temperature and pH at which the hydrolytic activity is at its peak. At 30-50°C and pH 5-7 are the optimal temperatures and pH respectively for hydrolysis. It has not been found yet whether or not the process of hydrolysis is improved below a pH of 7 (Huang, 2015).

2. Acidogenesis

Volatile Fatty Acids (VFA) and all other products are produced by acidogenic microorganisms by absorbing the products during the process of hydrolysis. This absorption takes place from their cell membranes.

The concentrations of the intermediates of the product in the acidogenesis stage majorly depend on the condition present in the digester. It has been observed that under different pH conditions, the VFA also fluctuates significantly (Huang, 2015; Wu, 2010).

Usually, the VFA production from amino acids is examined in protein-rich wastes such as sewage wastewaters. The process of anaerobic digestion can be inhibited by the presence of ammonia which can be formed after the deamination of amino acids at high concentrations (Kovacs E., 2013; Park J, 2014).

3. Acetogenesis

Acetate is produced through the step of acidogenesis, the original substrate is likely to be rendered into the substrate which is suitable for acetoclastic methanogenesis (Fournier G.P, 2008). The process of conversion of the larger quantities of VFAs along with other intermediates into acetate in which hydrogen is produced as well is known as acetogenesis (Hansen C.L., 2013).

Hydrogenotrophic methanogens present in the digester help to consume the additional hydrogen produced and also maintain the partial pressure at favorable levels for the continuation of acetogenesis which is done by creating exergonic reactions (Stams, A.J.M., 2009). While the partial pressure is being maintained, the lipids present go through a completely different process of acetogenesis via β-oxidation and acetogenesis. While acetogenesis leads to the production of acetate from glycerol and β-oxidation helps in the production of acetate from LCFAs (Cirne D.G., 2007).

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4. Methanogenesis

Methanogenesis is the final step of anaerobic digestion. So as to produce methane gas, the intermediates accessible are all consumed by methanogenic organisms present in the batch (Ferry, J.G., 2010) [27]. The group of obligate anaerobic archea represents the methanogenic microorganisms which are the testers for detecting acute sensitivity to oxygen levels. *Echinococcus vannielli* and *Methanococcus voltae* were the microorganisms that were found. 99% of these were killed within 10 hours of them being exposed to oxygen (Kiener A., 1983).

Roughly around ⅔rd of the methane production from acetate is accounted for by acetoclastic methanogenesis, while hydrogenotrophic methanogenesis accounts for the rest ⅓rd methane production. It has also been observed that methanol, methylamines, and formate also undergo methanogenesis (Belay N., 1986 & Lovley D.R., 1983).

When the biogas production stops, which can even take up to 40 days it is considered the end of the methanogenesis in the batch reactors (Theuerl S., 2019). The volatile solid content and the ability to dewater the sludge can help to evaluate the sludge's extent of digestion.

PROCESS PARAMETERS

Along with all the biological processes, the consistency of the conditions provided is important. There are several factors such as temperature changes, changes in the substrate concentration, etc. which can affect the production of biogas. The optimum condition for the microorganisms which affect the degradation of the microbes can depend on two different stages: the first stage is for hydrolysis/ acidification and the second stage is for acetogenesis/ methanation. This is stated, given that the entire process of degradation takes place in the same reaction system and that the appropriate conditions for methanogenesis must be fulfilled, because if they don't receive the optimal conditions then there is no chance of their survival within the mixed culture as their growth rate is also very less along with the high sensitivity to environmental factors.

1. Hydrogen Partial Pressure

So as to get an undisturbed process between the acetogenic bacteria producing H_2 and the H_2 consuming bacteria, a restricted spatial symbiosis is required. The concentration of hydrogen should be properly balanced: for the production of methane, methanogenesis requires a sufficient amount of hydrogen. However, it is proved that the partial pressure of hydrogen surrounding the acetogenic bacteria should be less, causing them to stop the production of hydrogen. The substrate and the bacteria present determine the maximum permissible hydrogen pressure.

2. Concentration of Microorganisms

Since the step of methanogenesis has a lower growth rate, biogas plants usually are considered to have a longer start phase. This can also take around 3 months in which the necessary amount of sludge which is inoculated, will start the plant at its full capacity as fast as it can, as the buildup is not available at the starting phase.

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3. Type of Substrate

The rate of anaerobic degradation is measured by the substrate, and it must be taken into consideration for the process of operation and technology. The microorganism's metabolism tends to run out once the substrate component runs short. Hence, it is important to feed the batch lacking substances (carbohydrates, fat, proteins, mineral substances, and trace elements) and also the substrate. For example, sugar hydrolyzes and acidifies in a really short span of time. Whereas cellulose is substantially slow and can take a pretty long time to degrade. Even medium- and heavy-weight degradable compounds hydrolyze and are eventually transformed to methane after 20 days or longer of residence.

4. Light

Methanogenesis is not killed by light, although it is substantially hampered by it. As a result, the creation of methane should take place in complete darkness.

5. Temperature

Mesophilic (35˚C) and thermophilic (55˚C) are the two different temperatures that are used for the process of anaerobic digestion. The mesophilic digesters are preferred over the thermophilic digesters even though the mesophilic digestion takes place at a much lower temperature and hence is slower than thermophilic digestion and yields less biomass. Mesophilic digesters are preferred due to their lower heater energy costs (Moset V., 2015). Thermophilic digestion takes place at a higher temperature compared to mesophilic digestion. Consequently, the rate of reaction is increased which leads to higher loading rates and hence an increase in the production of biogas (Moset V., 2015 & Shi, X. S., 2017). This thermophilic digestion is believed to have higher levels of pathogen clearance, which could be advantageous in some jurisdictions where pathogenic activity in effluents is regulated (Smith, 2005).

6. pH

There are no pH buffer ions which are released which degradation of the proteins and the hydrocarbons are also easier to acidify. Hence the value of pH is observed to decrease easily. As the degradation of carbohydrates and other substances takes place, the partial pressure of hydrogen increases. With the increase in the hydrogen pressure, the formation of acidic intermediate products is reduced. pH is kept in the neutral range by some natural processes in the fermenter.

Disturbance in the fermentation process can be checked by the drop in the pH and increase in levels of CO_2. The very first sign of acidification occurring is the increase of the propionic acid concentration.

7. Carbon and Nitrogen Ratio

During the process of anaerobic digestion, not much quantity of biomass is produced hence the need for nutrients is also comparatively low. The substrates which have a lesser C/N ratio will lead to an increase in the production of ammonia and inhibition of methane production. The high C/N ratio indicates the lack of nitrogen which leads to negative consequences on the protein formation mechanics and hence changes are observed in the structural, energy, and material of the microorganisms. Therefore, it is nec-

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essary to maintain the balance. For example: In China, a mixture of latrine waste and rice straw is used and in Nepal, co-fermentation occurs between human waste and elephant dung.

8. Biogas Removal

The degassing nature of the substrate is of utmost importance whenever the produced gases lead to metabolism inhibition. The transfer of materials from liquid to gaseous phase can be affected by any technical means. The $K_L a$ value of the produced biogas can be increased by 50% by this transfer from liquid to gaseous phase and inhibition of the microorganisms by H_2S can be reduced.

INHIBITORS

Composition of the substrate, concentration of inhibitors, and adaptation of bacteria to inhibitors help to determine the inhibition. Less quantity of inhibitors is needed for anaerobic bacteria as they degrade if high amounts of inhibitors are present. It has been observed that usually the anaerobic processes are mostly adaptable and hence relatively insensitive to inhibitors and also to toxic concentrations under other conditions.

REACTOR DESIGNS AND OPERATIONS

Below mentioned are the criterion which needs to be considered before choosing the optimum biogas reactor's design:

- Dry matter (DM) content of the substrate: Wet digestion (DM < 12%) and dry digestion (DM > 12%)
- Material feeding mode: semi-continuous (i.e. addition once per working day), intermittent (i.e. no addition of substrate during dwell period).
- Number of process phases: Single-phase (all steps take place in the same reactor) and two-phase (hydrolysis and methanogenesis take place in separate reactors)
- Process temperature: Psychrophilic (<25 °C), mesophilic (37 to 42 °C) and thermophilic (50 to (50 to 60°C) (Rohstoffe, 2012).

CONCLUSION

Various organic wastes can be easily treated by the process of anaerobic digestion and the production of biogas. There are several environmental and economic benefits of the biogas production. Production of biogas is affected by factors such as the type of organic waste, type of bioreactor, pH, temperature, levels of oxygen etc. The aim of this book chapter is to present a summary of the process of biomethanation, process parameters, and the factors for reactor designs and operations. A review of the literature suggests that there is a considerable prospect of increasing biogas generation in the field. The use of certain inorganic and organic additives to boost biogas production appears to be promising.

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Chapter 8
Algae as Superfood

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ABSTRACT

With the increasing population, there are problems like the shrinking of arable land, pollution, and feeding the entire growing population high-quality food and reaching consumer demand, and the list goes on. Novel superfoods are rich in nutrition and produced with the most sustainable methods, including algae, specifically microalgae. Microalgae have been studied for decades. Microalgae are one of the largest and most poorly understood organisms on planet earth, but in recent years, the research has been increased for the search of renewable and sustainable energy sources. Microalgae don't require much land for cultivation and thus do not compete with conventional agricultural land. Microalgae can double their size in 24 hours. CO2, solar rays, inorganic nutrients, and water are the basic requirements for them to grow. There are microalgae and macroalgae (e.g., seaweed) that grow in saline and hypersaline water. This chapter presents the promising potential of algae (microalgae and macroalgae) as a superfood.

BACKGROUND

In the year 1753 Carolus Linnaeus a Swedish botanist coined the term algae. Algae come under a division of the plant kingdom called Thallophyte which means, simple plants without roots stems, or leaves known as thallus. Algae are eukaryotic in nature but blue-green algae (cyanobacteria) are prokaryotic in nature. The composition of the eukaryotic algal cell consists of the cell wall, nucleus, endoplasmic reticulum, mitochondria, Golgi bodies, stigma or eyespot, vacuoles, flagella, and plastids.

DOI: 10.4018/978-1-6684-5269-1.ch008

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COMPOSITION OF ALGAE

The cell surface plays a very vital role in both the external and internal sides of the cell. Various functions of cell surface include cell interaction, cell signaling, excretion and secretion of various compounds, protection, and servers as an osmatic barrier. The cyanobacteria are enveloped by two parts of the gelatinous sheath and cell wall. The sheath is very slimy in nature which acts as a protective layer. Due to their slimness blue-green algae are also called my xophyceae. Algae also consist of a cell wall outside the plasma membrane, it presents a granular structure and lacks microfibrils. The cell wall is composed of Glycosamine, muramic acid and diaminopimelic acid. It has a structure of gram-negative type.

Protoplast

Cyanobacteria lack chloroplast but have Chromoplasm. Protoplasm is totally differentiated into two regions.

1. Centro Plasm (Central Area)

It is a clear and transparent region of the protoplast that contains chromatin or genetic material.

2. Chromoplasm (Peripheral Denser Region)

In this region, thylakoids are present which consists of chlorophyll A responsible for providing green color in blue-green algae, and several types of carotenoids are embedded into it. thylakoids are not restricted to peripheral reagion they are found scattered all over the cell (*Gloeotrichia* sp). phycobilins are on the surface of thylakoids. The best-known phycobilins are phycoerythrin and phycocyanin. Phycobilins are water-soluble whereas chloroplast and carotenoids are fat-soluble. phycocyanin C is the characteristic of blue-green algae and is responsible for the blue color in blue-green algae.

Gas Vacuoles

Tiny, transparent structures which are commonly found in bacteria and arches. Gas vacuoles are responsible for the floating of algae. Nitrogen and oxygen are majorly found gases but also have a minute amount of CO and CO2 which is liberated from metabolic activity. Gases are produced when there is not enough sunlight reaching the algae, especially in deep-underwater algae (*Trichodesmium sp*), they have low photosynthesis, low osmotic pressure, low production of carbohydrates and therefore increasing the density of the cell. Under these circumstances, gases are produced at a high rate and gain buoyancy.

Eukaryotic Cell Composition

The eukaryotic algal cell is simple, unicellular, motile, freshwater algae. It consists of membrane-bound organelles.

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Cell Wall

Most of the algae have a cell wall made up of multiple microfibrils like cellulose, callose, pectin, chitin, and hemicellulose. In a few marine forms of algae, lime and silica are found as cell wall components because of the environment they have adapted to grow.

Plastids

Plastids are pigments that contain photosynthetic pigments. They are the site for the manufacture and storage of chemical compounds. Plastids in algae are slightly different from plant plastids that is the presence of pyrenoids in algae and absent in plants. Plastids are differentiated based on the pigment they contain, and they are chloroplast and chromophore. Chloroplast consists of chlorophyll A and chlorophyll B (e.g., *chara* and *volvox*). Chlorophyll A is common for all the algae present for photosynthetic reaction. Chromophores have chlorophyll A and other carotenoids (*sargassum, laminaria*), and these carotenoids are responsible for a different color. Carotenoids are light-harvesting pigments, they capture the light and pass it on to the light reaction centre (chlorophyll A).

Flagella

It provides motility to the algae. These are emerging from the cytoplasm. Different variations of flagella can be found. Whiplash flagella (acronematic) have a smooth surface. While tinsel flagella (pantonematic) have a rough surface with many rows of minute hair-like structures grown to the main flagellum strand. Algae can be either of this flagellum are they may have both. isokont flagella are when flagella are of similar length and heterokont flagella are when flagella are not equal in length.

Eyespot

Photoreceptive organs are found associated with flagella and chloroplast (e.g., *Chlamydomonas*) and help the organism to sense the light and move in the direction of light.

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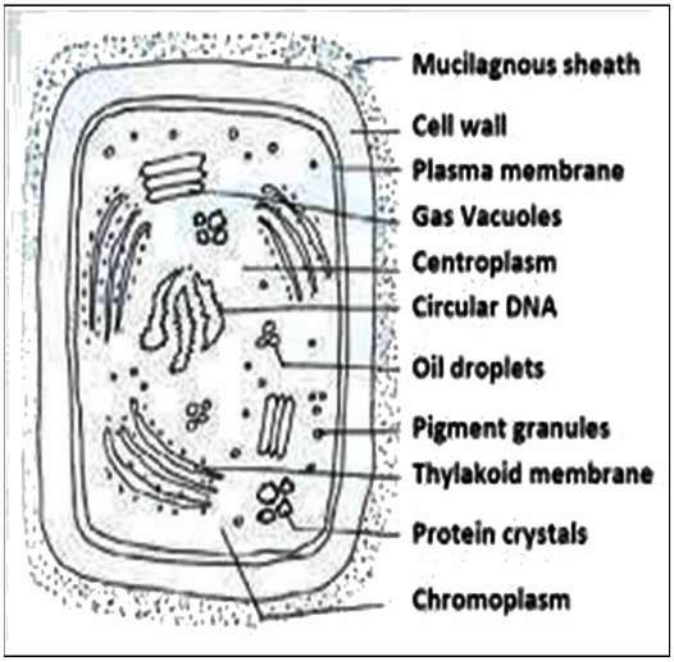
Figure 1. Cellular structure of algae



COMPOSITION OF SPIRULINA

The composition of spirulina is unique and extraordinary that it is called a superfood. Spirulina is composed of carbohydrates, protein, fats, vitamins, pigments and minerals. They also help in combating various kinds of diseases like anemia, vascular diseases, obesity, hepatotoxicity and certain cancers.

Protein

The protein content in spirulina is high which is 50 to 70 g/100 g of dry matter. Spirulina protein id totally different from other organisms which provide protein because spirulina doesn't contain any hard envelope like cellulose but has a very fragile envelope and has higher digestibility up to 83%-90% which makes it easier for people to consume and digest. It also contains Isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine are necessary amino acids that cannot be synthesized by the human body. People with a vegetarian diet don't usually have sufficient lysine and methionine. It is known a complete protein because of its amino acid composition as it has every amino acid (essential and nonessential). The protein content of various foods: Eggs 13%, Soybean 42%, Grain 29%, Peanuts 25.3%, Spirulina 60%-70%.

Carbohydrate

Carbohydrates are considered a great source of quick energy for our body. Spirulina consists of 10%-15% in the form of polysaccharides. Glucose, fructose, and sucrose - are found in little quantity. Carbohydrates

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offer 50-70 percent of the body's total energy requirements. Meso inositol phosphate is a carbohydrate present in larger and it consists of c phosphorus and inositol.

Lipids

Water insoluble lipids are soluble in organic solvents such as ether and chloroform. Dairy, cheeses, olives, walnuts, canola, soybeans, and flaxseed are the most prevalent sources of lipids. Spirulina has a lipid content of 5-6% of its dry mass. Many of the food sources which have fatty acids content are majorly meat products like beef, chicken, fish, egg, etc. spirulina is a vegetarian source that has the highest amount of fatty acid in it. Spirulina has gamma- linolenic, linoleic, and oleic acids in them. Gamma-linolenic acid is known to have the ability to suppress cancer growth.

Vitamins

Vitamins are organic compounds required by the body in the trace amount to perform a specific cellular metabolic activity to keep the body in functional condition. They cannot be synthesized by the human body, and therefore they must be supplied through the diet. Spirulina contains a vitamin known as vitamin B12 (COBALMINE) it is found in coordination complex with cobalt. Spirulina has the highest content of vitamin B12 compared to other seaweeds found in the water. Usually, vitamin b12 is prominently found in meat-based food, and people who consume a vegetarian diet lack this vitamin. Beta-carotene is abundant in spirulina., it contains700-1700 mg/kg. Recommended daily allowance of vitamin A per day is 1 mg per day. Consumption of 1-2g of spirulina is enough to gain enough beta-carotene.

Minerals

Minerals are essential components for the body to function properly and are required in trace amounts and are found in all kinds of food we consume. Particularly in iron, calcium, phosphorus and potassium are found in high amounts. Food with high iron content is hard to find, Cereals are considered one of the good sources of food with high iron content in them (150-250 mg/kg) but they also contain phytates, and oxalates are known to suppress the bioavailability of iron.

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Figure 2. Spirulina cultivated at lab scale level

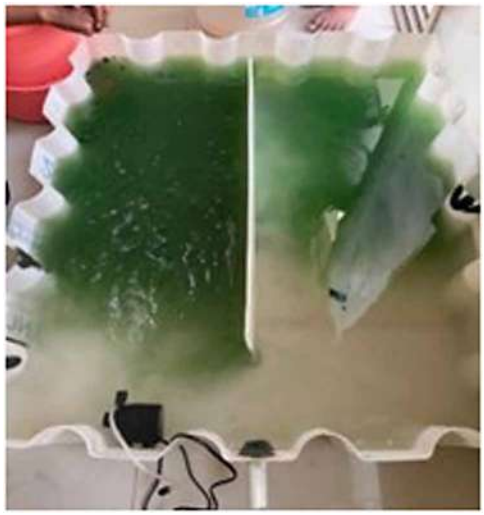

Table 1. Amino acid profile of spirulina

Amino Acids	% of Total Amino Acids
Alanine	7.9
Arginine	7.3
Aspartic Acid	10.1
Cystine	1.2
Glutamic Acid	14.7
Glycine	5.4
Histidine*	1.9
Isoleucine*	5.6
Leucine*	8.7
Lysine*	5.3
Methionine*	2
Phenylalanine*	4.9
Proline	4.2
Serine	5.2
Threonine*	3.4
Tryptophan*	1.6
Tyrosine	4.5
Valine*	6.1

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Algae are main producers that provide a rich source of several nutrients. While the high protein content of diverse species is one of the primary reasons for considering them as an unusual source protein, microalgae oils rich in several PUFAs appear to be especially good for youngsters, pregnant women, vegetarians, and people with seafood allergies. Because algae are a rich source of vitamins, minerals, antioxidants, and natural colorants, incorporating the entire biomass into food and feed might give color, increase nutritional value, and enhance texture and oxidation resistance.

Combining microalgae biomass into traditional foods is a means to create novel items that are both appealing and healthful. Even when used in small quantities in the nutrition of various animals, algae have been given credit with immune health, rising weight, the amount of eggs, reproductive performance, or reducing cholesterol, indicating the potential of future farming methods to improve the quality of meat and eggs. It's also not unexpected that they're important in aquaculture because they're food supply for these creatures. Finally, microalga Spirulina, humanity's greatest and most perfect resource of organic nutrition, has become a nutritious food globally due to its high protein content and many bioactive components.

heterotrophic. In a scientific sense, the term algae refer to a group of reduced plants that contain chlorophyll in their cells and are common in oceanic biotopes, since they are also found in other environments (Blazencic, 2007). They are divided into macroalgae (macroscopic algae) and microalgae (microscopic algae) based on their size.

Blue-green algae, often known as cyanobacteria due to their prokaryotic cell type, are a form of microalgae that has been identified as one of the most promising living organisms for the separation of new and biochemically active bioactive substances (Singh et al., 2005). Algae are grown and utilised as food all around the world. They are rich in vitamins and minerals, enzymes, protein, unsaturated fats, antioxidants, and other nutrients. (Pulz and Gross, 2004; Svircev, 2005; Blazencic, 2007; Gouveia et al., 2008b).

Chemical Composition of Algae

One of the key reasons for considering microalgae as an unusual source of proteins is their high protein content, and because they can synthesize all the amino acids, they could also be a supply of essential amino acids. Furthermore, in comparison to normal plant proteins, the general standard of most studied algae is comparable, if not greater (Becker, 2007). Dried Spirulina biomass, for example, provides all necessary amino acids and has roughly 68 percent protein, which is three times more than beef. Chlorella, another microalga, has 50-60% protein, which is equivalent to yeast, soy flour, and milk powder proteins in terms of quality (Blazencic, 2007). Milovanovic et al. are a group of researchers that came up with a novel way to solve Milovanovic et al. (2012) looked examined the protein content of dry biomass from different cyanobacterial strains from Serbia, and found that they all have very high levels, ranging from 42.8 percent to 76.5 percent. Algae also contain a lot of fibre. Polysaccharides such as agar, alginates, and carrageenan, which are widely utilized in the food sector as gelling or thickener agents in marmalade, ice - cream, and jellies, are the most commercially important products from algae. Because they boost the human immune system, some algal polysaccharides are also beneficial in pharmacology.

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ALGAE IS A SOURCE OF VITAMINS AND MINERALS

Algae is abundant in vitamins and minerals, making it a good choice for supplements. Most cultivated plants have lower vitamin content than chlorella species (Blazencic, 2007). Furthermore, the Spirulina genus has roughly ten times more -carotene than any other plant or animal nutritional source, including carrot (Mohammed et al., 2011), as well as much more vitamin B12. In comparison to green algae, leafy greens, and liver, this genus has the greatest vitamin E, thiamine, cobalamin, biotin, and inositol (Gantar and Svircev, 2008). Tocopherol is produced in large quantities by a number of microalgal species (T, the most physiologically active form of vitamin E). Rodriguez Zavala et al. (2010) reported that after 120 hours, the synthesis of -tocopherol in the heterotrophically cultivated microalga Euglena gracilis reached 3.70.2 mg/g, which is roughly 13, 18, 95, and 56 times more than sunflower, soybean, olive, and maize, respectively (some of the most common natural sources of vitamin E).

Algae as a Component of Functional Food

In industrialized nations, the food is rich in calories, which, when combined with a contemporary lifestyle, contributes to health issues such as obesity, heart disease, diabetes, and other ailments. As a result, there is a need for food items that can improve health by supplementing the diet with vitamins, minerals, PUFAs, and other nutrients, and consumers are increasingly preferring natural components over artificial ones. Algae are a unique natural supply of biologically active chemicals, yet they are under-explored. Beta1,3-glucan, a powerful immunological stimulant, free radical scavenger, and blood lipid reduction, appears to be the most essential ingredient in Chlorella (Spolaore et al., 2006). The antibacterial, antiviral, anticancer, and food additive properties of cyanobacteria have long been known (Singh et al., 2005), and drug development is the most potential element of microalgal biotechnology (Spolaore et al., 2006).

Spirulina is a freshwater and saltwater organism. It's a cyanobacterium, a single-celled microorganism that's also known as blue-green algae. Photosynthesis is a process through which cyanobacteria, like plants, may make energy from sunlight. Spirulina was first ingested by the Aztecs, but it gained popularity again after NASA claimed that it might be cultivated in space for astronauts to consume (1). Spirulina is usually taken in dosages of 1–3 grammes per day, however, amounts as high as 10 grammes have been shown to be helpful. The nutrients in this small alga are plentiful. A spoonful of dry spirulina powder (7 grammes) includes:

- Protein: 4 grams
- Vitamin B1 (thiamine): 11% of the RDA
- Vitamin B2 (riboflavin): 15% of the RDA
- Vitamin B3 (niacin): 4% of the RDA
- Copper: 21% of the RDA
- Iron: 11% of the RDA

It also has enough levels of magnesium, potassium, and manganese, as well as trace amounts of nearly all other nutrient you require.

Furthermore, the same quantity has just 20 calorie and 1.7 grammes of digestible carbohydrates. Spirulina may be the most nutrient-dense food on the world, gramme for gramme. Spirulina protein is believed to be of great quality, equivalent to that of eggs. It contains all the necessary amino acids that

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your body need. Spirulina is frequently said to contain vitamin B12, however this is untrue. It contains pseudo vitamin B12, which hasn't been proven to work in people.

Properties

Antioxidant and anti-powerhouse Chronic inflammation, which leads to cancer and other disorders, might be triggered by this damage. Spirulina is high in antioxidants, which defends the body from oxidative damage. Phycocyanin is the principal active ingredient. Spirulina's unusual blue-green hue is due to this antioxidant component. It has the ability to lower bad Low density lipoprotein and triglyceride levels. Cardiovascular disease is the biggest cause of mortality worldwide. A variety of risk factors have been linked to an increased risk of heart disease. Spirulina, it turns out, has a positive impact on a number of these traits. Total cholesterol, bad LDL cholesterol, and triglycerides can all be reduced, while good HDL cholesterol can be increased, for example. Two grams of spirulina each day significantly improved these indicators in a trial of 25 persons with type 2 diabetes. Several additional research have demonstrated that larger dosages of 4.5– 8 grammes per day had beneficial benefits. Oxidation of Bad LDL Cholesterol is prevented. Your body's fatty structures are vulnerable to oxidative damage. This is called as lipid peroxidation, and it is a major cause of a number of severe disorders. The oxidation of bad LDL cholesterol, for example, is a crucial phase in the development of cardiovascular disease. In humans and animals, the antioxidants in microalgae appear to be particularly efficient at reducing lipid peroxidation. 8 grammes of spirulina each day lowered oxidative damage indicators in 37 persons with type 2 diabetes in research. It also raised blood levels of antioxidant enzymes.

The Impact of COVID-19 on the Spirulina Market

Many economies throughout the world have been negatively impacted by the COVID-19 outbreak. Many businesses were badly impacted by government attempts to battle the COVID-19 epidemic, such as statewide lockdowns and quarantines. COVID-19 continues to have an influence on the majority of food manufacturers in the F&B industry. Many businesses have been forced to close across Asia, the United States, Europe, and the entire world due to social alienation, self-isolation, and town lockdowns. Consumer food and beverage producers are facing issues such as decreased demand and disturbed supply networks. The food sector has suffered a modest setback as a result of the trade embargoes implemented by a number of nations. This situation has had a detrimental influence on the confectionery sector since customers are only purchasing the most basic items. Furthermore, government agencies enacted a slew of rules and regulations governing the acquisition of basic items. The majority of customers are just purchasing necessities, which has resulted in a considerable reduction in sweets consumption. According to trade organizations in the nutraceutical industry, there has been a major effect on many locations in the global supply chain for functional foods and supplements, which has lowered spirulina biomass consumption among end-users and impacted the spirulina market's growth.

Humans have long ingested microalgae since Spirulina was gathered from Lake Texcoco and sold in Tenochtitlan markets as early as the sixteenth century (today Mexico City). Microalgae is now being used in a wide range of culinary products. Microalgae is used by the majority of these companies as a marketing technique or as a coloring agent. Spirulina (and chemicals produced from it) has the potential to be employed as components in the production of new functional meals, which is one of the most popular culinary trends. Numerous human intervention studies have shown that Spirulina has the potential to be

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employed in the prevention and treatment of metabolic syndrome illnesses. T. Spirulina consumption has been linked to a variety of health advantages, as well as some of one of most significant substances obtained from Spirulina.

Figure 3. Microscopic photo of cultivated spirulina

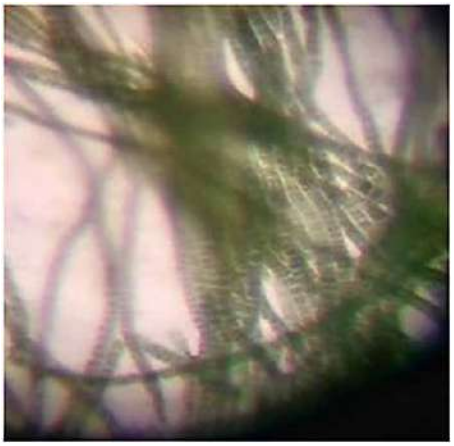

Table 3. The main fatty acids in two spirulina strains, Spirulina maxima and Spirulina platensis

Fatty Acids	Spirulina Platensis (% of Total Fatty Acids)
Alpha-linolenic (18:3 omega-3)	traces
Gamma-linolenic (18:3 omega-6)	40.1
Linoleic (18:2 omega-6)	12
Oleic (18:1 omega-6)	16.6
Stearic (18:0)	1.7
Palmitoleic (16:1 omega-6)	3.8
Palmitic (16:0)	25.8

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Table 4. Spirulina's vitamin content and adult daily vitamin needs

Vitamins	Content (mg/kg)	Daily Requirement (mg) (Adult, 24-25 Years)
C	traces	15-30
Biotin	0.05	0.1-0.3
Pantothenate	4.6-25	6-10
Folate	0.50	0.40
Niacin	130.00	20.00
B12	1.5-2.0	0.003
B6	30-46	1.80
B1	34-50	1.50

Figure 4. Chocolates made from spirulina cultivated at lab

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Table 5. Typical dry spirulina analysis

Minerals	Content in Spirulina (mg/kg)	Required Adult Daily Dose (47) (mg/day)
Potassium*	6400-15400	3500
Sodium	4500	500
Manganese	25-37	5
Chrome	2.8	0.5-2
Copper	8-10	1.5-3
Zinc	21-40	15
Iron*	580-1800	18
Magnesium	2000-2900	250-350
Phosphorus*	6700-9000	1000
Calcium*	1300-14000	1200

Table 6. Composition of Algae

Algae	Protein	Carbohydrates	Lipids
Aphanizomenon flos-aqua	62	23	3
Chlorella pyrenoidosa	57	26	2
Chlorella vulgaris	51-58	12-17	14-22
Porphyridium creuentum	28-39	40-57	9-14
Schizochytrium sp.	-	-	50-77
Arthrospira maxima	60-71	13-16	6-7

BIOMASS

Biomass is indeed the plant material produced by the photosynthetic process of CO2 in the air, water, and sunshine to make carbohydrates, which are the basic building block of biomass. In most cases, photosynthesis transforms less than 1% of available sunlight into chemical energy. The chemical bonds of the building elements of biomass store the sun energy that drives photosynthesis. Carbon is oxidized to generate CO2 and water if biomass is managed effectively, either chemically or biologically, by retrieving the energy contained in chemical bonds and the following 'energy' product mixed with oxygen. Because the co2 is again accessible to generate fresh biomass, the process is circular.

The physicochemical features of the big molecules that make up a specific form of biomass determine its value. For millennia, man has taken use of the energy held in these chemical bonds by igniting biomass as a biofuel and consuming plants for their sugar and starch nutritional content. Fossilized biomass has lately been mined as coal and oil. However, because the conversion of biomass to fossil fuels takes millions of years, they are not renewable on a timescale that humans can use.

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Burning fossil fuels turns old biomass into new CO2, contributing to the greenhouse effect and depleting a nonrenewable resource. Because replanting harvested biomass assures that Carbon dioxide is collected and regenerated for a loop of new growth, there is no additional carbon dioxide released into the atmosphere when fresh biomass is burned. The time lag between the initial release of Carbon dioxide from fossil fuel burning and its eventual absorption as biomass, which can take several years, is a significant element that is often disregarded when contemplating the use of biomass to help mitigate global warming. One of the difficulties that the industrialized world faces is the necessity to notice this time lag and take necessary measures to reduce the lag period. A similar issue confronts the developing world, which uses its biomass resources for fuel, but it does not conduct a replacement planting program. For commercial energy farming, a variety of plants have indeed been proposed or are being studied. Woody crops, dwarf varieties crops (all perennial crops), starch and sugar crops, and oilseeds are all potential energy crops.

Biomass: Impact on the Carbon Cycle and Greenhouse Gas Emission

Biomass has the longest history of any source of energy, yet it still delivers enough energy to supply 11% of the world's fundamental energy demands after 44 years. The majority of these are utilized in underdeveloped nations, where low-quality wood is used for cooking and heating, accounting for 38 EJ. The remaining 6 EJ are utilized in industrialized nations for current technology heating and power generating.

Heating was formerly the most popular way of extracting energy from biomass. However, technological advancements have made more efficient and sustainable energy conversion processes possible. Mineral oils may be converted into practically any use using solids, liquids, or gaseous biofuels.

The amount and frequency with which biofuels are utilized varies substantially. In example, biomass provides just 3% of total basic energy demands in industrialized regions, compared to more than 40% in undeveloped countries. Table II demonstrates the range of biomass contributions to energy demands in various nations, ranging from 1 percent in Japan, Ireland, and the Netherlands to 94 percent in Tanzania and Ethiopia. Finland, Sweden, and Austria are among the industrialized nations that get a lot of energy from bioenergy, thanks to the use of wood, wood waste, and grass as fuel.

Biomass Production by Using Microalgae

Microalgae may thrive in a variety of conditions, including clean, saline, and polluted water, and they can even be used to clean. Agricultural, municipal, and industrial wastewater discharge a variety of living and non-living organisms into the environment. These liquid waste streams can be repurposed as fertilizer sources for tiny algae growth systems. The symbiotic interaction between autotrophic growth and heterotrophic/mixotrophic microalgae can aid in improving water treatment efficiency.

This method minimizes carbon dioxide emissions from microalgae biofuels and biogas production technologies while saving power. The extraction of phosphorus and nitrogen from polluted water may be linked with microalgae extraction for the manufacture of biofuels, nutritional supplements, or other bio-based compounds. As a food supply for tiny algae culture while generating bioenergy and high-value bioproducts

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Biomass From Forests

Forests cover 38% of EU territory and provide a variety of ecosystem services, including carbon storage and depletion, accommodation, water management (quality, quantity, flow), air quality standards, soil depletion control, and recreation, wood, and non-timber goods. Forest growth differs greatly from that of the rest of the EU in several aspects, including site circumstances, soil, age formation, and species formation (70 percent of forests are made up of two or more tree species).

Forest management functions are driven by forest management principles, EU forestry variety, and the services they may give, impacting the potential supply of woody biomass to fulfil demand for wood-based goods and biofuels. Other elements have an impact on wood's integration inside the EU, which is crucial. Forest ownership is linked to data changes (60 percent of forests are owned by private individuals who are progressively moving to rural regions), forest area division (16 million forestland owners), and the economic advantages of forest management. This chapter focuses on biomass wood from European forests, as well as forest produce and markets. It's worth noting that forest biomass, which includes non-wood forest resources, plays a major role in the bioeconomy of several EU areas region in EU applied in this chapter to summarize forest-based sector data.

Biomass Properties

1. Moisture content
2. Calorific value
3. Proportion of fixed carbon and volatile matter
4. Ash / residue content
5. Alkali metal content
6. Cellulose / lignin ratio
7. Bulk density

Yields

The quantity of dry matter generated by each form of biomass in each production facility affects the available land area's potential manufacturing capacity, or yield. When the crop's production is quantified in dmt/ha and paired with HHV biomass, the crop's energy yield may be estimated. The highest energy output for biomass types.

The use of hybrid plants to increase biomass yields has been the subject of extensive study and development. Psychological tests on mixed popular species yielded 43 dmt / ha / an in in the US Pacific Northwest, and a reported eucalyptus harvest of 39 dmt / ha / an in Brazil (Hislop and Hall, 1996). High dmnt / ha varieties, which are correctly placed in a high-quality agricultural region, e.g., exception, and variety capable of generating high yields of dmt / ha, planted in a separate area, are anticipated to be the two primary types of variations developed for the cultivation of biomass power fields.

Biomass, a traditional third-world source of energy, has the potential to help the developed world reduce the environmental impact of burning fossil fuels to create power, but only if large-scale reforestation projects are implemented quickly. Biomass is a renowned source of renewable energy that is seen as a solution to help reduce global warming by replacing the use of fossil fuels: by 2010, renewable energy sources will provide up to 10% of the UK's electricity consumption.

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The principal forms of biomass of interest for generating power are woody plants, herbaceous plants, and grasses, with the C. plant species garnering the most attention. Plants store chemical energy in the form of cellulose, hemicellulose, and lignin, with the amounts varying depending on the kind of plant.

TYPE OF BIOMASS

Wood, sawdust, straw, seed debris, compost, paper waste, household trash, polluted water, and other materials are all examples of biomass. The majority of study is currently focused on unfavorable energy and the resulting power. Common fuels will be supplemented by biomass. The primary goal of this study was to examine the research background and biomass styles of renewable energy over the last 40 years (from 1978 to 2018) in order to aid the research community in understanding future trends, as well as the international context and context, all of which provided basic information that helps those in charge of scientific policy make better decisions. The world's population is growing at an alarming rate, with today's population tripling that of 1960 and anticipated to reach 9 billion by 2050. As a result of this condition, the global proportion of energy utilized has increased. Cities have grown significantly. Wood, sawdust, straw, seed debris, compost, paper trash, household waste, polluted water, and other items are all sources of biomass. Biomass resources have been used and are becoming more significant for their economic potential, since there are large yearly quantities of agricultural output, and their products may be used as a source of power and created as energy plants, specifically for this purpose. The main goal of this study was to examine the research environment and practices in biomass renewable energy from 1978 to 2018, in order to aid the research community in understanding the current situation and future trends, as well as the international situation, and to provide basic information to aid decision- making.

The USA, India, China, Germany, and Italy are among the major countries researching on biomass as a renewable energy source, as assessed by scientific output. The Chinese Academy of Sciences is the most productive institution in the sector, followed by the National Renewable Energy Laboratory, Danmarks Tekniske University, and the Chinese Department of Education. In addition, the study identified groups based on published terms discovered through book searches. There were six communities or groups discovered. The two most important ones are concerned with converting biomass to liquid fuel. Finally, eight clusters were established based on international collaboration and biomass research. All of this is focused on three countries that fall into separate categories: the United States, India, and the United Kingdom.

CLASSIFICATION OF BIOMASS

Based on their scope and functions, biomasses can be classified into many classes. There are no specific ways to the classification of biomasses; consequently, we can classify biomasses into several groupings by relying on their magnitude, composition, software, and natural existence. Typically, biomasses can be classified into distinct methods based on the goods, starting location, and characteristic of the biomass. Type can be determined by the types of biomass present in the environment as well as its application as a substrate. Woody biomass, herbaceous biomass, animal and human waste, and aquatic biomass are some of the most well- known classifications of biomass into distinct types.

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APPLICATION OF BIOMASS

Every quarter must recognize the three precepts that describe how to reduce, reuse, and recycle environmental waste rather than burning or discarding it. These methods not only aid in the control of all types of pollutants, but they also aid in the reduction of costs, as well as their availability and renewability. Biomass can be used in specialized sectors to meet the waste management hierarchy's requirements for energy, food, fertilizers, and a variety of other items. As the production of biomass increases every day, more appropriate and practical waste control measures are needed to help the ecosystem remain sustainable. Biomass can be used in the energy sector, for bio fertilizer production, for water treatment, In cement manufacturing, for the creation of thermal insulators, and for construction purposes, to name a few applications.

Application of Biomass for Biofuel Production

Biomass is one of the most effective and adaptable substrates for power generation. The provision of natural material, which includes Wood shavings, rice husk, sugarcane, timbers, bushes, leaves, and peanut shells, among other things, is the most important aspect in biomass electricity generation. Biomass is used for power generation in a wide range of applications, from small-scale use in homes for cooking to large-scale use in unique industries, such as boilers and powerhouses. Biofuel is a renewable energy source made from biomass that has been in great demand globally in recent years. One of the advantageous assets is biofuel. Biofuel is a sustainable energy source that helps to reduce air pollution, greenhouse gas emissions, and the reliance on carbon-based energy sources.

Ethanol and biodiesel are the two most common types of biofuels utilized today. Developed countries such as the United Kingdom and the United States rely on the production of bioethanol using marine yeast and seawater as a media. In the year 2019, America generated the most ethanol, Brazil placed second with 8.6 billion gallons, and the United States came in third with 15.8 billion gallons. In the year 2019, 530 million gallons of manufacturing will be produced., India is ranked fifth on this list. Biodiesel is commonly used in conventional diesel engines, either alone or in combination with petroleum diesel. Various feedstocks are used by different countries depending on their availability. The main distinction between ethanol and biodiesel Biodiesel is a type of oil, while ethanol is a type of alcohol. Ethanol is an alcohol made through the fermentation process that can be used instead of or in addition to gasoline, whereas biodiesel is made by extracting naturally existing oils from various flowers and seeds through a process called transesterification. The production of biofuel is targeted not only to meet the needs of decentralized energy generation, but also to meet the needs of shipping. This piques the interest of local organizations and entails the use of local groups' lands for the production of biofuels.

Bioprocessing Involved in Biofuel Production

Bioprocessing is the use of living things and their components, usually after various industrial processes, to produce a product that requires less energy and water and, as an outcome, produces a less affluent issue. Biofuels are a viable alternative to fossil fuels because they are non-poisonous, totally combustible, and environmentally sustainable. The production of biofuels involves a wide range of activities that are generally classified into two categories.: upstream and downstream approaches. Storage of liquid components, removal of inhibitory chemical compounds and particulates from the product, purification

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and sterilization, and many more procedures are included in the upstream procedures. Storage of liquid components, removal of inhibitory chemical compounds and particulates from the product, purification and sterilization, and many more procedures are included in the upstream procedures. The establishment of a microbial strain with the ability to synthesis the defined business value of a particular product is part of the upstream method. The pressure is then put through an enhanced routine in order to maximize its capacity to create the product at a low cost. Mobile isolation from the fermentation broth, purification and extraction of the desired product, and waste elimination or recycling are all examples of downstream processing. Several steps in fermentation procedure, including appropriate techniques for extracting, purifying, and characterizing the fermentation component in question. In addition to chromatography, a wide range of downstream processing options are available, including centrifugation and filtration. The chemical and physical qualities of the final product, as well as the intended grade, all influence these operations.

CONCLUSION

This study looked at the biomass's structure, composition, and classification, as well as its potential for bioenergy applications. Biomass is a mixture of biological and chemical substances that can be successfully converted into a value-added product. Biomasses can be divided into different divisions based on their applications and future possibilities. However, there are no specific criteria or methods for classifying biomasses, so we can classify biomasses into exceptional companies based on their fine, composition, application, and kind of lifestyles. Biomass can be divided into four distinct categories: woody biomass, herbaceous biomass, animal and human waste, and aquatic biomass. Exploring the relevance of lignocellulosic biomass and its utility over other current biomasses may have the capability in terms of environmental effect, bioprocessing involved in biofuel manufacture, liquid and gaseous biofuels derived from lignocellulosic biomass. The United States leads the way in researching biomass as a renewable energy source, as measured by scientific production, followed by China, India, Germany, and Italy. As a result, large nations with large populations are interested in adopting current renewable energy sources in conjunction with biomass.

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Chapter 9
Biofuels From Macroalgae: A Sustainable Alternative to Conventional Energy Resources

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ABSTRACT

The steep rise in human population together with the simultaneous growth of urbanization and industrialization has triggered overuse of fossil fuels in the recent years. However, they are non-renewable and release carbon dioxide causing global warming. In this context, biofuels come as excellent substitutes because they are renewable and eco-friendly. They are obtained from biological products and include biodiesel, biomethane, bioethanol, and biohydrogen. They are classified into first, second, and third generation fuels depending on the biological feedstocks being used. Of these, the third-generation fuels, obtained from marine resources like algae, have attracted special research interests because they do not rival the food crop production or depend on land and freshwater for their cultivation. However, the potentialities of macroalgae as a biofuel resource have not been investigated thoroughly. The chapter tries to outline all the aspects of biofuel production from macroalgae pointing out the scopes for future research.

INTRODUCTION

Overexploitation of the fossil fuels and the non-renewable energy resources like coal, petroleum and natural gas in the last few years has not only led to the depletion of resources but has also added carbon dioxide (CO_2) to the atmosphere. Needless to say, CO_2 is a major greenhouse gas (GHG) and causes global warming (Naik et al., 2010). The problem has been projected to rise in the years to come. This necessitates the development of alternative energy sources to meet the growing demands of the burgeoning popula-

DOI: 10.4018/978-1-6684-5269-1.ch009

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tion as well as reduce the adverse environmental effects (Mabee et al., 2005). According to Naik et al. (2010), eco-friendly and sustainable energy resources are the need of the hour for sustainable growth of the economy and industry of a nation. In this regard, several attempts have been taken by researchers to utilize plant biomass and/or organic wastes for extraction of energy. The lipid and carbohydrate contents of these substances have mainly been targeted by hydrolytic degradation, fermentation, pyrolysis and various other techniques to procure the fuels (Gao et al., 2020). They have been termed as 'biofuels' and have emerged as promising alternatives of conventional energy sources. Because of their renewability and sustainability they have been proposed as the 'future energy resources' (Reid et al., 2020). Their extraction from plant derivatives gives an added advantage of maintaining carbon balance in the atmosphere. According to Gao et al. (2020), plants help to sequester atmospheric CO_2 by photosynthesis. As such, burning of the fuels obtained from them does not become an additional source of CO_2 emission. Therefore, biofuels can combat global warming to a large extent (Kumar et al., 2016). Another advantage of biofuel production is its ability to generate new employment opportunities (Stevens & Verhe, 2004).

The biofuels sector is, however, in its stage of infancy because of several issues and challenges associated with their production that need to be addressed before they can be manufactured in large-scales (V. G. S. et al., 2021). The common challenge lies in the development of technological sophistication to extract the biofuels with minimum loses such that the final products do not exceed the price of conventional fuels. Otherwise, it will reduce their marketability (Gao et al., 2020). Currently, this field is being explored by various researchers in different parts of the world. The other problem is the direct confrontation of biofuel production with food resources because they have often been attempted to be derived from crop plants accelerating two pertinent issues – global water crisis and food scarcity (Naik et al., 2010). Land availability for cultivation of these crops is another problem under the current conditions of explosive population growth because of increasing land requirements for human habitation (Naik et al., 2010). Land is also required for augmenting crop production to meet the demands of the expanding population. Adequate freshwater supply is equally important to maintain agricultural productivity. Using freshwater for cultivation of terrestrial plants to be used as feedstock for biofuel manufacture will trigger competition between the biofuel and agricultural industries. These problems have encouraged the researchers to explore non-consumable raw materials (that are not terrestrial derivatives) for biofuel production (V. G. S. et al., 2021). Consequently, the marine resources primarily consisting of the micro and macroalgae have emerged as the preferred candidates for biofuel extraction. Some workers like Liao et al. (2021) have used the term 'blue bioeconomy' to describe the economy dependent on such biofuel resources. They offer several advantages over terrestrial plants the most important being their rich carbohydrate and lipid contents together with low (to almost zero) proportions of lignin facilitating the extraction of biofuels by employing cheaper technologies (Gao et al., 2020). Furthermore, they can be cultivated in unused, rejected or barren lands with saline water and wastewater and can withstand adverse environmental conditions (Gao et al., 2020). Despite these advantages, they have certain drawbacks that have prevented them from gaining popularity. They need further developments both in terms of technology and overcoming certain serious environmental issues associated with their production (Hughes et al., 2012; V. G. S. et al., 2021).

The current chapter tries to give a comprehensive review of the advantages of using macroalgae as the source of biofuels by going through some recent publications in this field. It discusses about the advantages and limitations of microalgae while referring to the potentialities of macroalgae as a biofuel resource. It also highlights about the various challenges and environmental issues of biofuel production

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from macroalgae that limits their acceptability. Last but not the least, it suggests some mitigation strategies by pointing out the research gaps in this field.

BIOFUEL GENERATIONS

The growing human population and its increasing demands puts severe stress on the fossil fuels that form the primary source of energy. According to V. G. S. et al. (2021), the fossil fuel requirements from 2010 to 2040 will augment by as much as 40%. However, they are non-renewable and tend to get exhausted if proper alternatives are not searched for. Additionally, they are also a cause of global warming, pollution and the emerging issue of global climate change (Biswal, 2021; 2022). These pressing conditions compelled the researchers to develop sustainable, renewable and eco-friendly energy resources time and again. Many workers have used the term 'green fuel' to describe them (Naik et al., 2010). They are primarily formulated from plant sources and depending on the raw materials used they have been classified into three generations.

First Generation Biofuels

Bioethanol, biodiesel and biogas form the three primary types of first generation biofuels. Bioethanol is a good alternative of gasoline and can be used in vehicles that are flexible with fuel use. It can be procured from fermentation of starch or sugars present in the plant biomass (Naik et al., 2010). Bioethanol can also be a source of ethyl tertiary butyl ether (ETBE) that blends well with gasoline. Additionally, vegetable oils together with residual oils and fat obtained from plant sources or wastes can be subjected to transesterification to obtain biodiesel. Another sustainable alternative of gasoline is biogas or biomethane that can be obtained by anaerobic breakdown of liquid manure and other plant sources or waste materials (Naik et al., 2010).

One major drawback of first generation biofuels is their dependency on food crops (e.g. wheat, rice, sugarcane and corn), edible oils and other food stuffs as raw materials. Since the growing population puts increasing demands on food production, utilizing food resources for biofuel production directly threatens the future food security (Naik et al., 2010). Many researchers have also claimed that utilization of food crops for biofuel production may inflate food prices (Gao et al., 2020). This has encouraged the cultivation of crops specially dedicated for biofuel production that can be grown on barren lands. In this context, oilseed crops have gained recognition because they can directly yield biodiesel while their lingnocellulosic biomass can be processed to manufacture second generation biofuels (Naik et al., 2010). However, their production has sparked controversy because they directly involve land use and also exerts negative impacts on biodiversity. Increased land use for fuel crops production has serious issues because increasing human population requires increased land supply for human habitation. Above all, according to many scientists, the production and utilization of first generation biofuels offsets carbon balance with negative impacts on the environment (Naik et al., 2010). Keeping these disadvantages in mind the second generation biofuels were developed.

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Second Generation Biofuels

The second generation biofuels use non-food substances, mainly the lingnocellulose materials of plants, as the raw material for fuel production (Zabaniotou et al., 2008). This places them ahead of the first generation biofuels by not competing directly with the food materials. Moreover, the raw materials are abundantly available from plants and are cheap. These fuels are also beneficial in terms of maintenance of CO_2 balance because the plant biomass used as the feedstocks fixed atmospheric CO_2 (by photosynthesis) during their lifetime. Thus, the CO_2 emitted from the ignition of the biofuels derived from them balances, to some extent, the removal and addition of atmospheric CO_2 (Naik et al., 2010). However, their potentialities have not been explored thoroughly despite the plant biomass being an immense source of liquid biofuels. Here, it is worth mentioning that the plant-derived raw material is primarily composed of 75% polysaccharides that make up the cell walls. Degradation of the polysaccharides followed by fermentation and/or gasification can give some important biofuels like bioethanol and biogas (Naik et al., 2010).

Residues from the wood industry (e.g. wood shavings), agriculture (e.g. straw), forestry (e.g. short rotation forestry crops like eucalyptus and willow) and perennial grasses (e.g. *Miscantus* sp.) are the most common sources of feedstocks for second generation biofuel production. It has been proposed that they have the ability to supply a large extent of the increasing demands for liquid fuels (Naik et al., 2010). Apart from the substitute of gasoline (i.e. bioethanol) they can generate FT-diesel (Fischer-Tropsch diesel) and BTL (Biomass-to-Liquids) which can completely replace diesel (Naik et al., 2010). Additionally, Bio-SNG (Synthetic Natural Gas) obtained by gasification of the plant biomass can be an alternative to gasoline but it requires slight modifications. Similarly, Bio-DME (Dimethyl Ether) [obtained from syngas] can be used as a used as a diesel-substitute with certain alterations (Balat, 2007). The syngas can also be transformed into bio-kerosene (Balat, 2007).

One of the greatest disadvantage of second generation biofuels is the cost involved in the technology required for the conversion of the lignocellulosic raw-materials into fuels. As such, the biofuels obtained become pricey compared to their conventional alternatives (V. G. S., et al., 2021). This lack of cost-effectiveness proves a barrier to their acceptability. The drawbacks prompted the development of third generation biofuels with inexpensive, non-consumable and renewable feedstocks that can be easily processed to give cost-effective fuels.

Third Generation Biofuels

Utilization of marine resources in the form of seaweeds and cyanobacteria made the way for greater production of biofuels with lesser investments (V. G. S., et al., 2021). Their non-dependence on agricultural lands and freshwater supplies make them the feedstock of choice for biofuel production (Gajaraj et al., 2018). Marine water, brackish water, saline water and even wastewater, which are unfit for cultivation of agricultural crops, may be used for cultivating most of the non-consumable algae. Animal, industrial and municipal runoffs can also be utilized for growing algae. Additionally, they can withstand adverse climatic conditions and can also be grown in dark using suitable sugar sources in the medium (Gajaraj et al., 2018). This makes their cultivation flexible. Furthermore, the biofuels derived from macroalgae have been found to be secure, recyclable and sulphur-free making them extremely useful alternatives of conventional fuels (Mat Aron et al., 2020).

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The algae are characterized by rapid rates of growth and high efficiency of fixing atmospheric CO_2 by photosynthetic processes (Peter et al., 2021). The macroalgae mainly consist of approximately 25% to 60% of carbohydrates, 5% to 47% of proteins, 18% to 55% of ash and lipid contents less than 5% (V. G. S., et al., 2021). The biochemical composition, however, varies with the biotic and abiotic factors of the environment where these algae are cultivated (Peter et al., 2021). The seaweeds have high productivity facilitating quicker generation of surplus raw materials for extraction of biofuels. The annual harvest of wet seaweeds across the globe has been recorded at 7.5 to 8 million tons (Subba Rao & Mantri, 2006). According to Jung et al. (2013) the brown algae, kelp (*Saccharina japonica*) and sea mustard (*Undaria pinnatifida*) have the highest productivity per unit area making them one of the best feedstocks for biofuel production. Eventually, the algal biofuels have emerged as promising alternatives to the earlier two generations and have gained popularity as the 'third generation biofuel'.

MACROALGAE AS A SOURCE OF BIOFUELS

The use of algae in biofuel production is recently gaining grounds because of their ability to fix carbon dioxide (CO_2) by photosynthesis. According to Aresta et al. (2005) their photosynthetic efficiency is high and approximately ranges from 6% to 8% whereas it is about 1.8% to 2.2% in biomass derived from terrestrial plants. Additionally, they do not compete with food production as was the case with first generation biofuels (Singh & Olsen, 2011). They are beneficial over second generation biofuels because of their low lignin content saving the cost incurred in its removal (Singh & Olsen, 2011). Poor quality lands, not suitable for crop cultivation, can be easily used for production of algae (Levine et al., 2010). They have rapid growth rates and the ability to utilize the nutrients (like nitrogen and phosphorus) present in wastewater, thereby, contributing to wastewater recycling as well (Singh et al., 2011).

Though both the microalgae and macroalgae have been recognized as prospective sources for biofuels, macroalgae is comparatively new and unexplored giving ample opportunities for research (Mata et al., 2009). The higher lipid contents and faster growth rates of microalgae have made them preferable over macroalgae for biodiesel production but their lower carbohydrate contents do not make them a good source for production of biomethane (Jin et al., 2013). The energy yield of macroalgae is also higher than the same amount of microalgae (Chen et al., 2015). It has been observed that the process of harvesting of macroalgae is simpler than microalgae because of their multicellular nature and plant-like features (Maceiras et al., 2011). The higher and good quality carbohydrates of macroalgae make them a good source of biofuels like bio-oils, bioethanol, and biogas.

There are three major groups of macroalgae depending on their photosynthetic pigments. They are green algae (Chlorophyta), red algae (Rhodophyta) and brown algae (Phaeophyta) (John, 2011; Kraan, 2013). The red algae is the most abundant group in terms of species followed by green algae (Jung et al., 2013). Brown algae has the least number of species compared to the other two and prefers cold water (Jung et al., 2013). Red algae flourish in the inter-tropical zones while the green algae can grow in all water types (Bucholc et al., 2014). Currently, macroalgae is cultivated extensively in Asia mainly as a food source, for production of fertilizers and for extraction of hydrocolloids (Roesijadi, 2010). Japan, Korea, China and Philippines account for approximately 72% of the world's share of macroalgae production (Chen et al., 2015).

Macroalgae has not received much attention for biofuel production but they have garnered industrial interests for extraction of hydrocolloids (Bucholc et al., 2014). Microalgae dominates the biofuel sector

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but their poor carbohydrate contents limit their versatility (Bucholc et al., 2014). The dry matter content of macroalgae amounts to about 10% to 15% and contains as high as 60% of carbohydrates (Roesijadi, 2010). Their cell wall is rich in carbohydrate sources like cellulose and/or starch (Roesijadi, 2010). Interestingly, their biochemical composition shows seasonal variation with the stored carbohydrates reaching its peak value in the autumn season (Kraan, 2013). Some of the major biochemical constituents of macroalgae have been represented in Table 1.

Table 1. Some major biochemical constituents of macroalgae (based on previous reports)

Macroalgae	Carbohydrates	Lipid	Protein	Reference(s)
Chlorophyta				
Ulva lactuca	11.6 to 13.2 (a)	9.6 to 11.4 (a)	11.4 to 12.6 (a)	Becker, 1994; Kaliaperumal et al., 2002; Suganya et al., 2016
Ulva intestinalis	367 ± 31 (b)	Not studied	90 (b)	Olsson et al., 2020
Enteromorpha compressa	24.75 (a)	11.45 (a)	7.26 (a)	Becker, 1994; Kaliaperumal et al., 2002; Suganya et al., 2016
Chaetomorpha linoides	27.00 (a)	12.00 (a)	9.45 (a)	Becker, 1994; Kaliaperumal et al., 2002; Suganya et al., 2016
Valoniopsis pachynema	31.50 (a)	9.09 (a)	8.78 (a)	Becker, 1994; Kaliaperumal et al., 2002; Suganya et al., 2016
Cladophora rupestris	399 ± 8 (b)	Not studied	184 (b)	Olsson et al., 2020
Phaeophyta				
Laminaria digitata	519 ± 42 (b)	Not studied	66 (b)	Olsson et al., 2020
Fucus serratus	287 ± 21 (b)	Not studied	71 (b)	Olsson et al., 2020
Chorda filum	292 ± 33 (b)	Not studied	63 (b)	Olsson et al., 2020
Saccharina latissima	557 ± 14 (b)	Not studied	69 (b)	Olsson et al., 2020
Ascophyllum nodosum	317 ± 17 (b)	Not studied	59 (b)	Olsson et al., 2020
Desmarestia aculeata	301 ± 101 (b)	Not studied	115 (b)	Olsson et al., 2020
Rhodophyta				
Laurencia papillosa	12.0 to 13.3 (a)	8.9 to 10.8 (a)	11.8 to 12.9 (a)	Becker, 1994; Kaliaperumal et al., 2002; Suganya et al., 2016
Hypnea valentiae	11.8 to 13.0 (a)	9.6 to 11.6 (a)	11.8 to 12.6 (a)	Becker, 1994; Kaliaperumal et al., 2002; Suganya et al., 2016
Acanthophora spicifera	11.6 to 13.2 (a)	10.0 to 12.0 (a)	12.0 to 13.2 (a)	Becker, 1994; Kaliaperumal et al., 2002; Suganya et al., 2016
Chondrus crispus	526 ± 35 (b)	Not studied	103 (b)	Olsson et al., 2020
Dilsea carnosa	477 ± 28 (b)	Not studied	152 (b)	Olsson et al., 2020
Brogniartella byssoides	240 ± 16 (b)	Not studied	158 (b)	Olsson et al., 2020

[(a) = biochemical constituent represented as % of dry weight; (b) = biochemical constituent represented as g/kg dry weight. For carbohydrates = Mean value ± S.D. (Standard Deviation)]

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The major polysaccharide content of green algae includes starch, cellulose, mannan and ulvan. Red algae are a good source of agar, carrageenan, lignin and cellulose. Alginate, laminarin, mannitol, cellulose and glucan form the primary polysaccharides of brown algae. These polysaccharides differentiate macroalgae from microalgae (Jung et al., 2013). Moreover, the poor lignin content (about 0.03 kg per kg of dry matter) of macroalgae facilitates their fermentation or anaerobic digestion for extraction of biofuels (Horn, 2000). The process becomes much cheaper because additional investments on pretreatments for lignin removal, which forms an integral step for extraction of second generation biofuels, can be avoided (Chen et al., 2015). Apart from the polysaccharides, the macroalgae have high proportions of alkali metals and halogen contents (0.5% to 11%) compared to the biomass obtained from terrestrial plants (1% to 1.5%) (Mautner, 1954). The nutrient contents of the macroalgae [mainly nitrogen (N) and phosphorus (P)] have been found to vary with their morphology as well as the nutrient enrichment of the aquatic medium (McHugh, 2003). The effects of these constituents on biofuel production have been discussed later in this chapter.

BIOFUELS FROM MACROALGAE

The following biofuels can be obtained from macroalgae by application of thermochemical and biochemical technologies.

Biogas

Sea weeds are excellent candidates for biogas production because of their high carbohydrate content with low proportions of cellulose and lignin. As such, they can easily be fermented to obtain biogas (Saqib et al., 2013). The process mainly involves anaerobic breakdown of the organic matters in sea weeds to liberate gases like carbon dioxide, methane and small quantities of hydrogen sulphide. The first step of biogas production involves microbial enzyme mediated hydrolysis of the organic constituents of macroalgae (i.e. proteins, lipids and carbohydrates) into a soluble organic extract which is subsequently treated by acidogenic bacteria. In the second step, the enzymes liberated by acidogenic bacteria trigger the process of acidogenesis resulting in the liberation of alcohols and volatile fatty acids (VFAs). Subsequently, in the third step, the acetogenic bacteria converts the alcohols and VFAs into acetic acid and hydrogen which are finally acted upon by methanogenic bacteria to yield methane and carbon dioxide (Romagnoli et al., 2011).

It is important to note that the high water content (80 to 85%) of macroalgae qualifies them for microbial degradation over the thermochemical processes. However, the production of biogas from macroalgae is not free from challenges. High nitrogen content of macroalgae inhibits the action of the microbes and generates excessive ammonia which is toxic to the methanogenic bacteria (Demirbas, 2010). The presence of alkaline metals also hinders the anaerobic digestion processes. Additionally their cell walls need to be broken down by physical or chemical pretreatments to access the organic matter during biogas production (Demirbas, 2010). This problem is usually overcome by co-digesting the algal biomass with a substrate having high C:N (Carbon:Nitrogen) ratios such as straw or waste paper (Yen & Brune, 2007; Vivekanand et al., 2012). Some common macroalgae utilized for biogas production and their yields have been shown in Table 2.

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Table 2. Some important biofuels and their macroalgal sources together with their respective yields (based on previous reports)

Biofuel	Macroalgal Source	Biofuel Yield	Reference(s)
Biogas	*Pilayella* + *Ectocarpus* + *Enteromarpha*	0.040 dm3/g - 0.054 dm^3/g of substrate	Grala et al., 2012
	Chaetomorpha linum	0.26 ± 0.045 L CH_4/g VS	Yahmed et al., 2016
	Fucus vesiculosus	144 ± 28 mL CH_4/g VS	Romagnoli et al., 2017
	Fucus vesiculosus + *Fucus serratus*	260 mL/g VS	Wu et al., 2019
	Ulva lactuca	498.75 mL/g VS	Hassaan et al., 2021
	Saccorhiza polyschides	227 ± 4 mL/g VS	Pardilhó et al., 2022
Bioethanol	*Sargassum* spp.	2.27 ± 0.04 g/L	Borines et al., 2013
	Gracilaria verrucosa	0.49 g/g	Shukla et al., 2016
	Sargassum latifolium	0.29 g/g of TRS	Soliman et al., 2018
	Ecklonia kurome	2.1 g/L	Sasaki et al., 2018
	Ulva linza	48.24 g/L	Greetham et al., 2020
	Ulva lactuca	0.41 g/g of Glucose	Allouache et al., 2021
Biodiesel	*Enteromorpha compressa*	90.6%	Suganya et al., 2013
	Caulerpa peltata	98.11%	Suganya & Renganathan, 2014
	Chara vulgaris	0.3 mL/g dry algae	Siddiqua et al., 2015
	Chaetomorpha linum	0.96 g; 4.85% dry wt.	Ameka et al., 2019
	Chaetomorpha antennina	0.48 g; 2.40% dry wt.	Ameka et al., 2019
	Cladophora sp.	4.58 g/200 g algal wt.	Naik & Naik, 2021
Biohydrogen	*Laminaria japonica*	67.0 mL H_2/g TS	Jung et al., 2011
	Padina tetrastromatica	78 ± 2.9 mL/0.05 g VS	Radha & Murugesan, 2017
	Ulva reticulata	63 mL H_2/g COD	Kumar et al., 2018
	Chaetomorpha antennina	74.5 mL H_2/g COD	Kumar et al., 2019
	Saccharina japonica	20.25 mLH_2/g VS_{added}	Yin & Wang, 2019
	Ulva sp.	1.52 mol H_2/mol Hexose	Margareta et al., 2020

[TRS - Total Reducing Sugars, VS – Volatile Solids, TS – Total Solids, COD – Chemical Oxygen Demand]

Bioethanol

Bioethanol is an example of liquid algal transportation fuel. Its production from algal biomass is facilitated by the presence of low proportions of hemicelluloses and lignin (Mutripah et al., 2014). Fermentation of algal biomass (e.g. with the yeast, *Brettanomyces custersii*) has been extensively explored for bioethanol production (Park et al., 2012). However, the algal biomass requires pre-treatments to enable the enzymes to act on the algal carbohydrates like carrageenan, laminarin and alginate (Wei et al., 2013; Chen et al., 2015). Pretreatment with acids like sulphuric acid (H_2SO_4) or hydrochloric acid (HCl) is most commonly employed for this purpose which facilitates the hydrolysis of structural polysaccharides (Han et al., 2007). Even though the process of acid hydrolysis is cheap, it generates by-products like

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levulinic acid and 5-hydroxy-methyl-furfural which impedes ethanol production (Klinke et al., 2004; Jeong et al., 2012). Enzymatic hydrolysis is a suitable alternative and at times essential. For example, digestion of laminarin (present in brown algae) requires enzymatic treatment with laminarinases (Lee et al., 2014). Similarly, cellulase enzyme is used to digest cellulose present in algal biomass (Singh & Trivedi, 2013). However, enzymatic hydrolysis is not a preferred method because it is very slow (Tan et al., 2013). Instead, a combined pretreatment method utilizing acids and enzymes has been found to be very productive (Daroch et al., 2013). Pretreatment using gamma radiation has also been found to be a sustainable method (Yoon et al., 2012). Interestingly, sea weeds like *Ulva pertusa* and *Gelidium elegans* are free from lignin and do not require any pretreatment (Yanagisawa et al., 2011).

Currently, the bacterium, *Zymomonas mobilis* and the yeast, *Saccharomyces cerevisiae* have gained popularity as the fermenting microorganisms for production of bioethanol (Horn et al., 2000). However, fermentative degradation of certain polysaccharides like mannitol requires the presence of oxygen. In these cases, the bacterium, *Zymobacter palmae* have been found to be very effective (Horn et al., 2000). Thus, the basic process of bioethanol production involves hydrolytic degradation of the algal polysaccharides into simple sugars followed by their fermentation to ethanol and separation by distillation in the final step. Examples of macroalgae commonly used for the production of bioethanol and their yields have been represented in Table 2.

Biodiesel

Biodiesel consists of a blend of monoalkyl esters of long chain fatty acids and can be used directly for running diesel engines (Demirbas, 2009). They can be derived from commercially obtained triglycerides from plant oils, animal fats and vegetables by subjecting them to transesterification reactions (Chen et al., 2015). However, the macroalgae have poorer lipid contents compared to microalgae resulting in lower yields of biodiesel. This makes microalgae, with oil productivity higher than the high yielding terrestrial oil crops, a raw material of choice for biodiesel production (Afify et al., 2010; Maceiras et al., 2011). Nevertheless, the macroalgal biomass can be converted into bio-oil consisting of a mixture of free fatty acids and lipids. The lipids can then be isolated and utilized in biodiesel production. Some common macroalgae used for biodiesel production and their yields have been enlisted in Table 2. The existence of large amounts of free fatty acids in bio-oil is, however, reported to hamper biodiesel production (Chen et al., 2015). Conversion of free fatty acids from algal oil into biodiesel has been attempted by Suganya and Renganathan (2014). Alternatively, glucose obtained from macroalgae (e.g. *Cladophora glomerata*) can be converted into free fatty acids followed by biodiesel production (Chen et al., 2015). In yet another method, the macroalgal biomass can be acted upon by oleaginous yeast to produce biodiesel while the free fatty acids obtained as the by-product could serve to nourish the yeast itself (Xu et al., 2014).

Proper dehydration of the algal biomass is essential prior to biodiesel production because presence of water lowers its yield by as much as ten times (Daroch et al., 2013). The algal biomass can then be mechanically compressed to extract the bio-oil though it is a time-consuming process and not much effective (Popoola & Yangomodou, 2006). Using organic solvents like hexane, acetone, benzene, chloroform, etc. to extract oils from algal biomass have been found to be more productive than the mechanical process (Topare et al., 2011). The extracted oil is then subjected to transesterification reaction using alcohols like butanol, propanol, ethanol, methanol, etc. However, use of methanol and ethanol is more preferred because they are cheaper and offer certain chemical and physical advantages over others (Surendhiran & Vijay, 2012). Presence of lipase enzyme or inorganic catalytic agents like acids and alkalis are equally essential

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for biodiesel production (Meher et al., 2006). Glycerol is an important by-product in these reactions and has to be removed from the reactor on a regular basis to prevent engine failure (Munir et al., 2013).

Biohydrogen

Electricity may be generated from the biohydrogen procured from algal biomass. It may also be used as a gaseous fuel (Behera et al., 2015). Photo fermentation and biophotolysis are two common methods for biohydrogen production (Shaishav et al., 2013). The red alga, *Gelidium amansii* can be anaerobically fermented to obtain biohydrogen (Park et al., 2011). However, the yield depends on adopting suitable pretreatment methods because acid hydrolysis of the algal biomass can produce 5-hydroxymethylfurfural which inhibits the production process reducing the output by almost 50% (Park et al., 2011). Dark fermentation of the fermentable sugars obtained from macroalgae by using mesophilic and thermophilic microbes has also been suggested as a suitable technique of biohydrogen production (Kumar et al., 2016). Furthermore, the carbohydrate content of the algae can be maximised by exposing them to stressed conditions during their cultivation. This can increase their biohydrogen yields (Kumar et al., 2016).

Heat treatment of the brown seaweed, *Laminaria japonica* at 170°C can produce hydrogen at the rate of 109.6 mL/g COD (Chemical Oxygen Demand) (Jung et al., 2011). Microwave pretreatment of *Laminaria japonica* at 160°C for about half an hour also gives good result (Yin et al., 2019). Macroalgae, on being treated with hydrogen peroxide, gives a good supply of hydrogen as well (Roy et al., 2014). Additionally, water can be subjected to biophytolysis using the photosynthetic properties of microalgae (Kumar et al., 2016). Table 2 represents some common macroalgal species from which biohydrogen can be obtained and their yields.

CHALLENGES OF BIOFUEL PRODUCTION

One of the greatest challenges of using terrestrial plants and/or their derivatives for biofuel production is the consumption of freshwater because many parts of the world are already facing challenges of potable water supply (Shilton & Guieysse, 2010). Under the growing negative impacts of climate change and non-sustainable anthropogenic activities, future availability of freshwater has already raised several questions (Biswal, 2021; 2022). These were the circumstances that prompted researchers to exploit the marine resources for generation of biofuels. Of the marine resources, the microalgae has been extensively experimented as biofuel producers (Behera et al., 2015). Their faster proliferating capabilities, high proportion of polyunsaturated fatty acids (often exceeding by almost 80% of their dry weight) and almost no lignin content make them highly efficient raw materials for generation of biodiesel (Mata et al., 2009; Jin et al., 2013). However, they are poorer in carbohydrates (compared to the macroalgae) restricting their versatility in biofuel manufacture (Chen et al., 2015). Their cultivation also has several constraints like availability of long periods of sunlight encouraging the use of photo bioreactors as an alternative. However, they did not gain acceptance because of the costs involved inflating the prices of the final product (Wilcox, 1977). The macroalgae, on the other hand, gives a good yield of biogas when their biomass is subjected to gasification (Wargacki et al., 2012).

At present, more than one hundred species of macroalgae are being used for commercial purposes (like pharmaceutical products, food and fertilisers) all over the world (Santelices, 2007). Knowledge regarding the life cycle of sea weeds and their cultivation in large-scales gained momentum in the twentieth

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century. It was China who first began to selectively breed sea weeds (beginning with *Laminaria* sp.) in the 1960s (Hughes et al., 2012). However, research shows that not even 1% of the available macroalgae have been explored properly (Budarin et al., 2011). They are good sources of bioethanol too (Hughes et al., 2012). Despite their potentialities, large-scale manufacture has not gained popularity because of the consequences on marine ecosystem due to their mass harvest (Springer et al., 2010; Kraan, 2013). It is to be remembered that the macroalgae, though non-consumable for humans, forms a support system for the marine environment. For instance, they have been found to grow profusely in the regions near the corals sheltering a variety of organisms together with the fishes and birds (Lorentsen et al., 2010). Massive harvesting of the seaweeds is also not eco-friendly because they take time to bounce back to their original conditions (Christie et al., 1998).

Cultivation of macroalgae can be carried out both offshore and onshore by several techniques. In the offshore methods, the lagoons are preferred for their growth and harvest while their onshore cultivation is operated along the coastal belts (V. G. S. et al., 2021). Comparison between both the cultivation techniques shows that offshore cultivation is more difficult and not sustainable (Hughes et al., 2012; V. G. S. et al., 2021). Hughes et al. (2012) have mentioned several environmental threats associated with large-scale cultivation of macroalgae in the marine environment (i.e. onshore cultivation). Since they grow in the water column just above the sea floor (also referred to as the seabed) they may severely impact the benthic flora and fauna. They may block the sunlight from penetrating deeper waters affecting the autotrophic organisms as well as competing with them for nutrients (Hughes et al., 2012). Sometimes inorganic fertilizers [e.g. nitrogen (N) and phosphorus (P)] are used to boost algae production which may lead to nutrient enrichment of marine waters and subsequent algal blooms. As such, the ecosystem along the coastline may be disrupted due to conditions of oxygen stress caused by algal overgrowth (Herbert, 1999; Springer et al., 2010). It may also result in terrestrial nutrient runoffs to the seabed beyond the photic zone resulting in nutrient enrichment of deep sea waters. This may make their habitats complicated. Accidental tearing or shredding of the macroalgae during their cultivation can create zones of organic enrichment (Cromey et al., 2009). The outcomes of these effects on marine organisms (e.g. cetaceans, fishes, etc.) have not been studied extensively raising doubts on the environmental impacts of macroalgae cultivation in the long run (Hughes et al., 2012). Despite the ability of the macroalgae to remove inorganic carbon from sea waters, a minor fraction may get converted to particulate organic matters and get settled on the sea floor with environmental consequences (Lüning & Pang, 2003). Several workers have pointed out that onshore cultivation often lowers the quality of the algae rendering them inappropriate for the chemical, pharmaceutical and the cosmetics industry (Hafting et al., 2015). This is due to lack of standardization and adoption of cost-effective sustainable strategies for macroalgal cultivation on large-scales (Hafting et al., 2015). Large-scale cultivation of seaweeds can affect the sedimentation patterns causing alterations in the local hydrodynamics (Hughes et al., 2012). However, according to some workers macroalgae cultivation in large-scales can enhance local biodiversity (Frank et al., 2012). Another important point that needs to be considered for biofuel extraction from macroalgae is the cost involved in their harvest and transportation to the factories. Since the seaweeds have a scattered distribution along the coasts the processes are quite expensive inflating the production costs of biofuels (Hughes et al., 2012).

Manufacture of biofuels require a steady supply of algal biomass which is difficult to manage because of the challenges involved in growing and harvesting of macroalgae. Since the bio-products obtained vary with the algal species (due to differences in their polysaccharide contents) the sorting process during biofuel manufacture becomes all the more complicated (V. G. S., et al., 2021). Further, the choice

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of the microbes for fermentation of the plant biomass is also an important and challenging task because the efficiency of the microbes differ with the raw materials and the conditions of reaction (V. G. S., et al., 2021). Pretreatment of the plant biomass is essential for maximum utilization of the polysaccharides in plant tissue. Therefore, this part also requires sufficient development to maximize the product output since many pre-treatment procedures may cause reduced outputs. Another major obstacle is the freshwater requirement in the bioprocessing stages which may augment the shortage of freshwater supplies in the future (Liao et al., 2021). Though seawater can be used in the hydrolysis and fractionation stages of macroalgae as well bioethanol production, their applicability on large-scales is still being worked upon (Joniver et al., 2021).

Often, the biochemical constituents of the macroalgae themselves hamper biofuel production. For instance, mixed sugar composition of the macroalgae decreases their efficiency as a biofuel source (Park et al., 2012). Additionally, the presence of large quantities minerals like calcium (Ca), magnesium (Mg), potassium (K) and sodium (Na) often inhibit the production of biofuels (Repérez, 2002). Apart from these, their pyrolysis for production of hydrocarbons, phenols, ketones, furanes, etc. involves huge energy expenditures adding to the production cost (Yanik et al., 2013). Keeping these constraints in mind, researchers from around the world are trying to develop this field by their continuous efforts (Bošnjaković & Sinaga, 2020; Fabris et al., 2020).

From the foregoing discussion it appears that besides technical challenges there are several environmental issues which contradict their claim of being eco-friendly. One more issue that often escapes observation is the role of geo-climatic conditions on the growth of macroalgae. It is well known that the geographical distribution of seaweeds largely depends on the prevalent climatic conditions. For instance, the red alga, *Porphyra* sp. grows commonly in Japan while the brown alga, *Laminaria japonica* is popular in China (Giugni & Giugni, 2010; Lam & Lee, 2019). This makes their distribution vulnerable under the effects of global climate change and will severely impact the location of the biorefineries (Zhou et al., 2010). The sustainability of bioeconomy also becomes a matter of doubt in the future (Zhou et al., 2010). Extinction of algal species or emergence of new invasive ones under the effect of climatic stress factors will require continuous research and developments in this field which will add to their production costs (Khan et al., 2018). According to experts, this augments the unpredictability of seaweeds which will affect the employment opportunities of the biofuels sector and availability of skilled labour because it will no longer remain a stable source of income. Indirectly, this will have strong impacts on biofuel production (V. G. S., et al., 2021). Furthermore, the environmental impacts of biorefinery construction along the ocean shores need to be thoroughly investigated to know their impacts in the future (V. G. S., et al., 2021).

FUTURE PERSPECTIVES

The biofuel sector is naïve and requires sufficient improvisation to increase their yields with lower investments. This provides several research opportunities in this area of which the environmental issues need to be properly addressed. Some current publications in this field show that the environmental issues that are discussed are mainly the ones involved with their direct combustion. Those related to macroalgae cultivation have, however, been poorly explored. This needs extensive research because of the proposed negative impacts on the marine ecosystem in the long run. Suitable mitigation strategies should be undertaken to resolve the issues failing which the sector will not only lose its sustainability but

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also its affirmation of being eco-friendly substitutes of conventional fuels. Regarding the technological advancements, importance should be given to the development of cost-effective techniques to make the biofuels cheap and readily available. This should not, however, compromise with their efficiency since that may restrict their applicability. The biofuel industry has a huge scope for recycling the wastewater by utilizing them in the cultivation of algae. Considering the growing amounts of wastewater production in the recent years, with increased urbanization and industrialization, developments in this sector can mitigate the problem of wastewater disposal as well.

CONCLUSION

Biofuels have tremendous potentialities to replace the conventional non-renewable energy resources and combat the production of greenhouse gases. This can not only solve the problem of future threats of energy availability but can also address the problem of global climate change. In this regard, the high carbohydrate contents and low proportions of lignin in macroalgae make them efficient sources of biofuels like bioethanol, biogas and biohydrogen. Biodiesel can also be extracted from macroalgae but microalgae gives better yield because of their higher content of unsaturated fatty acids compared to macroalgae. It is the versatility of macroalgae in terms of biofuel production that they are being considered to be more prospective biofuel source in the future. However, there are certain challenges, both technical and environmental, concerning their cultivation and biofuel extraction which have hindered their production in large-scales. Moreover, they have not been as extensively studied as the microalgae. Since, this sector is still in its developmental stages it gives ample opportunity for research. Resolution of the issues in this field can make macroalgae-derived biofuels one of the most promising alternative energy source in the future.

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Chapter 10
A Source of Future Energy: Gas Hydrates

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ABSTRACT

Natural gas hydrate (NGH) has emerged as a future source of energy for the world that can fulfill the future energy demand. The natural gas hydrates hold twice the amount of energy than all available fossil fuels. At present, the energy demand is growing, but these are not available commercially. Various field applications on the dissociation of the natural gas hydrates have been performed till now, and the output of these field applications confirms the possibility of dissociation of the natural gas hydrates. At distinct locations in the world, the huge deposits of hydrates have been identified, but the safe and economic dissociation of gas hydrates is not known yet. Hence, the evolution of new and inexpensive, suitable methods and technologies are required for the dissociation of gas hydrates. In this chapter, the authors reviewed several articles to compare the different dissociation techniques of gas hydrates and their field applications. The advantages and disadvantages of these production methods have also been discussed.

INTRODUCTION

Gas hydrates are non-stoichiometric solid formed naturally by the entrapment of gas within the crystal structure of water molecules. When water molecules entrap gas molecules at high pressure and low temperature, then create the cage-like structure of gas hydrates. It is also called combustible gas because it can be burned at ambient temperature and pressure. Gas hydrates can be formed by methane, ethane,

DOI: 10.4018/978-1-6684-5269-1.ch010

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hydrogen sulfide, carbon dioxide, nitrogen, propane, isobutane, n-butane, etc (Gabitto and Tsouris 2010). At equilibrium conditions, $1m^3$ of natural gas hydrates contain 160-180 m^3 of natural gas (Li et al. 2011). Naturally, the methane gas hydrates are made by combining the water and the methane gas released either by organic decomposition or by thermogenic gas leaking beneath the sea level or permafrost region (Zhou et al. 2009). The natural deposits of gas hydrates are classified as Class 1, 2, and 3 types of reservoirs. The Class 1 type of hydrate deposits are consisting of a layer of hydrate and an underlying free gas zone. The Class 2 deposits are consisting of a layer of hydrate and an underlying mobile water zone. The Class 3 type hydrate deposits are created with an isolated layer of hydrate with no underlying zone of free gas or mobile water. In Class 3 deposits, the whole layer of hydrates is in the stability zone and enclosed by the impermeable lower and upper burden layer (Huang et al. 2016).

Sir Humphrey Davy discovered the gas hydrates for the first time in 1810, but there is also a possibility that Priestley found it in 1778 (Sloan and Koh 1998). Natural gas hydrates can be found in the high altitudes/latitudes of permafrost or in the sediment layers, which occurs in deep water on the continental margins (Kvenvolden 1998; Kvenvolden and Rogers 2005). Small parts of red lakes, i.e., the continental land, seas of the Caspian Sea, Lake Baikal, and the Black Sea, also contain natural gas hydrates. These reserves were discovered in mountain permafrost regions or deep water, such as South China Sea, the Gulf of Mexico, Indian Ocean, Siberia, Arctic Alaska, multiple areas of Qilian and Mainland China (Ginsburg et al.1992; Ginsburg and Soloviev 1994; Lu et al. 2011). The curiosity for scientific research in gas hydrates increased exponentially when it became responsible for the blockage in oil and gas pipelines. Hence various research studies are in progress for the dissociation of the gas hydrates because it can solve the crisis of energy in the future and then become a potential source of energy in the world (Koh et al. 2009). Currently, the available methods for decomposing gas hydrates are chemical injection, depressurization and thermal stimulation (Kumari et al. 2020).

There is a need to find the cost-effective technologies for the economic dissociation of the gas hydrates, and the rate of dissociation will control this economic production of gas. Figure 1 depicts the available dissociation techniques of gas hydrates. Table 1 listed the list of some patents filed on new dissociation techniques of gas hydrates.

Figure 1. Dissociation techniques of gas hydrates
(Arora et al. 2015a)

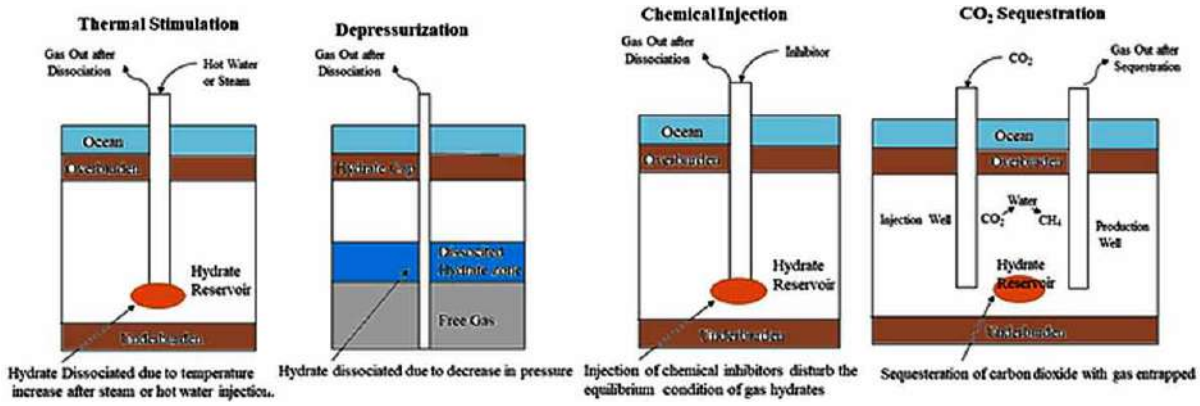

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Table 1. List of some Patents filed on New Techniques for the dissociation of gas hydrate.

Assignee	Description of Patent	References
Mobil Oil Corporation, Fairfax, VA (US)	Electromagnetic radiation can be applied for the recovery of gas from the gas hydrates.	(Heinemann et al. 2001)
ConocoPhilips Company, Houston, TX(US)	The substitution of the gas has formed a more stable hydrate structure with the releasing agent without melting the gas hydrates.	(Graue et al. 2007)
New Mexico Tech Research Foundation, Socorr, N.M.	Apparatus and methods for the transportation, generation, and the dissociation of hydrates	(Guo et al. 1995)
ConocoPhilips Company, Houston, TX(US)	By the application of electromagnetic radiation, methane can be recovered.	(Singhe 2013)
The University of Tulsa, Tulsa, OK (US)	The electric heater was used in the opening of hydrate rich subset sediment, and then the released gas is collected in an overhead receiver.	(Zhang et al. 2009)
Chevron U.S.A. Inc., San Ramon, CA (US)	A two-stage method has been employed to produce fluids from a Class 3 reservoir.	(Gullapalli et al. 2009)
Halliburton Energy Services, Inc., s s Duncan, Okla.	The method or the decomposition of hydrates is to combine the acidic liquid with basic liquid to form an exothermic chemical reaction and form a salt solution.	(Chatterji and Griffith 1998)

The main objective of this chapter is to review the various dissociation methods available for gas hydrates and compare them based on different parameters such as cost, suitability for the environment, and stability conditions. We also compare these methods by focusing on their importance and problems that arise by using them and investigated the stability parameters, i.e., temperature and pressure on which gas is dissociated. We will highlight the drawbacks of various dissociation techniques and introduce some new methods, which can be used. Also studied the implementation conditions for these methods. Finally, a section of conclusions, final remarks, comparisons, issues, and directions for future research has been discussed for all dissociation techniques of gas hydrates.

THERMAL STIMULATION

In the thermal stimulation dissociation method, the gas hydrate is dissociated into water and gas after an increase in the local temperature of the gas hydrates system. The increase in temperature will disturb the stability condition of the gas hydrate system (Fitzgerald and Castaldi 2013). Hydrate is dissociated due to the in-situ heat developed in the hydrate reservoir. The thermal energy has been provided by injecting hot fluids like brine, water, or steam from the upper surface or maybe by downhole processes like electromagnetic heating and electric or in situ combustion (Ning et al.2010). Various novel ideas have been developed due to the relatively high rate of production of gas, such as fire flooding, nuclear waste burial, electromagnetic heating, microwaves (Shashkov et al. 2012), downhole combustion (Castaldi et al. 2007). Still, these methods have not been used for field testing till now. The main technological challenges in the thermal simulation method are the thermal properties of the sediments and the self-preservation phenomena of gas hydrates. The heat transfer during the thermal stimulation method is characterized by thermal conductivity mainly, and the thermal conductivity of hydrates will increase by the presence of minerals in sediments. The drawback of this method is the self-preservation of ice

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around the hydrate after the dissociation below the freezing point of ice. The water extracted after the dissociation of gas hydrate can again form the ice because of the endothermic heat of dissociation and prevent further hydrate dissociation (Handa 1986; Shimada et al. 2005; Chong et al. 2016). Su et al. 2012 examined the production potential of the Huff and puff technique for the production of gas in the Shenu area on the northern continental slope of south China sea. They dissociated hydrate by producing fluids and injecting hot water alternatively. They suggested that the huff and puff technique through a vertical wall is not favorable and has not industrial potential (Su et al. 2012). Ning et al. 2010 proposed a technique to use renewable solar energy to provide energy for the dissociation of marine gas hydrates with the thermal stimulation method. In the first step, the conversion of solar energy to electric energy has proceeded. Then heat energy has been created by operating the downhole heaters such as electromagnetic waves and the microwave heaters (Ning et al. 2010). Yang et al. 2010 investigated the efficiency of the single well hot water cyclic injection method to recover the gas from methane hydrate-bearing sand. In this method, the overall temperature enhances with the injection of hot water and reduces with the production of gas. This technique is favorable for the hydrate with low permeability and high saturation (Yang et al. 2010). Phirani and Mohanty 2009 suggested that the injection temperature (323 K), pressure (2 & 4 MPa), and production pressure take an important part for the hydrate reservoirs of confined class 2 type. They concluded that when the pressure and the injection temperature of the production well are high, then warm water flooding is more suitable than depressurization (Phirani and Mohanty 2009). Castaldi et al. 2007 suggested that the downhole combustion technique is very energy efficient for gas production. This method uses local source of heat in the hydrate reservoir (Castaldi et al. 2007). Li et al. 2013 designed a three-dimensional pressure vessel to predict the dissociation of methane gas hydrate by steam-assisted gravity drainage method with different rates of injection of steam (Li et al. 2013). Kawamura et.al 2007 experimented with the steam injection to examine the thermal control system on the artificial sediment with and without methane hydrate. Approximately 44% of the hydrate origin gas was dissociated after the injection of steam (Kawamura et al. 2007). Sakamoto et.al. 2004 developed a special type of apparatus and experimented with showing the physical phenomena and production behavior of gas from hydrates by the injection of hot water. The production of gas from hydrate increases with time (Sakamoto et al. 2004). Sasaki, et al. 2007 proposed a novel system for the recovery of gas from methane gas hydrates layers by injecting hot water by manufacturing dual horizontal wells. The production rate of gas increases with the rate of hot water injection and the absolute permeability (Sasaki et al. 2007). Cranganu 2009 proposed that instead of using hot fluid injection from the surface, an air or gas fuel mixture can be injected into the combustion vessel from the upper surface and then into the gas hydrate zone directly (Cranganu 2009). Li et al. 2008 examined the dissociation behavior of gas hydrate using hot brine stimulation. With the doses of brine in the vessel, the dissociation of hydrate accompanied the decrease in temperature (Li et al. 2008).

The interaction characteristics between electromagnetic waves and disperse systems, the high-frequency energy, and microwave electromagnetic fields can be used to decompose gas hydrates (Fatykhov and Bagautdinov 2005). A heat source has been generated because of the interaction between high-frequency electromagnetic radiation and medium, and the energy of propagating electromagnetic wave converges to heat. This heat source distributed over the gas hydrate volume, and then phase transition occurs in the absence of temperature gradient. In the case of large zones of phase transition can occur, due to which the decomposition temperature of gas hydrate is attained, and the energy of the electromagnetic field has been extended to the dissociation of gas hydrates (Nasyrov et al. 1997). Islam 1994 proposed a new production technique for producing natural gas from Alaskan north slope reservoirs by using electro-

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magnetic heating with the vertical and horizontal wells. They also concluded that the porosity and the thickness of the gas hydrate reservoir are essential factors for the determination of the total amount of recovered gas (Islam 1994). Rahim et al. 2015 suggested that the plasma decomposition rate of methane to hydrogen production is faster than the methane hydrate decomposition by the microwave plasma method (Rahim et al. 2015). Liang et al. 2009 discovered that the decomposition rate is higher for the high power of microwave. However, a heat efficiency problem is raised as the temperature of the system increases. The temperature difference in the system resulted from the difference of dielectric properties of components (Liang et al. 2009). He et al. 2011 observed the decomposition behavior of gas hydrate in the unconsolidated sediment. They concluded that the sediment could supply an adequate amount of heat for decomposition, then the rate of hydrate decomposition is controlled by the intrinsic kinetics (He et al. 2011). Zhao et al. 2016 examined the dissociation of methane gas hydrates formed in the sediment by applying microwave stimulation (Zhao et al. 2016). Amit Arora 2015 proposed dissociation techniques of the gas hydrate by using microwaves (Amit Arora 2015).

Depressurization

This method is the first production method that has been applied outside the laboratory. The stability of gas hydrates is disturbed by exposing the hydrates to a low-pressure domain where the hydrates become unstable and then dissociated into water and gas. The released gas can be recovered by applying conventional technology (Ji et al. 2001; Moridis et al. 2005; Tsypkin 2005; Tang et al. 2007; Demirbas 2010a). The depressurization method is most favorable for those natural deposits where inescapable gas is found in a closure that forms the hydrate cap above it (Max and Cruickshank 1999). A well is extended and drilled into the hydrate layer and formed in the zone of free gas. The dissociated gas from this hydrate layer causes a reduction in pressure and then dissociation of the hydrate. The dissociation of hydrates in the Messoyakha fields has been done by the depressurization method (Makogon et al. 2007; Makogon 2010). There is no need for expensive continuous stimulation for the depressurization method. Hence it is an effective method for future gas hydrate dissociation et al. 2010).

Different methods have been suggested for the economic development of hydrates as an unconventional source of gas (Sawyer et al. 2000; Goel et al. 2001). The dissociation of free gas using traditional techniques generates a pressure drop. The hydrate becomes unstable because of this pressure drop and dissociates gas hydrates into the water and free gas. Three mechanisms involved in this method are dissociation, heat transfer with convection and conduction, and flow of water and gas (Goel et al. 2001; Kawamura et al. 2003). The most economical method for the dissociation of hydrates is depressurization because an external source of energy is not needed in this method as compared to other methods. But the drawback is that this method has a lower production rate due to slow reaction for the dissociation (Lee et al. 2010). In the depressurization method, the in-situ permeability or free gas fraction will affect the gas production rate. The depressurization method induced dissociation is limited by heat transfer and mass transfer, and free gas saturation is the critical factor for establishing the feasibility of gas production from marine deposits. The gas production at low pressure is fast compared to gas production at high pressure (Chong et al. 2016). Commercially this technique was used by the USSR to dissociate gas from gas hydrates in the Messoyakha field (Makogon 1997b). Kono et al. 2002 analyzed the rate of dissociation of methane gas hydrate using depressurization and also concluded that the properties of sediments could control the rate (Kono et al. 2002). Haligva et al. 2010 analyzed the decomposition of silica sand particle hydrate by depressurization method (Haligva et al. 2010). Ji et al. 2001 found out that the pro-

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duction rate of natural gas from a reservoir depends on well pressure, the reservoir's temperature, and the permeability of the reservoir zone (Ji et al. 2001). Kim et.al 2013 experimented on pressure cores, which were recovered from holes of UBGH (Ulleung Basin Gas Hydrate) (Kim et.al 2013). Moridis and Sloan 2007 evaluated gas recovery's potential from low saturation and dispersed hydrate formed in oceanic sediments. They also estimated the rate of gas and water production (Moridis and Sloan 2007). Lee et al. 2010 developed an experimental equipment to observe the dissociation behavior of gas hydrate in the porous rock (Lee et al. 2010). The depressurization technique was applied for field testing in Nankai Trough of Japan using two producer wells (Yu et al. 2019).

CHEMICAL INJECTION

In the chemical injection method, the equilibrium condition of hydrates must be displaced by the injection of liquid inhibitor or chemical towards the high pressure and low temperatures. This technique is also expensive, but it is less costly than thermal stimulation (Demirbas 2010a). The main factors to be considered in the chemical injection method are the concentration and temperature of chemicals, rate of injection of chemicals, pressure, and the interfacial area between hydrate and chemical. The heat of dissociation will be decreased after the chemical injection (Fan et al. 2006; Chong et al. 2016).

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Table 2. List of Studies on Gas Production from Gas Hydrates by Chemical Injection Method

S.No	Description	Chemical	Advantages	Disadvantages	References
1	Hydrate saturation affects the onset time, but it has less effect on the decomposition rate. The factors, which were considered in this study, are injection rate and saturation.	N2	An optimum N2 injection rate enhances the economic benefits. The N2 injection method is advisable for the exploitation of low hydrate saturation, and it is more advantageous than depressurization.	They did not consider the effects of deposit conditions of hydrates on dissociation by N2 injection.	(Zhang et al. 2017)
2	They analyzed the effect of brine salinity and recovery characteristics of gas from the injection of various concentrations of brine.	Brine	Permeability is an essential factor in determining the production of gas from hydrates. At a low concentration of brine, gas production is excessively high.	There is a significant reduction in the rate of production of gas because of the high concentration of brine.	(Lee 2010)
3	Experimented with the gas recovery from methane gas hydrate in the porous sediment by applying the different rate of injection of ethylene glycol and concentration in a one-dimensional experimental machine	Ethylene Glycol	The efficiency of gas production is disturbed by the concentration of chemicals and the rate of injection, and at last, it attains a maximum value.		(Li et al. 2007)
4	Conducted the dissociation experiment to synthesize the gas hydrate by adding ethylene glycol as an inhibitor in the reaction vessel	Ethylene Glycol	This study has importance not only for natural gas hydrate development but also for transportation and natural gas production.		(Fan et al. 2006)
5	Developed the model to observe the production behavior from the injection of methanol and combined the depressurization mechanism.	Methanol	They prove that the methanol content is the most sensitive parameter for the time taken for the complete dissociation of the well block.	When the injection is done after high production time, the injection effect is not expected to be much during the production.	(Sung et al. 2002)
6	Examined the characteristics of the dissociation process of the hydrate.	Ethylene Glycol and Methanol and	Include the effect of inhibitors on hydrate formation in the natural gas processing, transportation, and natural gas production.		(Sira et al. 1990)
7	Examined the dissociation behavior of artificially created hydrate core samples using an aqueous inhibitor solution.	Methanol	The advantage of an inhibitor was higher at low temperatures.	Gas production behavior at a temperature lower than 19°C has not been investigated.	(Kawamura et al. 2006)
8	Investigated the dissociation behavior of methane hydrates in ethylene glycol solution and silicone oil.	Ethylene Glycol and Silicone oil	Ethylene glycol solution helps motivate the dissociation process when required during the process, and silicone oil is favorable for the transportation of slurry and storage systems.	Silicone oil is not helpful for the dissociation of methane gas hydrate.	(Kawamura et al. 2003)

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In this method, various chemicals, for example, glycerin, methanol, glycol, and ethanol, are injected, which shifted the thermodynamic equilibrium stability curve of hydrates. The above techniques have been applied only in permafrost areas and till now have not been used in the dissociation of gas hydrates of marine areas (Williams et al. 2005; Hancock et al. 2005; Makogon 2010; Chong et al. 2016). These verifications are based upon several external factors such as marine and environmental, geological hazards. The advantages and disadvantages of some research work on chemical injection are listed in Table 2.

CO_2 SEQUESTRATION

The shortcomings of the chemical injection method thermal stimulation method, and depressurization method, is the low efficiency of heat transfer in a reservoir, which controls the decomposition rate of hydrates. The thermal conductivity of formation is relatively poor; hence the efficiency of the decomposition process cannot be compensated. The temperature in the hydrate decomposition region declines rapidly due to the huge absorption of heat. The above methods will decrease the reservoir's strength and further cause slope instability, seabed destruction because these methods depend on the principle of decomposition of hydrate (Khlebnikov et al. 2016). The CO_2 replacement method appears to be more profitable as it permits both recovery of gas from hydrates and sequestration of CO_2 as hydrates in the region of deep-sea sediments. This method is applicable where the ocean floor remains stable during the process and after the injection of CO_2 (Ors and Sinayuc 2014). The CO_2 sequestration process is the complex and immature technique for the dissociation of hydrates. The main drawback of CO_2 sequestration is the requirement of a high amount of CO_2, which can increase the process's cost (Ors and Sinayuc 2014). Several researchers have done work on this method for the decomposition of hydrates before and after the experiment. Studies were done by Yoon et al. 2004 revealed that the surface covering of CO_2 gas hydrate in the outermost layer is taken as an important part for deaccelerating the dissociation of methane gas hydrate within the core. But the water phase generated during the sequestration process allows the process to move forward rapidly by increasing the diffusion of carbon dioxide and methane, which results in absolute and comparatively fast production of methane gas (Yoon et al. 2004). Seo et al. 2013 suggested that various factors such as reaction kinetics, the environment of sediment, size of hydrate particle, and mass transfer should have to be considered for the replacement of CO_2 in natural gas hydrates deposits (Seo et al. 2013). Xu et al. 2017 worked for the replacement of different gas mixtures to produce methane gas from hydrates by the replacement method. Small molecules sized gases enhanced the rate of replacement by the stronger diffusivities in gas hydrates (Xu et al. 2018). Ota et al. 2005 found out that the mole fraction of CO_2 and CH_4 in the hydrate zone increases and decreases with time, respectively. They also provided a kinetic model for the exchange process by including the tendency of CH_4 re-generation in the S-Cage (Ota et al. 2005). Liu et al. 2016 investigated the thermodynamic and kinetic feasibility of CH_4 from hydrates by N_2/CO_2 mixture using the ab initio calculations and ab initio molecular dynamic simulations (Liu et al. 2016). Xu et al. 2015 examined the production of methane from a natural gas hydrate by CO_2 replacement by the estimation of similarity between various spectra of C-H, O-H, and C-O stretching vibrations in the different phases and gas hydrates with the help of Raman and NMR (Xu et al. 2015). Lee et al. 2014 developed an experimental system to obtain quantitative information for the exchange process of CO_2 and CH_4 (Lee et al. 2014). Espinoza and Santamarina 2011 designed an experiment to find the advantage of the contact- dependent sand stiffness on the formation of hydrate, CO_2 flooding and gas replacement, and dissociation (Espinoza and Santamarina 2011). Jung

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et al. 2010 evaluated the reaction rates, analyzed underlying phenomena, explored the region for the pressure-temperature for the optimal change, and anticipated the potential geomechanical implications the replacement in hydrates bearing sediments (Jung et al. 2010). Serrano et.al 2009 tested a continuous jet hydrate reactor in the seafloor process simulator for the CO2 replacement (Serrano et al. 2009). Pohlman et al. 2009 suggested that the decreased sedimentation rate increases the disclosure time of organic matter in sediment to anaerobic and aerobic degradation (Pohlman et al. 2009). Seo et.al 2016 experimented with investigating the replacement mechanism in the C_3H_8+CH_4 hydrate (sII) by injecting a gas mixture CO_2/N_2 (Seo et al. 2016). Yang et.al 2017 explored the potential for the production of methane from gas hydrate reservoirs by the injection of flue gas and form a carbon dioxide hydrate by the sequestration of CO_2 from flue gas (Yang et al. 2017). Wang et al. 2017 proposed a new dissociation technique for natural gas hydrate which improves the effect of replacement and reduces the cost of gas separation, but it decreases the partial pressure of methane gas in the gas phase. H_2 has an important role in disturbing the stability of methane hydrate (Wang et al. 2017b). Seo et al. 2013 suggested through the thermodynamic equilibrium studies that the composition of CO_2 in the hydrate zone and the chemical formula of mixed gas hydrate after the sequestration process can be determined at certain conditions (Seo et al. 2013). Lee et al. 2015 showed that the structural and thermodynamic properties of the mixed gas hydrates are sensible factors to determine the flow of heat in the sediments, for the required heat for the dissociation of hydrate and for the evaluation of the storage capacity of CO_2 in hydrate reservoir (Lee et al. 2015). Zhang et.al 2017 proposed a combined method using CO_2 replacement and the thermal stimulation to increase the recovery of methane gas from hydrates and CO_2 storage (Zhang et al. 2017). Ors and Sinayuc 2014 concluded the swap process of CH_4-CO_2 happens at the surface of the system mostly and the center of the system of unconsolidated sand pack accommodate CH_4 while surface contains CO_2 (Ors and Sinayuc 2014). Deusner et al. 2012 observed the production of methane from the methane gas hydrates by the injection of hot and supercritical CO_2. They concluded that the dissociation of methane gas hydrates is triggered by the heat addition, while thermally equilibrated CO_2 will react with pore water and reserves in the reservoir as immobile. They also studied the effect of temperature on the production process of methane gas from sediment by studying the effect of permeability (Deusner et al. 2014).

The above methods are not favorable for dissociating gas hydrates in marine sediments due to their limitations and disadvantages (Ning et al. 2010). Table 3 listed the advantages and disadvantages of available dissociation techniques of gas hydrates.

Table 3. Advantages and Disadvantages of different dissociation techniques of gas hydrates.

Technique	Advantages	Disadvantages	References
Depressurization	can reduce energy consumption	Advantageous for the widespread gas reservoirs in a closure	(Max and Cruickshank 1999)
Thermal Stimulation	Can overcome the problems associated with low gas production by depressurization	High loss of heat Low efficiency High consumption of energy	(Song et al. 2016; Zhang et al. 2017)
Chemical Injection	Less expensive than other methods	Not advantageous for the oceanic area Very expensive Environmental Pollution	(Demirbas 2010b; Ning et al. 2010)
CO2 replacement	----	The cost of CO2 supply is high. Efficiency and replacement speed is low.	(Lee et al. 2003; Ors and Sinayuc 2014)

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COMBINED PROCESS

The thermal Stimulation method can also be combined with the depressurization method to dissociate the gas hydrates. Various studies were reported to observe the effects of this combined method. Wu et al. 2012 used two methods (thermal stimulation and depressurization) under the equilibrium pressure for the dissociation of pure methane hydrates formed below the ice point (Wu et al. 2012). Song et.al 2015 provided the experimental investigation of the dissociation of gas from porous media by the injection of two-cycle warm water, depressurization, and combined method with different hydrate saturations. They concluded that the combined method is an effective and improved method for the dissociation of gas from methane gas hydrate formed in sediments. The combined process increases the average production rate of gas with increasing saturation (Song et al. 2015). Yuan et.al. 2013 studied hydrate dissociation using hot water, ethylene glycol, and brine solution with the continuous injection mode. The NaCl solution shows the highest amount of gas production (Yuan et al. 2013). Wang et al. 2014 examined the production behavior of methane gas hydrate by applying the three-dimensional pressure vessel and thermal stimulation method in the porous media. Using, the experimental conditions are manipulated by the set of scaling criteria for the gas hydrate reservoir (Wang et al. 2014). Wang et al. 2017 experimented with investigating the effect of thermal heat flow on the dissociation of gas hydrate by applying the depressurization method with the thermal stimulation (Wang et al. 2017a). Pang et al. 2009 showed the kinetic behavior of dissociation conducted by hot water. They showed that the rate-determining factors for hydrate dissociation are the heat transfer rate and the thermodynamic driving force of dissociation. The production of gas from hydrate can be increased by combing heating and depressurization (Pang et al. 2009). Liu et al. 2009 developed a mathematical model of dissociation of gas hydrate in the porous media by applying the depressurization and the thermal stimulation method. The Huff and Puff method was applied within the Prudhoe Bay of the Alaskan North Slope (Liu et al. 2009). The dissociation was carried using N_2-CO_2-CH_4 exchange to produce methane from the hydrate reservoir (Max and Johnson 2018).

ECONOMICS

The production of gas from hydrates is seemed to be more expensive as compared to conventional natural gas. Because of the higher cost of current dissociation techniques, it becomes essential to find the economically favorable dissociation techniques of gas hydrates based upon higher production rate, lower transportation cost to balance the higher wellhead cost as compared to the conventional gas production from gas fields (Cranganu and Nitzov 2009). Many researchers have suggested depressurization as the most economical dissociation techniques for gas hydrates (Makogon 1997a; Pooladi-Darvish 2004). Table 4 shows the economic comparison of different dissociation techniques of gas hydrates.

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Table 4. Comparison of different dissociation techniques of gas hydrates (Collett 2002; Cranganu and Nitzov 2009; Arora et al. 2015b)

Parameter	Depressurization	Thermal Stimulation	Conventional Gas Production
Investment (million Rs.)	166000	254200	157500
Annual Cost (million Rs.)	125500	160000	100000
Total Production (million m^3/year)	1557.43	1274.26	1557.43
Production Cost (Rs. million/m^3)	4025.9	6356.6	3213.6
Breakeven Wellhead Price (Rs. million/m^3)	2532.3	7945.8	3972.9

CONCLUSION

Natural gas hydrates are a significant source of energy all over the world. It can replace the various other sources of natural gas to fulfill the energy requirement of the world in future. Hence, natural gas recovery from gas hydrates became a significant research area in the present days. Various techniques are available for the dissociation of gas hydrates, but the economical production of gas from gas hydrates is a significant issue nowadays. Various safety issues are associated with the recovery of hydrate energy source for example seafloor subsidence and green house effects. The production testing of hydrate is in progress in Canada, The United States of America, Japan and India. This paper reviews the present dissociation techniques of gas hydrates and their advantages and disadvantages. Hence, to overcome these disadvantages, there is a need to explore the new dissociation techniques for the economical production of gas from gas hydrates. Hydrates can contribute significantly to the conventional future energy demand of the world.

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Chapter 11
Recent Advancements in Microalgae-Biofuel Generation Employing Nano-Additives

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ABSTRACT

Biofuels have gained significant interest as an alternative fuel in recent years owing to their environmental sustainability, cost-effectiveness, and the ability to blend with traditional fuels like gasoline without requiring engine modifications. The use of microalgae for biofuel production is universally preferred due to its energy efficiency and environmental sustainability; current research is geared towards enhancing the production of microalgae-biofuel from the initial stages to the final product as it is a cost-effective fuel option. The use of different types of nano-additives at different stages of microalgae cultivation and incorporation into biofuel produced noteworthy improvements in the final product. This chapter focuses on the prospective uses of nano-additives in microalgae cultivation, microalgal biomass conversion to biofuels, and biofuel combustion improvement for revolutionary advancements in biofuel technology.

INTRODUCTION

Photosynthetic microorganisms like green microalgae, diatoms, and cyanobacteria offer significant advantages over terrestrial plants providing an abundance of various biomolecules to be used for food, feed, and fuel applications. Microalgae or microphytes are microscopic algae. They are naturally found in freshwater bodies and oceans. Microalgae are unicellular species that can exist individually or in

DOI: 10.4018/978-1-6684-5269-1.ch011

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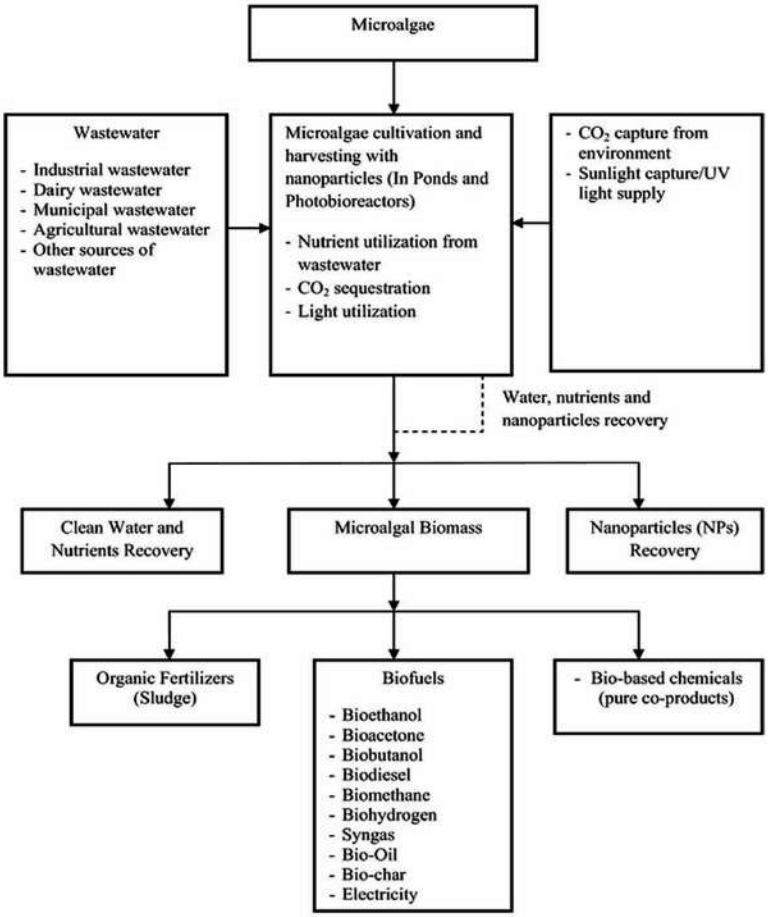
chains or groups. Microalgae can vary in size depending on the species, from a few micrometers (μm) to a few hundred micrometers. Microalgae are efficient photosynthesizers and are crucial for life on earth producing around half of the atmospheric oxygen it also performs its role as a photoautotroph consuming greenhouse gases like carbon dioxide to grow. Forming the base of the food web Microalgae and bacteria provide energy for all the trophic levels above them (Peter *et al.,* 2021). There exists a rich biodiversity of microalgae which represents an abundant, almost untapped resource. There are about 200,000-800,000 species in different genera of microalgae of which about 50,000 species are characterized and described. More than 15,000 novel compounds originating from algal biomass have been identified including carotenoids, antioxidants, fatty acids, enzymes, polymers, peptides, toxins, and sterols.

Microalgae form an abundance of carbon-rich biomass, that can be tapped for the production of biofuels, cosmetics, health supplements, and pharmaceuticals. They also have numerous applications in wastewater treatment and sequestration of atmospheric CO2. A wide range of bioproducts, including polysaccharides, lipids, pigments, bioactive compounds, proteins, vitamins, and antioxidants are also derived from microalgae. There is a rapidly growing interest in the use of microalgae as a renewable and sustainable feedstock for biofuels production fueling new research in the field of biorefinery using microalgae. The cultivation of microalgae on an industrial scale to produce biofuels and bioproducts has increased radically in the last couple of decades (Low *et al.,* 2021).

Figure 1. Different biofuels from microalgae and conversion processes
(Hossain et al, 2009)



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MICROALGAE AS A BIOFUEL

The increasing global demand for fuel energy, the intensive use of fossil fuels worldwide leads to its depletion and economic crises and global tension around the control of fossil fuels heralds the need for alternative, environment-friendly and sustainable fuel sources. Thus, biofuels are increasingly viewed as an alternative to fossil fuels. Some developed countries have already started the mass production of biofuels. Biofuels such as biodiesel and bioethanol are superb alternative fuels and can be produced from a number of sources of biomass, including garbage, food crops, crop wastes or fruits, woody parts of plants, and algae (Harun, Danquah and Forde, 2010; Ho, Chen and Chang, 2010). The advantages of the use of biofuels from biomass are renewability and a significantly smaller contribution to environmental pollution and global warming. The release of CO2 from the burning of fossil fuels is one of the main causes of global warming. 29 gigatons/year of CO2 is released with a total of 35.3 billion tons of CO2 released to date from the burning of fossil fuel (Paul *et al.,* 2014). Biofuels like algal fuels have oxygen levels of 10–45% and negligible levels of sulphur emission while petroleum-based fuels have no oxygen levels and considerable sulphur emission. Microalgae fuels are known for being environment-friendly, non-toxic, and capable of fixing global CO2. Gendy *et al.,* 2013 reported that 1 kg of algal biomass is capable of fixing 1.83 kg of CO2 additionally certain species are known to utilize and remove SOx and NOx along with CO2.

Various applications have been exhaustively studied for different species of microalgae. Table 1 summarizes various species of microalgae, ideal culture settings (cultivation environment and water quality), availability, and cellular requirements (Hossain, Zaini, Mahlia, and Azad, 2019). Carbohydrates, proteins and lipids are the primary constituents of complex biofuels (e.g., bio-oil, biodiesel, biobutanol, etc.,) (Harun and Danquah, 2011, Pathak, Singh, Kothari and Chopra, 2014). Microalgae contain large fractions of lipid, protein, and carbohydrates which are important for biofuel production. Table 2 details the cellular composition of prominent microalgae species used for biofuel production (Hossain *et al.,* 2019). Microalgae are ideal candidates for biofuel production owing to its feasibility to be grown and harvested on both land and water and the high lipid content. Nanotechnology comes into the picture because lipase immobilization and lipid extraction from microalgae are costly processes. To address this, nano-additives are increasingly being used in the production of microalgal-based biofuels.

Table 1. Prospective species of microalgae for the production of biofuel (Hossain et al., 2019).

Name of the Microalgae
Chlamydomonas reinhardtii
Symbiodinium sp.
Phaeodactylum tricornutum
Chlorella vulgaris
Saccharina japonica
Spirulina platensis
Ostreococcus tauri
Chlorella sp.
Nannochloropsis sp.
Botryococcus braunii
Chlorococum sp. and *Spirogyra* sp.

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Table 2. Microalgae species with optimal physiochemical composition for biofuel generation (Hossain et al., 2019).

Species of Microalgae	Total Sugars Content (%)	Protein Content (%)	Lipid Content (%)	Ash and Other Content (%)
Bellerochea sp.	3	24	15	3
Chaetoceros sp.	2	18	18	3
Chlamydomonas reinhardtii	48	17	21	14
Chlorella sp.	56	22	19	3
Dunaliella salina	57	32	6	5
Porphyridium cruentum	35	50	11	4
Rhodomonas sp.	9	74	15	2
Scenedesmus sp.	18	56	12	–
Spirogyra sp.	20	55	16	9
Spirulina platensis	60	12	8	20

Numerous biofuels like biodiesel, bioethanol, biomethane, bio-oil, bio-hydrogen, etc., are produced from microalgae (Brennan and Owende, 2010; Hossain *et al.,* 2019). Biodiesel is increasingly used as a replacement for fossil-based diesel as a sustainable biofuel. Despite the many benefits of using biodiesel, properties such as lower fuel atomization and high viscosity, result in BTE reductions. Innovation in the field of nanotech have resulted in the development of nano-molecules with improved thermal properties, which help in combustion, and increase volume-surface ratio and results in improved fuel characteristics and better engine performance and lower emissions. Nano-particles can be added during various stages of microalgal growth (e.g., cell suspension, cell separation, and cell harvesting), biofuel conversion stage and fuel application stage for increased total yield in each stage.

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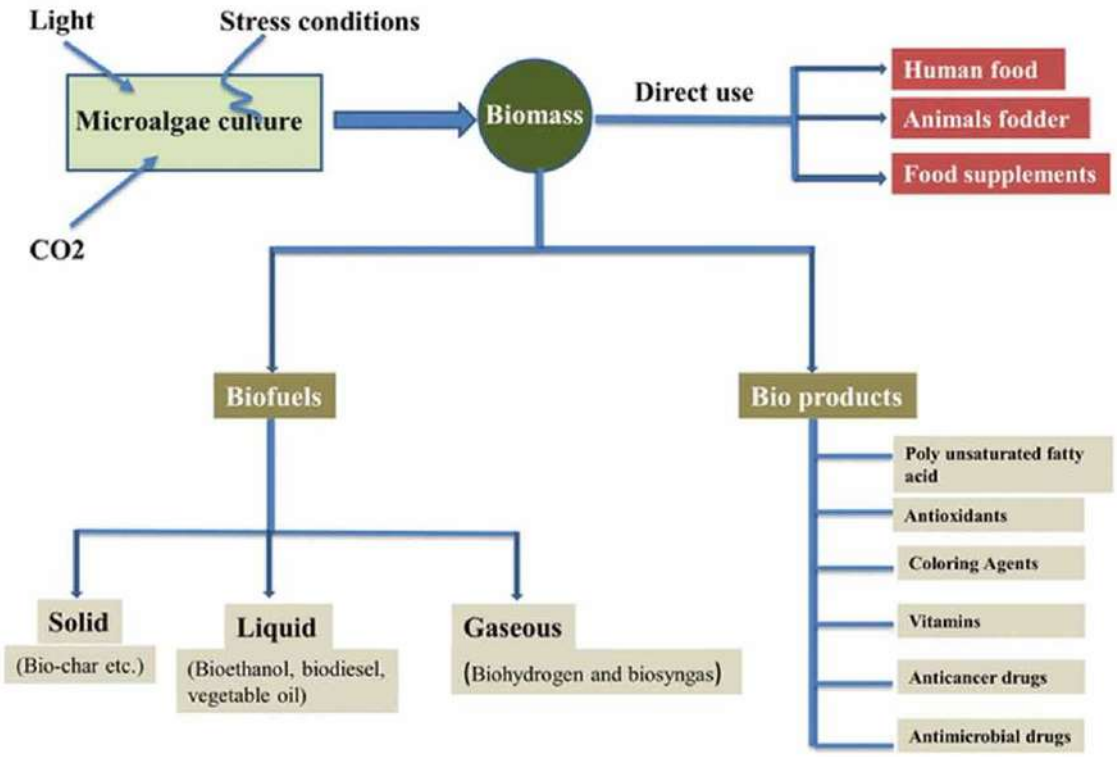
Figure 2. Microalgal conversion of atmospheric CO2 to carbohydrates, lipids and other valuable bio-products by using light
(Khan et al, 2018)



MICROALGAE-BIOFUEL WITH NANO-ADDITIVES

Nanoparticles and Nano-Additives

Nanoparticles (NPs) include a collection of materials that have at least one dimension less than 100 nm. Nanomaterials can be 0D (zero dimensional), 1D, 2D, or 3D depending on the general shape (Tiwari, Tiwari and Kim, 2012). NPs are usually made up of three layers i.e. (a) The surface layer- This can be made functional by using various small molecules, metal ions, surfactants, and polymers. (b) The shell layer- this has a different chemical composition from the core, and (c) The core- the central portion of the Nanoparticle (Shin, Cho, Kannan, Lee, and Kim, 2016). Due to these unique characteristics, nanomaterials garnered a great deal of intrigue and interest of researchers in various disciplines and fields. The NPs have applications in drug delivery (Lee, Lee, Kim, Kim, Hyeon, 2011), as biosenors and chemical sensing (Barrak, Saied, Chevallier, Laroche, M'nif, and Hamzaoui, 2019), gas sensing (Mansha, Qurashi, Ullah, Bakare, Khan, and Yamani, 2016; Rawal and Kaur, 2013), CO2 capture and sequestration (Ganesh, Hemalatha, Peng, and Jang, 2017; Ramacharyulu, Muhammad, Kumar, Prasad, and Mohanty, 2015) and various other applications (Shaalan, Saleh, El-Mahdy, and El-Matbouli, 2016). Nanomaterials are used as additives to improve the biofuel yield and facilitate biofuel utilization in petrol and diesel engines.

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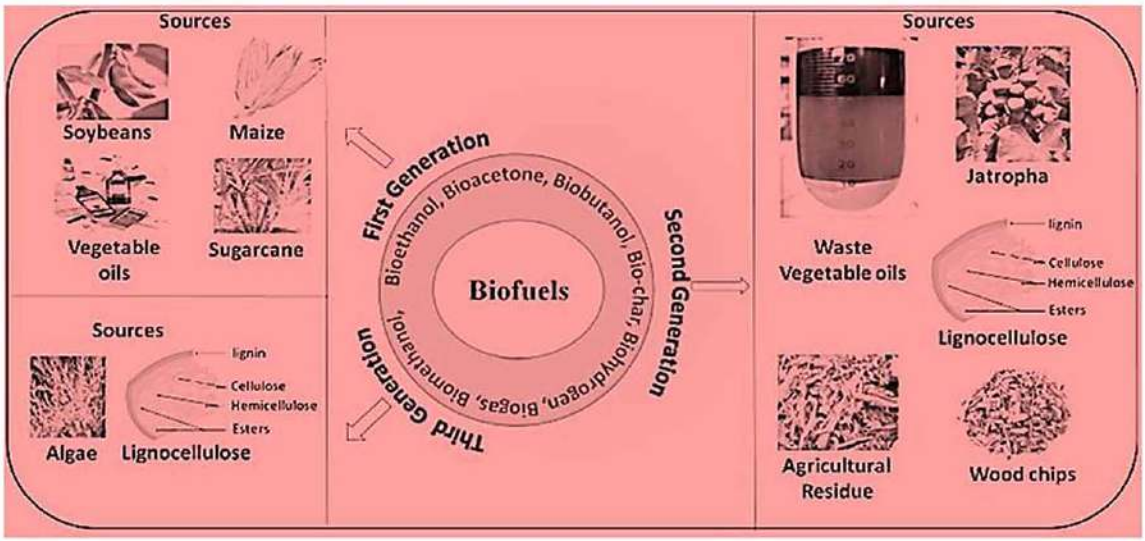
Nano-additives such as nano-magnets, nano-crystals, nano-droplets, nano-ðbres, etc., Figure 3 depicts the application areas of nano-additives in microalgae cultivation, and biofuel production.

Figure 3. Representation of biofuel types and their sources
(Arya et al, 2021)



Nanoadditives can be added at various stages of biofuel production from microalgae cultivation to biofuel application in engines due to their durability, recyclability, crystalline nature, adsorption efficiency, stability, catalytic activity, high storage capacity, superb biofuel yield, economical advantage and eco-friendly characteristics. (Hossain *et al.,* 2019). Use of Nano-technology has been shown to improve microalgae cultivation, maximize the yield of many microalgal biofuels and facilitate the use of such microalgal-biofuels in petrol and diesel engines and enhance the performance of biofuel-diesel hybrid fuels. Various nano-materials, e.g., nano-fibers, nano-sheets, nano-particles, nano-tubes and other nanostructures, have been investigated as effective nano-catalysts in biofuel production (e.g., bioethanol, biodiesel, biomethane, and others) and yield enhancement (Hasannuddin *et al.,* 2018; Karthikeyan and Prathima, 2017; Kim, Jia and Wang, 2006; Safarik, Prochazkova, Pospiskova, and Branyik, 2016).

Nano-Additives for Microalgal Growth

Microalgal growth rate remains a limiting factor in the lipid accumulation rate by microalgae and ultimately the mass production of biofuel from microalgae. Growth rates can be significantly accelerated by the use of nanomaterials. Sarma *et al.* (2014) studied the effects of Mg nanoparticles (NPs) on the microalgal growth rate, biomass production, and accumulation of intracellular compounds when Mg NPs were integrated into microalgae culture at different concentrations. They observed an improvement in their growth and lipid production upon use of the Mg NPs as nutritional additives. Nanoparticles added to wastewater may supplement nutritional deficits. Nanoparticles in culture media can also compete for nutrient absorption, inducing microalgae to absorb nutrients and accumulate lipids at a higher rate. An

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added benefit of using nanoparticles is that they possess antibacterial and antifungal properties to the culture media, inhibiting the growth of and killing bacteria and fungi which compete with microalgae for nutrients.

Nano-particles added during microalgae culture in a Photobioreactor (PBR) can boost and improve light conversion efficiency. Recently there has been an increased use of light-emitting diodes (LEDs) equipped with nano-materials in PBRs especially in the PBRs which use artificial light sources that lead to increased energy consumption and cost and where self-shading and biofilm formation on PBR surfaces during the growth of biomass lead to inadequate light penetration to the various parts of the culture. In order to circumvent this and to achieve desired illumination properties and photo-conversion efficiency in the PBR the nanoparticles were used. Pattarkine *et al.* (2012) reported the use of Gallium aluminum arsenide (GAA)-fabricated LEDs for algae cultures and the use of optical fibers which are cheaper alternatives. Steele, Grady, Nordlander, and Halas (2007) reported on another advancement in this field where metallic nano-particles (MNPs) integrated with localized surface plasmon resonance (LSPRs) were used to amplify the light scattering at a certain wavelength. Silver nano-particles' (Ag-NPs') suspended in plasmonic mini-PBRs were shown to backscatter blue light considerably which greatly improved the photosynthetic efficiency in green microalgae, *Chlamydomonas reinhardtii*, and blue–green microalgae, Cyanothece 51142, and a 30% increase in microalgal-biomass (Torkamani, Wani, Tang, and Sureshkumar, 2010). Increased CO2 absorption from the atmosphere, better CO2 sequestration, improvement in yield and boosted biomass growth was seen on the use of nano-particles in microalgae cultivation. Pattarkine *et al. (*2012), Zimmerman, Hewakandamby, Tesař, Bandulasena, and Omotowa (2009); Zimmerman, Tesař, and Bandulasena (2011) reported the use of stable nano-bubbles that help algal biomass float into the culture thus facilitating high mass transfer efficiency, improved biomass density, improved CO2 absorption, O2 stripping, and slight buoyancy. Nano-bubbles were shown to suspend the biomass in an airlift-loop bioreactor (ALB) and use less energy than micro-bubbles. For this purpose a uniform nonporous membrane of ALB was used which was capable of producing 100 nm sized bubbles. Safarik *et al. (*2016) delineated that nano-additives are very important for the flocculation and separation process before biofuel production besides microalgae harvesting.

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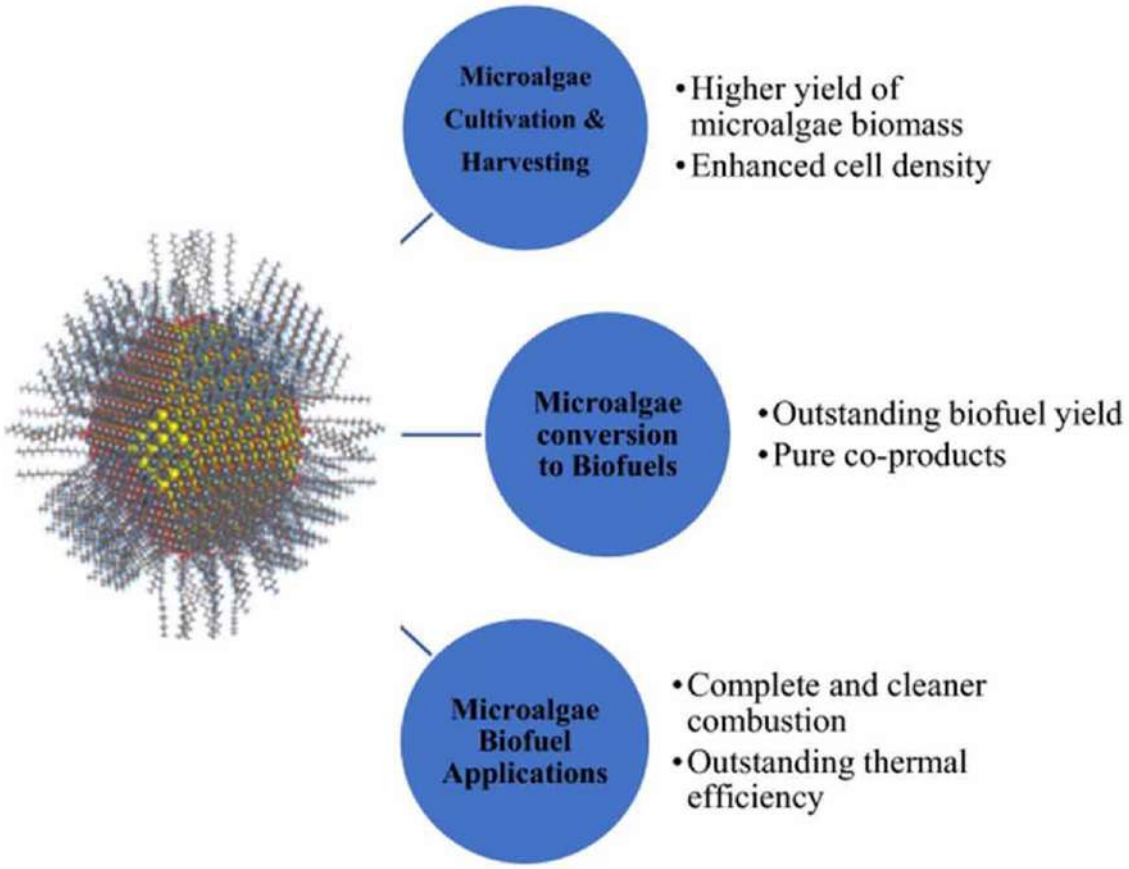
Figure 4. Nano-adhesives used for the enhancement of microalgae cultivation and biofuel production and implementation
(Hossain et al, 2019)



Nano-Additives for Microalgal Lipid Production

Microalgae typically accumulate large amounts of carbon sources when stressed in the form of pigments, starch, lipids, or moieties for self-protection from damage These results mainly from nutrient deprivation or shortage and can be exploited to abet the accumulation of vast amounts of carbon sources like sugars, starch, lipids and lipid moieties (Kadar, Rooks, Lakey, and White, 2012). These stress responses however result in decreased cellular growth. Nanoparticles added to microalgal culture media act as nutritional supplements to enhance growth and lipid accumulation. In the species *Trachydiscus minutes* a lipid increment from 26.20% to 35% was seen when 5.1 mg L−1 zero-valent iron nanoparticles (nZVI) were added (Pádrova *et al.* 2015). *Desmodesmus suspicious* showed a similar 38% increase in lipid accumulation when the identical concentration of nZVI was used. Ren *et al.* (2020) reported a similar occurrence when SiC nanoparticles were added to *Scenedesmus sp.* and the lipid content was enhanced. They also observed a biomass production of 3.18 g L−1 and lipid content of 40.26% with a 150 mg L−1 concentration of SiC nanoparticles under xenon light. Acetyl-coenzyme A carboxylase (ACCase) which is a key rate-limiting enzyme that catalyzes the first step in the biosynthesis of fatty acids was increased

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which likely increased the lipid content showing that SiC nanoparticles stimulate the activity of ACCase under xenon light, that lead to increased lipids synthesis. Several studies were conducted on the application of Mg, Se, Fe, Cu, C, Pb, Zn and Si nanomaterials in *C. vulgaris, S. obliquus, C. pyrenoidosa, T. minutus, S. rubescens, P. kessleri*, and *T. suecica* demonstrated NP application which led to accelerated microalgal growth, higher lipid contents and increased biomass production (as shown in Table 3) which can be exploited to achieve higher yields of biofuel (Sarkar *et al.* 2021).

Nano-Additives for Microalgae Biomass Conversion to Biofuels

Biodiesel, a form of microalgae biofuel, has gained in popularity as a commercial biofuel in the automotive fuel industry. Acidic and basic nano-catalyst spheres, which are recyclable and have a beneficial impact on the economy, can replace chemical compounds such as sodium methoxide in biodiesel generation by interacting with free fatty acids and oils. According to Trindade et al., 2011, using nano-catalysts minimises the amount of pollutant released into the environment generated by sodium methoxide, and the reactions may also be carried out at low temperatures and pressures. Silitonga, Atabani, Mahlia, Masjuki, Badruddin, and Mekhilef (2011); Trindade *et al.* (2011); Palaniappan *(*2017) and Nizami and Rehan (2018) described that Microalgae culture and growth may be considerably enhanced by using spherical nano-particles made of sand (silica) and calcium compounds without affecting harvesting. According to Chen, Mochizuki, Abe, Toba and Yoshimura (2014), a mesoporous silica nano-catalyst, Ti-loaded SBA-15, produced 10 times more free fatty acids (FFA) and tolerated water than any other catalyst for biodiesel generation from vegetable oil. It also outperformed other effective nano-catalysts like titanium silicalite-1 (TS-1) and titanium dioxide silicate (TiO2-S) and may thus be utilised to produce high-quality biodiesel from diverse sources.

Trindade et al. (2011) and Chen et al. (2014) described how Ti-loaded Santa Barbara Amorphous-15 (SBA-15) nano-catalysts reduced the chemical (alkaline catalyst, NaOH) cost of transesterification during biodiesel production, and how recycling the nano-catalyst made the process eco sustainable. Silva et al., 2017 demonstrated higher microalgae-biodiesel output when Niobia (N2O5) was combined with SBA-15 to produce biodiesel from biomass via esterification. Palaniappan et al. (2017) found that combining an enzyme extracted from the bacteria *Pseudomonas cepacia* with nano-particles such as polyacrylonitrile (PAN) nanofibre, Fe3O4, and nanoporous gold; lipase enzyme from *Rhizopus miehei* with silica nano-particles; ferric silica and the transesterification procedure used magnetic nanoparticles with Burkholderia sp. lipase and *Thermomyces lanuginosa* to create biodiesel from various bio-oils. Liu *et al.,* 2018 and Palaniappan et al. (2017) used a nano-magnetic biocatalyst of KF/CaO–Fe3O4, Li (lithium)-doped CaO, Fe2O3–CaO, sulphate (SO4) integrating Zi (zirconium), sodium titanate, and carbon-based nano-tubes and nano-particles from different kinds of biomass and biodegradable waste to produce 95 percent biodiesel. During the transesterification process, a zeolite NP (an alumina silicate mineral), has been employed as a commercial absorbent for increasing biodiesel output efficiency. Carrero et al. (2011) demonstrated the synthesis of biodiesel from *Nannochloropsis* microalga oil using a hierarchical h-Beta zeolite catalyst. In the process to produce FAMEs from Nannochloropsis oil, the catalyst had the maximum activity. The use of hierarchical catalysts demonstrates equal acid strength and number of acid sites for normal zeolites, but with a larger exterior surface area and the inclusion of a secondary micro-mesoporosity that minimizes lipid transport barriers to the zeolite acid active sites. Table 4 illustrates the use of nano-additives to enhance biodiesel yields during microalgae to biofuel conversion, as well as the relevant conversion procedures and efficiencies.

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Table 3. Lipid accumulation following Nano Particle treatment in several algal biofuels (Sarkar et al., 2021)

Microalgae Species	NP (Size in nm)		References
		Lipids	
C. vulgaris	Cu (100)	-	Mykhaylenko and Zolotareva. (2017)
C. vulgaris	Se (100)	-	Mykhaylenko and Zolotareva. (2017)
S. obliquus	C (<2)	8.9	He *et al*. (2017)
P. kessleri	Fe (-)	14	Pádrova *et al.,* 2015
C. pyrenoidosa	Fe_2O_3 (<50)	15.29	Rana *et al*. (2020)
S. obliquus	MgO (<50)	18.5	He *et al*. (2017)
S. rubescens	ZnO (50–70)	27.27	Aravantinou *et al*. (2020)
T. minutus	Fe (-)	33.58	Pádrova *et al.,* 2015
Scenedesmus. sp.	SiC (25–100)	36.46	Ren *et al*. (2020)
S. obliquus	Fe_2O_3 (<30)	39.6	He *et al*. (2017)
T. suecica	Fe (50)	41.9	Kadar *et al.,* 2012
C. vulgaris	Cu (89)	86.67	Sibi *et al*. (2017)
C. vulgaris	$MgSO_4$ (100)	118.23	Sarma *et al*. (2014)
C. vulgaris	Pb (76)	206.67	Sibi *et al*. (2017)
C. vulgaris	Zn (92)	333.33	Sibi *et al*. (2017)
C. vulgaris	Mg (82)	393.33	Sibi *et al*. (2017)

Table 4. Employment of nano-additives to improve biodiesel yield

Nano-additives Employed	Processes Involved	Conversion Efficacy	References
Blends of calcium oxide nanoparticles (CaO-NPs)	Catalytic transesterification	91%	(Pattarkine *et al.,* 2012)
KF/CaO–Fe3O4, Li(lithium)-doped CaO, Fe2O3–CaO, sulphate (SO4) infused Zi (zirconium), sodium titanate, and carbon-based nano-tubes and nano-particles	Transesterification	95%	(Palaniappan *et al.,* 2017; Liu *et al.,* 2018)
Ti-loaded SBA-15 mesoporous silica nanocatalyst	Transesterification	More than ten times the efficacy of other catalysts. 3 times more efficient than TS-1 and TiO2–S nanocatalysts	(Trindade *et al.,* 2011; Chen *et al.,* 2014)
SBA-15 integrated with Niobia (N2O5)	Esterification	Biodiesel yields have increased significantly.	(Silva *et al.,* 2017)
PAN nanofibre, Fe3O4 and integration of nanoporous gold, silica nanoparticles, integration of ferric silica and magnetic nanoparticles, transesterification of polyacrylonitrile nano fibre	Transesterification	Biodiesel productivity has increased significantly.	(Palaniappan *et al.,* 2017)

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Nano-Additives for Microalgal CO2 Absorption and Sequestration

Photosynthesis is one of the most common methods to utilize CO2 that has been sequestered. The largest contributors of biological CO2 fixation are microalgae. According to Huang et al. (2014), 1 tonne of microalgae can sequester 1.8 tonnes of CO2. Enhanced CO2 fixation resulted in improved nutrition supply to microalgae, resulting in raised biomass production during the log phase of development and product accumulation (Ling, Guo, Liu, Zhang, Wang, Lu, and Ng, 2015; Da Silva Vaz, da Silveira Mastrantonio, Costa and de Morais, 2019; Choi et al. 2012). Choi et al. (2012) revealed that when the quantity of dissolved gas in the liquid was enhanced well above natural balance of the algal culture, there was an elevation in CO2 fixation by microalgae. As adsorbent materials, nanoporous materials such as polymeric nanofibers have been increasingly popular in recent years (Vaz et al. 2018). Nanofibers are incredibly beneficial since they can be readily isolated from microalgal biomass, according to the researchers. In microalgae production, polymeric nanofibers can be employed as CO2 absorbents to enhance permeance in the medium, contact duration between the microalgae and the CO2, and CO2 mass transfer to the cells (Da Silva Vaz et al. (2019); da Silva Vaz, Costa and de Morais, 2020). Da Silva Vaz et al. (2019); Vaz, Costa and Morais (2018) revealed the synthesis of functional composite nanostructures by integrating metal nanoparticles or metal oxides into nanofibers, leading to improved adsorption efficiencies and molecule transport and interactions. The inclusion of nanoparticles to microalgal growth medium by Li et al. (2016) resulted in a microorganism-particle assembly for Co2 sequestration and in-situ conversion. The gas adsorbed on the nanofibers is progressively desorbed into the culture medium as a result of this. Vaz et al. (2018) investigated the impacts of different Fe2O3 nanoparticle concentrations and observed that 4 percent (w/v) Fe2O3 nanoparticles had a better CO2 adsorption capability. The smaller nanofibers and increased porosity are responsible for the enhanced CO2 adsorption/capture efficiency. When Chlorella fusca LEB 111 was grown in nanofibers with 4% Fe2O3 nanoparticles, a higher biomass concentration of 794 mg L−1 was observed, leading to the conclusion that nanofibers with Fe2O3 nanoparticles increased the specific surface area and CO2 adsorption capacity of the nanostructures, leading to greater gas availability in the microalgal medium, advancing production and enhance fixation.

Da Silva Vaz et al. (2019) investigated the addition of 4 percent Fe2O3 nanoparticles in their free form and encased in a stainless-steel screen support to *C. fusca* LEB 111 growth medium. Due to the desorption of the gas adsorbed on the nanostructures, the free form nanofibers supported a threefold buildup of CO2 in the medium, resulting in a 45 percent rise in fixation by the microalgae and an elevation in the (Dissolved inorganic carbon) DIC. Vaz et al, (2019) studied CO2 fixation in *C. fusca* LEB 111 grown in outdoor and indoor environments using polymeric nanofibers containing 4% Fe2O3 nanoparticles. Outdoor experiments showed increased CO2 fixation, which might be attributed to an increase in biomass content as the nanofibers-maintained CO2 for a longer period of time.

Nano-Additives for Microalgae-Biofuel Applications

Sinha et al. (2017) and Silitonga et al. (2018) reported enhanced fuel lubricity, catalytic efficiency, chemical processes, mass and heat transfer efficiency, cetane number, combustion rate, fire/flash point, water co-solvency, and reduced retention times, resulting in a more comprehensive and cleaner combustion of microalgae biofuel, while investigating solid nano-particles, nano-fluids, or nano-droplets with biofuel and fossil fuel. According to Trindade et al. (2011); Sinha et al. (2017); and Damanik, Ong, Tong,

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Mahlia, and Silitonga, (2018), nano-additives can improve microalgal-biofuel combustion efficiency while lowering smoke, NOx, soot, HC, CO2, and CO emissions into the atmosphere by 72 percent.

Karthikeyan and Prathima (2017), Sinha et al. (2017), Karthikeyan, Elango, Prathima and Raja (2014) and Karthikeyan and Dharma Prabhakaran (2018) reported an 11 percent rise in engine power, torque, and braking system thermal efficiency of biodiesel (derived from microalgae as well as other biomass) in CI and DI engines when solid nano-additives such as alumina (Al2O3) An research study with DI engines using nano-particles combined with biodiesel and also a diesel–biodiesel combination revealed outstanding performance and higher efficiencies than conventional catalysts (Prabu, 2018, Ong et al. 2018). Dhinesh and Annamalai (2018) found that nano-particles of CeO2 integrated into a biofuel emulsion employing sol–gel combustion technology functioned admirably in mono-cylinder 4 stroke direct CI and DI engines with no hardware modifications. In general, nanoparticles in biofuels boosted the calorific value of the fuel, expedited evaporation, enhanced brake-specific fuel consumption and thermal efficiency, and minimized greenhouse gases (GHGs) including CO, NOx, and smoke, as well as unburned hydrocarbons.

Scientists have successfully developed unique nanofluids by combining several nanoparticles. Because of their capacity to enhance the chemical and thermophysical characteristics of single-phase nanofluids, hybrid nanofluids have piqued the interest of many researchers (Hussain et al. (2020); Karisathan Sundararajan and Ammal, 2018). Selvan et al. (2014) investigated engine efficiency and emission of diesel-biodiesel-ethanol mixtures including cerium oxide nanoparticles and carbon nanotubes. To oxidise CO and diminish nitrogen oxides, cerium oxide nanoparticles served as oxygen supply catalysts. The activation of cerium oxide, which reduced carbon deposits in the cylinder, resulted in a considerable decline in HC and smoke emissions. As a result, the employment of both nanoparticles together can result in cleaner combustion and lower emissions.

Arunprasad, Sener, Thirugnanasambantham, Elango and Bothichandar (2022) conducted study on the performance and emissions of diesel engines incorporating a spirulina microalgae biodiesel mixture (B20) containing magnesium oxide nanoparticles. Because of its low calorific value, Spirulina microalgae biodiesel has poor brake thermal efficiency (BTE) and brake-specific fuel consumption (BSFC) fuel characteristics in general. Conversely, in their study, MgO nanoparticles coupled with spirulina methyl ester significantly enhanced its catalytic action, raising BTE and decreasing BSFC. This paves the way for including spirulina biodiesel enriched with magnesium oxide nanoparticles in diesel engines to substitute diesel without incurring any additional costs or necessitating hardware alterations. Thirugnanasambantham and Elango, 2020 investigated B20, a combination of biodiesel (20%) and diesel (80%), as a way to augment diesel fuel in IC engines with less expensive algal biofuels while lowering emissions. In a four-stroke diesel engine with no alterations, TiO2 nanoparticles added to the B20 fuel resulted in significant reductions in HC, CO, and smoke emissions.

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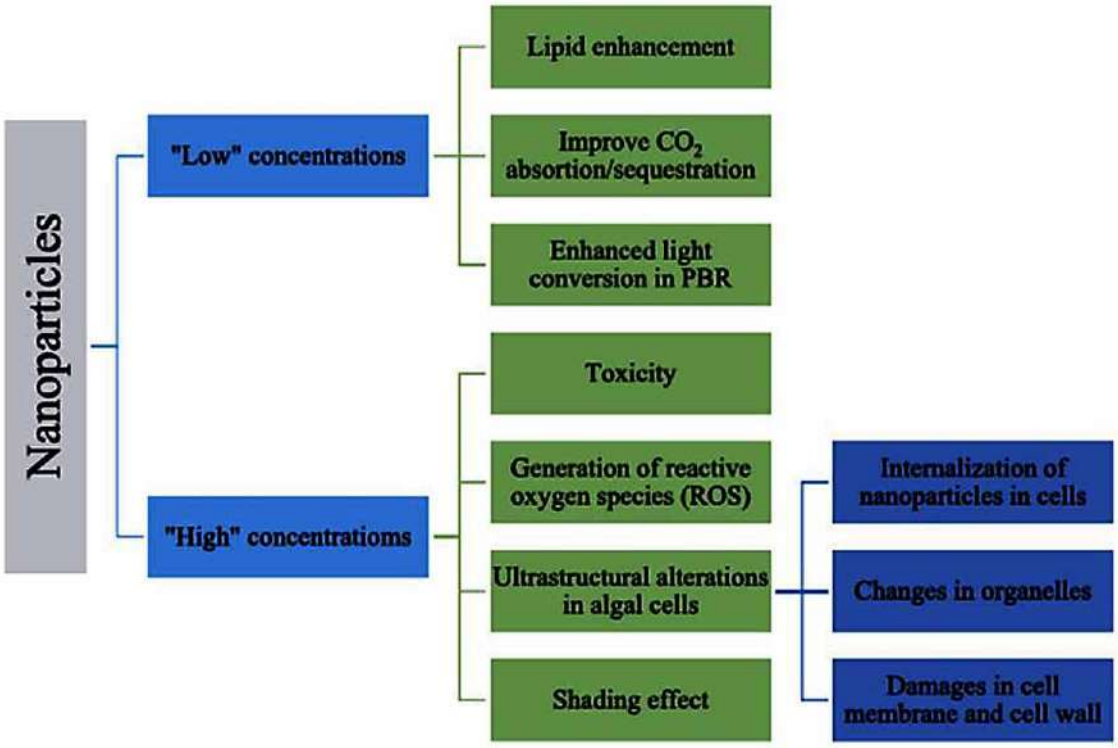
Figure 5. Effect of nano-particle addition to microalgae culture
(Vargas-Estrada et al, 2020)



CONCLUSION

One of the major advantages of employing microalgae for biofuel production is the abundance of diversified, lipid-accumulating microalgal species in nature. Large-scale microalgal production for biofuel and several other significant products, as well as the selection of suitable microalgal strains, may be achieved in the future by integrating conventional and cutting-edge processes. Biodiesel may be generated using heterotrophic microalgae as a feedstock. Unfortunately, the exorbitant expense of lipid extraction makes this method difficult. Nanomaterials can be intended to safeguard microalgal cells from damage, and nanoparticles may also be used to harvest microalgae. Because nanoparticles alter the lipid metabolism of microalgal cells, adding them to the culture medium can enhance lipid buildup. Nanotechnology might help enhance biodiesel synthesis from heterotrophic microalgae, however this process is still in its nascent phases. Future research might consider the effect of lipid buildup produced by the inclusion of nano silica and iron oxide to heterotrophic microalgae's growth medium. Strategies to gene manipulation that are careful and meticulous in their usage of genetically - modified strains offer a promising path to long-term and economical microalgal based biodiesel synthesis. The fabrication of nanomaterials different functional groups essential for lipid disintegration and cell wall breaking/weakening should be given more attention. Immobilization of whole-cell lipase on nanoparticles should be optimised and evaluated. There is a need for technological advancement, technical knowledge, and the identification of relevant microalgae species with optimal growth and production characteristics. Further changes to

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the development of genetically enhanced species should be made so that the appropriate quantity of biodiesel may be generated in a shorter period and with less energy usage.

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Chapter 12
Macroalgae-Based Bioethanol

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ABSTRACT

The chapter discusses the methods effective for the extraction of useful energy from the macroalgae biomass including liquefaction, anaerobic digestion, fermentation to biobutanol, trans-esterification to biodiesel, pyrolysis, direct combustion, fermentation to bioethanol, and gasification. However, if the algae are suited for the production of biodiesel, they can be studied from the content of their triacylglycerols (TAGs). Due to having high fatty acid content, they have a high conversion rate to biodiesel, and the lack of sulphur, phosphorus, and nitrogen also aids in the conversion. This chapter highlights the limitations and suitability of macroalgae for the conversion process in reference to chemical composition, process optimization, and cost effectiveness. It is concluded that bio-oils and bioethanol produced from wet macroalgae are considered over biodiesel production because of high lipid content of microalgae biomass. Moreover, the chapter considers electricity production from the dry mass as it would turn profitable, and this can be achieved from fast-growing macroalgae like "Ulva."

INTRODUCTION

Fossil fuel hydrocarbons are being overly used which results in their scarcity and being expensive. The focus of research on converting biomass to biofuels has shifted to third-generation biomass, such as algae. The first-generation feedstock is divisive due to the food-fuel conflict, whereas the high-cost lignin removal technique limits the second-generation biomass (A Bayu, 2018). As algae can repair greenhouse gases (CO_2) through photosynthesis, it is a promising renewable energy source. The typical photosynthetic productivity of marine biomass is 6–8%, which is significantly greater than the 1.8–2.2% of terrestrial

DOI: 10.4018/978-1-6684-5269-1.ch012

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biomass (Adams, J.M., 2009 & Junying, 2015). Marine macroalgae are the source of aquatic biomass which shows significant opportunities to become renewable energy sources. Aquatic biomass has 6–8% of photosynthetic efficiency, which is significantly greater than terrestrial biomass (1.8–2.2%). For the conversion of solar energy and production of biofuels, either marine macroalgae such as seaweed or microalgae might be employed (Hirano, A, 1997).

Macroalgae are large, fast-growing marine and freshwater plants with a maximum length of 60 metres. Due to the lack of water limitations, the marine macroalgae growth rates outpace the growth rate of terrestrial biomass (K. Sudhakar, 2018). The principal marine macroalgae have higher yearly production rates (grams C m2 yr1) than most terrestrial biomass. Phytocolloids like agar, alginates (derived from Phaeophyta) and carrageenan (derived from Rhodophyta), are mostly generated from seaweeds. The by-products of such processing can likewise be used as a renewable energy source (Kawai, 2016 & Notoya, M., 2010). Macroalgae are primarily of three kinds green (Phaeophyta), Red (Rhodophyta) and brown (Phaeophyta) depending upon their photosynthetic pigmentation differences (Chlorophyta). Red has the most species (6000), green (4500), and brown (2000) (Inn Shi Tan, 2020; Mohsen Ghadiryanfar, 2016). Red algae grow in the intertropical zones while the brown algae thrive in temperate to cold or extremely cold seas. Green algae may be found in a range of aquatic environments. In Asia, macroalgae are now farmed for food, fertilisers, and hydrocolloid extraction, (72%) majority-owned by Japan, Korea, Philippines and China worldwide yearly output. Macroalgae productivity varies from 150 to 600 t per hectare per year in fresh weight, with a total global production of 12 million tonnes of dry matter per year (Ashok Pandey, 2011; Huihui Chen, 2015). The extraction from macroalgae biomass of biofuels, on the other hand, received not much attention. Hydrocolloid extraction is presently the only significant industrial product derived from macroalgae. However, macro-algae may be used to extract a variety of high-value speciality goods.

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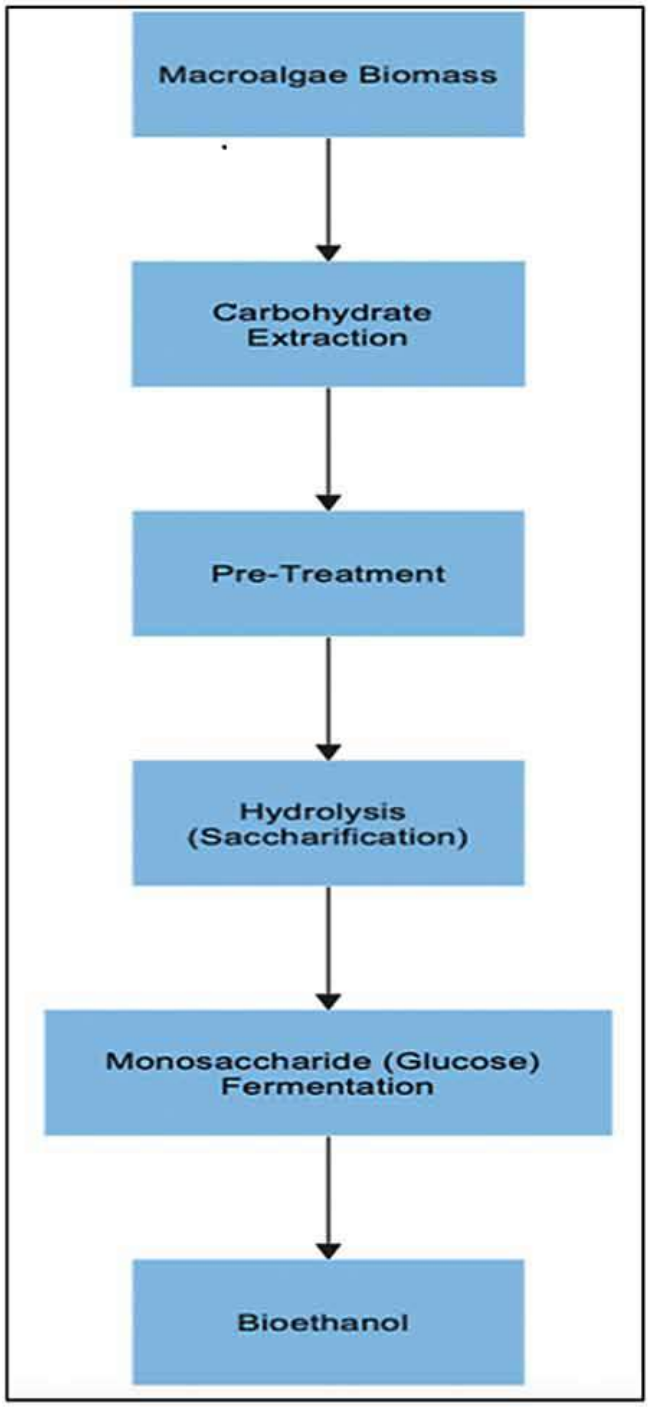
Figure 1. Stepwise representation of bioethanol production from algal biomass



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Chemical Composition of Macroalgae

Macroalgae have a dry matter content of only 10–15%. Large levels of carbohydrates may be found in dried macroalgae (approximately 60%). Carbohydrates are mostly obtained from cell wall components of macroalgae (Milledge, J.J., 2014). Red and green algae have an abundance of cellulose in them which is preferable for biofuel production. Seasonal changes in organic constitution and ash content differ correspondingly. For instance, the quantity of carbohydrates stored extends to the peak, in the fall. Mannan, ulvan, carrageenan, agar, laminarin, mannitol, alginate, fucoidin, fucose, and uronic acid distinguish macroalgae from microalgae and lignocellulosic biomass. Furthermore, lignin concentration as low as 0.03 g/kg of dry matter allows for downstream processing including fermentation or anaerobic digestion without the need for expensive pre-treatment.

Protein concentration in macroalgae can range from 7 to 31% dry weight, with lipid content of 2 to 13% dry weight. Macroalgae contain a significant quantity of carbohydrates (up to 32–60% of dry weight) (Gao, G., 2020). Macroalgae, being a photosynthetic organism is able to transfer into chemical energy from solar energy, usually as carbohydrates (mono- and polysaccharides). The intricacies of the aquatic environment, on the other hand, cause their metabolism to produce saccharides with distinct synthetic properties than those produced by terrestrial plants. For example, cellulose (35-50%) and hemicelluloses (20-35%), are made up of simple sugars such as ribose, glucose, mannose, xylose, galactose and arabinose, which are the most abundant carbohydrate components in all terrestrial plants. Furthermore, macroalgae contain a wide range of carbohydrates, including monomeric sugars of various kinds. Brown macroalgae have significant polysaccharides such as laminaran, fucoidan, and alginate, whereas red and green macroalgae have galactans (e.g. carrageenan and agar) and ulvan, respectively.

Carrageenan and agar are two polymers of modified galactose found in red macroalgae. Palmaria palmata has the greatest carrageenan content, with a concentration of 354 mg/g, and this high carrageenan content allows for a high bioethanol output. Brown algae, on contrary, is high in alginate and contains substantial amounts of laminarin, a significant amount of mannitol in the fall, and few commonly used sugars. The lipid content of brown and red algae is lower than 5%, which makes them unsuitable for biodiesel production. Green algae species vary in their cellulose content (Valonia types can have up to 70% cellulose) and starch content (20–30%). Furthermore, alkali metal and halogen levels in macroalgae are greater (0.5–11%) than in terrestrial biomass (1–1.5%). Nutrient content (N, P) is largely determined by macrophyte shape, followed by nutrient contamination in the environment.

Table 1. Algal sources suitable for bioethanol production with reference to their starch content

Algal Source	Starch Content of the Dry Matter	Reference
Ulva ohnoi	1.59% to 21.44% rising with nutrient starvation	(Meghanath Prabhu, 2019)
Saccharina latissima	50.0 (reserve food material)	(Adams, J.M., 2009)
Laminaria hyperborea	55.0 (reserve food material)	(Greetham, D., 2020 & Roesijadi, Guritno, 2010)
Chlamydomonas reinhardtii UTEX 90	53.0 (starch)	(Kim, M.S., 2006)
Chlorella vulgaris	12.0–17.0 (starch)	(Spolaore, P., 2006)
C. vulgaris	37.0 (starch)	(Horn, S.J., 2000)
Synechococcus sp.	15.0 (starch)	(Yusuke Sasaki, 2018)
Spirulina fusiformis	37.3–56.1 (starch)	(Rafiqul I.M., 2003)

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Cultivation

The quantity of bioethanol that may be produced, both in absolute and relative terms, varies greatly across algae families and strains within identical ones. This is due to the fact that algae species and strains differ significantly relating to prolificity and multiplication, nutrition and light requirements, capacity to collect lipids or other desired chemicals, ability to adjust to unfavourable circumstances, and so forth. As a result, strains that have the ability to produce the most bioethanol, either directly or via biomass assembly, are frequently chosen.

Brennan and Owende (2010) compiled a list of desired features for strains of algae regarded as biofuel prospects, including:

1. Have tolerance for a broad range of temperatures due to seasonal and diurnal changes;
2. In open pond production settings, capable of dominating wild strains;
3. High ability to absorb CO2;
4. Nutritional needs are limited;
5. Possibility of providing useful co-products;
6. Quick production cycle;
7. Photosynthetic efficiency is high, and
8. Self- flocculation

As starch is used in ethanol fermentation as a substrate, a strain providing a large quantity of renewable energy due to high starch concentration is the characteristic feature when screening for algal strain used in the production of bioethanol. The total dry weight of biomass may be calculated, and biochemical tests can be used to assess the biomass starch/cellulose content.

Direct harvesting of seaweed from the maritime environment, wrack collecting from the beach, and culture of chosen seaweed species are the three principal sources of macroalgal biomass for downstream processing. Offshore, onshore, and integrated seaweed farming are the various ways of seaweed cultivation. Direct culture of macroalgae in saltwater is done using tethered ropes off the coast. It's also possible to integrate it with onshore aquaculture and offshore wind farm locations. Offshore farming provides for more controlled growing and harvesting circumstances, resulting in better efficiency and output than natural growth. To extract nutrients directly from the saltwater, in sheltered bays or lagoons macroalgae can be cultivated. Traditional seaweed growing methods include long lines, permanent off-bottom farming, and rock-based farming. For biomass production, large brown kelps are generally favoured. Smaller species, comparatively, are preferred for high-value components.

Enclosed photobioreactors and open ponds are the most prevalent production technologies for algae culture. Controlling downstream processing features, growth parameters, pollution, productivity, capital and operational expenses, water evaporation and so forth are all different aspects of production systems.

Harvesting of Algal Biomass

Harvesting the grown biomass can be done in two ways. It has long been a custom in many coastal areas. Techniques for harvesting include:

- Manual (Hand)

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- Mechanical (Dredge, moving boat, mesh conveyor)

Three times a year, harvesting is usually carried out. Mechanical harvesting has a negative impact on marine ecosystems and stunts macroalgae development. In other cases, however, eliminating surplus nutrients from the environment by reducing prolific macroalgae growth is advantageous. Harvesting macroalgae blooms hasn't proven to be economically viable anywhere on the planet (Notoya, M., 2010).

Extraction of Carbohydrates

The extraction of energy from macroalgae may be classified in several ways, one of which being whether or not an initial drying phase is necessary. As a result, two separate groupings of processes emerge:

1. Energy extraction from dry macroalgae
    a. Pyrolysis;
    b. gasification (conventional);
    c. direct combustion;
    d. trans-esterification to biodiesel.
2. Energy extraction from wet macroalgae
    a. anaerobic digestion;
    b. fermentation to bioethanol or biobutanol;
    c. hydrothermal treatments.

Unfortunately, due to problems in processing technology and scaling up, bioethanol from second-generation feedstock has met with a lot of opposition. Cellulases degrade cellulose and hemicelluloses while lignin can prevent biodegradation, which is a major technical problem. Due to the lack of lignin in macroalgae, the conversion to ethanol from polysaccharides is more rapid in macroalgae. As a result, bioethanol determined from macroalgae are procuring popularity.

Brown, green, and red macroalgae are all forms of macroalgae that may be found in the ocean and have been used to make bioethanol. Because of their high carbohydrate content and ease of mass-cultivation, the predominant feedstock for bioethanol synthesis appears to be brown macroalgae. For example, a synthetic yeast platform can produce ethanol from brown macroalgal sugars at up to 83% of the theoretical maximum, but ethanol conversion from macroalgae at up to 50% of the theoretical maximum is regarded as ambitious (Elamathi Vimali, 2022).

Pre-treatment of the Seaweed Biomass and Hydrolysis of the Polysaccharides

Freshly harvested brown seaweed contains 15–20% carbs by weight, or roughly for every kg of wet weight it has 200 g carbohydrates, that is an acceptable concentration of substrate for conversion of microbial activities (Godvin Sharmila V, 2021). Moreover, seaweed does not require the pre-treatment that lignocellulosic biomass does to extract fermentable sugars since they don't contain lignin. Water is an easy way to extract laminaran and mannitol. Alginates (polymer blocks of Uronic and Guuronic acid) make up 30–40% of the macroalgae biomass. Calcium salt can be used to remove sodium alginate from the original extraction solution. This forms calcium alginate, which has a fibrous structure and is insoluble in water but may be isolated from it. To convert calcium alginate to alginic acid, the calcium alginate is

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separated and suspended in water along with acid. The fibrous alginic acid formed is easy to separate and mix with alcohol and the other sodium carbonate is slowly added to the paste to the endpoint when all alginic acid is transferred into sodium alginate. Sodium alginate paste is extruded occasionally into pellets, which are later dried and ground. Dilution with a substantial volume of water is necessary due to the high viscosity. Alginate concentrations of less than 2% are used in the procedure. Processes involving the use of alginate as a fermentation substrate cannot be done with such a dilution. Water should be avoided if at all possible, as it increases the downstream processing costs.

Enzymatic hydrolysis and fermentation can be used together to produce bioethanol from cellulosic sources. Hydrolysis is more difficult for wet seaweed as alginates do not extract easily, resulting in enzymatic destruction of untreated organic materials from macroalgae, and it will make hydrolysis a rate-limiting step in the fermentation method. As a result, hydrolysis should be included in the biomass pre-treatment process. This hydrolysis can be done physically using grinders and emulsifiers, chemically with acid or alkali, or enzymatically.

Alginates can be degraded by a number of alginate lyases discovered by marine microorganisms. There are several ways for partial or total decomposition of alginate in the biomass through mechanical pre-treatment. Chemical hydrolysis should be based on current technologies, such as modifying and adapting techniques used for wood biomass pre-treatment while combining with steam treatment using acid and alkali procedure (Adams, J.M., 2009).

Dihydroxy benzene-driven Fenton reaction and brown-rot fungus, are the other known techniques, which partly depolymerize oxidise phenols, carbohydrates and other fermentation inhibitors as they create highly reactive hydroxyl radicals and alike oxidants. Nonetheless, combining mechanical and enzymatic hydrolysis to pre-treat algal biomass is the simplest and most environmentally friendly method.

Algal Biomass to Bioethanol

There are three ways of producing bioethanol from algae. Algae may absorb a large quantity of starch/cellulose from the biomass, which is transformed into sugars used for fermentation, which can then be turned into bioethanol by ethanol production. During dark fermentation, certain algae can produce ethanol. The present techniques for the production of bioethanol from macroalgae speices: SHF (separate hydrolysis and fermentation) and SSF (simultaneous saccharification and fermentation) are two types of seaweed hydrolysis and fermentation. The fermentation procedure is broken down into many parts. Seaweed biomass is hydrolysed first and then fermented by bacteria or yeast in distinct units in the case of SHF. SSF, on the other hand, does both hydrolysis and fermentation in one step. One of the most common strategies for obtaining high sugar yields from lignocellulosic biomass is to use acid pretreatment. By acid hydrolysis (0.4 M H2SO4 at 125 1C for 25 minutes), red algae, Palmaria palmata, yielded glucose, galactose, and carbohydrates, which were subsequently fermented to ethanol.

Despite the fact that acidic hydrolysis of biomass has been claimed to be the most economical technology until today, glucose breakdown happened while in the process. The by-products of acid hydrolyses, such as 5-hydroxy-methyl-furfural and levulinic acid, have a significant influence on the production of ethanol capability. Enzyme hydrolysis, like acid hydrolysis of carbohydrates, has various drawbacks, including difficulties extracting the derived enzyme from the acquired products and a protracted hydrolysis period. Saccharification has recently been aided by the use of a two-step biomass treatment procedure that combines acid and enzymatic pretreatment. Pretreatment with 0.06% sulfuric acid at 170 1C for 15 minutes, followed by enzymatic pretreatment, resulted in a maximum glucan concentration of

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29.10%, which was four times greater than Saccharina japonica (raw). The digestibility of total biomass was 83.96%. At the moment, suitable enzymes are required for increasing hydrolysis effectiveness, and enzyme redemption and utilisation in biorefining processes also offer a guarantee. Separation of the membrane has been shown to be a reliable method for recovering enzymes.

Most polysaccharides and disaccharides are converted to monosaccharides after the effective hydrolysis phase; for example, sucrose can be catalysed by invertase enzyme into fructose and glucose, and subsequently, they are converted to ethanol by Saccharomyces ceveresiae. In red algae hydrolysates, the coexistence of glucose and galactose restricts total ethanol generation, while microorganisms in brown algae can't utilise several sugars at the same time as the capacity to utilise alginate is absent, therefore ethanol production is limited. As a result, the critical concern for bioethanol production from macroalgae monosaccharides through ethanol fermentation is an economical hydrolysis process.

CONCLUSION

In the coming future, research into prospective molecular processing using a range of different macroalgae species has found potential biochemical processing processes for the production of renewable energy. Macroalgae biomass varies depending upon the geographical area, species, and season, as do yields and productivity, which are heavily dependent on processing technology. As a result, macroalgae have the potential to make a large contribution to a sustainable and renewable economy. Conclusively, marine macroalgae are habitual of high salinity, huge temperature fluctuations, enormous nutrient dynamics, and extreme UV sunshine exposure. Due to the severe environment, their metabolism produces primary and secondary metabolites with distinct chemical structures in order to survive. The conversion of chemical energy from solar energy by macroalgae, mostly in the form of carbohydrates, is a normal photosynthetic organism. Because of their high carbohydrate content, they may be used to make alcohol or gaseous fuels. Furthermore, the distinctiveness of their chemical structure allows for the production of extensive carbohydrate-based compounds, including sulphated oligosaccharides and uncommon sugars, that have a potential market in the pharmaceutical and nutraceutical sectors. It also has the potential to be used in the synthesis of new materials with novel physicochemical features. Polyphenols, essential amino acids, and pigments are all found in macroalgae, as well as other important compounds. Integrating bioenergy production with valuable chemicals should make macroalgal bioenergy development more economically appealing since it provides possible additional revenue, high efficiency, and better environmental outcomes.

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Chapter 13
Microalgae Biofuels: Challenges and Potential

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ABSTRACT

Development of sustainable energy resources is a major concern in front of the scientific community and stakeholders in this field. Invariably, fossil fuels are utilized to meet the major part of energy demand all over the world, but their stocks are limited. Burning of fossil fuels also causes significant environmental pollution that results in drastic climate change. There is an emergent need of an alternative to fulfill the current energy demand in a sustainable way along with sorting out the pollution issues. Microbial biofuels could be the most appropriate choice in this regard because it bears enormous potential that needs to be exploited. But it has some challenging issues to employ it to meet out the current energy demands. This chapter illustrates the microbial biofuels, harvesting techniques, different process, and potential to utilize them as sustainable energy sources along with considerable challenges that need to be addressed to make it an eco-friendly and economic fuel choice to pave the path of sustainable development.

DOI: 10.4018/978-1-6684-5269-1.ch013

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INTRODUCTION

The scarcity of fossil fuel resources and the catastrophic environmental consequences caused by their use are major global concerns today. Fossil fuels have resulted in massive and long-term damage to our environment, including acid rain, global warming, and climate change. Greenhouse gas emissions continued to increase, and in 2010, they reached up to 30.6 gigatonnes (GT). The OECD (Organization for Economic Co-operation and Development) suggested that without implementing more effective policies than in force today, it will increase by another 50% by the end of 2050. It is anticipated that without taking appropriate measures, the atmospheric concentration of greenhouse gases would be reaching 685 ppm CO_2-equivalents by 2050. This is well above the 450ppm level of CO_2 required to have at least a 50% chance of increasing the global average temperature to 2°C. As per OECD predictions, the global average temperature is likely to exceed an increment of 2°C by 2050 and rises to 3 to 6°C greater than the pre-industrial levels by the end of this century. The United Nations Environment Programme (UNEP) "Emission Gap Report 2019", unless the greenhouse gas emissions must fall by 7.6% each year from 2020 to 2030, the world will fail to meet the 1.5 °C temperature reduction goal of the Paris agreement. (UNEP, 2019). Such a high rate of temperature rise would continue to change rain patterns and the melting of glaciers, leading to rising sea levels and worsening weather events. It could also exceed some critical "tipping points", resulting in dramatic natural changes resulting in irreversible or catastrophic events for natural systems and society (OECD, 2012). Also, fossil fuel reserves are depleting very fast. The growing economy needs fossil fuels to function, from the production of plastics and agricultural fertilizers to the energy requirements for heating, lighting and transportation services. Rapidly growing industrialization and the exploding world population are the driving force to become increasingly dependent on fossil hydrocarbons for economic growth (Ahmed et al., 2021). The fossil fuel demand is predicted to grow by 40% from 2010 to 2040 (Bhore et al., 2019). Fossil fuel reserves are insufficient to meet rising demand at the current consumption rate and will be exhausted soon. An overreliance on fossil fuel supplies challenges the world's energy security resilience. It will lead to a dwindling of fossil fuel resources and is expected to exhaust in less than 80 years (Rafa et al., 2021). The majority of the world's energy demand is fulfilled by fossil fuels (approximately 80%), with biofuels (11.3%), nuclear energy (5.5%), and other renewable energy sources, such as wind, filling in the gaps (2.2%) (Kopplaar, 2012). According to the International Energy Agency (IEA), biofuels will be contributing more to total energy sources by 2030. It could be expanded significantly if new petroleum fields are not accessed or substantial new alternatives are not identified (Hannon et al., 2010). Biofuels are among the most sustainable options. Biofuels and other renewable energy sources, such as wind and solar power, have attracted researchers, industrialists, and administrations to address energy shortages, rising crude oil costs, global climate change, and land and water degradation (Alam et al., 2015). This chapter entails a detailed description of microbial biofuels and their potential and contemporary challenges to make their most appropriate economically viable and sustainable choice of energy demands.

Biofuels

Biofuels have a zero-carbon footprint and are renewable fuels that are essential for environmental and economic sustainability. Any solid, liquid, or gaseous fuel derived from biomass (plants, animal waste, manure, sludge, etc.) stores solar energy through physical and chemical processes. This energy source can replace fossil fuels (Mobin & Alam, 2018). The usages of biofuels have several over conventional

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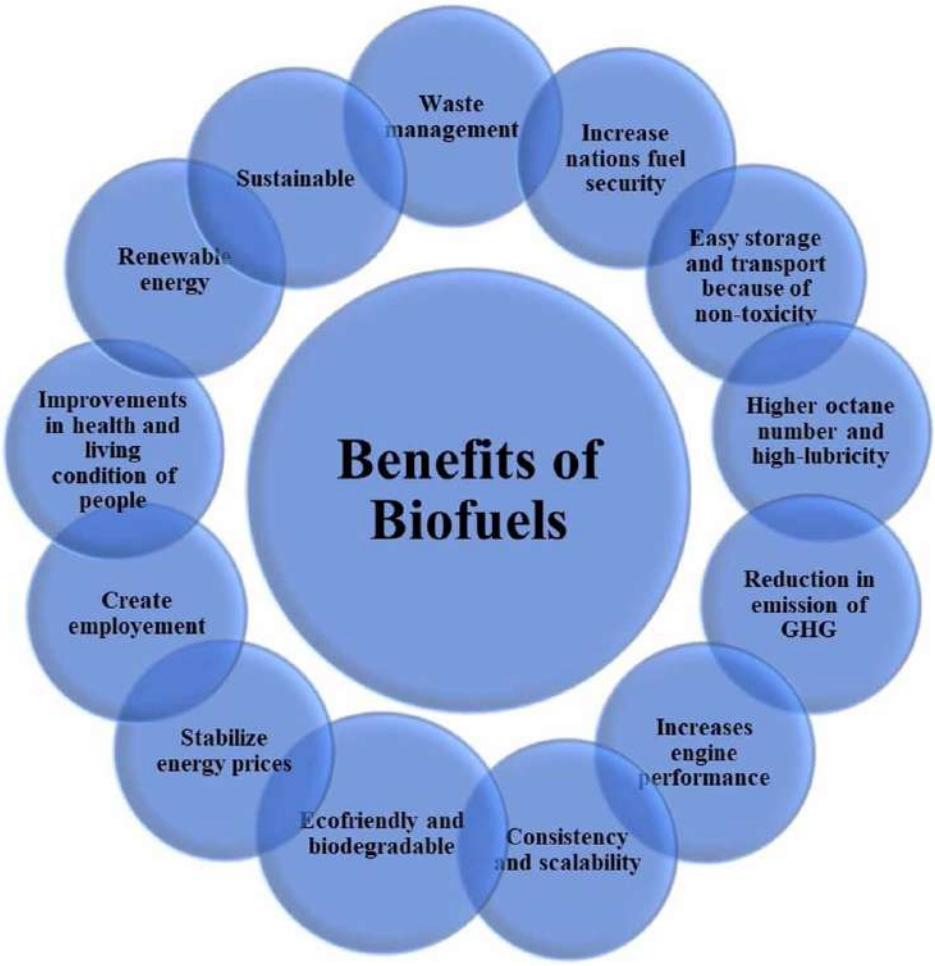
fossil fuels, as shown in **Fig. 1**. Biofuel can be converted to another variety of customized fuels as per the requirement. As reported by IEA in 2017, the annual global production of terrestrial and marine biofuels is estimated to be around 1,324 million tons of oil equivalents (International Energy Agency, 2017).

Figure 1. Benefits of biofuels over fossil fuels



Classification of Biofuels

The classification of biofuels is commonly categorized into two groups, namely primary (1þ) biofuel and secondary (2þ) biofuel. There is a further classification of secondary biofuel into three groups (as shown in **Fig. 2**) (a) 1stgeneration biofuel, (b) 2ndgeneration biofuel and (c) 3rdgeneration biofuels. Biofuels produced from oil crops have great potential as renewable and carbon-neutral alternatives to fossil fuels. But biomass, in the case of oil crops, used to produce biofuels, is part of human food stock that competes with the human food chain and causes a shortage of food, especially in developing countries (Alam et al., 2012)

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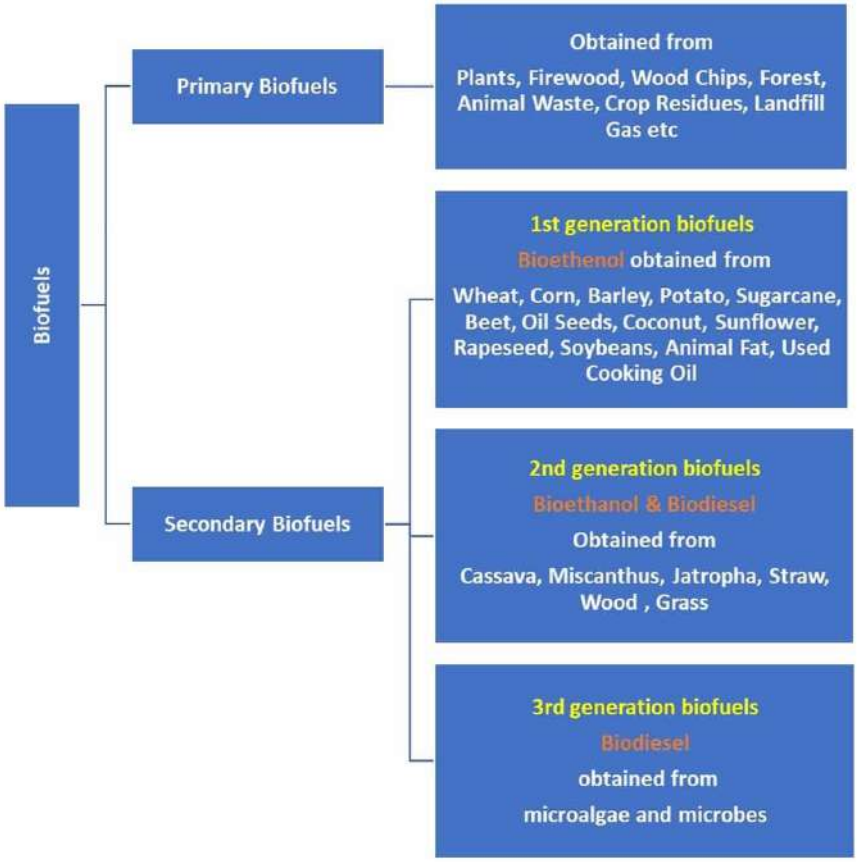
Figure 2. Types and sources of Biofuels
(Modified from Dragone et al., 2010)



Microalgae

Algae are photosynthetic autotrophs found in various water basins, including rivers, lakes, and oceans. Microalgae are unicellular or multicellular microorganisms found in marine and freshwater habitats. In comparison to plants, microalgae have basic structures and organs. It can undertake photosynthesis using sunlight, carbon dioxide, and water due to the existence of chlorophyll pigments involved in photosynthesis. Microalgae have a substantially greater average photosynthetic efficiency (6–8%) than terrestrial biomass (1.8–2.2%) (Aresta et al., 2005). Growing microalgae have various advantages, as shown in **Fig. 3**.

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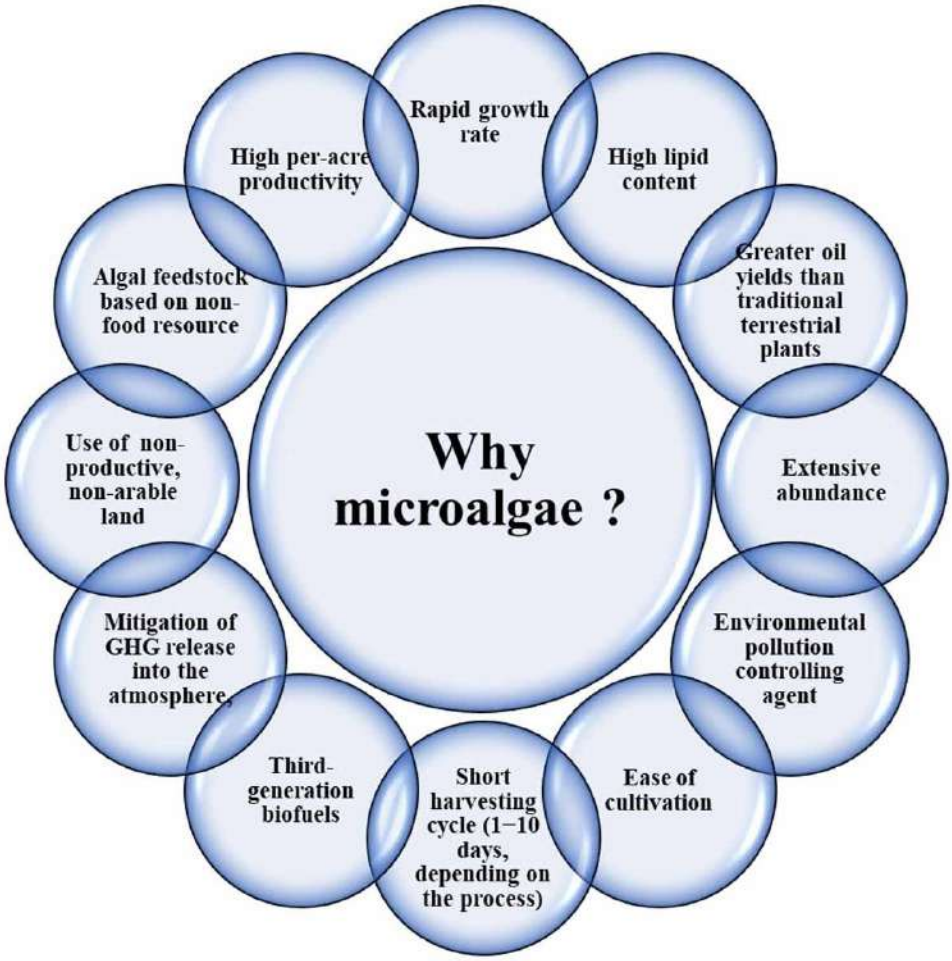
Figure 3. Advantages of growing microalgae as biofuel resources



It is microscopic and found in aquatic and terrestrial environments in a large community that can survive varied environmental conditions. Microalgae can grow faster under various metabolic conditions, including autotrophic, heterotrophic, mixotrophic, and photoheterotrophic, and demand three essential components: sunlight, water, and carbon dioxide. Geographically, microalgae strains can be exploited to produce biomass grow in tropical and subtropical climates with abundant year-round solar radiation and water (either from the sea or as groundwater) (Mobin & Alam, 2018). So, the tropical countries have a relatively more significant advantage in growing microalgae as a sustainable source for biofuels production. Because of their high lipid content, simplicity of cultivation, rapid growth rate, and volume at which they can be cultivated, microalgae have become appealing oil sources for the production of biofuels (Chisti, 2007). Producing microalgae has several benefits, including the feature that it does not compete with human or animal crop production and may be produced in non-agricultural fields as well as in saline water. Microalgae are an excellent replacement for industrial raw materials for biofuels production because of their high photosynthetic capability and ability to assemble many bio-products in their cells (Li & Yu, 2011). Microalgae have been employed as a biodiesel feedstock since the 1970s due

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to their widespread abundance and higher oil content than regular terrestrial plants (Demirbas, 2009). A comparative account of microalgae with various other biofuels sources is given in **Table 1**.

Table 1. The table shows a comparative account of different sources of biofuels and their oil content(modified from Najafi et al., 2011).

S. No.	Crops	Oil Content (L/hac)	Oil Yield Relative to Microalgae (%)
1	Rubber seed	100	0.07
2	Corn	172	0.13
3	Soybean	446	0.33
4	Safflower	779	0.57
6	Camelina	915	0.67
7	Sunflower	952	0.70
8	Peanut	1,059	0.77
9	Canola	1,190	0.87
12	Jatropha	1,892	1.38
13	Karanj	2,590	1.89
14	Coconut	2,689	1.96
15	Oil palm	5,950	4.35
16	Microalgae (30% oil by wt)	58,700	42.88
17	Microalgae (70% oil by wt)	136,900	100.00

It is well clear from the table that the oil content (L/ha) in microalgae is much higher than other feedstocks available such as oil palm, coconut, jatropha, avocado and rapeseed/canola (Maity et al., 2014).

Types and Composition of Microalgae

Algae are divided into three main classes, i.e., Chlorophyceae, Phaeophyceae and Rhodophyceae. Chlorophyceae are also known as green algae due to chlorophyll a and b, e.g. *Chlamydomonas* and *Volvox*. Phaeophyceae are also known as brown algae. They possess chlorophyll a, Chlorophyll c, carotenoids and xanthophylls. Rhodophyceae is also known as red algae because of red pigment, i.e., r-phycoerythrin. Marine algae are commonly known as seaweed. Algae are featured as microalgae due to their microscopic size. Microalgae are single cellular photosynthetic cells. Microalgae are either prokaryotic such as cyanobacteria *(Chloroxybacteria),* or eukaryotic such as green algae *(Chlorophyta)*(Mathushika & Gomes, 2022). At least 30,000 species of microalgae have been identified. (Mathushika & Gomes, 2022). Different types of associations of microalgae form large structures called colonies. Colonies may be heterogeneous groups formed by eukaryotic species like diatoms and bacteria and prokaryotic organisms like cyanobacteria.

Microalgae species can produce up to 58,700 L of oil per hectare (Mobin & Alam, 2018). Subsequent studies have identified the micro-algal species, such as *Botryococcusbraunii*, *Scenedesmus obliquus, Chlorella vulgaris, Chlamydomonas reinhardtii, and Nannochloropsisoculata,* that are recognized as

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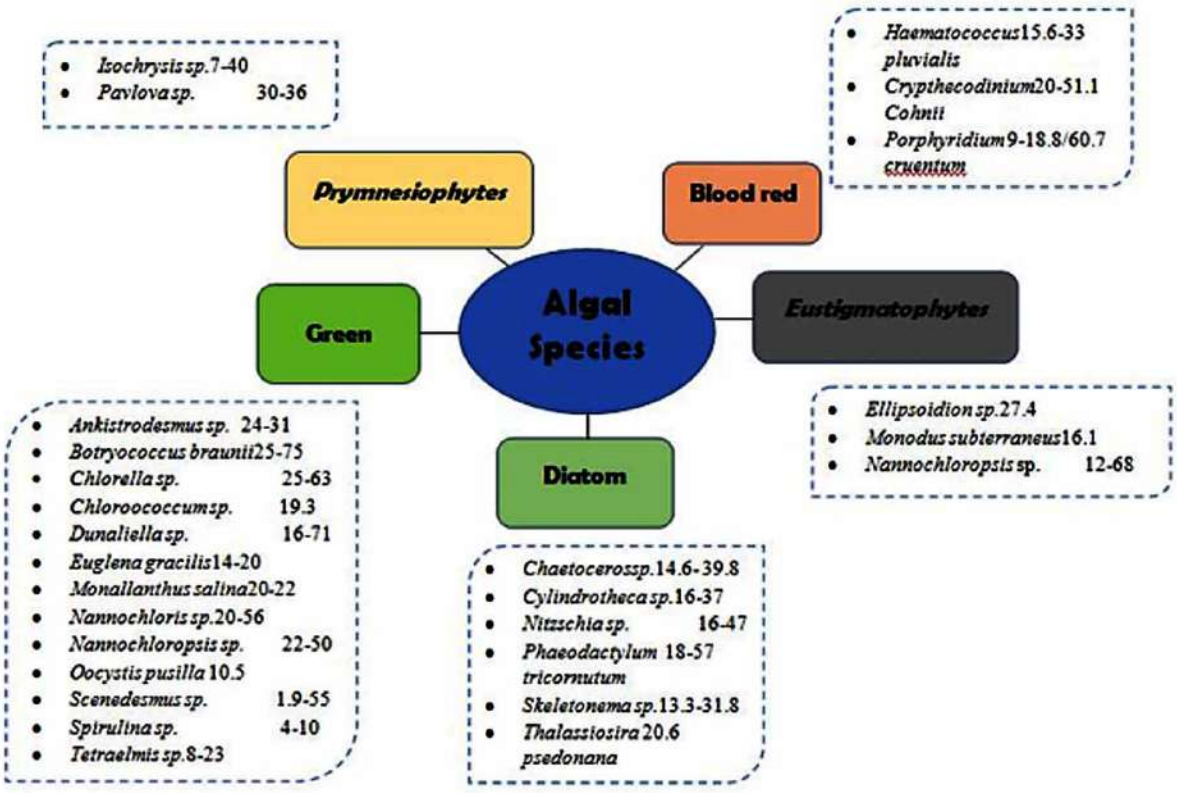
promising species for the production of biofuel (Veeramuthu & Chawalit Ngamcharussrivichai, 2020). All three biochemical fractions of microalgae (lipids, carbohydrates, and proteins) can be converted into fuels (Ghasemi et al., 2012). The fatty acid profile of microalgae species greatly favours biodiesel production (Qari et al., 2017). The major constituents of microalgae are lipids varying from 2–23%, proteins (6–52%), carbohydrates(5–23%) and some fat (Brown et al., 1997). Green micro-algae have a significantly higher lipid synthesis rate (mg/L/day) than red or blood-red micro-algae (Mobin& Alam, 2018). Microalgae lipid fraction consists of both neutral lipids and polar lipids. It accumulates triacylglycerols under stress conditions. Microalgae are rich in polar lipids in the exponential stage of development. Some microalgae have high protein concentrations in terms of quantity, up to 58% for *Chlorella vulgaris* on a dry weight basis. Carbohydrates (complex polysaccharides) are the main structural characteristics of the cell wall. The energy storage products of microalgae are glucose, starch and fat. Microalgae contain both saturated and unsaturated fatty acids. The lipid content of microalgae species varies from 20% to 50% of dry biomass (w/w). The most productive and cultivable microalgae and their respective lipid content are in **Fig. 4.**

Figure 4. Figure depicting different algal species and their respective lipid content (in percent)
(modified from Maity et al., 2014)



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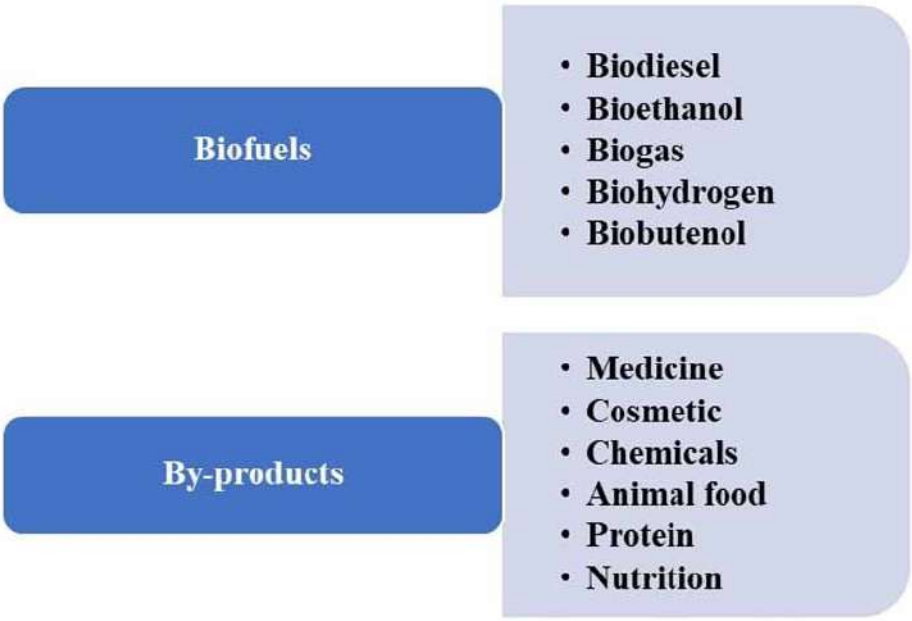
Complementary Algal By-Products

Along with the production of biofuels, microalgae have a variety of by-products with significant economic value. Following lipid extraction, the residual microalgae, i.e., dry biomass/cake, can be used as livestock feed, organic fertilizer due to its high nitrogen and phosphorus content, or merely burned for fuel co-generation (Jain et al., 2004). In addition, the resulting dry algal cake can be used to reduce the quantity of coal needed in thermal power plants. Furthermore, algal biomass has a better calorific value and lower SO_x concentration in flue gas when compared to coal. The calorific value of algal biomass is around 28,000kJ/kg, whereas the calorific value of typical high-quality coal is 27,000kJ/kg (Sudhakar & Premalatha, 2012). The algal by-products are used in different sectors, as shown in **Fig. 5.**

Figure 5. Different kinds of biofuels and by-products are produced from microalgae



Direct photolysis, indirect photolysis, and an ATP-driven pathway have all been developed as methods for producing hydrogen. The single-cell green algae *Chlamydomonas reinhardtii* has been studied extensively for photo-biological hydrogen generation. In sulfur-limited and anaerobic conditions, the starch in algal cell walls is converted to hydrogen. Because of the quantity of carbohydrates/polysaccharides and thin cellulose, algae are a better source of bio-ethanol production (Mathushika & Gomes, 2022), e.g. *Chlorococcumlittorale*, marine green algae produce ethanol under dark fermentation. Brown algae are remarkable feedstock for the development of ethanol because they have high carbohydrate content. Algal bioethanol prevails over the disadvantages of first-generation and second-generation bioethanol.

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Current Tools and Techniques to Grow and Harvest Microalgae

Currently, industrial production of microalgae is small, estimated to be around 6000 tons (dry weight) per year worldwide (Maity et al., 2014).

The equation below shows that microalgae require essential environmental components to grow and synthesize raw material for biofuels.

$$\text{Light} + CO2_{+} H2O_{+} \text{nutrients lipids+proteins+carbohydrates}$$

Essential requirements of nutrients from the environment are carbon, phosphorus, nitrogen, and iron, and some minor are calcium, potassium, magnesium, manganese, sulphur, copper, zinc, and cobalt (Chisti, 2007). The temperature requirement must be within the range of 20°C- 30°C. The microalgae cultivation methods are mainly categorized into ways 1. Suspended cultivation. It includes 1a) open ponds and 1b) closed photobioreactors 2. Immobile cultivation. It includes 2a) immobile matrix cultivation and 2b) bioðlms cultivation. Different types of techniques used to cultivate microalgae are illustrated in Fig**. 6. The** industrial-scale of microalgae production utilizes a continuous suspended culture technique during the daytime. This method needs to feed a new culture and continuously remove the same quantity of culture from the system. The biomass is generated during the day; out of that, approximately 25%biomass lost during nighttime due to respiration failure and sufficient unavailability of growth nutrients, temp. and sunlight (Chisti, 2007). Open ponds or Raceways are also the most widely used methods of microalgae production on a large scale because this system is a shallow and open, equipped circulating wheel which provides adequate mixing and circulation of nutrients. These setups are economically suitable to build and run but face relatively lower production due to uncontrolled conditions(Mata et al., 2010). These systems have been utilizing microalgae biomass production for the last sixty years (Tredici & Materassi, 1992). The open ponds are shallow, not deeper than 30 cm, to allow sunlight for microalgae growth. Therefore they become easy to operate and economically viable but confront some disadvantages (Chisti, 2007). A paddlewheel creates mixing and circulation in raceways, and it works continuously to prevent sedimentation. However, open systems are generally simple to operate and use solar energy as an energy source; they have several drawbacks, including the need for a vast area to cultivate, unregulated environmental conditions which change seasonally and diurnally, water evaporation, comparatively unequal carbon dioxide and light distribution in the system, and high exposure to contamination or infection that is difficult to control. To grow in such fluctuating environmental conditions, the extremophilic strain of microalgae is required to cultivate. Normal species having a high production rate face low productivity in the case of an open pond system.

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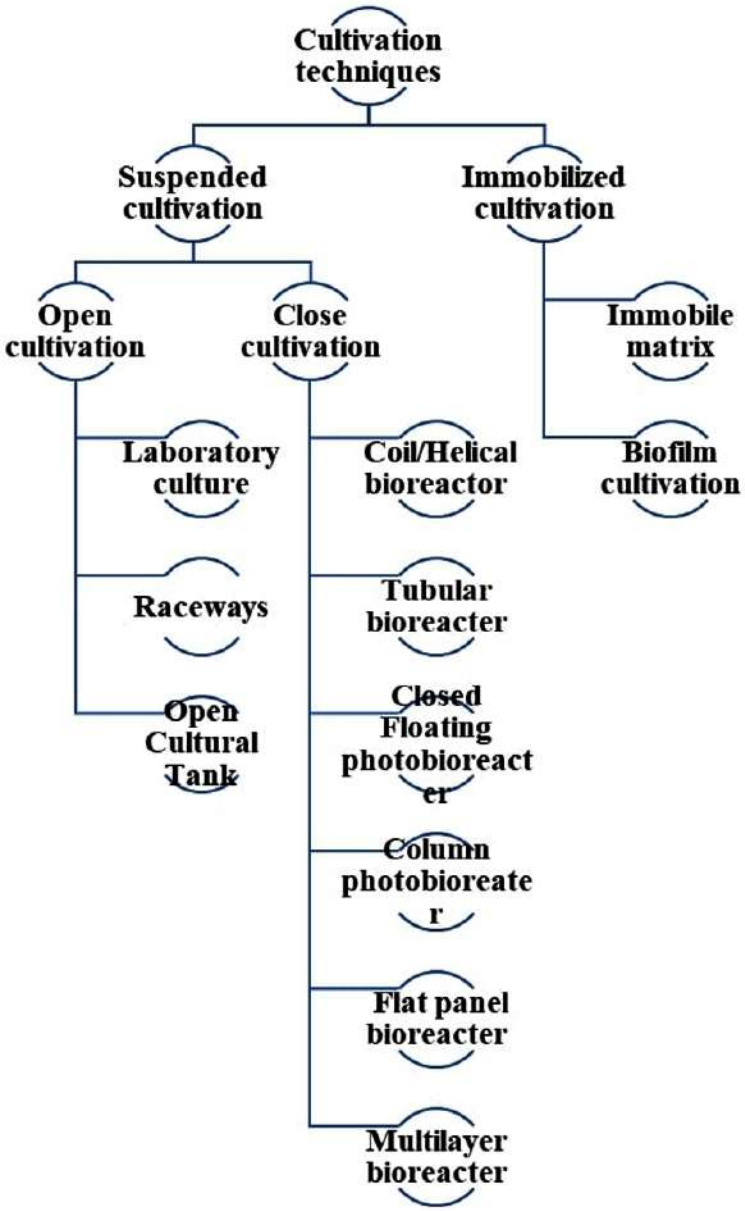
Figure 6. Techniques used to grow microalgae



Photobioreactors are considered to be most productive for microalgae cultivation as compared to open pond or raceways systems because of better controlled medium parameters like temperature, pH, nutrient addition, efficient mixing, better control over contamination or infection, low water evaporation and a large number of cells per unit area (Lundquist et al., 2010, Carvalho et al., 2006, Kunjapur & Eldridge, 2010, Darzins et al., 2010, Lam et al., 2019). Photo-bioreactors can also be customised per the specific strain of microalgae to enhance productivity in every possible way and better control environmental fluctuation. But these reactors are energy-intensive and expensive to build, making biofuels economically infeasible (Mata et al., 2010). Photobioreactors are made up of transparent glass or

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plastic tubes with £ 0.1 m in diameter. Based on different closed structures, photobioreactors are of the following type 1) vertical, in that tubes are parallelly arranged vatical to ground. 2) Horizontal, in that tubes or plates are parallel to one another but horizontal to the ground 3) helical, in that tubes are making coiled structure around a supporting frame. They are considered easy to increase the production, up to 13 times more than raceways (Chisti, 2007). But every system has its advantages and disadvantages. To increase the production of microalgae, the surface area needs to be improved, increasing sunlight exposure. This could be achieved by making tubes thinner and plates broader, increasing the surface area to volume ratio. Zemke et al., 2016 reported that a doubling of volume had doubled the microalgal biomass production, but built-up cost increases only 59% of the initial. In the case of phototropic open culture doubling surface area increases the capital cost by 100%, but it has the advantage of using free energy; otherwise, it requires a significant investment cost to provide it (Zemke et al., 2016). The significant disadvantages of photobioreactor are the recovery of biomass, but this issue was resolved up to a great extent by biofilm or immobilized matrix cultivation system (Alam et al., 2015, Hoffmann, 1998). Open ponds or raceways are also preferable for microalgae cultivation due to their low capital cost and relative ease of operation. The current technologies are not economically suitable and capable enough to compete in the market. To cut down its production cost and improve harvesting and processing techniques, optimising the utilization of wastewater as algal culturing feed is required. Various studies have considered the most appropriate environment-friendly and economically viable method to produce biofuel and other complementary products with significant economic value.

But the use of wastewater as microalgae feed faces significant challenges, which include high turbidity causes improper penetration of sunlight in the medium, overdose of nutrients which also hinder the algal growth, the suitable strain of microalgae required to cope with up wastewater environment, and essential requirement of freshwater to dilute the waste etc. Different techniques, methods, and strategies are described (Mobin & Alam, 2014, Dragone et al., 2010, Chisti, 2007, Scott et al., 2010, Kumar & Bharadvaja, 2020). Harvesting has also been a challenging task after cultivating microalgae because it costs up to 30% of the total production cost of microalgae biofuel(Grima et al., 2003). Various methods presently employed to harvest microalgae include mechanical, physical, chemical, biological and electrical processes such as centrifugation, ultra-filtration, floatation, auto flotation and flocculation, etc. (Dragone et al., 2010). The flocculation is utilized when algae size is comparatively small. Flocculants like ferric chloride, aluminium sulphate and ferric sulphate are used—moreover, different combinations are employed for efficient harvesting. Centrifugation is used to separate suspended microalgae, and the medium is decanted. This is considered the most reliable method for separating microalgae from the medium(Christenson & Sims, 2011). Suspended culture is passed through the filter in filtration until a thick layer of microalgae biomass is formed. An electrical method is based on separating algal cells under an electric field, but the low-cost method is required to do this task efficiently.

Different Processes to Produce Microalgae Biofuel

Several processes are available to convert microalgae biomass into different types of energy. It has the following method (1) direct combustion, (2) chemical reaction, (3) thermochemical conversion (4) biochemical conversion. The different processes of microalgae biomass conversion and resulting products are shown in **Fig.7**_(Wang et al., 2008). Along with the production of biofuel, some other complementary products also can be produced, and their detailed description can be obtained from these

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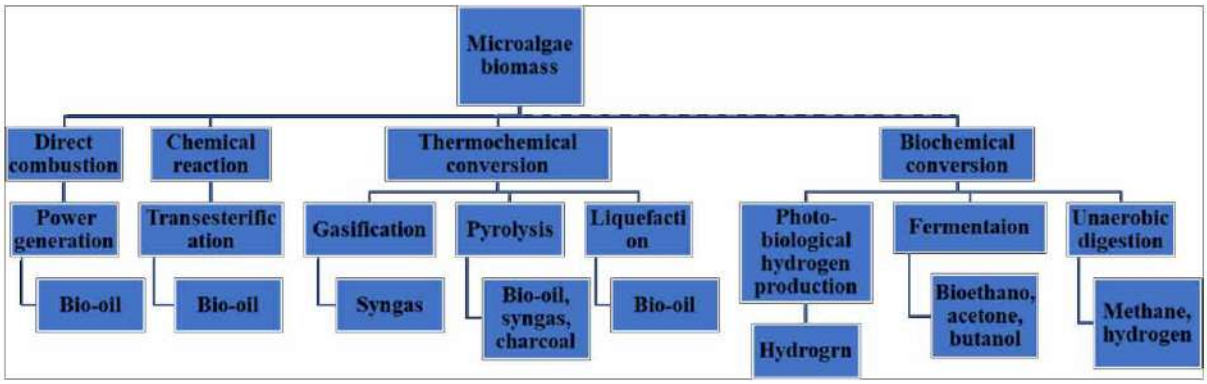
sources(Dragone et al., 2010, Spolaore et al., 2006, Benemann et al., 1987, Eriksen et al., 1998, Harun et al., 2010, Mendes-Pinto et al., 2001).

Figure 7. The different processes of microalgae biomass conversion and resulting products
(Modified from Dragone et al., 2010)



Potential and Challenges

Microalgae can utilise CO_2 emitted from thermal power plants with higher than 10% enhanced photosynthetic efficiency compared to 0.5% of terrestrial plants (Khan and Rashmi, 2008).

It is estimated that microalgal biomass of one kilogram can fix 4.8 kg of CO_2 (Baral et al., 2015). So, microalgal cultivation with sequestration of CO_2 emitted from the thermal power plant and production of value-added products, including biodiesel, make them an efficient and carbon-neutral alternative for energy production. Several pilot studies of microalgae cultivation using emitted CO2is going on worldwide, including Stanwell Power Plant in Queensland, Loy Yang Power Plant in Victoria, and Eraring Power Plant in New South Wales. Due to technical challenges and higher capital costs of solar and wind energy utilization, third-generation algal biofuel can play an essential role in filling the partial gap left by reducing fossil fuel utilization. However, some fundamental limitations must be solved to obtain the maximum potential of third-generation biofuels derived primarily from microalgal biomass.

The following are real challenges:

1. Technological constraints in microalgae selection, culture, harvesting, processing, and distribution techniques. The development of microalgae strains that are high yielding, broad temperature tolerant, and salinity tolerant is a challenge. Commercial-scale microalgal production lacks a cost-effective, consistent culture and harvesting process and system. The available mechanical and chemical technologies for extracting biofuels are not economically sustainable. Compared to conventional methods, the expense of infrastructure for biofuel distribution and retailing is also a challenge.
2. Microalgae cultivation also faces significant obstacles in terms of land availability and agricultural inputs. Commercial-scale microalgae cultivation cannot wholly utilise arable land and freshwater resources, which would pose a danger to human and animal food demand. Excessive use of agricultural additives (inorganic and organic fertiliser) in algal mass production can lead to eutrophication when phosphate and nitrate levels rise.

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3. Gene modification remains a barrier, even though detailed information can assist. Comprehensive perception of microalgal biology, genetic manipulation, and gene cloning are all challenges that must be addressed to improve photosynthetic efficiency, metabolic activity, temperature tolerance, and algae biomass output and oil content.
4. Wastewater utilization for microalgae cultivation remains a concern due to the lack of a well-developed and cost-effective procedure for screening out toxic and undesired substances that can interfere with algal growth.
5. A significant amount of investigation and innovation is necessary to use value-added by-products such as bio-fertilizer, animal feed, cosmetics, colouring agents, chemical and pharmaceutical commodities, biogas masses, and biofuel generation.
6. Currently, biofuels' production cost is significantly higher than fossil fuel prices. So, it is highly challenging for biofuel to be competitive in the market.

CONCLUSION

Sustainable energy sources for the growth and development of the world are a mindboggling task in front of the scientific community. Fossil fuels are not environment friendly and have limited stocks, which will be exhausted soon. Biofuels are emerging areas of sustainable, potential energy sources that need to be exploited to meet growing energy demands in this chaos. Microalgae are a flexible biofuels source with significant potential to produce different biofuels. Microalgae are considered a carbon-neutral fuel due to the capability to utilize wastewater as a feed to grow, co2 from a thermal power plant with enhanced photosynthetic capacity, and the production of various complementary by-products with significant economic benefits values. Still, multiple gaps need to be addressed to commercialise fuel production using microalgae, including efficient technologies that harvest, process, and convert it into economically viable biofuels. Systematic and comprehensive research is required in this regard. Presently, microalgae biofuels face significant challenges, including high performance, broad range tolerating and high yielding microalgae strains, improved culturing techniques, reduction in production cost, utilisation of waste to compete in markets, and improved harvest and processing techniques to make economically viable source.

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Chapter 14
Microbial Cellulase in the Production of Second Generation Biofuels: State-of-the-Art and Beyond

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ABSTRACT

Bioethanol from inedible cellulose-rich biomass is the most promising candidate to replace fast depleting, environmentally polluting fossil fuels. Hydrolysis of cellulose to glucose is the crucial step in its biotransformation to bioethanol. Enzymatic hydrolysis is favored over acid hydrolysis, as enzymes are eco-friendly biocatalysts with high substrate specificity and superior catalytic efficiency in mild reaction conditions. Complete hydrolysis of cellulose is achieved by cellulase. Higher cellulase production yield, stability, and catalytic efficiency are the main attentive points for the successful implementation in industrial production of bioethanol. This chapter will highlight general characteristics of microbial cellulases and their role in the bioconversion of cellulose to biofuels, economic sustainability of cellulose-based biofuels, and the latest innovations in cellulase immobilization as the most comprehensive strategy for improvement of enzyme stability, activity, and reusability for cost-effective large-scale application.

INTRODUCTION

In 2016 the use of bioethanol-gasoline blends in transportation reduced the emission of carbon dioxide by 43.5 million tons, the equivalent of removing 9.3 million cars from the road for the entire year (Robak & Balcerek, 2018). Lignocellulose biomass is the most abundant and renewable source of carbohydrates in the biosphere. It is obtained from inedible energy crops, agricultural and industrial waste (Kricka et al., 2015; Verma & Kumar, 2021). Bioethanol from lignocellulose biomass is a second-generation biofuel, whose production does not compete against food supplies, in contrast to the first-generation bioethanol,

DOI: 10.4018/978-1-6684-5269-1.ch014

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produced from edible seed-derived starch. Accordingly, second-generation bioethanol is the most promising candidate to replace conventional gasoline in the near future (Hoffman et al., 2021; Horn et al., 2012). Enzymes are green biocatalysts with remarkable substrate specificity and catalytic efficiency in mild reaction conditions. They can be genetically or chemically modified to meet the specific requests of the biotechnological process in which they are employed (Ejaz et al., 2021; Trbojević et al., 2013). Enzymatic hydrolysis of cellulose to glucose is the critical step in production of the second-generation bioethanol. Complete hydrolysis of cellulose is achieved by concerted activity of endoglucanase, exoglucanase (cellobiohydrolase) and β-glucosidase, which form a multi-enzyme cellulase complex. Mesophilic and thermophilic bacteria and fungi are the major industrial cellulase producers (Acharya & Chaudhary, 2012; Bhardwaj et al., 2021; Contreras et al., 2020; Horn et al., 2012; Menendez et al., 2015; Srivastava et al., 2018; Verma & Kumar, 2021). Commercial cellulase cocktails have contributed to the significant cost-reduction of cellulose bioconversion to bioethanol (Bhardwaj et al., 2021; Contreras et al., 2020; Lamichhane et al., 2021). However, further decrease in cellulase price is necessary by improving cellulase production yield, stability, specific activity and catalytic efficiency for economically viable bioethanol production.

Immobilization is the strategy of choice for improving catalytic performance of industrial enzymes, since it encompasses significant biocatalyst stabilization and reusability. Enzyme reusability significantly reduces the cost of the biocatalyst application at industrial scale and can be achieved exclusively by immobilization (Milosavic et al., 2016; Trbojević et al., 2013). With myriad of possibilities regarding immobilization methods and the choice of immobilization support, cellulases can be tailored for cost-effective and optimum catalytic performance. This chapter is a comprehensive overview of current state-of-the-art in the industrial application of cellulases in biofuel production. It will provide detailed fundamental understanding of microbial cellulases and their critical role in bioconversion of cellulose to bioethanol. Furthermore, the techno-economical aspect of the cellulose-derived biofuels will be discussed, with additional analysis of COVID-19 impact. The separate section of the chapter will be dedicated to cellulase immobilization and its effect on enzyme stability, activity and reusability. It will specifically deal with nanomaterials as innovative immobilization carriers, having in mind their increasing popularity in this area of research and application.

BACKGROUND

Lignocellulose biomass is a complex mixture of carbohydrate and non-carbohydrate compounds. Cellulose is the dominant component (40-60% of the total biomass weight), followed by hemicellulose (20-40%) and lignin (20–30%). It also contains proteins, lipids, pectin, minerals and soluble sugars (Adegboye et al., 2021; Horn et al., 2012; Kricka et al., 2015). Structure of major constituents of lignocellulose biomass is represented in Figure 1.

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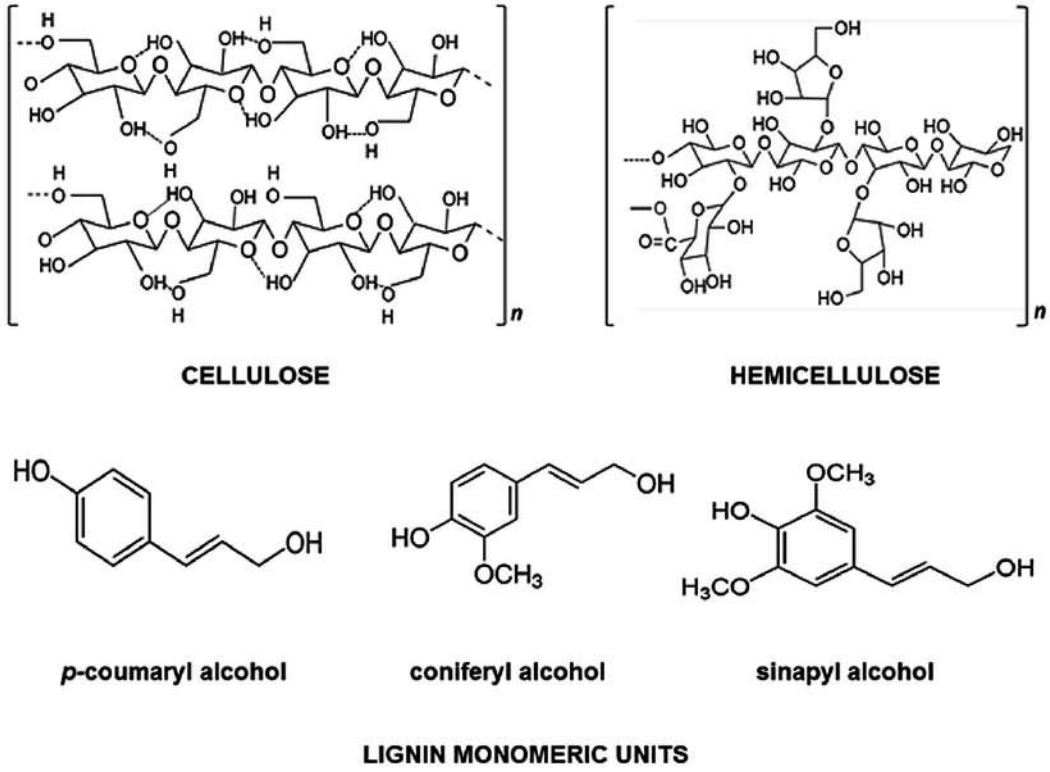
Figure 1. Constituents of lignocellulose biomass
Source: (Adegboye et al., 2021; Horn et al., 2012; Kricka et al., 2015; Mohanram et al., 2013; Zoghlami & Paës, 2019)



Cellulose is a linear homopolysaccharide of β-D-glucopyranose units linked by β-(1,4)-glycosidic bond. Its structure is stabilized into microfibrils by numerous hydrogen bonds and van der Waals forces. Each microfibril contains up to 36 glucan chains with thousands of glucose units (Mohanram et al., 2013). Consecutive sugars along chains in crystalline cellulose are rotated by 180°, meaning that cellobiose is the fundamental building block (Kricka et al., 2015). Cellulose can be crystalline or amorphous. Individual glucan fibrils in crystalline cellulose are tightly packed and hardly accessible to enzymes or solvents (Adegboye et al., 2021). Depolymerization of cellulose induces changes in its crystallinity and porosity (Zoghlami & Paës, 2019).

Hemicellulose is heteropolysaccharide, composed of xylose, arabinose, rhamnose, galactose and mannose. It can also contain glucuronic, galacturonic and methylgalacturonic acid (Adegboye et al., 2021). Common hemicelluloses are xylan, mannan and xyloglucan. Hardwood xylans contain β-(1,4)-linked xylose backbone with a high amount of methylgalacturonic acid, while grass xylan is more complex with high amounts of arabinose and ester modifications with hydroxycinnamic acid. Hydroxycinnamic acids can also cross-link adjacent xylan chains, building a more complex and rigid structure (Horn et al., 2012). Degree of hemicellulose polymerization is lower than of cellulose, therefore hemicellulose is amorphous with little physical strength (Zoghlami & Paës, 2019). Lignin is hydrophobic heteropolymer of coniferyl, sinapyl and p-coumaryl alcohol, linked *via* C-C and C-O-C links. In lignocellulose biomass lignin is cross-linked with carbohydrates by ether or ester linkages which provides structural integrity

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of plant cell walls and hinders the enzymatic hydrolysis of biomass, a phenomenon known as biomass recalcitrance (Adegboye et al., 2021; Horn et al., 2012; Verma & Kumar, 2021).

Endoglucanase (EC 3.2.1.4, systematic name: 1,4-β-D-glucan-4-glucanohydrolase), exoglucanase (EC 3.2.1.91, systematic name: 4-β-D-glucan cellobiohydrolase; EC 3.2.1.74, systematic name: 4-β-D-glucan glucohydrolase) and β-glucosidase (EC 3.2.1.21; systematic name: β-D-glucoside glucohydrolase) form multi-enzyme machinery for complete hydrolysis of cellulose. Aerobic bacteria and fungi produce individual cellulases, which are further combined in biocatalytic cocktails with a precise share of each individual enzyme for efficient hydrolysis (Acharya & Chaudhary, 2012). Microbial cellulases have a two-domain structure, consisting of cellulose-binding domain and catalytic domain, connected by flexible hinge (Ejaz et al., 2021). Hinge region is rich in proline, threonine and serine and enables the enzyme to move along cellulose fibril in a caterpillar-like mode. Detailed data of microbial cellulase structure were obtained from model systems: endoglucanase from *Thermoascus aurantiacus* (Cel5A), cellobiohydrolase from *Phanerochaete chrysosporium* (Cel7D) and β-glucosidase A from *Bacillus polymyxa*. Polypeptide chain of Cel5A is organized into a very compact eight-fold β/α-barrel with short loops and it contains only catalytic domain. Its active site contains acid/base glutamate 133 and nucleophilic glutamate 240. Substrate-binding site of Cel5A is shallow and long, delineated with tryptophans 170, 174, 273, 278 and 279 (Lo Leggio & Larsen, 2002). Catalytic domain of Cel7D is organized in a β-sandwich with antiparallel β-sheets, stabilized by hydrophobic interactions and disulfide bridges. In comparison to *T. reesei*, active site is more open, however not as open as in endoglucanase. Nucleophilic glutamate 207, acid/base glutamate 212 and proton donor aspartate 209 form catalytic triad of Cel7D, located in the tunnel-shaped active site, delineated with tryptophan and additional aromatic residue from tyrosine 47 at the tunnel entrance (Muñoz et al., 2001). β-Glucosidase from *B. polymyxa* is octamer, organized into α/β barrel, stabilized by fourteen hydrogen bonds. Loops A, B and C connect individual parts of the α/β barrel. Length and chemical nature of these loops governs substrate specificity of β-glucosidase. Most significant differences were observed in the loop C, a part of the substrate-binding cavity whose end is in a close proximity to the non-reducing end of cellobiose. Substrate-binding cavity of β-glucosidase A is wider and more extended, so that it can accommodate larger substrates than cellobiose. Interesting mechanistic attribute of glucosidases, including β-glucosidase, is the ability to catalyze synthesis of disaccharides and oligosaccharides from glucose in transglucosylation. Hydrolysis *vs* transglucosylation is regulated by the water content in the reaction mixture (Pavlović et al., 2013; Veličković et al., 2014). Concerted action of individual cellulases in cellulose hydrolysis is depicted in Figure 2.

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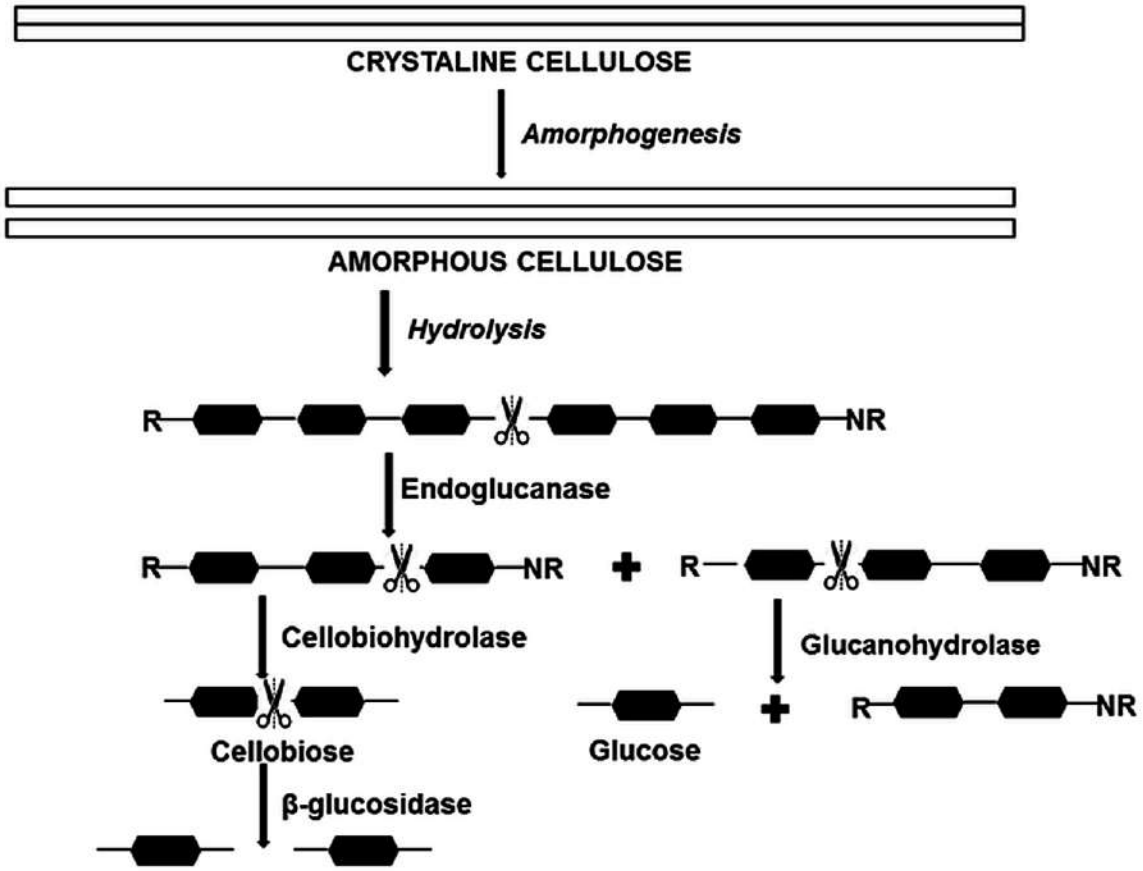
Figure 2. Enzymatic hydrolysis of cellulose
Source: (Adegboye et al., 2021; Barcelos et al., 2015; Ejaz et al., 2021; Horn et al., 2012; Mohanram et al., 2013; Tiwari et al., 2018)



Cellulose hydrolysis is initiated by random attacks of internal sites in amorphous regions in cellulose by endoglucanase, resulting in rapid depolymerization (Barcelos et al., 2015; Ejaz et al., 2021). Exoglucanases then attack non-reducing or reducing ends of the glucan chain, releasing cellobiose or glucose, respectively (Adegboye et al., 2021). Exoglucanases acting on non-reducing ends are called cellobiohydrolases and they are the dominant component of cellulase cocktails *(*Adegboye et al., 2021; Horn et al., 2012*)*. Exoglucanases, acting on reducing ends are called glucanohydrolases (Mohanram et al., 2013). In the final step, β-glucosidase attacks the non-reducing end of cellobiose, yielding glucose. Hydrolysis of cellobiose is the rate-limiting step of the overall process, making β-glucosidase the key regulatory enzyme in cellulose hydrolysis (Tiwari et al., 2018). β-Glucosidase is strongly inhibited by glucose, with the exception of β-glucosidase from *Aspergillus oryzae* (Barcelos et al., 2015). Synergism of cellulases is influenced by physical and chemical heterogeneity of the substrate, access of the substrate to the active site of the cellulose-processing enzymes and ratio of individual cellulases in the enzyme cocktail. Low content of endoglucanase and high content of cellobiohydrolase provide the best synergy in the catalytic process and the highest glucose yield (Contreras et al., 2020).

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Natural and genetically modified fungi and bacteria have been heavily exploited for production of industrial cellulases. Fungal cellulases are more common, due to high productivity of engineered strains (>100g protein/liter of cell culture), which secrete the enzyme to the medium for easy extraction and purification (Mohanram et al., 2013; Srivastava et al., 2015). On top of that, *Escherichia coli*, the most common host for heterologous expression of proteins, suffers from several significant limitations in cellulase production. Thick outer membrane of *E. coli* or impaired transport across the membrane due to degradation of linker sequence result in limited secretion of the target enzyme. Formation of inclusion bodies further complicates isolation and purification of the target enzyme and often leads to its inactivation. Solution can be found in genetic engineering of cellulolytic bacteria, *Bacillus subtilis* or *Clostridium thermocellum* for homologous expression of cellulases. However, high production costs and need for enriched media limit this approach (Menendez et al., 2015). On the other hand, a significant drawback of fungal systems is the deficiency of a specific cellulase. *Hypocrea jecorina* (*T. reesei*) cellulase mixture is rich in cellobiohydrolase, but low in β-glucosidase. The opposite is true for *Aspergillus niger*. Therefore, in order to produce the complete and effective cellulase system, several fungal strains need to be employed, which is not user-friendly nor cost-effective (Srivastava et al., 2015). Apart from that, bacteria have higher growth rate than fungi and produce cellulases of higher stability (Mohanram et al., 2013).

Submerged and solid-state fermentation are the two main methods for large-scale production of cellulases, using lignocellulose biomass as a growth medium for inducible cellulolytic microbial producers. Submerged fermentation is a well-established technology for large-scale production of fungal cellulases in which water is abundant in fermentation mixture, resulting in reduced oxygen, temperature and nutrient concentration gradients and facilitated enzyme recovery. However, low enzyme yield and additional costs of dewatering during enzyme purification are key limitations of this strategy. Solid state fermentation is essentially a one-pot system for cellulase fermentation and production of bioethanol from lignocellulose biomass. It is performed without free water in the system, but with ample amount of moisture. Accordingly, solid state fermentation is cost-effective and time-saving method for high-yield production of bioethanol in lower reactor volume, lower enzyme loading and lower requirement for sterile conditions. The major bottleneck of this method is reaction temperature: in saccharification step temperature is maintained at 50^{o}C, while during bioethanol production temperature is maintained between 28 and 37^{o}C. Therefore, thermotolerant enzymes are necessary to endure this process (Bhardwaj et al., 2021; Dey et al., 2020). Table 1 summarizes the leading market suppliers and characteristics of commercial cellulase preparations.

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Table 1. Commercial cellulase preparations

Market Name	Supplier	Source	Characteristics
Accellerase® 1500	Genencor-Danisco, USA DuPont Industrial Biosciences	*T. reesei*	82mg/mL cellulase 51mg/mL xylanase Stable at 50-65°C and pH 4.0-5.0
Bio-feed beta L	Novozymes, Denmark	*Trichoderma longibrachiatum* *T. reesei*	<5 filter paper units/mL 12U/mL β-glucosidase
Biocellulase A	Quest Intl., USA	*A. niger*	32U/mL β-glucosidase High stability at pH 5 and 55°C
Cellubrix (Celluclast)	Novozymes, Denmark	*T. longibrachiatum* *A. niger*	56 filter paper units/mL 136U/mL β-glucosidase
Cellic (CTec2)	Sigma Aldrich, USA	*T. reesei* *A. niger*	Variable efficiency
Cellulase 2000L	Rhodia-Danisco, France	*T. longibrachiatum* *T. reesei*	10 filter paper units/mL
Cellulyve 50L	Lyven, France	*T. longibrachiatum* *T. reesei*	24 filter paper units/mL
Cellulase AP 30 K	Amano Enzyme Inc., Japan	*A. niger*	60U/mL β-glucosidase High stability at pH 4.5 and 60°C
Cellulase NLT	Zhongbei Bio-Chem Industry Co., Ltd, China	*Trichoderma spp.*	10 000U/g cellulase Optimum activity at pH 4.5 and 40–50°C
Energex L	Novozymes, Denmark	*T. longibrachiatum* *T. reesei*	<5 filter paper units/mL 19U/mL β-glucosidase
GC220	Genencor-Danisco, USA	*T. longibrachiatum* *T. reesei*	116 filter paper units/mL 215U/mL β-glucosidase
GC440			<5 filter paper units/mL 70U/mL β-glucosidase
GC880			<5 filter paper units/mL 86U/mL β-glucosidase
Novozyme 188	Novozymes, Denmark	*A. niger*	1116 filter paper units/mL
Rohament CL	Rhom-AB Enzymes, Finland	*T. longibrachiatum* *T. reesei*	51 filter paper units/mL 28U/mL β-glucosidase
Shearzyme Plus	Novozymes, Denmark	*A. oryzae* *T. reesei*	250U/g xylanase 350U/g cellulase β-glucosidase side activity
Spezyme CP	Genencor-Danisco, USA	*T. longibrachiatum* *T. reesei*	49 filter paper units/mL
Ultraflo L	Novozymes, Denmark	*T. longibrachiatum* *T. reesei*	<5 filter paper units/mL 20U/mL β-glucosidase
Ultraflo XL		*Humicola insolens* *Bacillus amyloliquefaciens*	1500U/g xylanase 500U/g β-glucanase α-amylase side activity
Viscoferm		Not defined	222U/g β-glucanase Other activities: cellulase, xylanase Active at 30-80°C and pH 5.5-6.5
Viscozyme L		*T. longibrachiatum* *T. reesei*	<5 filter paper units/mL 23U/mL β-glucosidase
Viscostar 150L	Dyadic, USA	*T. longibrachiatum* *T. reesei*	33 filter paper units/mL 111U/mL β-glucosidase

Source: (Acharya & Chaudhary, 2012; Contreras et al., 2020; Srivastava et al., 2015)

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MAIN FOCUS OF THE CHAPTER

In this section following topics will be discussed in detail:

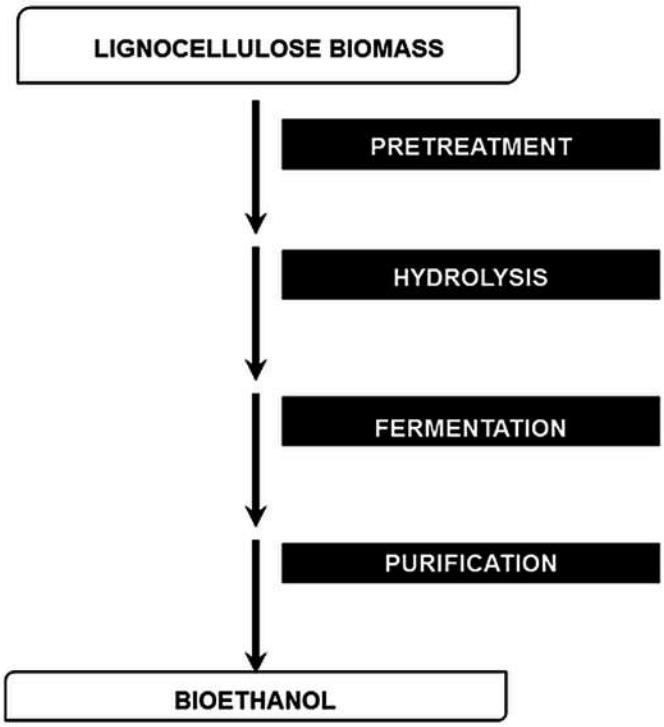
- Bioconversion of lignocellulose biomass to bioethanol
  - Fundamental aspects and the main phases of bioethanol production
  - Process parameters influencing lignocellulose bioethanol yield
- Techno-economic analysis of bioethanol as an alternative fuel
  - Economic segments of global bioethanol market
  - Impact of COVID-19 on economic viability of bioethanol
  - Co-relation of global cellulase market and global bioethanol market.

Accordingly, this section will address actual issues, which need to be taken into an account from both scientific community and industry when considering bioethanol as transportation fuel of the future.

Bioconversion of Lignocellulose Biomass to Bioethanol

Bioconversion is a complete transformation of complex lignocellulose biomass to bioethanol as a final product. It is a multiphase process, as presented at Figure 3.

Figure 3. Bioconversion of lignocellulose biomass to bioethanol
Source:(Devi et al., 2022; Dey et al., 2020; Mohanram et al., 2013; Robak & Balcerek, 2020)



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Pretreatment of Lignocellulose Biomass

The main purpose of pretreatment is to reduce the biomass recalcitrance by removal of lignin and hemicellulose and to promote amorphogenesis of cellulose to make it more accessible for hydrolysis. Methods of lignocellulose biomass pretreatment can be physical, chemical, physicochemical and biological, as indicated in Table 2.

Table 2. Pretreatment of lignocellulose biomass

Type of Pretreatment	Method	Reaction Conditions	Remarks
Physical	Mechanical comminution	Chipping, grinding, milling	Reduction of particle size to 0.2-2mm
	Pyrolysis	Intense heating above 300°C, followed by cooling and condensation	Rapid decomposition of cellulose to H_2, CO and char Leaching of char to obtain fermentable sugar
	Microwave pretreatment	High heating in a microwave oven	Inefficient separation of hemicellulose and lignin from cellulose Energy consuming and expensive for industrial applications
	Electron-beam irradiation	Continuous change of magnetic field	
Chemical	Alkaline hydrolysis	Dilute NaOH or $Ca(OH)_2$, aided with H_2O_2 60°C for NaOH, 120°C for $Ca(OH)_2$	Sequestration of lignin and hemicellulose into alkali-soluble fraction Reduction of cellulose crystallinity Lower reactor cost than in acid hydrolysis
	Acid hydrolysis with diluted inorganic acids	0.75–5% H_2SO_4, HCl, or HNO_3 120–200°C 1 MPa 2–10min	Lignin is not hydrolyzed pH neutralization is necessary High production of toxic inhibitors
	Acid hydrolysis with concentrated inorganic acids	10-30% H_2SO_4 170–190°C	Equipment corrosion Acid recovery is necessary Environmentally challenging High production of toxic inhibitors
	Organosolv	Organic solvents mixed with water, inorganic salts and inorganic acids >120°C, 20-60min	Removal of lignin and hemicellulose Environmentally challenging
	Ionic liquids	/	Efficient solubilization and removal of lignin Low hemicellulose and cellulose loss High price of ionic liquids
Physicochemical methods	Liquid hot water	170–230°C >5MPa 1-45min	Cellulose depolymerization Partial lignin solubilization High xylose recovery No toxic inhibitors
	Steam explosion (autohydrolysis)	160–300°C 0.69–4.85MPa Several seconds to minutes	High solid loads Formation of inhibitors Lignin is not solubilized High degree of hemicellulose hydrolysis
	Ammonia fiber explosion	1–2kg ammonia/kg dry biomass 90°C 1.12–1.36MPa 30min	Ammonia recovery is required No inhibitors Partial lignin solubilization
Biological methods	Enzymatic delignification	Enzymatic cocktail of laccase, lignin peroxidase and manganese peroxidases from white rot fungi	No inhibitors formed Mild reaction conditions Environmental friendly Long pretreatment time

Source: (Dey et al., 2020; Robak & Balcerek, 2020)

Mechanical comminution, alkaline hydrolysis, steam explosion and liquid hot water method are the most common physical, chemical, or physicochemical methods of lignocellulose biomass pretreatment,

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respectively. The most significant limitation of all pretreatment methods, except biological, is formation of toxic inhibitory byproducts: phenolic compounds, furfural, 5-hydroxymethylfurfural, formic, acetic and levulinic acid. These compounds interfere with microbial growth, inhibit yeast alcohol dehydrogenase, induce cell membrane damage and chromatin destruction, compromising fermentation productivity and bioethanol yield. Hence, it is of utmost importance to detoxify the substrate mixture prior to hydrolysis by membrane filtration, absorption on activated charcoal, solvent extraction or ion-exchange (Dey et al., 2020; Robak & Balcerek, 2020).

Hydrolysis of Polysaccharides

Hydrolysis of cellulose and hemicellulose is otherwise known as saccharification, indicating depolymerization and hydrolysis of polysaccharides into a mixture of corresponding monosaccharides (fermentable sugar). Hydrolysis can be chemical and enzymatic. In chemical hydrolysis polysaccharide mixture is treated with inorganic acids. Diluted acid hydrolysis facilitates step-by-step separation of hemicellulose and cellulose monomers, whereas hydrolysis with concentrated acids mainly yields hemicellulose monomers. Acid hydrolysis is characterized by high saccharification yield (70-100%, depending on the acid concentration). However, environmental concerns, equipment corrosion and glucose decomposition are significant limitations of this method (Dey et al., 2020).

Enzymatic hydrolysis provides selective conversion of lignocellulose polysaccharides in mild reaction conditions with a saccharification yield of 75–85%. Depending on the pretreatment technique, cellulases or mixtures of cellulases and hemicellulase are necessary for complete hydrolysis. Hemicellulases are complex enzyme cocktails that hydrolyze hemicellulose to a mixture of pentoses (xylose, arabinose) and hexoses (galactose, glucose, mannose). Commercial development of hemicellulases is not as advanced as for cellulases, since the predominant substrate for enzymatic hydrolysis is the biomass pretreated with diluted inorganic acids, where hemicellulose is removed before saccharification (Mohanram et al., 2013). Large-scale enzymatic saccharification of lignocellulose polysaccharides is most commonly performed in fed-batch bioreactor, which relies on sequential time-dependent addition of substrates for complete hydrolysis in each step. Most common disadvantages of this method are batch-to-batch oscillations, frequent startup and shutdown problems, high cost of labor and problem of enzyme recovery (Dey et al., 2020).

Thermostable cellulases are ideal biocatalysts for cellulose bioprocessing, because higher temperatures increase the reaction rate, diffusion coefficient, bioavailability and solubility of the substrate, while decreasing viscosity and minimizing the risk of microbial contamination (Srivastava et al., 2015; Trbojević Ivić et al., 2016). Thus far, *Thermoascus aurantiacus*, *Humicola grisea* var *thermoidea*, *Chaetomium thermophilum*, *Geobacillus* sp. HTA426, *Sporotrichum thermophile*, *Thermomonospora curvata*, *Scytalidium thermophilum*, *Clostridium staminisolvens* and *Pyrococcus furiosus* were reported as producers of thermostable cellulases (Ejaz et al., 2021).

Fermentation of Monosaccharides to Bioethanol

Industrial bioethanol fermentation is usually performed with *S. cerevisiae* and bacteria *Zymomonas mobilis* (Robak & Balcerek, 2020). Significant disadvantage of *S. cerevisiae* is its inability to ferment pentoses, therefore, for efficient fermentation of mixed pentose and hexose substrate, a microbial co-culture would

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be preferred (Lamichhane et al., 2021; Verma & Kumar, 2021). Also, with the upgrade of fermentation techniques implementation of thermostable microorganisms can result in better bioethanol yield.

The typical method of bioethanol production in batch fermentation is the Melle-Boinot process. It consists in broth preparation (pH adjustment, 14–22% by mass) and sterilization, followed by yeast fermentation. Fermented broth is centrifuged and bioethanol is separated from supernatant, while the yeast biomass is recycled for the next fermentation. The process can be repeated until the activity and viability of yeast cells is lost after which fresh inoculum is added (Bušić et al., 2018). From this basic set-up, several upgraded technologies emerged, summarized in Table 3.

Table 3. Advancement in bioethanol fermentation techniques

Fermentation Method	Characteristic	Remark
Separate hydrolysis and fermentation	Independent enzymatic hydrolysis and fermentation	Each stage is performed under its optimal conditions Enzyme hydrolysis is unaffected by bioethanol inhibition Surfactants must be added to reduce β-glucosidase inhibition by glucose High cost of separate bioreactor system
Simultaneous saccharification and fermentation	Simultaneous enzymatic hydrolysis and fermentation	Lower production cost Reduced enzyme inhibition by simultaneous end product removal Lower requirement for sterile conditions (bioethanol is produced immediately with glucose conversion) Thermotolerant microbes must be used
Simultaneous saccharification and fermentation with short pre-saccharification	Pre-hydrolysis at 50°C, followed by fermentation	Reduced viscosity of fermentation substrate Higher productivity of fermentation
Simultaneous saccharification and co-fermentation	Simultaneous saccharification and fermentation of pentose/hexose mixture	High bioethanol yield Pentoses and hexoses can be fermented Recombinant thermotolerant microbes, capable of fermenting both monosaccharides are necessary
Consolidated bioprocessing	One-pot production of hydrolytic enzymes, hydrolysis of pretreated biomass and fermentation of all types of soluble sugars	Cost-effective More efficient hydrolysis Reduced risk of microbial contamination Specialized microbes must be used (recombinant *E. coli* or *Fusarium oxysporum*)

Source: (Dadwal et al., 2020; Dey et al., 2020; Hoffman et al., 2021; Lamichhane et al., 2021; Liu et al., 2016).

After fermentation bioethanol is extracted from fermentation broth and purified. Distillation is the conventional method for separation and partial purification of bioethanol from aqueous fermentation broth, based on the difference of the boiling points of bioethanol and water (78.3°C and 100°C, respectively). Continuous distillation column system is conventionally used in large-scale processes (Robak & Balcerek, 2020). Lately, membrane technology has been employed in bioethanol recovery. Pervaporation was used to purify bioethanol from bioethanol-water mixture, but also from more complex lignocellulose broth (Abels et al., 2013; Gaykawad et al., 2013). Membrane extraction of bioethanol from fermentation broth

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was performed with around 80% of the product recovery (Chang et al., 1992). High purity bioethanol (99.7% v/v) is obtained only by dehydration (Robak & Balcerek, 2018).

Factors Influencing Bioethanol Yield

Composition of lignocellulose biomass, enzyme and substrate loading and mixing are the major factors to consider in the hydrolysis step. Lignin and hemicellulose are the main contributors to biomass resistance to hydrolysis, therefore better results could be achieved by the careful selection of raw material with lower percentage of lignin and hemicellulose, such as rice husk, saw dust or pinewood (Preethi et al., 2021). Higher enzyme and solid loadings favor higher saccharification yield. However, high solid loading can also increase viscosity, heat and mass transfer limitations and inhibition by toxic components during fermentation (Dey et al., 2020; Robak & Balcerek, 2020). Inappropriate mixing will negatively affect saccharification yield, either due to insufficient aeration when the mixing is poor, either due to enzyme denaturation by the shearing force. Non-ionic surfactant Tween 80, non-catalytic protein bovine serum albumin or polyethylene glycol prevent non-productive adsorption of cellulase to lignin, with no effect on enzyme catalytic activity and are common additives during hydrolysis and fermentation (Robak & Balcerek, 2020).

Fermentation requires more complex regulation, since it must provide optimum conditions for growth of microbial cell mass and utilization of fermentable sugars. Temperature and pH range of industrial fermentation are adapted to *S. cerevisiae* and have to be optimized for any other microorganism. Higher glucan loading exhibits positive dose-dependence to the bioethanol yield. However, high sugar concentrations induce osmotic pressure on yeast and plasmolysis. Steering speed and reaction time are inversely proportional variables: at higher agitation speed fermentation will be completed in shorter reaction time. Too intense agitation can cause microbial cell disruption. At a low biomass density (optical density at 600 nm=1) the yeast cell viability is compromised by the substrate's inhibitory effect, while at the excessively high biomass density (optical density at 600 nm=8) yeasts consume more glucose, which is converted into the microbial biomass instead of bioethanol (Robak & Balcerek, 2020). Bacterial contamination during fermentation is a common cause of low bioethanol yield. Antibiotics are traditionally used to control contamination; however, they can be detected in the final product. Alternatively, microbial contamination can be prevented by using antimicrobial natural products, lysozyme and bacteriophages and by the proper selection of fermentation method (*i. e.* simultaneous saccharification and fermentation, Table 3) (Lamichhane et al., 2021; Verma & Kumar, 2021).

Techno-economic Analysis

Bioethanol market is segmented by feedstock type, application, and geography. In 2020 approximately 100 billion liters of bioethanol were produced globally, with projected compound annual growth rate (CAGR) >4.5% between 2021 and 2026 (Mordor Intelligence, 2016).

Bioethanol Market by Feedstock Type

Majority of global bioethanol production arises from food crops: maize (60% share, main producer USA), sugarcane (25% share, main producer Brazil), molasses (7% share, main producer India), cereals and sugar beet (4%, main producer the European Union) (Flach et al., 2019; Ibeto et al., 2011). Global

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production of lignocellulose-derived second-generation bioethanol is 400 million liters per year, but is expected to increase in the future (Mizik et al., 2020). Significant step forward is the opening of processing plants for production of bioethanol from bagasse, rice straw, wood chips, corn stover and straw in Finland, Italy, Australia and Serbia, respectively (Dey et al., 2020; Martinov et al., 2015).

The price of feedstock represents 40-75% of the total bioethanol production costs. According to Devi, only 3% of global annual production of lignocellulose biomass accounts for all of its derivatives (bioethanol, bio-based chemicals, non-food products) (Devi et al., 2022). Paradoxically, food crops are more cost-effective feedstock than lignocellulose biomass (US$0.2 - US$0.3/L for bioethanol from sugar cane, compared to US$0.8 – US$1.2/L for bioethanol from lignocellulose material) (Bušić et al., 2018). The rationale behind this is the non-uniform composition of the lignocellulose biomass components, depending on the source. Biomass with higher lignin content requires additional costs of pretreatment for lignin removal, than biomass in which cellulose and hemicellulose are more abundant (Preethi et al., 2021; Verma & Kumar, 2021).

Bioethanol Market by Application

Approximately 80% of globally produced bioethanol is used as transportation fuel, followed by the food, pharmaceutical, and cosmetics sector (Mizik et al., 2020). As a transportation fuel, bioethanol is mixed with gasoline at volume fractions 5, 10 or 85% of bioethanol (fuel codes E5-85). Only E5 and E10 can be directly used in the existing motors without modifications, hence they represent the largest share in the market (Bušić et al., 2018). Recently, efforts are also being made to implement bioethanol as bio-jet fuel in aviation transport (Han et al., 2019).

Bioethanol Market by Geography

The leading global producers of bioethanol are USA, Brazil, the European Union, China, India and Canada (Bušić et al., 2018; Mordor Intelligence, 2016). In Europe, Sweden, Germany, the United Kingdom, France and Italy have primacy in bioethanol production. Collectively, European countries produce annually more than 2 billion liters of bioethanol, while in the USA production capacity is 66 billion liters bioethanol/year (Robak & Balcerek, 2018). The USA and Brazil are the leading global consumers of bioethanol, whereas the top three European bioethanol consumers are Germany, the United Kingdom and France (Flach et al., 2019). The Asia-Pacific region is considered to be the fastest-growing market, with China and Japan as regional leaders.

Impact of COVID-19 on Global Bioethanol Market

The COVID-19 pandemic has affected the bioethanol economy dramatically. From April-June 2020 global consumption and bioethanol price decreased by 40%, or US$3.4 billion (Renewable Fuels Association, 2021). The USA was most seriously affected, with US$2.9 billion losses in the bioethanol sector. Pandemics had no measurable impact on the European bioethanol market (Mizik et al., 2020). This interesting observation can be explained by the regional differences in feedstock for bioethanol production: USA relies predominantly on corn, while in Europe cereals and sugar beet are the dominant raw material. Reduction in biofuels demand has also sharply increased market prices of bioethanol value-added by-products as a result of a cutback in supplies (Renewable Fuels Association, 2021). The negative effect

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of the pandemics is even more pronounced in the middle-income countries where agriculture is a more significant contributor to the GDP, however the long-term effect of the pandemic will be comprehended only after the COVID-19 crisis passes (Shruthi & Ramani, 2021).

Co-relation of Global Cellulase Market and Global Bioethanol Market

It is estimated that the global biofuel enzymes market will reach US$2.0 billion by 2026. Cellulase market follows the similar pattern as bioethanol market. Around 40–49% of the total cost of bioethanol production is attributed to cellulase. Novozymes and Genecor, market leaders in the production of industrial enzymes, have contributed to the significant decrease in price of industrial cellulases (Srivastava et al., 2015). Nevertheless, to improve bioethanol production economy, further decrease of the price is necessary to the projected value of US$0.026/L (Acharya & Chaudhary, 2012). Cost-reduction can also be achieved by the careful choice of the fermentation method. By switching from separate to simultaneous saccharification and fermentation (Table 3) costs could be reduced from US$1.2/L to US$0.6/L (Dey et al., 2020).

SOLUTIONS AND RECOMMENDATIONS

Solutions and perspectives for the issues presented in the previous section will be considered in this section from the following aspects:

- Increasing the economic value of lignocellulose biomass
- Tailoring of cellulases for efficient production of bioethanol.

Increasing the Economic Value of Lignocellulose Biomass

As discussed previously, the price of lignocellulose raw material contributes most significantly to the overall price of bioethanol. These expenses can be compensated by production of other valuable by-products. Accordingly, subject of great attention in recent decades is design and development of biorefineries for integrated production of bioethanol and other valuable products from cellulose, hemicellulose and lignin, as a base of circular bioeconomy. Other than bioethanol, cellulose is a source of sorbitol, levulinic and succinic acid, additives in food and pharmaceutical industry. Lactic acid, xylitol, xylo-oligosaccharides, furfural, hydroxymethylfurfural and polyhydroxyalkanoates, obtained from hemicellulose, are used in pharmaceutical, chemical and food industry. Furfural and hydroxymethylfurfural are precursors of pesticides and fertilizers. As mentioned previously in this chapter, these two compounds are inhibitors of cellulose hydrolysis. Therefore, their sequestration represents a double gain. Lignin can be used directly as fuel to boilers for pulp production, but it is even more important as a source of lignol and vanillyl alcohol (Devi et al., 2022). Vanillyl alcohol exhibits antioxidant and antimicrobial activity, but also can be used as a flavoring agent in food and cosmetic industry. In a recent study it was used for synthesis of capsinoids, small molecules with wide array of physiological activity (Trbojević-Ivić et al., 2017).

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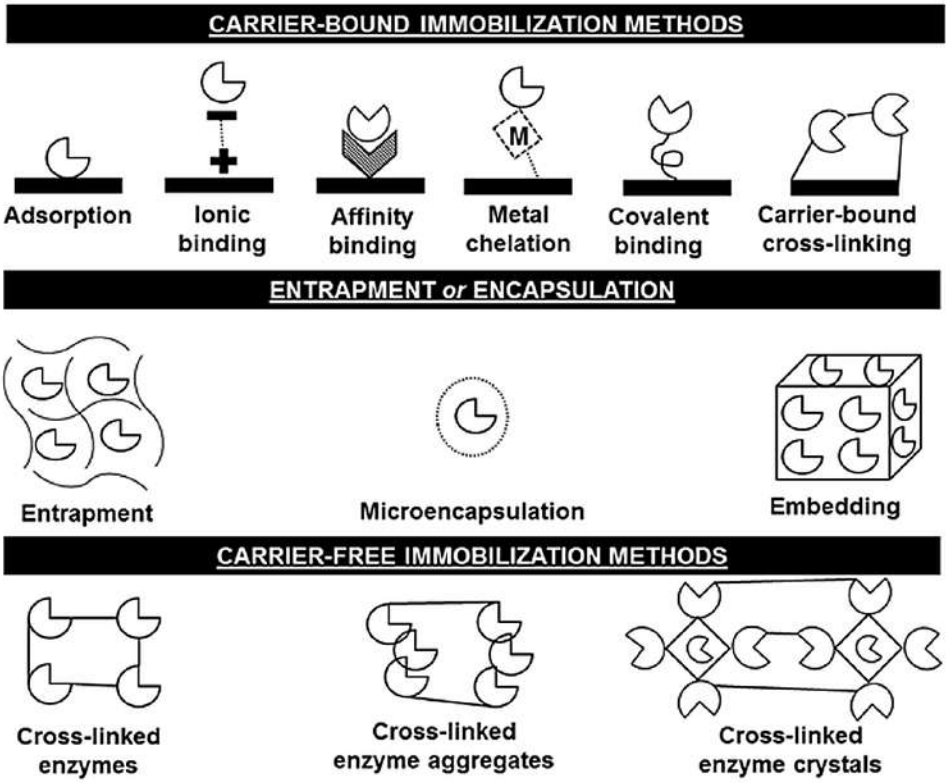
Tailoring of Cellulases for Efficient Production of Bioethanol

Biorefinery process requires biocatalysts with high catalytic efficiency on insoluble substrate, stable at higher temperature and pH, and resistant to end-product inhibition (Mohanram et al., 2013). This section will highlight how these requirements can be met by enzyme immobilization.

Fundamental Aspects of Enzyme Immobilization

Enzyme immobilization is sequestration of enzymes in a limited, well-defined space with preservation of its catalytic activity. This is unique technique of biocatalyst tailoring that combines simultaneous stabilization, reusability and easy separation of the enzyme from the reaction mixture. Additionally, multiple enzymes with different catalytic activities can be simultaneously immobilized into unique biocatalyst machinery for one-pot biotransformation. An overview of enzyme immobilization methods is represented at Figure 4.

Figure 4. Techniques of enzyme immobilization
Source: (Datta et al., 2013; Sheldon, 2007)



Carrier-bound techniques and entrapment have the most practical significance. In physical adsorption, ionic and affinity binding and metal chelation enzymes are reversibly bound to the carrier *via* weak non-

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covalent bonds. Enzyme immobilization carriers can be of different origin, chemical structure, particle morphology and size. Natural immobilization carriers, polysaccharides and proteins, are biocompatible and non-toxic, which is relevant for medical applications, pharmaceutical or food industry (Trbojević-Ivić et al., 2018). Eupergit-C, Sepabeads-EC, Amberlit and Purolite A109 are frequently used synthetic polymers for enzyme immobilization (Mihailović et al., 2016; Milosavić et al., 2012; Trbojević-Ivić et al., 2018; Žuža et al., 2009). Among inorganic materials, silica-gel, metal oxides and ceramics are most common. Recently, immobilization of lipase from *Candida rugosa* on hydroxyapatite *via* metal chelation resulted in an improved biocatalyst with interesting novel applications (Trbojević-Ivić et al., 2017; Trbojevic Ivic et al., 2016). Entrapment is an irreversible immobilization method, in which an enzyme is confined inside the carrier matrix but the two entities do not interact chemically. Alginate gels are the most popular matrixes for entrapment (Dimitrijević et al., 2011). In microencapsulation concentrated enzyme solution is confined in micelles or reverse micelles, which enables better catalytic efficiency (Trbojević-Ivić et al., 2018).

Immobilization efficiency is governed by the immobilization method (Figure 4) and immobilization carrier characteristics. In covalent immobilization, enzymes are irreversibly chemically attached to the carrier so enzyme leakage is minimized. However, problems arise if enzymes are bound *via* an active site. Physical adsorption is a popular technique, because high immobilization yield is achieved in a short time. However, a significant limitation of this method is enzyme leakage. In encapsulation and entrapment enzyme leakage is minimum, however these methods are unsuitable for enzymes processing polymeric substrates, such as cellulases.

Nanomaterials in Enzyme Immobilization

Nanomaterials are next generation of immobilization carriers with high surface area, distinctive morphology, architecture and porosity (Altinkaynak et al., 2016). Enzyme is bound covalently or non-covalently to silica-based, polymer-based, metal-based or carbon-based nanomaterials, forming nanobiocatalyst system. Magnetic nanoparticles are most common in practice. They consist of magnetic core (iron, nickel, cobalt), which enables easy separation in magnetic field, while chemical coating provides functional groups for enzyme immobilization and additional functionalization. Polymer-based nanomaterials can be easily produced at high quantities and have plethora of functional groups for immobilization and modification. However, their mechanical stability is low. Carbon-based nanomaterials are suitable for physical adsorption, but can also be used for covalent immobilization after additional functionalization (Misson et al., 2015).

Cellulase Nanobiocatalyst Systems

Nanomaterials have become an essential tool in tailoring cellulase activity for production of bioethanol. Strategies to immobilize particular cellulase or mixture of several cellulolytic enzymes are described in the literature (Husain, 2017; Perwez et al., 2017; Zanuso et al., 2022). Among individual cellulases, the most common are those from *T. reesei*, *A. niger*, *A. fumigates*, *A. nidulans* or *T. viride* and commercial cellulases Cellusoft CR, Cellic CTec2 (Husain, 2017; Jabasingh, 2011; Lupoi & Smith, 2011; Simon et al., 2018). In multi-enzyme preparations, endoglucanase is co-immobilized with β-glucosidase, cellobiohydrolase and xylanase, while β-glucosidase can also be co-immobilized with glucose oxidase or cellobiohydrolase. Silica-based, magnetic and metal-oxide nanoparticles are the most common carri-

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ers for immobilization of cellulases. Functionalization of these materials with polymers, polyalcohols, glutaraldehyde, carboxyl and amino groups enables efficient covalent binding or physical adsorption. Nanomaterials with small pores and encapsulation are unsuitable for cellulase immobilization, due to significant diffusional limitations of bulk substrate, such as pretreated biomass (Husain, 2017).

Efficiency of the applied immobilization protocol is determined through immobilization yield, temperature and pH stability and optima and reusability. In most cases, regardless of the method and support choice, immobilization yield was between 60–95% (Husain, 2017; Jabasingh, 2011; Manasa et al., 2017; Sillu & Agnihotri, 2020; Simon et al., 2018). Immobilization commonly resulted in the shift of the optimum pH and temperature to higher values and improved temperature stability (Lupoi & Smith, 2011; Manasa et al., 2017; Sillu & Agnihotri, 2020). The most prominent feature of immobilized cellulase is reusability: immobilizate could be used in more than ten consecutive cycles of carboxymethyl cellulose hydrolysis without significant loss of activity (Husain, 2017; Jabasingh, 2011; Lupoi & Smith, 2011; Manasa et al., 2017; Zanuso et al., 2022). Storage stability of cellulase was also significantly improved at 4^{o}C upon immobilization (Simon et al., 2018; Zanuso et al., 2022).

Industrial potential of immobilized cellulase was assessed by hydrolysis of lignocellulose biomass after different pretreatments. *T. reesei* cellulase covalently immobilized on zinc ferrite nanoparticles effectively hydrolyzed sonified and alkali-pretreated sun hemp biomass, with hydrolysis yield of 53% (Manasa et al., 2017). Cellic CTec2 cocktail, immobilized on chitosan-coated magnetic nanoparticles, hydrolyzed hydrothermally pretreated corn cob with hydrolysis yield of 65% (Zanuso et al., 2022). Cellulase-halloysite-metal-oxide nanobiocomposites hydrolyzed bagasse extracted with ionic liquids with 50.2% hydrolysis yield (Sillu & Agnihotri, 2020), while cellulase from *T. viride*, physically adsorbed on silica nanoparticles, was successfully implemented in simulation of simultaneous saccharification and fermentation of bioethanol from microcrystalline cellulose (Lupoi & Smith, 2011).

FUTURE RESEARCH DIRECTIONS

Bioethanol is technologically, economically and environmentally viable alternative to fossil fuels. However, major issue is the production of bioethanol at a competitive price. Efficient selection and pretreatment of raw material, together with circular bioeconomy concept will reduce the total cost of lignocellulose bioethanol production by co-generating other valuable products. However, a good biorefinery is the one that leaves minimum or no waste during a process. Therefore, zero-waste biorefinery needs the skills and proficiency from different interdisciplinary fields such as chemistry, biochemistry, chemical engineering, biology, biomolecular engineering and materials sciences. Genetic modification of microbial producers of cellulase and biocatalyst tailoring are aimed at reducing biocatalyst price by increasing its catalytic efficiency and stability for industrial reaction conditions. Immobilization provides an endless field of possibilities to improve cost-effectiveness of cellulases in bioconversion of lignocellulose biomass to second-generation bioethanol. Plethora of well-established immobilization protocols, innovative immobilization carriers and development of nanotechnology will lead to design of reusable nanobiocatalysts of high stability and activity for large-scale processes. It is believed that these solutions combined will significantly contribute to price competitiveness of bioethanol on the global fuel market.

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CONCLUSION

Modern society must find a long-lasting solution to depletion and environmental concerns caused by extensive use of fossil fuels. Lignocellulose biomass is the most abundant raw material in the biosphere, giving rise to second-generation bioethanol, the most promising alternative to fossil fuels. Bioconversion of lignocellulose to bioethanol is a complex process of selection and pretreatment of biomass, hydrolysis of cellulose, fermentation and purification of bioethanol. Hydrolysis of cellulose is the key phase of the process and is governed by synergistic activity of cellulase multi-enzyme complex. Mesophilic and thermophilic fungi and bacteria are the main industrial producers of cellulases. Price of raw material and cellulase affect the overall cost of bioethanol production from lignocellulose biomass and need to be reduced to make bioethanol more concurrent on the global fuel market. Reducing cellulase price is considered crucial to increase bioethanol market competitiveness. Important feature, which contributes to cost-effectiveness is biocatalyst recycling or reusability. This can be achieved only by enzyme immobilization. Immobilization of enzymes is a well-known and well-established strategy with a myriad of elaborate protocols and immobilization methods available to choose from. By proper selection of the method and carrier a superior catalyst can be designed with high stability and activity, which can be reused many times without significant activity loss and can be easily separated from the product. Recently, nanomaterials are considered as innovative immobilization carriers with unique characteristics and a whole new field of research and application of nanobiocatalysts has emerged. Silica, magnetic and metal-oxide nanoparticles are the most common carriers for cellulase covalent and non-covalent immobilization. Upon immobilization, temperature stability and reusability of cellulases were significantly improved, which resulted in successful pilot-scale implementation of immobilized cellulases in hydrolysis of biomass with glucose yield above 50%. Issues addressed in this chapter can provide a good starting point to research and applied sectors in devising a production strategy for more efficient and cost-effective production of cellulosic biofuels.

ACKNOWLEDGMENT

This research was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia [Contract number: 451-03-68/2022-14/200288].

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KEY TERMS AND DEFINITIONS

Cellulase: A multi-enzyme complex, consisting of endoglucanase, exoglucanase, and β-glucosidase, which act synergistically for efficient hydrolysis of cellulose to glucose.

Enzyme: A protein of unique structure, which accelerates chemical reactions in biological systems with high substrate specificity. Also referred to as biocatalyst.

Enzyme Immobilization: Confinement of enzyme in a limited, well-defined space, with preserved catalytic activity, resulting in enzyme stabilization, activation, and easy continuous use.

Enzyme Reusability: Continuous use of enzyme in more than one reaction cycle without significant loss in activity. The most significant parameter of efficiency of the enzyme immobilization method.

Fermentable Sugar: Mixture of monosaccharides, obtained after hydrolysis of carbohydrate polymers in lignocellulose biomass. They are converted to bioethanol by metabolic machinery of specialized microorganisms in well-defined, tightly controlled conditions.

Lignocellulose Biomass: A complex material, composed of lignin, cellulose, and hemicellulose, which strengthens and protects plant cells from pathogens and mechanical damage. The most abundant and renewable raw material for production of bioethanol.

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Nanobiocatalyst: An assembly of enzyme immobilized on nanomaterial with high catalytic efficiency, operational and storage stability, which can be easily removed from the reaction mixture and reused in the next reaction cycle.

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Chapter 15
Comparative Studies on Neem and Jatropha Oil-Derived Biodiesels

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ABSTRACT

Sustainable development is a key aspect of modern-day development. Reducing fuel cost and emissions is considered as the most important research area in sustainable development. Biodiesel has the potential to reduce the requirement of petroleum diesel if blended in suitable proportion. Various feedstocks are being explored for biodiesel production. Oils derived from neem and jatropha can be utilized for biodiesel production. In the current investigation, optimum parameters for the production, namely catalyst proportion, temperature, and oil-to-alcohol ratio were optimized. Experiments were carried out at different blend proportions to study fuel properties, namely kinematic viscosity, aniline point, diesel index, flash and fire points, specific and API gravity, cetane number, and ASTM distillation characteristics.

INTRODUCTION

Sustainable development is key aspect of modern-day development. Reducing fuel cost and emissions is considered as the most important research area in sustainable development. Most commonly fuels used across the world for vehicles, railways, boilers and majority of industrial applications are derived from fossil fuel. These fossil fuels are non-renewable. Alternative fuel sources are being explored such as wind and solar energy, hydrogen fuel, tidal energy and biodiesel from renewable feedstocks like

DOI: 10.4018/978-1-6684-5269-1.ch015

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vegetable oil, cellulose, fats and chitosan. Viability of biodiesel synthesis depends on raw material and biodiesel properties. Waste food, vegetables, nonedible crops are suitable raw materials for biodiesel synthesis. Advantages of biodiesel include reduced toxicity, improved emission, rapid degradation and high flash point (Romano and Sorichetti, 2011). Predojevic et al. utilized Linoleic and oleic sunflower oils for biodiesel by (Predojevic et al.,2012). They used calcium oxide as solid base catalyst (Predojevic et al.,2012). Low-cost feed stocks are important for economic viability of biodiesel synthesis. Waste minimization through energy synthesis can serve the sustainable development goal (Ray et al., 2013; Stephane Bungener, 2016). Selection of appropriate catalyst is also very crucial aspect. Use of strong basic catalyst such as zeolite is being explored as promising method for transesterification (Ejikeme et al., 2010). Sodium methoxide and sodium hydroxide found to have good catalytic properties(Foon et al., 2004). Use of waste cooking oil for biodiesel can serve twin purpose, one of disposal and other of the raw material (Raqeeb and Bhargavi, 2015).

The fatty acid alkyl esters have characteristics very similar to diesel derived from fossil fuel (European Tech. and Innovation Platform, 2022). Acid or base catalysts can be used for the transesterification (Patel and Shah, 2015). The functional group of the catalyst attacks the carbonyl carbon. Catalytic and non-catalytic pathways are available for transesterification (Patel and Shah, 2015). Transesterification temperature depends on the raw material (Mekala et al., 2014). High temperature favors reaction but need to be controlled to avoid denaturation.

Neem is a tree in a mahogany family found in abundance in India (Elkadi et al., 2013). It has pesticidal and insecticidal application. Neem seeds find application in remedial therapy. Neem oil is extracted from the fruits and seeds of the neem. This neem oil is being explored for its use as a feedstock for synthesis as the alkyl ester by transesterification process. The composition of the oil depends on processing method. The neem oil yield that can be obtained from neem seed kernels varies widely from 25% to 45% (Anya et al.,2013; Sathya and Manivannan, 2013). Jatropha grows widespread in world in the tropical and subtropical areas. Jatropha oil finds applications in soapmaking. *Jatropha curcas* oil plays important role in cosmetic industry (Pandey et al., 2012). For transesterification, homogeneous catalysts such as NaOH, KOH can be employed (De Oliveira et al., 2009; Wang et al., 2006, Berchaman et al., 2010). The two-step method with an acid and basic-catalysed reactions can be used to produce biodiesel from oil containing high amount of free fatty acid (FFA) such as Jatropha oil (Choudhury, 2013; Ngo et al., 2008). Before the investigations, parameters for the production, namely catalyst proportion, temperature, oil to alcohol ratio were optimized. Experiments were carried out at these optimized parameters with different blend proportions to study fuel properties namely kinematic viscosity, aniline point, diesel index, flash and fire points, specific and API gravity, cetane number and ASTM distillation characteristics.

METHODOLOGY

Oil Extraction

Neem Oil

Transesterification by alkali catalyst is one of the most effective methods (Ibrahim et al., 2021.; Ramasubramanian *et al.,* 2017). Hexane was used as solvent and Soxhlet apparatus was used for extraction. 200 ml of hexane and neem powder (70 gm thimble) was taken in Whitman paper. Round bottom flask

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and condenser were installed in Soxhlet apparatus. The solvent was heated to maintain the temperature of 65-70 ^{0}C. As the liquid boils, the vapors are condensed. The condensate of n-hexane falls down and wets the thimble. n-hexane extracts the neem oil. The mixture n-hexane and oil is collected in the same round bottom flask. Four cycles were repeated for extracting more neem oil. The oil was separated from the solvent by using distillation. This oil was used for the transesterification process. Oil was heated in a three neck round bottom flask to 110þC for removal of water and then cooled. 0.5 gm of KOH and 300 gm of methanol were added to the oil. The mixture was stirred to blend with KOH. The oil was warmed to 60þC & mixed with potassium methoxide. The reaction was carried out for 5 hours at temperature 67þC. The resultant mixture changed to light green shade. The fishy scent indicated formation of ester and glycerol layers. Traces of glycerol, unreacted methanol and impurities were removed by adding 200 ml of warm water. Finally, a clear light biodiesel was obtained. Neem oil biodiesel blends with diesel were prepared. The blends were analyzed for specific gravity, density, kinematic viscosity, aniline point, pour point, flash point and cetane number.

Jatropha Oil

Pellets of potassium hydroxide were taken in a measuring cylinder and methanol was added in required proportion. Proper blending of oil with the methanol was ensured by shaking, warming and stirring. Blending and warming was continued for 2 hrs at temperature of 64 ^{0}C. The mixture changes to a turbid orange tan color in 5-10 minutes. Then it changed to a reasonably transparent dark shade. Sweet smell indicated the finishing stage. Blending and warming was continued for another 60 minutes. Two distinct phases are formed once blending and warming is halted. In the ester phase, likely impurities are glycerol, unreacted methanol and traces of soap. These impurities are removed by adding 100 ml of warm water to the ester. Then it was passed through the calcium hydroxide pellet for removing moisture content properly. The other phase was glycerol as a byproduct. The jatropha ester was blended with petroleum diesel fuel in several proportions and the analysis was carried out. The blends were analyzed for acid value, specific gravity, density, kinematic viscosity, aniline point, pour point, flash point and cetane number.

As indicated in table 1, optimum parameters obtained from optimization experiments were used to carry out experiments for studying properties of oil derived biodiesels and their different blends.

Table 1. Optimum parameters

Parameter/Factor	Parameter Value
Neem oil	
process temperature	67^{o}C
Catalyst concentration	0.5 gm
Oil: methanol ratio	1:6.5
Jatropha oil	
process temperature	64^{o}C
Catalyst concentration	0.35 gm
Oil: methanol ratio	1:5.5

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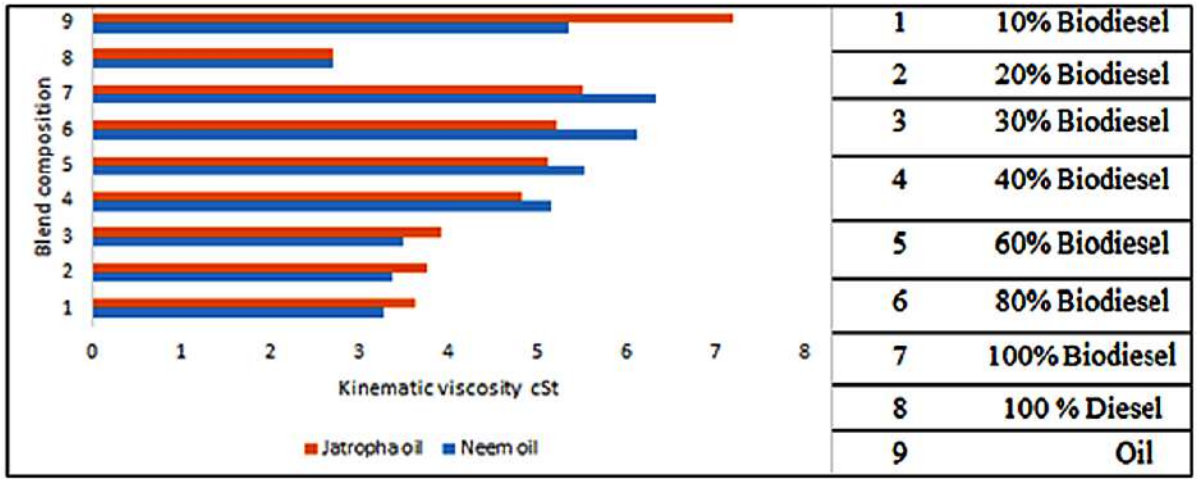
RESULTS AND DISCUSSION

Calorific values for neem oil biodiesel and jatropha oil biodiesels were 7439.91 kcal/kg and 9121.8 kcal/kg respectively. Blends of these biodiesels with petroleum diesel can be used with improved calorific values. The diesel fuel has calorific value 10800 kcal/kg. The BIS specified range is 9900-10,500 kcal/kg. Other fuel properties are discussed in following sections or biodiesels and their blends.

Kinematic Viscosity

The kinematic viscosity at 40 ^{0}C for high-speed diesel must 2.5 to 7.5 cSt (centistokes) as per the standard specification of BIS. For pure neem oil, kinematic viscosity was found to be 5.34 cSt, well within the permissible range. Pure diesel has kinematic viscosity 2.75 cSt while neem oil biodiesel, 6.32 cSt. The viscosity of 10%, 20%, 30%, 40%, 60%, 80% blends of methyl ester of pure neem with diesel are 3.28, 3.36, 3.48, 4.53, 5.15, and 6.12 cSt respectively(Figure1). Viscosity increased with increase in percentage of biodiesel in the blend. For pure biodiesel, viscosity was within the range, but high. To avoid improper ignition, lower blends (20%, 30%) having the kinematic viscosities bellow 4 cSt can be used. The kinematic viscosity of the jatropha oil methyl ester was 5.5 cSt, higher compared to diesel(2.7 cSt). The viscosities were observed to be 3.63, 3.76, 3.91, 4.81, 5.11 and 5.22cSt for 10%, 20%, 30%, 40%, 60% and 80% blends respectively (figure 1). First three blends had suitable viscosity for fuel application. It can be observed from the figure that pure biodiesel from neem oils had higher viscosity. Need oil biodiesel blends had lesser viscosity than corresponding jatropha oil biodiesel for 10, 20 and 30% biodiesel blends. For higher blends, jatropha oil biodiesel had lesser viscosity than neem oil biodiesel.

Figure 1. Kinematic viscosity for different blend compositions



Aniline Point

High aniline point indicates that the fuel is highly paraffinic and has high diesel index indicating good ignition quality. High speed diesel has an aniline point of 68 ^{0}C whereas 100% biodiesel has an aniline

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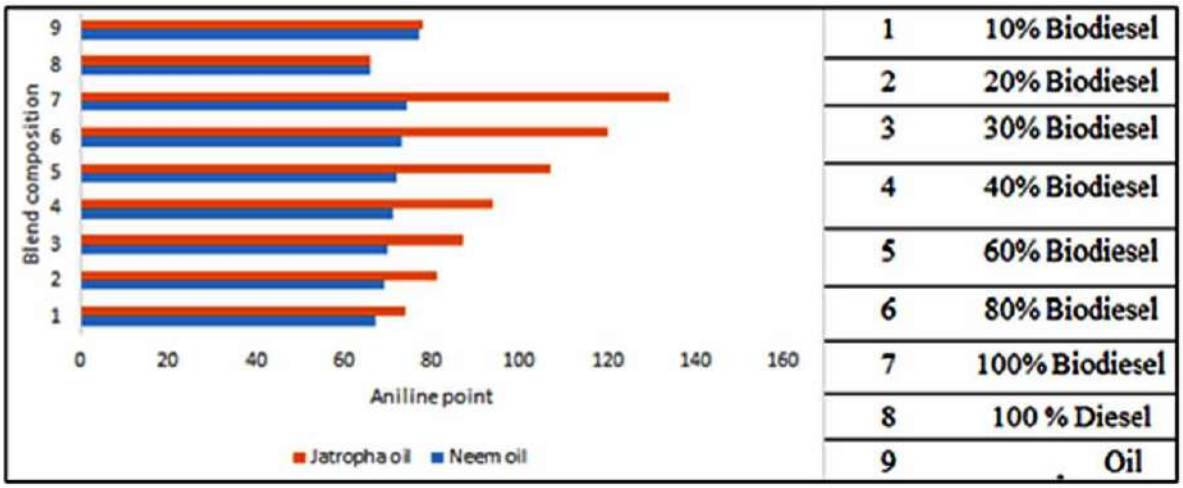
point of 77 ^{0}C. Blends of neem oil methyl ester and high speed diesel have a higher aniline point when compared to high speed diesel. It can be seen from Figure 2 that the aniline point increases with biodiesel percentage in the blend. Thus the blending is advantagious as per aniline point characterization.

In case of jatropha oil derived biodiesel, 100% biodiesel had high aniline point (134 ^{0}C). The blends of jatropha methyl ester and its blends had higher aniline point compared to high speed diesel. Aniline points for various blends have been shown in figure 2. Larger the aniline point, larger the diesel index, which leads to superior quality of ignition in diesel engine. When the fuel is rich in aromatic content, it has low values of aniline point and the ignition is poor. Jatropha oil derived biodiesel is better alternative from ignition point of view.

Figure 2. Aniline point for different blend compositions



Diesel Index

The BIS specified value of diesel index is 45. The high speed diesel has a diesel index of 57.14. The diesel index of the neem oil is approximately 37.75 which is far below the prescribed limit of BIS specification. Consequently utilization of pure neem oil as a fuel will bring about an extremely poor ignition quality. A diesel index of 51.72 is obtained by using 100% methyl ester of neem oil, above the BIS specified of 45. By blending different amount of diesel and methyl ester from neem oil, diesel indices were found to decrease slightly. The diesel index for blends containing 10%, 20%, 30%, 40%, 60% and 80% biodiesel were 53.65, 57.40, 56.80, 56.50, 55.86 and 55.53 respectively, well above the specified value,45.

Pure jatropha biodiesel diesel index is approximately 81.03. For the different blends of 10%, 20%, 30%, 40%, 60% and 80% of methyl ester, the obtained diesel index values are above specified value and hence had better ignition property(figure3). Jatropha oil biodiesel was better choice than neem oil biodiesel considering diesel index.

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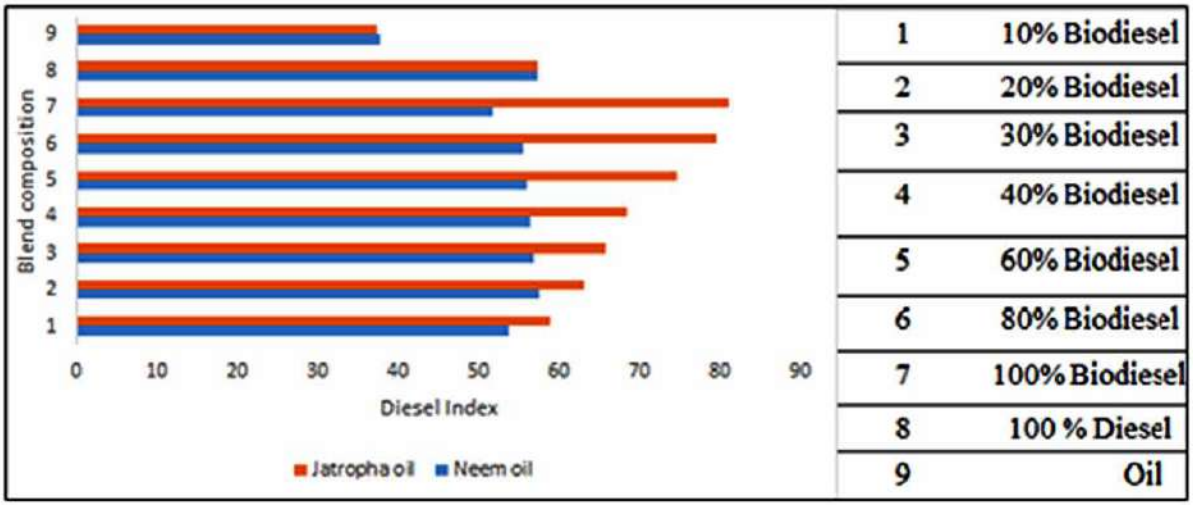
Figure 3. Diesel index for different blend compositions



Flash and Fire Point

The flash and fire points indicate possible fire hazards during transportation, handling and storage of diesel fuel. For the diesel fuel, the flash and fire points was 68 ^{0}C and 71^{0}C respectively. For 100% neem oil methyl ester, these value were 181^{0}C & 184^{0}C.As the blend percentage increases, the flash and fire point also increases. For 10, 20, 30, 40, 60 & 80%, the values of flash and fire point was obtained as 79 & 82, 89 & 92, 105 & 108, 126 & 128, 142 & 146, 162 & 165^{0}C respectively. The blending of biodiesel in petroleum diesel improves the flash point properties which is needed for better performance of fuel in terms of storage, handling and transportation.

Flash and fire points of jatropha biodiesel was 153 & 156 ^{0}C respectively. Increase in flash and fire point temperature is observed as jatropha biodiesel concentration in diesel blend increases. Flash and fire points for 10%, 20%, 30%, 40%, 60% and 80% blends were 81 ^{0}C & 84 ^{0}C, 87 ^{0}C & 89 ^{0}C, 92 ^{0}C & 95 ^{0}C, 98 ^{0}C & 101 ^{0}C, 118 ^{0}C & 120 ^{0}C, 130 ^{0}C & 133 ^{0}C respectively. The flash point study suggests that use of jatropha oil methyl ester in diesel will improve fuel efficiency. Jatropha oil biodiesel had lower flash and fire points compared to corresponding neem oil biodiesel blends. Figure 4 and 5 depicts flash and fire points for biodiesel derived from two oils.

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Figure 4. Flash point for different blend compositions

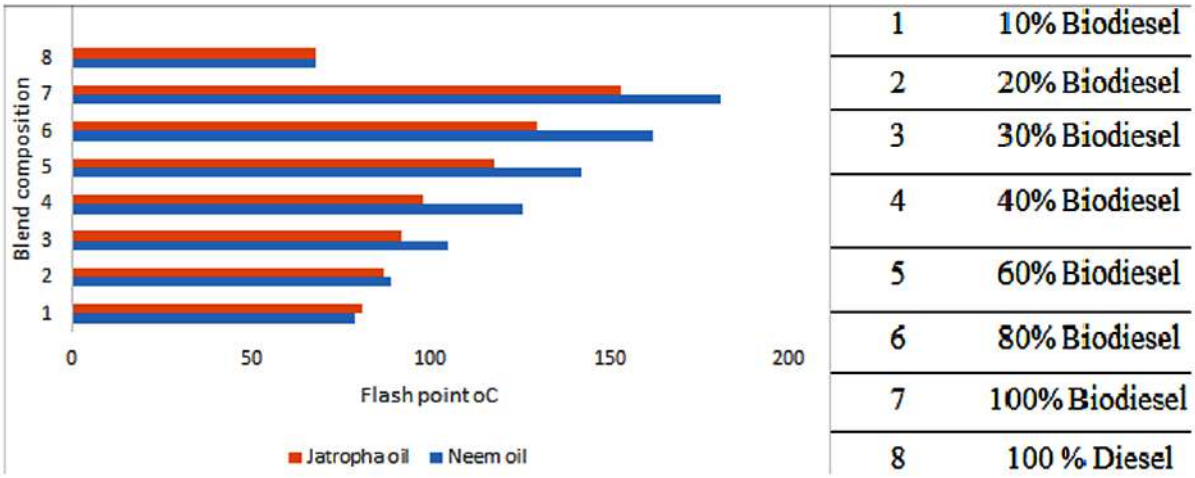
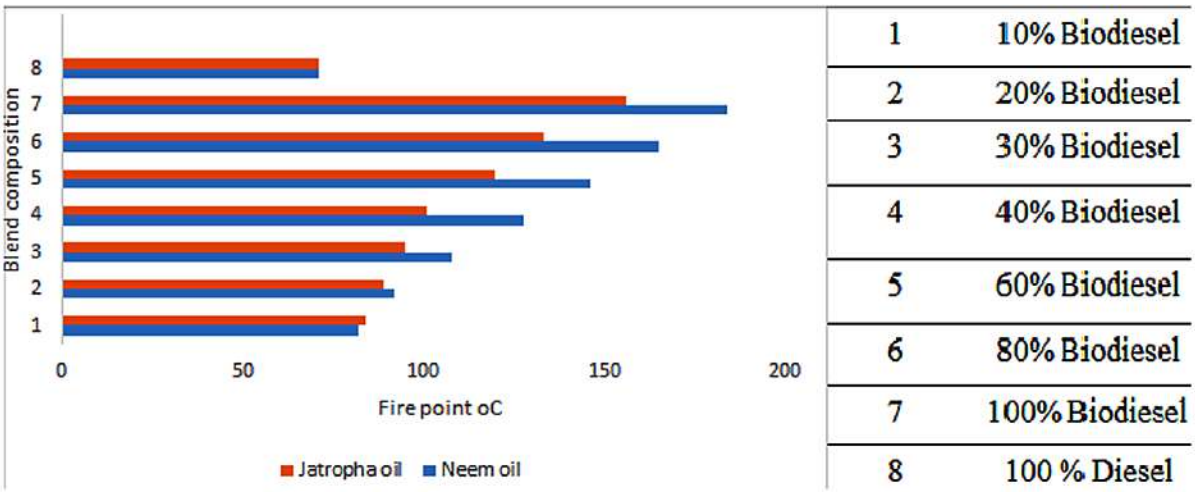


Figure 5. Fire point for different blend compositions



Specific and API Gravity

The specific gravity of the diesel was noted 0.835. Its value for 100% jatropha oil methyl ester was 0.89 and that for neem oil biodiesel was 0.87. The specific gravity values increased marginally with the blends proportion. Values for 10, 20, 30, 40, 60 and 80% biodiesel blend in diesel were found to be 0.838, 0.842, 0.846, 0.855, 0.862, 0.872 and 0.839,0.841,0.845,0.848,0.852,0.855 respectively for jatropha and neem oil biodiesel(figure 6 and 7). The specific gravity values are used for calculating API gravity. High specific gravity indicates high diesel index.The API gravity and aniline point values are useful for calculating diesel index by well-known relation

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Diesel Index=(0.018×Aniline point °C+0.32)×API gravity

For neem oil, The API gravity pure biodiesel was found 37.96 and 31.14.Here the reduction of API gravity with blend proportion rise were observed. Jatropha oil biodiesel was better option considering this property.

Figure 6. Specific gravity for different blend compositions

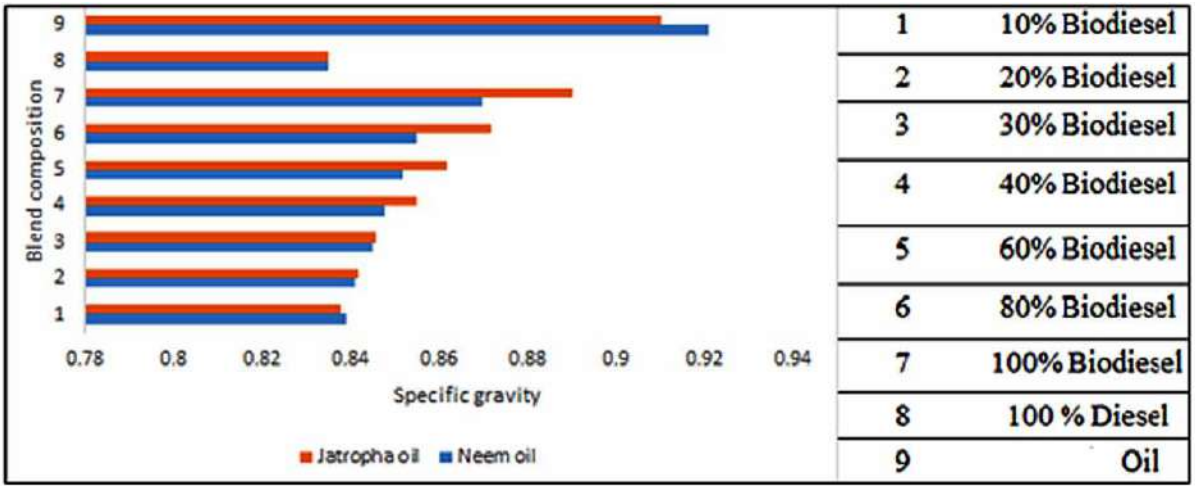
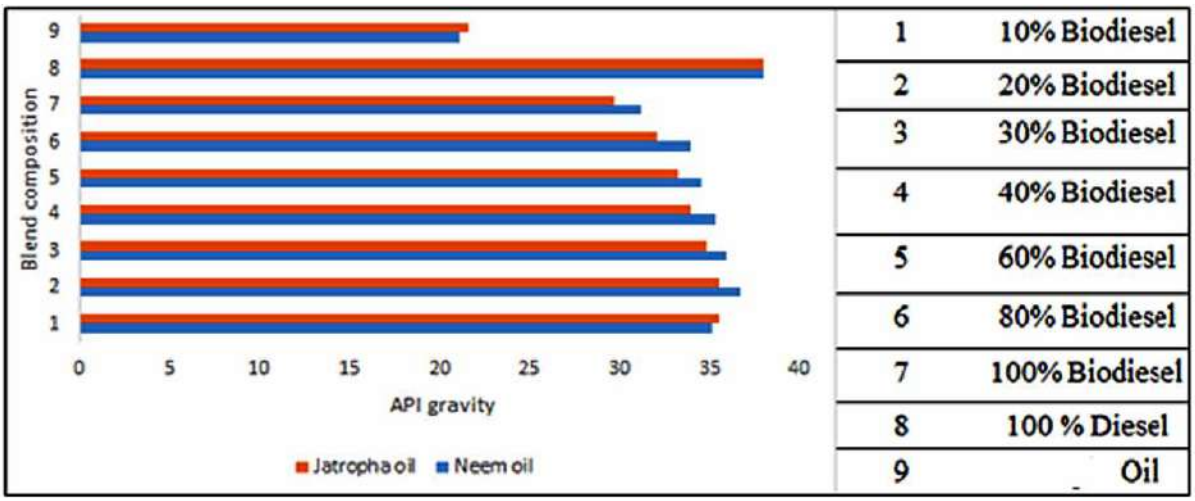


Figure 7. API gravity for different blend compositions



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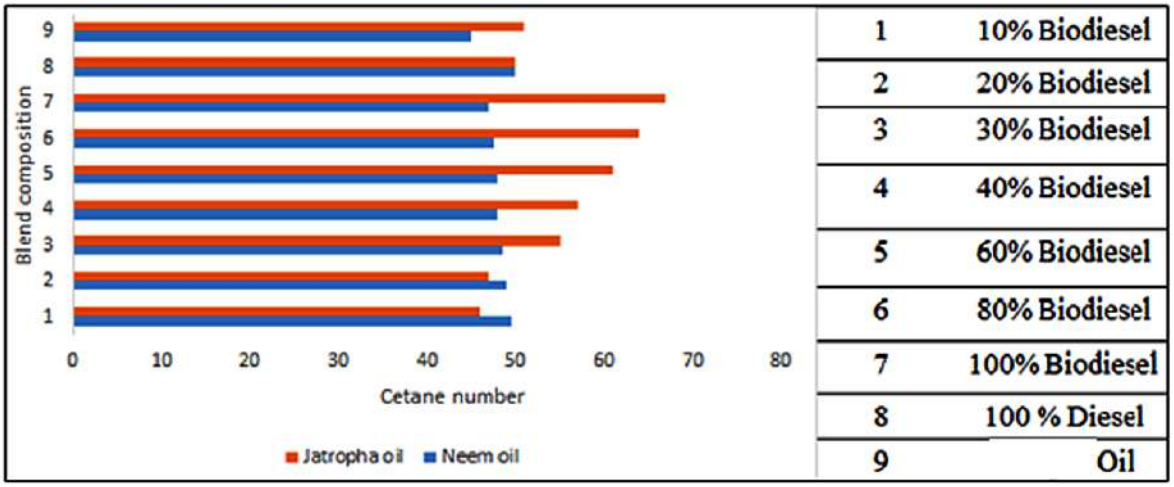
Cetane Number

The Cetane number is directly proportional to diesel index. The cetane numbers for pure diesel and neem oil biodiesel were 50 and 47 respectively(figure 8). For jatropha oil biodiesel it was 67. As the blend percent increased from 10 to 80%, Cetane values decreased. Cetane numbers for neem oil biodiesel almost remained same for all blends. Cetane number for all blends was satisfactory compared to pure diesel.

Figure 8. Cetane number for different blend compositions



Initial Boiling Point

Initial boiling point(IBP) was 92 ^{0}C for 100% diesel and 125 ^{0}C for 100% biodiesel (neem oil methyl ester). It was 132^{0}C for jatropha oil biodiesel. As shown in figure 9, initial boiling point increased as the proportion of biodiesel increased. The ease of engine start-up is governed by initial boiling point for the 10% recovery in ASTM distillation. Lower IBPs are preferred for cold climates. Very low IBPs are responsible for vapor locking tendency in the diesel engine. IBPs for all the blends are in the acceptable limits for diesel fuel.

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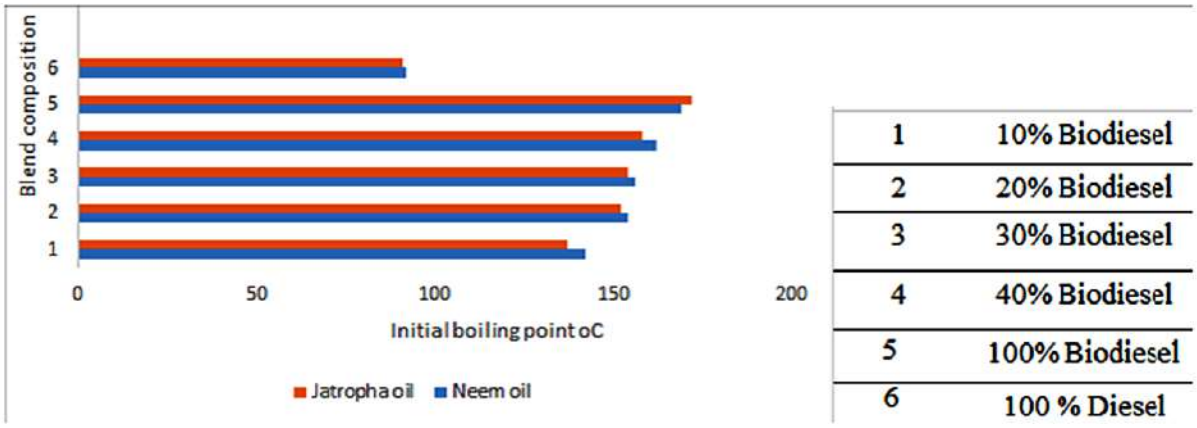
Figure 9. Initial boiling point for different blend compositions

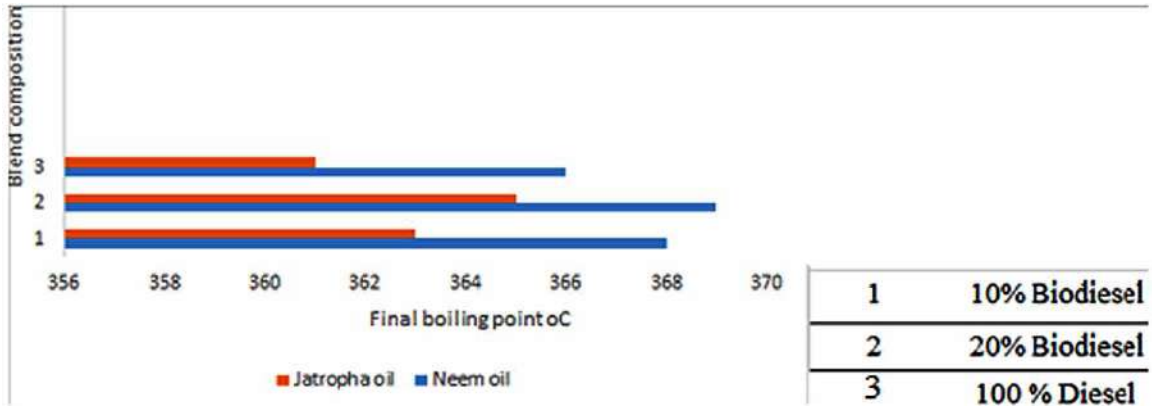


Final Boiling Point

Need oil biodiesel blends for both 10% biodiesel and 20% biodiesel yielded 92% recovery and the final boiling points(FBP) 368 and 369^{0}C respectively indicating good distillation characteristics(Figure10). While for higher blends and pure neem and jatropha oil methyl esters, the FBP was not obtained due to cracking.

From the experimental study it was observed that for 10% jatropha biodiesel blend, the FBP was 363^{0}C with distillate recovery 92%, for 20% biodiesel blend, FBP was 365 ^{0}C with distillate recovery 92%(figure 10). For higher blends cracking was observed. As per ASTM distillation technique only 10% and 20% jatropha oil methyl ester blends in diesel is feasible.

Figure 10. Final boiling point for different blend compositions



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Comparison With Past Work

Many investigators have investigated biodiesel synthesis using different oils. Table 2 summarizes significant past work for synthesis and characterization of biodiesel for different types of oils. The biodiesel synthesized using waste cooking oil can be made suitable for vehicles with use of deep eutectic solvent (DES) in the local moderate temperature climate (Khan et al.,2021). Biodiesel production from edible oil faces challenges due to cost of oil. Use of low cost oil can reduce this problem to considerable extent (Abdelrahman, 2020; Shaah et al., 2021).Feedstocks such as vegetable oils, nonedible oils, oleaginous microalgae, fungi, yeast, and bacteria have been utilized in various investigations(Chintagunta et al., 2021). Management of waste frying oil can be additional benefit of its use as starting material for biodiesel synthesis(Topi, 2020).Various aspects of biodiesel synthesis such as process design and simulation, sustainability evaluation, optimization and supply chain management needs to be studied and investigated further to render viability to the oil derived biodiesel(Pasha et al., 2021).There is higher emphasis for sustainability in fuel synthesis in the production of the third generation of biofuels(Vignesh et al, 2021). Advanced oil extraction technologies for biodiesel along with supercritical and microwave-assisted transesterification and novel catalysts are being explored(Zulqarnain et al., 2021; Jamil et al., 2021; Pikula et al., 2020).

CONCLUSION

Utilizing 100% methyl ester of neem oil or jatropha oil in diesel motor is unrealistic. Studies in recent past indicate that provide up to 40% oil yield per seed based on weight(Riayatsyah et al., 2022).Blends of 10% and 20% methyl esters of neem oil and jatropha with diesel meet the kinematic viscosity, pour point, diesel index and ASTM refining temperatures according to BIS values. So utilizing 10% and 20% blends of methyl ester of neem oil or jatropha oil with diesel may reduce 20% diesel requirement of the nation. Blends of 30% methyl ester of with diesel meet the kinematic viscosity(neem and jatropha oil biodiesel) and pour point(neem oil biodiesel, ASTM refining properties(jatropha oil derived biodiesel) according to BIS. However, it doesn't meet the diesel index(neem oil derived biodiesels), kinematic viscosity(jatropha oil biodiesel) and ASTM refining properties(neem and jatropha oil biodiesel). Use of 20% biodiesel blends of both types of biodiesels(neem oil and jatropha oil) can reduce petroleum diesel requirement by 20%.

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Table 2. The past work for synthesis and characterization of biodiesel for different types of oils

Oil	Parameters			Biodiesel Characterization				Authors
	Oil: Alcohol Ratio	Catalyst Loading	Temperature and Time	Specific Gravity	Kinematic Viscosity	Flash point /fire point	Cetane Number/ Calorific Value	
Waste cooking oil (WCO)	1:8	1% by wt.	reaction time 90 minutes	----------	----------	----------	----------	Elkady et al., 2015
Second-used cooking oil	1:6	1%	temperature 66.5 °C	0.90	4.9 cSt	flash point 180.5 °C	----------	Kawentara and Budiman 2013
Waste cooking oil (WCO)	1:8	1%	reaction time90 minutes	----------	----------	--------	----------	Degfie et al., 2019
Canola cooking oil	1:1	0.5%	temperature 55 °C, time 160 minutes	----------	----------	--------	----------	Hossain and Mekhled, 2010
Soyabean oil	1:6	0.5%	reaction temperature 60 °C, 100 minutes reaction time	----------	----------	--------	----------	Churasia et al. 2015
Soyabean oil	1:6	1%	reaction temperature 40 °C, 180 minutes reaction time	----------	----------	--------	----------	Hossain and Al-saif 2010
Sunflower oil		1 gm/l	Temperature 49 °C	0.68	6 cSt	----------	47	Thirumarimurugan et al. 2012
Rapeseed	1:9	0.7 w/w	60 to 80 °C temperature, 90 to 100 minutes time	0.86 to 0.87	4.7 to 4.9 cSt	flash point 175 to 178 °C	43-44	Encinaret al. 2018
Palm oil	1:6	1%	60 to 80 °C temperature	0.85 to 0.89,	3.05 to 6.2 cSt	flash point from 56 to 164 °C	Calorific value 42800 to 38050 kJ/kg	Mamilla et al.2012
Jatropha oil	1:6	1%,	reaction temperature 20 to 25°C (room temperature)	0.87	4 to 5.3 cSt	flash point 93 to 95°C	48-50	Kywe and Oo 2009
Cotton seed oil		----------	----------	0.89	3.6 cSt	flash point 155 °C	50.4, Calorific value 40000 kJ/kg	Singh et al.2015

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Chapter 16
Characterization and Comparison of Various Blends of Honge Oil Methyl Ester (Biodiesel) With Diesel Fuel

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ABSTRACT

The Honge oil, known as pongamia oil, is one of the most widely used lamp oils around the world. It is also used in leather tanning, in soap making, and as a lubricant. The Honge oil methyl ester can be used as an alternative fuel for diesel. The transesterification of methanol and Honge oil in the presence of base catalyst results in Honge oil methyl ester. In the current work, the Honge oil methyl-ester known as biodiesel was produced. It was mixed with petroleum diesel fuel in different proportions. Various physical and petroleum properties were investigated for Honge oil biofuel and also for various diesel and ester mixtures. The optimum parameters were temperature was 65^{o}C, catalyst quantity, 0.4 grams and oil: alcohol ratio, 1:6. The blends of 10%, 20%, and 30% compositions were most suitable as potential alternative fuel. Different proportions of biodiesel ester in diesel fuel have been also compared with petroleum diesel fuel to identify a suitable blend having good engine fuel potential.

DOI: 10.4018/978-1-6684-5269-1.ch016

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INTRODUCTION

Worldwide economy runs on energy and with increase in population, the fuel demand is increasing rapidly (Natarajan and Venkatesh, 2012; Ahmad et al. 2009; Kumar et al., 2013; Patil et al., 2008; Hayyan et al., 2010). These needs, coupled with environmental changes, increasing costs and exhaustion of fossil fuel have motivated investigators to discover new energy assets (Georgianna and Mayfield, 2012; Ramadhas et al., 2004; A. Kumar and P. Kumar, 1984; Khan et al., 2007). Biodiesel has been considered to be one the best option forfuture fuel needs.Itcan be renewed because of its biodegradability (Bobade and Khyade, 2012; Santana et al., 2012; Ma and Hanna, 1999). Biodiesel creation from a renewable source can fulfill goals of a balanced environment and manageable monetary quality (Bobade and Khyade, 2012; Hossain and Salleh, 2008). Advantages of biodiesel are, its biodegradability and no requirement of alteration of engine to accommodate the fuel (Ahmad et al. 2009). Biodiesel can be smoothly blended (Anya et al., 2012; Saribiyik et al., 2010; Hribernik and Kegl, 2007). Any alternative fuel should be characteristically achievable, agreeable and financially feasible.

The source of Honge oil is the seeds of the Millettia pinnata tree. Karanja oil (in Hindi), Honge oil (in Kannada), Kanuga oil (in Telugu) and Pungai oil (in Tamil) are different terms used for this oil, Pongamia being the generic name. The Pongamia tree is around 40 feet tall after complete growth. Pink and white flower are grown on their branches. These mature and develop into brown seed pods (Allen O and Allen E., 1981). They grow in temperature range 0 to 40°C.Also they can survive in 5–25 cm annual rainfall. This tree grows wild on soil that is rough and hard (Meher, et al., 2004). The plant is nonedible, has harmful properties and can cause harmful effects on body if injected orally. However, it finds applications in the traditional medicines via its fruits, flowers and seeds (Bobade and Khyade, 2012). Honge plant derived oil and juices are effective on skin disease and have pest control abilities. Complete growth of this plant takes 10 years and it takes 5 years only to develop pods. A single tree can yield oil in the range of 10 to 50 kilograms depending on its size (Meher et al., 2004). Different methods like extraction and pressing (expeller and cold) can be used for Pongamia oil for its extraction from seeds. Pongamia oil is antiseptic and resistant to pests. The bitter flavonoid constituents results in disagreeable taste and odor and also has a high content of triglycerides.

In the recent years Pongamia oil industry has caught great attention in the global market. India, being one of the largest Pongamia producers, has a very important role in it. Pongamia pinnata (the Derris Indica trees) has been identified a definite source of next generation fuel. This plant can grow on barren and unused land. The plant has no food applications and hence its use as a source of biodiesel doesn't affect food requirement. High oil recovery is another important advantage of this source. All these advantages makes Honge oil, even more attractive alternative. This plant can minimize nitrogen requirement of soil as it can fix nitrogen from atmosphere.

Meng et al. (Meng et al., 2008) optimized the reaction temperature at 65^0C whereas Freedman et al. (Freedman et al.., 1986) suggested that optimal catalyst concentration is generally between 0.5 wt. % to 1.0 wt. %. The effect of ratio of oil to alcohol was also optimized experimentally at 1:6 by Alia and Taya (Alia and Taya, 2013). The major objective of the current investigation is to prepare the biodiesel from Honge oil and to study its petroleum properties, compare with pure petroleum diesel. Initially parametric studies were performed and parameters were optimized for experiments on different blend compositions. Prepared biodiesel and its blends were analyzed by comparing their properties with pure diesel. The words Honge oil and Pongamia oil are used in the investigation interchangeably.

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MATERIAL AND METHOD

Material

Honge oil alkyl ester was synthesized with base catalyzed transesterification reaction. The oil was obtained from market and was provided by Jinendra Scientifics, Jalgaon. Potassium hydroxide used was manufactured by Hi-Pure Rankem Chemicals and Methanol provided was for laboratory use only of AR grade.

Method

The setup used for the synthesis of biodiesel is shown in figure 1. The synthesis of ester was carried out in the equipment using methanol and KOH. Optimum values of the parameters are indicated in table 1. By using waste vegetable oil(WVO), WVO oil to methanol ratio 1:12, 1% wt/wt of NaOH, reaction time 90 minutes were found to be optimum parameters by Elkady et al. (Elkady et al.,2015). Degfie et al. obtained 1:8 WCO(waste cooking oil) oil to methyl alcohol, 90 minutes reaction time, 0.01 weight fractionpotassium hydroxide loading rate as optimum parameters(Degfie et al., 2019). In another investigation, maximum yield was obtained at alcohol to oil molar ratio 6.18:1, 1% wt/wt KOH as catalyst and 66.5^0C(Kawentara and Budiman, 2013). The catalyst was properly shaken and mixed with the alcohol using stirrer. Proportion of hydroxide pellets and methanol was obtained after analysis of effect of various proportions on the yield. The Honge oil was exposed to high temperature to remove moisture content and mixed with methanol and potassium hydroxide mixture. The mixture was heated and agitatedfor 180 minutes and at 63 to 67 ^{o}C (Average 65 ^{o}C). The reaction mixture became turbid and changed to orange colour after 15 to 18 minutes. The colour became transparent with time. Completion of the reaction was identified from a sweet fragrance of the mixture. The mixture was then agitated and subjected to high temperaturefor 60 minutes more. The mixture was taken into a separating funnel to obtain layers ester and glycerol. Warm water was used to separate glycerol, soap and other impurities from the mixture. It was then treated with calcium hydroxide medium to minimize moisture level. The ester portion is the Honge oil biodiesel. The petroleum properties were studied of pure Honge oil ester and blends of this ester and diesel sample. The performance of blended samples was analyzed and compared with the pure diesel fuel properties.

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Figure 1. Setup for biodiesel preparation

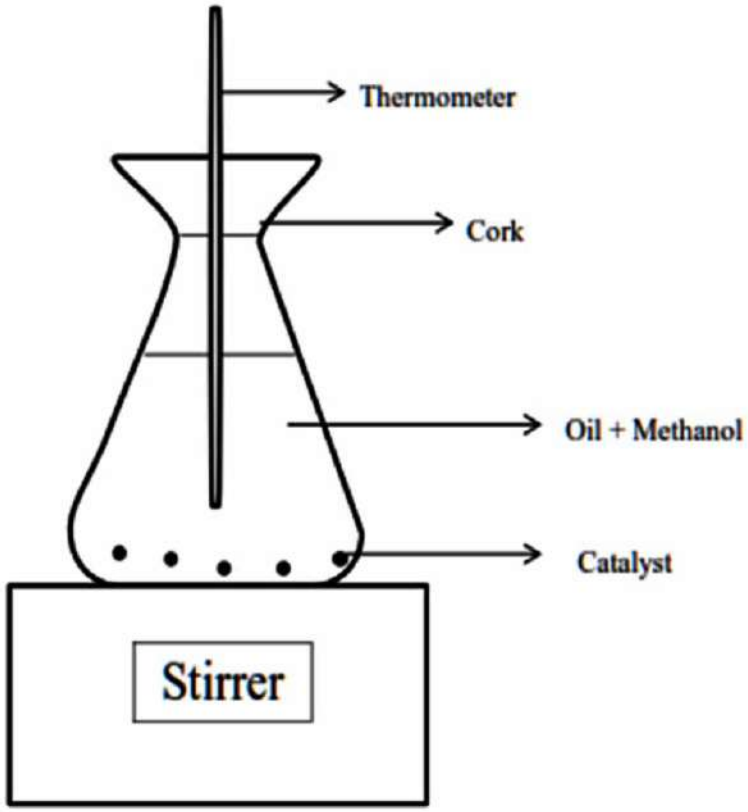

Table 1. Parametric optimization

Parameter/Factor	Optimum Value
catalyst	KOH, maximum yield 81.3%
time	3 hrs.
catalyst concentration	0.4 gm
process temperature	65 ^{o}C
oil-methanol ratio	1:6

Analysis of Biodiesel

The analysis of biodiesel and its various blends with diesel thus obtained is important in order to understand the properties and to compare them to diesel fuel in order to know its feasibility and viability. Various properties were studied from the analysis of biodiesel obtained from Honge oil. These include kinematic viscosities, diesel index, aniline point, flash and fire point, ASTM distillation properties,

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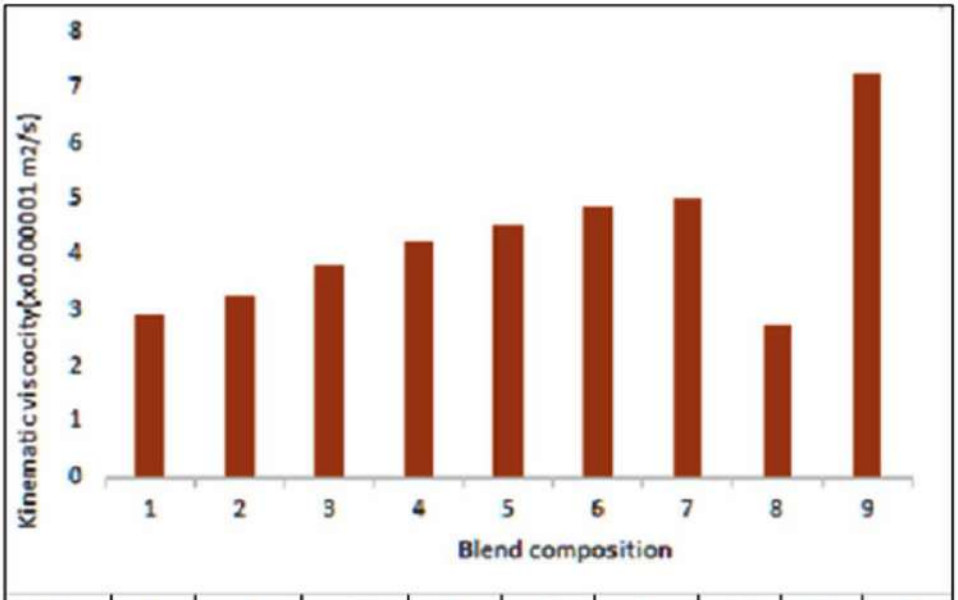
specific gravity, API gravity. The determinations of these properties and their values along with the methodologies have been described extensively in the result and discussion section.

RESULT AND DISCUSSION

Evaluating Kinematic Viscosity Requirement

Kinematic viscosities for different blends are shown in figure 2. For any fuel used in diesel engine, the desired range of viscosity is necessity and prime requirement since it directly affects the ignition characteristics. According to ASTM D6571, the liquid fuel need to have the viscosity in the specified limit. The limit for diesel for its efficient and safe use is viscosity 1.9mm^2/s minimum to 6.0 mm^2/s maximum. The kinematic viscosities of diesel and biodiesel samples were determined by viscometer. The diesel has a kinematic viscosity of 2.5 mm^2/s at 40^{0}C while pure Honge oil has a viscosity of 7.25 mm^2/s. Viscosity of the Honge oil methyl ester was comparatively less than vegetable Honge oil (5.03 mm^2/s). The viscosities of 10%, 20%, 30%, 40%, 60%, 80% blends of methyl ester of Honge with diesel were obtained as 2.91mm^2/s, 3.26 mm^2/s, 3.81mm^2/s, 4.22 mm^2/s, 4.52 mm^2/s and 4.87 mm^2/s respectively. Up to 30%, Honge oil ester blends have comparable viscosity with Petro diesel.

Figure 2. Change in kinematic viscosity for change in biodiesel composition



No.	1	2	3	4	5	6	7	8	9
Blend Composition	10% Biodiesel	20% Biodiesel	30% Biodiesel	40% Biodiesel	60% Biodiesel	80% Biodiesel	100% Biodiesel	100 % Diesel	Pongamia Oil

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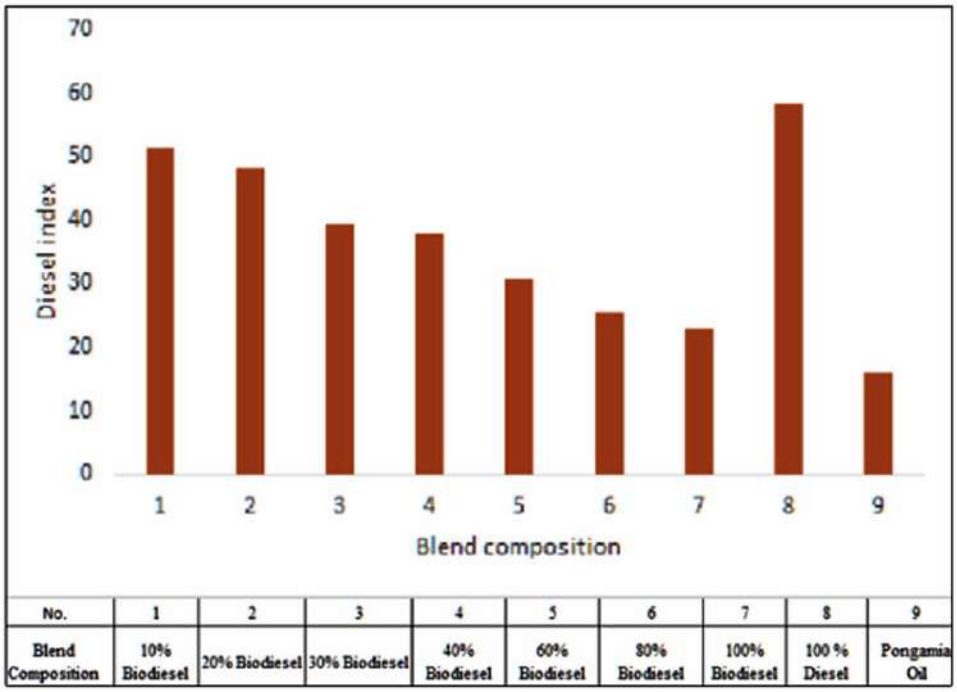
Evaluation of Ignition Quality With the Help of Diesel Index

Ignition property of the liquid fuel is well represented by the diesel index. The optimum value should be closed to 45 as per mentioned specification of BIS. By knowing aniline point and specific as well as API gravity, diesel index can be calculated as:

Diesel Index value = Aniline point^{0}F x degree API Gravity / 100

Market purchased diesel fuel has a diesel index between 58 to 60 which is well above the specified value of 45. On the other hand, the diesel index of the Honge oil is approximately 16.2 which is far below the specification. Diesel index values for different blends are plotted in figure 3. A diesel index of 23.1 was observed for pure ester synthesized from Honge oil. Thus, use of pure Honge oil or its methyl ester in place of diesel will result in very poor ignition characteristics. Hence, Honge oil and its methyl ester alone doesn't have appropriate fuel characteristic from ignition perspective. It can be blended with diesel fuel. An addition of 10% methyl ester of Honge oil results in diesel index to 51.27 and 20% methyl ester of Honge oil has a diesel index of 48.1 which is above the specified value, indicating superior ignition property. For the different blends of 60% and 80% of methyl ester, the obtained diesel index is below the specified value and hence will result in very poor ignition property.

Figure 3. Change in Diesel Index for change in biodiesel composition



No.	1	2	3	4	5	6	7	8	9
Blend Composition	10% Biodiesel	20% Biodiesel	30% Biodiesel	40% Biodiesel	60% Biodiesel	80% Biodiesel	100% Biodiesel	100 % Diesel	Pongamia Oil

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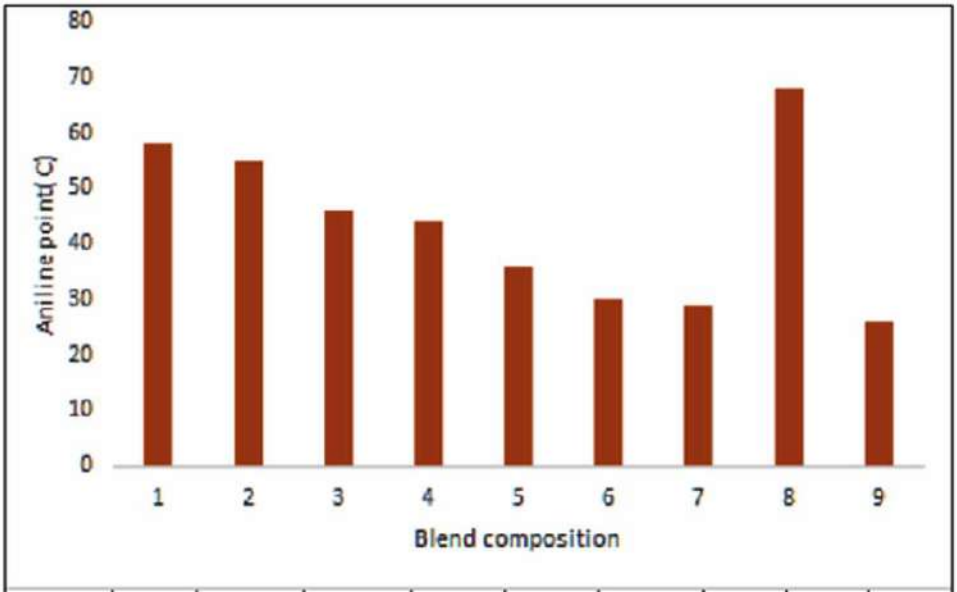
Evaluation of Aniline Point Property

Aniline point was determined to study fuel properties. A test tube with thermometer and stirrer was filled with dry sample and aniline. Sample and aniline mix completely. After cooling, the mixture become opaque. For determination of the aniline point, 5 ml of a carefully dried sample and aniline were filled in a test tube fitted with a thermometer and stirrer. On stirring, the sample and aniline are completely miscible. The apparatus is then cooled until the mixture is opaque. Then the temperature in increased slowly until the thermometer bulb is visible. Aniline point is the lowest temperature at which equal volume proportion mixtures of aniline and oil are completely miscible. Aniline point is indicator of aromatics present. Lower the aniline point, higher are aromatic compounds in the oil.

High values of aniline point temperature indicates that the fuel has high paraffinic hydrocarbon and hence has high diesel index, which is good ignition quality(figure 4). Determining aniline point is important in determining diesel index properties. Aniline point of diesel is 68 °C whereas that of 100% biodiesel is 29 °C. Aniline points of the blend of Honge oil methyl ester and high-speed diesel are lower compared to high-speed diesel. Compared to pure biodiesel obtained from Honge oil, these values were higher.

Figure 4. Change in Aniline point for change in biodiesel composition



No.	1	2	3	4	5	6	7	8	9
Blend Composition	10% Biodiesel	20% Biodiesel	30% Biodiesel	40% Biodiesel	60% Biodiesel	80% Biodiesel	100% Biodiesel	100 % Diesel	Pongamia Oil

Evaluating Pour Point Properties

The pour point temperature evaluation is necessary for fuels to analyze their behavior in cold weather climatic conditions and their storage handling. The flow characteristics of liquid vanishes at pour point and liquid ceases to flow. A tube with flat bottom with dimensions 3 cm diameter and 12 cm height is

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used for measuring pour point characteristics. It is kept in air jacket. The jacked in placed in a jar that contains the freezing mixture (Ice + $CaCl_2$). The tube is filled up to 50 percent height. A thermometer is inserted in the tube through cork. The temperature falls with cooling due to freezing mixture. For every 1 ^{0}C drop, the mixture is taken out and observed for cloudiness. Cloudiness is indicator of decrease in flowability. After every 3^{0}C drop in temperature, the sample was titled to check oil flow characteristic. Pour point is identified when the oil doesn't flow, even after complete horizontal tilting of the tube for 5 seconds.The Honge oil biodiesel at the minimum temperature of ambient condition will be free flowing. Pour point of Honge oil was -5°C which is far below the requirements. Pour point of methyl ester of Honge oil (100%) and 10%, 20%, 30%, 40%, 60% and 80% blends of methyl ester of Honge oil with diesel were experimentally obtained as 2°C, 5°C, 4°C, 4°C, 4°C, 3°C and 3°C respectively. Thus, increasing amount of Honge oil biodiesel in diesel, decrease in the pour point temperature was estimated. So, values of any blend of pour point temperature can be used since all values are close to diesel pour point value which is 5°C.

Evaluating ASTM Distillation Properties

Using standard ASTM Distillation apparatus, the initial and final boiling points and the temperatures for various percent recoveries of distillates were determined. Initial boiling temperature for diesel was 88 ^{0}C while for Honge oil biofuel it was 128 ^{0}C. There was rise in initial boiling point for 10 to 40% Honge oil biofuel blends in diesel (figure 5). The initial boiling temperatures were 130^{0}C,140^{0}C,142^{0}C and 146^{0}C for 10 to 40% Honge oil biofuel blends respectively. The initial boiling temperature represents ease of starting of an engine. The lower value of initial boiling temperature is desirable especially in chilled weather climates. However, it causes vapor locking problems. Greater values of Honge oil biofuel blends protect the diesel engine from vapour locking. For the 10% blend of Honge oil biofuel, the final boiling temperature was 362^{0}C (recovery 92%), for 20% blend, the temperature was reduced to 348 ^{0}C at same distillate recovery and for 30% biofuel blend, maximum boiling temperature was found slightly higher 352 ^{0}C (recovery 90%). For the next proportion of 40 percent Honge oil biodiesel blend, 8% recovery of distillate was recorded at temperature 278^{0}C. For higher honge oil methyl ester blends, cracking phenomenon was observed. Only the lower blends of honge oil ester would have proper ignition in diesel engine. Figure 5 and 6 depict initial and final boiling points for different blends.

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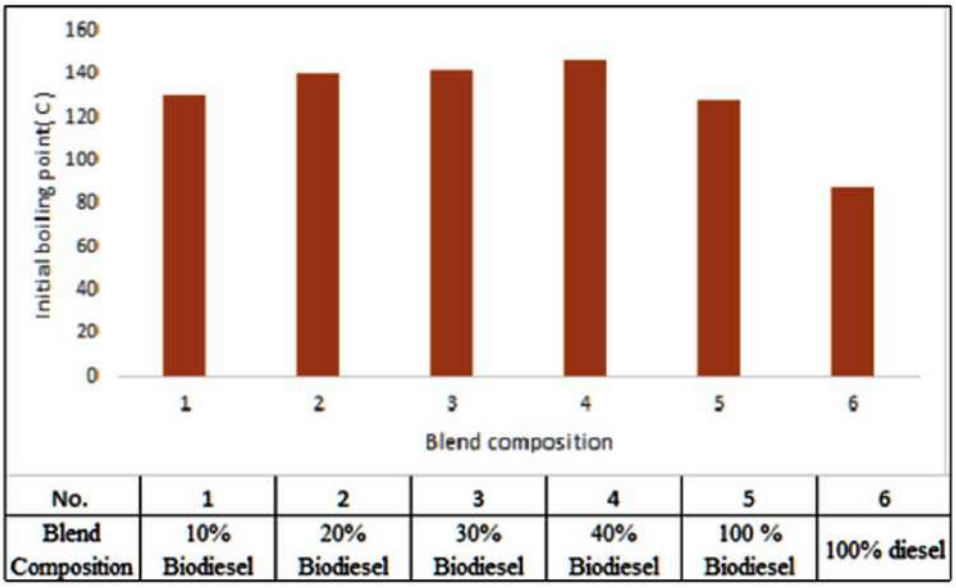
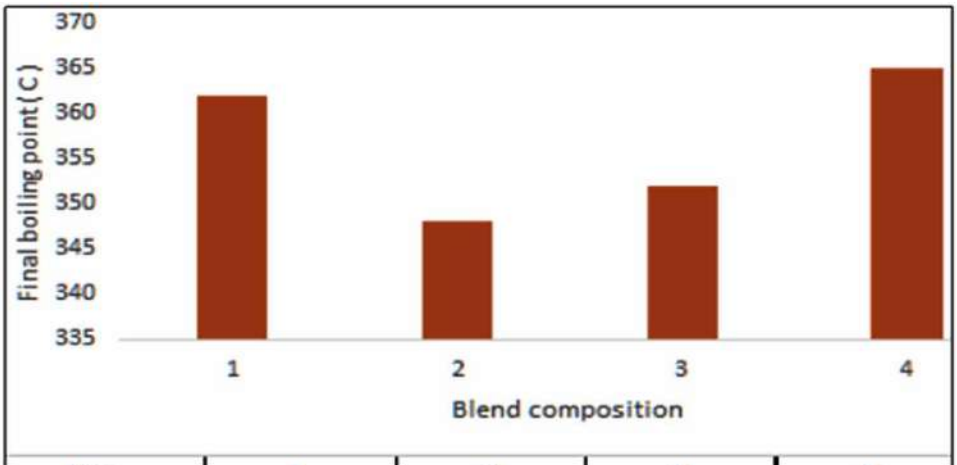
Figure 5. Change in Initial Boiling Point for change in biodiesel composition



No.	1	2	3	4	5	6
Blend Composition	10% Biodiesel	20% Biodiesel	30% Biodiesel	40% Biodiesel	100 % Biodiesel	100% diesel

Figure 6. Change in Final Boiling Point for change in biodiesel composition



No.	1	2	3	4
Blend composition	10% Biodiesel	20% Biodiesel	30% biodiesel	100 % Diesel

Flash Point and Fire Point

Flash and fire points for different blends are shown in figure 7 and figure 8 respectively. The oil sample

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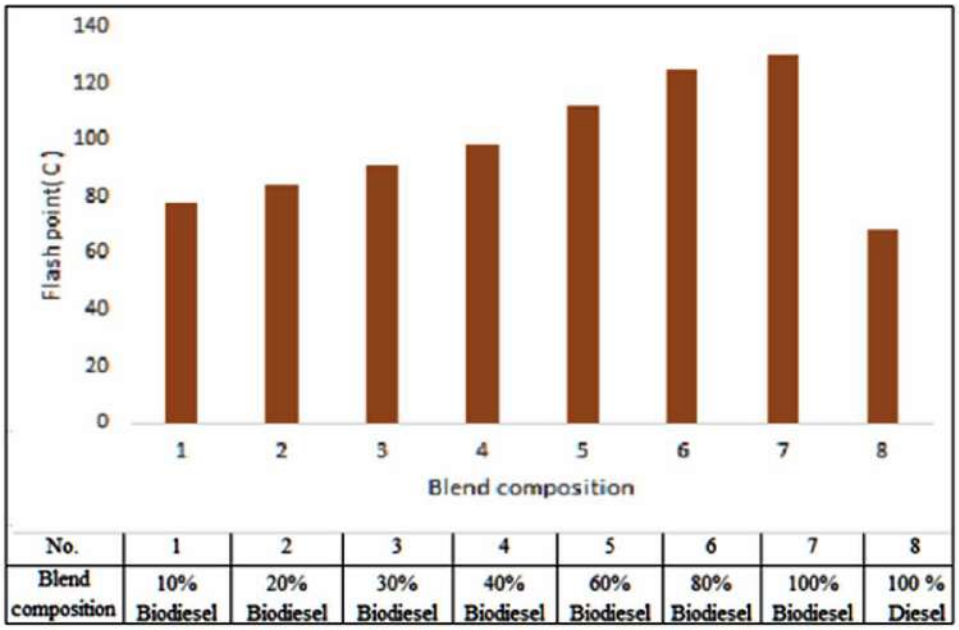
under test is filled up to the mark in the oil cup. It was heated in the air bath by a burner. Flame was introduced for a moment, for every degree rise in temperature. The flash point is identified by appearance of distinct sound and light. The test flame is applied and heating is continued for some more time. The fire point of the oil is obtained by observing the test flame which burns continuously for minimum five seconds. Flash point of oil is about 5 to 40 ^{0}C lower than its fire point. The diesel fuel flash and fire points by open cup method were 68^{0}Cand 70^{0}C respectively.

The pure Hong oil biodiesel had higher values of 130^{0}C and 134^{0}C respectively. The flash and fire point values increased as the Honge oil methyl ester blend percent in diesel increased by every ten percent like 78^{0}C and 81^{0}C, 84^{0}C and 87^{0}C, 91^{0}C and 95^{0}C, 98^{0}C and 101^{0}C, 112^{0}Cand115^{0}C, 125^{0}C and135^{0}C respectively. Flash and fire points are indicators of possible fire hazard during the transportation of the fuel material and also in the storage and handling condition.

Figure 7. Flash points for different blend compositions



No.	1	2	3	4	5	6	7	8
Blend composition	10% Biodiesel	20% Biodiesel	30% Biodiesel	40% Biodiesel	60% Biodiesel	80% Biodiesel	100% Biodiesel	100 % Diesel

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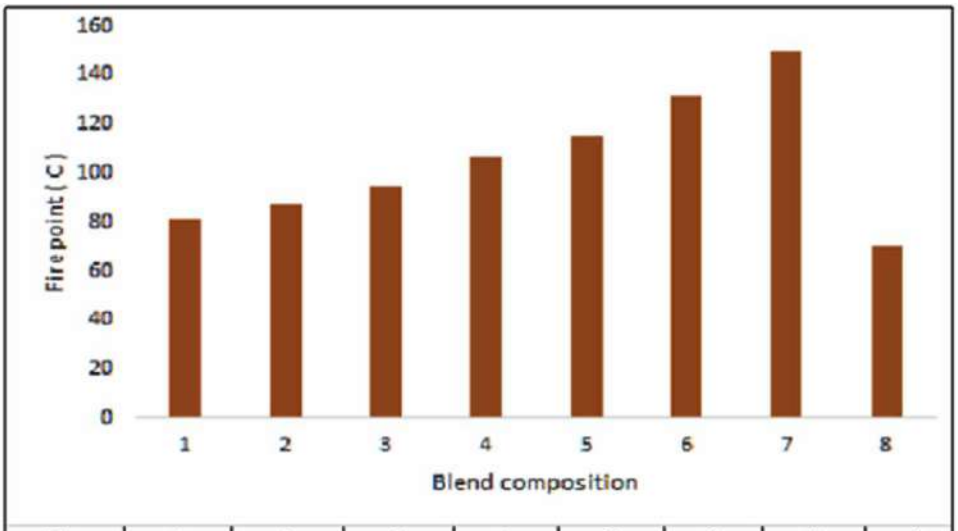
Figure 8. Fire points for different blend compositions



No.	1	2	3	4	5	6	7	8
Blend composition	10% Biodiesel	20% Biodiesel	30% Biodiesel	40% Biodiesel	60% Biodiesel	80% Biodiesel	100% Biodiesel	100 % Diesel

Specific Gravity and API Gravity Measurement

The specific gravity of diesel and Honge oil ester was 0.836 and 0.890 respectively. Figure 9 depicts specific gravity at different blend compositions. Specific gravities obtained for the blends were 0.837,0.841,0.853,0.867,0.878 and 0.890 in increasing order of their compositions(for every ten percent addition of ester in petrodiesel like 10,20,30,40,60 and 80 percent respectively). Higher values of specific gravity leads to higher values of diesel index. 10 and 20% blends were most suitable from diesel index analysis. The API gravity for diesel was 37.75 and for pure biodiesel it was 27.42. There was decrease in API gravity with increase in biodiesel proportion in the blend (Figure 10).

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Figure 9. Specific gravity for different blend compositions

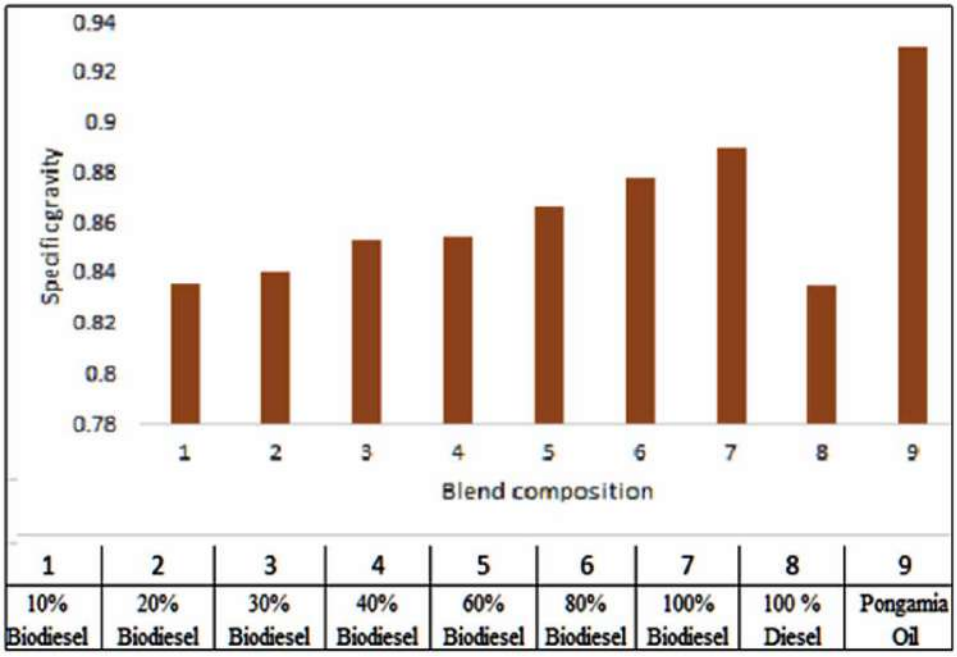
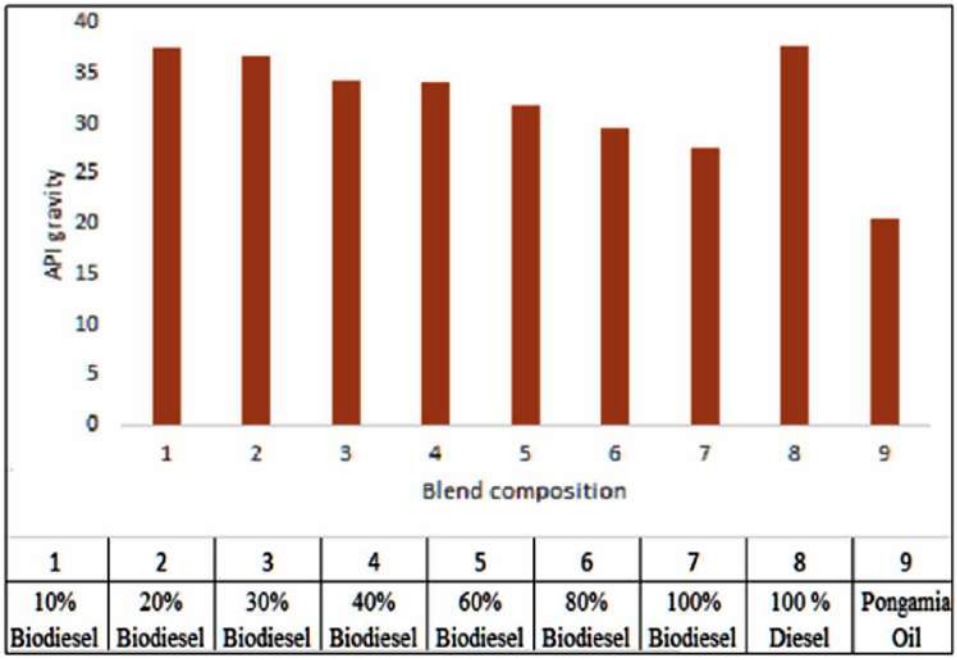


Figure 10. API gravity for different blend compositions



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Cetane Number and Calorific Value Analysis

The samples were analysed for cetane number and calorific value(Table 2). These properties are very important as identification of good fuel.

Table 2. Cetane number and Calorific value

Cetane Number	52.1
Calorific Value	3780 kcal/kg

Cetane number, indicates speed of fuel burning. It also indicates the amount of compression required. For satisfactory operation of diesel engine, cetane values should be in the range, 40 to 55. Vegetable oil sources yield esters which have a cetane number range of 46 to 52. Size of the carbon chain is indicator of the calorific value. There is direct relation between the Cetane number and diesel index.Pure diesel has Cetane number of 50. Cetane values decreased with increase in biodiesel proportion in the blend(figure 11). For first four samples, up to 40% biodiesel content, the cetane number was found to be within satisfactory range(near 40).

Figure 11. Cetane numbers at various blend compositions

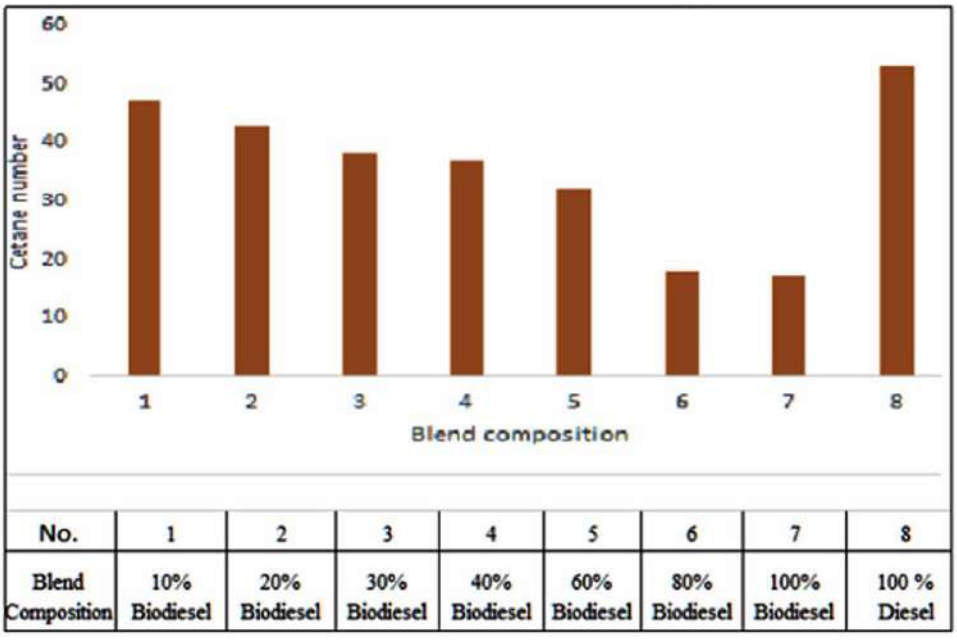

Table 3 summarizes the past work for synthesis and characterization of biodiesel for different types of oils. It can be observed that the optimum parameters and biodiesel characteristics are in the range of

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the values obtained in these investigations. This indicates that the biodiesel synthesis and characterization is satisfactory and Honge oil is very good source of biodiesel.

Table 3. The past work for synthesis and characterization of biodiesel for different types of oils

Oil	Parameters	Biodiesel Characterization	Authors
Waste cooking oil (WCO)	WCO oil to methanol ratio 1:8, Catalyst loading 1% by wt., reaction time 90 minutes	----------	Elkady et al., 2015
Waste cooking oil (WCO)	1:8 WCO oil to methanol, 90 minutes reaction time, 1% by weight of catalyst loading rate	----------	Degfie et al., 2019
Second-used cooking oil	1:6.18 oil to methanol ratio, 1% KOH, temperature 66.5 °C,	Specific gravity 0.90 kinematic viscosity 4.9 mm^2/sec, flash point 180.5 °C	Kawentara and Budiman, 2013
Soyabean oil	1:6 molar ratio oil to methanol, catalyst concentration 0.5%, 100 minutes reaction time, reaction temperature 60 °C	----------	Churasia et al., 2015
Soyabean oil	1:6 molar ratio oil to methanol, catalyst concentration 1%, 180 minutes reaction time, reaction temperature 40 °C	----------	Hossain and Al-saif, 2010
Sunflower oil	Temperature 49 °C, 1 gm/l catalyst loading	Kinematic viscosity 6 mm^2/sec, Cetane number 47, specific gravity 0.68	Thirumarimurugan et al., 2012
Rapeseed	Methanol to oil ratio 1:9, 0.7 w/w catalyst concentration, 60 to 80 °C temperature, 90 to 100 minutes time	Flash point 175 to 178 °C, Cetane index 43 to 44, specific gravity 0.86 to 0.87, viscosity 4.7 to 4.9 mm^2/sec.	Encinaret al., 2018
Canola cooking oil	catalyst concentration of 0.5%, temperature 55 °C, time 160 minutes, 1:1 molar ratio of methanol to oil	----------	Hossain and Mekhled, 2010
Jatropha oil	Methanol: oil ratio 1:6, potassium hydroxide catalyst concentration 1%, reaction temperature 20 to 25°C (room temperature)	Cetane number 48 to 50, flash point 93 to 95°C, specific gravity 0.87, kinematic viscosity 4 to 5.3 mm^2/s	Kywe and Oo, 2009
Palm oil	1:6 ratio of oil to alcohol, 60 to 80 °C temp., 1% catalyst concentration	For 20 to 100% composition specific gravity varied from 0.85 to 0.89, Kinematic viscosity varied from 3.05 to 6.2 mm^2/sec, Flash point from 56 to 164 °C, Calorific value 42800 to 38050 kJ/kg	Mamilla et al., 2012
Cotton seed oil	----------	Specific gravity0.890, Viscosity 3.6 mm^2/sec, flash point 155 °C, Cetane number 50.4, Calorific value 40000 kJ/kg	Singh et al., 2015

CONCLUSION

Use of pure Honge oil methyl ester as an alternative fuel for diesel will not be advisable as 100% Honge oil biodiesel because of viscosity. It can be used as 10, 20 and 30 percent blends and can be very useful alternate fuel. The higher blends like percent 40 and above have high values of viscosity and can create

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ignition difficulties. The results obtained for kinematic viscosity, ASTM properties, specific gravity, aniline point, cetane number, flash point, fire point were in agreement with earlier investigations.(Natarajan and Venkatesh 2012; Ahmad et al. 2009; Kumar et al. 2013;Mamilla et al.2012). As per diesel index analysis and aniline point determination, the 10 and 20% blends were most useful. The specific gravity, flash and fire point and pour points of all the Honge oil ester blends were within the limits. The distillation property also recommends the use of lower blends up to 20 percent. The 20% Honge oil biodiesel has potential to reduce diesel usage by an equivalent amount and minimize environmental footprints.

ACKNOWLEDGMENT

Authors express their gratitude towards their affiliated institutes for their support and cooperation. Both the institutes (KBC NMU University Institute of Chemical Technology and Gharda Institute of Technology) have played motivational role in experimentation and preparation of this book chapter.

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Chapter 17
Explicit Conceptual Design Approach to Adapt a Biomass-Fed Anaerobic Digester and Status Indicators in Semi-Arid Areas

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Sokoine University of Agriculture, Tanzania

ABSTRACT

The mitigation of adverse climate change requires holistic strategies. The strategies include the proper handling of biomass wastes such as cow dung. The handling of the wastes has to be efficient using appropriately designed anaerobic digesters. In addition, the operating status of these digesters has to be monitored for detection of any fault of the digesters. Unfortunately, existing approaches for designing and monitoring the working condition of digesters have limitations. This chapter presents the innovative and explicit conceptual design approach of adapting the biomass-fed anaerobic digester and the digester operating-status-indicators in semi-arid areas. The approach is recommended to the renewable energy system designers. Finally, the appropriate indicators are recommended to be applied by the biogas plant operators or users.

INTRODUCTION

The performance improvement of biomass-fed anaerobic digesters for sustainable bioenergy generation is essential. Several ways of employing engineering design approaches are available for digester design improvement. In addition, the approaches are useful in adapting the anaerobic digester (AD). Adaptation refers to modifying the existing AD design to get its improved design with high performance (Divya *et al.*, 2015). The existing designs of the biogas plants have some pitfalls, which render poor plant performance. The pitfalls oblige the adaptation of the biomass-fed AD design. The adaption process involves

DOI: 10.4018/978-1-6684-5269-1.ch017

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comprehensive engineering designing procedures, including conceptual design explained by Andreasen *et al.* (2015). The shortcomings noted in the methodologies presented by researchers, including Wu (2013) and Al *et al.* (2019), who applied the approaches for AD designing, impose the investigation of the innovative but explicit conceptual design approach. The faults of biogas plants harm the financial base of users, e.g. failing to attain the predetermined net present value, and polluting the environment. Additionally, the pollution of the environment due to the biogas plant faults, like biogas leakage, exacerbate the adverse climate change. Wu *et al.* (2019) and others have proposed several methodologies of monitoring the biogas plant working conditions intending instantly to track the faults. However, the methodologies have some limitations, including late detection of the faults resulting in the search for novel biogas plant working-status-monitoring methodology with appropriate indicators. Therefore, this chapter aims to demonstrate i) the development and application of the innovative but explicit conceptual design approach for adapting biomass-fed digesters and ii) the establishment of the novel biogas plant working-status-monitoring appropriate indicators.

BACKGROUND

Biomass is a renewable energy resource that harvests solar energy and stores that energy in the form of chemical energy. It includes animal, agricultural, and municipal wastes. Various ways are available to convert biomass into bioenergy, e.g., biogas. The conversion of biomass may be done through the anaerobic digestion process. The process is run without air while employing microbial degradation to produce biogas, among other by-products. The process is a proven technology. Several factors, including temperature, influence the technology of sustainable biogas generation and the state of feedstock applied (Theuerl *et al.*, 2015). Other factors affecting the efficient production of biogas from anaerobic digesters (ADs) include substrate pH, Carbon-Nitrogen ratio of feedstock, feedstock loading rate and particle size, hydraulic retention time and additives. The essential and critical factor, especially in batch-fed ADs, is temperature. It significantly affects biogas generation and other factors (Adekunle and Okolie, 2015). Here below is the information on the temperature relative to the biogas generation. The mesophilic state requires AD temperature to be within 20°C to 45°C. However, keeping the AD at a constant temperature, around 35°C, increases biogas generation. The biogas microbes survive optimally at a temperature variation of 0.5°C/day; henceforth, keeping ADs at temperature variations approaching this value is important (Rowse, 2011). Usually, biogas microbes can sustain temperature variation of ±3°C/day. Therefore, devising a simple temperature control system to reduce temperature variation of biogas digester toward 0.5°C/day is of paramount importance for sustainable biogas generation.

The feedstock employed in biogas generation may consist of dry matter of up to 15% wet basis for the liquid state digestion, i.e. liquid anaerobic digestion (LAD) or above 15% wet basis for the solid-state digestion, i.e. high solid anaerobic digestion (HSAD) (Li *et al.*, 2011). The HSAD feedstock has a much longer retention time and lacks control over the biological process (Li *et al.*, 2011). Therefore, it produces more biogas and needs less or no addition of water compared to the LAD feedstock. However, the HSAD feedstock needs a large amount of inoculum for exciting biogas generation (Yang *et al.*, 2015).

Biogas generation technology is essential for the sustainable energy supply to communities and conserving the environment (Surendra *et al.*, 2014). The technology is relevant in rural areas of economically growing countries of most sub-Saharan African regions. In these countries, Tanzania inclusive, the energy insufficiency rate, especially for cooking, is increasing (Kusekwa, 2011). The energy insufficiency is

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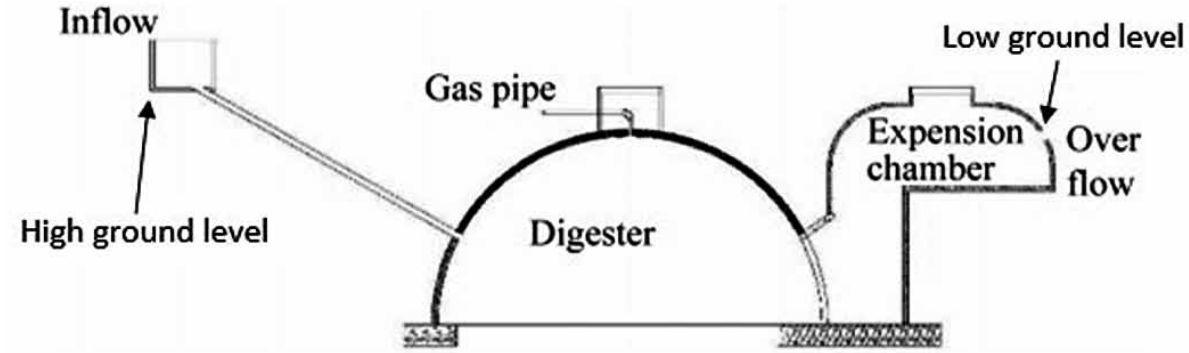
caused by dwindling forest acreages resulting from the following: an expanded search for fuelwood, a traditional source of energy; enormous pressure exerted by population growth for habitation, and the surging expansion of agricultural and pastoral land (Ordway *et al.*, 2017). The effects of energy insufficiency are exemplified in rural semi-arid areas (RSAAs). The areas are found in central regions, for the case of Tanzania.

Strategies to abate the effects of the energy insufficiency in Tanzania involved introducing biogas technology in RSAAs under a subsidy scheme facilitated by non-governmental organisations (Wawa, 2012). The introduced technology was the continuous fed underground fixed dome digester ranging from 4 to 6 m^3 in size. It was a modified Chinese-type digester design, mainly known in Tanzania as the Centre for Agricultural Mechanisation and Rural Technology (CAMARTEC) type digester (Mrema *et al.*, 2011). Figure 1 illustrates this design. In addition, CAMARTEC has two biogas plant designs, one for humid regions and another for semi-arid areas. Troubleshooting faults, like leakages, in this biogas plant is challenging as they are underground installed. Besides, they are fed with feedstock of biomass and water at a ratio of one to one (Oloche *et al.,* 2017).

Figure 1. CAMARTEC digester design found around the study site
(Melinda et al., 2013)



Apart from the large-sized digesters, portable small-sized digesters are available in Tanzania. For example, Kusekwa (2011) reported the portable small-sized plastic digester design of about 200 litres capacity developed by the Dar-es-Salaam Institute of Technology in Tanzania. However, its performance and dissemination of information are not available. Furthermore, in 2012 a Netherland-based company, SimGas BV with Silafrica Company, introduced in Tanzania the small-sized biogas system, alias GesiShamba (Figure 2), with a minimum capacity of 2 m^3 and fed continuously with biomass and water at a 1:1 ratio (SimGas, 2018). The GesiShamba is made of cheap plastic material, making it portable, unlike the conventional biogas plant.

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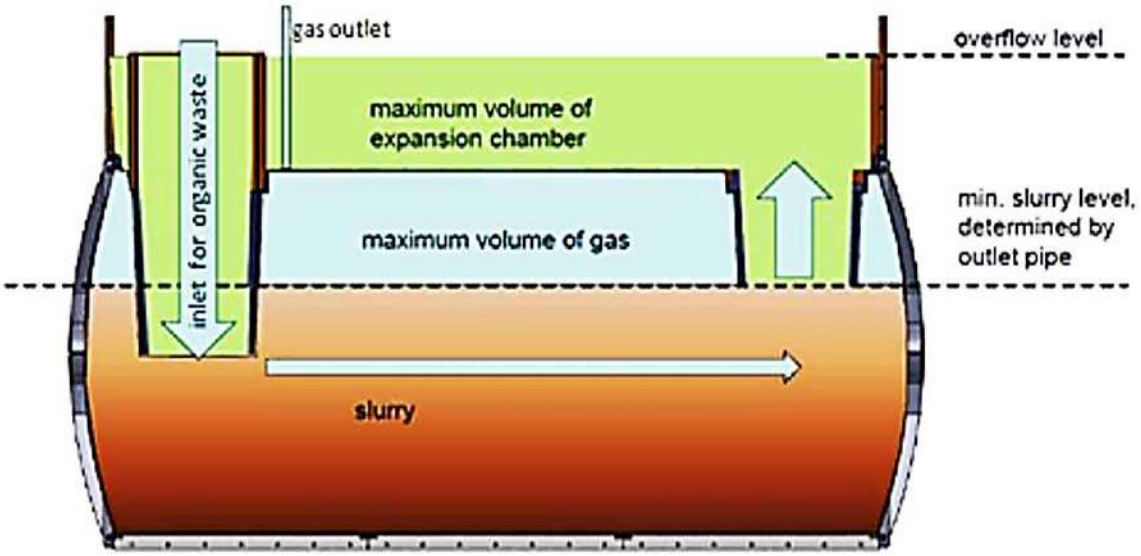
Figure 2. GesiShamba system design
(WB, 2015)



Aside from the designs mentioned above, other AD designs are used in Tanzania and other parts of the world. They include a floating drum (India type), separate storage (Sri-Lanka type), and plug flow digesters (Mrema *et al.*, 2011). Furthermore, by mode of operation, digesters are classified into batch-fed, semi-batch-fed, and continuous-type digesters. The batch-type digester for handling HSAD is simple and cheap in design compared to the others, although it has clogging problems (Kranert *et al.*, 2012).

The biogas, among others, contain methane (CH_4) content, which indicates biogas quality (Poulsen and Adelard, 2016). The high CH_4 content means high quality of biogas (Siefers *et al.*, 2010). Su *et al.* (2015) described techniques for improving the quality. The techniques include reducing impurities (Carbon dioxide, hydrogen sulphide, water vapour and ammonia) from biogas. The biogas plant auxiliary components for reducing the impurities include water traps and scrubbers filled with water, steel wools or other organic and inorganic materials like steel wools and activated carbon (Kulkarni and Ghanegaonkar, 2019; Awe *et al.*, 2017). Affordable auxiliary components facilitate the uptake of biogas technology in low-income communities in RSAAs. Enormous-sized biogas plants, for industrial application, of 100-m^3 capacity and 10 000-kPa pressure, support the biogas' purification up to 95% CH_4 content (Achinas *et al.*, 2017). The small domestic biogas plants of fixed dome or floating drum type mostly operate at low pressure, which may hardly reach 16 kPa because of being open to the atmospheric pressure at the substrate outlet (Zalm, 2017). However, for a batch-fed AD design, which is well sealed and the biogas outlet valve is closed, its pressure may rise above 16 kPa. Furthermore, the biogas flow rate in the low-pressure systems may decrease to 0.025 m^3/h. On the other hand, most applied standard biogas cooking facilities operate at a moderate biogas flow rate of 0.45 m^3/h (Zalm, 2017). Therefore, applying the low biogas flow rate directly while cooking is challenging. Therefore, cheap biogas storage is vital when operating small domestic biogas plants at low pressure and flow rate due to the prevailing weather conditions in semi-arid areas.

Several researchers have adapted the biogas plant designs using strategies explained by Divya *et al.* (2015). The adaptation involves connecting the mixing and recycling of leachate in the digester, adding

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fixed-film on the surface inside the digester periphery walls, and/or adding pieces of inert materials (plastics, nylon, and sponge) in the feedstock to increase the contact surface area of biogas microbes' growth. In addition, it includes minimising the abrupt changes in temperature inside the digesters. Furthermore, the digester designs alteration for biogas quality increment and handling may be done by adding improved auxiliary components like a water trap for carbon dioxide gas and water vapour screening, a scrubber for hydrogen sulphide trapping, and separate robust biogas storage for releasing the biogas efficiently (Muñoz *et al.*, 2015). However, some of these adaptations are expensive, energy-intensive, and result in biogas plant operating challenges (Karellas *et al.,* 2010). Moreover, on-ground installed small-sized (200 - 1000 litres) ADs operated in individual households are unavailable in RSAAs to address the above-stated developmental and research gaps. Besides, limited literature is available on the small-batch type system to easily release biogas from the interior of substrates of high solid anaerobic digestion. Therefore, adapting batch-type AD for domestic purposes, which addresses the fore-explained challenges, is considered.

The system adaption process involves two main design procedures: detailed and conceptual design approaches (Al *et al.,* 2019). The systems designers that deal with various types of ADs apply both approaches. However, the approaches presented by Sánchez and Cardona (2012) are obscure, involve sophisticated and complicated tools (Wu, 2013), and models which do not adequately reflect the existing physical condition of the system being developed (Al *et al.,* 2019). In some cases, results based on these approaches are prone to biases by the designers. Moreover, understanding the monitoring of biogas plant working conditions reduces the risk of plant upset to generate the biogas sustainably. Several researchers have proposed methodologies for monitoring the status of ADs (Wu *et al.,* 2019). They proposed methods to monitor the digester conditions, including checking the substrates properties, percentage of biogas constituents, physical condition of the plant, and pressure variation. Other methods include checking the physical condition of the plant for damages or biogas leakage. However, the methods are time-consuming before identification of the faults, while some are difficult to implement depending on the design and installation of the biogas plant (Iswanto *et al.,* 2021). In addition, there is a lack of standard digester pressure curves indicating the leakage and the proper indicators of biogas microbes' activities inside the small-sized digesters. The challenges related to the adaptation process necessitate developing an adaptation approach for the digester design that is relatively clear, simple but effective and efficient biogas to facilitate sustainable biogas generation. In addition, the approach, which indicates the biogas plant's working condition at the appropriate time, is essential.

Therefore, this chapter presents and demonstrates the explicit conceptual design approach of adapting biomass-fed AD and the novel working condition indicators of the adapted anaerobic digestion system. The adapted AD may fit into any other renewable energy system to produce useful energy and bio-slurry.

EXPLICIT CONCEPTUAL DESIGN APPROACH OF ADAPTING THE BIOMASS-FED ANAEROBIC DIGESTER AND THE DIGESTER OPERATING-STATUS INDICATORS

There are several ways of adapting biomass-fed AD to produce biogas and bio-slurry. However, the approaches for adapting the digester based on the engineering design methodology presented by many researchers have several shortcomings, as noted in Sánchez and Cardona (2012) and Al *et al.* (2019). Additionally, a quick and reliable approach to identifying the faults of biomass-fed ADs facilitates a rapid

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remedying of malfunctioning ADs. However, systematic methods proposed for monitoring the digester operating status have limitations, as Wu *et al.* (2019) and Iswanto *et al.* (2021) explain. Furthermore, there is a lack of standard digester pressure curves indicating the leakage and appropriate indicators of biogas microbes' activities inside the digesters. These indicators are vital, as they closely reflect the working condition of the digester. The solutions to these shortcomings and limitations reinforce the theme of Biomass and Bioenergy Solutions for Climate Change Mitigation and Sustainability.

With that briefing, this chapter provides the explicit conceptual design approach of adapting the biomass-fed AD and demonstrates its application. The approach's demonstration involves adapting the batch-fed AD for effective cow dung degradation for sustainable biogas generation. Besides, the appropriate digester operating-status indicators are established and presented.

The research area of this study included the Department of Engineering Sciences and Technology at Sokoine University of Agriculture (SUA), located in Morogoro, Tanzania. In addition, it involved Chamwino District, Dodoma region Tanzania. The region represents semi-arid areas.

Formulation and Demonstration of Conceptual Design Approach of Adapting the Biomass-Fed Anaerobic Digester

After a thorough review of literature related to engineering design approaches and frameworks, intuitively, the AD adaption approach was formulated. Figure 3 illustrates the approach. The approach employs the adapted engineering procedural and research techniques. These guide the adaptation process of the biomass-fed AD design. Authors executed several tasks during the adaptation process in the field than in laboratories and workshops of research centres. In addition, the approach involves input, processing and output phases. The inputs phase includes reviewing the literature on the functionality of various existing designs of digesters, developing design criteria to be achieved by the intended components or system to be adapted/re-designed, synthesising the working principles (WPs) or concepts as per design criteria, and selection of the best working principle (WP).

The processing phase comprises the conceptual design of the selected best WP, fabrication, fixing of the fabricated components, and assembly of the digester system using materials and tools available in the workshop. Finally, the assembled system enacted the output phase as the intended biomass-fed digester; the digester was adapted batch AD for this study.

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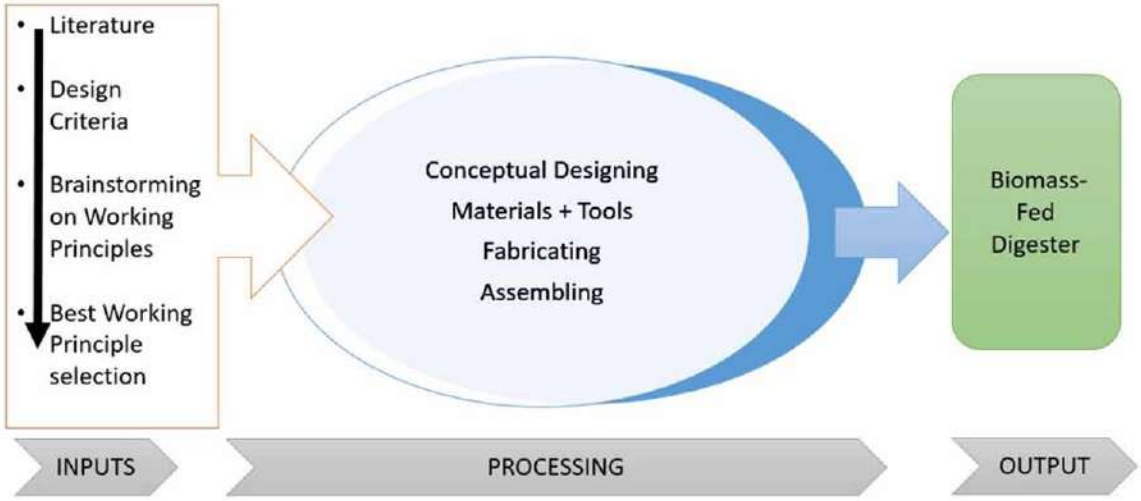
Figure 3. The framework of adapting the biomass-fed AD



The Inputs Phase of the Adaption Framework

After a thorough literature review of various digesters' designs and operations (Garfi *et al.,* 2016), the authors selected the batch-fed anaerobic digestion system for use. The design simplicity, low cost and ease of operation guided the selection of the system. However, this system needed an adaptation to offer the intended service of increased generation of quantity and biogas quality. The basis of achieving this result was the design criteria explained in the subsequent sub-sections and the approach shown in Figure 3.

The Batch Anaerobic Digestion System Design Criteria

The criteria formulated as a basis for devising the intended system are the ability to withstand the pressure of about 16 kPa; biogas tight seal capacity/airtight sealing by 100%; self-mixing during operation; possible self-leachate re-circulation during operation, and increased contact surface area of biogas microbes. Other criteria include easy passage of biogas from the interior of substrates; easy to operate; ensuring no biogas outlet clogging; availability of raw materials locally; less cost involved; more durable and easy manufacturability.

Synthesis and Selection Approach of Best Working Principle

1. Synthesis of the Working Principles

Based on the criteria, the authors generated WPs of all batch anaerobic digestion system components. The components include the digester, the water trap, the scrubber, biogas storage and the piping system. Table 1 shows details of the generated digester WPs. The unscaled drawing in Figure 4 illustrates the generated digester WP with various types of top cover.

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Table 1. Generated digester working principles

S/N	Working Principle	Volume (litres)		Drum Material	Seal Used	Other Components
1	DWP1	240		Plastic	Plastic sheet and lid	None
2	DWP2	240		Plastic	Tube rubber thread and lid	Sealed central core
3	DWP3	240		Plastic	Flexible plastic tube and lid	Perforated central core
4	DWP4	240		Plastic	2 mm thick gasket rubber and lid	The perforated four-winged central core
5	DWP5	500		Plastic	Screwed top lid	The perforated central core and four wings
6	DWP6	240		Plastic	2 mm thick gasket rubber, lid and 2 mm thick flat bar frame	The perforated four-winged central core

Figure 4. Unscaled drawing of the digester working principle

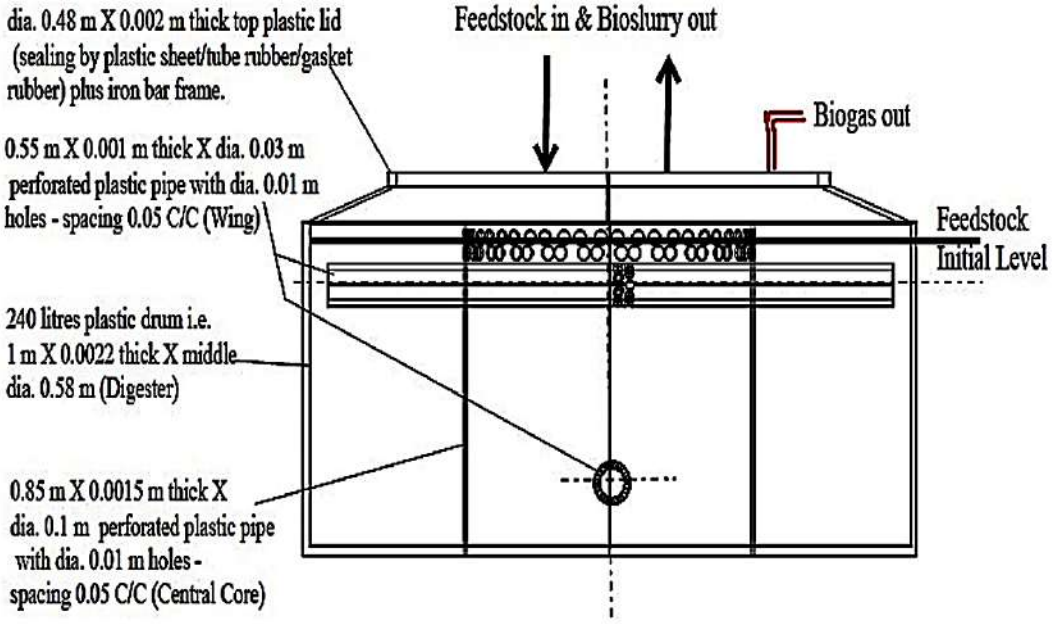

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Table 2. Generated water trap working principles

S/N	Working Principle	Volume (litres)	Main Part	Important Dimension	Other Components
1	WWP1	5	Plastic jerry-can	1 mm-thick-wall	None
2	WWP2	3	Plastic-bucket	1.5 mm-thick-wall	None
3	WWP3	0.6	IPS Plastic-pipe	Length 500 mm and diameter 40 mm	None
4	WWP4	5	Plastic-can	0.7 mm-thick-wall	None
5	WWP5	12	Plastic-can	0.7 mm-thick-wall	None
6	WWP6	10	Plastic-bucket	1 mm-thick-wall	None
7	WWP7	20	Plastic-bucket	1 mm-thick-wall	None
8	WWP8	10	Plastic-jerry-can	1 mm-thick-wall	None
9	WWP9	20	Plastic-jerry-can	1 mm-thick-wall	None
10	WWP10	3	Plastic-bucket	1 mm-thick-wall	Fastening 2 mm-thick iron bar frame

Table 3. Generated scrubber working principles

S/N	Working Principle	Volume (litres)	Main Part	Important Specification	Other Components
1	SWP1	0.5	IPS-plastic-pipe	Length 400 mm and diameter 40 mm (monolithic)	None
2	SWP2	0.5	IPS-plastic-pipe	Length 400 mm and diameter 40 mm (non-monolithic)	50 mm-diameter IPS-Connector
3	SWP3	1.5	Plastic-bottle	0.5 mm-thick-wall	None
4	SWP4	1	Plastic-bottle	0.5 mm-thick-wall	None
5	SWP5	2	Plastic-jug	1 mm-thick-wall	None
6	SWP6	1	Plastic-jug	1 mm-thick-wall	None
7	SWP7	0.8	Iron-pipe-size IPS-plastic pipe	Length 400 mm and diameter 50 mm (monolithic)	None
8	SWP8	0.8	IPS-pipe	Length 400 mm and diameter 50 mm (non-monolithic)	40 mm-diameter IPS-Connector

The WPs of biogas storage thought of are the following: inflatable plastic bag (BSWP1), inner tyre tube - of various sizes (BSWP2) and inflatable plastic bag covered with clothes (BSWP3). The consideration of the WPs of the piping system was to comprise one, two or three types of pipe. Authors of this chapter assumed a total length of 1.7 m of the piping system without including fittings for computing the percentage of the length of the type of pipe in the whole system. The percentages and combinations of types of pipes differentiate the WPs generated. Table 4 shows the intuitively generated WPs of the piping system.

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Table 4. Generated piping system working principles

S/N	Working Principle	Pipe Type Length Percentage to the Total Length of the Piping System			Fittings Material Types
		Horse Pipe (%)	Another Flexible Pipe (%)	IPS Plastic Pipe (%)	
1	PWP1	100	-	-	IPS-plastic-and-galvanised-steel
2	PWP2	70	30	-	IPS-plastic-and-galvanised-steel
3	PWP3	-	-	100	IPS-plastic-and-galvanised-steel
4	PWP4	70	30	-	IPS-plastic-and-galvanised-steel
5	PWP5	70	25	5	IPS-plastic-and-galvanised-steel

2. Selection Methods of the Best Working Principle

Best concept selection techniques are available. They involve Decision Matrix Analysis (DMA)/grid analysis, ABC analysis, and Pareto analysis; Analytic Hierarchy Process (AHP) based approach, anonymous voting technique, cost-benefit (CB) analysis, and Failure Modes and Effects Analysis (FMEA). Other concept selection techniques include Plus-Minus and Interesting (PMI) analysis, Kano model, nominal group technique, Sticking dots, and "Strength, Weakness, Opportunity and Threat" (SWOT) analysis (Mardani *et al.*, 2015). The following criteria are applied in choosing an appropriate selection technique of the best WP: its simplicity in application, ability to single out one best option, quick sorting of more than three options and diverse application in different disciplines, which include designing renewable energy systems.

With the procedure mentioned above, the authors found that CB analysis, SWOT analysis and Kano model are discipline-specific for financial, business, and marketing analysis, respectively. Additionally, the ABC analysis utilisation intends to prioritise one among the three options only and for controlling inventories. Furthermore, Pareto analysis uses the 80/20 rule, i.e. only 20% of options influence 80% of the same options. Therefore, it renders the analysis unable to single out one best option. The Analytic Hierarchy Process (AHP) based approach is complex to apply. It requires special software tools to perform the calculations, which involve the concept of Eigen-vectors, to get the best option.

The anonymous voting technique is very subjective and based on individuals' opinions. Additionally, FMEA was found to fit in identifying potential failure modes, determining their effect on the operation of the product, and identifying actions to mitigate the failures (Rebernik *et al.*, 2008). However, it is complex to apply, as it is impossible to anticipate every failure mode. The PMI analysis employs finding positive, negative and interesting points of an idea leading to its decision being entirely subjective regarding the most appropriate idea screening technique. The Nominal Group Technique is useful for prioritising ideas and projects rather than choosing one best option. At the same time, the Sticking dots analysis found is the quick method for determining priorities by voting. The Sticking dots analysis is useful for collecting opinions early in the idea selection process.

The review of DMA showed that the technique evaluates an idea as per criteria (Mardani *et al.*, 2015). It is simple and allows quick sorting of options/concepts by identifying their relative strengths and weaknesses. The DMA is applied in different areas of study, including designing renewable energy systems (Abu-Taha and Daim, 2011). However, the careful setting of scoring criteria is necessary for

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the expected useful results (Abu-Taha and Daim, 2011). Therefore, this study opted for the application of the DMA method.

3. Application of the DMA Method

The best WPs were selected using the DMA method as applied by Olabanji and Mpofu (2014). In this procedure, the weight values assigned to design criteria are based on their importance to ensure the component or the system needs to meet the requirement set. Experts in renewable energy, system design, and mechanical engineering assigned a weight to the design criteria. The selection of experts to participate in the weight values assignment exercise was made intuitively by considering the expert's experience to design and operate renewable energy systems and machines involving fluid systems. The authors contacted twenty experts to participate in this exercise, but only thirteen were effectively ready. Therefore, eight renewable energy system developers and five mechanical engineers were involved based on their readiness to participate in this task.

The fore-mentioned weight assignment process produced the weight values in brackets. The presentation of assignment results is as follows: the ability to withstand the pressure of about 16 kPa (0.21), biogas tight/airtight at 100% sealing (0.4), and self-mixing during operation (0.01). Others were the following: possible self-leachate re-circulation during operation (0.01), the increased surface area of contact for biogas microbes (0.03), easy passage of biogas from the interior of substrates (0.08), easy to operate (0.1), ensuring no biogas outlet clogging (0.05), availability of raw materials locally (0.04), less cost involved (0.03), more durable (0.02), and easy manufacturability (0.02).

Moreover, factors with their corresponding rating on Likert factor scale values in the bracket needed to determine the possibility of the WPs meeting each design criteria were intuitively set as follows: not at all able (1), lowly able (2), moderately able (3), highly able (4), and very highly able (5). Furthermore, the weighted score of the WPs relative to each design criterion was determined as the product of design criteria weight and the rating on Likert factor scale values. Finally, the total weighted score for each WP was determined by summing up the weighted scores.

Above mention analysis produced plots of the total weighted score against the WPs. The authors selected the WP with the highest total weighted score as the best. However, for those WPs that did not produce conclusive weighted scores, testing results interpretation of the prototypes was a basis for their selection. For instance, this happened when WPs assignment of the same rating of at least three for the highly weighted design criterion that weighs more than 0.21. The authors fabricated the prototypes as no cheap and reliable alternative way to evaluate them was feasible at that particular time and place.

4. Selection Results of the Best Working Principle

In this subsection, the authors present the results of selecting the best WP of the ADS components. The tested fabricated prototypes are the water trap, scrubber, and biogas storage, regarding the purpose of the best option selection. This exercise considered the leakage problems where the prototype was filled with air to a safe pressure of 8 kPa. Any sign of leakage was detected by immersing the prototype in water or pouring water on it. The selected best options resisted 100% of the leakage at the stated pressure. If more than one option passed the test, the authors applied the prototypes' total weighted score for the selection. Additionally, the authors considered another new WP for conceptual designing if all options failed the test.

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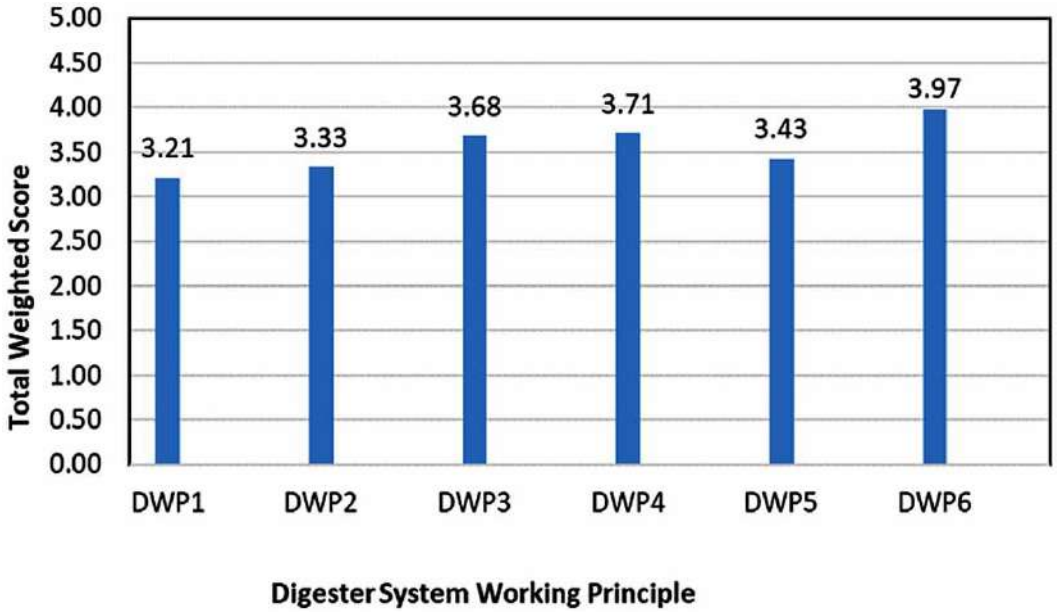
1. Selection of the working principle of the digester

The results obtained and shown in Figure 5 reveal that a working principle DWP6 secured the highest total weighted score of 3.97 and was then selected, meaning it was the best of all WPs. Note that a frame of flat iron bars encases the plastic drum.

Figure 5. Decision matrix analysis results for digester working principles



The score of 3.97 by DWP6 was based on the high likelihood of the following: the gasket rubber to offer an airtight sealing and withstanding 16 kPa pressure, and the central core and its four wings to allow easy passage of biogas from the interior of the substrates. The ease of passage of biogas from the interior reduces the possibility of building up a biogas mass at the bottom of the digester, pushing the substrate mass up, and leading to system clogging.

The central core offers a space for releasing the leachate into the bottom centre of the digester, as it is perforated and not filled with cow dung during digester feeding. Additionally, the leachate may rise to wet the above substrates through the perforated wall of that central core and its wings as the digestion process continues. Authors assumed that as the leachate mass finds space in the central core, the solid substrates drop down to occupy its space. In doing so, kneading solid substrates by the central core wings is possible.

Moreover, the provision of the central core and its wings results in increased contact surface area for the growth of the biogas-generating microbes in the central mass of the substrate. There was a likelihood of increased biogas microbes as they would be away from the possible rapid changes of temperature near the wall of the digester. The rapid temperature changes affect the performance of the microbes adversely.

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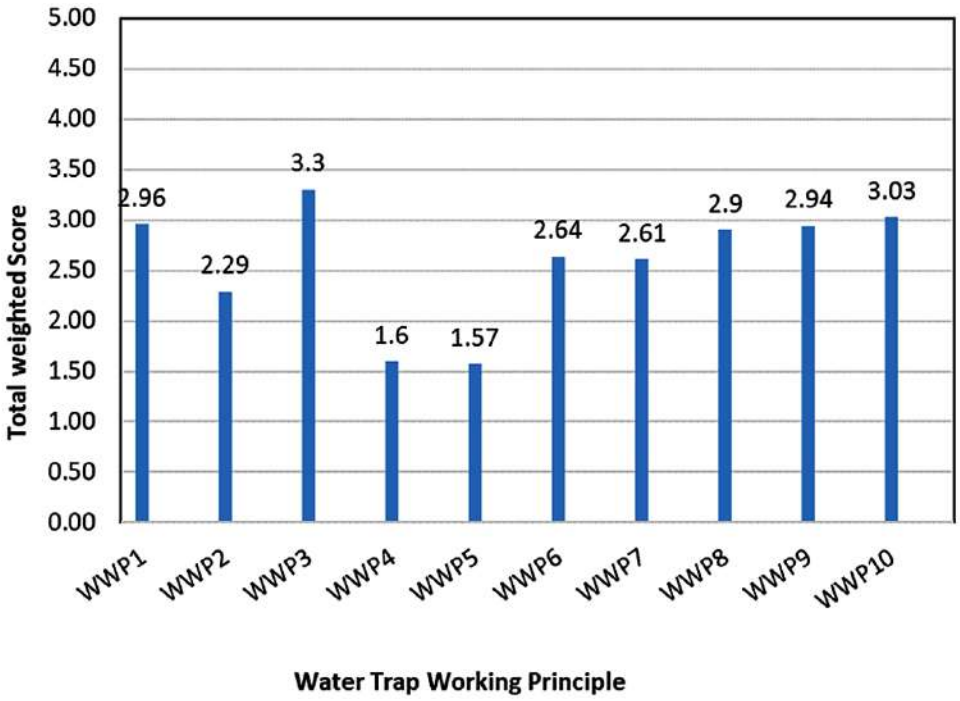
The iron bar frame supports the gasket for airtight sealing as it holds together the top lid and the plastic drum of the digester, even above 16 kPa pressure. According to Tubecon (2018), a flat bar may withstand more than 16 kPa under tension, a factor of safety exclusive.

2. Selection of the water trap working principle

The DMA results were inconclusive regarding the high-weighted design criterion for the water trap WP. Therefore, the authors fabricated the three water-trap prototypes for leakage testing. The concepts in question are WWP1, WWP3, and WWP10. All three concepts had equal-weighted scores for the high-weighted design criterion of withstanding airtight sealing. Therefore, WWP3 was best option selected for sealing the leakages by 100% and having the highest-scoring of the total weighted score after the leakage test, as shown in Figure 6.

Figure 6. Decision-matrix analysis results for water trap working principles



The authors filled the water trap with a 140 ml volume of freshwater. Then, the authors applied trial and error methods to determine the volume while ensuring no water and air inflow to the digester and outflow beyond the water trap on the accidental opening of the system to air. As a result, water enhances moisture and Carbon dioxide (CO_2) trapping. Note that, by assuming the annual mean atmospheric pressure of 101.9 kPa at the study site (TDA, 2018) and pressure and temperature of both 6 kPa and 28°C, respectively, in the water trap, the authors considered the solubility of CO_2 in water as 15 ml of CO_2 per one gram of water (Diamond and Akinfiev, 2003). Therefore, a process removes about 2.1 litres of CO_2 from the 7 litres of biogas (assuming biogas contains 30% of CO_2) passing through the water trap.

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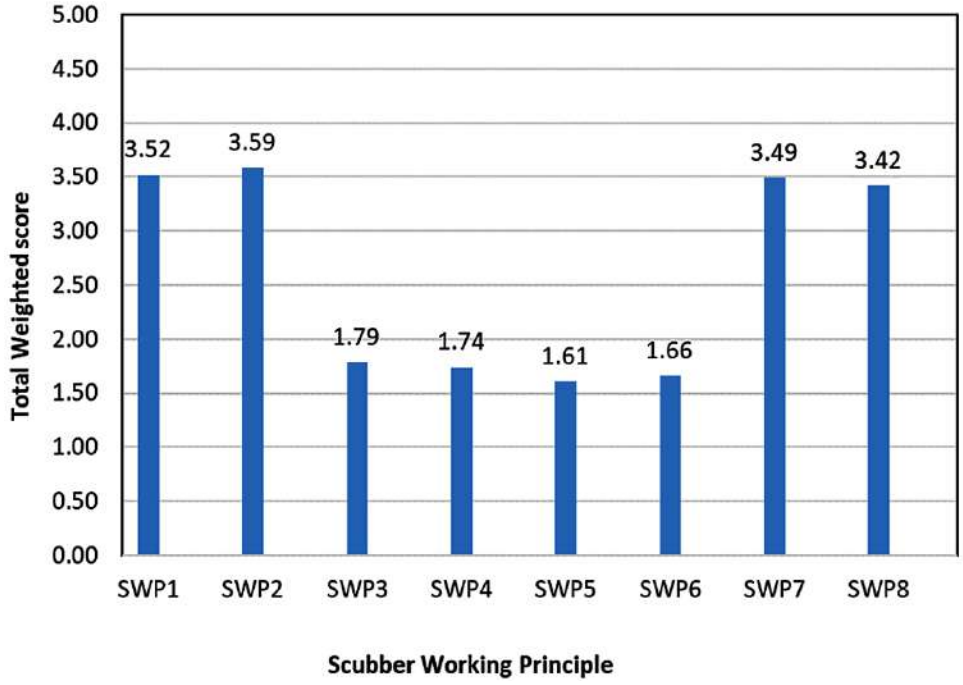
3. Selection of the working principle of the scrubber

The authors subjected the SWP1 and SWP2 to the leakage test while parked with iron wools. Both concepts were not leaking at all under testing conditions. Therefore, the selection is based on the total weighted score shown in Figure 7. Following these results, the authors selected the SWP2, with a score of 3.59, as the best scrubber design to be incorporated in the intended anaerobic digestion system.

Figure 7. Decision matrix analysis results for scrubber working principles



4. Selection of the working principle of the biogas storage

Three concepts of biogas storage facility under the subjection of the DMA produced results shown in Figure 8. The two concepts, the inflatable plastic bag (BSWP1) and the inner tyre tube (BSWP2), further passed leakage tests after fabrication prototypes. However, the BSWP1 performed poorly; therefore, the authors selected the BSWP2 as biogas storage's best option and included it in the list of components of the ADS. The used tyre tube sizes were 7.5-15 (TR174A), 8.25-16 (TR177A), 9-20 (TR177A) and 12-24 (TR177A.).

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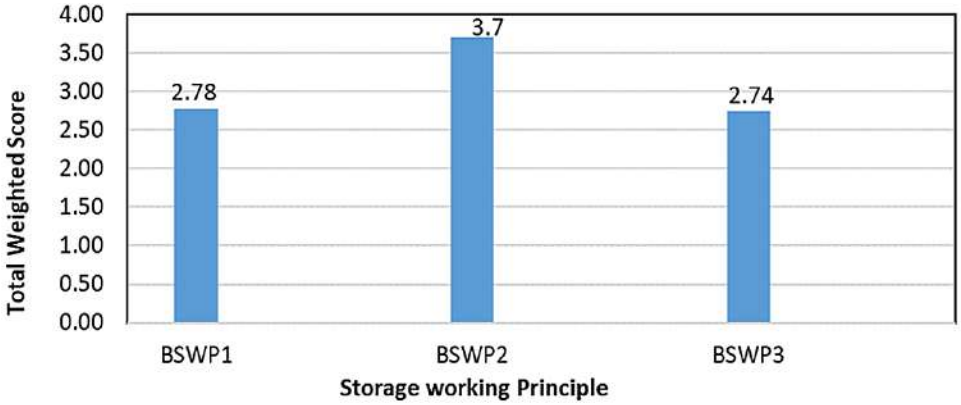
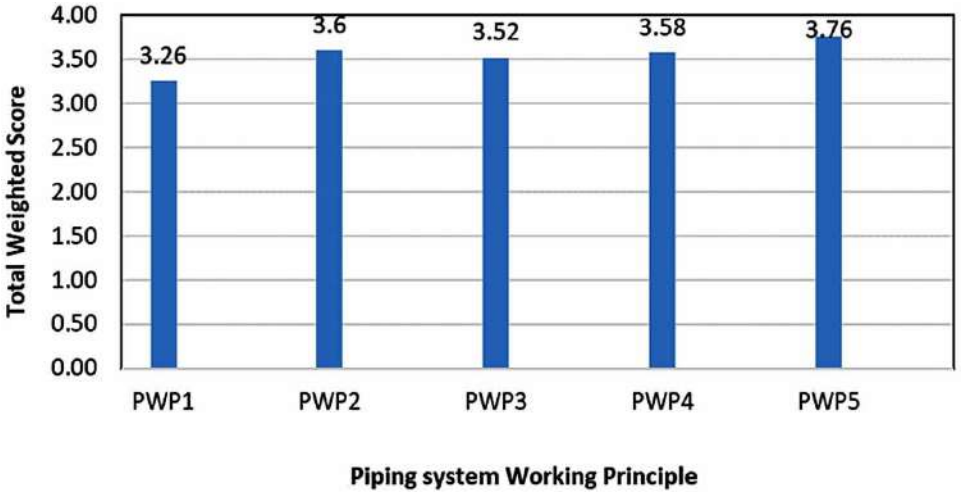
Figure 8. Decision matrix analysis results for biogas storage concepts



5. Selection of the working principle of the piping system design

The results from the DMA, as shown in Figure 9, guided the selection of the best piping system option. The option PWP5 (IPS plastic pipe (70%) + other flexibles (25%) + horse pipe (5%) + IPS fittings) secured the total weighted score of 3.76, which was the highest, leading to its selection as the best option.

Figure 9. Decision matrix analysis results for piping system concepts



The Processing and Output Phases of the Adaption Framework

The authors did the conceptual designing of the selected WPs in the processing phase. After that, using predetermined materials and tools, they fabricated and assembled all the conceptual designed components to produce the wholly anaerobic digestion system. Finally, the fully assembled system activated the output phase.

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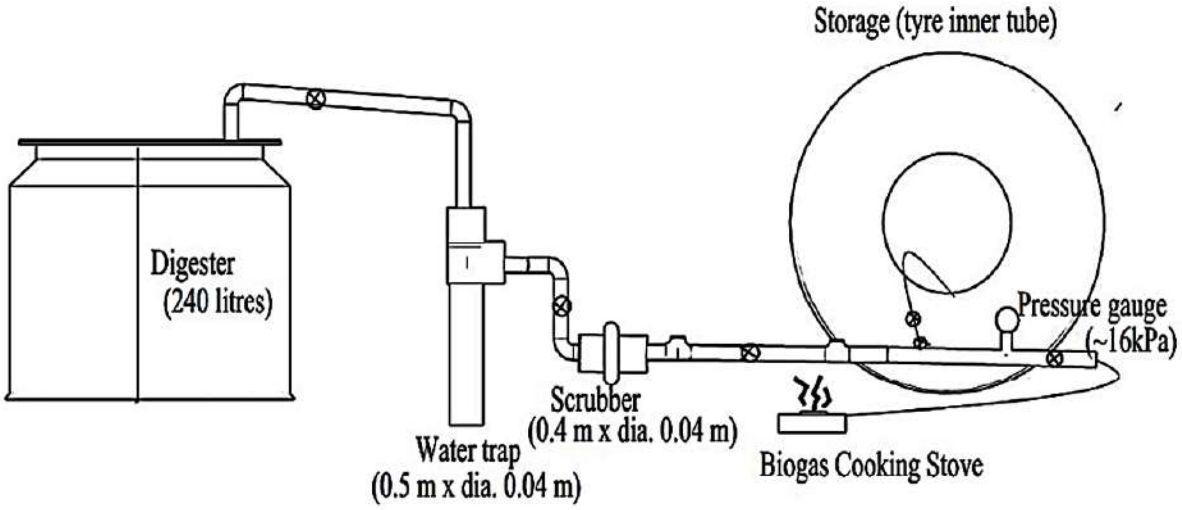
1. Materials and Tools Applied for Fabrication of the System

The materials used were fabrication consumables and items, including IPS plastic pipes (50 mm, 38 mm, and 13 mm diameter); standard plastic pipes (25 mm and 100 mm diameter. Other materials were the 240-litre plastic drum (2.2 mm wall-thickness), IPS fittings (13-38 mm diameter), bolt and nuts of N10 (8 mm diameter), flexible tube clamps, and a Grade S235JR - 25 x 2 mm flat bar (EU standard); 13 mm diameter flexible water pipes and thread seal tapes. Other materials used were putty sealants, gasket rubbers, plastic sheets, and pieces of inner tyre tubes. Additional materials were the 2.5 mm square wires and butyl tyre inner tubes (Korean Standard, made in India) of sizes: 8.25- 16, 9-20, and 12-24, all with valves type TR177A and 7.5-15 with valve type TR175A. The metalwork and plumbing workbench tools were used during the fabrication.

2. Fabrication and Assembly of the ADS and Its Components

Authors fabricated all selected components of the system except those selected during the second stage of selecting the WP, as their prototypes were utilised directly. Then the final finishing of all components was done. Afterwards, they were assembled by connecting the batch-fed digester, water trap, scrubber, biogas storage and biogas cooking stove to produce the whole ADS. Next, the steel wool and 140 ml of soft water were filled into the scrubber and water trap to end the processing phase. Finally, the complete ADS with the digester, the water trap, the scrubber, the storage, and the piping system was ready for use in the output phase of the approach as the adapted batch-fed anaerobic digestion (ABFAD) system. Figure 10 illustrates the complete connected ABFAD system ready for use or incorporated into the integrated renewable energy system.

Figure 10. Assembled adapted batch-fed anaerobic digestion system sketch



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The Formulation and Demonstration of Digester Operating-Status Indicators

The authors reviewed the literature related to the assessment of the working condition of the biogas plants to collect information regarding the challenges of the monitoring process of the plant indicators applied. In addition, they considered the application of biomass-fed digesters in the semi-arid areas of least developing countries. The review outputs resulted in selection variables representing the indicators for demonstration using the assembled ABFAD system. The selected variables are piping system and digester temperatures, system pressure, methane content, pH and dissolved oxygen (DO).

Furthermore, the authors demonstrated the application of the ABFAD system by conducting experimental testing involving data collection. The testing involved using biomass waste, which is cow dung. The cow dung had the following properties: dry matter (DM) and Carbon Nitrogen ratio (C/N ratio) of 228.93 g/kg of feed (standard deviation of 5.21 g/kg) and 19.30 (standard deviation of 0.56), respectively.

Instrumentation and Measurements for ABFAD System Testing

A list of essential instruments and tools used during this study include the following:

1. UGT Humidity and temperature (thermocouple Type-K) sensor with Relative Humidity (RH) measuring range of up to 100%, humidity accuracy of ± 2% and temperature measuring range of -30.00 - +70.00°C with the accuracy of 0.01°C. The sensor connects, picks signals, and transfers them to the UGT DL 386 data logger. The authors set the sensor to sample ambient air temperature values at the one-hour interval.
2. Micro Footprint Dissolved Oxygen (DO) monitoring subsystem equipped with the DO circuit of Version 4.0 for hardware and firmware (*Atlas-Scientific.com*), with a full range of DO readings from 0.01 to +35.99 mg/L and the accuracy of ± 0.2, set to sample data after 15 minutes interval,
3. The device applied to measure the acidity or alkalinity of the ageing substrate in the digester was the Micro Footprint pH-monitoring subsystem. The device is equipped with a pH circuit Version 4.0 for hardware and firmware (*Atlas-Scientific.com*), with full range pH reading from 0.01 to 14.00 and accuracy within two significant figures (XX.XX); set to sample data after 15 minutes interval.
4. Ultrasonic biogas flow meter Version BF-2000 with a flow range of 0.025 – 4.000 m^3/h, methane (CH_4) content percentage to the total volume of the biogas measurement range of 30-100% (accuracy is 0.01%), temperature measurement ranges from -20°C to 60°C (accuracy being 0.01°C), the working pressure of less than 100 kPa and explosion-proof grade of Ex ibiiat4 Gb; CH_4 content was measured at three-hour interval, and
5. Simgas pressure gauge encased in plastic material with a measuring range up to 16 kPa with an accuracy of 0.5 kPa.

A Solar PV module with a battery bank powers all electronic instruments. Furthermore, the authors calibrated all the instruments for measuring pH, DO, digester temperature, room temperature, ambient air temperature, CH_4 content, pressure and piping system temperature and fixed them to the ABFAD system. In addition, the authors inserted probes for measuring pH, DO, and the digester temperature deep to 0.7 m from the top of the digester and axially 0.1 m away from the central core. The probe insertion was followed by feeding the digester with cow dung—Figure 11 illustrates the experimental setup.

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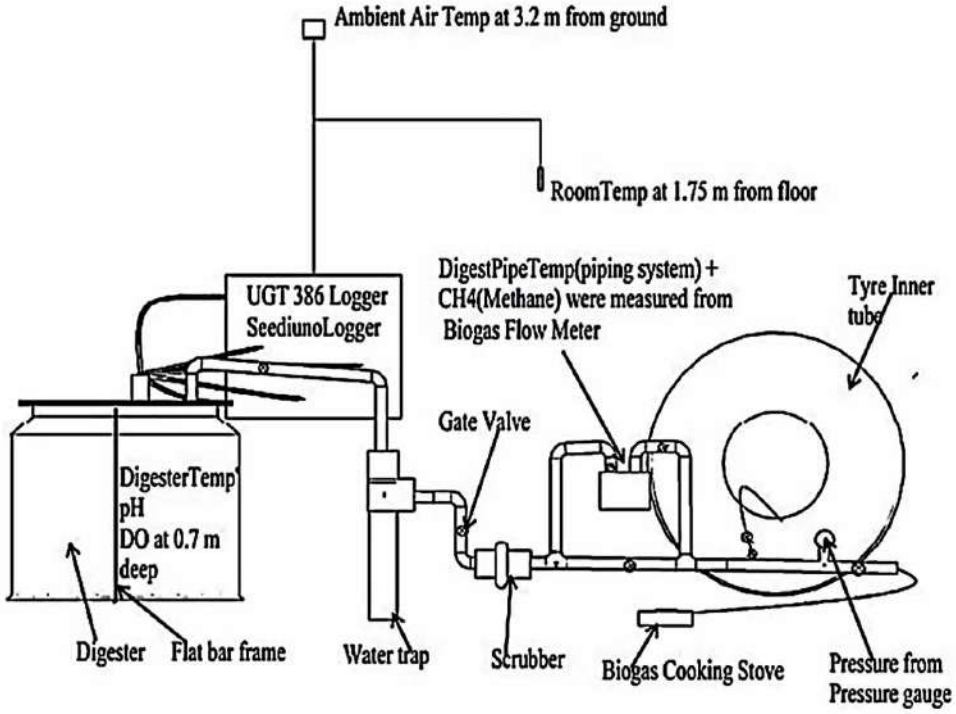
Figure 11. Experimental set up of adapted batch anaerobic digestion system



A room with a wall thickness of 0.3 m made of muddy materials (sandy clay soil) and lined with cement mortar accommodated the experiment. The inside, front and part of the outside left sides' room surface were lined. The room was slant roofed by corrugated iron sheets (gauge 30) with low and high heights of 2.5 m and 3.1 m, respectively. Its floor plan had 3.7 m by 5.1 m dimensions. The room had a screening floor (0.15 m thickness). Furthermore, the room had a window (0.6 m by 0.5 m dimensions) made of wooden frames and fitted with glass (2 mm thick). The room door (1.8 m by 0.8 m in dimensions) was made of timber and a corrugated iron sheet (gauge 30).

Furthermore, the collected data was analysed to get the average temperature, standard deviations, and rate of change of temperature (^{o}C/h) values. The rate of temperature change was determined by dividing the standard deviation temperature by the mean of a given factor by 24 hours of a day.

Experimental Test Results and Discussion

The results include the independent variables: ambient air temperature, temperature response variables of room, digester and piping system. In addition, the results included the CH_4 content in the biogas, pH, DO inside the digester and pressure of the whole system. These parameters are essential for the technical evaluation and running of the system.

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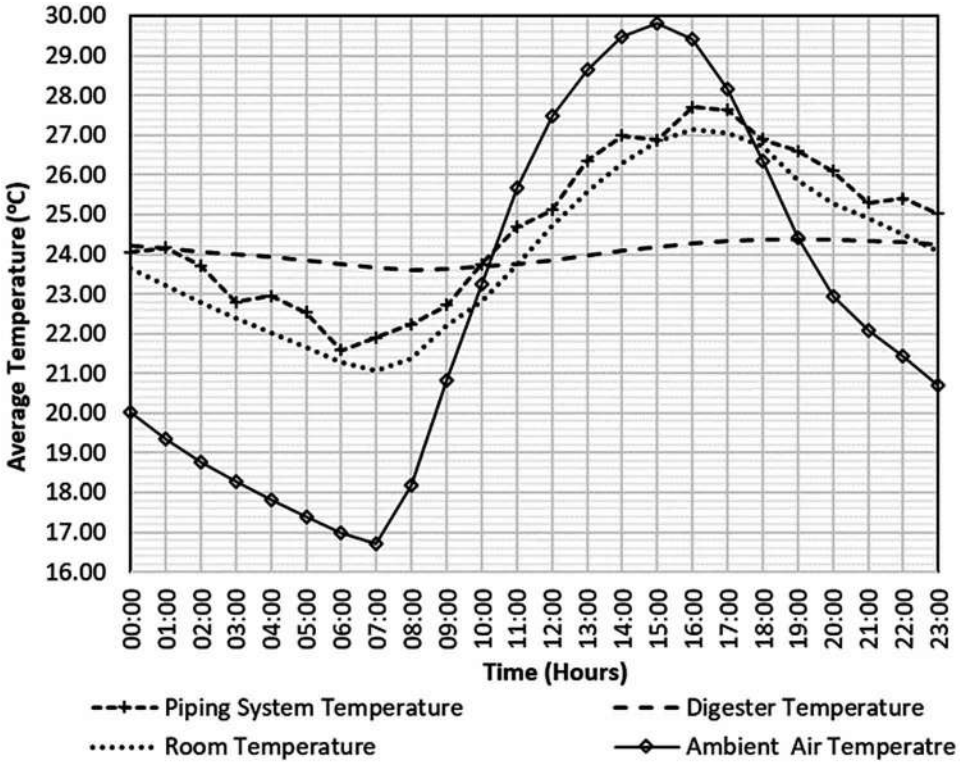
Temperature and CH_4 Content of the ABFAD System

The similar trends of the proportional relationship among temperature of the piping system, room, the digester and ambient air are shown clearly in Figure 12. The figure shows an analysis of the temperature data on an hourly basis within a day. Moreover, the figure shows that the piping-system temperature curve is not smooth compared to the other curves. The piping-system temperature curve indicates that temperature oscillates as it follows the trend of room temperature. The oscillation may be due to the intermittent flow of varying amounts of biogas at a low-temperature level through the piping system from the digester. Usually, the digester has a lower temperature compared to the piping system most of the time.

Figure 12. Hourly average temperature variations in ABFADS (Jun 16 - Oct 1/ 2017)



The authors computed the temperature change rate based on standard deviation from the mean temperature of the piping system, room, digester and ambient air. Table 5 shows the values of factors obtained for each of the ABFADS parameters. It shows that the temperature change rate inside the digester was the lowest with the value of 0.01°C/h, while ambient air was highest at 0.18°C/h. This revelation indicates that any ADS installed outside must be away from ambient air temperature variation, which has been the case for all biogas plants installed outside. The high-temperature variation affects biogas microbes adversely.

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Table 5. Temperature rate of change and other factors of ABFADS's parameters

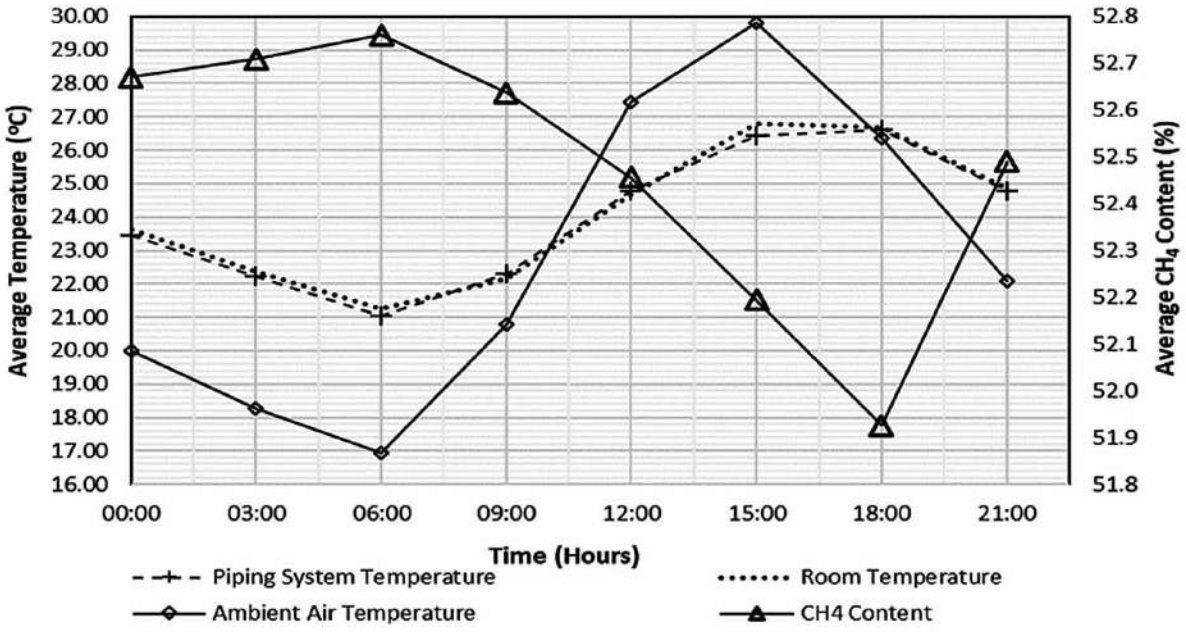
Factor (unit)	Parameter			
	Piping System	Digester Temperature	Room Temperature	Ambient Air Temperature
Mean temperature (°C)	24.71	24.04	24.05	22.67
Standard deviation (°C)	1.86	0.26	1.95	4.38
Temperature rate of change (°C/h)	0.08	0.01	0.08	0.18

Additionally, the average digester temperature of 24.04°C shown in Table 5 indicates that the AB-FADS was operating in the mesophilic phase of temperature, i.e. 20-39°C of anaerobic digestion. The optimum temperature for mesophilic microbes' performance is 35°C.

The digester's low temperature change rate indicates that the substrate mass exhibited insulation behaviour towards the center of the digester. This situation seems true, as the temperature probe for measuring digester temperature was close to the central digester core. Moreover, the room walls and air-insulated the digester from an extreme variation of ambient air temperature regarding the experimental setup. The phenomenon is evident from the closeness of temperature trends of room and digester piping systems and their rate of change (Figure 12 and Table 5).

The ambient air, room, and piping system temperatures are inversely proportional to CH_4 content between 00:00 and 15:00 hrs (Figure 13). Furthermore, the temperatures decrease at the different slopes with CH_4 content between 15:00 and 18:00 hrs before the ambient temperature decreases fast than a room and piping system temperatures. In contrast, CH_4 content increases fast to 52.65% in a range of 18:00-00:00 hrs.

Figure 13. CH_4 content and piping system, room and ambient air temperatures (Jun 25- Oct 1/ 2017)



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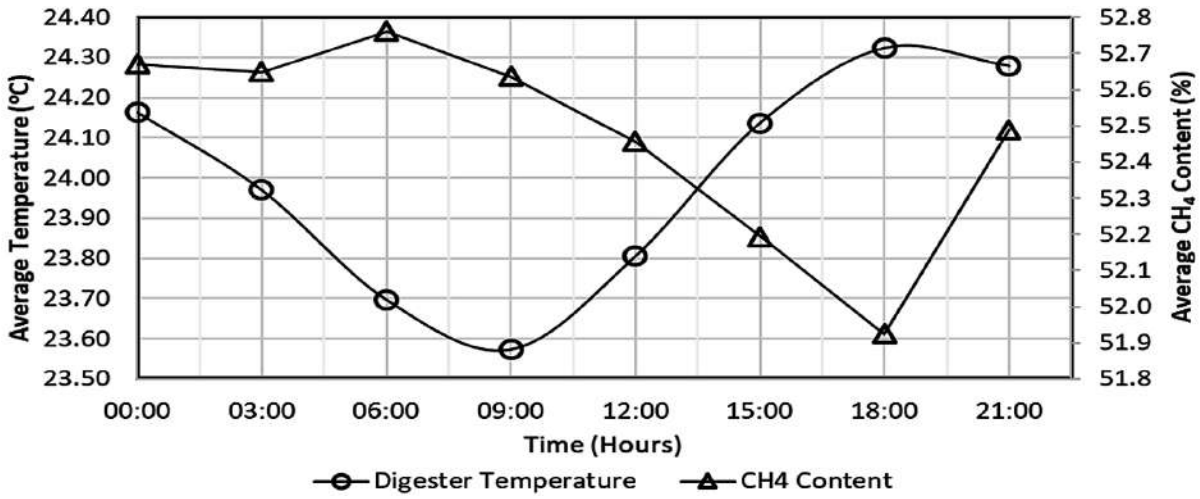
The digester temperature is directly proportional to CH_4 content in the 00:00 - 3:00 hrs and 6:00 -9:00 hrs. Between 18:00 and 00:00 hrs, the digester temperature slowly descends to 24.15°C while CH_4 content quickly ascends to 52.65%. In the remaining time ranges in Figure 14, digester temperature and CH_4 content are inversely proportional.

Figure 14. CH_4 content and digester temperature relationship (Jun 25- Oct 1/ 2017)



The average CH_4 content in generated biogas from 25/06/2017 to 01/10/2017 was 52.4%, with a standard deviation of 2.9%. Figure 14 shows that the room temperature and digester piping system increased as the average methane content decreased and vice versa.

CH_4 content was higher (averagely 52.8%, with a standard deviation of 0.3%) in the morning when the temperature was very low. Then CH_4 content decreased to 51.9% at 18:00 hours when the temperature inside the digester and the digester piping system was in the high range. This phenomenon may be due to the high solubility of carbon dioxide (CO_2), one of the principal constituents of biogas in the water at low temperatures. The solubility is given as moles of solute, i.e. CO_2 per 100 grams of solvent, e.g. water. Wu *et al.* (2006) reported that CO_2 has half of the solubility at 60°C compared to the same quantity of CO_2 when the temperature was 35°C and found this phenomenon after a laboratory-scale experiment. The aim was to assess the influence of temperature fluctuation on thermophilic anaerobic digestion of municipal organic solid waste. Therefore, this study's relationship between CH_4 content and temperature is supported scientifically.

Pressure, DO, and pH in the ABFAD System

There are various indicators of the functionality of the anaerobic digestion system, including pressure inside the digester. Pressure can indicate whether the system is leaking or not, as it was described by Oloche *et al.* (2017) on testing the underground ADS. In addition, pressure indicates the amount of biogas in the system. Figure 15 shows pressure variations of the ABFADS when the system leaks due to the failure of some pipe fittings. Pressure rose to maximum levels between 15:00 and 18:00 hours

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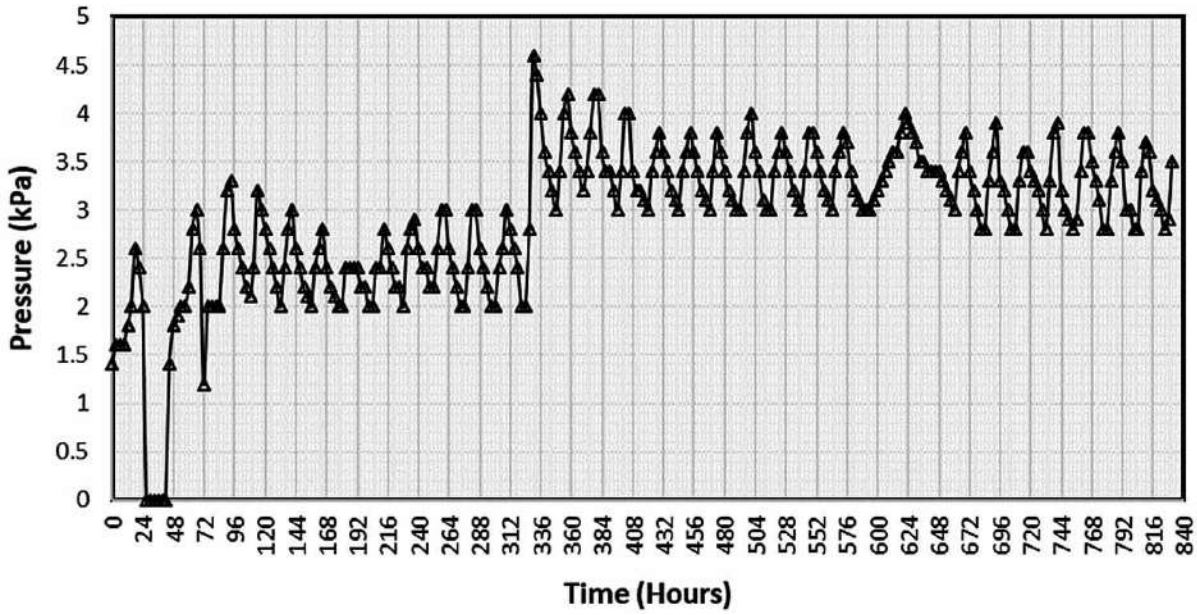
and then decreased afterwards before rising again from 9:00 hours. This phenomenon is indicated by the peak and trough of the pressure curve in Figure 15. Besides, the phenomenon is shown clearly in the expanded view in Figure 16. The curves in both figures (Figures 15 and 16) indicate leakage of biogas from the ABFADS, calling for immediate remedial measures.

Figure 15. Pressure variation of ABFADS (08/07/2017 (21:00 hours) - 10/08/2017)



Furthermore, the rising pressure in the daytime at high temperatures indicates more biogas generation than biogas leakage from the system. This condition may also imply that biogas generation microbes are very active, producing biogas at high temperatures, as shown in Figure 16. As temperature decreases, the generation of biogas declines. It implies the reduced activity of microbes to generate biogas, while the rate of biogas leakage is higher than the rate of biogas generation; hence, the pressure in the system drops. In the phenomenon of system leakage, the pressure was hardly above 4.5 kPa.

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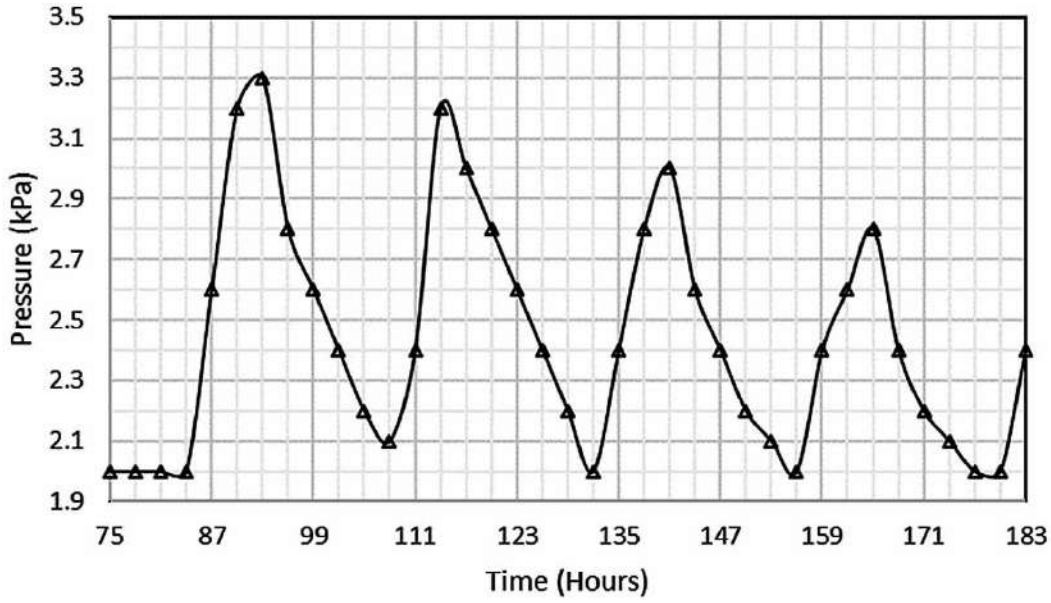
Figure 16. Pressure variation of ABFADS (12/07/2017 (00:00 hours) - 16/07/2017)



Window 24-48 hours, 75-81 hours, 321-327 hours, and 588-594 hours in Figure 15 indicate the time of remedying the fault pipefittings identified after troubleshooting. The pressure rise after window 321-327 hours indicates the improvement of sealing the biogas leakage. However, rising and falling pressure levels continued afterwards. Finally, at 831 hours, the pressure recording was stopped, and the piping system of ABFADS was disassembled and reassembled to replace all of its faulty pipe fittings. Generally, both pressure curves in Figures 15 and 16 have a shape similar to a sinusoidal curve presented by Kuhi *et al.* (2019).

Figures 17 and 18 illustrate the pressure variation in the ADS after fixing the faults causing leakage. The leakage occurred on the top lid. The rubber gasket of 2 mm thickness was replaced with that of 4 mm thick. In this case, a pressure drop did not occur without releasing biogas for the boiling test and replacing the biogas storage. The sudden drop in pressure seen in Figure 17 indicates the event of releasing generated biogas from the storage or replacing the biogas storage. In Figure 17, the pressure drops within windows of 12-24 hours and 228-240 hours were due to the change of biogas storage with the empty biogas storage. At the same time, the rest occurred due to boiling tests. However, the pressure rising rate decreased as the pressure level increased in the ABFADS (Figure 18). The situation might have been caused by the biogas compressibility, digester's biogas generating rate, and or physical elasticity property of the biogas storage. The biogas storage, i.e. inner tyre tube, was proven to safely withstand a pressure of up to 10.8 kPa (Figure 17 at 228 hours). Generally, the shape of increasing pressure curve before the pressure drop in the system is similar to that of the logarithmic curve (Figures 17 and 18). Grossman-Ponemon *et al.* (2019) have described the logarithmic curve.

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Figure 17. Pressure variation of ABFADS (12/09/2017(03:00 hours) - 01/10/2017)

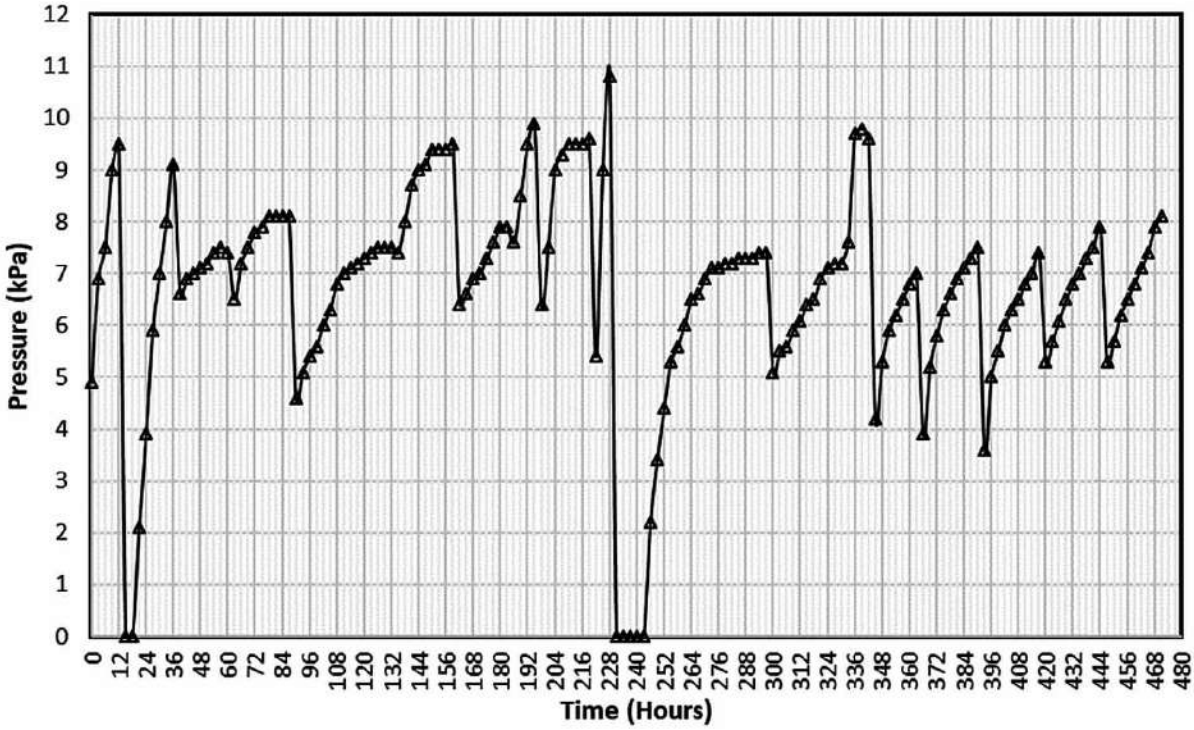
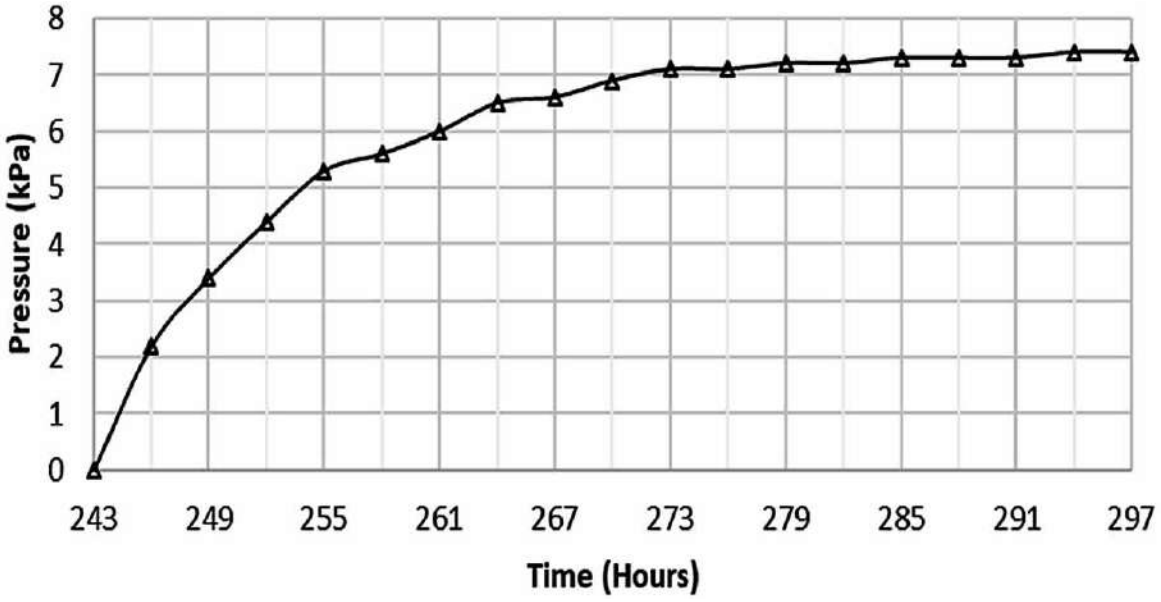


Figure 18. Pressure variation of ABFADS (22/09/2017(06:00 hours) - 24/09/2017)



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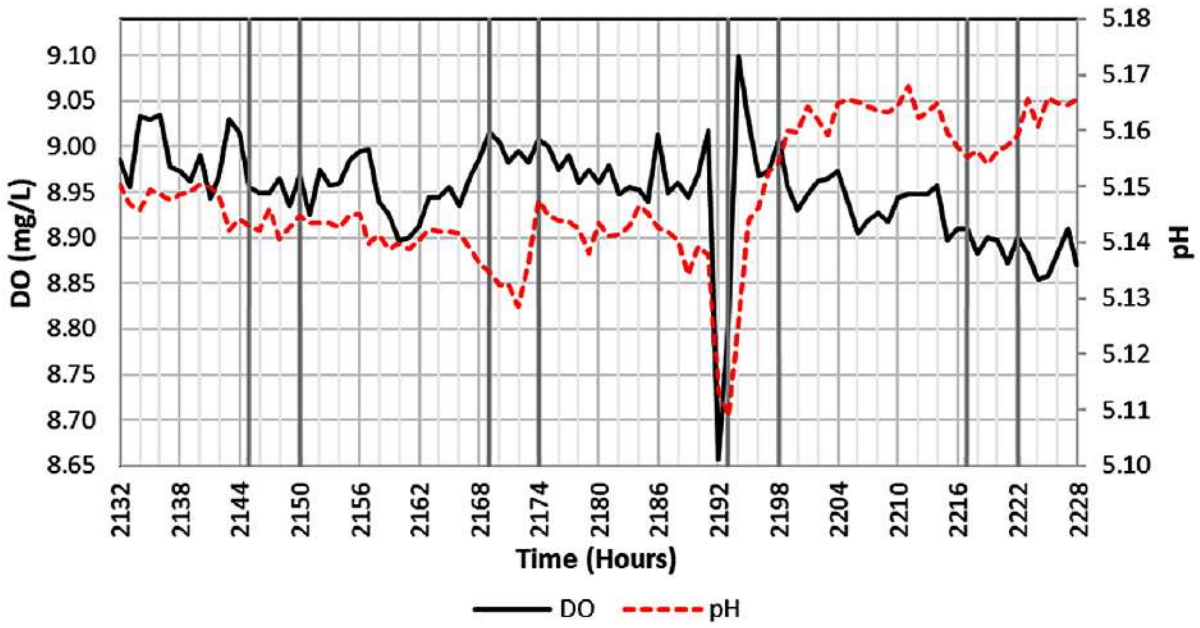
Generally, Figure 19 shows that pH dropped when the temperature was high in the afternoon. This condition is shown in windows 2144-2150 hours, 2168-2174 hours, 2192-2198 hours, and 2216-2222 hours, indicated with bolded vertical lines in the same figure. However, DO was not constantly dropping in the afternoon as pH did. This phenomenon is shown in windows 2192-2198 hours and 2216-2222 hours of Figure 19. Referring to the same figure and Figure 19, both DO and pH values were high when the digester temperature was low, i.e. from 19:00 to 11:00 hours. The digester's pH variation appeared to be influenced more by the digester temperature than the temperature did to DO. Generally, DO in the digester during testing was between 8.8 and 9.0 mg/L while pH was 5.11-5.15 range.

Figure 19. DO & pH versa time of ABFADS (13/09/2017 (00:00 hours) - 17/09/2017)



Although not tested, the durability of the ABFADS was envisaged to be more than 15 years if kept away from ultraviolet radiation and physical damage. However, the replacement of a valve made of iron-based material, located between the water trap and digester of ABFADS, is expected to occur after at least seven years (Sun and Nesic, 2007). The replacement is needed because biogas contains a trace of Hydrogen sulphide (H_2S) gas, which corrodes iron materials. The corrosion is caused by the reaction of iron with Sulphur found in H_2S.

SOLUTIONS AND RECOMMENDATIONS

The formulated approach of adapting the biomass-fed digestion system enables the development of the small-sized (with a digester of 0.24 m^3) ABFAD system. The system addresses designs and operational constraints when applying conventional biogas generation systems in RSAAs. Additionally, the system has the following features: no clogging of system biogas flow; easy handling of its auxiliary components

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like replacing of iron wool and portable biogas storage; withstanding appreciable pressure (10.8 kPa), and self-squeezing capability for easy release of quality biogas with a methane content of 52% ready for use.

The biogas leakage detection in the system using the pressure curves is reliable. The sinusoidal curve shape indicates system leakage, while the logarithmic curve form indicates no system leakage. The pressure values can be measured instantly using pressure gauge tools. The online pressure data collection and curve generation are done with the simple Arduino integrating circuit available in the market. Dissolved Oxygen and pH are indicators of the working condition of the ABFAD system's digester. However, pH is a more reliable indicator of the microbial activities in the system than DO, as the pH. Nevertheless, the DO-value tool can be applied in the absence of the pH-value indicator tool. Simple tools/devices are available for instant online measurement of pH and DO values inside the digester. Therefore, the pressure curves and pH values are appropriate for indicating the working condition of the biogas plant.

The following are recommended to system designers: applying the formulated approach of adapting the biomass-fed digestion system and integrating other renewable energy systems (solar and wind energy-based systems) with the ABFAD system for cooking energy and electricity generation. Also, the households in RSAAs to utilise the ABFAD system. Other recommendations are applying the ABFAD system's pressure in the sinusoidal-curve shape (leaking) and the logarithmic-curve form (not leaking) for identifying the system's operating status, and the system operators to utilise the pH parameter as a reliable indicator of the operating status of the ABFAD system or similar systems

FUTURE RESEARCH DIRECTIONS

The focus of this chapter unlocks future research potential on biomass and climate change issues. The research potentials are investigating the simple-cheap materials, optimising their amount fed in the auxiliary parts of the ABAFAD for improving the quality of the biogas generated, and studying the established operating status indicators of the ABFAD system using other types of biomass.

CONCLUSION

The challenges related to the existing design of the biomass-fed digester and the digester operating status indication are considered in this chapter. In addition, the solutions to address the challenges are devised and demonstrated. The solutions are the formulated explicit conceptual design approach for adapting the biomass-fed digester and the appropriate indicators of the operating condition of the digester for immediate fault detection. The solutions support the theme of Biomass and Bioenergy Solutions for Climate Change Mitigation and Sustainability. The solutions are recommended to the designers and operators of the digester, similar to the ABFAD system.

ACKNOWLEDGMENT

The German Federal Ministry of Education and Research (BMBF) and the Federal Ministry for Economic Cooperation and Development (BMZ) supported this research through the Trans-SEC project, with Project ID No.: 031A249 A-G. The above are ushered with gratitude for this vital support. However,

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the views expressed in this chapter are purely those of the author and, in any circumstances, may not be regarded as stating an official position of BMBF or BMZ.

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KEY TERMS AND DEFINITIONS

Adaptation: The reference to a process of varying the system or structure design to get its improved design with high performance for the betterment of the user or community.

Conceptual Designing: An act of extensive outlining of purposes and forms of a system or structure to be established for facilitating the conception of a clear user interface to understand and interpret the system or structure.

Detailed Designing: The process in which the generated system design is refined where plans, specifications and estimates are created and ready for manufacturing or construction.

Rural Semi-Arid Areas: In the sub-Saharan Africa context, a land of marginal crop production potential, quick maturing cereals, and carrying natural vegetation of Acacia-Themeda association but including dry Brachystegia woodland. It is a potentially productive grazing area, limited by bush encroachment, leached soils, and inadequate water.

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Chapter 18
Pretreatment of Lignocellulosic Biomass and 2G Ethanol

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ABSTRACT

Rapid depletion of fossil fuel-based energy sources increased the demand for alternate energy sources. Lignocellulosics-based 2G ethanol can be used as an alternative sustainable source that presents in ample amount. Sources of lignocellulose biomass are wood, food-agriculture wastes, and forest residues. Cellulose, hemicellulose, and lignin are the core components of lignocellulosic biomass. Cellulosic and hemicellulosic biomass are enzymatically hydrolyzed to produce the monomer sugar (such as glucose or xylose) which is further converted into ethanol using fermentation process. The presence of lignin provides physical barrier that limit the access of enzymes required for saccharification. Pretreatment helps in removing the lignin from biomass and reducing recalcitrance. Pretreatment can be done by conventional methods, which are chemical, physical, and biological. This study covers the different methods of pretreatment including their disadvantages and benefits along with saccharification and fermentation processes.

INTRODUCTION

Globally, the rapid use of energy causes the rapid reduction of fossil fuel-based energy sources. Also, the chief concern with its frequent use is that it releases harmful (unfavourable) gases like methane, CO, CO_2, nitrous oxide, etc. which are known as greenhouse gases (Houghton, 2005). Since these energy sources are vanishing, we need to emphasis on finding its sustainable alternative source. The goal should be to develop a long-term sustainable energy source that emphasizes “recovery and reuse” of bio-derived raw materials.

DOI: 10.4018/978-1-6684-5269-1.ch018

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Lignocellulosic biomass (LCB) has acknowledged a lot of interest as a renewable, sustainable energy source. LCB has the potential for meeting future energy requirements while posing no environmental risks (Ahmad *et al*.,2020). Wheat straws, rice husk, sugarcane bagasse, forest and agricultural wastes, industrial and urban trash might all be good sources of lignocellulosic biomass (LB) (bioethanol) (Zheng *et al*., 2014; Amin *et al*., 2017).

Traditionally, sugar and starch-based feedstocks including sugarcane juice and molasses, as well as corn, have been used to make bioethanol (Balan *et al*., 2013). Bioethanol is currently being generated on a commercial scale using lignocellulosic feedstocks (Balan *et al*., 2013). LBM is the most abundant raw material found in nature which includes sources like hardwood, grasses, softwood, agricultural wastes, etc. Newsprint, office paper, municipal solid wastes, and other raw materials might be of interest as well. Starch is a simple substrate, whereas lignocellulosic biomass is more complicated. Second generation (2G) ethanol is less trending when compared to the first generation ethanol, and it is still less economical due to its high cost (Furlan *et al*., 2013; Macrelli *et al*., 2014). Several research of sugarcane bagasse-based 2G ethanol production techniques have been conducted. In one study, enzymatic hydrolysis of sugarcane bagasses after diluted acid pretreatment produced fermentable sugar for high yield bioethanol production (Benjamin *et al*., 2013; Albuquerque *et al*., 2013).

Lignocellulosic biomass is made of three key components which are cellulose, hemicellulose, and lignin. In which cellulose is the polysaccharide present in great amount therefore known as the most abundant LBM. Cellulose is the linear chain of glucose linked via β-1,4 glycosidic linkages. Hemicellulose is branched heteropolymer whereas coniferyl alcohol, sinaphyl alcohol and p-coumaryl alcohol make form the three-dimensional macromolecule lignin. Lignin causes the recalcitrance in the LBM (Alonso *et al.,*2012).

Since this recalcitrance of lignocellulose biomass is being the hurdle in bioethanol production, so, it should be reduced to get efficient yield of bioethanol. The recalcitrance of biomass can be reduced by using numerous pretreatment methods like chemical pretreatment, biological and physical pretreatment. By doing pretreatment we can increase the enzyme accessibility and hydrolysis of cellulose and hemicellulose polysaccharides, which further leads to higher bioethanol yield.

LIGNOCELLULOSIC BIOMASS

Lignocellulosic biomass is ubiquitous and sustainable resource which is composed of polysaccharides (cellulose and hemicelluloses) and an aromatic polymer (lignin) (Fengel *et al*., 1984). The percentage of cellulose, hemicellulose and lignin varies with respect to sources (Table 1).

COMPONENTS OF LIGNOCELLULOSIC BIOMASS

Lignin, hemicellulose and cellulose together makes the lignocellulose biomass. The main chain of cellulose is made up of D-glucose subunits joined by β-1,4 glycosidic linkages (Fengel *et al.*, 1984). Cellulose is present in the cell wall of the plants and is the most abundant biomass. Cellulose is 'bundled' into cellulose fibrils or cellulose bundles (fig 1) and are interlinked to each other through van der Waals and hydrogen bonds (Fengel *et al*., 1984). These cellulose fibrils are generally self-contained and have just a weak hydrogen bonding connection (Fengel *et al*., 1984).

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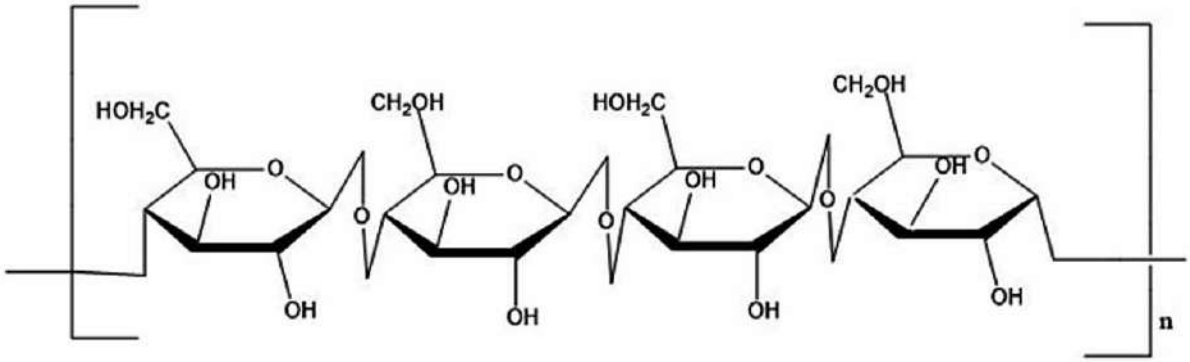
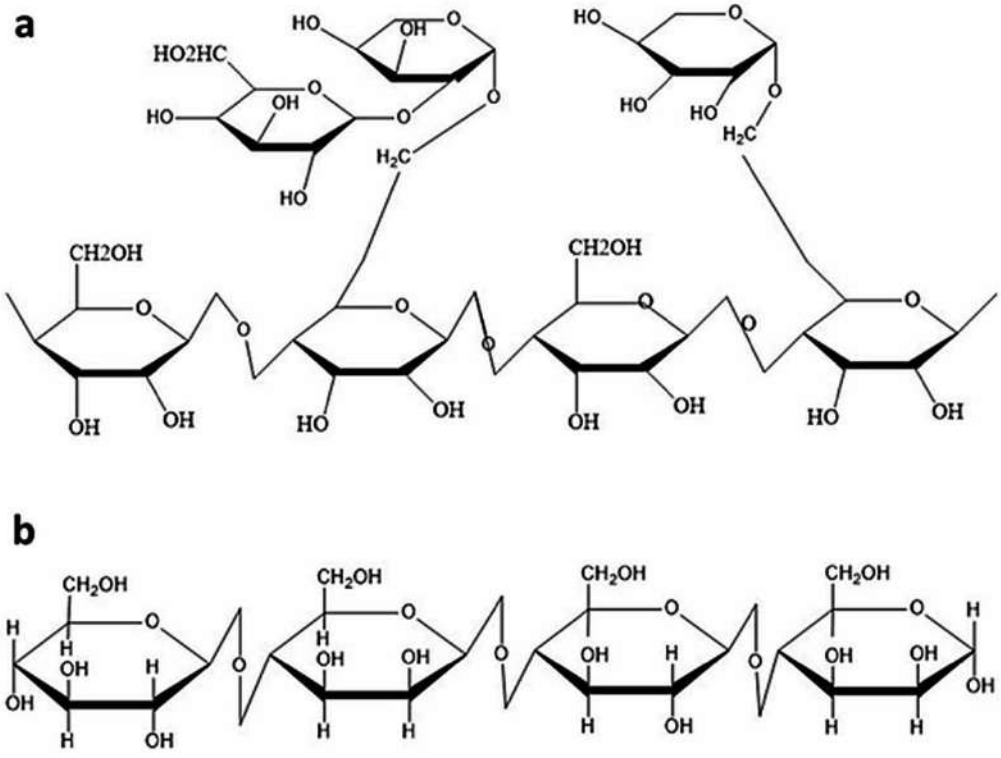
Figure 1. Structure of Cellulose



Xylose, arabinose, and other pentoses, as well as hexoses like mannose, glucose, and galactose, along with sugar acids make form the complex carbohydrate structure known as hemicellulose (fig. 2) (Grabber, 2005). In hardwood and agricultural plants like grasses and straw, xylan is the most prevalent kind of hemicellulose., whereas glucomannan is the most abundant component in softwood (Grabber, 2005). Lignin and cellulose fibers are connected with hemicellulose and this linkage increases the stiffness of the entire cellulose–hemicellulose–lignin complex (Levan et al., 1990; Winandy, 1995). Hemicelluloses are the more thermally reactive compared to cellulose and lignin, because, It's solubility increases as the temperature rises. (Levan et al., 1990; Winandy, 1995).

Figure 2. Structure of Hemicellulose a) Xyloglucan b) Glucomannan



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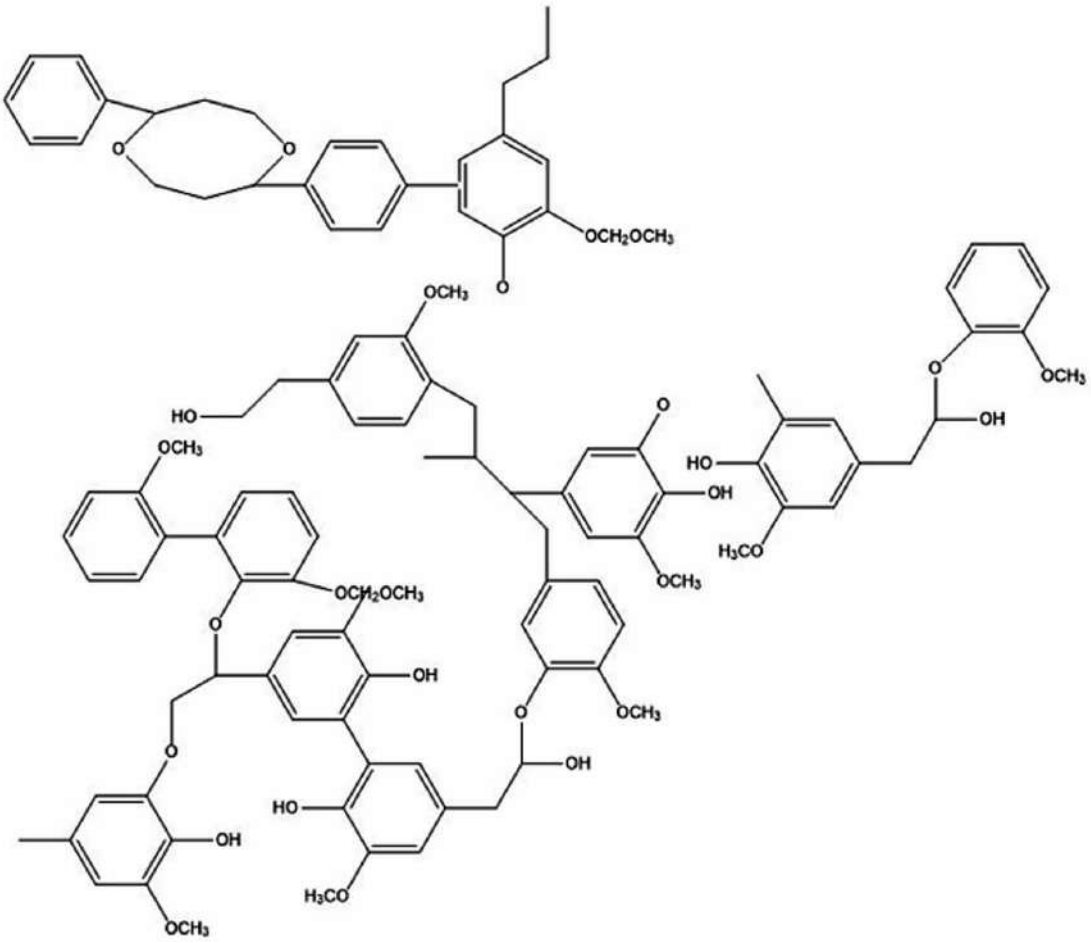
Plant cell walls include lignin, which is one of the plentiful polymer in nature (fig.3), along with hemicellulose and cellulose. Three different phenylpropane units (p-coumaryl, coniferyl, and sinapyl alcohol) together forms the amorphous heteropolymer, which are joined together by various connections (Fengel *et al*., 1984). The key role of lignin in plant cell wall is to provide support to maintain the structure of cell walls and to maintain the impermeability and oxidative stress also, it gives resistance to microbial assault (Grabber, 2005). Under neutral circumstances, lignin dissolves into water at 180^0C (Bobleter,1994). But the lignin's precursor decides whether it dissolves in an acidic, neutral, or alkaline environment (p-coumaryl, coniferyl, sinapyl alcohol, or mixtures thereof).

Figure 3. Structure of Lignin



SOURCES OF LIGNOCELLULOSIC BIOMASS

To date, the majority of ethanol produced as a biofuel has come from edible crops, either through the fermentation of sugars or the conversion of starch from corn (Hill et al. 2006; Chandel and Singh 2011). The most accessible renewable resource on earth is the cell wall of a plant. A long-term option for the

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production of biofuels is therefore thought to be the manufacture of ethanol from non-crop plant cell walls or non-edible plant parts. Forages, in particular cultivated grasses, can be considered as significant prospects for the generation of cellulosic biomass, and they look to be very promising as a future biomass sources among the numerous kinds of biomass accessible for ethanol synthesis (Hill et al. 2006; Chandel and Singh 2011). The predominant herbaceous energy plants that have been chosen for the production of bioethanol (*Pennisetum purpureum*) include switchgrass (*Panicum virgatum*), miscanthus (*Miscanthus spp. Anderss*.), canary grass (*Phalaris arundinacea*), giant reed (*Arundo donax L.*), alfalfa (*Medicago sativa L.*), and Napier grass (Hill et al. 2006; Chandel and Singh 2011). Willow, eucalyptus, silver maple, eastern cottonwood, green ash, black walnut, sweetgum, and sycamore are a few examples of fast-growing, short-rotation forest trees that can be utilised as a feedstock for biofuels (Hadar et al., 2013). Sources of woody materials include natural forest wastes, forestry wastes including sawdust from sawmills, wood chips, and branches from dead trees, as well as short rotation energy forest plantations that use a variety of fast-growing tree species (Hadar et al., 2013). Municipal Solid Waste (MSW), which consists of paper and cardboard, food scraps, and garden waste, can be converted into biofuel, but it is not the best feedstock because of the variety of MSW components, as well as the incomplete source separation and contamination (Li et al., 2007).

Rice straw, wheat straw, maize stover, and sugarcane bagasse are the agricultural wastes that are most readily available in terms of biomass (Kim *et al*., 2004). One of the most significant agricultural crops in countries with tropical climates is sugarcane. Bagasse, the byproduct of sugarcane obtained after crushing the sugarcane to get the juice, is required for the production of sugar and ethanol. It has an advantage over other crop leftovers since it saves the time and resources (Chandel *et al*., 2012).

Table 1. Percentage of hemicellulose, lignin and cellulose in different LCB sources.

Sources	Lignin %	Hemicellulose%	Cellulose %	References
Sugar cane	20–42	19–25	42–48	Kim *et al.,*2011
Rice straw	12–14	23–28	28–36	Saini *et al*.,2015
Corn fiber	8.4	16.8	14.28	Mosier *et al.,*2005
Switch grass	12	31.4	45	Sun *et al.,*2005
Cattle manure	2.7–5.7	1.6–1.33	16–4.7	Sánchez *et al.,*2009
Wheat straw	17–19	26–32	33–38	Saini *et al*.,2015
Hardwood	18-30	25-40	40-50	Sun *et al.,*2005
Alfalfa stalks	16.6	6.5	48.5	Chan *et al.,*2007
General MSW	10–14	49.9–16	33	Li *et al.,*2012
Napier grass	9	20	32	Saxena *et al*.,2009
Food waste	11.4	7.2	55.4	Schmitt *et al.,*2012
Miscanthus	24.1-25	18-24.3	38.2-40	Brosse *et al.,*2010
Newspaper	18-30	25-40	40-55	Howard *et al.,*2003
Corn stover	7-19	24-26	38-40	Zhu *et al*.,2005
General grasses	10-30	25-50	24-40	Saini *et al*.,2015

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PRETREATMENT

Hydrolysis of polysaccharide components to soluble sugars, which may then be fermented to produce desired end products, is used to produce fuels from renewable lignocellulosic sources (Singh *et al*., 2015). Because such materials are only partially digestible in their natural state, some sort of pretreatment is usually necessary to make the carbohydrate component amenable to enzymatic hydrolysis (Singh *et al*., 2015). Degradation of lignocellulosic biomass is difficult due to its intrinsic recalcitrance. Pretreatment disrupts the lignocellulosic biomass's tightly packed structure and reveals the fibres made of cellulose and hemicellulose (Singh *et al.,* 2015). Using a mix of chemical and structural changes to the lignin and carbohydrates, the lignocellulosic material is processed in order to overcome recalcitrance (Singh *et al.,* 2015). The diverse composition and refractory nature of the cellulosic fraction of the biomass provide major challenges in the conversion strategy. Pretreatment is used to render cellulosic and hemicellulosic biomass susceptible to enzyme activity (Bharat *et al*., 2013). The main factors influencing accessibility and digestibility are cellulose crystallinity, hemicellulose disruption, accessible surface area (porosity), lignin protection, and cellulose-hemicellulose-lignin linkage.

Hemicellulose and lignin are ubiquitous polymers, while cellulose is the primary contributor to the crystalline component. Lignin serves as a barrier to prevent the deteriaration of cellulose and hemicellulose. Because lignin and hemicellulose are chemically related through covalent bonding, removing lignin will result in the elimination of hemicellulose as well. To improve the specific surface area and crystallinity of cellulose, it requires pretreatment to eliminate these amorphous polymers (Y.P *et al*.,2013).

Fractionation and delignification are two popular pretreatment methods for this lignocellulosic material. Fractionation is a process for separating the components of lignocellulosic biomass by disturbing the biopolymer pattern to enhance access to polysaccharides in lignocellulosic biomass. Delignification is used to remove lignin, although it can also separate hemicellulose fractions. To extract lignin for exposing cellulose to enzymatic hydrolysis, delignification is usually incorporated in the fractionation process. Physical, chemical, biological and physicochemical methods are some of the pretreatment methods (fig 4). Physical pretreatment method includes size reduction, microwave irradiation, etc. Chemical pretreatment method includes alkaline, acid and organic solvent methods. Physicochemical pretreatment methods are hot water, steam explosion whereas biological pretreatment methods deal with microorganisms. Advantages and disadvantages of different types pretreatment methods are shown in table 2.

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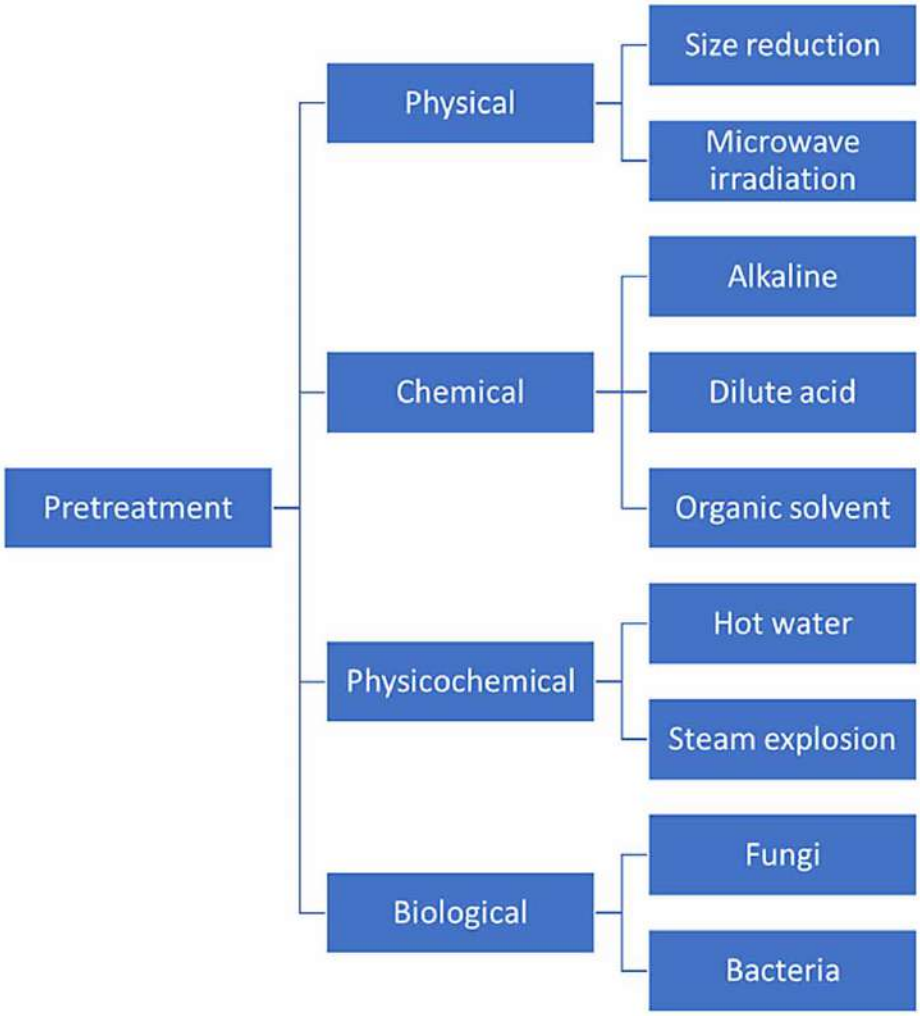
Figure 4. Types of pretreatment methods



1. Physical Pretreatment Methods

These techniques are employed to change the size of the biomass and break the crystal structure of LBM.

a) Size Reduction Pretreatment Method

Size reduction is a common approach used for pretreatment of lignocellulose biomass through which polymerization and cellulose crystallinity is lowered. The size of biomass can be reduced by employing various physical size reduction methods like milling (using a ball or hammer and vibratory rod/ball milling), shredding, chipping and grinding (J.Y *et al*., 2013). The amount of power required for mechanical size reduction is determined on the basis of the moisture content and the size of the biomass. As a result, particle size has an impact on specific energy consumption. Because of the need for high mass and heat transfer efficiency, the design of handling, transportation, and conversion facilities will

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certainly be determined by the selection of an appropriate particle size of the biomass (Barakat *et al*., 2013; J.Y *et al*., 2013).

b) Microwave Irradiation Pretreatment Method

Microwave irradiation for lignocellulosic biomass is another type of physical preparation. The remarkable heating efficiency and simplicity of this pretreatment method, which has been improved over time, are well known. Physical, chemical, and biological processes speed up as a result of heat and many collisions brought on by polar molecule oscillation and ion movement. The lignocellulosic material's performance is influenced by its dielectric properties. The ability of a material to convert electromagnetic energy into heat is measured by the dielectric loss factor, as opposed to a material's capability to store electromagnetic energy (H. Ma *et al*., 2009).

2. Chemical Pretreatment Methods

The recalcitrant structure of lignocellulose materials can also be changed by treating with chemicals. These pretreatment methods majorly include alkaline method, dilute acid method and organic solvent method to reduce the crystallinity.

a) Acid Pretreatment Method

Acid pretreatment is used primary to remove hemicellulose from lignocellulose biomass. Enzymes are more easily accessible to cellulose after pretreatment of biomass using an acidic method (Velmurugan *et al.,* 2011). Sulfuric acid, acetic acid, and phosphoric acid are the three acids that are used for pretreatment the most frequently (Rezania *et al.,*2020). Acid pretreatment can be carried out in two distinct ways: concentrated acid pretreatment at comparatively lower temperatures, and diluted acid pretreatment at higher temperatures (Rezania *et al.,*2020). By-products of acid pretreatment include 5-hydroxymethyfurfural and 2-furfuraldehyde. Peracetic acid pretreatment can also successfully remove lignin and produce hemicellulose breakdown, revealing cellulose. Acid pretreatment eliminates almost all carboxylic acid changes, including acetyl groups and uronic acids. (Park *et al*., 2012; Zhao *et al*.,2008). It removes the hemicellulose component and promotes the crystallinity of the biomass (Zhao *et al*.,2008). The residence time might last anywhere between a few seconds and minutes, depending on the pretreatment's temperature. In a stainless-steel reactor, an aqueous substrate solution is heated to the necessary temperature and then processed with warmed sulfuric acid.

b) Alkaline Pretreatment Method

For the pretreatment of biomass, hydroxides of potassium (K), sodium (Na), ammonium (NH_4), and calcium (Ca) are used in alkaline pretreatment method. This pretreatment technique causes the biomass to swell, increasing its internal surface area while decreasing its level of polymerization and cellulose crystallinity (Chandra *et al.,* 2007). Alkaline pretreatment alters the structure of lignin and removes the bonds that bind it to the other carbohydrate fractions, making the heteromatrix's carbohydrates more accessible. As the lignin is eliminated, the efficiency of polysaccharide enzyme hydrolysis rises (Galbe *et al.,* 2007). Alkali pretreatments also get rid of acetyl and other uronic acid substitutions on hemicellulose

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that make the surface of the cellulose less accessible to enzymes (Mosier *et al.,*2005). When, compared to acid pretreatment with HCl and H_2SO4, alkaline pretreatment with NaOH generally removes more lignin (Zhao *et al*.,2008). There are no by-products of the alkaline treatments. It results in increased lignin removal, biomass porosity, and cellulose swelling. Almost all carboxylic acid substitutions will be eliminated by alkaline pretreatment (Garlock *et al*., 2011; Park *et al*., 2012; Zhao *et al*.,2008).

c) Organic Solvent Pretreatment Method

The organosolv procedure is another appealing chemical pretreatment. This method has been widely used to extract high-quality lignin. Greater biomass conversion results from the removal of lignin from the biomass because this makes the cellulose fibres available to enzymes for hydrolysis and limits the amount of lignin that cellulolytic enzymes can digest. (Wormeyer *et al*.,2011). In this process mixture of aqueous organic solvent (ethanol, methanol, ethylene glycol, and acetone) is added with or without use of catalyst (Acid, base, or salt) along with high temperature. By doing so, linakages of hemiceelulose and lignin are cleaved due to solubilization which improves the surface area overall for enzymatic hydrolysis of cellulose (Hage *et al*.,2009 ; Wormeyer *et al*.,2011).

3. Physicochemical Pretreatment Methods

a) Liquid Hot Water Pretreatment Method

This pretreatment method uses H_2O at high temperature to reduce the crystallinity of lignocellulosic biomass. Using this technique, lignin is removed and the hemicellulose is hydrolyzed along with the cellulose making them easily accessible to the enzymes. The hemiacetal linkages are broken down followed by acid liberation during the hydrolysis of biomass by hot water (Antal,1996). Because lower temperatures are used, there is less chance of degradation products forming. And since, the pretreatment solvent in this case is water, there is no need for a final washing or neutralising step. For large-scale applications, the solvent's low cost is also a benefit.

b) Steam Explosion Pretreatment Method

In Steam explosion process, before being physically treated with high-pressure saturated steam in the steam explosion process, biomass is chipped, crushed, or just left raw. Because of the high temperature, the hemicellulose breaks down and lignin is transformed, enhancing the possibility for cellulose hydrolysis (Agbor *et al*., 2011). Poplar plant chips processed by steam explosion showed enzymatic hydrolysis efficiency of 90% in 24 hours, while untreated chips showed only 15% hydrolysis efficiency (Grous *et al*.,1986). The increase in enzymatic efficiency gave the clear idea about the role of steam explosion in the LBM's pretreatment.

4. Biological Pretreatment Methods

Biological pretreatment methods include the use of Fungi and bacteria. Fungi like White-rot fungi (*Dichmitus squalens, Phlebia radiata, Rigidosporus lignosus, Phanerochaete chrysosporium,* and *Jungua separabilima*), brown-rot fungi (*Serpula lacrymans, sulfur shelf, Fomitopsis pinicola and Phae-*

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olus schweinitzii), soft-rot fungi (*Chaetomium, Ceratocystis,* and *Kretzschmaria deusta*), and bacteria (*Clostridium thermocellum, Caldicellulosiruptor saccharolyticus, Caldicellulosiruptor bescii* (Putro *et al., 2016)* are used to change chemical composition of LBM to make it more enzymatically digestible. Fungi degrade lignocellulosic biomass in different ways. White-rot fungus breakdown lignin more aggressively, whereas brown and soft rots fungi target cellulose and showed less effect on lignin (Putro *et al., 2016*). Degradation of lignocellulosic materials has been explored using many microorganisms with thermostable or thermophilic activity such microorganisms are called thermophilic microorganisms. These microorganisms provide a number of benefits, including a faster hydrolysis time, great tolerance to low and high pH, reduced contamination risk, and cheap energy costs.

Table 2. Advantages and Disadvantages of various pretreatment methods.

Pretreatment Methods	Advantages	Disadvantages	Reference
Size reduction	Disrupt biomass crystallinity, reduces the degree of polymerization	Larger the particle size lowers the crystallinity.	Barakat *et al*.,2013; J.Y *et al*., 2013.
Microwave irradiation	Facilitate the disruption of recalcitrant structures	High cost	H. Ma *et al*., 2009
Alkaline method	Lignin structure is disrupted	Highly costly and used for small scale only.	Park *et al*., 2012; Zhao *et al*.,2008
Organic solvent method	Hydrolyses hemicellulose and lignin.	High cost	Hage *et al*.,2009
Acid method	High yield of end product (sugars)	Degradation products are generated.	Zhao *et al*.,2008
Hot water method	No need to reduce size of biomass, chemicals	Toxic compounds are formed.	Bobleter, 1994
Steam explosion method	Alters lignin and solubilize hemicellulose	Partially degrade hemicellulose	Mes-Hartree *et al.,* 1988
Biological method	No requirement of chemicals. Causes delignification	Process is slow and not very efficient.	Putro *et al., 2016;* Maurya *et al.,*2015

2G ETHANOL

The biofuel with the greatest potential to replace oil-based fuels is second-generation (2G) ethanol made from lignocellulosic biomass (Seabra *et al*.,2010). The stages involved in producing ethanol from lignocellulosic biomass are as follows: 1) pretreatment of lignocellulosic biomass 2) saccharification 3) fermentation as shown in fig 5.

Because LBM's high resistant structure restricts the accessibility of cellulose and hemicelluloses to hydrolytic enzymes and prevents the release of sugars, it hinders the enzymatic hydrolysis process. To, achieve effective and quick enzymatic hydrolysis of carbohydrates, biomass must be pretreated to change its structure (Mosier *et al*., 2005). Lignocellulosic ethanol production involves the enzymatic degradation of cellulose and hemicelluloses into fermentable sugars (Pan *et al*., 2005). The composition and structure of the feedstocks, the pretreatment technique utilised, and the kind of enzymes used are all factors that affect this stage. Enzyme costs and catalytic efficiency are a key stumbling block to enhancing the bioethanol industry's profitability. Optimizing enzyme mixtures for enzymatic hydrolysis

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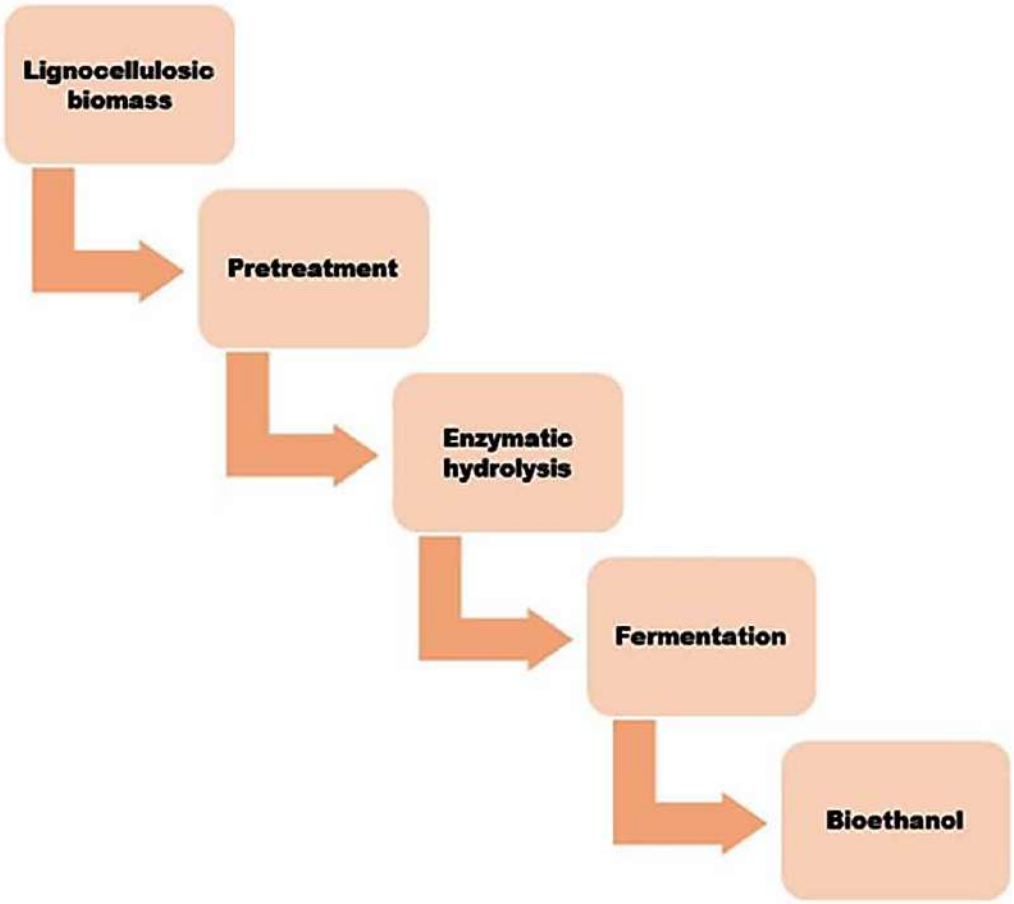
is crucial for the economic viability of lignocellulosic ethanol production. Now in case of saccharification and fermentation, it can be done separately or simultaneously.

Figure 5. Production of Bioethanol



a) Hydrolysis of LBM

Fermentable sugars from hemicellulose and cellulose is obtained through the hydrolysis process. Hydrolysis process is of two types namely acidic and enzymatic. The process in which acid is used is called acid hydrolysis and enzymatic hydrolysis involves enzyme (Jatoi *etal.,*2021). In order to acquire a higher yield of fermentable sugars, which are then turned into ethanol, hydrolysis is one of the important steps in the manufacturing of ethanol.

i) Enzymatic Hydrolysis

Either commercial enzyme systems, which are more feasible and often used on an industrial scale, or microorganisms that release enzymes into the media can produce enzymatic hydrolysis. (Klein *et al.,*

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2012). But for industrial utilization the only drawback is its higher enzyme cost (Klein *et al.,*2015). Hemicelluloses, have complex structure in comparison to cellulose. While cellulose is not that complex structure but still there is need of several enzymes for complete hydrolysis. Hemicelluloses include xylans, mannans, xyloglucans, and glucomannans, as well as β 1-3,1-4 glucans which are hydrolysed by their respective enzymes like xylanase, mannanase and β-glucnase etc., whereas cellulose is hydrolyzed by cellulase enzyme, which includes endoglucanase, exoglucanase, and glucosidses.

ii) Acid Hydrolysis

Acid hydrolysis is called the chemical hydrolysis. Acid hydrolysis method is advantageous in comparison to enzymatic hydrolysis since reaction rate achieved is higher than enzymatic hydrolysis (Cheah *et al.,*2020). Dilute acid hydrolysis is commonly used to break down hemicellulose and to prepare cellulose for enzyme digestion. This hydrolysis can be achieved by two ways 1) concentrated acids, low temperature, long reaction time 2) high temperature, dilute acids, short reaction time (Balat *et al.,*2011). The major drawback with concentrated acids is its toxicity and corrosive properties that can reduce the fermentation yield (Balat, 2007). While dilute acid provided by low temperature is favorable for depolymerization of hemicellulose and cellulose at high temperature (Rezania *et al.,*2018). Acids like hydrogen sulphate, phosphoric acids with appropriate concentrations and optimum required temperatures are often used in this acid hydrolysis method.

b) Fermentation of LBM

The hydrolysis step releases the simple sugars such as glucose, xylose and mannose etc. in the optimized condition. This simple sugar can be converted to bioethanol using fermentation techniques. Yeast strains such as *Sacchromyces cerevisiae*, *Pichia stipitis*, *Kluyveromyces fagilis* were used for bioethanol production from different types of monomer sugars. *S. cerevisiae*, one of these yeast varieties, has been widely employed in industry sector for the manufacture of bioethanol since it can withstand a wide pH range. (Azhar et al., 2017).

There are various ways through which fermentation is done namely continuous, batch, and fed-batch fermentation processing (Jatoi *et al.,*2021). Type of fermentation process is dependent or can say determined by feedstock origin and by nature and type of microorganisms used (Huang *et al.,*2018).

Advantage of Fed-batch fermentation is that if critical process parameters are maintained then maximum viable cell concentration is achieved as a result (Balat *et al.,*2011). One downside of this method is that Feed-rate limits productivity, which is restricted by concentration of cell mass. Continuous fermentation process gives more output in comparison to batch processing.

The two fermentation techniques are submerged fermentation and solid state fermentation. Water to lignocellulose ratios of 1:1 to 10:1 are used to wet the biomass by depositing a thin layer of water on its surface. (Lee *et al.,*1997). Fermentation process can be done separately or can be done simultaneously with saccharification. Separate fermentation is time consuming while simultaneous saccharification and fermentation is less time consuming. Still the research is going on this topic, through which we can reduce time and cost of process.

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CONCLUSION

Lignocellulosic biomass is available at an affordable price, has a high carbohydrate content, is adaptable, and is not competitive with food and feed. The scientific and technological aspects of producing lignocellulosic ethanol have been extensively studied in recent years. However, the economic applicability of LBM is still constrained by the exceedingly challenging ethanol conversion process. Several parameters, including cellulose crystallinity, accessible surface area, and lignin concentration, restrict the biodegradability of lignocellulosic biomass. To, increase ethanol production, numerous pretreatment procedures for lignocellulosic biomass have been described. The hydrolysis of cellulose and hemicellulose is restricted by the existence of lignin and the crystallinity of cellulose. Lignin removal and crystallinity reduction are accomplished using a variety of pretreatment techniques, including physical, chemical, and biological ones. Pretreatment method is followed by saccharification and fermentation, these both processes can be done simultaneously or separately. saccharification is done by using different enzymes such as cellulases, xylanase, mannanase and β-glucnase etc. These enzymes depolymerize the ccomplex sugars and convert it into simple sugar. This simple sugar then subjected to fermentation to get ethanol as final product. *Sacchromyces cerevisiae*, *Pichia stipitis*, *Kluyveromyces fagilis* are the yeast used for conversion of sugar to ethanol. Thus, by using these techniques (pretreatment, saccharification and fermentation) lignocellulose biomass can be converted into bioethanol that can overcome the energy requirement and can a best alternative of fossil fuel-based energy source.

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her B.Tech program. This intrigued her to further understand the topic and initiate research in developing alternative fuels from bioresources for the future. Furthermore, she developed an interest in studying the process and decided to enroll in an internship program on fermentation technology.

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Abhishek Dutt Tripathi
Kianoush Khosravi-Darani
Suresh Kumar Srivastava *Editors*

Novel Food Grade Enzymes

Applications in Food Processing and Preservation Industries

Springer

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ISBN 978-981-19-1287-0 ISBN 978-981-19-1288-7 (eBook)
https://doi.org/10.1007/978-981-19-1288-7

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Preface

Enzymes play a significant role in pharma, agriculture, and other allied industries. In the modern era, there is significant application of enzymes in food processing industries and our proposed book will give deeper insight into food applications of enzymes. This book covers all the aspects of enzyme including its classification, kinetics, microbial production, biosynthetic pathways, commodity-wise industrial applications, and its downstream processing strategies. The broad focus of this book will be on the application of various classes of enzymes in dairy, fruits and vegetable, cereals and oilseeds, meat and poultry, brewing and food packaging industries. The first chapter of this book gives a brief introduction about enzymes classifications and their kinetics. Chapters 2, 3, 5, 6, 7, and 8 provide information regarding enzyme applications in different food commodities like fruits and vegetables, dairy, beverage, and meat processing industries. These chapters also focus on different food grade enzyme biosynthesis mechanism in simpler and illustrative way. Besides that, this book will also allow the readers to understand the biosynthesis, production, optimization, and purification of certain less known enzymes such as CGTase and naringinase. Chapters 9 and 12 of this book will be giving some novel information related to application of enzymes specifically for improvement of flavor, color, organoleptic attributes, shelf-life extension, and consumer acceptability of value-added diversified food products. Chapters 10 and 11 also cover some of the very interesting aspects of enzymes application in the food packaging and development of biosensors which will be highly beneficial for those researchers and students who are interested in learning the food safety issues and indulged in product quality improvement. Enzymes play a significant role in the development of GM (Genetically Modified) food production. Chapters 13 and 14 provide detailed information related to enzymes application in GM foods. Chapter 15 highlights the various enzyme infusion strategies adopted in the food processing industries that will enable the students and entrepreneurs to understand the basic concept of enzyme activity and stability after infusion in food. Future prospects of enzymes and their nanotechnological applications have been discussed in Chapters 16 and 17 of this book. The illustrated case studies related to certain novel enzymes will allow the academicians and researchers to broaden their knowledge and will provide an opportunity to carry out extensive research work pertaining to the applications of such enzymes. The authors contributing to this book have already done extensive

research work in the proposed area and the contributed chapters will also be highlighting some of the salient research work already published in the reputed journals by internationally recognized authors in the proposed area. The flowchart described in certain chapters will provide all the necessary information related to the production, optimization, and characterization of food grade enzymes in simpler manner. This book will not only cover the basic enzyme kinetics part but also provide information related to the efficient enzyme recovery and purification process. It also gives broad information on the factors affecting enzyme activity and stability as well as involvement of enzyme in the development of functional food combating chronic diseases and disorders.

As per our survey, only few books are available on the role of enzymes in food processing although this is a prominent area of study and needs to be covered in detail. The earlier books primarily focused on the general enzyme properties and its application. However, no deep insight knowledge pertaining to the commodity-wise application of food grade enzymes has been previously illustrated. We have tried to cover all the aspects of food grade enzymes including its general properties, kinetics, wide application in all the food processing and packaging industries. Although numerous books on medical and therapy-based application of enzymes are available in the market, no books on wider perspective of food grade enzymes applications are available till now. The simpler language, clearly illustrated concepts, and broadly discussed case studies related to various food grade enzymes will enable the readers to strengthen their concept and knowledge. This book will be beneficial for the faculty members, undergraduate, postgraduate, and PhD students of Microbiology, Food Technology, Dairy Technology, Horticulture, Biotechnology, Biochemistry, and other allied discipline of Life Science. This book will also be beneficial to those authors who are indulged in the research activities related to the above-mentioned subject areas such as food science, enzyme and fermentation technology, bioprocess technology, and biochemical engineering. The involvement of foreign authors and editors will provide deeper insight about the subject knowledge and will improve the quality of book content making it readable and acceptable at global level. I hope the readers will enjoy reading this book and will develop interest in the area of enzyme technology.

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Tehran, Iran — Kianoush Khosravi-Darani
Varanasi, Uttar Pradesh, India — Suresh Kumar Srivastava

Acknowledgments

I am grateful to my co-editors, Prof. Suresh Kumar Srivastava, School of Biochemical Engineering Indian Institute of Technology (IIT), Banaras Hindu University, India, and Prof. Kianoush Khosravi-Darani, National Nutrition and Food Technology Research Institute of Iran for providing the immense support in compilation of this book. I am thankful to all my authors from India, Iran, and Nigeria who contributed different chapters for this book as per their expertise. I am grateful to Springer Nature group for selecting our book proposal and permitting us to complete this task. I am thankful to external reviewers who approved the book proposal after thorough evaluation. I pay my sincere gratitude to our Director and Esteemed Dean for their constant encouragement. I am grateful to my Head of the Department Prof. Dinesh Chandra Rai, Department of Dairy Science and Food Technology, Institute of Agricultural Sciences, Banaras Hindu University, India, for his immense motivation and providing necessary facilities required for compilation of this book. I would like to pay my sincere thanks to my parents Shri Vishnu Dutt Tripathi and Mrs. Urmila Tripathi for their encouragement and support. I would like to pay my sincere thanks to my beloved wife Mrs. Priyanaka Upadhyay and my kids Master Ojas and Baby Prajul for their kind support and cooperation. Lastly, I am thankful to almighty who blessed me with the completion of this task.

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Food Enzymes: General Properties and Kinetics

1

S. M. Khade, S. K. Srivastava, L. H. Kamble, and J. Srivastava

Abstract

Enzymes are the biocatalysts having a catalytic power that speeds up a chemical reaction without changing the equilibrium of the reaction. Almost all enzymes are protein in nature and the biocatalytic power lies in the integrity of their structural conformation. The enzymes are highly specific with their substrate molecules that convert into the product by decreasing the activation energy, without getting changed itself. However, the biocatalytic power of the enzymes also depends on several physico-chemical parameters such as temperature, pH, salt concentrations, etc. of the reaction. The enzyme catalysis can be quantitatively revealed by the enzyme kinetics mechanism which measures the reaction rates and the affinity of enzymes towards the substrates and inhibitors. The enzymes can be isolated from the various biological factories such as plant, animal, or microbial cells depending upon the type of enzyme. However, in order to produce at large scale, the microbial sources are the best choice which can effectively reduce the production and purification cost of enzymes. The enzymes are widely used in various sectors such as agriculture, environmental, leather tanning, paper and pulp, chemical and pharmaceutical, detergent, food and beverages, etc. In this chapter, we are mainly focussing on the role of enzymes in the food industry.

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A. Dutt Tripathi et al. (eds.), *Novel Food Grade Enzymes*,
https://doi.org/10.1007/978-981-19-1288-7_1

Keywords

Enzyme · Biocatalyst · Substrate · Physico-chemical parameters · Enzyme kinetics · Inhibitors

1.1 Introduction

An enzyme is a biomolecule which acts as a biocatalyst that increases the rate of a chemical reaction without altering the equilibrium of the reaction. The enzyme transforms a substrate into a product, without getting changed itself. Enzymes are produced by the living cells. The term 'enzyme' was first named by Kuhne in 1878 for the catalytically active substances earlier called **ferments**. However, a German biochemist Eduard Buchner in 1897 was the first to discover the enzyme zymase from the yeast which is involved in the fermentation of sugar into alcohol. In 1907, he was awarded the Nobel Prize for the biochemical investigations of non-cellular alcoholic fermentation (Kohler, 1971). Most of the metabolic reactions are catalysed by the enzymes in the living cell. All the enzymes are not present at all the time in the cell, but are produced whenever the cell needed from the blueprint is present in DNA. There are nearly 75,000 enzymes that are functional in the human body which are divided into three types: metabolic enzymes (responsible for anabolic and catabolic processes), digestive enzymes (mainly extracellular responsible for the digestion of food), and food enzymes from raw foods (responsible for initiation of food digestion) (Barnes-Svarney & Svarney, 2014).

General Properties:

1. Almost all enzymes are protein in nature except a small group of catalytic RNA molecules. The catalytic activity of the enzyme relies on the structural integrity of native protein conformation.
2. Enzymes are very specific to their substrates.
3. Enzymes possess huge catalytic power which increases the rate of a chemical reaction by reducing the activation energy without altering the equilibrium position of the reaction.
4. The biocatalytic power of the enzyme depends on the optimum physio-chemical parameters of the reaction such as pH, temperature, and salt concentration.
5. Enzymes are highly regulated in nature.

Generally, the enzymes are classified as

(a) *Simple enzymes*: The enzymes which are entirely made up of the proteins constituting the amino acids only, e.g. trypsin, pepsin, etc. (Bisswanger, 2017).
(b) *Conjugated enzymes*: The enzymes which contain the non-protein part called **cofactor** along with the protein part, called **apoenzyme**. In these enzymes, the apoenzyme part is generally biologically inactive, if the cofactor is removed. The complete biologically active conjugated enzyme (apoenzyme and cofactor)

is called **holoenzyme**. The cofactor may be inorganic or organic in nature that linked either covalently or non-covalently to the apoenzyme. The inorganic cofactors are simple metal ions, whereas the organic cofactors are complex organic groups called **coenzymes**. Also, the organic cofactors which are firmly attached to the apoenzyme are called **prosthetic group**. The conjugated enzymes are alkaline phosphatase, horseradish peroxidase, etc. (McComb et al., 2013).

1.2 Nomenclature and Classification

Most of the enzymes are named with the suffix –ase; however, some old names are exceptions, e.g. trypsin, ptyalin, pepsin, chymotrypsin, renin, etc. Also, some of the old names indicate the source rather than the action, e.g. papain from Papaya and bromelain from Pineapple (Bromeliaceae family). Many enzymes are named based on substrate and the chemical reaction they catalyse with the suffix –ase, e.g. pyruvate kinase that helps in the synthesis of pyruvate from the phosphoenol-pyruvate, whereas pyruvate dehydrogenase enzyme which converts pyruvate to acetyl-CoA. To avoid the confusion arose due to the common names, an International Commission on enzymes was established for the systematic nomenclature of enzymes.

The enzyme commission (EC) has set a regulation for the nomenclature of enzyme. According to EC, each enzyme is classified and named based on the type of chemical reaction, and for each enzyme, number with four parts is given, e.g. EC 1.7.3.3 (Uricase); EC 3.5.1.1 (Asparaginase). The first three numbers denote major class, subclass, and sub-subclass, respectively, and the last number is a serial number in the sub-subclass, which indicates the order in which each enzyme is added to the list (Boyce & Tipton, 2001) (Table 1.1).

All the enzymes are classified into six classes based on the type of chemical reaction they catalyse. The first integer in the EC number denotes the class of enzymes.

Table 1.1 Common name and EC numbers of some enzyme

Common name of an enzyme	EC number	Reference
Hexokinase	EC 2.7.1.1	Dai et al. (1995)
Lactate dehydrogenase	EC 1.1.1.27	Vanderlinde (1985)
α-Amylase	EC 3.2.1.1	Janeček (2009)
Pectinase	EC 3.2.1.15	El Enshasy et al. (2018)
Lipase	EC 3.1.1.3	Kouker and Jaeger (1987)
Invertases	EC 3.2.1.26	Vu and Le (2008)
Xylanase	EC 3.2.1.8	Huhtanen and Khalili (1992)
Alcohol dehydrogenase	EC 1.1.1.1	Heinstra et al. (1989)
Maltase	EC 3.2.1.20	Nawaz et al. (2015)

1.2.1 EC 1 Oxidoreductase

The enzymes which catalyse the oxidation-reduction reaction are classified under this class. The enzymes are involved in the transfer of electrons from one molecule (oxidant) to another molecule (reductant) (Table 1.2).

$$X_{oxi} + Y_{red} \rightarrow X_{red} + Y_{oxi}$$

1.2.2 EC 2 Transferase

The enzymes which catalyse the transfer of groups from one molecule to another are classified under transferase class. The groups involved in the transfer are phosphoryl ($–PO_3^{2-}$), methyl ($–CH_3$), carboxyl (–COOH), amino ($–NH_2$), acyl (–RC=O), and carbonyl (–C=O). The common names for these enzymes start with prefix *trans* with exceptions of kinases, phosphorylases, etc. (Table 1.3).

$$X - A + Y \rightarrow X + Y - A$$

Table 1.2 Some examples of enzymes under oxidoreductase class

Oxidoreductase	Type of reaction	Reference
Peroxidases	Utilize H_2O_2 as an electron acceptor	Gramss and Rudeschko (1998)
Dehydrogenases	Oxidize a substrate by reducing the electron acceptor other than oxygen, e.g. NAD^+	Pumirat et al. (2014)
Oxygenase	Directly incorporate oxygen into the substrate	Huang et al. (2008)
Oxidases	Use molecular oxygen as an electron or hydrogen acceptor	Kiess et al. (1998)

Table 1.3 Some examples of enzymes under transferase class

Transferase	Type of reaction	Reference
Transaminases	Transfer amino group from amino acids to keto acids	Smit et al. (2009)
Kinases	Transfer of phosphate group from ATP to substrate	Martín et al. (2010)
Methyltransferases	Transfer of methyl group from methyl donor to the substrate	Shixian et al. (2006)
Acyltransferases	Transfer of acyl group to the substrate	Kuhajda et al. (2011)

1.2.3 EC 3 Hydrolases

These are the enzymes which catalyse the cleavage of a covalent bond by adding water (Table 1.4).

$$X - Y + H_2O \rightarrow X - H + Y - OH$$

1.2.4 EC 4 Lyases

These are the enzymes which cleave C–C, C–O, C–N, C–S, and other bonds without hydrolysis or oxidation, but by means of elimination, which results into the formation of double bond or a new ring or contrarily adding groups to double bonds (Table 1.5).

$$X = Y + SP \rightarrow X - S + Y - P$$

1.2.5 EC 5 Isomerases

The isomeric structures are created by means of intramolecular arrangements that convert one isomer to another (Table 1.6).

$$X\ Y \rightarrow Y\ X$$

Table 1.4 Some examples of enzymes under hydrolases class

Hydrolases	Type of reaction	Reference
Esterases	Cleave esters into an acid and an alcohol	Faulds et al. (2004)
Phosphatases	Remove phosphate group from a substrate	Morisseau et al. (2012)
Peptidases	Cleave amide bonds present in the proteins	Perrier et al. (2005)

Table 1.5 Some examples of enzymes under lyases class

Lyases	Type of reaction	Reference
Decarboxylases	Removal of CO_2 by elimination reactions	van der Werf et al. (1994)
Dehydratases	Formation of double or triple bond by elimination of water	Shibata et al. (2010)
Ferrochelatases	Addition of divalent metal cations to the tetrapyrrole structure	Gazel and Yildiz (2016)

Table 1.6 Some examples of enzymes under isomerases class

Isomerases	Type of reaction	Reference
Racemases	Interconversion of L and D stereoisomers	Staudigl et al. (2014)
Tautomerases	Interconversion of keto- and enol-groups	Orita et al. (2001)
Mutases	Intramolecular transfer of functional groups	Rhimi et al. (2008)

Table 1.7 Some examples of enzymes under ligases class

Ligases	Type of reaction	Energy source	Reference
Carboxylases	Catalyse the formation of C–C bond by using CO_2	ATP	Portevin et al. (2005)
T4 DNA ligases	Catalyse the formation of phosphodiester bond	ATP	Wilson et al. (1997)
E. coli DNA ligases	Catalyse the formation of phosphodiester bond	NAD	Nandakumar et al. (2007)

1.2.6 EC 6 Ligases

These are the enzymes which join the two molecules by forming a chemical bond like C–C, C–N, C–O, and C–S. The energy required for these is gained by the cleavage of ATP or NAD. Ligases are also called synthetases, because of synthesis of new molecule (Table 1.7).

$$X + Y + ATP\ (NAD) \rightarrow X - Y + ADP\ (NAM)$$

1.3 Role of Enzyme

The collision of an atom, ion, or molecule of one substance with an atom, ion, or molecule of other results into a chemical reaction between the two substances. After this collision, the intermediate product is produced, having higher chemical energy than the chemical energies of both the reactants together. The transition state in the reaction is achieved with the help of some of the energy entering the reaction apart from the chemical energy of the reactants. The transition state is the stage with highest free energy. The difference in free energy between the transition state and the reactants is known as the Gibbs free energy of activation or only the activation energy.

The enzyme plays an important role in lowering the activation energy of a reaction which results in increase in the rate of reaction in both the directions. However, the enzyme cannot alter the relative energies of the initial and final states. In addition, the catalyst does not alter the position of equilibrium of a chemical

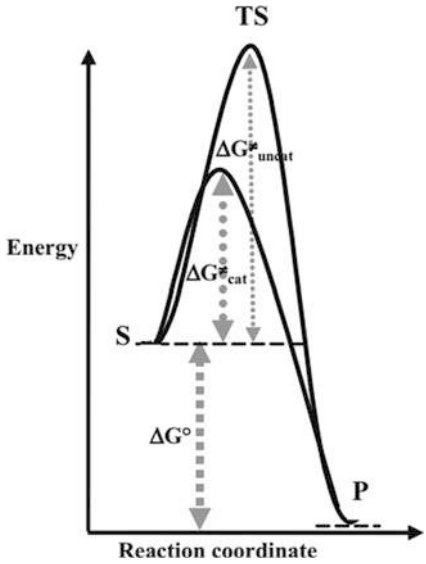

Fig. 1.1 Free energy profile of uncatalysed vs. catalysed reactions. The standard free energy of reaction (ΔG^0) and free energy of activation for uncatalysed ($\Delta G^{\#}_{\text{uncat}}$) and catalysed ($\Delta G^{\#}_{\text{cat}}$) reactions are shown here (Punekar, 2018)

reaction, but only increases the rate of a reaction by lowering the activation energy (Fig. 1.1).

The biocatalysis of a chemical reaction begins with the binding of enzyme and the substrate, the substrate to be catalysed. The substrate specifically binds at the specialized region in the enzyme called active site with multiple weak non-covalent interactions such as hydrophobic, hydrogen bond, ionic interaction, or reversible covalent bonds. Upon binding of the enzyme and substrate, a large amount of free energy is released, called binding energy, which is used to lower the activation energy. The general catalysed reaction between enzyme and substrate may be represented as

$$\mathrm{E} + \mathrm{S} \leftrightharpoons \mathrm{E} - \mathrm{S} \rightarrow \mathrm{E} + \mathrm{P}$$

where E is enzyme, S is substrate, the complex E–S is the enzyme-substrate complex, and P is the product formed.

The binding of substrate at the active site of the enzyme has been proposed with two models.

1.3.1 Lock-and-Key Model

This model assumes that the binding of substrate at the active site of enzyme is highly compact, which may be due to the high degree of similarity between the shape of the substrate and the geometry of the binding site, i.e. active site on the enzyme. This binding of substrate and enzyme is just like a lock and a specific key (Fig. 1.2).

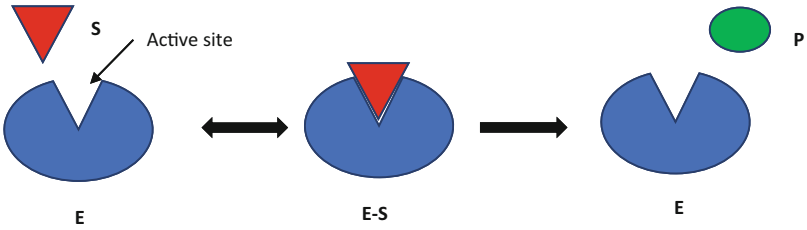

Fig. 1.2 Lock-and-key model of enzyme-catalysed reaction

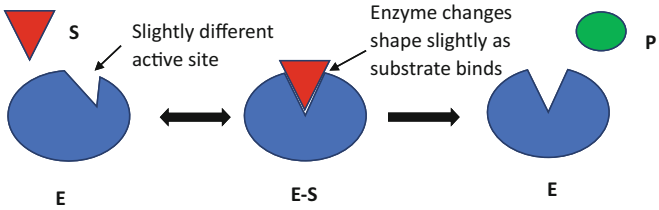

Fig. 1.3 Induced-fit model of enzyme-catalysed reactions

1.3.2 Induced-Fit Model

This model describes the behaviour of protein with some three-dimensional flexibility. Based on this model, the conformational change occurs in the enzyme upon binding of the substrate which leads to complementary fit as soon as substrate is bound. However, prior to substrate binding, the active site has slightly different three-dimensional shape. After binding of the substrate, the enzyme-substrate complex with transition state is formed which subsequently converts into product and free enzyme (Fig. 1.3).

1.4 Enzyme Kinetics

Enzyme kinetics is the quantitative study of chemical reactions catalysed by the enzymes. The kinetic study helps to determine the reaction rates and the affinity of enzymes for the substrates and the inhibitors. Let us consider a chemical reaction $X + Y \rightarrow P$, where X and Y are the reactants and P is the product obtained, the rate of reaction can be determined by either the rate of disappearance of one of reactants or the rate of formation of product. The rate of disappearance of X is $-\Delta[X]/\Delta t$, where Δ represents change, [X] is the concentration of A in mol/L, and t denotes time. Similarly, the disappearance of another reactant Y is $-\Delta[Y]/\Delta t$ and the rate of formation of product P is $\Delta[P]/\Delta t$. So, from these, the rate of reaction can be measured which is related by the stochiometric equation for the reaction.

$$\text{Rate} = -\frac{\Delta[\text{X}]}{\Delta t} = -\frac{\Delta[\text{Y}]}{\Delta t} = \frac{\Delta[\text{P}]}{\Delta t}$$

1.4.1 Kinetics of Enzyme-Catalysed Reactions

Leonor Michaelis and Maud Menten in 1913 proposed a model for the kinetics study of enzyme-catalysed reaction of hydrolysis of disaccharide sugar and sucrose by invertase enzyme into monosaccharide sugars, glucose, and fructose units. Michaelis-Menten assessed the relationship between the reaction velocity and the substrate concentration. The results of this disaccharide hydrolysis into the two monosaccharides by the invertase are represented with the following reaction:

$$\text{E} + \text{S} \underset{k_{-1}}{\overset{k_1}{\leftrightharpoons}} \text{E} - \text{S} \xrightarrow{k_2} \text{E} + \text{P}$$

Here, k_1 represents the rate constant for the formation of enzyme-substrate complex, ES after the binding of substrate S with the enzyme E; the rate constant k_{-1} denotes the reverse reaction of dissociation of enzyme-substrate complex to the free enzyme and the substrate; whereas the rate constant k_2 represents the conversion of ES complex to product P and the free enzyme.

As per Michaelis-Menten, the rate or velocity of enzyme-catalysed reaction depends upon the substrate concentrations [S], when measured at different substrate concentrations with keeping all the physio-chemical parameters constant throughout the course of experiment. At the low concentration of substrate, the initial velocity (V_0) increases at a linear rate with the increase in the concentration of substrate to the point beyond which the velocity does not increase further called maximum velocity (V_{max}) with increase in the substrate concentration (Fig. 1.4).

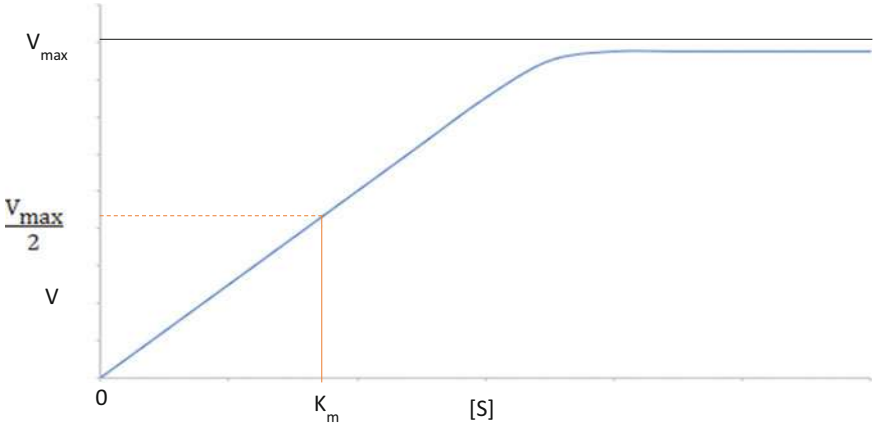

Fig. 1.4 Kinetic study of substrate-saturation curve

1.4.1.1 Lineweaver–Burk Plot

In Lineweaver–Burk plot or doubsle reciprocal plot, this graph was plotted in between reciprocal of velocity against the reciprocal of substrate concentration as in the following equation:

$$\frac{1}{v} = \frac{K_m}{V_{max}} \cdot \frac{1}{[S]} + \frac{1}{V_{max}} \tag{1.1}$$

In this plot, x and y intercepts represent $\frac{-1}{K_m}$ and $\frac{1}{V_{max}}$, respectively (Fig. 1.5).

1.4.1.2 Eadiae-Hofstee Plot

The errors in kinetic parameters in Double reciprocal plot were minimized by Eadiae-Hofstee plot which was plotted in between the rate of reaction (v) and v/[S] and represented by the following equation:

$$v = -K_m \cdot \frac{v}{[S]} + V_{max} \tag{1.2}$$

where V_{max} is the y-intercept and $\frac{V_{max}}{K_m}$ is the intercept on x-axis (Fig. 1.6).

1.4.1.3 Hanes-Woolf Plot

The kinetic parameters were determined by the advanced plot called Hanes-Woolf plot. The graph was plotted between the substrate concentrations vs. the rate of reaction 'v'. The following equation was used for the plot:

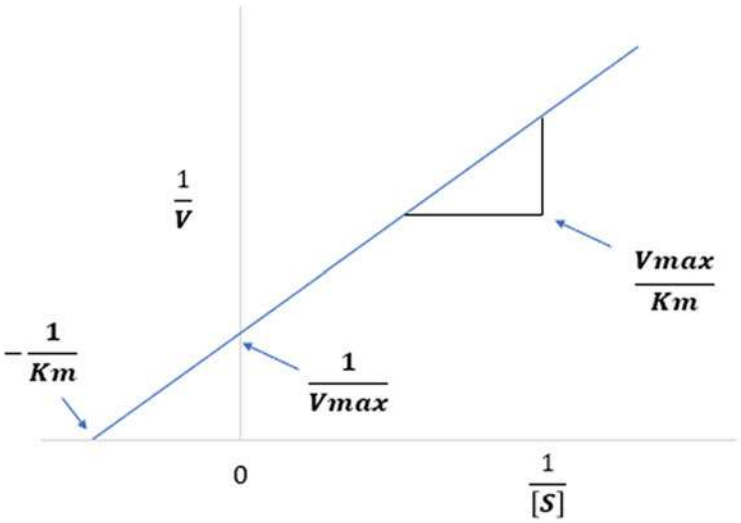

Fig. 1.5 Lineweaver–Burk plot to determine kinetic parameters

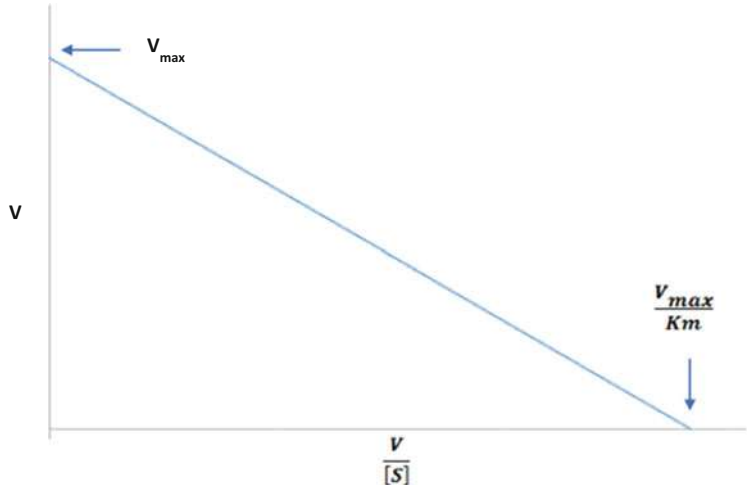

Fig. 1.6 Eadiae-Hofstee plot to determine kinetic parameters

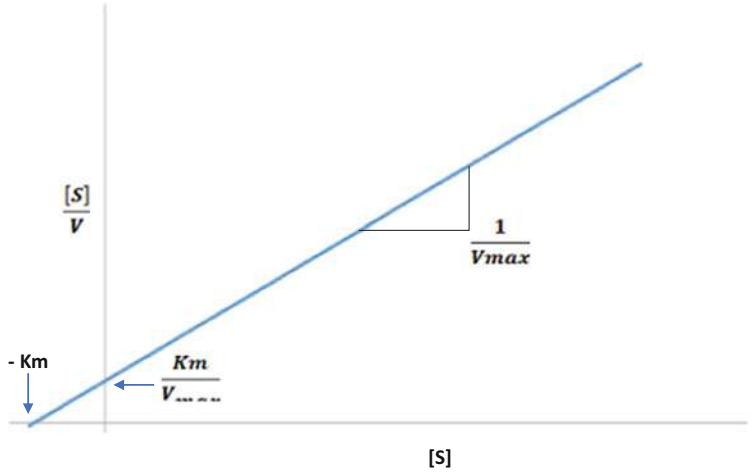

Fig. 1.7 Determination of kinetic parameters by Hanes-Woolf plot

$$\frac{[\mathrm{S}]}{v} = \frac{[\mathrm{S}]}{V_{\max}} + \frac{K_{\mathrm{m}}}{V_{\max}} \tag{1.3}$$

The straight line graph will yield $\frac{1}{V_{\max}}$ as the slope, whereas $\frac{K_{\mathrm{m}}}{V_{\max}}$ as the y-intercept and $-K_{\mathrm{m}}$ as the x-intercept (Fig. 1.7).

1.5 Applications of Enzymes in Food Industries

The enzymes play a vital role either in the food processing or additives in the food industries. The major applications of food enzymes are in the baking industries, dairy industries, starch processing, juice industries, brewing industries, etc.

1.5.1 Baking Industry

To produce a valuable high-quality bakery product, the baking industry utilizes yeasts and enzymes since long ago. The starch containing foods can be processed by the addition of amylolytic enzymes like amylases to improve the softness, freshness, and shelf life qualities of food. The other enzymes, such as xylanases, oxidoreductases, lipases, and proteases, are also used to improve the dough stability, gluten strength, stability of the gas cells in the dough, and by reducing the protein content in the flour, respectively (Li et al., 2012).

1.5.2 Dairy Industry

The enzymes employed for the processing of dairy products become essential in the improvement of the quality and physiologically health benefits. The enzymes, chymosin (coagulation), lipases (low-down fat level), and lysozymes (to avoid late blowing), are used in the production of cheese (Kilcawley et al., 1998; Marseglia et al., 2013). In addition, β-galactosidases and lactases enzymes are predominantly employed in the hydrolysis of lactose sugar in milk into glucose and galactose to avoid lactose intolerance.

1.5.3 Starch Industry

The α-amylase is the prominent enzyme used in the baking industry to act on the starch present in the dough of the bread into the dextrins, which enhances the fermentation process. The released extra sugar in the dough improves the taste and textural properties of the bread (Souza, 2010).

1.5.4 Juice Industry

The enzymes, amylases and glucoamylases, are mainly used in the clarification of cloudy juices. Pectinases are used in the hydrolysis of structural heteropolysaccharide of fruit cell wall pectin, leading to increase in the juice production. Naringinases are mainly employed to reduce the bitterness of the citrus juices (Tapre & Jain, 2014).

1.5.5 Brewing Industry

The α-amylase hydrolyses the starch into maltose and glucose units with reduced viscosity. β-Glucanases hydrolyse glucans to reduce the viscosity and smooth wort separation. Proteases are used to process the malt which allows favourable content for yeast growth. Xylanases are used to enhance the 5-C pentose utilization in malt, barley, and wheat with extraction and beer filtration (Li et al., 2012).

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Plants- and Animal-Derived Enzymes and Their Potential Application in Food Processing and Preservation

2

Mahmoud Aminlari

Abstract

Enzymes are biological catalysts which increase the rate of biochemical reactions in living cells. It is important that enzymologist understands the specific action of particular enzyme in a plant or animal tissue and applies these properties in vitro and in a food product. Most enzymes can be used as processing aids and as protection agents against microbial and deteriorative processes. Although the advent of recombinant DNA technology and advances made in food applications by microorganisms are more practical and economic, some of these enzymes are sufficiently abundant in their natural sources to make them amenable to large-scale production (for example, egg-white lysozyme and plant proteases). In this chapter, several plant and animal enzymes, their occurrence, and potential applications in food industry will be presented. Emphasis will be made on enzyme working on carbohydrates, proteins, and lipids. A section is devoted to miscellaneous enzymes used in food industry, such as phenylalanine ammonia lyase of wheat seedling which metabolizes Phe, thereby rendering foods suitable for PKU patients. In the final section of this chapter, examples of chemical modification of enzymes to improve their properties will be discussed and examples of the studies on modification of chicken egg-white lysozyme to enhance its functional and antimicrobial activities, performed in the laboratory of this author, will be presented.

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A. Dutt Tripathi et al. (eds.), *Novel Food Grade Enzymes*,
https://doi.org/10.1007/978-981-19-1288-7_2

Keywords

Plant enzymes · Animal enzymes · Food application · Antimicrobial · Bioactive peptides · Chemical modification

2.1 Introduction

Enzymes perform many biochemical functions in living cells. They participate in multitude of biochemical reactions which are vital for cell well-being, growth, and production of various biochemical products. As such, enzymes are indispensible in any living system. Some enzymes are unique to particular cell type, while others are present in many different types of cells. It is the responsibility of the food enzymologist to determine and adapt the functionality of a particular enzyme in a plant or animal tissue to a functional property in food systems.

Enzymes from plants and animals have been utilized in the food industry for millennia. The maturation of sugar to liquor by yeast is an earliest illustration of a biotechnological cycle. Enzymes present in various raw materials have been used to produce traditional foods such as cheese, yoghurt, and bread and fermented beverages such as wine, beer, and vinegar for many thousands years (Poulsen & Buchholz, 2003). As early as 1783, it was shown that pepsin gastric juice could digest meat in vitro. The first industrial application of enzymes involved using crude protease mixture from animal pancreas as laundry detergents in the beginning of twentieth century.

In view of abundance of starting raw material the greater part of the first intracellular enzymes came from yeast and skeletal muscle. Starting late 1940s, significant improvements in the separation techniques such as chromatography applicable to proteins resulted in preparation of large quantities of industrially important enzymes (Aehle, 2007). With the advent of recombinant DNA technology, it became possible to transfer and express the genes encoding the enzymes of interest to selected host microbes for production at industrial scale. Today, molecular biology and genetic engineering play major roles in both the discovery of novel enzymes and the optimization of existing enzymes and are the basis for the production of the majority of industrial enzymes.

The grade or degree of purity of enzyme preparation depends on its application. For many industrial applications, partially purified enzyme preparations can be used, provided they do not contain interfering contaminating activities.

They are less costly and, therefore, preferred to highly purified products. They are commonly produced in large scale worldwide and are mostly obtained from animal or plant sources with few purification steps. Examples include proteases and enzymes working on carbohydrates. Enzyme destined for analytical purposes must be pure and do not contain unknown by-products that can interfere with enzymatic analyses, for example, glucose oxidase and restriction endonucleases. Finally, clinically important enzyme which must be highly purified is usually sold in crystalline form and must be devoid of any contaminating activity. A very famous example of

this group of enzymes is asparaginase which is used for treatment of childhood leukemia (Chahardahcherik et al., 2020). The last two types of enzyme are produced from microbes, plants or animals. These enzymes are produced in mg-g quantities worldwide using sophisticated purification procedures.

Food applications of enzymes represent wide and highly diverse fields including baking, dairy, fruit juice, vegetable processing, and meat. Several factors must be taken into consideration when one attempts to employ an enzyme in foods; reaction specificity, requirement for mild conditions, possibility of formation of an unacceptable by-product by a chemical process, and relative cost-benefit.

Uses of exogenous enzymes are used in the production of many commodities (glucose, high-fructose corn syrup, invert sugar, and other sweeteners), protein hydrolysates, and modified lipids; modification of components inside a food matrix (such as brew adjustment, cheese making, meat tenderization, citrus juice debittering, and crumb relaxing), process improvement (such as cheese maturing, juice extraction, juice clarification, fruit and oil seed extraction, drink filtration, quicker batter blending, baked product raising, and adjustment), process control (for example, online biosensors), and food analysis. The enzymes are utilized to acquire various advantages, like more proficient cycles, prompting decreased utilization of raw materials, improved or consistent quality, substitution of synthetic food-additives and evasion of potential destructive side effects in the food, and enhancements of nutritional attributes.

There are some regulations in food industry which allow adding certain chemical agents to foods to achieve particular goals, such as oxidizing and reducing agents (cysteine, bisulfites, and potassium bromide) in baking industry and preservatives such as benzoate and sorbates in different foods. However, in recent years, consumer demand for "natural" foods has driven development of products without additives. In order to meet this demand, much attention and interest have been directed toward identification and application of naturally made compounds. In many cases, the beneficial effect of enzymes to replace chemical agents can be demonstrated.

This chapter aims to present an overview of recent developments in identification of novel plant and animal enzymes with potential application in the food industry. The aim is to increase awareness of and stimulate interest in developing novel enzyme technologies to meet the new and changing needs of the food industry. The activities of many endogenous enzymes continue postharvest and postmortem, and in most cases, contribute to the food quality deterioration, such as excessive ripening of fruits by pectic enzymes, hydrolytic rancidity of milk lipids due to lipase activation, and extra meat tenderization by calpain system. However, the aim of this chapter is to exclusively address the application of exogenously added enzymes of plant and animal origin for their beneficial effects. Because of space limitation, this chapter focuses on enzymes working on major food constituents (i.e., carbohydrates, proteins, and lipids). A few enzymes with specific action in foods will be briefly described under the heading "miscellaneous enzymes". A section is devoted to production of bioactive peptides through use of plant and animal proteases. In the final section of this chapter, examples of chemical modification of enzymes to improve their properties will be discussed and some of the studies done on lysozyme

in the laboratory of this author will be presented. Table 2.1 summarizes the use and suggested use of enzymes in food industry.

2.2 Enzyme Working on Carbohydrates

Most of the enzymes working on food carbohydrates are hydrolase, i.e., use water as one of the substrates. These enzymes are commonly referred to as glycosyl hydrolases or glycosidases.

They catalyze the hydrolysis of glycosidic bonds in complex sugars. They are amazingly normal enzymes with roles in nature including degrading of complex biomass like starch (amylase), cellulose (cellulase), hemicellulose, and as antibacterial safeguard methodologies (e.g., lysozyme), in pathogenesis components (e.g., viral neuraminidases), and in ordinary cell work (e.g., managing mannosidases associated with N-connected glycoprotein biosynthesis). Along with glycosyltransferases, glycosidases' are the major synergist apparatus for the synthesis and breakage of glycosidic bonds (Bourne & Henrissat, 2001). Glycosidases hydrlyze α-1,4 or α-1,6 glycosidic bonds. Glycosidases can be classified either as "retaing" or "invering" types, in light of the destiny of the anomeric configuration (α or β) of the hydrolyzed glycosidic bond. One more broad differentiation among glycosidases is whether they are "endo" or "exo" splitting. Exo-acting works for the most part on the non-reducing terminal of the substrate, while endo-acting types rondomly assault internal glycosidic bonds of the substrate. Naming glycosidases as "α" and "β" (as in amylases and glucosidases) perceives the anomeric configuration of the freed reducing group as being axial and equatorial, respectively (Whitaker, 1994). Specialty applications for various glycosidases continue to emerge. Glycosidases account for about half of enzyme used as processing aids in the food industry, primarily for the production of low molecular weight sweetener and bulking or thickening agents (dextrins) from starch (such as amylases), and for carbohydrate modification in baking and fruit products (such as cellulases and pectic enzymes) (Parkin, 2017). Most of these enzymes are commercially available. A summary of the types and classification of glycosidases of most importance in foods is provided in Table 2.2.

2.2.1 Amylases

2.2.1.1 α-Amylases

The amylases are utilized to hydrolyze starch into lower molecular weight dextrins and consequently "thin" starch suspensions. α-Amylase is an endo- splitting glycosidase that hydrolyses α-1,4 glycoside bond. The initial product is dextrin and the final main products are maltose or maltotriose. The α-1,4 bonds close to α-1,6 branches are impervious to hydrolysis. Broad hydrolysis of amylopectins with α-amylases produces "α-limit dextrins," as cleavage stops at the α-1,6 bonds. Figure 2.1 shows the impacts of various glycosidases on starch molecule (Wong & Robertson, 2007;

Table 2.1 Some uses and suggested uses of enzymes in food industries (from Whitaker, 1994)

Enzyme	Food	Purpose or action
Amylases	Baked goods	Increase in maltose content for food fermentation
	Brewing	Conversion of starch to maltose for fermentation, removal of starch turbidity
	Cereals	Conversion of starch to dextrins and maltose, increase water absorption
	Confectionary	Recovery of sugar from candy scraps
	Fruit juices	Removal of starch to increase sparkling properties
	Jellies	Removal of starch to increase sparkling properties
	Pectin	Aid in preparation of pectin from apple pomace
	Syrup	Conversion of starch to low molecular weight dextrins (corn syrup)
	Vegetables	Hydrolysis of starch as in tenderization of peas
Cellulase	Brewing	Hydrolysis of complex carbohydrates cell walls
	Coffee	Hydrolysis of cellulose during drying of beans
	Fruits	Removal of graininess of pears, peeling of apricot, tomatoes
Invertase	Artificial honey	Conversion of sucrose to glucose and fructose
	Candy	Manufacture of chocolate-coated soft-cream candies
Lactase	Ice cream	Prevention of crystallization which results in grainy, sandy texture
	Milk	Stabilization of milk proteins in frozen milk by removal of lactose. Hydrolysis of lactose, permitting use by adults deficient in lactase in intestinal and infants with congenital lactase deficiency
Tannase	Brewing	Removal of polyphenolic compounds
Pentosanase	Milling	Recovery of starch from wheat flour
Naranginase	Citrus	Debittering citrus juice by hydrolysis of the glucoside naringin
Pectic enzymes	Chocolate, cocoa	Hydrolytic activity during fermentation of cocoa
	Coffee	Hydrolysis of gelatinous coating during fermentation of beans
	Fruits	Softening
	Fruit juice	Improving yield of press juice, prevention of cloudiness, improving concentration process
	Olive	Extracting oil
	Wines	Clarification
Proteases	Baked goods	Softening action in dough, cut mixing time, increase extensibility of dough, improvement in grain, texture, loaf volume, liberate β-amylase
	Brewing	Body, flavor, and nutrient development during fermentation, aid in liberation and clarification, chill-proofing
	Cereals	Modification of proteins to increase drying rate, improve product handling characteristics, manufacture of misu and tofu
	Cheese	Casein coagulation, characteristic flavor during aging
	Chocolate, cocoa	Action on beans during fermentation

(continued)

Table 2.1 (continued)

Enzyme	Food	Purpose or action
	Eggs	Improve drying properties
	Meat, fish	Tenderization, recovery of proteins from bones and trash fish, liberation of oils
	Milk	In preparation of soy-milk
	Protein hydrolysate	Condiments such as soy sauce, specific diets, dehydrated soups, gravy powder, processed meats, production of bioactive peptides, aids in digestion of proteinous foods
	Wines	Clarification
Lipase	Cheese	Aging, ripening, and general favor characteristics
	Oils	Conversion of lipids to glycerol, fatty acids, and monoglycerides
	Milk	Production of milk with slightly cured flavor for use in milk chocolates

Table 2.2 Catalytic properties of some glycolytic enzymes important in food technology (from Whitaker, 1994; Parkin, 2017)

Enzyme	Bond selectivity	Product selectivity	Products
α-Amylase (endo)	α-1→4 glucose	Retaining α→α	Initial major product dextins, final product maltose, maltotriose
β-Amylase (exo)	α-1→4 glucose	Inverting α→β	β-Maltose
Pullulanase (endo)	α-1→6 glucose	Retaining α→α	Acts on pullulan to give maltotriose and on starch to give linear dextrins
Glucoamylase	α-1→4 (α-1→6) glucose	Inverting α→β	β-Glucose
Cyclomaltodextrin transferase	α-1→4 glucose	Retaining α→α	Acts on cyclodextrins and linear dextrins to give maltose and maltotriose
Cellulase	β-1→4 glucose		1,4-β-dextrins, mixed 1,3–1,4-β-dextrins
Invertase	β-1→2 fructose	Retaining β→β	Glucose and fructose
β-Galactosidase	β-1→4 galactose	Retaining β→β	Galactose and glucose
β-Gluctosidase	β-1→4, β-1 aglycan, glucose	Retaining β→β	Aglycan and glucose
Polygalactourinase	α-1→4	Inverting α→β	Galactouronic acid
Xylanase	α-1→4 xylose	Retaining β→β	
Lysozyme	α-1→4 NAM-NAG	Retaining α→α	

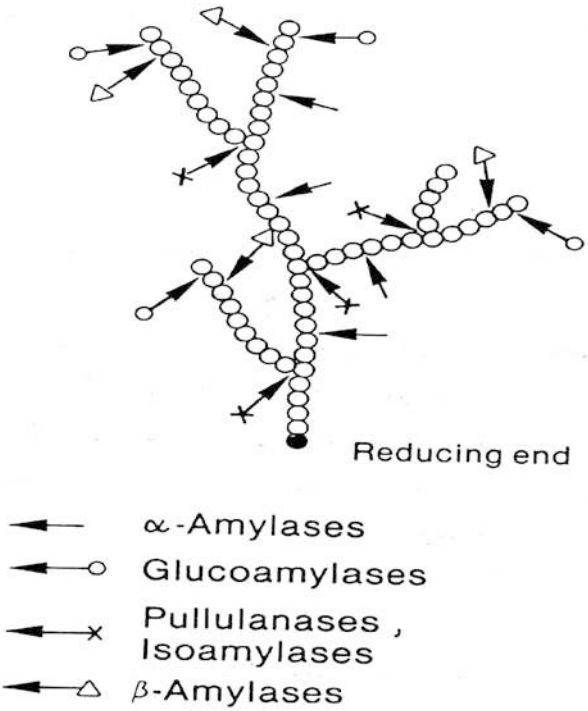

Fig. 2.1 The action of different glycosidases on starch molecule. (From Wong & Robertson, 2007; Saini et al., 2017)

Saini et al., 2017). α-Amylase is an important enzyme in carbohydrate metabolism of microorganisms, plants, and animals. The active site requires a substrate of no less than three glucose units long. The normal final results of α-amylase activity are α-limit dextrins and maltooligosaccharides of up to 12 glucose units. The random action of this enzyme on starch results in rapid decrease in the viscosity of starch solution (Fig. 2.2). The average molecular mass of α-amylase from different sources is 50–70 kDa. α-Amylases requires Ca^{2+} for activity. Ca^{2+} is firmly bound and serves to widen the pH optimum of the catalyst to between pH 6 and 10 as displayed in Fig. 2.2. α-Amylase from different sources are stable at temperatures in the range of 30–130 °C (Pandey et al., 2000). There are many commercial sources of α-amylases available, the majority of which are microbial, despite the fact that malt (grain or wheat) amylases are also accessible. The α-amylase routinely is purified from barley using different purification steps, including salt precipitation, centrifugation, ion exchange, and affinity column chromatographic methods such as CM Sepharose CL-6B column and cyclohexaamylose (CHA)-Sepharose affinity column. The enzyme can be also expressed in yeast and then purified from yeast extract as described above (MacGregor & Morgan, 1992).

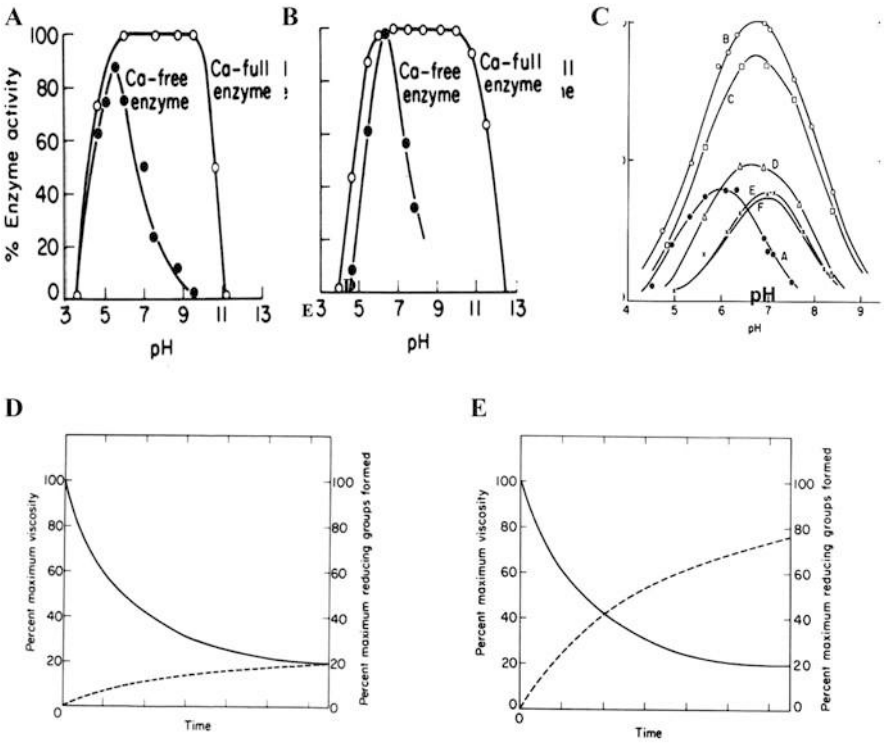

Fig. 2.2 Effect of pH, calcium ion, and salts on α-amylases: (**a**) pancreatic, (**b**) *Bacillus subtilis* α-amylases, (**c**) effect of different salts on α-amylase activity (A: No salt, B: 0.04 M NaCl, C: 0.04 M bromide, D: 0.04 M iodide, E: 1.0 M nitrate, F: 1. 0 M chlorate), effect of β-amylase (**d**) and α-amylase (**e**) on viscosity and reducing sugar content during starch hydrolysis. (From Wong & Robertson, 2007; Saini et al., 2017)

Food Application of α-Amylases

Starch Hydrolysis

Ordinary applications include preparation of starch syrup, dextrose, liquor, brewing, and bakery. α-Amylase is utilized economically in the liquefaction of starch to create dextrins which are further saccharified by glucoamylase to yield glucose feed stock for corn syrup, fuel ethanol, or alcoholic beverages. Heat stable α-amylase in the presence of calcium ion and a 105 degree centigrade is used to produce a 30-40% starch slurry.

This yields a mixture of linear and branched dextrins (maltodextrins) with extent of hydrolysis ranging from 8 to 15 DE (DE: dextrose equivalence). The product of this reaction is used for the production of 15–40 DE maltodextrins (corn syrups, used as thickening, bulking, and viscosity building), sweetener production, and a 95–98% glucose syrup (95 DE) in which other enzymes such as glucoamylase (which is often used as an immobilized enzyme column), with or without pullulanase, might also be

used. The glucose syrup can then be treated with an immobilized glucose isomerase column to generate a high-fructose corn syrup of 42% fructose (52% glucose) (Wong & Robertson, 2007; Van der Maarel et al., 2002).

In future, improving starch handling and change will zero in on broadening optimum pH (to pH 4–5), heat stability and decreasing Ca^{2+} prerequisite of α-amylase, thereby improving starch processing and transformation, and improving the capacity to process crude starch by β-amylases. For all enzymes included, improving heat stability is of significance as this will increase efficiency in handling just as advancing a single-step process. Likewise, improvement of determinants of selectivity is also important.

Baking and Baked Goods Industries

Over the course of bread making, proteins have consistently assumed a significant role. Indeed, even the old Egyptians utilized proteins present endogenously in the flour, in spite of the fact that they might not have known about the efffect.

The first application of enzymes in baked goods was supplementation of α-amylase by addition of malt to correct the concentration of endogenous α-amylase in the flour. Today, a whole range of enzymes is available for end users of flour. These make it possible to correct suboptimal concentrations of endogenous flour enzymes. Virtually all of the glycosidases presented in Table 2.2 have been added for some benefit in baking applications. The most widely used enzyme in baking industry in terms of amount and value is α-amylase. Initially, amylases were believed to function primarily by mobilizing fermentable carbohydrate for yeast.

The essential impact of amylase supplementation is getting satisfactory gassing power by degradation of damaged starch granules in the mixture, which helps maltose production by endogenous β-amylase. It is presently perceived that amylases added straightforwardly to the dough will decrease batter viscosity and further develop portion volume, bread softness(antistaling), and color. The majority of these impacts can be considered to be due to incomplete hydrolysis of starch during baking as the starch gelatinizes. Decrease in viscosity advances volume and texture by permitting the reactions engaged with mixture molding and baking to happen quicker (mass transfer effect) (Christophersen et al., 1998).

The most broadly utilized α-amylase in baking industry is the alleged α-amylase from fangus or Taka-amylase from *Aspergillus oryzae*. The most well-known other options, malt amylases from grain or wheat malt, have higher protease contamination and higher thermostability, and this makes them more inclined to negative incidental effects when used too much. The second important goal for use of amylases in baking is antistaling, i.e., improving the fresh keeping of baked goods.

Staling is a highly complex phenomenon, but it is generally accepted that retrogradation of amylopectin is the main contributor to bread staling (Morgan et al., 1997). This happens during the first hour of cooling subsequent to baking, the initial crumb structure is set by amylose gelation, which makes an organization in which the gelatinized starch granules are implanted. Recrystallization of amylopectin side chains leads to rigidification of the starch granules and to a general

reinforcing of the crumb structure, considered as an increment in crumb firmness. An inter- and intragranular amylose recrystallization is additionally associated with the staling process. Estimates of worth of lost baked goods due to staling in the United States in 1990 was about US $1 billion. All the more as of late, the maltogenic types of α-amylases as and β-amylase have been perceived as being far better antistaling agents, since they will generally produce smaller maltooliogosaccharides (DP 7–9) and larger dextrins (which are plasticizers) (Hebeda et al., 1991).

Brewery and Fermentations

One of the primary uses of enzymes in brewing industry is preparing the materials for fermentation and whether the enzyme is endogenous in kernel or been added from outer sources, a profound knowledge and examination of those enzymes is required for better production and better overall quality. Fermenting is one of the most established food processes done by man. Brewing is characterized as the course of beer production where the sugars in starch are converted to ethanol through the activity of yeast. These days, brewing is one of the lead food industries in many countrie. Use of enzymes is one of the fundamental mainstays of brewing industry (Gomaa, 2018).

The four most normal enzymes utilized in brewing are β-glucanase, protease, α-amylase, and β-amylase. The shared factor in the beer production is the conversion of starch in the grains to ethanol. In the first stage, enzymes hydrolyze starch into fermentable sugars. In a subsequent interaction stage, these sugars are changed over to ethanol and carbon dioxide by yeast. The customary sources of enzymes utilized for the change of cereals into beer lager is malted grain, and malted barely is a vital factor in brewing. Commercial enzymes can be utilized for additional quality ascribes, for example, clarification, color, texture or flavor, and it is obligatory to utilize the enzymes from outer source since the amylases endogenous to malted grain are insufficient to use all of the fermentable starch because of low, absence of heat stability, as well as presence of endogenous inhibitors in the grains (Bamforth, 2009).

Thus, α- and β-amylases are added to maximize the availability of fermentable carbohydrate. Auxiliary enzymes such as glucoamylase, pullulanase, glucanases, xylanases, and cell wall hydrolyzing enzymes are added to complete the degradation of starch to α- and β-limit dextrins that the thermally labile malt amylases cannot achieve, to render limit dextrins fermentable and to hydrolyze glucans (similar to cellulose, but with β-1,3 and β-1,4 linkages) and xylans. The remaining limit dextrins provide body to the final product. Exogenous enzymes are added during (or right after) the “mashing” step, which is conducted at moderate temperatures (45–65 °C), and they are destroyed during the subsequent boiling stage. A range of exogenous enzymes, such as glucanases, acetolactate decarboxylase, and prolyl endopeptidase, are available for enhancement of the existing brewing process (Bamforth, 2009).

2.2.1.2 β-Amylases

β-Amylase is an exoglucanase that catalyzes the hydrolysis of 1,4-α-D-glucosidic bonds in polysaccharides to separate progressively maltose units from the nonreducing end of α-1,4-glucans like starch and glycogen. Sweet potato contains a bountiful β amylase which represents 5% of the total soluble proteins in the tuber; while only little α-amylase is available. The activity of this endogenous catalyst brings about production of maltose which renders a pleasant sweet flavor to potato (Thacker et al., 1992).

Properties and Applications

There are several similarities and differences between β-amylase and α-amylase. Both enzymes cleave α-1,4 glucosidic bonds in amylose and amylopectin. β-Amylase cleaves successively maltose units from the nonreducing end. Both have an average molecular weight of about 50 kDa. While α-amylase is widely distributed in nature, β-amylase is only found in plants.

Seeds of higher plants and sweet potatoes are the essential source of β-amylase. β-Amylase is liable for the pleasantness of aged natural product since it hydrolyses starch into maltose during ripening of fruits. pH 4.0–5.5 is optimum for the hydrolytic action of β-amylase. The product in α-amylase activity is an oligosaccharide with α configuration, and in β-amylase, it is β-maltose.

α-Amylase is an endo-splitting glycosidae, while β-amylase is exo-splitting. α-Amylase does bypass the branch point (i.e., α-1,6 glucosidic bonds) and β-amylase cannot bypass. Both enzymes cause reduction in viscosity as demonstrated in Fig. 2.2d, e, α-amylase acts more rapidly than β-amylase (Das & Kayastha, 2019).

The amino acid sequences of β-amylases from different plants, including sweet potato, soybean, barley, wheat, maize, rye, cowpea, alfalfa, and several species of bacteria, have been deduced from their respective gene sequences. The sequences between plant and bacterial β-amylases show only 30% homology (Thalmann et al., 2019).

β-Amylase has been prepared in a crystalline form from a variety of plant sources including germinated barley, sweet potato, and soybean. Purification mostly involves multisteps fractionation by acetone, ethanol, or ammonium sulfate precipitation followed by cation/anion exchange chromatography and gel filtration and the affinity chromatography using cyclodextrin Sepharose 6B column.

β-Amylase has been used for various research and industrial applications. β-Amylase is employed by the starch industry in the production of high maltose syrups. Compared with glucose syrups, maltose syrups have a higher viscosity and lower hygroscopicity, less tendency to crystallize, and more resistance to browning. These characteristics make them particularly suitable for use in the confectionery and baking industry (Aiyer, 2005).

2.2.2 Pectic Enzymic

2.2.2.1 Introduction

Pectins or pectic substances are collective names for a group of closely associated polysaccharides present in plant cell walls, where they contribute to complex physiological processes like cell growth and cell differentiation and so determine the integrity and rigidity of plant tissue. They also play an important role in the defense mechanisms against plant pathogens and wounding. Commercial pectins are polymers of α-D-galactopyranosyluronic acids with various contents of methyl ester groups and are obtained from citrus peel and apple pomace. Pectins have a unique ability to form spreadable gels in the presence of sugar and acid or in the presence of calcium ions and are used primarily in these types of applications. By definition, preparations in which more than half of the carboxyl groups are in the methyl ester form are classified as high-methoxyl (HM) pectins, the remainder of the carboxyl groups will be present as a mixture of free carboxyl group and their sodium salt. Preparations in which less than half of the carboxyl groups are esterified with methanol are called low-methoxyl (LM) pectins. The percentage of carboxyl groups is the degree of esterification (DE) or degree of methylation (DM) (Benen et al., 2003; Schols & Voragen, 2003; Yapo, 2011).

The pectin degrading enzymes or pectic enzymes are classified into three general groups, namely polygalacturonase, pectate and pecyin lyases, and pectin methyl esterase. The specific responses brought about by these three pectinase activities are displayed in Fig. 2.3. The depolymerizing enzymes are hydrolases and lyases. The methylesteraseases hydrolyze the methylesterase at O6 of a galacturonicacid residues, though acetylesterases hydrolyze the acetylester at O2 or potentially O3 of a galacturonic acids. These enzymes are commonly found in plants and microorganisms (particularly in fungi like *A. niger*) and have been purified and widely studied (Benen et al., 2003).

Pectic enzymes are normally present in many vegetables and fruits (endogenous enzymes), yet they are also added as processing aids as exogenous enzymes. In higher plants, especially pectin methyl esterase and polygalacturonase, both endo- and exo-asplitting are present. Pectin acetylesterase and pectate lyase have been found in citrus products of plants. The endogenous enzymes are expected to assume significant parts in plant improvement and during maturing. The cutting edge genomic approach will empower us to more readily comprehend their functions in plants in various isoforms and their role in plant improvements. Taking into account that pectic enzymes alone record for around one fourth of the world's food enzymes production, one can securely conclude that the technological innovations and applications designed to reduce economical costs and increase the productivity of these enzymes will be of great value (Alkorta et al., 1998; Whitaker, 1990).

2.2.2.2 Polygalacturonases

Polygalacturonases hydrolyze the α-1,4-D-galacturonosidic linkage (Fig. 2.3). Whereas endo-polygalacturonases hydrolyze the polymer substrate randomly, the exo-polygalacturonases are confined to cleave off galacturonic acid monomers or

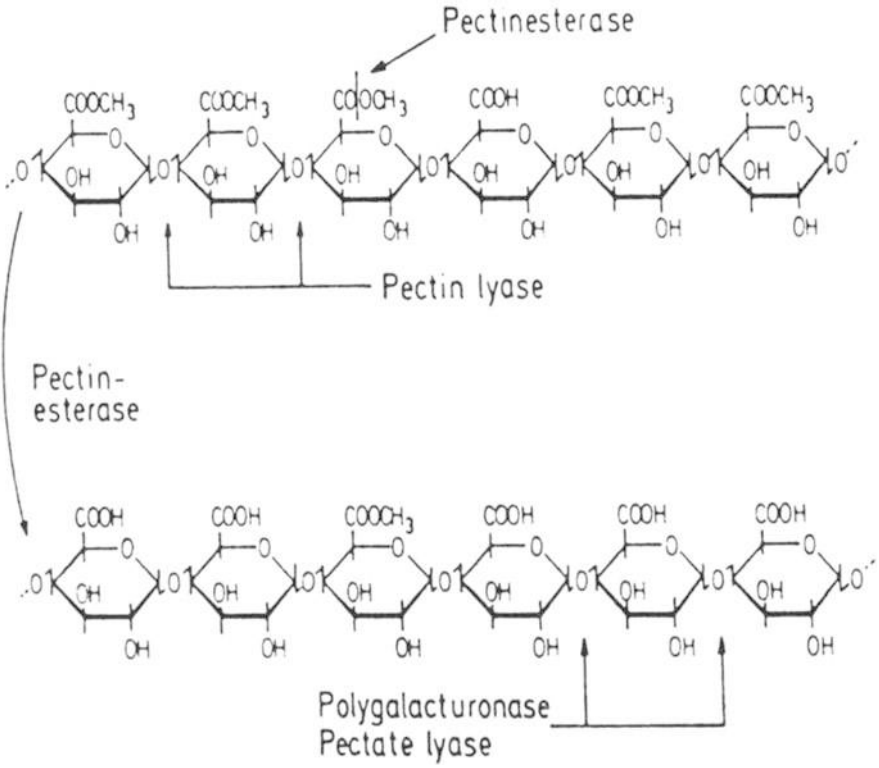

Fig. 2.3 Site of action and reaction mechanism of pectin-degrading enzyme. (From Whitaker, 1994)

digalacturonides from the nonreducing end. The enzymes, as found for the pectate lyases, use both polygalacturonic acid and low to moderate degree of esterification of galactouronic acids as their substrate (Benen et al., 2003). Polygalacturonases are widely distributed in higher plants and are believed to contribute to fruit softening of pears, peaches, and avocado.

These enzymes assume significant parts in, food technology, for example, vegetable and fruit juice extraction, such as, apples, grape wine, strawberries, grapes, raspberries, and so on, degumming of plant fiber, waste water treatment, oil extraction, coffee and tea aging, paper and pulp industry, and animal feed. To clarify fruit juices, a combination of amylases and pectinases is utilized. The time of filtration is decreased up to half. Tomato is a rich source of polygalactouronase. Brilliant practices are utilized in the tomato processing to heat inactivate the enzymes momentarily ("hot break" process) to prepare thick juice liked by the purchaser and the high-consistency concentrated juice (tomato pase) utilized for sauces, soups, catsup, and comparable items. At the point when tomatoes are utilized for color and flavor, and consistency is obtained by different agents like starch, thiner, "cold break", juice is the beginning material for paste production. A holding time is applied

between smashing and heat treatment to guarantee breakdown of the pectins by joined pectin methylesterasease/polygalacturonase activity (Benen et al., 2003). To the food enzymologist, the most intriguing endogenous polygalacturonases are those present in the parts of the plant that are utilized as or in food. The pernicious impact of endogenous polygalacturonases, i.e., diminishing in consistency of the homogenate during aging, has become progressively clear. To prevent the activity of these enzymes, tomatoes are homogenized after heat pretreatment to inactive the enzymes and yield the purported hot break paste (Javed et al., 2018).

2.2.2.3 Pectin Methylesteraseases

Pectin Methylesteraseases have been industrially utilized in the commercial sector for wine and fruit juice industry since 1930. Apart from the presence of many genes encoding putative pectin methylesteraseases, these enzymes have also been detected in various plants by their activity, which confirms their wide distribution (Gupta et al., 2015; Dixit et al., 2013). Pectin methyl esterases are of major importance for the preparation of pectins for specific applications. These enzymes have been identified in higher plants and vegetables. Within each species, multiple forms of pectin methylesterases can be present. These enzymes, along with polygalacturonases, are used in clarification of juices in fruit juice industry. The break down of pectins additionally decreases the viscosity of juices and facilitates their filtration and concentration. One more utilization of pectin methylesterases in natural product juice industry includes liquefaction of natural product mashes. In such an application, the pectinases are joined with cellulases and hemicellulases for the total interruption of the cell walls. In this multitude of enzymatic applications, degradation of pectin facilitate the process and increase juice yield. Likewise, the favorable aroma of juices is increased and amount of waste material decreases. Pectin methylesterasease from *Datura stramonium* in blend with polygalacturonase expanded clearity of orange, apple, pomegranate, and pineapple juices by 2.9, 2.6, 2.3, and 3.6 fold, respectively (Dixit et al., 2013).

2.2.2.4 Pectate Lyases

Pectate lyases cleave the α-1,4-D-galacturonosidic linkage by β-elimination, resulting in the formation of a Δ4,5-unsaturated newly formed nonreducing end as is shown in Fig. 2.3b. Pectin lyases perform the same reaction. However, the latter enzymes require the galacturonic acid residues adjacent to the scissile bond to be methylesteraseified. Pectate lyases have been identified in plants (Uluisik & Seymour, 2020). The first clear cut demonstration of plant pectate lyase activity was reported in the plant zinnia eleganse (*Zinnia elegans*) (Semenova et al., 2006; Domingo et al., 1998). Pectate lyases have not found many applications in food industry because of their harsh reaction conditions (Semenova et al., 2006).

2.2.3 Cellulases

Cellulose is the most plentiful biopolymer on the planet. It is mostly created in higher plants in which it shapes the inflexible skeleton of the plant. Cellulose is a homopolymer comprising of up to 1000 β-1,4-linked-anhydroglucopyranoside units. Single glucose polymers are packed onto one another to shape an exceptionally crystalline fibrillar material in which the singular cellulose chains are held together by hydrogen bonds. Cellulose microfibrils likewise contain some amorphous areas, the level of which depends on the source. The most crystalline cellulose is produced by algae, and the least crystalline by plants (Klemm et al., 2005).

Cellulases belongs to the group of β-glucan hydrolases that can break down cellulose. Several microorganisms, including filamentous organisms, yeast, and microbes existing in the digestive tract and colon of monogastric, can hydrolyze cellulose to oligosaccharides and in the long run to glucose. Ruminants can completely degrade cellulose in their rumen by the wide range of microorganisms present. Cellulases have generally been classified into two specific groups endoglucanases and exoglucanases, which act in the middle or at either ends of the cellulose chain, respectively (Tenkanen et al., 2003). Cellulases are produced by fungi, bacteria, protozoans, and animals. There are additionally a few reports on the presence of cellulases in plants. Up to this point, just the endoglucanases (1,4-β-D-glucan-4-glucanohydrolase) have been found in plants. This enzyme catalyzes random cuts in cellulose chains, subsequently creating more shorter cello-oligomers, which can be additionally degraded by exoglucanases. Endogenous plant endoglucanases have been recommended to assume a part in plant development, for example, in fruit maturing and leaf abscission, or in the rearranging of polysaccharides in developing cells. The producion of microbial cellulase in transgenic plants, for example, sugarcane and corn, has been reported. These advances can offer one potential utilization of plant biomass as biofuel harvests or remaining horticultural waste as a sustainable fuel source (Hefferon, 2017).

Cellulases are not among the central enzymes used in the food industries. They are, nevertheless, applied in treatment of grain-based beverages and food assortments like beer and bread. Cellulases are used in rather immense totals in animal feed (Zhang & Zhang, 2013). Cellulase has a wide scope of utilizations in industrial biotechnology and is the second most utilized modern enzyme after protease. Cellulases are utilized in the textile industry, in cleansers, pulp and paper industry, further developing edibility of animal feeds, and food industry. In numerous food applications, cellulases are not utilized alone, but are added to help the activity of other enzymes. In collaboration with enzymes, such as pectinases and hemicellulases, cellulases disrupt the underlying rigidity of the plant cell walls and upgrade the extraction efficiency of the molecules of interest. Thus, they are utilized in any process of handling plant-based materials. Cellulases are utilized in wine and production of fruit juice to facilitate the maceration, and, the extraction of color and flavor compounds, fermentable sugars and the extraction of juices. Macerating enzymes mix containing cellulases are likewise utilized in fruit juice production. Furthermore, the utilization of these enzymes offers benefits in the treatment of

waste of the fruit juice industry by further developing the extraction yield and the general process efficiency. In baking industry, cellulase is utilized to degrade gums in the dough, improving bread structure. Added enzymes are utilized progressively for improving the processes and final quality of products. Obviously, the biggest modern utilization of cellulases is outside the food and feed area but in material handling of fibers made out of cellulose (Zhang & Zhang, 2013; Autio et al., 1996). Cellulases are likewise utilized for the extraction of nutraceuticals from plants. Nutraceuticals are compounds from normal sources with extra medical advantages, other than a nutritious agent (Fernandes, 2018). Cellulases and hemicellulases can be utilized for the separation of pectin from citrus fruits to separate the cell wall, expanding the pectin yield. Cellulases can be utilized alone or in blend with other cell wall degrading enzymes in all processes in which significant mixtures like juice, oil, polysaccharides, protein, and so on are removed from the plant material (Laurikainen et al., 1998).

2.2.3.1 β-Glucosidases

β-Glucosidases are enzymes with great practical significance to natural systems. These are divided in various glycoside hydrolase families in light of their catalytic mechanism and amino acid sequences. Most investigations completed on β-glucosidases are centered around their applications rather than their endogenous capacity in the their natural environment (Singh, 2016). In a large number of leafy foods tissues, significant flavor compounds are glycosylated. β-Glucosidases catalyze the hydrolysis of alkyl and aryl β-glycosides disaccharides and short-chain oligosaccharides. β-Glucosidases have an extraordinary potential to be utilized in different biotechnological processes from freeing flavours, fragrances, and isoflavone aglycons to the biosynthesis of oligosaccharides and alkylglycosides. The utilization of glycosidases to deliver flavor compounds from glycosidic compounds was at first analyzed in wines. β-Glucosidases are omnipresent, generally dispersed in nature, and can be found in microorganisms, plants, and animals (Romero-Segura et al., 2009; Zhu et al., 2017).

One more attribute of glucosidases critical to food processing and quality is the presence of cyanogenic glycoside in many plants that can potentially produce hydrogen cyanide during preparation or by eating. The cyanogenic glucoside linamarin in cassava roots and leaves (widely used as food staple in tropical areas of Africa, Asia, and South America), lima beans, bitter almonds, and flax seeds are examples of these glycosides. There are roughly 25 cyanogenic glycosides referred to, for example, linamarin in cassava and lima beans; dhurrin in sorghum; amygdalin in almonds, peaches, and apricot pits (Hughes, 1999). The enzyme rhodanese is a ubiquitous enzyme active in all living organisms from bacteria to man.

It is a multifunctional enzyme, yet appears to have important role in cyanide detoxification. This enzyme is likewise broadly present in plants (Chaudhary & Gupta, 2012). The primary source of this enzyme was reported to be the liver of animals. Nonetheless, broad examinations on the distribution of rhodanese in our research have exhibited that different organs, specifically the gastrointestinal system, is more prominent source of rhodanese, which show that cyanide delivered from

cyanogenic glycosides is mainly detoxified in the GI prior to being absorbed (see Aminlari & Vaseghi, 2006).

2.2.3.2 Xylanases

After cellulose, xylan is the most abundant polysaccharide in nature. Xylans are a major hemicellulose component, and together with cellulose, they comprise the bulk of the cell wall material in botanical products. Xylanases are β-retaining glycosidases capable of hydrolyzing linear β-1,4-linked polymers of xylose (with various substitutive groups such as arabinose). Multiple isoforms exist and these enzymes can be endo- or exo-acting (endo-acting are more important in foods). The xylanases are ubiquitous in nature and their presence is observed diversely in a wide range of organisms (Biely, 2003). They are in plants (especially important in cereals), bacteria, and fungi, and they typically range in molecular mass from 16 to 40 kDa. Xylanolytic enzymes also occurring in plants participate in the process of cell wall extension, cell division, seed germination, and other morphological and physiological events in plants.

During germination, endogeneous xylanases catalyze hydrolysis of these polysaccharides to eliminate the actual boundary imposed by cell wall on the free dispersion of starch and protein degrading enzymes through the germinating grain. Enzymes responsible for breaking down hemicellulose polysaccharides are likewise reported to be available in wheat grain and wheat flour (Kumar et al., 2017).

Xylanase enzymes are beneficial to food handling by depolymerizing water-unextractable arabinoxylan pentosans, which have high water-holding capacity This increases the viscosity and thickness of the dough, gluten strength, and final bread volume. The mixed xylanases are especially significant in the preparation of frozen dough. Endoxylanases are one of the hemicellulases that are utilized in fruit and vegetable processing. Xylanases are likewise utilized in brewing to lower viscosity of the wort in preparing, easeing partition/filtration steps, decreased haze formation, and marginally further developed process yields (Kumar et al., 2017; Bhardwaj et al., 2019).

2.2.3.3 Glucoamylase

Glucoamylase catalyzes the hydrolysis of α-(1,4), α,β-(1,1), α-(1,6), α-(1,3), and α-(1,2) glycosidic connections between adjoining glucosyl residues, arranged by their decreasing rates of hydrolydid. It starts hydrolysing from nonreducing end of starch-based substrates to liberate glucose. Glucoamylas assume a significant function in food processing to saccharify starch in cereals and produce glucose syrups from maltodextrins after the activity of α-amylase. Glucoamylas is produced by certain eubacteria, some archaea, various yeasts, and numerous filamentous fungi. Despite the fact that there are reports of animal and plant glucoamylas, these seem, by all accounts, to be essentially various enzymes with analytic properties that crossover those of genuine glucoamylas. Thus, and notwithstanding wide utilization of this enzyme in food industry, this catalyst won't be additionally discussed in this section.

2.3 Enzymes Working on Proteins

Proteolytic enzymes, proteinases, or proteases are used interchangeably to refer to enzymes that hydrolyze peptide bonds in proteins and peptides, according to the following equation:

$$-\mathrm{NH}-\mathrm{CHR}_1-\mathbf{CO}-\mathbf{NH}-\mathrm{CHR}_2-\mathrm{CO}-\ +\ \mathrm{H_2O}$$
$$\rightarrow -\mathrm{NH}-\mathrm{HR}_1-\mathrm{COOH}+\mathrm{N}+\mathrm{H}_3-\mathrm{CHR}_2-\mathrm{CO}-$$

Proteases are found in all living life forms (creatures, plants, and microorganisms). They are fundamental for separating of proteins to peptides and amino acids. In the human digestive system, the proteolytic enzymes pepsin (stomach) and several proteolytic enzymes in small intestine (trypsin, chymotrypsin, carboxypeptidases, leucine aminopeptidases, tripeptidases, and dipeptidases) convert the proteins of ingested food varieties to amino acids.

Proteases are by far the best-portrayed enzymes as far as their fundamental roles in the human digestion and early commercialization (the originally normalized calf rennet for cheddar making was promoted in 1874) is concerned (Parkin, 2017). Peptidases breakdown food proteins in situ or are added exogenously to cause protein change.

2.3.1 Specificity

The investigation of protease specificity provide information on active site structure and functionality, protein-protein association, controlling intracellular and extracellular pathways, and evolution of protease and substrate genes (Diamond, 2007). Proteolytic enzymes are specific as far as being endoproteases or exoproteases. The endoproteases, like trypsin, chymotrypsin, and chymosin, hydrolyze peptide bonds inside of the polypeptide chains. The exoproteases follow up on peptide bonds at the N-terminal (aminopeptidases) or the C-terminal (carboxypeptidases) of the protein. A few proteases are said to have broad specificity, i.e., they can hydrolyze peptide bonds regardless of the side chain group adjoining the scissile peptide bond (pepsin and bacterial subtilisin from different *Bacillus* spp). Majority of proteolytic enzymes, nonetheless, are totally specific for one or few amino acid residue at the scissile peptide bond. The scissile bond of the peptide substrates is assigned as that connecting residue R1 and R2 in above equation. For instance, trypsine attacks peptides on the C-terminal side of lysine and arginine amino acid residues. The residues for chymotrypsine and elastase are aromatic amino acids, in particular phenylalanine, tyrosine, or tryptophan and small hydrophobic amino acids like alanine, respectively (Parkin, 2017; Grahn et al., 1999). The protease binding sites are chemical and spatial complements of the substrate amino acid. This specificity for the substrate is due to the favorable binding interaction of the substrate amino acid side chain with residues that form the binding site of the protease.

$$\begin{array}{c} \ldots\ldots S3 - S2 - S1 - S1' - S2' - S3' \ldots\ldots \\ \text{-------}NH - CHR - \mathbf{CO} - \mathbf{NH} - CHR - CO\text{------} \\ \ldots\ldots P3 - P2 - P1 - P1' - P2' - P3' \ldots\ldots \\ \uparrow \end{array}$$

In the protease active site, S1, S1′, ... refer to the amino acids that provide the chemical and spatial environment that complements the substrate amino acids referred to as P1, P1′, ... Arrow shows the scissile peptide bond. These sites have been mapped and characterized for many proteases (Parkin, 2017; Vizovišek et al., 2018).

2.3.2 Classification

There are four distinct kinds of proteases, specifically, the serine proteases, the cysteine proteases, the aspartic proteases, and the metalloproteases, in light of the active site group of the enzyme engaged with the nucleophilic attack at the carbonyl carbon of the scissile peptide bond of the substrate. These are portrayed below (Whitaker, 2003).

2.3.2.1 Serine Proteases

Serine proteases share the presence of the side chain of a serine residue in the active site which function in their catalytic activity. Among the serine proteases, the most notable are trypsin, chymotrypsin and elastase, and the nine proteases engaged with blood coagulation and collagenase. The serine protease subtilisin is from *B. subtilus*. Most enzymes in this group have molecular masses of 25–35 kDa, and they are featured by a surface or internal cleft as substrate binding site. The pH ideal of these compound is around 8 (Whitaker, 2003; Hedstrom, 2002; Di Cera, 2009).

2.3.2.2 Aspartic (Acid) Proteases

Aspartic proteases are portrayed by two profoundly conserved ASP residues as the catalytic unit, with optimum pH close to 3–4. Most natural enzymes from this group are pepsin, calf chymosin (additionally called "rennin" or "rennet," utilized in cheese making), cathepsin (engaged with after death meat tenderization), and the chymosin-substitute peptidases from *Mucor* spp. These endopeptidases are typically 34–40 kDa in mass. The main mechanism for hydrolysis includes a dyad of conserves ASP residues acting as general acid/base (Whitaker, 2003; Ulusu et al., 2016; Nair & Jayachandran, 2019).

2.3.2.3 Cysteine (Sulfhydryl) Proteases

Cysteine proteases are a large group of enzymes (more than 130 known) present in animals, plants, and microorganisms. Most of these enzymes are among the papain family, and others being chymopapain (various isoforms) and papain from tropical

tree, Carica papaya (papaya), actinidin from kiwi fruit, ficin from fig latex, bromelain from pineapple, and lysosomal cathepsins from animal tissues. Among them, papain, bromelain, and ficin address 5% of the worldwide sale of proteases (Illanes, 2008; Verma et al., 2016).

The most studied cysteine protease is papain, which will be examined later in light of its significant uses in the food industry for chill proofing of brew and meat tenderization, and for synthesis of peptides and other organic compounds. An interesting cysteine protease in muscle is calpain (numerous isoforms) that is activated by Ca^{2+} and plays a part in after death tenderization of muscle. Commonly, the enzymes of this group are 24–35 kDa in mass, are active at pH 6.0–7.5, and can endure temperatures up to 60–80 °C (due in some extent by three disulfide bonds) (Whitaker, 2003; Otto & Schirmeister, 1997).

2.3.2.4 Metalloproteases

A large group of the metalloproteases are exopeptidases. These are the carboxypeptidases A and B, glycyl-glycine dipeptidase, carnosinase (work on β-alanyl-L-histidine and related compounds), amino-acyl-histidine hydrolase, and cytosolic aminopeptidase (α-amino-acyl-peptide hydrolase). All require Zn^{2+} as cofactor. Some of these enzymes, for instance, prolidase (where proline or hydroxyproline is the carboxyl terminal residue) and iminodipeptidase (in which proline or hydroxyproline is the N-terminal residue) require Mn^{2+}. Other metalloprotases are endo-acting thermolysin (from *Bacillus thermoproteolyticus*) and the nreutal endoprotease from *Bacillus amyloliquefaciens* (Tavano et al., 2018).

2.3.3 Application of Proteases in Food Industry

Commercial proteases are available at various levels of purity, and some contain multiple proteolytic agents. Some of the important commercial applications of proteolytic enzymes are shown in Table 2.3 and are described below (Whitaker, 2003).

2.3.3.1 Production of Protein Hydrolysates

Hydrolysis of food proteins utilizing proteases, like pepsin, trypsin, chymotrypsin, papain, and thermolysin, change their functional properties, for example, nutritional, flavor/sensory, textural, and physicochemical (solubility, foaming, emulsifying, gelling) and decreased allergenicity. Broad hydrolysis by nonspecific proteases, for example, papain, causes solubilization of even inadequately soluble proteins. Such hydrolysates ordinarily contain low molecular weight peptides with two to four amino acid residues. Several functional properties of proteins, like gelation, foaming, and emulsifying properties, suffer from excessive hydrolysis. Typically, a protein is treated by an endopeptidase in a batch process for a couple of hours, after which the added enzyme is inactivated by heat treatment. This treatment brings about fast reductions in normal molecular weight of proteins, whereas exopeptidases are utilized to hydrolyze small oligopeptides to composite amino acids. Proteins

Table 2.3 Use of proteolytic enzymes in food processing

Food	Purpose of action
Baked goods	Softening action in doughs. Cut mixing time, increase extensibility of doughs. Improve texture, elasticity, and loaf volume. Liberate β-amylase.
Brewing	Body, flavor, and nutrient development during fermentation. Aid in filtration and clarification. Chill-proofing.
Cereals	Modify proteins to increase drying rate, improve product handling characteristics. Production of miso and tofu.
Cheese	Casein coagulation. Characteristic flavor development during aging.
Chocolate, cocoa	Action on beans during fermentation.
Egg, egg products	Improve drying properties
Feeds	Waste product conversion to feeds. Digestive aids, particularly for pigs.
Fish	Solubilization of fish protein concentrate. Recovery of oil and proteins from inedible parts.
Legumes	Hydrolyzed protein products. Removal of flavor. Plastein formation.
Meats	Tenderization. Recovery of protein from bones.
Milk	Coagulation in rennet puddings. Preparation of soybean milk.
Protein hydrolysates	Condiments such as soy sauce and tamar sauce. Bouillon. Dehydrated soups. Gravy powders. Processed meats. Special diets. Antinutrient factor removal. Specific protein inhibitors of proteolytic enzymes and amylases. Phytate. Gossypol. Nucleic acid.
Wines	Clarification
In vivo processing	Conversion of zymogens to enzymes. Fibrinogen to fibrin. Collagen biosynthesis. Proinsulin to insulin. Macromolecular assembly.

(ordinarily meat, milk, fish, wheat, vegetable, vegetable, and yeast sources) might be exposed to a pretreatment that renders them to some degree of denaturation as this improves peptidase access and hydrolytic activity. Protein hydrolysates can improve functional properties, for example, emulsifying capacity, foam capacity and stability, antioxidative effect, water absorption capacity, and nutritional properties (Chalamaiah et al., 2012; Thiansilakul et al., 2007; Sinha et al., 2007; Clemente, 2000). Protein hydrolysates constitute an alternative to intact proteins and elemental formulas in the development of special formulations designed to provide nutritional support to patients with different needs. The production of extensive protein hydrolysates by sequential action of endopeptidases and exoproteases coupled with the development of post-hydrolysis procedures is considered the most effective way to obtain protein hydrolysates with defined characteristics (Rocha et al., 2009). Protein levels in production of protein hydrolysates are often 8–10%, provided there are no limitations on solubility, and the amount of enzyme added is generally ~2% on a protein basis, depending on purity. Protein:enzyme levels are sufficiently high so that the enzyme reacts at nearly Vmax with limited autodigestion of the enzyme, although product inhibition by accumulating peptides may attenuate reactivity. The hydrolysates can be characterized by the degree of hydrolysis (DH), sequence of produced peptides, functional properties such as solubility, emulsifying properties,

etc. DH can be easily evaluated with *o*-phthaldialdehyde (OPA) method which determines the number of free amino groups (Mirzaei et al., 2016). Typically, a DH value of 3–6% corresponding to peptides of 2–5 kDa is desired for physicofunctional properties, while DH of 8% or more means 1–2 kDa peptides, demonstrating optimal solubility for use in sports and clinical nutrition products. More exhaustive DH (as high as 50–70%) yields small peptides and amino acids of <1 kDa which are useful for infant and hypoallergenic foods and savory flavoring ingredient preparations (soups, gravies, sauces). One problem associated with the greater DH is the possibility of production of bitter peptides (small and hydrophobic), thereby necessitating to control this potential flavor defect (Chalamaiah et al., 2012).

Proteases can likewise be utilized to detach remaining muscle protein from bones of fish and land animals as protein hydrolysates, and this normally includes heating at 55–65 °C for 3–4 h. The final product blend from enzyme hydrolysis of proteins might require posttreatment refining as well as separation to get deravatives appropriate for the planned application (Chalamaiah et al., 2012; Mizani et al., 2005).

2.3.3.2 Application of Proteolytic Enzymes in Dairy Industry

Milk is an entirely perishable food item and therefore there has been a strong motivation to change it over to more stable items, which is facilitated by specific properties of milk. Traditionally, milk has been safeguarded by fermentation, normally with addition of salt (cheddar, fermented milks, butter). More current safeguarding strategies incorporate drying, pasteurization/sterilization, and freezing. A few qualities of milk additionally render it entirely amiable to modification by enzymes. The reality that milk is a fluid facilitate enzyme addition. Cheese manufacturing is presumably the oldest, as yet the biggest, utilization of exogenous enzyme in food processing.

Since the principal components of milk are proteins (3:5%), lipids (3:6%), and lactose (4:8), the principle enzymes used in dairy technology are proteinases and peptidases, lipases, and β-galactosidase (lactase). However, several oxidoreductases have significant applications. In this section, we will focus on the proteases. Other enzymes will be dealt with in other sections. Proteolytic enzymes are the most widely used enzymes in dairy technology. Applications include cheese manufacturing, modification of functional properties, production of protein hydrolyzates, and for nutritional and other applications (Fox, 1993; Shah et al., 2014).

Cheese Manufacturing

The main gastric enzyme of neonatal ruminants is chymosin or rennet. Chymosin has low broad proteolytic action, but high milk-coagulating action. Calf chymosin (rennet) and chymosin substitutes (see beneath) are added to milk to cause the underlying milk-clotting reaction prompting cheese formation. The rennet coagulation of milk is a two-stage process. The first (essential) stage includes specific hydrolysis of the PHE105-MET106 bond of κ-casein and creation of "para-casein" and TCA (trichloroacetic acid)-soluble peptides (glycomacropeptides), while the

next stage includes the Ca-induced gelation of para-casein at a temperature of 30–35 °C. Proteolysis is essentially complete before the beginning of coagulation. The remaining from k-casein, which is referred to as para-k-casein, stays in the micelle, yet the hydrophilic peptide, called the caseino(glyco)macropeptide, diffuses into the whey and thus the micelle-settling properties of k-casein are lost. Starter cultur are added to milk at 40–45 °C to cause a pH reduction to 5.8–6.5, whereupon chymosin is added to start clotting. Because of ensuing steps of cheese manufactur, some enzyme activity stays in the curd and contributes to cheese aging and flavor improvement during aging. Proteases in the starter cultures additionally add to support proteolysis and flavor improvement during aging.

The gene for calf chymosin has been cloned in chosen microbes, yeasts, and molds. A recombinant chymosin from *E. coli* has been created. Microbial recombinant chymosin contain no pepsin, though 5 to 50% of the milk clotting action of calf rennets might be because of pepsin (Kawaguchi et al., 1987; El-Sohaimy et al., 2010).

Expanding world production of cheddar (3% each year throughout recent years), accompanying with a diminished stock calf neonatal, the stockpile of veal rennet has been deficient for a long time, which has led to a quest for rennet substitutes. During the last 40 years, the expanding utilization of an assortment of milk-clotting catalysts has turned into a significant specialized pattern in cheese production. Although numerous proteinases can coagulate milk, just few have been viewed as pretty much adequate as rennets. These are cow, porcine, and chicken pepsins; rennet (watery concentrates produced using the fourth stomach of calves, kids, sheep, goat, and cow); microbial rennets made by *Endothia parasitica*, and *Mucor* species (*Mucor miebei* or *M. pusillus*) and *Cryphonectria parasitica*, and plant proteases (Shah et al., 2014; Kim et al., 2004a).

Chicken pepsin is the most unreasonable of pepsins and is utilized uniquely in extraordinary conditions. Bovine pepsin gives commonly palatable outcomes regarding cheese yield and quality; numerous commercial "calf rennets" contain a significant extent of bovine pepsin. Although the proteolytic specificity of the three normally utilized fungal rennets on αs1- and β-caseins is extensively different from that of calf chymosin, they by and large yield acceptable cheese and were broadly utilized in the United States before the presentation of microbial recombinant chymosin (Nelson, 1977; Green, 1977).

Milk-Clotting Enzymes from Plants

Although several properties of microbial milk-clotting enzymes such as high proteolytic activity and high resistance to heat denaturation made them suitable for cheese manufacturing, plant protease have attracted considerable attention and interest in cheese industry since they are easily available and their purification is simple. As discussed above plant protease are classified into several groups depending on the mechanism of catalysis in the hydrolytic step. Aspartic, serine and cysteine protease are the three major milk clotting enzymes from plants (Shah et al., 2014).

Aspartic protease are present in many plant species and have aspartic acid residues in their active site. They participate in many activities in plants. Examples include:

1. Cardosin from flowers of the artichoke thistle (*Cynara cardunculus*)
2. Cynarase from flowers of globe artichoke (*Cynara scolymu*)
3. A Cardosin A like enzyme from thistle (*Cynara humilis*)
4. An enzymatic extract from the thistle *Silybum marianum* flowers
5. Oryzasin from seeds of rice (*Oryza sativa*)
6. Enzymatic extract from flowers of moringa (*Moringa oleifera*)
7. Onopordosin from cotton thistle (*Onopordum acanthium*)
8. Cirsin JN703462 from flowers of common thistle (*Cirisum vulgare*)
9. Enzymatic extract from cell suspension of red star-thistle (*Centaurea calcitrapa*)

Serine proteases have in their active sites serine residues. Their main role in plants is almost the same as the aspartic proteases, with some additional features. Serine proteases are widespread in plants and belong to several taxonomic groups. They are extracted, purified and characterized from several parts of plants, especially fruits. Some of the enzymes in this group are (Di Cera, 2009):

1. Dubiumin from seeds of *Solanum dubium* (Solanum, also called bittersweet or woody nightshade) (from Wikipedia, the free encyclopedia).
2. Cucumisin from *Cucumis melo* fruits (Muskmelon is a species of melon that has been developed into many cultivated varieties. These include honeydew, cantaloupe, and Persian melon) (from Wikipedia, the free encyclopedia).
3. Lettucin from leaves of lettuce, *Lactuca sativa* (from Wikipedia, the free encyclopedia).
4. Religiosin from *Ficus religiosa* (Sacred fig is a species of fig native to the Indian subcontinent and Indochina) (from Wikipedia, the free encyclopedia).
5. Streblin from stems latex of *Streblus asper* (a tree known by several common names, including Siamese rough bush, khoi, serut, and toothbrush tree).

Cysteine proteases or thiol-proteases have a catalytic mechanism that involves a cysteine group in their active site. Plant extracts, or their purified proteases, have been extensively characterized in many studies, for their potential use as milk coagulants. Following is a list of examples of cysteine proteases from plants (Amal Ben Amira et al., 2017; Lo Piero et al., 2011):

1. Enzymatic extract from seeds of *Albizia lebbeck* (a species of *Albizia*, native to Indomalaya, New Guinea, and Northern Australia and widely cultivated in tropical and subtropical regions). It is often called lebbek tree siris, frywood, koko, and woman's tongue tree.
2. Enzymatic extract from seeds of sunflower (*Helianthus annuus*).

3. Ficin from branches latex of figs tree *Ficus carica sylvestris* and an enzyme from stem latex of *Sideroxylon obtusifolium* (*Sideroxylon* is a genus of trees in the family *Sapotaceae*. They are collectively known as bully trees).
4. Actinidin from kiwi (*Actinidia chinensis)* fruits.
5. Calotropain from latex of crown flower *Calotropis gigantean.*

Recently, we reported on application of actinidin to the production of cheese from cow's milk. Comparison of actinidin and chymosin indicated that the former could be a potential alternative to the latter in application as milk coagulant (Alirezaei et al., 2011).

In general, the major drawback of most plant rennets is the development of an increased bitterness and the appearance of cheese texture defects during storage and/or ripening. These defects are mainly due to excessive proteolytic activities and low ratios of milk-clotting activity/proteolytic activity. For this reason, the evaluation of enzyme activities and their comparison with those of commercial rennet (chymosin) is an important first step in selecting a suitable plant rennet.

Taken together, the utilization of various sorts of plant proteases in cheese indudrty impacts the hydrolysis level of the protein matrix of milk, prompting differeneces in sensory properties of cheese. The vast majority of plant rennets produce cheeses with harsh flavors because of too much proteolytic activity, which restricts their industrial application. Hence, the determination of a proper plant coagulant and the control of various gelation properties are vital to acquire a superior quality of end product.

2.3.3.3 Application of Enzymes in Meat Industry

Meat and meat product utilization (beef, pork, lamb, goat, and poultry) has expanded, especially in nonindustrial nations. The world utilization of meat products will arrive at 40 kg per capita in 2020. The platability of meat is affected by a few factors, among which meat tenderness is viewed as the main determinant for consumers. The processes associated with the transformation of muscle to meat are complex. The chemical and physical properties of muscle tissue and the related connective tissue are determinant of meat quality. Of the relative multitude of characteristics of meat quality, consumers rate tenderness as the most significant. Tenderness is a quality attribute coming about because of the interaction of actomyosin of myofibrillar proteins, the foundation of connective tissue, sarcomere length, and the bulk density of fat. Meat tendeerness likewise relies on the degree of protrolysis of muscles (Arshad et al., 2016; Marques et al., 2010).

There are multiple ways of tenderizing meat, by chemical or physical means, which basically decrease the amount of recognizable connective tissue without causing broad breakdown of myofibrillar proteins. The myofibrillar breakdown begins after initiation of the enzymatic system and involves the proteins tropomyosin, troponin T, troponin I, C-protein, connectin, desmin, vinculin, dystrophin, nebulin, and titin. The first change in meat tenderness is because of the activity of complex endogenous calpain-calpastatin system, which acts in muscle tissue after slaughter. In muscles, these proteolytic enzymes play a huge part in after death

proteolysis and meat tenderization. Cathepsins were the main enzymes utilized in meat tenderization, after which calpain was applied owing to its action in changing the Z-line thickness found in post-mortem, despite the fact that it was not at first connected with meat tenderization. Calpains are calcium-dependent proteases that break down myofibrillar proteins. Calpastastin, on the other hand, inactivates calpains, decreases the myofibrillar degradation, and hence lessens the tenderness. Calpastatin effect is finished after being inactivated by cooking. The amount of the enzymes changes among species, which affect the degree of meat tenderness, because of expanded or diminished proteolysis of myofibrillar proteins (Lian et al., 2013).

Meat tenderness relies upon the kind of muscle, pre- and post-mortem variables, and after death pH and temperature. The research for significant proteases with unique specificity for industrial applications is consistently a continued challange. Proteolytic enzymes from plant sources have gotten extraordinary attention for being active over a wide range of temperatures and pH's. Treatment by proteolytic enzymes is one of the most well-known techniques for meat tenderization. The utilization of exogenous proteases for meat tenderization is a rather modern strategy to further develop meat quality. There are few exogenous proteolytic enzymes, plant proteases (papain, bromelain, ficin, and actinidin), and proteases from *Aspergillus oryzae* and *Bacillus subtilis*, which have been considered as generally regarded as safe (GRAS) for use in the meat. Papain and other sulfhydryl endopeptidases (bromelain and ficin) are applied to muscle or meats that don't turn out to be sufficiently tendered during post-mortem aging. These enzymes are powerful in this application since they can hydrolyze collagen and elastin, connective tissue proteins that cause toughness in meat. These proteolytic enzymes are blended in the meat to break down the proteins in muscle and hydrolyze collagen and elastin, which helps in meat tenderization. Papain and bromelain are the most regularly involved plant enzymes for meat tenderization. As meat tenderizers, proteolytic enzymes are the most ideal for breakdown of collagen and elastin in connective tissue at somewhat low pH and low temperature. The structure of myosin and actin fibers is impacted by the plant proteases. The tenderness of meat is deteremined by enzyme activity assessment, myofibrillar fragmentation index, hydroxyproline estimation, and examining by electron microscopy. The ideal meat tenderizer would be a proteolytic enzyme with specificity for collagen and elastin in connective tissue, at the generally low pH of meat, which would act either at the low temperature at which meat is stored or at the high temperature accomplished during cooking (Arshad et al., 2016; Lian et al., 2013).

Papain

Papain is a nonspecific thiol protease and the significant protein constituent of the latex in the tropical plant Carica papaya. It is an important plant protease. The latex is obtained by scoring, and afterward permitting it to dry on the fruit, and a crude material is resulted. In the food industry, papain is regularly utilized for meat tenderization, production of protein hydrolysate, and the clarifying of juice and beer. Papain is also used in the baking and dairy industries (in cheese production),

and for the extraction of flavor and color compounds from plants. Papain is prepared by decersing contaminants and further extraction. The enzyme has high thermal and pressure resistances, requiring exceptional process conditions for sufficient inactivation (to accomplish 95% inactivation of papain at 900 MPa and 80 °C, 22 min of processing is required). The physiological job of papain in plants is to protect them from insects. Papain tends to over tenderize the meat surface, making it "soft". Papain is utilized at a portion of 300 units/kg to release free amino acids in dry fermented frankfurters. The three-dimentional structure for papain is reported. Wide range enzymatic activity has been shown by papain in the pH range 5.9–7.5 and the temperatures of 70–90 °C with optimum of 65–75 °C. Under these conditions, papain has superb activity in hydrolysis of myofibrillar proteins and moderate impact on hydrolysis of collagen (Istrati, 2008; Gokoglu et al., 2017).

Papain can be utilized in the fruit juice and beer clarification. In beer, an imperfection alluded to as chill-haze might be brought about by the combination (complexing) of tannins and proteins. Papain has for some time been utilized to hydrolyze protein and limit cloudiness, despite the fact that bromelain and ficin just as other bacterial and fungal proteases may now be utilized for this reason. The endopeptidase is added after aging and preceding final filtering. Papain is finally inactivated by pasturization, and unnecessary activity of papain might cause loss of foam stability. Controlled or evaluated proteolysis in brew is important, since some remaining protein is important to preserve specific quality attributes (Mosafa et al., 2013).

Bromelain

Bromelain (or bromelin) enzymes are present in huge amounts in fruits, leaves, root, and stems of the Bromeliacea family, of which pineapple (*Ananas comosus*) is the best known. The juice of various pieces of the plant contains bromelain in soluble form. Its high proteolytic action has made a wide interest in various applications, principally in tenderization, food, detergents, and the textile industry. Bromelain is commercially accessible in powdered form. It is assessed that 95% of the utilized proteases in the United States are derived from plant proteases like papain and bromelain, though microbial tenderizers are not utilized broadly. Reverse micellar extraction is utilized for isolation and purification of bromelain from pineapple. There is high bromelain recovery and purification in this strategy. This enzyme, as different proteases, breakdown myofibrillar proteins and collagen, causing overtenderization of meat. Bromelain can be utilized in beef at 10 mg/100 g meat and ideal tenderization happens after 24 h at 4 °C. Following this time span, the enzyme can be inactivated by heating at 70°. pH range for enzymatic activity for bromelain is 4–7 and optimum pH is 5–6. The range of temperature for activity is at 50–80 °C and optimum is 65–75 °C. Under these conditions, bromelain has moderate hydrolysis activity of myofibrillar proteins and good effect on hydrolysis of collagen (Arshad et al., 2016; Gokoglu et al., 2017).

Ficin

Due to the problems raised by the use of animal or microbial recombinant proteases, the use of ficin is becoming increasingly popular. Ficin is an outstanding example of proteases from plants. This enzyme can be applied for protein hydrolysis, the production of bioactive peptides, meat tenderization, milk coagulations in cheese making, or peptide synthesis. Ficin does have huge potential and brilliant prospect in the near future. Ficin is a sulfhydryl or cysteine protease commonly obtained from *Ficus carica* (fig tree) that enhances the solubility of muscle proteins (Englund et al., 1968; Morellon-Sterling et al., 2020). Ficin has a molecular weight of 44.5 kDa and shows maximal activity at pH 5.0–9.0 and optimum pH is 7.0. The enzyme is fully active at 45–75 °C with optimum temperature of 60–70 °C. Under these conditions, ficin has moderate activity in hydrolysis of myofibrillar proteins and excellent effect on hydrolysis of collagen. These properties make ficins a beneficial class of plant proteases for use. We used ficin to tenderize beef used for manufacture of sausages. Results indicated that solubility of meat proteins increased and SDS-PAGE results showed the disappearance of several protein bands in ficin-treated meat. Ficin-tenderized meat substantially improved water-holding capacity and emulsion stability. The results of this study indicated that some quality attributes of meat products can be improved by enzymatic modification of protein sources in the manufacture of meat products (Ramezani et al., 2003).

Actinidin

Actinidin is a novel sulfhydryl protease extracted from gooseberry or the kiwi fruit. Actinidin is the predominant enzyme in kiwifruit and can play a role in aiding the digestive process. It has a molecular weight of 32 kDa. It is used commercially in meat industry to tenderize meat. The ability of pre-rigor infusion of kiwifruit juice to improve the tenderness of lamb was investigated. It was shown that actinidin in kiwi fruit juice enhances proteolytic activity, resulting from the infused kiwifruit juice in carcasses, and is associated with significant degradation of the myofibrillar proteins, appearance of new peptides, and activation of m-calpain during post mortem aging. Thus, kiwifruit juice is powerful and easily prepared meat tenderizer, which could contribute efficiently and effectively to the meat tenderization process (Boland, 2013; Morton et al., 2009).

Actinidin has many applications in the food industry because of its advantages over other plant proteases such as papain and ficin. Actinidin shows mild tenderizing activity even at high concentrations, preventing surface mushiness. It has a relatively low inactivation temperature (60 °C), which makes it easier to control the tenderization process without overcooking. A group in our lab studied the effect of actinidin on beef for use in manufacture of sausage. Actinidin from kiwi fruit was partially purified by precipitation with ammonium sulfate, followed by DEAE-Sephadex column chromatography. The effect of purified actinidin on the protein solubility (nitrogen solubility index [NSI]), water-holding capacity (WHC), texture, and SDS-PAGE pattern of beef was studied and the quality attributes of a sausage product were evaluated. Actinidin significantly increased NSI and WHC of beef; the highest NSI and WHC (approximately 20% and 8% increase, respectively) was

observed when beef was incubated with 0.9 unit enzyme/g beef. Texture analysis indicated increased tenderization (10% decrease in shear force) when slices of beef were treated with actinidin at 37 °C for 2 h. SDS-PAGE results indicated appearance of several low molecular weight bands (<10 kDa) after treating beef with different levels of actinidin for 30 or 60 min. Slight changes in protein band in the range of 100–120 kDa and 13–25 kDa were also observed. Use of actinidin-tenderized beef significantly improved emulsion stability, texture, and organoleptic properties of the sausage product (Lewis & Luh, 1988). We applied actinidin to tenderize camel meat and beef and used the tenderized meats in the manufacture of emulsion type sausages (Gheisari et al., 2008). The properties of sausages made from the meat of both species were similar. Emulsion satiability, folding, texture, taste, and overall quality of sausages produced from actinidin-tenderized meat were superior than untreated samples (Aminlari et al., 2009). The actinidin has less tenderization property as compared to other traditional plant proteases and still not approved as GRAS by FDA (Lewis & Luh, 2007; Toohey et al., 2011).

Calpains

Endogenous proteolytic systems are responsible for modifying proteinases as well as the meat tenderization. Abundant evidence has shown that calpains and calpastatin (CAST) have the closest relationship with tenderness in livestock. They are involved in a wide range of physiological processes including muscle growth and differentiation, pathological conditions and postmortem meat aging (Lian et al., 2013). Meat tenderness undergoes changes after slaughtering due to the activity of the endogenous calpains and calpastatin. These calcium-dependent proteases degrade the myofibrillar proteins. The calpain system consists of three members—m-calpain, μ-calpain, and calpastatin, which is the calpain-specific endogenous inhibitor. Both m-calpain and μ-calpain are cysteine proteases, and their proteolytic activity is affected by oxidation, which can influence the quality of fresh meat. In the presence of calcium, calpains autolyze, and this autolysis is indication of their proteolytic activation during postmortem changes in muscles (Zhang et al., 2013). Calpain is an important enzyme that is chiefly used for degradation of myofibrillar proteins. It also aids in meat tenderizing and improves water holding capacity during postmortem aging.

2.3.3.4 Conclusion

In meat, tenderness is the most important factor associated with meat palatability and consumer satisfaction. Different plant proteases like papain, bromelain, actinidin, and ficin have been used for tenderization of meat and meat products. These enzymes are effective in this application because they can hydrolyze collagen and elastin, connective tissue proteins that cause toughness in meat. Antemortem application of enzyme is possible, as a fairly pure solution in saline can be injected intravenously into animals 2–10 min before slaughter, sometimes after stunning; this helps distribute the enzyme throughout the muscle tissues. Injection of inactivated papain (disulfide form) obviates any discomfort among animals, since the enzyme

becomes activated by the reducing conditions that soon prevail postmortem (Arshad et al., 2016).

2.3.4 Bioactive Peptides

Bioactive peptides have been defined as specific protein fragments that have a positive impact on body functions or conditions and may influence health (Sánchez & Vázquez, 2017). In recent years, peptides with known sequences have been identified which have been shown to contain biological activities such as opiate-like, mineral binding, immunomodulatory, antimicrobial, antioxidant, antithrombotic, hypocholesterolemic, and antihypertensive actions. These bioactive peptides benefit the human physiological system through the cardiovascular, nervous, gastrointestinal, and immune systems (Choi et al., 2012). Most of the bioactive peptides are inactive in the native protein sequences and become active only when released when the parent proteins are hydrolyzed in vivo by the action of proteolytic enzymes during digestion or in vitro by intentionally added proteases (Korhonen & Pihlanto, 2006). The activity of these peptides is based on the inherent amino acid composition and sequence. Bioactive peptides usually contain 3–20 amino acid residues per molecule (Bhat et al., 2015), but in some cases may consist of more than 20 amino acids. These peptides may be used as components of functional foods or nutraceuticals because of their health-enhancing potential and safety profiles. There is increasing commercial interest in the production of bioactive peptides from various sources. Some of rich sources of bioactive peptides include milk and egg, meat of various species of animals, fish, many plants including soy bean, chickpeas, rice, and many other food-proteins and nonconventional protein sources (Mazorra-Manzano et al., 2018; Sabbione et al., 2016).

Proteolytic enzymes have the ability to modify proteins through limited or extensive cleavage, releasing free amino acids, peptides, or polypeptides with physicochemical properties different from the original protein. Proteolytic enzymes from proteolytic system of starters, proteases endogenous to food, or added enzymes (e.g., rennet) differ in their specificity and therefore in their capacity to release bioactive sequences (Korhonen & Pihlanto, 2006; Sabbione et al., 2016). Most hydrolytic processes use one protease at a time; however, a combination of two enzymes with different specificity has also been explored. Sometimes, proteins partially hydrolyzed are further treated with pepsin and trypsin to simulate gastrointestinal digestion. Other proteases widely used for bioactive peptides' production include commercial preparations such as Alcalase, Neutrase, Flavourzyme, Thermolysin derived from bacteria and fungi (Rui et al., 2012), and ficin (Shahidi et al., 2018).

We have studied the production of peptide fragments produced from goat's milk whey proteins using trypsin and ficin and evaluated the bacterial growth inhibitory activity of peptides. Goat's milk whey proteins were subjected to enzymatic hydrolysis and peptides were purified by ultrafiltration followed by reverse-phase high-performance liquid chromatography (RP-HPLC). Growth inhibitory activities of

hydrolysates ranged from 4.67% to 87.46% for *E. coli* and 3.03% to 98.63% for *B. cereus*. Among all peptide fragments, permeate containing 3 kDa peptides produced by trypsin showed maximum inhibition against Gram-positive and Gram-negative bacteria. This fraction was further purified by HPLC. Fourteen peptide fractions were collected and evaluated for their growth inhibitory activities. Two fractions showed the highest growth inhibitory activities with MIC50's of 383 ± 8 and 492 ± 10 μg/mL against *E. coli* and *B. cereus*, respectively. In a similar study, we used trypsin and ficin to generate antibacterial peptides from goat milk caseins. The peptide obtained by ficin with MW of <3 kDa showed the highest antimicrobial activity and was selected for further purification by reversed-phase high-performance liquid chromatography (RP-HPLC). Twenty-seven peptide fractions were separated, and their antimicrobial activities were evaluated. The results showed that one of the fractions (No. 14) possessed the highest activity against *Escherichia coli* and *Bacillus cereus* (Esmaeilpour et al., 2016, 2017). The authors of both papers suggested these novel antibacterial peptides can potentially replace synthetic food preservatives in food industries.

In another research project, the yeasts *Kluyveromyces marxianus* and *Saccharomyces cerevisiae* protein hydrolysates were prepared by trypsin and chymotrypsin hydrolysis and the peptides purified by reverse-phase high-performance liquid chromatography (RP-HPLC). The antioxidant and ACE (angiotensin-converting enzyme) inhibitory activities of the generated peptides were determined. From *K. marxianus* two new peptides, LL-9, MW 1180 Da and VL-9, MW 1118 Da, were identified. These peptides were sequenced and their functional properties studied. Both peptides exhibited significant ACE inhibitory activity (IC50 of 22.88 mM for LL-9 and 15.20 mM, for VL-9). Molecular docking studies revealed that the ACE inhibitory activities are due to interaction with the His513, His353, Glu281 and Tyr520, Lys511, Gln281 pockets of ACE by LL-9 and VL-9, respectively. In the case of *S. cerevisiae*, a fraction with molecular weight of <3 kDa exhibited the highest activity. RP-HPLC resolved this fraction into five fractions, one of which (fraction F3) with amino acid sequence of Tyr-Gly-Lys-Pro-Val-Ala-Val-Pro-Ala-Arg (MW: 1057.45 Da) exhibited ACE inhibitory ($IC_{50} = 0.42 \pm 0.02$ mg/mL) and antioxidant activities (26.25 ± 0.13 μM TE/μg protein). Taken together, the results of these studies showed that *K. marxianus* and *S. cerevisiae* proteins contain specific peptides in their sequences which can be released by enzymatic hydrolysis.

These peptides have excellent bioactive properties that can potentially replace the antioxidant and antihypertensive agents with chemical origin (Mirzaei et al., 2015, 2017).

The use of plant proteases in the production of bioactive peptides is still scarce. Cysteine proteases such as papain, ficin, and bromelain are currently the most used plant proteases. However, serine proteases such as zingibain, cucumisin, and actinidin obtained from ginger rhizome, melon, and kiwifruit, respectively, are three new emerging plant proteases which have been considered recently (Nafi et al., 2013).

The search for novel specialized proteolytic enzymes with preference for specific peptide bonds for the selective release of bioactive peptides requires further study in order to improve process efficiency. The various plant proteases, the protein substrates used, and the bioactive properties of peptides produced and their potential to prevent or treat disorders such as hypertension, diabetes, obesity, and cancer have been extensively reviewed (Mazorra-Manzano et al., 2018).

2.4 Enzymes Working on Lipids

2.4.1 Lipases

Lipases are key enzymes involved in fat digestion in vertebrates by converting insoluble triacylglycerols into more soluble products, fatty acids, and monoacylglycerols, which can easily be assimilated by the organisms' intestinal absorption. Most lipases have a basic pH optimum. Lipases act only at the oil–water interface (Schmid & Verger, 1998).

While activity of endogenous lipases is often associated with acylglycerol hydrolysis and problems with lipid degradation and/or hydrolytic rancidity (or leading to oxidative rancidity since liberated fatty acids tend to be more prone to oxidation), exogenous lipases are used for beneficial purposes. Lipases for food processing are obtained from edible forestomach tissue of calves, kids, or lambs, and animal pancreatic tissues as purified edible tissue preparations or as aqueous extracts (NAS, 1996). The porcine pancreatic enzyme is a glycoprotein composed of 450 amino acids with a calculated MW of ~50 kDa (Wong, 2003). Industrial lipases are also produced by the controlled fermentation of *Aspergillus niger* var., *Aspergillus oryzae* var., *Candida rugosa*, and *Rhizomucor miehei* as a powder or liquid (Parkin, 2017).

Currently, commercial uses of lipases involve liberating flavoring (short-chain) acids from lipids and rearranging fatty acyl groups along the glycerol backbone to create highly valued and functional triacylglycerols from low-value lipids. Typical applications in the food industry include: flavor generation in cheese. Using pregastric lipases from goat, lamb, and calf, selective hydrolysis of short-chain (C4–C8) fatty acids from triacylglycerols of milk fat can be achieved (Parkin, 2017). The lipase in papaya latex is selective for the sn-3-glycerol position, but since papaya latex contains papain, it would not be suitable in cheese. Lipases are also used for modification of lipids, manufacture of dairy products and confectionery goods, and development of flavors in processed foods. These applications are founded on selecting lipases with reaction selectivities required to yield the desired products. Selectivity of lipases involves selectivity toward fatty acylgroup, ester position along the sn-glycerol backbone, size of the glyceride (mono-, di-, or triacylated), as well as interactions among these factors, which confer characteristic stereoselectivity. The types of selectivity are exhibited by many of the commercially relevant or promising lipases from over 100 characterized sources (Shahidi et al., 2018).

Another major use of lipases is production of "structured lipids" in which the fatty acyl groups are rearranged by lipases to yield a novel distribution along sn-glycerol to create high-value lipids from low-value acylglycerol (Gunstone, 1999).

Lipases are used in baking industry and are added to bread doughs to supplement endogenous cereal grain lipases and are added as dough improvers. This functionality results in increased bread volume, more uniform crumb and air cell size, and lesser tendency to stale, hydrolysis of lipids, thereby producing mono- and diacylglycerolipids which function as emulsifying agents in the dough (Sonnet & Gazzillo, 1991; Parkin, 2017).

2.4.2 Lipoxygenases

Lipoxygenase is a naturally existing and inexpensive enzyme widely distributed in plant and animal cells and is abundantly found in legumes. Lipoxygenase is an oxidoreductive enzyme catalyzing many oxidative reactions (Song et al., 2016). The substrates of lipoxygenase in plants are mainly linoleic acid and linolenic acid. Arachidonic acid is the primary substrate for lipoxygenase in animal cells. Lipoxygenase catalyzes the dioxygenation of polyunsaturated fatty acids (PUFA), converting them to diene hydroperoxy fatty acids which further decompose to different chemicals and volatile compounds (Gigot et al., 2010). Lipoxygenase utilizes atmospheric oxygen to react with PUFA and forms potentially useful and valuable chemicals such as leukotrienes and lipoxins. The enzyme has many potential usages in the food industry; however, it is also associated with some unwanted reactions, which include color change and nutrient deterioration (Parkin, 2017). The flavor and aroma compounds produced by lipoxygenase activity on fatty acids affect the food flavor. In one aspect, the aroma compounds might improve the desirability of foods, and on the other hand, the oxidized compounds produced might give rise to off-flavor of foods. By studying and understanding the properties and reaction mechanism of LOX, food researchers could well control and utilize LOX as a natural food additive (Shi et al., 2020). In the baking industry, it functions as an effective baking conditioner and flour treatment agent. It is a potential substitute for potassium bromate and benzoyl peroxide, which are commonly used as strengthening and bleaching agents, respectively. Dough strengthening is probably through affecting disulfide cross-links within the gluten. LOX has been widely used in the food and beverage industries as a biocatalyst to produce aroma compounds at a low price and large quantity (Parkin, 2017).

It appears that in the future, the enzyme will play a very important role in the food industry. By better understanding the mechanism of action of the enzyme, controlling the undesirable activities of the enzyme by inhibiting it allows food producers to improve the quality of food.

2.4.3 Phospholipases

Mammal, plant, and microbial phospholipases are continuously being studied, experimented, and some of them are even commercially available at industrial scale for food industry. Phospholipases form a large class of enzymes with wide diversity (Mansfeld, 2009). They are generally classified as acyl hydrolases and phosphodiesterases. These enzymes are further divided into different groups depending on the site of action within the phospholipids (PL) molecule. The acyl hydrolases include the phospholipase A1 (specifically hydrolyzes 1-acyl ester bond of PL) to release lyso-phospholipids (lyso-PL) and free fatty acids (FFA), phospholipase A2 (catalyzes the hydrolysis of fatty acids at the sn-2 position of PL, releasing lyso-PL and FFA), phospholipase B (which does not discriminate between the two positional acyl ester bonds), and lysophospholipase A ½ (partially hydrolyzes PL) (Parkin, 2017). The phosphodiesterases are represented by phospholipase C and D (which cleave the phosphorus–oxygen bond between glycerol and phosphate, releasing diacylglycerol and phosphate esters). Except phospholipases A2, most of these phospholipases are not widely available at large quantities for industrial purposes. The use of secreted PLA 2 from porcine pancreas or snake and bee venoms has a very long tradition in food industry for modification of PL, such as egg yolk production for emulsification in mayonnaise, sauces or salad dressings, baking industry, or refinement of vegetable oils by degumming (Mansfeld, 2009).

The most representative examples of the use of phospholipases in food industry can be found in the production of edible oils, dairy, and baking products or emulsifying agents. Phospholipases are incorporated in processes such as the degumming of vegetables oils during refinement for removing undesirable compounds, the manufacture of cheese for yield increasing, or the production of bread as bakery improvers for reducing the inclusion of emulsifying compounds (Ramrakhiani & Chand, 2011). Lipases and phospholipases offer the opportunity to generate compounds with technological effects and emulsifier characteristics in the bread making, with the main final advantage of allowing the reduction or replacement of added emulsifiers in bakery products. On the other hand, superior emulsifying properties of released lysolipids can be obtained, leading to improved dough rheological properties (Zhao et al., 2010; Casado et al., 2012).

2.5 Miscellaneous Enzymes

2.5.1 Catalase

The enzyme catalase is known to catalyze the breakdown of hydrogen peroxide into oxygen and water. It is an oxidoreductase enzyme as it plays a crucial role in quenching the reactive oxygen species (ROS), i.e., hydrogen peroxide, often produced as a by-product of aerobic respiration. Hence, it acts as an antioxidant and protects the cell against oxidative stress (Kaushal et al., 2018).

Catalase is widely distributed in nature. It is found in all aerobic microorganisms and in all plant and animal cells. The catalase activity of mammalian cells varies. It is highest in liver and kidney and low in connective tissues. Catalase has been used in the food processing industry to determine the adequacy of blanching (70–105 °C) of vegetables and fruits to destroy microorganisms and enzymes to preserve color, texture, flavor, taste, and aroma of the products when stored frozen up to 2 years. Early in the application of blanching for stabilization of frozen foods, catalase was used especially for peas (24–26), the best on the market (Williams et al., 1986; Wong & Whitaker, 2003). In the dairy industry, catalase activity is a sensitive and easily detected indicator of contamination of milk by neutrophil granucytes. Also, the use of catalase has proven to be a very cost-efficient and also a comparatively environmental friendly method of getting rid of excess hydrogen peroxide from milk samples for cheese production. H_2O_2 is used in the cold pasteurization process of milk for cheese manufacture. The residual H_2O_2 is converted to water and molecular oxygen by catalase, thereby avoiding its toxicity. Catalase enzymes are typically obtained from bovine livers or microbial sources (Kilcawley et al., 1998).

2.5.2 Amino Oxidases

Biogenic amines and polyamines are present in variety of foods, such as fish, meat, cheese, vegetables, and wines. These are organic bases with aliphatic, aromatic, and heterocyclic structures. They are produced by decarboxylation of amino acid substrates catalyzed by enzymes from contaminating bacteria. The most common biogenic amines found in foods are histamine, tyramine, cadaverine, 2-phenylethylamine, spermine, spermidine, putrescine, tryptamine, and agmatine (Ruiz-Capillas & Herrero, 2019). The formation of biogenic amines in food can result in consumers suffering allergic reactions, characterized by difficulty in breathing, itching, rash, vomiting, fever, and hypertension. Several methods for prevention of the production or removal of biogenic amine in food have been practiced, primarily by limiting microbial growth through chilling and freezing. However, for many socioeconomic and technological reasons, such approaches are not always practical. Therefore, alternative measures to prevent biogenic amine formation in foods or to reduce their levels once formed are needed. One approach might be application of enzymes which metabolite these amines (Naila et al., 2010).

Amine oxidase catalyzes the reaction with mono-, di-, or polyamine as substrate (Ito & Ma, 2003):

$$RCH_2NH_2 + O_2 + H_2O \rightarrow NH_3 + RCHO + H_2O_2$$

The activity of these enzymes has been detected in *Arthrobactor*, yeast *Candida boidinii*, *Asp. niger*, pea seedling, bovine plasma, and bovine lung. In human and animals, the enzyme plays an important role for the metabolism of biogenic monoamines in the central nervous system and peripheral tissues. Pea seedling amine oxidase, a copper containing diamine oxidase, catalyzes the oxidative

deamination of histamine to the corresponding aldehyde, i.e., imidazole acetaldehyde concomitant with the reduction of dioxygen to hydrogen peroxide. The antihistamine properties of this enzyme make it an attractive candidate in fish industry to combat the allergic responses elicited by consumption of fish. However, the observed variation in the efficacy of the enzyme has been attributed to the diversity and distribution of the enzyme inhibitors present in the variety of fish species (Ebrahimnejad et al., 2013; Stránská et al., 2007).

2.5.3 Phenylalanine Ammonia-Lyase

Phenylketonuria (PKU) is an autosomal recessive genetic disorder affecting on average incidence of about 1–3 case in 10,000 Caucasian live births. It is caused by deficiency of the hepatic enzyme phenylalanine hydroxylase (PAH) which is responsible for converting phenylalanine to tyrosine using molecular oxygen and tetrahydrobioptrin as necessary cofactor to perform its catalysis (Al Hafid & Christodoulou, 2015). PAH deficiency leads to hyperphenylalaninenemia, a dramatic increase in blood phenylalanine concentration ($<$120 to $>$1200 μM) with concurrent appearance of phenylalanine, phenylpyruvate, and phenylacetone in urine. High levels of accumulated phenylalanine in PKU patients are toxic to human body if left untreated and are associated with an abnormal phenotype presenting with growth failure, microcephaly, seizure, and mental retardation. Children with severe PKU can have normal cognitive development when dietary treatment is initiated in early infancy and the blood phenylalanine level is maintained at near normal or normal levels (Ding et al., 2004). The established treatment for PKU resides in a phenylalanine-restricted diet, which has known deficiencies of several nutrients and unsatisfactory organoleptic properties, making long-term compliance a major challenge (Levy, 1999).

Phenylalanine ammonia lyase (PAL) is an enzyme that catalyzes a reaction converting L-phenylalanine to ammonia and *trans*-cinnamic acid (which is excreted as hippurate in urine). PAL is found widely in plants with isoenzymes existing within many different species. It has a molecular mass in the range of 270–330 kDa. A large number of plant species were screened for PAL activity and it was found that enzyme activity was highest in grain seedlings, with maximal enzyme activity in 7-day-old red spring wheat (Goldson et al., 2008; Camm & Towers, 1973). We have studied the stability of wheat seedling PAL during storage at different temperatures and found a first order kinetic for inactivation of PLA with half-life of 30 and 18 days at −18 and 4 °C, respectively. Activity of PAL increased while germination occurred at 25 °C up to 8 days. These results can be used to develop appropriate strategies for storing PAL containing materials with retained PAL activities (Aminlari et al., 2010).

PAL has recently been studied for possible therapeutic benefits in humans afflicted with phenylketonuria. The use of phenylalanine ammonia-lyase (PAL) is an attractive alternative to the dietary treatment of PKU and both pharmacologic and physiologic proofs of principle have been established using recombinant PAL for

enzyme substitution therapy in a murine PKU model (Sarkissian et al., 1999). A large amount of active recombinant PAL from parsley *(Petroselinum crispum)* has been prepared and chemically modified to render PAL nonimmunogenic and stable in the circulation (Kim et al., 2004b). Oral therapy with phenylalanine ammonia lyase (PAL), naturally encapsulated in plant cells, may provide a potential alternative treatment for patients with PKU (Goldson et al., 2008). One significant challenge in application of PAL for PKU therapy is protecting PAL from proteolysis and/or denaturation and against immunologic responses. Different physical and chemical methods have been investigated to address this problem. These include PAL immobilization in semipermeable, microcapsules, the entrapment of PAL in silk fibroin, and the immobilization of recombinant PAL in expression bacteria such as *Escherichia coli* or *Lactobacillus lactis*, and modification with PEGylation (Bell et al., 2017; Ikeda et al., 2005).

2.5.4 Lysozyme

In recent years, consumer demand for 'natural' foods has driven development of products without additives. In order to meet this demand, much attention and interest have been directed towards identification and application of naturally made compounds such as antimicrobial agents, in food and pharmaceuticals (Branen & Davidson, 2004). Some naturally occurring proteins such as lactoperoxidase, lactoferrin, and lysozyme have received much attention and are being considered as potential antimicrobial agents to replace the currently used synthetic food preservatives (Demain, 2009).

Lysozyme, also known as muramidase or *N*-acetylmuramide glycanhydrolase, is an antimicrobial enzyme found in many different sources, from viruses to vertebrates, and has been subjected to extensive scrutiny, both as a protein model and a natural antimicrobial and pharmaceutical agent. It forms part of the innate immune system. Lysozyme is abundant in secretions including tears, saliva, human milk, and mucus. Chicken egg white has the highest content of lysozyme (it constitutes 3.5% of the total egg white proteins), from which this enzyme is purified and is commercially produced (Proctor & Cunningham, 1988). Lysozyme is a glycoside hydrolase that catalyzes the hydrolysis of 1,4-beta-linkages between *N*-acetylmuramic acid and *N*-acetyl-D-glucosamine residues in peptidoglycan, which is the major component of gram-positive bacterial cell wall. In general, lysozyme shows in vitro antimicrobial activity against some Gram-positive bacteria such as Staphylococcus aureus, *Micrococcus luteus*, *Bacillus stearothermophilus*, and *Clostridium tyrobutyricum*, but little action against Gram-negative bacteria (Cunningham et al., 1991). Gram-negative bacteria, including foodborne pathogens, resist lysozyme due to steric hindrance posed by the outer LPS layer, hence lysozyme cannot access the peptidoglycan (Ibrahim et al., 1994). Thus, modification of lysozyme, which can broaden its antibacterial properties against both Gram-negative and Gram-positive bacteria, can also increase the usefulness of lysozyme (Seo et al., 2013).

Lysozyme has received considerable interest as a food preservative (Cegielska-Radziejewska & Szablewski, 2013). In many countries, lysozyme is used as a preservative in many types of foods (including vegetables, sea foods, soy bean products, meat products, and semihard cheeses), and as a component of pharmaceutical products. In the European Union, its use in specific products such as hard cheeses and in wine making to control infection (E number E 1105 as a food additive) is allowed (Losso et al., 2000). Proctor and Cunningham (1988) and Cunningham et al. (1991) have reviewed the use of lysozyme as a food preservative.

During the last two decades, extensive researches in the authors' laboratory and others have been directed toward modification of lysozyme in order to improve its antimicrobial properties. Several types of these modifications are summarized in Table 2.4. The results of modifications of lysozyme using its conjugation with

Table 2.4 Modified lysozymes and their properties (from Aminlari et al., 2014)

Modified lysozymes	Effect on functionality
Palmitic acid	Enhanced antimicrobial against *E. coli* (WT-3301)
Short and middle chain saturated fatty acids	Enhanced antimicrobial against G-positive bacteria
Glucose–stearic acid monoester	Enhanced activity against *E. coli* and *E. tarda* (G8104)
Perillaldehyde	Enhanced activity against *E. coli* K12 and *S. aureus*
Cinnamaldehyde	Enhanced activity against *E. coli* and *S. aureus*
Glucosamine	Improved solubility at different pHs and temperatures, increased heat stability, emulsion activity and stability, and foam capacity
Caffeic acid–cinnamic acid	Antimicrobial activity against *E. coli* (ATCC 8739), decreased activity against *S. aureus* (ATCC 6538)
Dextran	Enhanced activity against *E. coli* and *S. aureus* in cheese
	Enhanced activity against *E. coli* and *S. aureus* in milk
	Treatment of bacterial isolates from cows with mastitis
	Preparation of a lysozyme–dextran nanogel
	Increased heat stability, higher emulsifying property
Galactomannan	Antibacterial emulsifier
	Emulsifier, antibacterial against G-negative pathogen *E. tarda*
Chitosan	Enhanced bactericidal action against *E. coli* K12
	Lysozyme-composite film with activity against *E. coli*, *L. monocytogenes*, and *S. faecalis*
Cellulose	Preparing a textile with potential barrier to microbial invasion
Gum Arabic	Enhanced activity against *E. coli* and *S. aureus* in mayonnaise
Xanthan gum	Enhanced activity against *E. coli* and *S. aureus*
Dextran sulfate	Enhanced activity against *E.* coli and *S. aureus*
Barley beta-glucan	Enhanced activity against *E.* coli and *S. aureus*
Tragacanth	Enhanced activity against *E. coli*, *S. typhimyrium*, *B. cereus*, and *S. aureus*
Inulin	Improved functional properties
Trypsin and ficin digestion of	Enhanced activity of peptides *E. coli* and *B. cereus* dextran-conjugated lysozyme

different small molecule and polysaccharides by application of a Maillard-based reaction, as well as modifications using proteolytic enzymes, have revealed that these types of modifications have not only increased the functional properties (such as solubility and heat stability), but also extended the antimicrobial activity of lysozyme (Aminlari et al., 2014). We have shown that it is possible to broaden the antimicrobial effect of lysozyme by several types of modifications, which consequently make these lysozyme derivatives excellent food preservatives. Table 2.3 summarizes the effect of different types of modifications on lysozyme.

2.6 Conclusions

Nature provides us with remarkably vast array of enzymes with extraordinary catalytic properties. In their natural reservoirs, these so-called "endogenous" enzymes perform essentially all biochemical reactions vital to the living organisms. When these enzymes are removed from their natural habitat, they continue to catalyze same reactions when they have access to their substrate. At this stage, they are indeed "exogenous" enzyme. Food scientists have used these exogenous enzymes as food additives for many years. In this chapter, the properties of several enzymes from plant and animal sources were presented and their application or potential application in food industry was discussed. Over the years, it has become clear that despite advances in experimental mutational studies, a quantitative understanding of enzyme catalysis will not be possible without computer modeling approaches (Frushicheva et al., 2014). With the advent of new technologies such as information technology, rational molecular design, and DNA enzyme (deoxyribozyme) technologies (Breaker, 1997; Woolcock, 2016), it is expected that new doors for application of these technologies will open to food enzymologists in the near future. The discussion is by no means complete, and with the advent of knowledge of enzymology, the endeavor will continue. For those of us who (as Arthur Korenberg, the discoverer of DNA polymerase once said) are in love with enzymes, the challenge has just began.

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Role of Enzymes in Fruit and Vegetable Processing Industries: Effect on Quality, Processing Method, and Application

3

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Abstract

The significance of enzymes and their application in food processing industry is increasing rapidly. Different kinds of enzymes are extensively used based on their effective application. In fruits and vegetables processing, several endogenous enzymes and newly developed enzymes are used. Enzymes present in fruits and vegetables play a huge role in determining the texture, colour, flavour, and taste attributes of the processed products. The continued enzymatic activity in fruits and vegetables affects the storage quality, shelf life, and palatability of the product. Therefore, several processing methods such as grinding, crushing, slicing, juices, or preservation are used to prolong the shelf life and reduce wastage of fruits and vegetables. Hence, it is very important to control the stability and activity of endogenous enzymes present in fruits and vegetables during food processing. Apart from the conventional techniques of thermal processing such as blanching, heating, ohmic, and microwave, new and highly advanced processing techniques like high hydrostatic pressure (HHP), high-pressure homogenization processing (HPHP), pulsed electric field (PEF), and ultrasound processing have been introduced successfully. The major advantage of these new techniques is the use of non-thermal technology, which helps in retaining the sensory attributes and nutritional content of the product. These non-thermal processing techniques are effective at ambient or sub-lethal temperatures and minimize the adverse thermal effects on the nutritional content and quality of fruits and vegetables. Nonetheless, multiple advantages are rendered by these techniques in food processing industry over conventional thermal techniques which affect not only the enzymes, but also the texture, taste, and colour of the product compelled for further investigation and improvement.

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A. Dutt Tripathi et al. (eds.), *Novel Food Grade Enzymes*,
https://doi.org/10.1007/978-981-19-1288-7_3

Keywords

Enzymes · Fruit and vegetable processing · HPP · PEF · Shelf life extension

Abbreviations

CAGR	Compound annual growth rate
HG	Homogalacturonan
HHP	High hydrostatic pressure
HPHP	High-pressure homogenization processing
HTLT	High temperature-long time
HTST	High temperature-short time
OH	Ohmic heating
PEF	Pulsed electric field
PME	Pectin methylesterase
POD	Peroxidase
PPO	Polyphenol oxidase
RG-II	Rhamnogalacturonan-II

3.1 Introduction

Enzymes play a very critical role in the growth and maturation of fruits and vegetables. They also make significant contributions during the post-harvest phase in maintaining stability of raw food materials and improving their quality attributes like aroma, colour, texture, flavour, and nutritional quality. Apart from these, enzymes also have the ability to act as catalysts in transforming raw materials to food products during processing. They are also found to have extensive applications in food processing and production by enhancing the nutritional, safety, functional qualities, and overall acceptability of ingredients and processed products. Moreover, enzymes are also known for their substrate specificity, effectiveness in catalytic reactions, and enhancement rate under controlled conditions of moisture, pH, and temperature (Berg et al., 2010).

Due to rapid growth of population and fast changing lifestyle, the production and demand of convenience and processed food products have also been gradually increasing. As per the study of Markets and Markets (2020), the fruit and vegetable processing enzyme global market is expected to touch around $41.39 billion by 2022 at 6.7% CAGR from 2016 to 2020. During this corresponding period, the Asia-Pacific region enzyme market is projected to grow at the highest CAGR due to high growth of the food and beverage industry in big emerging economies like India and China. Therefore, productive usage of the existing enzymes and the development of new enzymes have become very significant in meeting the ever-increasing demands

in food processing industries. Hence, rapid advancement in enzymology is given prime focus in the food processing sector.

Currently, new enzymes based on their sources (fungi and bacteria) and types (amylase, pectinase, xylanase, and cellulase) have been isolated, characterized, and developed commercially for productive usage either in enhancing the desirable or delaying the unfavourable characteristics of foods during processing. Nonetheless, the importance and significance of endogenous enzymes in the present scenario of food processing industries cannot be overlooked for their productive usage in meeting the desired colour, texture, flavour, appearance, and stability of foods during industrial processing.

In light of the importance of enzymes in food processing, the uses of enzymes and their application in fruit and vegetable processing industries are indispensable in order to increase the productivity and shelf life, reduce cost of production, and also develop new products.

3.2 Fruit and Vegetable Composition

Knowing the composition of fruit and vegetable is the first step in determining the benefits and drawbacks of them. Fruits and vegetables are mainly composed of cellulose (7.20–43.60%), hemicelluloses (4.26–33.50%), pectin (1.50–13.40%), lignin (15.30–69.40%), and starch (3–21%) (Dhillon et al., 2013). The primary cell walls in the young plants comprise largely of cellulose and tend to thicken and become higher in hemicelluloses and lignin as they grow (Albersheim et al., 1996). Now, let's discuss in detail the different types of composition in fruits and vegetables.

3.2.1 Cellulose

Cellulose is one of the main components in fruits and vegetables ranging from 7.20% to 43.60%. It is a linear polymer of D-glucose present in the primary cell wall. It forms microfibrils, which give a stiff shape, structure, and tensile strength to the cell wall. It also helps to form a resistance against the degradation of this polymer (Szymańska-Chargot et al., 2017). These microfibrils are coated with hemicelluloses and help in binding firmly to their surface (Carpita & Gibeaut, 1993). Cellulose is known for its diverse applications in food processing industry. It is used as an emulsifier, bulking agent, texturizer, and as a fat substitute (Grassino et al., 2016). However, the application of celluloses during the extraction process needs extra precaution in order to avoid breaking down the cellulose network in maceration processes as it decreases the quantity of juice production.

3.2.2 Hemicellulose

Hemicellulose is another major component in fruits and vegetables with a variable range of 4.26–33.50%. It is a matrix polysaccharide often present along with cellulose in the cell walls and comprises of xyloglucan, arabinoxylan, glucomannan, and galactoglucomannan. Xyloglucan is the most abundant hemicellulose with similar structure as D-glucose backbone in cellulose. Hemicelluloses are bound via hydrogen bonds, forming a network of cross-linked microfibrils between pectin and cellulose. Such interconnections play a major role in the integrity of the pectin cross-linking network. Vincken et al. (1994) demonstrated that the key structure in the apple cell wall is xyloglucan and it helps in breaking down cell-wall embedded in cellulose if it gets hydrolysed. In apple cell wall matrix, xyloglucan accounts for 24% of the total amount of sugar, whereas the cellulose–xyloglucan complex accounts for 57% approximately. In food processing industries, hemicelluloses are primarily used as enhancing agents in viscosity, stabilizing, and gelling. Moreover, it is also widely used in technical and pharmaceutical fields as coatings, films, gel materials, and adhesives (Wang et al., 2010).

3.2.3 Pectin

Pectin is one of the least present components in fruits and vegetable ranging from 1.50% to 13.40%. It forms a class of complex polysaccharides commonly found in the cell walls of higher plants. It provides structure and firmness to the plant tissue in the primary cell wall and middle lamella component involved in intercellular adhesion (Thakur et al., 1997). There are three pectic polysaccharides, namely, homogalacturonan, rhamnogalacturonans, and substituted galacturonans isolated from primary cell walls. Homogalacturonan (HG) is a homopolymer, a linear chain of 1,4-linked α-D-galactosyluronic residues, known for its gel forming ability. In HG, some of the carboxyl groups are methylesterified at the C-2 and C-3 positions. Similarly, the backbone of Rhamnogalacturonan-II (RG-II) is composed of 1,4-linked D-galactosyluronic residues like HG, with a non-saccharide and an octa-saccharide side chains attached to C-2 and two disaccharides attached to C-3 of the backbone residues. RG-II is significantly used in winery and fruit juice industries because of its exceptional quality of binding heavy metals, thereby reducing the toxicity level in the final product. It also has immunomodulating activities (Grassin & Coutel, 2009). Galarturonan fractions are generally separated from other high molecular weight pectin fractions by degrading purified pectins specifically present in the galacturonan backbone either enzymatically or chemically. In fact, as explained above, the different types of pectins perform certain specific functions, but pectins in general are largely used as gelling and thickening agent in dairy and baking. Also, pectins are being widely used as a carrier of drug delivery system in cosmetic and pharmaceutical industries (Kollarigowda, 2015).

3.2.4 Starch

Starch is another main component in fruits and vegetables which is known very commonly. It is a polysaccharide composed of amylose and amylopectin. Amylose has linear chains of about 840–22,000 units of α-D-glucopyranosyl residues linked by 1→4-α-D-glucan, whereas amylopectin is usually comprised of highly branched α-1→6 and α-1→4 glucosidic linkages. Starch is a form of carbohydrate or energy reserve mostly found in storage organs, seeds, unripe fruits, and vegetables. The most important quality of starch is its water holding capacity which depends on the specific shape and size of each granule. For example, a potato granule can hold approx. 5–100 μm water/glucose unit (Jobling, 2004). Moreover, each granule contains 'blockets' of amylopectin which are crystalline and amorphous in nature. They are responsible for absorption of water, swelling, lose crystallinity, and seep amylose. However, the swelling capacity tends to be lower with higher amylose content and thus results in reduction in gel strength (Li & Yeh, 2001). Therefore, because of its inconsistent natural properties due to the vagaries of weather and agricultural conditions, most of the suppliers constantly try to make uniform starches as functional ingredients. In food processing industry, the usage of starch is manifold. It acts as an innate natural ingredient with different added functionalities like an emulsifier, stabilizer, water binder, thickener, and gelling agent.

3.2.5 Lignin

Lignin is one of the major components present in fruits and vegetables ranging from 15.30% to 69.40%. It represents a class of natural aromatic polymers of 4-hydroxyphenylpropanoids units connected by ether and carbon-carbon linkages. Lignins are generally considered a part of dietary fiber. They are mainly present in cereals, fruit, and vegetables, in which wheat bran has the richest source of lignin. Lignins have efficient antioxidant properties, mainly scavenging superoxide and hydroxyl radicals. It also inhibits the activity of xanthine oxidase, glucose-6-phosphate dehydrogenase, and non-enzymatic and enzymatic lipid peroxidation (Lu et al., 1998). In addition to this, its concentration and composition influence the adsorption capacity of cell walls. Therefore, due to these properties, lignins are considered as efficient adsorbers of hydrophobic heterocyclic aromatic amines (Funk et al., 2006). Although lignin has many applications in other different industries, in food industry it is mainly used as an additive, provides roughage to foods, and as sequestering agents.

3.3 Enzymes Used in Industrial Processing of Fruits and Vegetables

As discussed, the role of enzymes in the overall natural growth, maturation, and ripening of fruits and vegetables is very critical. This very importance of enzymes remains the same or even more in the industrial processing of fruit and vegetables. Another important positive aspect for enzymes is their ability in keeping the quality of fresh fruits and vegetables post-harvest and during storage conditions, which ultimately plays a very pivotal role in the industrial food processing.

In industrial processing, the ripening process with the application of enzymes is a major step, wherein several changes like alterations in the cell wall, middle lamellae, and membrane occur resulting in softening of tissues. Naturally, most of the enzymes in fruits and vegetable tissues are important for the maintenance of metabolism; however, they are also associated with undesirable effects on colour, flavour, odour, texture, and nutritional value. For example, in some vegetables, flavour and odour development is affected by lipoxygenase, lipase, and peroxidase (Fleuri et al., 2016). Furthermore, phenol oxidases result in discolouration and unfavourable effects on the taste and nutritional quality of fruits and vegetables. Also, fruits and vegetables containing pectic substances and α-amylases have major effects on their textural integrity (Berg et al., 2010; Fleuri et al., 2016).

With the advancement in enzymology, different types of new enzymes have been developed and are being used in improving the quality of products, development of new products, and in processing aids such as peeling, extraction of juice, and clarification, thereby increasing the efficiency in processing operation. For example, amylases, cellulases, and pectinases facilitate maceration, liquefaction, and clarification in processing of fruit juice, and hence they are cost-effective and increase the yield. Moreover, different enzyme extracts from plant tissues, bacteria, yeast, and fungi are applied in fruit and vegetable processing industries for the same purpose (Leadlay, 1993). In Table 3.1, some of the important microorganism origin enzymes used in industrial processing and its application are summarized. In Table 3.2, different types of enzymes, product type, and its application in fruit and vegetable processing are highlighted.

Enzymes are classified broadly into four types: Pectinases, Cellulase, Xylanase, and Amylase. In the proceeding paragraphs, we will be discussing in brief the different types of enzymes in fruit and vegetable processing industry.

3.3.1 Pectinases

Those enzymes whose primary role is to break down pectin, a structural heteropolysaccharide found in primary cell walls of fruits and vegetables, cereals, and fibers, are known as pectinases (Singh et al., 2003). The pectin substances are of high molecular weight acidic heteropolysaccharide primarily composed of α-(1–4)-linked D-galacturonic acid residues (Kavuthodi & Sebastian, 2018). Pectic acids are water-soluble substances having variable degrees of methyl ester groups and for

Table 3.1 Enzymes derived from microorganisms and their application in fruits and vegetable processing

Enzyme	Microorganism	Action	Application
Pectinase	*Aspergillus* spp., *Penicillium funiculosum*	Pectin hydrolysis	Degradation of pectins, increases the overall juice production, fruit juice clarification
Pectinesterase	*Aspergillus* spp.	Remove methyl groups from galactose units of pectin	Increase firmness of vegetables and also used with pectinase depectinisation technology
Protopectinase	*Kluyveromyces fragilis*, *Galactomyces reesei*, *Trichosporon fragilis*, *Bacillus subtilis*	Catalyse pectin solubilization	Clarification and reduction of viscosity in fruit juices
Hemicellulase	*Aspergillus* spp., *Bacillus subtilis*, *Trichoderma reesei*	Hemicellulose hydrolysis	Helps extraction of fruit juices, vegetable oils, and aromatic compounds, acts on hydrolysis of soluble pectin and cell wall components with pectinases, lowers viscosity and texture
α-Amylase	*Aspergillus* spp., *Bacillus* spp., *Microbacterium imperiale*	Random hydrolyses α-1,4 bounds to rupture starch and produce maltose	Hydrolysing starch to reduced viscosity, liquefying adjunct, helps in sugar production, for softness and increases volume of fruit juices and vegetables
Glucoamylase	*Aspergillus niger*, *Rhizopus* spp.	Hydrolyse dextrin from starch in glucose	Fruit juice extraction and also used for corn syrup and glucose production
Glucose isomerise	*Streptomyces rubiginosus*, *Streptomyces lividans*, *Actinplanes missouriensis*, *Bacillus coagulans*	Conversion of glucose to fructose	Helps in high-fructose corn syrup production (beverage sweetener)
Cellulase	*Trichoderma* spp., *Aspergillus niger*	Hydrolyses cellulose	Liquefaction of fruit in juice production

Note: Adapted from "Enzymes in food and beverage processing", Fleuri, L. F., Delgado, C. H. O., Novelli, P. K., Pivetta, M. R., Do Prado, D. Z., & Simon, J. W., 2015, p. 257, London, New York: CRC Press, Taylor & Francis Group

neutralization, which form gels with the addition of sugars and acids under favourable conditions (Guo et al., 2014). Pectic substances are classified into protopectins, pectic acids, and pectin which are partially soluble in water (Uneojo & Pastore, 2007). These substances are generally degraded by the enzyme pectinases. They are further classified into different sub-types, namely,

Table 3.2 Enzymes used in fruit and vegetable processing

Name of fruit	Product type	Enzyme	Application
Apple (*Pyrus malus* L.)	Juice	Amylase	Decrease starch concentration to improve durability
Banana (*Musa sapientum* L.)	Juice	Pectinase	Decrease turbidity and viscosity
Blueberry (*Vaccinium myrtillus* L.)	Juice	Pectinase	Improve juice yield and anthocyanin level
Citrus (*Citrus sinensis* L. Osbeck)	Juice	Pectinase and cellulase	Decrease the turbidity of juice
Cherry (*Prunus avium* L.)	Juice	Pectinase and protease	Decrease turbidity and increase stability
Cloudy ginkgo (*Ginkgo biloba* L.)	Juice	Amylase and protease	Reduce hydrolysis time to improve stability
Grape (*Vitis vinifera* L.)	Juice	Pectinase	Decrease turbidity and soluble solids
Lemon (*Citrus sinensis* L. Osbeck)	Soft drink punch	Pectinase	Clarification of peel extract to produce soft drinks
Pineapple (*Ananas comosus* L.)	Juice	Pectinase and cellulase	Improve soluble solids and aromas
Pomegranate (*Punica granatum* L.)	Juice	Pectinase	Improve concentration of antioxidants and decrease turbidity
Carrot (*Daucus carota* L.)	Juice	Pectinase	Improved nutritional properties as the content of polyphenols and flavonoids
Date syrup (*Phoenix dactylifera* L.)	Syrup	Cellulase and pectinases	Reduce turbidity and increase the extraction of soluble solids
High-fructose corn syrup (HFCS—*Zea mays* L.)	Syrup	Amylase	Conversion of glucose to fructose
Olive oil (*Olea europaea* L.)	Oil	Pectinase, cellulase, and hemicellulase	Oil extraction from olive residue and improve soluble solids on oil extraction
Sunflower oil (*Helianthus annuus* L.)	Oil	Pectinase	Improve oil yield

Note: Adapted from "Enzymes in food and beverage processing", Fleuri, L. F., Delgado, C. H. O., Novelli, P. K., Pivetta, M. R., Do Prado, D. Z., & Simon, J. W., 2015, p. 258, London, New York: CRC Press, Taylor & Francis Group

methylesterases (based on mechanical action), polygalacturonases, and lyases (based on their mode of action).

Then, polygalacturonases are further sub-classified into endo-polygalacturonases (E.C. 3.2.1.15) and exo-polygalacturonases (E.C. 3.2.1.67). Similarly, lyases are also sub-classified into three types, namely, pectin methylesterases (E.C. 3.1.1.11),

pectatelyases (E.C. 4.2.2.9 and E.C. 4.2.2.2), or pectin lyases (E.C. 4.2.2.10) (Kc et al., 2020).

As per a recent study, it is reported that pectinases from microbial origin account for about 25% of global industrial enzymes market which is projected to reach USD 6.3 billion by 2021 (Oumer, 2017; Oumer & Abate, 2018). This clearly highlights the significance of microorganism origin enzyme as a reliable source of industrial enzyme production. Pectinases or pectinolytic enzymes are also naturally produced by many other organisms like insects, bacteria, nematodes, and protozoans (Khairnar et al., 2009). Some of the other commonly used microorganisms for extensive production of pectinases are *Aspergillus* spp., *Bacillus* spp., *Erwinia* spp., and *Penicillium* spp. (Oumer, 2017).

In food processing industries, especially in fruits and vegetables processing, pectinases have multiple usage. They are mainly used during processing of citrus juice. Moreover, pectinases are also generally recommended for use with the combination of other enzymes such as cellulases and hemicellulases. Such combinations of enzymes are generally used in facilitating the process of maceration, liquefaction, and clarification. It ultimately helps in increasing the extraction yield and enhancing the concentration of acids, colourants, and flavourings (Oumer & Abate, 2018). Apart from these, pectinases are also widely used in wine clarification, concentration, and fermentation of tea, cocoa, and coffee. Further, these enzymes are also used regularly in pickling, preparation of jam and jellies, syrups, starches, and vegetable oil extraction (Kubra et al., 2018).

3.3.2 Cellulases

Cellulases are enzymes that promote the process of hydrolysis of "cellulose", a fibrous, tough and water-insoluble polysaccharide and a homopolymer comprising of several glucose units joined by β-1,4 linkages. They act synergistically as a biocatalyst to release the sugar molecules, which helps in converting into ethanol and organic acids (Fleuri et al., 2016). Cellulases are generally produced as a multi-enzyme system comprising of glucosidase (β-D-glucoside gluco-hydrolase, E.C. 3.2.1.21), β-1,4-endoglucanase (1,4-β-D-glucan 4-glucan hydrolase, E.C. 3.2.1.4), and cellobiohydrolase or exoglucanase (1,4-β-D-glucan cellobiohydrolase, E.C. 3.2.1.91) (Jecu, 2000). Cellulases are produced by bacteria and fungi (Sharma et al., 2017). However, for commercial preparation of cellulases, different filamentous fungi such as *Trichoderma reesei* (Megazyme) and *Aspergillus niger* (Cellulocast from Novozyme) are used. Some other common cellulases producing fungi are *Aspergillus*, *Chaetomium*, *Fusarium*, *Humicola*, *Neocallimastix*, *Penicillium*, *Piromonas*, *Thermoascus*, *Trichoderma,* etc. (Singhania et al., 2010; Bansal et al., 2011).

In food processing industry, majorly in beverage industry, cellulase enzymes are extensively used in the production of fruit juices and wine processing. It facilitates the extraction of juice and maceration process by breaking the cellulose chains present in the plant cells. It also helps in extracting pigments and flavouring

substances present in the grape skin. Besides releasing the flavouring substances and improving the aroma and flavour of beverages, it also breaks the unpleasant-tasting compounds present in the fruits and vegetables (Juturu & Wu, 2013).

3.3.3 Hemicellulase and Xylanase

Hemicellulase is an enzyme complex that breaks down the backbone of xylan and arabinose side chains and releases pentoses (xylose and arabinose) (Yang et al., 2017). Similarly, xylanases (endo-1,4-β-D-xylanohydrolase; E.C. 3.2.1.8) are hydrolytic enzymes involved in depolymerization of xylan. They are usually present in superior plants, such as cereals, grasses, and trees that present noncellulosic polysaccharides, such as D-glucose, L-arabinose, D-xylose, D-mannose, D-galactose, D-glucuronic, and D-galacturonic acid (Cunha & Gandini, 2010). These enzymes also degrade hemicellulose polymers, including acetyl xylan esterase (E.C. 3.1.1.72), α-arabinofuranosidase (α-L-arabinofuranosidase, E.C. 3.2.1.55), arabinase (endo-α-L-arabinase, E.C. 3.2.1.99), endo-xylanase (endo-1,4–βxylanase, E.C. 3.2.1.8), feruloyl esterase (E.C. 3.1.1.73), α-glucuronidase (α-glucosiduronase, E.C. 3.2.1.139), and β-xylosidase (xylan-β-1,4-xylosidase, E.C. 3.2.1.37) (Juturu & Wu, 2013). Polymers such as xylan and arabinoxylan are completely hydrolysed by the synergistic action of several xylanolytic enzymes: endo-1,4-β-D-xylanases. The hydrolysis of these polymers degrades the β-D-xylan linkages; β-D-xylosidases release a xylose monomer from the cleavage of the non-reducing end of xylo-oligosaccharides and xylobiose (Terrasan et al., 2010).

Currently, commercial xylanases are produced on a large scale in many countries, such as United States, Japan, Finland, Germany, Ireland, Canada, and Denmark, by fermentation processes using bacteria, yeast, and fungi (Polizeli et al., 2005). Substantial increase in the production of xylanases has been observed after the development of improved microbial strains and efficient fermentation techniques and recovery systems. It has several applications in food industries, agriculture as well as in human health.

Furthermore, during processing of beer, the cellular wall is generally hydrolysed releasing long chains of arabinoxylans which in turn increase the viscosity of beer rendering it “muddy” in appearance. Then, xylanases are added to hydrolyse arabinoxylans in order to lower oligosaccharides, thereby reducing the viscosity and consequently improving its appearance (Dervilly et al., 2002). Xylanases in combination with amylases, cellulases, and pectinases provide multiple advantages such as increase yield of juice, stabilization of fruit pulp, and increased recovery of flavours and aromas, essential oils, mineral salts, vitamins, etc.

3.3.4 Amylases

Amylases are enzymes that act as catalysts in the hydrolysis of starch into sugars such as glucose and maltose (Sundarram & Murthy, 2014). These enzymes are

extensively found in plant, animal, and microbial kingdoms. Amylases are classified into exo-amylases and endo-amylases based on their action. Exo-amylases are involved in hydrolysis of α-glucan into maltose and glucose, whereas endo-amylase hydrolyses α-glucan-forming oligosaccharides. Starch which is a polysaccharide that is an essential factor of structure, consistency, and texture in foods is hydrolysed by β-amylase. Moreover, the non-reducing terminals in starch polysaccharides and malto-oligosaccharides are also hydrolysed by β-amylase (Fleuri et al., 2016). Such enzymes are responsible for the degradation of starch and its related polymers to yield products characteristic of individual amylolytic enzymes.

Amylase enzymes have some common types, namely, amylolytic, α-amylase, β-amylase, and glucoamylase. These enzymes are largely present in a wide range of organisms, including plants, animals, and microorganisms. *Aspergillus* spp. is the most extensively used fungi for the production of amylolytic enzymes. The major sources of α-amylase enzymes are *A. niger*, *Aspergillus oryzae*, *B. circulans*, *Bacillus amyloliquefaciens*, *B. subtilis, B. licheniformis,* and *B. stearothermophilus*. β-Amylase enzymes are obtained from species like *Bacillus* spp., *Pseudomonas* spp., and *Streptomyces* spp. However, out of these enzymes, the production of α-amylases accounts for about 30% of enzymes in world market for their extensive use in food industrial processes, such as in baking, brewing, fruit juices, syrups, starch, etc. It is also widely used in the production of drugs and other pharmaceutical products (van der Maarel et al., 2002).

3.4 Significance of Enzymes in Food Quality

In recent years, the significance of enzyme application in food processing industries has been increasing. It is due to the diversified role played by them in increasing the rate of biochemical processes. But, sometimes enzymes are also liable to unfavourable biochemical and physiological changes in fruits and vegetables. These changes alter the colour, texture, aroma, and flavour in them. Therefore, it is important to understand and characterize the interrelationships between the quality components and the associated enzymes. It also helps in determining the optimal post-harvest handling procedures and processing techniques to provide high-quality products to consumers.

3.4.1 Enzymatic Reaction on Colour of Fruits and Vegetables

The colour of a product is the primary assessment factor of quality more than any other factor. So, maintaining the natural colour of food is very critical. The colour of food products is affected during pre-harvest and post-harvest factors which are coupled by enzymatic reactions. The main enzymes responsible for changes of colour in fruits and vegetables are polyphenol oxidase (PPO), peroxidase (POD), and β-glucosidase (Zabetakis et al., 2000). Among the various processing changes, enzymatic browning causes major problem in food processing (Terefe et al., 2014).

It is due to the oxidation of phenolic compounds to quinones via polymerization reactions resulting in the production of dark colour compound called melanin (Marshall et al., 2000). Either to inhibit or delay this particular phenomenon, the fruits and vegetables are treated with anti-browning agents prior to further processing. The most commonly used anti-browning agents are citric acid, ascorbic acid, and calcium chloride. They can be used either singly or in combination with other chemicals. Ascorbic acid and citric acid are extensively used as anti-browning agents since they act as a reducing agent on the enzymatic reaction of PPO. Similarly, calcium chloride ($CaCl_2$), also known for PPO inhibitors, reduces enzymatic browning by acting on pH and as a chelating agent. However, combined treatments were found to be more effective to prevent the browning effect.

Polyphenol oxidases (PPOs) are a group of copper containing enzymes, capable of catalysing the oxidation of several phenols to *o*-quinones (Oliveira et al., 2011). These *o*-quinones in turn react with molecules which undergo non-enzymatic secondary reactions, resulting in the formation of melanin, brown complex polymers, and cross-linked polymers with protein functional groups (Rolff et al., 2011). PPOs are abundantly present in various fruits and vegetables, such as, apple, peach, banana, potato, mushroom, coffee bean, microorganisms, etc. (Eisenmenger & Reyes-De-Corcuera, 2009). Conversion of phenolics substrates into *o*-quinones occurs in two oxidation steps. In the first step, hydroxylation of *ortho*-position adjacent to an existing hydroxyl group, "monophenolase" or "monophenol oxidase", is generally referred to as cresolase or hydroxylase activity. The second step is the oxidation of *o*-dihydroxybenzenes to *o*-benzoquinones, "diphenolase activity" or "diphenol oxidase", referred to as oxidase or catecholase activity (Yoruk & Marshall, 2003).

The oxidation of phenolics compounds by polyphenol oxidase is the main reason for most of the enzymatic browning in foods occurred during harvesting, handling, storage, and processing (Eisenmenger & Reyes-De-Corcuera, 2009). It was reported that the PPO activity in apple, tomato, and tobacco is encoded by multiple genes and regulates tissue damage (Kim et al., 2001). In another study, expression of PPO response was observed in both damaged and non-damaged leaves (Constabel & Ryan, 1998). The expression of PPO in peaches was observed for 48 h of storage and it was found that there was a slight or gradual decrease in the PPO activity in intact tissues. On the other hand, in damaged tissues, there was a rapid increase in PPO activity (Tourino et al., 1993). In peaches, enzyme inhibitors such as ascorbic acid, sodium metabisulfite, β-mercaptoethanol, and cysteine were found to be effective against PPO activity (Belluzzo et al., 2009).

Furthermore, enzymatic browning can also be controlled by several processing techniques, such as freezing and heating coupled with natural and synthetic inhibitors (Marshall et al., 2000). However, many PPO inhibitors are associated with off-flavours, food security, and sustainability. For example, sulphites as PPO inhibitors are reported to cause allergies and thus seek natural alternatives (Loizzo et al., 2012). 4-Hexylresorcinol is a natural compound; when used along with ascorbic acid, cysteine, or kojic acid, it resulted in the reduction in PPO activity and changes colour in mango puree and apple juice (İyidoğan & Bayındırlı, 2004).

Kojic acid is another natural compound; it chelates metal ions such as Fe^{3+} and Cu^{2+} in addition to impounding of free radicals (Kim & Uyama, 2005).

Proteases are another group of enzymes that catalyse the hydrolysis of proteins which are degraded to peptides and amino acids (Omaña-Molina et al., 2013). It inhibits PPO activity through proteolysis or binding to specific sites. According to a study report, it was found that the browning reaction was decreased in apple when papain was used (Labuza et al. 1992). Also, when pineapple juice and high-pressure technique were applied, the browning reaction was reduced in apple slices. Such inhibitory effect is due to the presence of bromelin, sulfhydryl groups, citric, or malic acid (Perera et al., 2010). Similarly, the prevention of browning effect was also observed in peeled banana when pineapple juice was used (Chaisakdanugull et al., 2007). However, in some studies, bromelin was found to be ineffective in preventing browning of apple juice (Tochi et al., 2009).

Peroxidases (POD) are generally isolated from plants, animals, and microorganisms. These are oxidase enzymes which use hydrogen peroxide as a catalyst in the oxidation reaction of polyphenols, aminophenols, monophenols, and diphenols (Fatibello-Filho & Vieira, 2002). They are heat-stable and hence used as a parameter to increase the efficiency in bleaching (Aguero et al., 2008). POD lead to undesirable changes in colour, flavour, texture, and nutritional values in foods (Gonçalves et al., 2007). The browning of sugar cane juice is due to the presence of enzymes, POD and PPO, which oxidize phenolics compounds (Qudsieh et al., 2002). The enzymatic activities of POD and PPD enzymes were studied in different cultivars of grapes. In these studies, the POD extracts showed similar activity in both the soluble and bound fractions, and highest PPD activity was observed in cultivar Ruby. PPD and POD activities in cultivars Benitaka and Ruby decrease when juice extracts were treated with higher temperature and longer duration. It was observed that enzyme inactivation was achieved at 85 °C with 10 min exposure time, but the thermal treatments were not sufficient to inactivate the enzymes completely. For example, the thermal treatment in jam, jellies, and juices causes reduction in PPO and POD activities, but not sufficient to inactivate the enzymes (Freitas et al., 2008). Similar activities were also reported in processing of guavas (Zanatta et al., 2006).

In other studies, it was shown that apple peel from Fuji and Gala cultivars when compared to its pulp had elevated enzymatic activity both for PPO and POD. When heat treatment was done in the concentrated extracts of pulp and peel, there was a decline in PPO enzyme activity and total inactivation was also achieved after 10 min of heat treatment at 75 °C. However, such case of total inactivation was not observed for POD enzyme activity (Valderrama et al., 2001). Studies have shown that the enzymatic activity of PPO in fresh broccoli was higher compared to bleached broccoli. In case of POD, the activity was found to be lower in bleached broccoli, indicating that bleaching was partially effective in denaturation of these enzymes (Lopes & Clemente, 2002).

3.4.2 Enzymatic Reaction on Texture of Fruits and Vegetables

Texture of fruits and vegetables is an important indicator in determining the quality of the product. It is dependent on the cellular structure of cell walls. Any changes in the composition and structure of cell wall polysaccharides result into changes in the firmness (Alkorta et al., 1998). It influences the keeping quality such as storage time, handling damages, and consumer acceptance of fruits and vegetables after harvesting. Degradation in the textural quality may be due to several factors such as growth of microorganisms, infestation by insects, rodents, and other environmental conditions.

The texture quality of fruits and vegetables is also affected by a number of enzymes. Pectin which is an important cell wall polysaccharide helps in maintaining the texture by avoiding the breakdown of cellular structure. The enzymatic effect on the texture of fruits and vegetables happens when enzymes such as pectinases, cellulases, and hemicellulases break the cell walls. So, the inactivation of these enzymes during processing would help in preserving the texture of the food. Generally, pectin is used as a gelling and texturizing agent. It also improves the taste and appearance of processed foods like jams, jellies, and marmalades (Alkorta et al., 1998). However, pectins also have some major disadvantage in food processing, especially in juice processing. Soluble pectins in fruits are responsible for cloudiness or haziness in different fruit juices. Hence, the texture of such final juice product may or may not have positive feedback from consumers. But, these days, cloudy juices from tropical fruits are generally accepted and their market is growing. Nonetheless, clear fruit juices from apples, grapes, oranges, etc. are still the preferred choice of the consumers as they have appealing texture. Therefore, the enzymatic application on such hazy or cloudy juices becomes important for improving their texture and ultimately increasing consumer acceptability in the market. In order to overcome this issue, clarification process is conducted for the degradation of pectins by the application of enzyme pectinases (Alkorta et al., 1998). Different types of pectinases are used in this process and some names are pectin lyase, pectin esterase, and polygalacturonase (Kant et al., 2013). But, the pectinolytic enzymes from A. niger are commonly used in fruit and vegetable processing to improve the texture of the final products (Dinu et al., 2007).

Transglutaminases is another important enzyme which has been used extensively for food texture and new product development in food processing industries. The enzyme helps in the catalytic cross-linking reactions between proteins which transfer the glutamine residue, namely, γ-carboxyamide of one protein to the ε-amino group of lysine residue of the same protein or another protein (Nandakumar & Wakayama, 2015). This results in the improvement in food properties, such as firmness, elasticity, viscosity, and water-binding capacity. The main microbial sources of enzymes for improving the texture in food are *Bacillus* sp., *Streptomyces* sp., and *Corynebacterium* sp. (Zhang et al., 2012; Placido et al., 2008). Glutaminase is another enzyme that helps in texture by improving the protein properties, thereby increasing its solubility, gelation, emulsification, etc. (Nandakumar & Wakayama, 2015).

3.4.3 Enzymes in Flavour and Aroma Production

Taste and flavour are the two major attributes in determining the overall quality of various fruits and vegetables. Taste is determined by the contents of sugar, tannins, phenols, organic acids, and other compounds. Analysis of flavour compounds has given us an inclusive knowledge on the chemical compounds responsible for flavour sensations of fruits and vegetables. However, different enzymes have multiple effects on these attributes of fruits and vegetables. Enzymes such as peroxidases and lipoxygenases are responsible for off-flavour formation in fruits and vegetables (Bhowmik & Dris, 2004). The enzyme peroxidases are responsible for deterioration in colour, flavour, texture, and loss in nutritional qualities in raw or processed fruits and vegetables. The off-flavour is often linked with the oxidation of phenolic compounds and indigenous lipids. It was observed that enzyme inactivation was achieved at 70 °C with 15 min exposure time (Sessa & Anderson, 1981). Therefore, inhibition of such enzymes during processing is a must in order to retain or improve the aroma and flavour.

Another enzyme that has a major effect in flavour and aroma is lipogenase. This enzyme produces free radicals and conjugated unsaturated hydroperoxy acids by catalytic oxidation of polyunsaturated fatty acids. Then, these free radicals interact with other constituents like proteins, vitamins, phenolics, etc. present in fruits and vegetables which helps in enhancing the flavour and aroma of the product. However, these aromatic compounds may produce off-flavours in *Brassicaceae* family. According to the reports of Sheu and Chen (1991), increase in colour losses and development of off-flavour were observed in broccoli and asparagus during storage of non-blanched and under-blanched products.

Furthermore, the volatile compounds such as aldehydes, alcohols, ketones, esters, lactones, etc. are related to flavour and aromatic characteristics in foods (Beaulieu & Baldwin, 2002). For example, the compounds present in alcohols and aldehydes are extensively used as food additives due to the aroma referred to as ‘green touch’, a characteristic observed in freshly harvested fruits (Schwab et al., 2008). These volatile compounds are synthesized using various substrates like amino acids, fatty acids, and carotenoids (Goff & Klee, 2006). The main enzymes involved in the synthesis of volatile compounds from fatty acids are lipoxygenase, alcohol dehydrogenase, hydro peroxide lyase, and (3*Z*): (2*E*)-enal isomerise (Schwab et al., 2008). These enzymes are used in the extraction from fruits and vegetables such as banana, soy, tomato, and olive where they subsequently react with fatty acids to produce volatile compounds (Akacha & Gargouri, 2009).

In order to improve the taste and flavour in citrus fruits, enzymes limoninase and naringinase can be enzymatically tailored by degrading the bitter taste compounds such as limonin and naringin (Ribeiro et al., 2010). The formation of limonin can also be prevented by using limonoate dehydrogenase, as it catalyses the oxidation of its precursor lactone-A-ring to 17-dehydrolimonoate, a non-bitter derivative which cannot be changed into limonin (Merino et al., 1997). Bitter compounds in citrus fruits can be reduced by using adsorbing polymers, such as Amberlite XAD-16HP and Dowex Optipore L285 resins. Besides acting as a debittering agent, Dowex

Optipore L285 can also induce other modifications in juice processing like reduction of total titratable acidity (TTA), increasing total soluble solids (TSS), the ratio of TSS to TTA, pH, etc. (Kola et al., 2010).

3.5 Application of Enzymes in Fruit and Vegetable Processing

The significance of enzymes and its application in food processing industry is increasing rapidly. Different kinds of enzymes are used extensively based on their effective application. In fruits and vegetables processing as well, several endogenous enzymes and newly developed enzymes are used because of the following advantages:

- Low temperature requirements
- Low energy requirements during processing
- Increased product yield especially in juice processing. They make the juice stable without the addition of additives
- Less by-product formation during processing
- Improved product quality (Grassin & Coutel, 2009)

In the following paragraphs, detailed analyses are discussed on the use of enzymes in different fruit and vegetable processing techniques.

3.5.1 Fruit Firming

Texture is one of the important quality attributes of fruits and vegetables (Pan et al., 2014; Grassin & Coutel, 2009; Guillemin et al., 2008; Jensen et al., 2004; Degraeve et al., 2003). However, the texture of processed fruits and vegetables, especially the softer ones, such as strawberry, raspberry, and tomatoes, is adversely affected by thermal processing treatments like blanching, sterilization, freezing or pasteurization, and other mechanical method which may result in softening (Grassin & Coutel, 2009; Guillemin et al., 2008; Degraeve et al., 2003).

The texture and structural integrity of fruits and vegetables depend on the composition of cell walls present in them. These cell walls are composed of an interlinked fibrous structure of cellulose embedded in a matrix of pectin, hemicelluloses, and celluloses (Guillemin et al., 2008; Sila et al., 2008). The enzyme pectin methylesterase (PME) bound to the cell wall is a pectin degrading enzyme which results in demethoxylation of pectin (de-esterification of the methylated carboxy groups of polygalacturonic pectin), releasing methanol and forming carboxylated pectin. This carboxylic acid is said to interact with calcium, resulting in firmness of fruits by strengthening the cell wall (strong pectate network with added calcium) (Pan et al., 2014; Sila et al., 2008; Guillemin et al., 2008; Degraeve et al., 2003). This process is known as chelation, and it helps to overcome the

adverse effects of thermal and mechanical processing treatments resulting in reduced fruit damage and disintegration (Grassin & Coutel, 2009; Degraeve et al., 2003).

Studies were conducted as early as 1965 and 1972 to assess the role of PME in increasing the firmness of canned tomatoes and potatoes, respectively. In both the studies, heat treatments such as blanching in case of tomatoes and preheating of potato slices in water were used to assess the role of PME with firmness (Bartolome & Hoff, 1972; Hsu et al., 1965).

As per a study conducted on peaches, vacuum infiltration of blanched peaches with PME of citrus fruit for 2 h along with calcium chloride increased the firmness of peaches (Javeri et al., 1991). Another study also demonstrated that vacuum impregnated PME (*Aspergillus niger*) along with calcium chloride increased the firmness in pasteurized fruits, namely, apples, strawberries, and raspberries (Degraeve et al., 2003). Similarly, a study demonstrated enzymatic firming of red pepper by using exogenous pectinesterase (Rheozyme from Novozyme A/S) and retention of texture even after freezing or heating (Jensen et al., 2004). Thus, many fruits and vegetables may be processed by using PME and calcium, thereby retaining the texture of the products (Grassin & Coutel, 2009).

3.5.2 De-Skinning (Enzymatic Peeling)

De-skinning means removal of peel/skin of fruits and vegetables. It was initially done mostly by different methods such as manually, chemical, steam, mechanical, or freeze method. But, these methods involved high labour cost, high water consumption, and peeling losses (Toushik et al., 2017; Pretel et al., 2007a, 2010). Now, considering their disadvantages, these methods have been replaced almost by enzymatic peeling in processing industry. In this method, the product is infused in enzyme preparation of pectic substances such as pectinases, hemicellulases, and cellulases (Berry et al., 1988; Bruemmer et al., 1978). Once infused, the vacuum is applied which helps the enzymatic solution to enter the intercellular spaces (Pretel et al., 2008). In the process, cellulase releases pectin from albedo, while pectinases break down the cell wall by hydrolysis of polysaccharides (Ben-Shalom et al., 1986).

Nowadays, many commercial enzymatic preparations are available and pectolytic preparation (mixture of pectinases, cellulase, and hemicellulase) is one of them. It is obtained from *Aspergillus species* fungi. It has been observed that the enzymatic solution easily diffuses when there are large intercellular spaces in albedo, resulting into easy peeling (Baker & Bruemmer, 1989). However, many studies have found that the efficiency of enzymatic peeling effect depends on the treatment methods such as heat (scalding), vacuum infusion, temperature, and pH (Pagán et al., 2005; Suutarinen et al., 2003; Toker & Bayındırlı, 2003; Rouhana & Mannheim, 1994). Details of some recent studies are given in Table 3.3.

Furthermore, the role of enzymes in the peeling process of vegetables such as potato, carrot, onion, and Swedish turnips was examined by Suutarinen et al. (2003). It was observed that enzymes did help in peeling of carrot and onions; however, potato and Swedish turnips could not be peeled properly due to the presence of cutin

Table 3.3 Studies assessing role of enzymes in enzymatic peeling

Reference	Fruit/ vegetable	Pre-processing condition	Enzymatic solution	Result
Prakash et al. (2001)	Grapefruit	Scalding, 1–4 min	Polygalacturonase, cellulase and pectin methylesterase, incubated for 12 min	Peeling improved
Suutarinen et al. (2003)	Potato, carrot, Swedish turnip, onion		Cellulase, polygalacturonse	Difficulty in removing peel of potato and Swedish turnips
Liu et al. (2005)	Mandarins		Pectinase, polygalacturonse, and cellulase, 20 min	Peeling efficiency improved
Pretel et al. (2007b)	Orange, Thomson, and Mollar	Hot water bath (45 °C)	Pectinase, polygalacturonse, and cellulase, 40 min	Peeling improved
Pretel et al. (2007a)	Orange, Sangrina	Hot water bath (45 °C)	Pectinase, polygalacturonse, and cellulase, 30 min	Peeling improved

or suberin in vegetable surface (Suutarinen et al., 2003). As per a study by Toker and Bayindirli, it was observed that the enzymatic peeling was easier at relatively moderate temperatures such as 44–47 °C for nectarines, 41–46 °C for peaches, and 45 °C for apricots with a pH ranging from 3 to 4.1 (Toker & Bayındırlı, 2003). The steps involved during enzymatic peeling are illustrated in Fig. 3.1.

The first step is selection of fruits or vegetables and washing them with water. At times, hot water treatment or scalding is done prior to enzymatic peeling to improve the peeling process and have a high quality end product (Pretel et al., 2008, 2010). A study on grapefruit as early as 1974 demonstrated dipping the fruit in a water bath of 60 °C for 30–35 min resulted in a good quality product (Bruemmer et al., 1978). The advantage of scalding is that it reduces the viscosity of pectin as well as enhanced the ability of the peel to absorb the enzyme solution, thereby helping in enzymatic peeling process (Pretel et al., 2008) (Fig. 3.1).

Therefore, enzymatic peeling has wide range of applications in fruits and vegetables such as grapefruit, orange, mandarin, apricot, peaches, potato, carrot, and Swedish turnips (Suutarinen et al., 2003; Toker & Bayındırlı, 2003). This method is a significant alternative in food processing industry because of its advantages in improving the quality of products, reducing wastage, minimal water use, reduction in contamination, and being cost-effective (Toker & Bayındırlı, 2003).

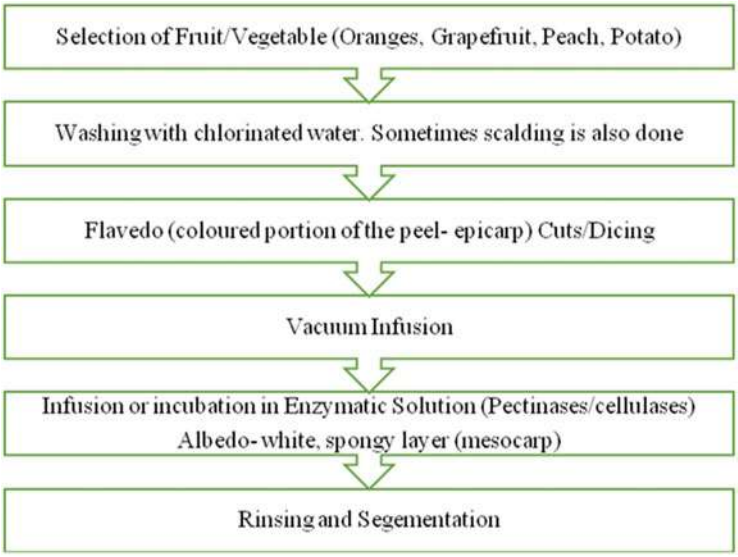

Fig. 3.1 Steps in enzymatic peeling. (Adapted from Pretel MT, Sánchez-Bel P, Egea I & Romojaro F. (2010). Enzymatic peeling of citrus fruits. In Bayindirli (Ed.), Enzymatic processing of fruits and vegetables: Chemistry and engineering applications (pp. 145–174). CRC Press, Taylor and Francis Group)

3.5.3 Application of Enzymes in Fruit and Vegetable Juice Processing

The natural liquid substance contained in fruits and vegetables is commonly known as juice. It can be obtained or prepared directly by extraction, pressing, or diffusion from fruits or vegetables and such juices are meant for direct consumption. They can be broadly categorized as juices without pulp (clarified/cloudy) or juices with pulp such as purees, nectars, and pulps (Ceci & Lozano, 2010; Cautela et al., 2010). In juice processing, different enzymes are extensively used in fruit and vegetable for higher yield, clarification, and improvement in filtration, resulting in higher quality of juices (Fleuri et al., 2016). Some of the commonly used enzymes in fruit and vegetable juice processing are pectinases, hemicellulases, and cellulases. In juice processing of fruits and vegetables, multiple steps are involved and below are the standard procedures. The steps involved in fruit juice processing are also illustrated in Fig. 3.2.

3.5.3.1 Selection of Fruit, Followed by Washing and Peeling

Enzymatic preparations can be used for peeling of fruits such as oranges, lemon, or vegetables such as pumpkin and beet (Toushik et al., 2017) as discussed in Sect. 3.4.2. This is followed by juice extraction.

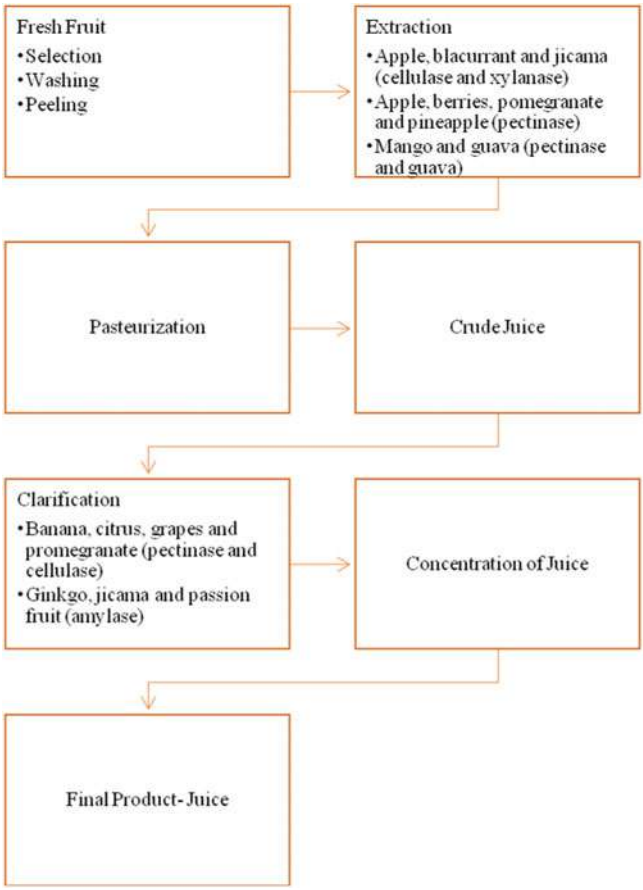

Fig. 3.2 Steps in juice processing. (Adapted from Fleuri, L. F., Delgado., C. H. O., Novelli, P. K., Pivetta, M. R., do Prado, D. Z., & Simon, J. W. (2016). *Enzymes in food and beverage processing* (pp. 255–280). CRC Press, Taylor and Francis Group)

3.5.3.2 Juice Extraction

Juice can be extracted by a number of methods—centrifugation, diffusion, extraction, and ultrafiltration. Enzyme preparations help in breakdown of cell wall and release the juice contained within the structure in fruits such as apples, grapes, berries, pears, and citrus fruits (Ribeiro et al., 2010). Among apples, the activity of endogenous enzymes is low; therefore, commercial pectinases from *Aspergillus* species are added so they can be macerated before pressing (Aehle, 2007). The extracted juice is pasteurised for microbial stability (Grassin & Coutel, 2009). A study on processing of blackberry juice observed that application of pectinolytic enzymes during pressing resulted in an increase in juice extraction as high as 81.73% in three cultivars as compared to 53.79% in the control arm (Granada et al., 2001).

Another important aspect in juice processing is the discolouration of juices during pasteurization due to presence of anthocyanins. This issue can be prevented by the use of β-glucosidase which hydrolyses anthocyanins into anthocyanidins, thereby minimizing discolouration due to its low colour intensity and solubility (Villena et al., 2007; Palma-Fernandez et al., 2002).

3.5.3.3 Clarification and Fining

Turbidity in juices is due to scattering of light caused by insoluble substances such as cell fragments that come from pulpy tissue (pectin from cell walls) in suspension. It is usually undesirable except when present in citrus fruits (Ribeiro et al., 2010). Pectin is the cause of turbidity in juices especially apple juice (Aehle, 2007). Another reason for the haziness in extracted juices is due to presence of starch in unripe fruits (Ceci & Lozano, 2010). At this stage, the role of enzymes becomes critical as enzymes like pectinase help in reducing turbidity by attracting pectin and forming clusters (aggregation of cloud particles). Then, these particles usually settle down and can be easily removed by filtration or centrifugation (Uneojo & Pastore, 2007). Apart from enzymes, flocculating or fining agents such as gelatin and bentonite may be added to aid in clarification process (Ceci & Lozano, 2010). Similarly, citrus juices, especially lemon juice, are also clarified by pectinolytic enzyme preparations (Fleuri et al., 2016).

Apple juice contains about 15% of starch which results in turbidity, slow filtration rate, and gelling after concentration. The addition of amylase along with pectinase hydrolyses this starch and removes the haze or cloudiness (Ceci & Lozano, 2010; Carrín, 2004). Sometimes, haziness occurs in juice, so adding of fining agents can prevent post-bottling haziness, thereby optimizing fining and ultrafiltration process. Similarly, araban present in certain fruits may be responsible for post-concentration haze in juice, and to prevent such haze formation, arabanase is added to the juice (Ceci & Lozano, 2010).

3.5.4 Role of Enzymes in Wine Processing

The role of enzymes in the production process of alcoholic beverages such as brewing of beer and winemaking is very important (Toushik et al., 2017; Gómez-Plaza et al., 2010; Aehle, 2007). Different enzymes, namely pectinases, amylases, glucanases cellulases, glycosidases, lyzozymes, and ureases, are used. They help in improving the quality and yield of the product (Toushik et al., 2017; Gómez-Plaza et al., 2010; Aehle, 2007).

3.5.4.1 Enzymes Used in Processing of Wine

Wine is a type of alcoholic beverage made from fermentation of fresh grapes (Bruchmann & Fauveau, 2009). Pulp and skin of the grapes account for about 75–85% and 6–7%, respectively. The cell wall of grapes is composed of hemicelluloses, pectin, and structural proteins like chitinase. Also, the cellulose and pectin present in cell walls provide rigidity to the grapes, thus hindering juice

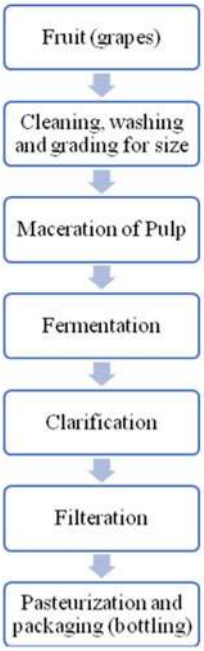

Fig. 3.3 Steps in wine-making. (Adapted from Fleuri, L. F., Delgado., C. H. O., Novelli, P. K., Pivetta, M. R., do Prado, D. Z., & Simon, J. W. (2016). *Enzymes in food and beverage processing* (pp. 255–280). CRC Press, Taylor and Francis Group)

extraction, clarification, and filtration (Claus & Mojsov, 2018). Moreover, the skin and cell walls of grapes contain several essential compounds such as anthocyanins and tannins. These compounds are responsible for colour and structure of wine (Gómez-Plaza et al., 2010; Bruchmann & Fauveau, 2009).

The natural enzymes present in grapes and those obtained from yeast, fungi, and bacteria collectively play an important role in wine making process (Gómez-Plaza et al., 2010). Usually, the manufacturers extend the action of endogenous enzymes by adding exogenous enzymes during production (Gómez-Plaza et al., 2010). The commonly used commercial enzymes include pectinases, glucanases, glycosidases, lysozymes, and ureases (Gómez-Plaza et al., 2010). The application of these exogenous enzymes and yeast strains during processing increases the rate of production, yield, and quality of the wine (Toushik et al., 2017).

The standard steps in wine processing are illustrated in Fig. 3.3.

These steps can be broadly categorized in three phases, namely pre-fermentation, fermentation, and post-fermentation.

Pre-fermentation

This phase involves crushing the fruit and extraction of juice. In red wine preparation, the skin is not separated, while it is separated in white wine. During the processing of red wine, pulp, skin, and seeds of grapes are kept together after crushing and during fermentation in order to extract colour and flavour (Byarugaba-Bazirake, 2008). The colour of red wine is due to the presence of anthocyanins and tannins in grape skin and seed (Gómez-Plaza et al., 2010). In

this phase, exogenous enzymes derived from *Aspergillus* species such as pectinases, cellulases, and hemicellulases (xylanases and galactanases) are employed, which help in expediting the extraction of juice and colour control (Espejo, 2020; Toushik et al., 2017; El Darra et al., 2016; Kelebek et al., 2007; Bautista-Ortín et al., 2005). These macerating enzymes also modify the stability, taste, structure, and mouth feel of red wine (Gao et al., 2019).

Furthermore, maceration of the cell walls by pectinases results in the liberation of liquid and other phenolic compounds (Espejo, 2020; Gao et al., 2019). It has been observed in different studies that wines prepared by enzyme treatment tend to have phenolic and tannin content, as well as colour intensity (Romero-Cascales et al., 2008; Revilla & Gonzalez-San Jose, 2003). Sometimes, maceration is accompanied by pressing (wine press extract must or wine from crushed grapes), resulting in better yield (Gómez-Plaza et al., 2010). Another important attribute in wine making is its aroma; reductases such as glycosidases and polyphenol reductase help in aroma extraction and polyphenol reduction, respectively (Espejo, 2020). Glycosidases improve the aroma of wines by releasing aromatic compounds from their non-aromatic precursors or non-odouriferous sugar compounds (Zhu et al., 2014). As per a study, treatment of Albarino wine with pectinolytic preparations and glycosidase resulted in an improved aroma (Armada et al., 2010). However, in industrial processing, usually exoglycosidases and β-glucosidase from *Aspergillus niger* are used (Gómez-Plaza et al., 2010).

Fermentation

In this phase, yeast is added to the must in red wine to initiate fermentation (Byarugaba-Bazirake, 2008). During the process, the sugars present in the juice are converted into alcohol and release carbon dioxide. In this period, pectin plays an important role by preventing the diffusion of intercellular components such as phenolic and aroma compounds into the must (Claus & Mojsov, 2018; Bruchmann & Fauveau, 2009).

Post-fermentation

This phase involves different steps such as clarification, filtration, and microbial stabilization with the help of different enzymes. Proteases are used at the time of clarification, while pectinase and β glucanase are used during filtration and lysozyme for microbial stabilization (Espejo, 2020). After pressing, the grape must is quite turbid and is rich in solid particles (Armada & Falqué, 2007). Thus, clarification is an important step to improve the quality of wine, especially in white wine (Gómez-Plaza et al., 2010). Moreover, activated carbon, bentonite, and pectinase may be added to aid in clarification or physical removal of suspended matter in the must (Gómez-Plaza et al., 2010). Commercial enzymes that help in clarification are:

(a) Pectin lyase which results in destabilization of the cloud, thereby reducing viscosity.
(b) Pectin methylesterase (PME) results in demethylation of pectin, aiding polygalacturonase in hydrolysis of pectin and leading to cloud flocculation

Table 3.4 Enzymes and their roles in wine processing

Step	Enzyme	Action
Clarification	Pectinase	Removal of suspended particles
Maceration	Pectinases, hemicellulase, cellulase	Degradation of pectin
Maturation	Pectinases and glucanases	Ageing results in mouth feel and is done at the end of fermentation
Filtration	Pectinases and glucanases	Glucanases are applied to the must to shorten the glucan chains preventing problems during filtration
Addition to wine/ must	Urease	Elimination of urea and preventing the formation of ethyl carbamate
Addition to wine/ must	Lysozyme	Elimination of bacterial
Late phase of fermentation (white wine)	Beta glucosidase	Increased aroma by splitting glucose residue from odourless precursors
Stabilization/haze formation	Proteases	Stabilization of wine, reducing betonite demand

Note: Adapted from Gómez-Plaza et al., 2010. Use of enzymes in wine production. In Bayindirli (Ed.), Enzymatic processing in fruits and vegetables: Chemistry and engineering applications (pp. 215–243). CRC Press, Taylor and Francis Group

and clarification of must (Gómez-Plaza et al., 2010; Bruchmann & Fauveau, 2009).

Furthermore, during ageing or maturation, cellulases and β-glucanases are used to improve the mouthfeel (Espejo, 2020; Gómez-Plaza et al., 2010). Other than the above mentioned advantages of the macerating enzymes, studies have also observed that pectinases are found to improve haze condition, increase pressability of grapes to reduce filtration time, and increase must volume (Sharma et al., 2017; Garg et al., 2016). Table 3.4 illustrates different enzymes and their actions during wine processing.

Other important enzymes used in commercial wine production are cinnamyl esterase, cinnamyl decarboxylase, glucanase, urease, and lysozyme. The yeast-derived enzymes, cinnamyl esterase and cinnamyl decarboxylase, may be present in commercial pectin preparations as a side activity and can cause off-flavours due to volatile vinyl phenols (Bruchmann & Fauveau, 2009). At times, grapes infected with *Botrytis cinerea* result in formation of glucans. These glucans prevent the natural sedimentation of cloud particles and cause filtration problems as well. Thus, glucanase enzyme is added to prevent the development of such glucans during wine making process (Gómez-Plaza et al., 2010). Urease is another enzyme that is added occasionally during wine preparation. This enzyme hydrolyses urea into ammonia and carbon dioxide and prevents the formation of ethyl carbamate in wine which is carcinogenic at high concentration (Gómez-Plaza et al., 2010).

Lysozyme is used in winemaking to prevent or delay malolic fermentation and sulphur dioxide is added to the must or wine (Gómez-Plaza et al., 2010).

3.6 Effect of Processing Methods on Enzymes and Vegetables

Enzymes present in fruits and vegetables play a huge role in determining the texture, colour, flavour, and taste attributes of the processed products (Oey, 2010). The continued enzymatic activity in fruits and vegetables affects the storage quality, shelf life, and palatability of the product. Therefore, several processing methods such as grinding, crushing, slicing, juices, or preservation (pickling) are used to prolong the shelf life and reduce wastage of fruits and vegetables (Oey, 2010). Hence, it is very important to control the stability and activity of endogenous enzymes present in fruits and vegetables during food processing (Oey, 2010).

Apart from the conventional techniques of processing such as blanching, heating, or freezing, new and highly advanced processing techniques like high hydrostatic pressure (HHP), pulse electric field (PEF), and ultrasound have been introduced successfully. The major advantage of these new techniques is the use of non-thermal technology, which helps in retaining the sensory attributes and nutritional content of the product (Oey, 2010; Jaiswal & Sharma, 2016). In HHP technique, it employs high pressure range of 100–600 MPa, resulting in enzyme and microbial inactivity which may affect the shelf life of the products (Briones-Labarca et al., 2015). In PEF technique, being a non-thermal processing, it uses a series of short and high voltage pulses to inactivate microbes or enzymes (such as peroxidases and polyphenol oxidases) in food (Segovia et al., 2015). However, in ultrasound processing technique, high frequency short pulses are used for inactivation (Jaiswal & Sharma, 2016). These non-thermal processing techniques are effective at ambient or sub-lethal temperatures and minimize the adverse thermal effects on the nutritional content and quality of fruits and vegetables (Tiwari et al., 2009).

However, these non-thermal processing techniques still have several drawbacks, such as high equipment and processing cost, tedious to operate, hazardous, requires stringent process control operations, etc. (Jaiswal & Sharma, 2016). Nonetheless, multiple advantages are rendered by these techniques in food processing industry over conventional thermal techniques, which affect not only the enzymes, but also the texture, taste, and colour of the product compelled for further investigation and improvement (Oey, 2010).

3.6.1 High-Hydrostatic Pressure (HHP) Processing

HHP, also known as High-Pressure Processing (HPP), is based on the Le Chatlier principle, i.e. pressure is equally applied in all directions (Augusto et al., 2018; Terefe et al., 2016; Keenan et al., 2012). As the name implied, this technique uses very high pressure for application of uniform pressure to a product or food, leading to the inactivation of certain microbes and enzymes in the food (Augusto et al.,

2018). This technique can be applied in solid, semi solid liquid, or particulate food products (Augusto et al., 2018).

The high pressure (HP) may increase the shelf life of fruits and vegetables, especially when combined with temperature by resulting in enzyme activity or inactivity and pressure-induced gel formation (Guerrero-Beltrán et al., 2005). In HHP technique, enzyme inactivation may be affected by the type of food, pH, temperature, and duration of treatment (de Castro Leite Júnior et al., 2017; Guerrero-Beltrán et al., 2005).

The HHP unit consists of a pressure vessel, pressure generator, temperature control, and pressure handling system. The HHP processing can be of two types—batch (closed vessel system) or semi-continuous (number of closed vessels). In this process, the hydrostatic pressure at a given point is transformed rapidly and uniformly in all directions. The intensity of HHP is determined by the process parameters like pressure, treatment duration, and temperature. The entire HHP processing cycle takes a few minutes and it can be performed at pressures as high as 1400 MPa and temperatures between less than 0–150 °C (Oey, 2010) (Augusto et al., 2018; Oey, 2010). The maintenance of a product inside a vessel at a high-pressure results in molecular changes affecting the enzymes (de Castro Leite Júnior et al., 2017).

Several studies have been conducted to determine the effect of HHP (at times combined with thermal processing) on enzyme activity in various fruits and vegetables as shown in Table 3.5. Enzymes like PME, POD, and PPO are considered baroresistant with POD and PME being the most and least resistant, respectively (Augusto et al., 2018). Hence, some studies have found that PPO and POD are not affected substantially by HHP (Augusto et al., 2018).

3.6.2 High-Pressure Homogenization Processing (HPHP)

HPHP, also called Ultra High-Pressure Homogenization, is a non-thermal processing technique, especially used in fruit juice processing. In this method, the fluid is pressurized with high or ultra-high pressure to flow through a narrow valve and the shear stress distribution across the product helps to inactivate microbes and enzymes present in the fluid (Augusto et al., 2018; de Castro Leite Júnior et al., 2017). It is a technique which can be used only in fluids. HPH is a continuous process and is often called dynamic high-pressure processing (Augusto et al., 2018; de Castro Leite Júnior et al., 2017). The high shear and sudden drop in pressure, turbulence, and caviation result in changes in processed foods (de Castro Leite Júnior et al., 2017).

In HPH, factors such as enzyme structure, fluid, equipment, media, and process parameters determine its effect on enzyme activity (Augusto et al., 2018). To ascertain its efficacy, HPH was used to treat oranges and PME activity was measured at five pressures (0–250 MPa) and three inlet temperatures (22, 35, 45 °C). A reduced activity of 70% can be achieved at 45 °C and 250 MPa (Welti-Chanes et al., 2009). Similarly, HPH has also been found to result in complete inactivity of

Table 3.5 Effect of HHP on enzyme activity

Reference	Fruits/ vegetables	Enzyme	Processing	Enzyme stability after application of HHP processing
Andreou et al. (2016)	Tomato juice	PME	800 MPa, 15 min, 65 °C	70% reduction in enzyme activity
		PG	500 MPa, 10 min, 55 °C	Complete inactivation of enzyme activity
Terefe et al. (2016)	Pear	POD	600 MPa, 3 min, 40–100 °C	Enzyme activation by 23% at 40 °C, while reduction by 92% at 100 °C
		PPO	600 MPa, 3 min, 100 °C	Reduction in activity by 90%
		PME	600 MPa, 3 min, 100 °C	Reduction in activity by 83%
Marszałek et al. (2015)	Strawberry puree	POD	500 MPa, 15 min, 50 °C	Reduction of about half of enzyme activity
		PPO	500 MPa, 15 min, 50 °C	Reduction of 72% of enzyme activity
Castro et al. (2008)	Green bell pepper	POD	100–200 MPa, 10 and 20 min, 18–20 °C	70% of reduction in enzyme activity
	Red bell pepper	POD	100–200 MPa, 10 and 20 min, 18–20 °C	40% of reduction in enzyme activity
	Green bell pepper	PPO	100–200 MPa, 10 and 20 min, 18–20 °C	50% reduction in enzyme activity
	Red bell pepper	PPO	100–200 MPa, 10 and 20 min, 18–20 °C	No effect

the enzyme pectate lyase at pressures more than 150 MPa (Calligaris et al., 2012). Therefore, it is not recommended to use HPH independently and preferably; it needs to be coupled with other processing methods for microbial or enzyme inactivation (Augusto et al., 2018; de Castro Leite Júnior et al., 2017; Calligaris et al., 2012; Welti-Chanes et al., 2009).

3.6.3 Ultrasound Processing (Ultrasonication)

In ultrasound processing technique, ultrasonic waves are employed with frequencies above the hearing range of humans. The ultrasonic waves are of two types: high frequency ultrasound (2–20 MHz) corresponding with low sound intensity (0.1–1 W/cm^2) and power ultrasound (20–100 kHz) with high sound intensity (10–1000 W/cm^2) (Feng et al., 2008). These techniques are used in processing industry for improving the shelf life of fruits and vegetables by inactivating the enzymes like PME, PPO, and POD present in the food (Bourke et al., 2010). But,

when the technique, high power ultrasound with low frequency, is combined with temperature/heat, the process is known as thermosonification or pressure (manosonification) (Bourke et al., 2010). The combination of heat and ultrasound helps to ensure product stability and inactivation of enzymes and microbes, thereby resulting in retaining the quality and extending the shelf life of fruits and vegetables, especially juices (Rojas et al., 2017; Saeeduddin et al., 2015). The inactivation of microorganisms or enzymes in this technique may be due to physical factors such as caviation or mechanical effects (Bourke et al., 2010; O'Donnell et al., 2010).

Ultrasonic processing in fruits and vegetables focuses on the inactivation of endogenous enzymes which are more resistant to heat treatments (Feng et al., 2008). Studies have It has been observed that ultrasonic processing either in combination with heat or pressure has minimal effect on quality of fruit juices such as orange juice, guava, and strawberry juice (Bourke et al., 2010). Further, it has been observed that high ultrasound and longer processing times may be required for enzyme inactivation as the pulp complicates the inactivation process (Rojas et al., 2017). Hence, ultrasound technique is usually combined with a thermal process to increase the rate of inactivation of enzymes in juices (Chen et al., 2019; Rojas et al., 2017; Anaya-Esparza et al., 2017; Abid et al., 2014). PME was also found to be inactivated in lemon juice (Knorr et al., 2004) and mousambi (Siwach & Kumar, 2012) by using ultrasonic processing along with heat. Likewise, peroxidase enzyme present in watercress was also found to be inactivated by the use of thermosonication (Cruz et al., 2006).

3.6.4 Pulsed Electric Field (PEF)

In PEF processing technique, electrochemical effects and ohmic heating are responsible for changing the structure and function of enzymes present in the food. At times, large specific energy inputs are required to inactivate enzymes (Poojary et al., 2017). Enzyme activity can be affected by a number of factors such as properties of the enzyme, treatment parameters, processing conditions as well as the condition of the medium (Poojary et al., 2017).

The PEF unit consists of a treatment chamber, pulse generator, a pulse monitoring system, and a temperature monitor. It can be conducted in two ways—continuous or batch processing. The unit also contains a fluid handling system for liquid foods in continuous PEF processing (Min et al., 2007). The design of the treatment chamber plays a key role in distribution of uniform temperature inside the PEF chamber (Oey, 2010). In PEF, the duration of processing takes substantially lesser time (micro to milli seconds) as compared to other methods (Oey, 2010).

However, in PEF processing, the activity on enzyme may either limit its activation/inactivation or may not be affected at all (Poojary et al., 2017). In this process, multiple factors such as food matrix, pH, enzyme dissolving medium, and certain treatment conditions affect the enzyme treatment in fruits and vegetables (Poojary et al., 2017; Oey, 2010). Two important parameters that affect the intensity of PEF processing are electric field intensity and the total duration of treatment per energy

(Oey, 2010). A higher electric field and treatment duration may result in enzyme inactivation, while a lower electric field and duration may result in enzyme activation (Poojary et al., 2017). Also, increasing pulse width lowers enzyme activity (Poojary et al., 2017).

Furthermore, several studies have been conducted to assess the effect of PEF processing on various food enzymes such as polyphenol oxidase, lipoxygenase, peroxidase, and pectin methyl esterase (Poojary et al., 2017; Aguiló-Aguayo et al., 2009; Noci et al., 2008; Espachs-Barroso et al., 2006) and are given in Table 3.6. The findings from these studies indicate that a longer treatment duration, higher electric field intensity, and pulse width result in enzyme inactivation.

3.6.5 Thermal Processing

In spite of advancement in alternative non-thermal treatments in food processing industry, thermal treatments are still widely considered the most cost-effective and simple method to ensure enzymatic and microbial inactivity in foods (Rawson et al., 2011). However, these treatments have many drawbacks as well, such as slow in conduction and convection of heat transfer, loses in nutritional and functional properties, reduction in sensory attributes, etc. (Baysal & Icier, 2010; Gonzalez & Barrett, 2010). In addition, food matrix, complexity of products, and the microorganism also affect the efficacy of thermal processing (Chen et al., 2013). As a result, optimization of thermal treatment becomes important to preserve the sensory attributes, nutritional quality, and safety of the product.

The common thermal treatments (conventional and non-conventional) employed during processing of fruits and vegetables are High temperature-long time (HTLT), High temperature-short time (HTST), and Ohmic and Microwave heating (Lee et al., 2015). Brief explanations of these treatments are discussed below.

High temperature-long time (HTLT) is a conventional thermal treatment. In this, the processing of juice and beverages is done at temperature 80 °C with >30 s holding time (Miller & Silva, 2012). It inactivates or reduces the enzymatic activities of polyphenoloxidase (PPO), pectin esterase (PE), peroxidase (POD), etc. (Marszałek et al., 2017). During the treatment, it was observed that antioxidant activity and PME enzymatic activity were reduced to 75% and 83% in mombin juice and litchi beverage, respectively (Swami Hulle & Rao, 2016; de Carvalho et al., 2015).

High temperature-short time (HTST) is another conventional thermal treatment used in food processing. It is carried out at temperature ≥80 °C with ≤30 s holding time. Here, priority is given for destruction of microorganism than nutrient degradation (Achir et al., 2016). For example, HTST treatment reduces PME and PPO activity by 95.3% and 90.9% in apple juice, respectively (Aguilar-Rosas et al., 2013). In another study based on apricot nectar, complete inactivation of POD, PPO, and PME was observed (Huang et al., 2013).

Ohmic heating (OH) is a common non-conventional thermal treatment. This treatment is primarily used in fruit juices processing for its effectiveness. Fruit juices

Table 3.6 PEF and effect on enzyme activity

Reference	Fruits/ vegetables	Enzyme	Processing	Enzyme stability after application of HP processing
Aguiló-Aguayo et al. (2009)	Tomato juice	Lipoxygenase	Electric field: 35 kV/cm for 1000, pulse width: 1–7 μs, frequency: 50–250 Hz	An increase in frequency or pulse width reduced enzyme activity
	Strawberry juice	Polygalacturonase	Electric field: 35 kV/cm for 1000 μs, treatment time: 1700 μs, frequency: 100 Hz, pulse width: 4 μs	Enzyme activity inactivated by 26%
	Strawberry juice	PME	Electric field: 35 kV/cm for 1000 μs, treatment time: 1700 μs, frequency: 100 Hz	Enzyme inactivation
Noci et al. (2008)	Apple juice	PPO	Electric field: 40 kV/cm, pulse width: 1 μs, treatment time: 6000 μs, frequency: 15 Hz, no. of pulses: 100	About 50% reduction in enzyme activity
Espachs-Barroso et al. (2006)	Carrot	PME	Pulses of 13.2–19.1 kV/cm for 40 μs, time: 1.6 ms, frequency: 0.5–5 Hz	PME inactivation—45% banana, 83% carrot, 87% orange and tomato. Enzyme inactivity increased with increased treatment time and electric field and pulses
	Banana			
	Tomato			
	Orange			

that contain water and ionic salts in higher amount are found to be more effective with this treatment (Miller & Silva, 2012). In other foods, this process has many advantages like uniform and rapid heating which ultimately helps in nutritional and sensory attributes of the processed products. As per the study by Somavat et al. (2013), *B. coagulans* (ATCC 8038) in tomato juice when treated with OH at 60 Hz and 10 kHz expedites the inactivation process as compared to conventional treatment (Somavat et al., 2013). However, microwave heating is another common

non-conventional treatment which is mainly used in urban areas. Even though it is a thermal treatment, minimal thermal exposure is required for enzymatic inactivation (Arjmandi et al., 2016).

3.7 Regulatory Aspects of Food Enzymes Used in Fruit and Vegetable Processing

The regulation of enzymes for its application in food industry is very complex and differs from county to country. The European Union (EU) classifies food enzymes as processing aids of food additives. Majority of the enzyme preparations used in food processing are categorized as processing aids by European Union since they have a role in the technological aspect of food processing stage and not in the final product (Aehle, 2007). The processing aid may not be labelled (Bruchmann & Fauveau, 2009). The enzyme lysozyme used in wine processing is considered as a food additive (Bruchmann & Fauveau, 2009) since it has a role to play in the final product and is regulated under Food Additives Directive (95/2). A vertical legislation of EU on fruit juices (Council Directive 93/77/EEC) allows pectinolytic, proteolytic, and amylolytic enzymes, while another regulation (Council Regulations 82/87/EEC) allows only pectinolytic enzymes on wines (Aehle, 2007). The Organization Internationale de la Vigne et du Vin (OIV) regulates the enzymes used in wine processing in EU (Gómez-Plaza et al., 2010; Bruchmann & Fauveau, 2009; Aehle, 2007) and has recognized the important role of pectinases, hemicellulases, cellulose, β-glucanase, and glycosidase. The European Commission Regulation 1493/1999 authorises the use of pectinases from *Aspergillus niger*, β-glucanase from *Trichoderma harzanium* and urease from *Lactobacillus fermentum*. Pectinases are assigned a GRAS status by United States Food and Drug Administration (Bruchmann & Fauveau, 2009). In India, Food Safety and Standards Authority of India (FSSAI) regulates the use and safety of food enzymes. As per the regulation Food Safety and Standards (Food Products Standards and Food Additives) (Amendment) Regulations, 2015, the food regulator had permitted the use of processing aids in bread. The enzymes permitted for usage are glucose oxidase, lipase, and xylanase. They are obtained from various microbial sources.

In some EU countries, namely Denmark, France, and United Kingdom, approval is required for the use of any food enzymes (Aehle, 2007). However, in countries like Poland, China, Japan, Australia, New Zealand, Canada, Brazil, and Mexico, approval is needed for the use of enzymes produced traditionally, especially if they are new enzymes (Aehle, 2007). On the other hand, countries such as Thailand, Korea, and Taiwan require registration prior to their enzyme use, while USA requires a GRAS (generally regarded as safe) assessment, GRAS notice, or Food additive (Aehle, 2007). Even though countries have different set regulations, enzyme preparations must comply with specifications recommended by Joint FAO/WHO Expert Committee on Food Additives (JECFA) and by Food Chemical Codex (FCC) for food enzymes (Bruchmann & Fauveau, 2009; Grassin & Coutel, 2009).

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4 Production of α, β, and γ-Cyclodextrin Gluconotransferase (CGTase) and Their Applications in Food Industry

Rizul Gautam and Shailendra Kumar Arya

Abstract

Cyclodextrins (CD) are cyclic oligosaccharides which can be produced by the enzymatic degradation of starch and have the ability to form inclusion complex (IC) with variety of molecules. Formation of inclusion complexes with cyclodextrins (CDs) is known to enhance guest solubility in aqueous medium. CD has α-(1–4) linkages forming a cone shaped structure with a hydrophilic outer surface which makes them water-soluble. The rate of hydrolysis increases in the order of α-CD < β-CD < γ-CD. Gamma cyclodextrins have been proved to retain the maximum retention properties than the three cyclodextrins. Cyclodextrins are also used to retain colours of food substances. Three common types of CDs are α-CD, β-CD, and γ-CD with 6, 7, and 8 sugar-membered ring molecules. This book chapter will discuss the preparation and properties of three types of CDs with their applications in food industry.

Keywords

α-Cyclodextrin · β-Cyclodextrin · γ-Cyclodextrin · Food industry · Applications

4.1 Introduction

Cyclodextrins (CDs) are a family of cyclic oligosaccharides that show the ability to form inclusion complex (IC) through variety of molecules (like Allyl isothiocyanate (AITC)) (Del Valle, 2004). These molecules are produced through the action of

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A. Dutt Tripathi et al. (eds.), *Novel Food Grade Enzymes*,
https://doi.org/10.1007/978-981-19-1288-7_4

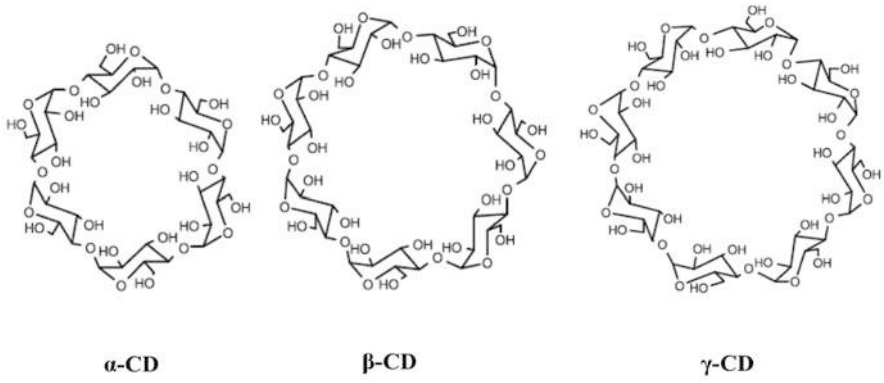

Fig. 4.1 Basic structure of alpha, beta, and gamma cyclodextrins (Radi & Eissa, 2010; Das et al., 2013; Astray et al., 2009)

cyclodextrin glycosyltransferase (CGTase) enzyme on starch or starch derivatives. This enzymatic activity sometimes results in changing the properties of many molecules like their stability, reactivity, bioavailability, and solubility (Larsen et al., 1998). On the basis of number of D-glucose units attached to CDs, they are classified as six-α-cyclodextrin, seven-β-cyclodextrin, and eight-γ-cyclodextrin (Blackwood & Bucke, 2000; Singh et al., 2002). Cyclodextrins cannot be formed if less than six glucopyranose units used because of steric hindrances (Qi & Romberger, 1988). Cyclodextrin possesses a hydrophobic central and hydrophilic outside cavity. The α-(1–4) linkages of CD help in the formation of a cone-shaped structure that results in its hydrophilicity. The central cavity of the cone is hydrophobic in nature because of the presence of carbon and oxygen atoms of the glucose residues. This arrangement of a hydrophilic exterior with a hydrophobic interior design of CDs helps cyclodextrins to make inclusion complexes or compounds with hydrophobic guest molecules. α-, β- and γ-CD are 6, 7, and 8 sugar-membered ring molecules, respectively, and are the most common CD types (Del Valle, 2004), as shown in Fig. 4.1 (Del Valle, 2004; Radi & Eissa, 2010; Das et al., 2013). Cyclodextrins have number of applications in food industry due to their ability of encapsulations of molecules and formation of inclusion complex with hydrophobic compounds (Gawande et al., 1998). Cyclodextrins are preferred due to the following reasons: they are made from renewable raw material such as starch or starch derivatives. They are relatively cheap. They are toxic-free and beneficial for human body. Applications of cyclodextrins in food industry are expressed in many different ways, like for encapsulation of flavours, used as food preservative, used as an eliminator of bitter tastes from food, used against oxidative degradations, heat- and light-induced changes, and for modification of taste and odours of foods (Astray et al., 2009).

Among all the three cyclodextrins, gamma cyclodextrin proves to be more suitable to be used in the field of pharmaceutical and food industries because of its properties like water solubility, size of internal cavity, etc. (Li et al., 2007). However,

the most marketed utilized CD is β-CD and to lesser extent α-CD. The production of gamma CD is less due to high cost and low production associated with it. The CGTases producing cyclodextrin of single type are of interest as it is difficult to isolate the CD of interest from a complex mixture of CDs. Cyclodextrins are hydrophobic molecules and their solubility increases with ethanol concentration. The spectroscopic studies imply that the confirmation of cyclodextrins in solution is similar to that in crystalline state; however, β-CD has a perfect regularity, whereas alpha and gamma are slightly distorted (Astray et al., 2009).

Cyclodextrins are useful because of following reasons: They are made from renewable material (starch). They are relatively cheap. Due to their biodegradability, they do not produce any harmful effect to the environment. They are nontoxic for human body (Duca & Boldescu, 2008). The stability of the cyclodextrins follows $\alpha > \beta > \gamma$. The cyclodextrins are as stable as sucrose in solid state and are resistant to degradation and can be stored for several years (Kurkovsergey & Loftssonthorsteinn, 2013). These are homogenous, crystalline, and non-hygroscopic substances (Stella & Rajewski, 1997). The alpha cyclodextrin has six glucopyranose rings, beta has seven, and gamma has eight glucopyranose units (Astray et al., 2009). The rate of hydrolysis accelerates in the order of $\alpha\text{-CD} < \beta\text{-CD} < \gamma\text{-CD}$. Gamma cyclodextrins have been proved to retain the maximum retention properties than the three cyclodextrins. Cyclodextrins are also used to retain colours of food substances (Duca & Boldescu, 2008; Lopez-Nicolas et al., 2007a, b). The hydrophobic interior of the cavity possesses some lewis base characteristics due to the presence of high electron density.

4.2 History

First time cyclodextrin was discovered by Villiers in 1891 from starch using a strain of *Bacillus amylobacter* (Das et al., 2013; Szejtli, 1990). The following table represents the discoveries of cyclodextrins according to time passage and work done on cyclodextrins in different fields (Del Valle, 2004; Dardeer, 2014).

Year of working	Work done on cyclodextrin	References
1891	First time CDs produced from starch by digesting it with *Bacillus amylobacter* strain by scientist Villiers. Villiers also produces cyclodextrin from impure culture by using strain *Bacillus macerans*.	Singh et al. (2002)
1903	Schardinger produces two crystalline products and named it as dextrin A and dextrin B, but the strain from which they are produced are not maintained.	Eastburn and Tao (1994)
1904	Schardinger discovered a new organism that has capability of producing ethyl	Eastburn and Tao (1994)

(continued)

Year of working	Work done on cyclodextrin	References
	alcohol and acetone from sugar and starch-containing plant materials.	
1911	Schardinger produced large amount of cyclodextrin from strain *Bacillus macerans* about 25–30% and named it α-CD and β-CD.	Eastburn and Tao (1994)
1935	Schardinger discovered γ-CD. After that, Freudenberg and Cramer explained the existence of large cyclodextrin molecules.	Loftsson and Brewester (1996), Matsuda and Arima (1999), Mabuchi and Ngoa (2001)
1942	Schardinger demonstrated the structures of β-CD and α-CD using X-ray crystallography.	Buschmann and Schollmeyer (2002)
1948	γ-CD structure is determined by using X-ray crystallography. At that time, it is observed that cyclodextrins can form inclusion complexes.	Buschmann and Schollmeyer (2002)
1961	Some evidences show the existence of natural cyclodextrins like ξ, δ, ζ, and even η-cyclodextrin.	Qi and Romberger (1988), Hirose and Yamamoto (2001)
1965	A method is developed in this year for isolation of larger homologues structures of ζ-cyclodextrin and η-cyclodextrin, method named fractionation method.	Kainuma (1984)
1970	In 1970, several numbers of microorganisms are considered as a source for cyclodextrin glucanotransferases and glucanotransferase was purified and named during the exploration period. Applications of cyclodextrins in different industries started in this period.	Kainuma (1984)
1980	Development in biotechnology in the mid of 1980s, improved the purification and production of cyclodextrins and led to increases in their value for commercial uses.	Crini (2014)
1982	In 1982, first book on cyclodextrins was published, written by Szejtli.	Crini (2014)
1992	In this period, Hedges R.A. published a book on cyclodextrin under a title Cyclodextrins and Their Applications in Biotechnology.	Hedges (1992)
2003	In this period, Tomasik P. published a book on cyclodextrins, under a title Cyclodextrins Chemical and Functional Properties of Food Saccharides.	Szejtli (2004a)
2004	In this year, review articles were published on data related to cyclodextrin and its	Szejtli (2004b)

(continued)

Year of working	Work done on cyclodextrin	References
	future aspects and also on application of cyclodextrins.	
2006	Helena Dodziuk published a book on Cyclodextrins and Their complexes: Chemistry, Analytical Methods and Applications.	Kainuma (1984)
2007	Investigation of cyclodextrin glucanotransferase production using alginate-immobilized cells of alkalophilic *Bacillus* sp. in an airlift reactor.	Kunamneni et al. (2007)
2010	Biochemical characterization of α-cyclodextrin glycosyltransferase and its extracellular expression from *Paenibacillus macerans* have been published by Zhaofeng Li et al.	Zhaofeng et al. (2010)
2011	Encapsulation of ethylene gas into α-cyclodextrin and characterization of the inclusion complexes.	Ho et al. (2011)
2012	Effects of cyclodextrins on the antimicrobial activity of plant-derived essential oil compounds.	Liang et al. (2012)
2013	Optimization of reaction of γ-cyclodextrin glycosyltransferase and isoamylase for enhanced production of c-cyclodextrin.	Wang et al. (2013)
2014	Cyclodextrins in capillary electrophoresis: Recent developments and new trends.	Escuder-Gilabert et al. (2014)
2015	Experimental setup and kinetic study of ultrasonic-assisted transesterification of waste cooking oil over sulfonated carbon catalyst derived from cyclodextrin.	Maneechakr et al. (2015)
2016	Use of cyclodextrins to recover catechin and epicatechin from red grape pomace.	Lopez-Miranda et al. (2016)

4.3 Properties of Cyclodextrins

CD have hydrophobic cavity and the ability to form reversible host–guest complexes or termed as supramolecular complex via weak forces like, dipole–dipole interactions, Van der Waals interactions, and hydrogen bonding with molecules (Escuder-Gilabert et al., 2014). CDs have the ability to solubilize hydrophobic materials and thus can entrap volatile components by forming inclusion complexes with organic compounds, which results in enhancing their physical and chemical properties. They have the property of selective recognition of a chiral compound and enantio recognition of enantiomers of chiral compounds (Del Valle, 2004; Steinbrunn & Wenz, 1996). Low toxicity and immunogenicity of CDs allow for

their use as a transport system into the living systems. Also, CDs have the capacity to form pseudorotaxanes and rotaxanes through number of linear species. Rotaxanes are the compounds that consist linear and cyclic species and are linked together by non-covalent interaction (Isnin & Kaifer, 1993; Buston et al., 2001). As inclusion bodies, CDs help in increasing the stability, solubility, and bioavailability of lipophilic organic molecules, improve the stability, increase the permeability of water-insoluble compounds, and diminish the drug toxicity by manufacturing the compound effective at lesser doses (Del Valle, 2004; Kamimura et al., 2014).

4.3.1 CGTase Synthesis

Cyclodextrin glucanotransferases (EC 2.4.1.19, CGTases) using starch and its derivatives are used for the production of cyclic α-(1,4)-linked oligosaccharides commonly known as cyclodextrins (CDs) via the cyclization reaction (Tonkova, 1998). CGTase is isolated from *Bacillus* sp. and degrade the starch from raw substrate (like potato) to form cyclodextrin. It was reported that immobilization increases the activity of CGTase enzyme, as when it was immobilized in mixed gel beads, its activity increases. This fermentation process is carried out in a continuous stirred tank reactor (CSTR) and a packed bed reactor (PBR) for commercial production. Enzyme preparation is done according to production process required. Generally, CGTase is isolated from *Bacillus* sp. C26 and inoculated into medium and then incubated at 37 °C at 200 rpm for 48–50 h. Centrifugation is done for 15 min to remove the cells and insoluble material at 4000 × *g* and at 4 °C. Precipitation of enzyme is done by adding the cell-free supernatant with ammonium sulfate and keeping them for overnight in controlled conditions at 4 °C temperature and pH is maintained at 7. Product purification is done by dialysing the solution obtained after overnight incubation with molecular cut-off 8000 Da. Enzyme activity is monitored by using phenolphthalein colourimetric method, and to measure the enzyme protein concentration, folin ciocaltue method is used. It was reported that CGTases are an extracellular enzyme produced by number of microorganisms, for example, bacteria (mostly from *Bacillus* species) (Leemhuis et al., 2010).

B. circulans DF 9R was isolated from rotten potatoes (Ferrarotti et al., 1996). Culture was then transferred to medium containing cassava starch, $(NH_4)_2SO_4$, phosphate buffer, $MgSO_4$, and $FeSO_4$. Culture was grown aerobically until the absorbance at 620 nm becomes 0.3 and is then used as inoculum. The medium is then transferred to 1000 mL Erlenmeyer flask containing medium. Then the mixture is centrifuged and the supernatant obtained is used as a crude enzyme (Rosso et al., 2002; Kunamneni et al., 2007). The microorganism producing β-CGTase is alkalophilic *Bacillus* species (*Bacillus* sp. AK11) that has the capacity to grow in high alkaline condition when cultivated in air lift reactor. Alkalophilic bacilli are more trending and more commonly in use due to broad range specificities of pH and temperature (Kunamneni et al., 2007; Park et al., 1999; Vassileva et al., 2005). Microorganism is then transferred to growth medium containing starch, peptone, KH_2PO_4, and $MgSO_4$ and medium is adjusted to pH 8 using sodium carbonate and

maintained at a temperature of 37 °C. Cells were immobilized using sodium alginate method (Kunamneni et al., 2007; Higuti et al., 2004). The alginate beads were loaded into the reactor containing the production medium and allowed to stay for 24 h to activate cells. The broth obtained was drained and further replaced with production medium which contained different molar concentrations of the added ingredients. The production medium is then constantly added so that there is continuous mixing of the solution and overflow is regulated by peristaltic pump and finally its activity can then be measured spectrometrically. It was observed that with the increase in the air flow rate, the production of CGTase increased. The concentration of carbon and nitrogen needs to be maintained for effective CGTase production using immobilized beads (Kunamneni et al., 2007).

However, it was observed that the average volume productivity increased during immobilization as compared to free cells (Kunamneni et al., 2007). The concentration of α-CD production is measured by observing the decrease in absorbance at 507 nm. The decrease in absorbance is due to formation of methyl orange α-CD complex (Kunamneni et al., 2007; Higuti et al., 2004).

One unit of CGTase is defined as the amount of enzyme catalysing the production of 1 μmol of β-CD per minute under the reaction conditions. The cyclising activity of CGTase was tested by phenolphthalein method. In this test, the production of β-cyclodextrin is measured on the basis of its ability to form inclusion complex with this dye which is then measured by taking absorbance at 550 nm (Kunamneni et al., 2007; Goel & Nene, 1995). Phenolphthalein is diluted in ethanol and the pH is adjusted to 11 using Na_2CO_3. The activity assay consisted of substrate solution containing 1% (w/v) maltodextrin in tris-HCl buffer at 50 °C, taking water as a blank.

The concentration of γ-CD was measured at 630 nm due to formation of inclusion complex with bromcrescol green (Goel & Nene, 1995; Natalia et al., 2007) taking water as a blank. In this case, the reaction mixture consists of 1% (w/v) soluble starch in gly-NaOH buffer, pH 10, and enzyme solution diluted by buffer incubated at 40 °C for 10 min. Then 0.1 N HCl was used to stop the reaction. Finally, the citric acid–citrate sodium buffer (pH 4.2) were added to the reaction mixture, and then the absorbance is taken at 630 nm.

Methods for CD-complex formation: The forces that are responsible for interaction of these inclusion complexes include Van der Waals forces, hydrophobic interactions and hydrogen bonds (as shown in Fig. 4.2). These inclusion complexes change the chemical and physical properties of the molecule. The energy of Van der Waals is proportional to molecular polarizability and molecular refraction. The stability of the complex increases with the increase in the electron donor ability of the substituents (Astray et al., 2009).

Cyclodextrins can also form complexes with the gases such as CH_4, C_2H_6, C_3H_8, Cl_2, Xe, etc. Alpha cyclodextrin due to its smaller diameter (470–530 pm) can form stable complexes with guest molecules having five carbon atoms or less. However, ethylene gas is more suitable for formation of complexes with alpha CDs. The encapsulation of ethylene into alpha cyclodextrin was done by dissolving alpha cyclodextrin powder in water at 25 °C. After the powder is completely dissolved,

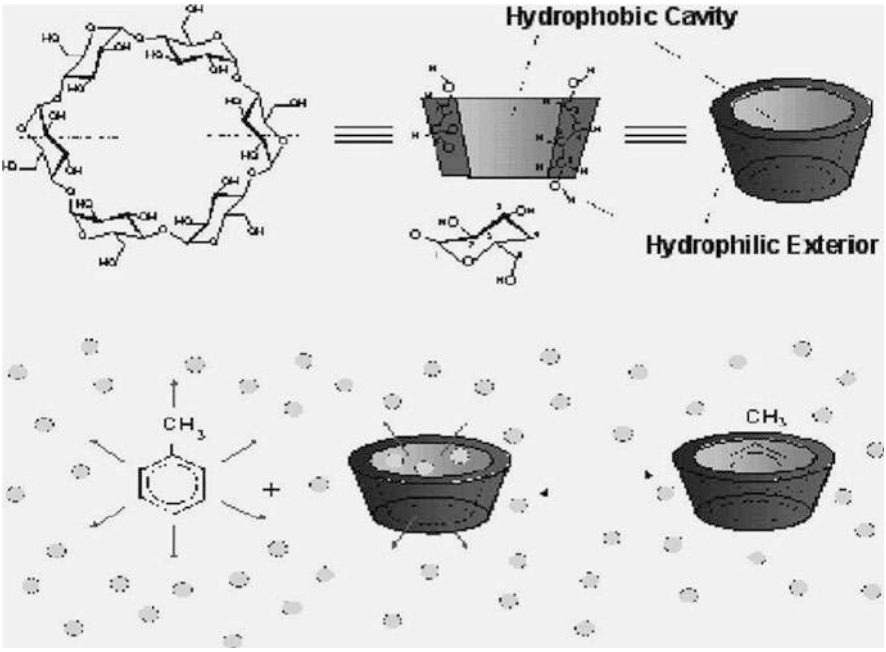

Fig. 4.2 Cyclodextrins structure and inclusion complex formation (Schmid, 1989; Gomes et al., 2014; Garnero et al., 2010)

solution is then stirred for complete 5 min. Saturated alpha cyclodextrin obtained is added to plastic container and ethylene gas was then pressurized at 0.2, 0.5, 1, and 1.5 Pa at 25 °C. Afterwards, crystal precipitates formed are collected by vacuum filtration and then analysed for ethylene gas concentrations and further characterized for their physiochemical properties (Ho et al., 2011).

CDs have hydrophobic cavity. CDs have the ability to form host–guest complexes via weak forces, like Van der waal interactions, dipole–dipole interactions, and hydrogen bonding with molecules (Szejtli, 1988). CDs can solubilize hydrophobic materials and thus entrap volatile components by forming inclusion complex with organic compounds, resulting in enhancing their chemical and physical properties. CGTase production at large scale by using *Bacillus* sp. depends upon the kinetic model of cyclodextrin-glucanotransferase (Burhan et al., 2005). Advantages of CD to form IC are: higher thermal stability, high solubility, bioavailability of hydrophobic guests, control volatility, masking off unpleasant odours, and controlling release of drugs and flavours.

4.3.2 CGTase Sources

This table represents the various sources of CGTase from different spices, their mode of production, and their optimum parameters like temperature and pH.

Sources of CGTase	Production	Optimum temperature and pH	References
Bacillus macerans IFO 3490	*Bacillus macerans* IFO 3490 mainly produces α-CGTase. Fermentation medium is soluble starch, corn steep liquor.	Optimum temperature is 55 °C and pH 5.2–5.7.	Fujiwara et al. (1974), Kitahata et al. (1974), Rimphanitchayakit et al. (2005)
Bacillus circulance E 192	*Bacillus circulance* E 192 produces mainly β-CGTase. Agar-immobilized cells are used for high productivity of CGTase. *B. circulance* requires alkaline pH for an efficient CGTase formation.	Optimum temperature 60 °C and pH 5.5–5.8.	Charoenlap et al. (2004), Vassileva et al. (2005)
Paenibacillus	Main product is β-CD.	Optimum temperature is 50 °C and pH is 6.0–8.0.	Larsen et al. (1998)
Klebsiella pneumonia	The CGTase from *Klebsiella pneumonia*. Main product is α-CD.	It can be preferred at pH 6–8, temperatures of 40–50 °C.	Gawande and Patkar (2001)
Bacillus stearothermophillus	CGTase from *Bacillus stearothermophillus* ET1 is a potential antistaling enzyme with cyclodextrin (CD)-producing activity. Main product is α- and β-CD.	Its optimum activity at 80 °C temperature.	Rahman et al. (2004)
Bacillus lehensis	*Bacillus lehensis* is isolated from cassava starch wastewater. Main product is β-CD. Its CGTase activity is 18.9 μm/L	Optimum temperature is 55 °C and pH 8.0.	Blanco et al. (2014)
Micrococcus	*Micrococcus* variant M 849 produces α and β-CD as main products.	Its optimum activity temperature is 55–65 °C and pH is 5.0–9.0.	Kim et al. (1997)
Thermoanaerobacter sp.	*Thermoanaerobacter* sp. ATCC 53627 mainly used for the	Its optimum activity temperature is 90–95 °C.	Starnes et al. (1992)

(continued)

Sources of CGTase	Production	Optimum temperature and pH	References
	production of β-CGTase.		
Alkalophilic *Bacillus* sp.	Alkalophilic *Bacillus* sp. 38-2 mainly produces β-CD.	Optimum temperature is 45 °C.	Sian et al. (2005)
Bacillus amyloliquefaciens	CGTase is synthesized by *B. amyloliquefaciens* by immobilized method in batch and continuous cultures.	Growth medium is maintained at pH 6.5 and microorganism is maintained at 4 °C on potato dextrose agar slants.	Andel-Naby et al. (2000)

4.3.3 Methods for Production of Inclusion Complexes

In this table, methods for CDs complexes or inclusion complexes formation have been explained and the manufacturing of CDs.

Methods for CD-complex formations	Process	References
Precipitation method	Precipitation method is most widely used for inclusion complex production. According to Sapkal et al., by using co-precipitation method, a poor aqueous solubility guest molecule is combined with β-cyclodextrin to form inclusion complex. In this method, drug and CD are dissolved in water and solvent (like acetone) is heated at 75 °C. Stirring is done for 1 h at 75 °C to obtain concentrated, viscous, and translucent liquid and then cooled at room temperature. Precipitates are filtered or separated than dried and stored to get solid inclusion complex.	Das et al. (2013), Sapkal et al. (2007)
Kneading method	Conventional kneaders like low and high shear mixers are used to prepare CD paste, paste is prepared with small amount of water in which guest molecule is added (without a solvent) in a small amount of ethanol, and then after grinding, solvent is evaporated and powdered form cyclodextrin-inclusion formed.	Das et al. (2013), Miller et al. (2007)
Spray drying	In this method, first cyclodextrin is dissolved in solution that is already alkalinized with 25% aqueous ammonia, then guest molecule is dissolved in 96% ethanol alcohol solution in 100 mL, both solutions are then mixed, and finally sonicated. The l solution obtained is then spray-dried to get inclusion complexes; this is done at	Das et al. (2013), Arias et al. (2000)

(continued)

Methods for CD-complex formations	Process	References
	equilibrium state which is attained by removing the solvent by spray drying.	
Neutralization method	The guest molecule is dissolved in basic or acidic aqueous cyclodextrin solution. Cyclodextrin and drug are dissolved separately in 0.1 N NaOH solution, mixed and stirred for half an hour; pH is maintained by 0.1 N HCl at 7.5; at this pH, complexes precipitate and then residues are separated and washed until free from chlorine and then dried at 250 °C for 24 h	Das et al. (2013), Choi et al. (2001)

4.3.4 Production Methods

(a) Batch process: Generally CDs are produced through batch process which is quite simple and easy to control process. But there are several limitations of this method, like long operation hours, high labour cost, and high enzyme concentration requirement that leads to switching to other production method (Sakinah et al., 2014; Lia et al., 2014).

(b) Enzymatic membrane reactor (EMR): EMR is basically a stirred tank reactor combined with a separation membrane that recirculates the reaction mixture through a membrane module placed outside the enzymatic reactor. As compared to batch process, this is a continuous process method and is more practical and economical. Also, reusability of the enzyme results in high yield productions of cyclodextrins as compared to batch process (Lia et al., 2014). In this process, product (CD) is separated from the enzyme and substrate through a semi-permeable membrane, which is composed of an ultra-thin separation layer widely used in enzyme separation. During the separation process, CDs penetrate through the membrane and the enzymes get retained by the membrane within the reaction reactor (Bodalo et al., 2001).

The working principle is such that the pressure difference across the membrane, also called the transmembrane pressure (TMP), impels the product through the membrane, while the unreacted substrate and free enzyme are recirculated to the enzymatic reactor (Sakinah et al., 2009). Advantages of enzymatic membrane reactor over batch reactor are its high production flow rates; reductions in cost, energy, and waste products by the recycling practice; easy reactor operation and control; and the high yields of pure material (Katzir, 1993). But the main limitation of this method is fouling of membrane as the substrate gets deposited onto the membrane surface and enzyme precipited within the membrane pores (Hughes & Field, 2006; Balakrishnan & Dua, 2000; Yuan & Zydney, 1999; Bansal et al., 2006). However, this problem can be overcome by employing a high cross-flow velocity (CFV), but this condition requires large amount of

energy and sometimes reduces the enzyme activity (Sakinah et al., 2014; Gugliemi et al., 2007; Priyananda & Chen, 2006).

4.4 Alpha Cyclodextrin Production

α-Cyclodextrin containing six 1,4-linked D-glucose units makes it cyclic polysaccharide, which differentiates it from other two, β (seven) and γ (eight)-cyclodextrins. It is commercially produced from starch (used as a substrate) by an enzymatic production process. α-Cyclodextrin has variety of application in food industry due its small internal cavity and high resistance to its enzymatic hydrolysis. α-Cyclodextrin is less important than other CDs because of its low production yield and high cost. α-Cyclodextrin is generally used as a dietary fibre food. α-Cyclodextrins are used in food industry due to following reasons: α-CDs has both nutritional and technological properties (Lia et al., 2014). It acts as a carrier for natural flavour, colours, and vitamins, and as a stabilizer of oil in water emulsions. It is also used as a solubilizer of lipids.

A number of applications for α-cyclodextrins have been published in a variety of papers. Some of the important applications are: α-cyclodextrin have been used in bakery products: like biscuits, rolls, and cakes; snack food: like salty snacks, etc.; dairy desserts: such as yoghurt and dessert mixes; beverages and powders: like coffee whitener, vegetable and fruit juice, soy drinks, and soft drinks (diet); grain-based foods: like pasta, maggie, macaronis, noodles, and instant rice dishes; and so many other food items like chewing gum, sauces, fat, and oil products like mayonnaise and dressing. So these all are the proposed applications of cyclodextrins in food products by some users in particular papers (Lia et al., 2014).

4.4.1 Production

CDs' production can be divided into four stages as follows:

1. Production of CGTase in the reaction medium from the cultivation of the microorganism.
2. Extracting the enzyme through separation, concentration, and purification method.
3. Converting pre-hydrolysed starch into cyclic and acyclic dextrin with the action of enzyme.
4. Separation, purification, and crystallization of CDs (Katzir, 1993; Moriwaki et al., 2014; Astray et al., 2009).

Cyclodextrins are produced from starch or their derivatives because of enzymatic conversion that is catalysed by cyclodextrin glycosyltransferase (CGTase) (Rios et al., 2004). In simple words, cyclodextrins are produced by cyclodextrin glucosyltransferase (CGTase) through cyclization reaction of gelatinized starch

(Rakmai et al., 2015; Iyer et al., 2003). The result is a mixture of α-, β-, and γ-cyclodextrins, containing trace amounts of cyclodextrins (Sakinah et al., 2014).

1. α-Cyclodextrin: It has small internal cavity and high resistance to enzymatic hydrolysis, which make it suitable for application in the food industry. But because of its low production yield and high price, its market share is currently smaller than that of β-cyclodextrin. Thus, the production of α-cyclodextrin can be improved by modifying the properties of CGTases.

4.4.2 Properties of α-Cyclodextrin

α-CD has the lowest hydrogen bond strengths between the 3-OH and 2-OH groups around the wider rim. Diameter of internal cavity is 4.7–5.3 Å and the cavity volume is about 66% or 41% of that of β- or γ-cyclodextrins, respectively (Li et al., 2007). It typically forms inclusion complexes with benzene derivatives because of Van der Waals interactions. α-Cyclodextrin has modest solubility in water which means it is almost eight times greater than that of β-cyclodextrin, but 1.6 times lower than that of γ-cyclodextrin at 25 °C. Also, it is more resistant to hydrolysis in acid solutions. It was observed that even after 3 h of incubation period in extremely acidic conditions (pH = 2.4) at 100 °C, no breakdown of α-cyclodextrin is recognized (Jodai et al., 1984; Saha & Zeikus, 1992). But α-cyclodextrin can't be hydrolysed by human salivary and pancreatic amylases for its commercial production (Kondo et al., 1990). α-CGTases from *Bacillus macerans* or *Paenibacillus macerans* are most commonly used. It is also effective at solubilizing free fatty acids (Qi & Zimmermann, 2005).

4.4.3 Enzymatic Production of α-Cyclodextrin

There are two types of processes that result in the production of cyclodextrins:

Non-solvent process: In this process, cyclodextrin mixture is produced without the addition of any complexing agents and the mixture produced can be further separated by chromatographic procedures.

Solvent process: This process requires an organic complexing agent that helps in selecting one type of cyclodextrin and thus directs the enzymatic reaction to produce the cyclodextrin of interest. This method is commonly used for the production of α-cyclodextrin on industrial scale as shown below in the Fig. 4.3 (Flaschel et al., 1984; Rendleman, 1997).

4.4.4 Applications

Cyclodextrins are used as browning inhibitors in different fruit juices. The oxidation of volatile precursors present in freshly squeezed pear juices can be prevented by

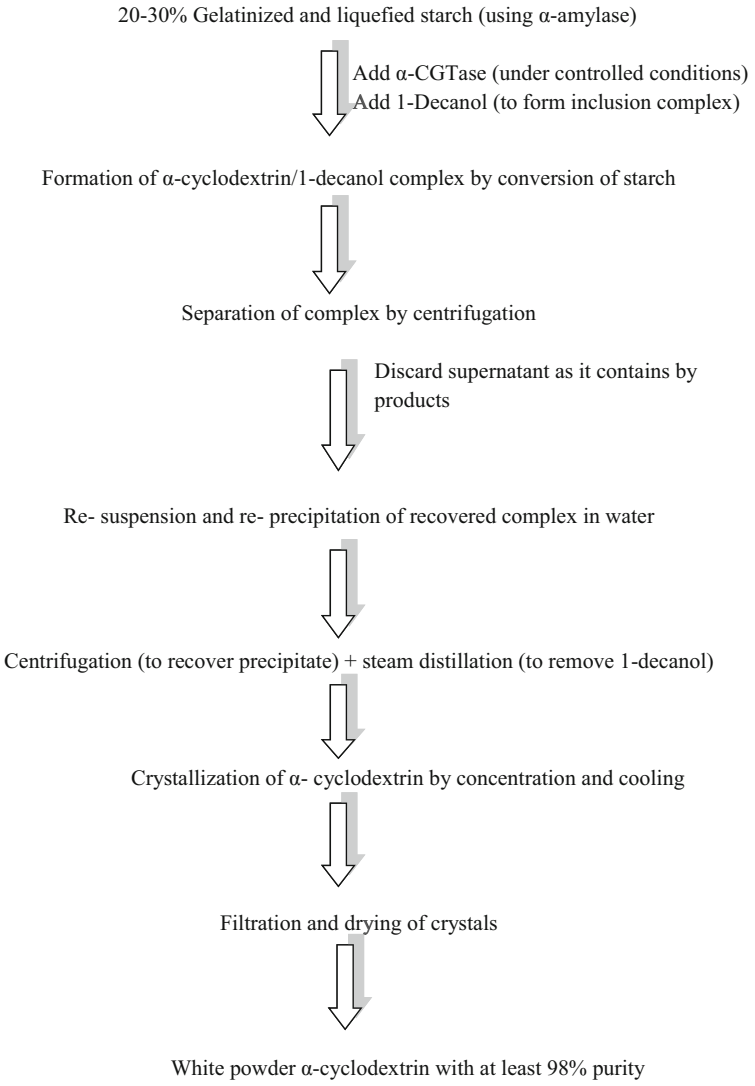

Fig. 4.3 Flow chart is showing the production of α-CD (Lia et al., 2014; Rendleman, 1997)

adding α-cyclodextrin (90 mM). It is one of the most suitable agents for encapsulating flavours that are encapsulated in powder form by spray drying with α-cyclodextrin (Shiga et al., 2004). α-Cyclodextrin is also a dietary fibre as it forms complex with triglyceride at a ratio 1:1 that is typical for dietary fibres and form a stable complex with dietary fat (Ferrari et al., 2013; Threapleton et al., 2013; Zhang et al., 2013a, b). It reduces the absorption and bioavailability of dietary fat, which makes it practical as a weight loss supplement (Suzuki & Sato, 1985). α-Cyclodextrin is effective in reducing and/or maintaining body weight with

increasing energy intake for obese patients with type 2 diabetes (Gallaher et al., 2007; Grunberger et al., 2007). It is effective in improving metabolic syndrome by reducing serum triglyceride and leptin levels and increasing insulin sensitivity and faecal fat excretion in rats. α-Cyclodextrin may also be useful as an ingredient for reducing the glycaemic impact of foods (Penninga et al., 1995).

4.4.5 Alternative Method for the Production of α-Cyclodextrin

Alpha CGTase from *Paenibacillus macerans* is most commonly used for the production of alpha cyclodextrin. However, the production of enzyme can be increased by expressing it in *E. coli* which can bring a benefit in reducing cost of alpha cyclodextrin. Alpha CGTases produced in *E. coli* are accumulated in the cytosol in the form of inactive inclusion bodies. In this case, alpha CGTase from *P. macerans* JFB05-01 is expressed extracellularly in *E. coli*. The recombinant enzyme is then purified and characterized with respect to cyclization activity, including optimum conditions effect of metal ions, etc.

4.4.6 Steps for the Production of Alpha Cyclodextrin

The plasmid pET20b is used for the production of alpha cyclodextrin. At 25 °C, very little recombinant alpha CGTase was secreted into the medium. But after 90 h of incubation period, the production rate increased rapidly and the cyclization activity of alpha CGTase also increased which was 42 times higher than the parent strain. A single colony of *E. coli* BL21cells harbouring plasmid CGT/pET20B was inoculated into LB medium containing ampicillin. For inducing the expression of α-CGTase, different amounts of isopropyl 1-thio-b-D-galactopyranoside (IPTG) were added. The recombinant alpha CGTase contains a histidine sequence on a C-terminal domain which can be separated by metal binding affinity. To overcome the heterogeneity, the recombinant enzyme is purified by chromatographic separation techniques which include Q-sepharose and phenyl-superose chromatography. The purified protein obtained is then separated by SDS page. From the SDS PAGE, it was found that alpha CGTase migrated as a single band with molecular mass of approximately 72 kDa. The optimum temperature of alpha CGTase recombinant enzyme was 45° which was below that of the native enzyme which is 50 °C. The recombinant and native CGTase both showed the best cyclization activity at pH 5.5 at a pH value; above or below this, the cyclization activity decreases. Enzyme is stable in Na_2HPO_4/NaH_2PO_4 at the same pH. The cyclization activity of alpha CGTase is inhibited completely by Hg^{2+} and slightly by Fe^{2+} or Co^{2+}. The addition of 2 mM Ca^{2+}, 2 mM Ba^{2+}, or 0.1 mM Zn^{2+} activated the cyclization activity considerably. It is also observed that the growth medium has a great impact on the secretion of proteins in *E. coli*. However, TB medium was considered the best for extracellular production of alpha CGTase as TB medium has beneficial effect on cell growth because of its relative high buffering capacity. Also, the low IPTG

concentration (0.01 mM) favoured the extracellular enzyme production. The extracellular productivity of the recombinant enzyme was 40 fold higher than the parent enzyme *P. macerans* JFB05-01. The metal chelator like EDTA doesn't show any effect on the activity of α-CGTase enzyme even at relatively high concentration (10 mM), which indicates that a metal cofactor was not required for the function of the enzyme. Furthermore, α-CGTase could be activated in the presence of Ca^{2+}, which was probably because of the binding of Ca^{2+} with its binding sites which are located at the active site of the protein molecule, resulting in stabilizing the substrate binding cleft of CGTase (Eastburn & Tao, 1994). Following table represents number of the sources of alpha cyclodextrin production, optimum temperature, optimum pH, and main products (Stella & Rajewski, 1997; Buschmann & Schollmeyer, 2002; Hirose & Yamamoto, 2001).

Source of α-CD production	Optimum pH	Optimum temperature (°C)	Main product	References
CGTase produced from *Klebsiella pneumoniae* M5	6–8	40–50	In this method, alpha-cyclodextrin is the main product, produced by enzymatic degradation of starch, starch used as a substrate. The yield of alpha-cyclodextrin is lower by the enzymatic method.	Flaschel et al. (1984)
CGTase produced from *Micrococcus* variant M 849	5.0–8.0	55–65	Main product is α-CDs, studied by purification and characterization of cyclodextrin glycosyltransferase from *Micrococcus* variant M 849.	Larsen et al. (1998), Yagi et al. (1980)
CGTase produced from *Klebsiella oxytoca* M5A1	5.0–7.5	–	By using this sp., CGTase produces α-CDs as a main product. Purification and characterization of CGTase are done in this article.	Larsen et al. (1998), Bender (1983)
CGTase produced from *Bacillus macerans* IFO 3490	5.2–5.7	55	In this paper, we study the production of α-CDs by using sp. *Bacillus macerans*.	Larsen et al. (1998), Fujiwara et al. (1974)

The concentration of α-CD production is measured by observing the decrease in absorbance at 507 nm due to formation of methyl orange α-CD complex.

4.5 Beta-Cyclodextrin

Beta-cyclodextrin (β-CD) has the ability to encapsulate molecules that are hydrophobic in nature which helps in improving their aqueous solubility and also reduces their volatility (Lin et al., 2016).

Production of β-CD can be achieved in both batch and continuous process. But continuous process is preferred over batch because of its advantages like greater process control, high productivity, and improvement of quality and yield. Continuous process includes the combination of both CSTR (continuous stirred tank reactor) and PFR (plug flow reactor) and is considered to be more suitable for long-term use. Also in case of industrial scale production, CSTR is preferred over batch process because of its properties like high stability and low by-product formation, but the disadvantage of using CSTR is the undesired thermal gradients in case of PBR, poor radial mixing, and low mass transfer rate (Santos et al., 2015; Rakmai & Cheirsilp, 2016; Galan et al., 2011; Kittikun et al., 2008).

Gram-positive microorganisms *Bacillus firmus* strain and *Bacillus sphaericus* strain 41 were immobilized on a loofa sponge and are used as the direct source for the CGTase and CD production (Moriwak et al., 2014). The CGTase produced by *Bacillus* sp. C26 has been reported to have high activity for β-cyclodextrin production; immobilized CGTase is then applied for the production of β-cyclodextrin (Rakmai et al., 2015) (Fig. 4.4).

4.5.1 Alternative Method

CGTase was produced by bacillus firmus isolated from brazillian soil of oat culture. The medium contained KH_2PO_4, $MgSO_4$, and Na_2CO_3 and the pH was maintained at 10.3. Then the medium is transferred to a 5 L medium and proceeded for cultivation for 5 days at 37 °C. The specific enzyme is isolated using affinity column chromatography and the protein concentration is estimated using Bradford method using bovine serum as standard, and using SDS-PAGE, molecular mass of protein is estimated. The CGTase activity is measured by replacing maltodextrin by cornstarch and potato starch. The maximum activity of enzyme is seen at a pH greater than 5 and less than 10 and at a temperature of 50 °C (Natalia et al., 2007).

Production of β-CD (10 whole till last): Immobilization of CGTases is more commonly used as it reduces the production costs and makes it feasible to be used in various food industries (Garcia-Galan et al., 2011). The enzyme immobilization has a lot of advantages as the enzyme can be reused, makes downstream processing easy, and increases thermo-stability of enzyme (Rodrigues et al., 2013; Natividade Schöffer et al., 2013). Chitosan derivative of chitin has required properties for enzyme immobilization (Muzzarelli, 1980). Chitosan has reactive amino and hydroxyl groups in its glucosamine chain (Chiou & Wu, 2004). Chitosan also exhibits mechanical stability, rigidity, and biocompatibility which permit its use in food industries (Krajewska, 2004). The following table represents the sources of β-cyclodextrins from various papers and its optimum pH, temperature, and fermentation methods.

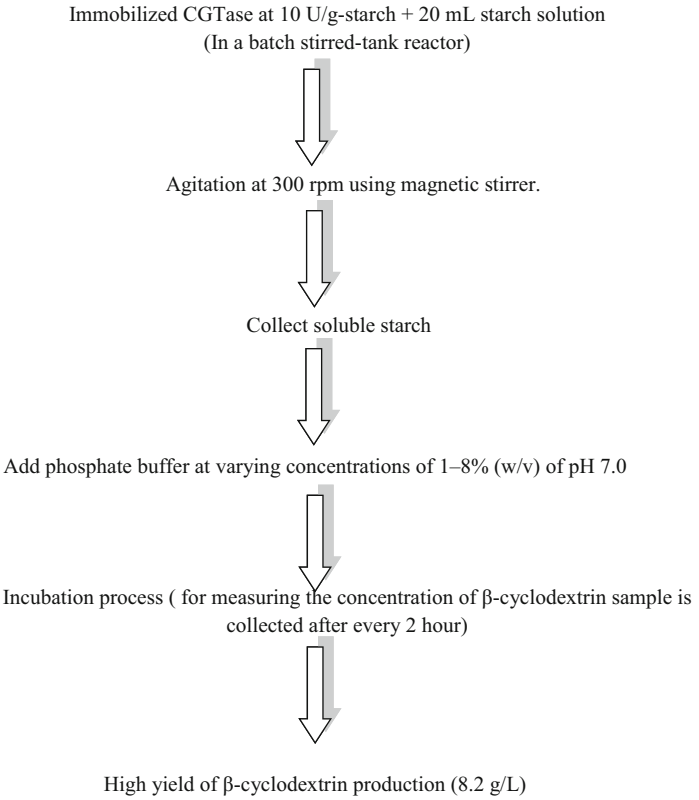

Fig. 4.4 Flow chart showing production of β-cyclodextrin using entrapped CGTase (Rakmai et al., 2015; Muria et al., 2011)

Source of β-CD production	Optimum pH	Optimum temperature	Main product and fermentation medium	References
CGTase produced from *Bacillus circulans* (ATCC 21783)	9.8–10	40 °C	In this article, cyclodextrin glucanotransferase is produced by immobilized and free cells of *Bacillus circulans* ATCC 21783 in various types of bioreactors. Main product is β-cyclodextrin.	Vassileva et al. (2007)
CGTase produced from *Bacillus* sp. (Alkalophilic bacteria)	6–7	40–90 °C	*Bacillus* sp. G1 is isolated from soil. Inoculum for *Bacillus* sp. G1 contains 1% (w/v) tapioca starch,	Mora et al. (2012)

(continued)

Source of β-CD production	Optimum pH	Optimum temperature	Main product and fermentation medium	References
			0.5% (w/v) yeast extract, 0.5% (w/v) peptone, 0.1% (w/v) KH_2PO_4, 0.02% (w/v) $MgSO_4$, and 1% (w/v) Na_2CO_3. Its main product is β-cyclodextrin.	
CGTase produced from *Bacillus firmus* strain 37	8.0	Different temperatures 37, 50, 60, and 70 °C at different substrate concentrates.	*B. firmus* strain 37, isolated from the soil. Composition of culture medium (%, w/v) is such that it contains 1.0 soluble starch, 0.5 polypeptone, 0.5 yeast extract, 0.1 K_2HPO_4, 0.02 $MgSO_4{\cdot}7H_2O$, 0.01 Congo red dye, 1.0 Na_2CO_3, and 1.5 agar. The plates were incubated at 37 °C for 48 h. Main product is β-CD.	Mazzer et al. (2008)
CGTase partially purified from *Bacillus circulans* (TISTR 907) by ammonium sulfate precipitation.	4.5–5.0	55–60 °C	Composition of medium: 10 g/L soluble starch, 5 g/L yeast extract, 0.2 g/L $MgSO_4{\cdot}7H_2O$, 5 g/L peptone, 1 g/L K_2HPO_4, and 10 g/L NaCl at pH 10.0 and incubation temperature is 37 °C for complete 24 h with shaking at 200 rpm. Main product is β-CD.	Charoenlap et al. (2004)
CGTases produced by *Bacillus* sp. C26	5–8.5	60–65 °C	Composition of medium 1% w/v soluble starch, 0.5% w/v yeast extract, 0.5% w/v peptone, 0.02% w/v K_2HPO_4, 0.02% w/v $MgSO_4{\cdot}7H_2O$, and 1% w/v Na_2CO_3. Its main product is β-cyclodextrin.	Cheirsilp et al. (2010)

4.5.2 Applications of β-Cyclodextrin

β-Cyclodextrin is used in inhibiting the browning of potato crude extract and different fruit juices: β-Cyclodextrin acts as an anti-browning agent in potato extract, and in case of fruit juices, it helps in stabilizing the flavour, improves mouth feel and also a mild antimicrobial effect, antioxidant properties in grape juice, improves retention of volatiles in pear juice, and helps in elimination of off-flavours in lemon oil (Santos et al., 2015).

4.6 Gamma Cyclodextrin

Gamma cyclodextrin is produced from microbial strain *B. thuringiensis* GU-2. The optimum pH and temperature for γ-CD production are pH 8.5 and 37 °C, respectively, in the presence of 1.0% starch substrate which results in 95% production of product with high yield (Goo et al., 2014). γ-CD has the premier solubility and largest interior cavity. Bioconversion of gamma cyclodextrin (γ-CD) from starch in the presence of cyclodextrin glycosyltransferase is using a technique aqueous two-phase system (ATPS). This is a low pollution technique, simple, economic, and is used for purification and production process in single step (Lin et al., 2016).

γ-CD production was to a great extent enhanced with the use of cyclododecanone, 5-cyclohexadecen-1-one, and glycyrrhizic acid. Optimum pH for reaction conditions is between 7 and 10 using 15% potato starch. All these parameters resulted in the corresponding increase in the yield of γ-CD from 21.2% to 41.6%. Optimum temperature range for the reaction is between 40° and 65°. Higher enzyme dosages had a detrimental effect on the CD yield, probably because excess CGTase might have hydrolysed CDs to produce glucose and high quantities of malto-oligosaccharides, which might have facilitated coupling reaction with the CDs, thus severely degrading them. A CD was not detected. When soluble starch or potato starch as a source was used, it becomes very difficult to detect the amount of CD. Soluble starch and potato starch are responsible for the production of highest percentage of γ-CD among the total CDs (nearly 88%), when compared with the other two starches (Loftsson & Brewester, 1996).

Source of γ-CGTase	Optimum pH	Optimum temperature (°C)	Main product	References
Bacillus subtilis strain 313	8.0	65	γ-CD	Li et al. (2007), Kato and Horikoshi (1986)
Bacillus sp. strain AL-6	7.5–10.5	55	γ-CD	Li et al. (2007), Fugita et al. (1990)
Bacillus firmus strain 290-3	6–8	60	γ-CD	Li et al. (2007), Veen et al. (2000)
Bacillus cereus	8.0	37	γ-CD	Li et al. (2007), Lin et al. (2016), Ng et al. (2011)

4.6.1 Applications

Cyclodextrins are used in food due to the following reasons:

1. *Cyclodextrins are used to preserve the natural flavours, vitamins, and natural colours of foods*
   Manufacturing, packaging, and other factors modify the flavours by reducing aroma. There are wide techniques and processes used for retaining the properties and flavour of the food like spray drying, freeze-drying, fluidized bed coating, and crystallization. However, those involving the formation of flavour/CD molecular-inclusion complexes offer great potential for the protection of volatile substances throughout many rigorous food-processing methods such as freezing, thawing, and microwaving. The addition of β-CD has proved to be most effective in retaining the flavour against heat, evaporation, and also against thermal degradation (Jouquand et al., 2004). The flavour enhancing complexes consists of many components, thus it is important that there is proper incorporation of these components in the complex without causing any change in its composition (Astray et al., 2009). Natural and synthetic coffee flavours have also been retained by the use of beta cyclodextrins. When these complex bound flavours come in contact with water molecules, they get released immediately and thus responsible for the taste or flavour (Astray et al., 2009; Szente & Szejtli, 1986). In addition to coffee, β-CD is also used for tea aromatization (jasmine, peppermint, and cinnamon) and also results in stability as well as efficient storage.
2. *For the removal of undesired taste, flavour, microbial contamination, and other undesired products*
   For the rejection of various food items, bitter taste is responsible. It has been found that there are two classes of chemical compounds like flavonoids and limonoids that are responsible for bitterness. Fresh citrus juice turns bitter in case of storage which is dependent on pH and temperature. It is highly desirable to remove the bitter components without the addition of any other substance to the fruit juice. Therefore, cyclodextrins are used for masking the undesired components from food. When the cyclodextrins are added to the fruit juice which has a peculiar odour and an undesirable taste, it forms complexes and retains the flavour of the components. For example, this process is used for deodourizing soybean milk and soy protein which removes the peculiar fish odours. A mixture of cyclodextrins alpha, beta, and gamma is ordinarily used. Rice which gives an unpleasant odour when stored for a long time can be made odour-free by cooking the rice in the presence of 0.01–0.4% CD (Sakakibara et al., 1985). The taste of cooked ice can be improved by addition of maltosyl beta CD. When CDs are complexed with sweetening agents such as aspartame; it helps in stabilizing and improving the taste of product. This complexation process also helps in eliminating the bitter aftertaste of other sweeteners like stevioside, glycyrrhizin, and rubusoside. CD itself is a promising new sweetener (Singh

et al., 2002). When these cyclodextrins or cyclodextrin-complexed antimicrobial agents are incorporated into food packaging plastic films, they help in effectively reducing the loss of the aroma substances and also improve the microbiological preservation during storage. Various CD complexes can be utilized in food as an antiseptic or conserving agents; 0.1% iodine-β-CD inhibits putrefying for 2 months at 20 °C in fish paste or in frozen seafood products (Hara & Hashimoto, 2002). Incorporating fungicide CD complexes into films-for example, packaging of hard cheeses-significantly elongates the shelf life of the product by inhibiting the rapid development of mould colonies on surface of the packaged cheese. The characteristic colour of muttons and fish and bad odour of bone powder can be removed by the use of cyclodextrins. The bitter taste of grape juice also reduced considerably when 0.3% of β cyclodextrin was added prior to heat treatment of canned juice. Empty beta cyclodextrin has also been used in food industries to remove undesirable compounds such as bitter taste from tea and coffee (Shiga et al., 2004) and fruit juices (Ferrari et al., 2013; Threapleton et al., 2013).

3. *To protect against oxidative degradation, heat-induced changes, and light-induced decomposition*

   The lipophilic components present in food which are sensitive to light- or heat-induced degradation can be protected because of the formation of inclusion complexes with CDs. The formation of inclusion complexes with cyclodextrins removes the polyphenol oxidase which converts the colourless polyphenols to coloured compounds which then leads to browning reaction in case of preparation of fruit juices. Cyclodextrins remove the polyphenol oxidase by forming complexes (Del Valle, 2004).

   In case of apple and pear juice, the maltosyl beta cyclodextrin can enhance the ability of ascorbic acid to prevent enzymatic browning due to protective effect of maltosyl beta cyclodextrin against ascorbic acid oxidation. Hence, maltosyl beta cyclodextrin is considered as secondary antioxidant reducing apple and pear juice browning reaction. During industrial food processing when these inclusion complexes are heated, they exhibit high thermal stability, are stable, and last for a longer period of time. This can be supported by taking an example such as in case of aromatizing substances which are sensitive to some kind of radiation, like citral (responsible for fresh citrus odour) cyclizes under exposure to UV irradiation. This can be prevented by complexation with beta cyclodextrins. It has been found that complexation is more effective (protective) in solid state than in aqueous solution. Another application of cyclodextrins is that since they can form inclusion complexes with the substances, they can be used to retain or scavenge substances such as odour, lactose, and cholesterol, thus retaining the aroma, colours which could enhance shelf life, and also the quality of the packaged product (Zhang et al., 2013b; Artiss et al., 2006; Suzuki & Sato, 1985).

4. *Food preservation*

   This is the most important application of cyclodextrins in food. Cyclodextrins help in reducing the volatile organic contaminants present in packaging materials

and also improve the barrier properties (diffusion rate and transmission rate), thus maintaining the food quality (Suzuki & Sato, 1985). Cyclodextrins can release antimicrobials and antioxidant compounds using CDs as carriers. As the humidity levels in freshly cut vegetables or fruits increase, they can lead to spoilage; this can be reduced by using cyclodextrins. At high relative humidity, high water–CD interaction weakens the host guest interaction and consequently there is a release of antimicrobial molecule which should protect the molecule against microbial growth (Astray et al., 2009; Ayala-Zavala et al., 2008).

5. *Cyclodextrins as stabilizers*
   Cyclodextrins are used for the preparation of stable water in oil emulsion such as mayonnaise and salad dressing due to their hydrophobic cavity and hydrophilic outer surface. In tomato ketchup, natural food colouring components can be stabilized by the addition of beta cyclodextrins. This ketchup when prepared by heating at 100° for 2 h did no discolouration, while the control did. Also, the addition of cyclodextrins to emulsified cheese increased the water retention and shelf life. However, the control discoloured when heated to this temperature (Szente & Szejtli, 2004). Cyclodextrins also help in processed meat products in improving water retention and texture (Ota & Takeda, 1981).
6. The browning or caking formation in the solid compositions containing sugars and amino acids can be prevented by addition of oligosaccharides such as cyclodextrin
   By the addition of 20% of β-CD to powdered juice containing anhydrous glucose, aspartic acid, d-alanine, citric acid, etc., the shelf life and also stability of the product are enhanced. Even after 30–35 days at 40 °C, no change in colour was observed (Fujimoto, 1981). The control started to discolour even on the second day and turned brown on fourth day (Szente & Szejtli, 2004). Starch gelatinization is the process in which heat treatment is given to starch and water, resulting in swelling of starch granules. However, addition of β-CD considerably improves the gelatinization of wheat flour. Addition of 1.5% beta cyclodextrin improves the solubility and swelling properties of starch granules. It has been observed that the adding up of 1.5% of beta cyclodextrin augmented the viscosity of starch paste to 4%. Milk casein hydrolysate is a readily digestible protein and its bitter protein can be removed by addition of 10% of beta cyclodextrin (Specht et al., 1981). Similarly, the bitter taste of ginseng extract and propylene glycol can also be eliminated by the use of beta cyclodextrin (Akiyama & Miyao, 1979).
7. *Extraction efficiency is increased with the use of beta cyclodextrin*
   When the roasted coffee beans are extracted using beta cyclodextrin, the extraction becomes more effective and the complex containing beta cyclodextrin and coffee can be used for effectual preservation of volatile components and also provide better aroma of the product (Wagner et al., 1988). Low cholesterol butter is also made by the addition of beta cyclodextrin. Molten butter is blended with crystalline beta cyclodextrin, which forms stable complexes with triglycerides/cholesterol and the cholesterol/β-CD is easily removed from molten butter.

Ninety percent of cholesterol in fat milk can also be separated by the addition of beta cyclodextrin; the butter obtained after this process does not retain any of the cyclodextrin (Szente & Szejtli, 2004). Cyclodextrins are widely used in order to modify the natural properties of materials, for example simply eggs have been produced in USA which means a low cholesterol egg which is produced by the addition of cyclodextrins to reduce cholesterol.

8. β-Cyclodextrin is more extensively used in food applications due to its availability at low cost, chemically stable structure, and non-hygroscopic, non-toxic, and edible nature. Ethyl vinyl alcohol copolymers are widely used in food industry due to their properties like gas barrier to oxygen and organic compounds and high transparency (Sanchez-Chaves et al., 2007; Lopez-de-Dicastilloa et al., 2010). On comparing all the three forms of cyclodextrins, γ-cyclodextrins show the highest flavour retention capacity among the other three.

4.6.2 Properties

S. no.	Properties	α-Cyclodextrin	β-Cyclodextrin	γ-Cyclodextrin	References
1.	Form complexes with	Benzene derivatives	Aromatic or heterocycles	Macrocycles or steroids	Threapleton et al. (2013)
2.	Water solubility	Modest solubility (14.5)	Lowest solubility (1.85)	Highest solubility (23.2)	Threapleton et al. (2013), Arya et al. (2006)
3.	Hydrolysis by human salivary and pancreatic amylases	Resistant to hydrolysis	Resistant to hydrolysis	Completely digested by amylase	Threapleton et al. (2013)
4.	Production from halophilic α-CGTase	1 (produced in much higher amount)	0.6	0.3	Threapleton et al. (2013)
5.	No. of glucopyranose units	6	7	8	Qi and Romberger (1988), Radi and Eissa (2010), Arya et al. (2006)
6.	Molar mass (g/mol)	972	1135	1297	Arya et al. (2006)
7.	Inner cavity diameter (Å)	4.7–5.2	6.0–6.4	7.5–8.3	Arya et al. (2006)
8.	Outer cavity diameter (Å)	14.6	15.4	17.5	Arya et al. (2006)
1. 9.	Cost per production			Highest	Threapleton et al. (2013)

(continued)

S. no.	Properties	α-Cyclodextrin	β-Cyclodextrin	γ-Cyclodextrin	References
10.	ΔH° (ionization), kcal/mol ΔH° (solution), kcal/mol	8.36 7.66	9.98 8.31	11.22 7.73	Arya et al. (2006)
11.	ΔS° (solution), kcal/mol ΔS° (ionization), cal/mol/K	7.67 −28.3	8.31 −22.4	7.73 −17.6	Arya et al. (2006)

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Enzyme in Milk and Milk Products: Role and Application

5

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Abstract

Enzymes are biocatalysts that catalyse a desired chemical reaction. Enzymes are specific in their action and yield into products. The enzymes that are utilized in the dairy industry for processing milk and milk products, like yoghurt, cheese, and fermented milks, are commonly known as dairy enzymes. These enzymes mostly aid in coagulation, cheese production, and enhancing shelf life of various dairy products. The most used dairy enzymes include lactase, amylase, lipases, transglutaminase, protease, catalase, and rennet. The functions of enzymes vary with the kind of the product to be processed. Both endogeneous and exogeneous enzymes are important for dairy application to provide functionality and safety to the product along with shelf life extension.

Keywords

Enzymes · Dairy products · Cheese · Fermented products

5.1 Introduction

Enzymes, also known as biocatalysts, are proteins that immobilize chemical reactions (Godfrey & Reichelt, 1983). Almost all the processes that exist in nature require enzymes in order to occur at significant rates. During an enzymatic reaction, the substrate molecules present on the onset are converted by the enzymes into different molecules, called products (Whitehurst & Van Oort, 2009).

The nomenclature of enzymes depends upon the reaction carried out by them or the substrate that they act upon as enzymes are highly specific for their substrates,

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A. Dutt Tripathi et al. (eds.), *Novel Food Grade Enzymes*,
https://doi.org/10.1007/978-981-19-1288-7_5

and hence, of various reactions; they catalyse only some of the many possibilities. Generally, the suffix 'ase' is added to the substrate name (e.g. glucose-oxidase, an enzyme which oxidizes glucose) or the type of reaction (e.g. a polymerase for a polymerization). However, pepsin, rennin, and trypsin are enzymes that were studied originally, and hence, are the exceptions to this rule. Enzymes can be categorized based upon the reactions they catalyse. These include, EC 1 oxidoreductases, enzymes that immobilize oxidation or reduction reactions (e.g. oxidases or dehydrogenases); EC 2 transferases enzymes (e.g. transphosphorylases and phosphomutases, example of a phosphate group), transfer a functional group such as a methyl or phosphate group; EC 3 hydrolases (e.g. hydrolases, including carbohydrases, deaminases, and proteases; hydrases such as fumarase, enolase, and carbonic anhydrase) are enzymes which catalyse the process of hydrolysis of different bonds (involving mostly, addition or removal of water); EC 4 lyase enzymes split bonds by methods other than hydrolysis and oxidation or form a C=C bond; EC 5 isomerases stimulate isomerization modifications within a molecule (e.g. glucose-isomerase); and EC 6 ligases combine two molecules using covalent bonds (Whitehurst & Van Oort, 2009).

All enzymes reduce the energy required by a reaction to take place. Different enzymes have different mechanisms to catalyse reactions, which involve creating conditions in which the transition state is stabilized in order to reduce the activation energy. This can be accomplished by providing a substitute way which involves bonding, and consequently, stabilizing the transition-state conformation of the substrate. For example, temporary reaction with the substrate in order to form an intermediate enzyme–substrate complex, where an enzyme plays an indispensable role. Enzymes possess an interesting property which is their specificity. For example, certain enzymes display absolute specificity which implies that they immobilize only one specific reaction or are selective for a certain kind of chemical bond or functional group. Generally, there exist four types of specificity which are: Absolute specificity; here, highly specific enzymes stimulate only one reaction; Group specificity; here, enzymes that are group-specific act only on molecules with certain functional groups, such as phosphate, amino, or methyl groups; Linkage specificity, where enzymes act on chemical bonds of specific nature, regardless of the molecular structure; and, Stereochemical specificity, where enzymes act only on a certain steric or optical isomer and not on their isomeric counterparts (Whitehurst & Van Oort, 2009). These biocatalysts are highly potent, as even in a very small quantity, they are capable of augmenting the rate of reactions to several folds. For example, orotidine-5′-phosphate-decarboxylase is an enzyme that facilitates a reaction to occur in milliseconds which would generally take millions of years (Callahan & Miller, 2007; Radzicka & Wolfenden, 1995). As a result, enzymes have advanced as a vital component for the sustenance of life. Evidently, over 5000 types of biochemical reactions have been found to be catalysed by enzymes (Schomburg et al., 2013).

Enzymes take part in several metabolic functions of living organisms. For example, they (kinases and phosphatases) are crucial for processes like signal transduction and cell regulation. They are also responsible for inducing movement, with adenosine triphosphate (ATP) which hydrolyses myosin in order to produce

contraction in the muscles and to also mobilize cargo around the cell as part of the cytoskeleton (Macrae, 1985). They also serve a vital role in the 'digestive systems' of various mammals and animals. For example, amylases cleave starch molecules and proteases cleave protein molecules (Whitehurst & Van Oort, 2009). Besides, enzymes have also been utilized at an industrial scale for antibiotic production. Moreover, there are certain enzymes which are used in household products like detergents that help in cleaving several stains on clothes. Additionally, as a result of the ability of enzymes to alter and enhance the nutritional, functional, and organoleptic properties of various ingredients and products, they have also found a widespread application in the food industry. They are employed in the food industries to manufacture bread, alcoholic drinks, and milk-based products like cheese and yoghurt, and to improve the flavour and texture of dairy-based products (Qureshi et al., 2015).

The enzymes that are utilized in the dairy industry for processing milk and milk products like yoghurt and cheese are commonly known as dairy enzymes. These enzymes mostly aid in coagulation, cheese production, and enhancing shelf life of various dairy products. The most used dairy enzymes include lactase, amylase, lipases, transglutaminase, protease, catalase, and rennet. Lactase enzyme is mostly utilized for hydrolysing lactose molecules to glucose and galactose sugars and augment the solubility and sweet flavour in various dairy products. Amylases are biocatalysts which break down starch by immobilizing the process of hydrolysis of internal α-1,4-glycosidic bonds of polysaccharide sugars like maltose, glucose, dextrin, and maltotriose, while preserving the α-anomeric configuration in the products (Takata et al., 1992). Lipase is another commonly used enzyme which is employed mostly to mature cheese and improve flavours. Another dairy enzyme is transglutaminase, commonly known as TGases, which plays a pivotal role in the enhancement of protein functionality in dairy products. Similar to lipases are different types of protease enzymes which are also utilized in manufacturing of cheese to increase the rate of cheese aging, as a functional property, and modifying milk protein to lower the allergic effects in cow milk-based infant food products (Fox, 2002). Another frequently used dairy enzyme in cheese production is catalase. It is employed in producing some cheeses in lieu of pasteurization (e.g. Swiss cheese) to save regular milk proteins that are responsible for providing certain benefits to finished product and flavour enhancement of the cheese (Fox, 2002). Rennet, which is also known as rennin, is a combination of pepsin and chymosin extracted from animals as well as microbiological sources. It is also employed in the cheese manufacturing industry to curdle milk (Merheb-Dini et al., 2010).

This chapter aims to emphasize on various endogenous and exogenous dairy enzymes like lactase, amylase, lipases, transglutaminase, protease, catalase, and rennet which are used widely, not only in the production of milk and milk-based products, but also lactose-free products. It briefly delineates the structure, sources, and applications of these enzymes in the dairy industry. Additionally, the chapter also meticulously explains the use of these dairy enzymes in the production of cheese, lactose-free milk, fermented milk as well as in packaging.

5.2 Endogenous Enzymes

All biochemical processes and metabolic pathways are propelled and modulated by a group of enzymes mobilizing a series of reactions. These enzymes catalyse reactions and improve the product yield up to several folds. However, in their absence, the rate of reaction decelerates, and eventually reaches impasse. Enzymes, on the basis of their source, can be broadly classified into two categories—exogenous enzymes and endogenous enzymes (EHEs). Enzymes secreted by animals are known as exogenous enzymes. These are used to enhance the process of digestion in animals. Optimizing the conditions required for exogenous enzymes is a tiresome job, which include optimum temperature, pH, and enzyme-substrate concentration. Additionally, these enzymes are expensive, and hence, increase the ultimate cost of the by-product. Contrariwise, EHEs are enzymes that are added to the animal feed. The efficacy, ease of use, unique properties, and profitability of these enzymes make them an appealing candidate for industries for the production of numerous by-products (Ramawat & Mérillon, 2015).

5.2.1 Lactase

β-Galactosidase, also known as lactase, is an endogenous enzyme which is accountable for hydrolysing lactose, a disaccharide composed of subunits of galactose and glucose (Carrara & Rubiolo, 1994; Felicilda-Reynaldo & Kenneally, 2016; Vandenplas, 2015; Lukito et al., 2015). This enzyme is located in the brush border of the small intestine of humans and other mammals. It plays a crucial role in the absorption of lactose. Lactase cleaves lactose by breaking the bond that links the two monosaccharide sugars—galactose and glucose and eventually resulting in glycolysis.

This enzyme also results in transgalactosylation of lactose to allolactose, which is then split into monosaccharides. The binding of allolactose to lacZ repressor modulates the amount of lactase in the cell by generating positive feedback (Pivarnik & Senecal, 1995).

5.2.1.1 Structure

Lactase or β-Galactosidase is a tetramer, that is four identical polypeptide chains. Each chain comprises 1023 amino acids that amalgamate to create five distinct structural domains. Of the five domains, one is a jelly roll barrel, whereas the rest comprise β-sandwich, fibronectin, and a central domain. The central domain with TIM-type barrel is made of tetramer subunits and acts as the active site (Huber et al., 1976). However, cleavage of tetramer to dimers causes the active site to inactivate. The α-peptide present at the n-terminal of lactase is involved in α-complementation and contributes in subunit interface (Corral et al., 2006).

5.2.1.2 Sources

Lactase can be found in fungi, bacteria as well as in yeasts. It can be mostly found in plants, like apples, apricots, peaches, and almonds. However, for industrial and commercial scale, lactase is usually acquired from fungi like *Aspergillus* and *Kluyveromyces* (Zhou & Chen, 2001).

Bacterial Sources

Lactase derived from bacterial sources possesses high activity, stability, and ease of fermentation, and hence, it is utilized to hydrolyse lactose. Studies have reported *Lactobacillus* and *Bifidobacterium* species to be an efficacious probiotic. As a result, they are commonly used to obtain lactase. It has been reported that lactase derived from bacterial strains like *Bifidobacterium longum* strain CCRC 15708, *Bifidobacterium infantis* strain CCRC 14633, and *Bifidobacterium longum* CCRC 15708 possess high enzyme activity (Hsu et al., 2007).

Yeast Sources

Lactase is extracted from yeast to produce lactose-free products. *Kluyveromyces lactis*, a variety of yeast found in dairy environment, is one of the most commercially used sources of lactase (Pivarnik & Senecal, 1995). The optimal pH of lactase obtained from a yeast lies between 6.0 and 7.0. Studies have reported that *Kluyveromyces marxianus*, another variety of yeast, can be utilized to produce homologous enzymes of lactase and other heterologous proteins that have the ability to thrive on a variety of substrates with lactose as their only source of energy. It has been found that a cold-active acidic lactase extracted from *Guehomyce spullulans*, a strain of psychrophilic yeast species, can be utilized for whey and milk hydrolysis in food industries (Saqib et al., 2017).

Fungal Sources

Fungi have been known to yield enzymes that are highly stable. Lactases obtained from fungal sources have an optimal acidic pH range of 2.5–5.4. This makes them highly potent for hydrolysing lactose of an acidic material such as whey. The most commonly used fungal sources to produce lactases that have been accepted as "generally recognized as safe" (GRAS) by FDA are *Kluyveromyces lactis*, *Kluyveromyces fragilis* (*Saccharomyces fragilis*), and certain *Aspergillus* species. Lactase extracted from *Aspergillus oryzae* is used on commercial scale and has been reported to be an effective source for whey utilization. Lactase generated from another fungus, that is, *Aspergillus niger*, is used to remove galactose residues from oligosaccharides and polysaccharides that are obtained from plant (Kazemi et al., 2016).

Plants

Lactase can also be obtained from a variety of plant sources like apple, tomato, mango muskmelon, avocado, coffee, kiwifruit, and Japanese pear plants (Seddigh & Darabi, 2014). These enzymes contribute to plant growth, ripening of fruits, and hydrolysis of lactose. It has been reported that this enzyme reduces galactosyl

content of cell wall of persimmons, causes cell wall hydrolysis in papaya, and facilitates the process of fruit ripening (Saqib et al., 2017).

5.2.1.3 Applications in Dairy Industry

Lactase has multiple industrial applications. They are used to produce lactose-free food products for patients suffering from lactase deficiency (Karasova et al., 2002). As a result of the hygroscopic nature of lactase, which leads to crystallization in foods, it is utilized for hydrolysing lactose to avert crystallization of lactose in desserts that are frozen and concentrated. This helps in reducing the lactose content of milk to be consumed by individuals with lactase deficiency (Champluvier et al., 1988). Additionally, on a commercial scale, this enzyme is also used to dispose whey. Whey disposal is a grave environmental concern as it is disposed into water streams, which leads to water pollution (Brandão et al., 1987). Hence, lactase is utilized to convert whey into useful products that have a range of applications in food industry such as ethanol and sweet syrup (Zhou & Chen, 2001).

Production of Hydrolysed Milk Products

A significant percentage of the world's population has been known to develop lactase deficiency which causes lactose intolerance (Sitanggang et al., 2016). The burden of lactose intolerance among the Asian population is nearly 90%. Individuals suffering from lactose intolerance can consume food products with either no or least amount of lactose in them. A solution to this problem is to treat milk or milk-based products with lactase. Lactase helps in pre-hydrolysing lactose in these products, thus reducing the concentration of lactose in them (Ramana Rao & Dutta, 1978).

Besides, lactase is also preferred to decrease the crystallization in frozen and condensed milk and milk products which occurs as a result of high concentration of lactose. Lactase also helps in improving the texture of milk and milk-based foods and in making them even more digestible. The by-products obtained from lactose hydrolysis (i.e. glucose and galactose) have been reported to be fermented easily. This helps in minimizing the total time required to attain the optimal pH in various milk-based foods like cottage cheese and yogurts. Additionally, lactase has been reported in reducing the calorie content of the end product by reducing the requirement of adding extra sweeteners (Domingues et al., 2005).

Whey Utilization

Cheese industry has been known to yield a massive amount of whey as a derivative. The chief constituents of whey include lactose, proteins, and minerals. The lactose in whey is associated with chemical oxygen demand and biochemical oxygen demand. Therefore, despite the fact that in developing countries a substantial amount of whey is used to produce permeates and protein concentrates, whey, when disposed in water streams, leads to severe water pollution. Nevertheless, whey, when converted into beneficial products such as ethanol as well as lactase, can be utilized for the production of lactose-free food products (Kokkiligadda et al., 2016). Additionally, treating whey with lactase can help in converting it into a substrate that is readily available for cell cultivation (Parashar et al., 2016). Another application of lactase

includes recovering of highly functional whey proteins like lactalbumin through ultrafiltration and subsequently hydrolysing them to manufacture numerous pharmaceutical intermediates (Domingues et al., 2005).

Production of Galacto-Oligosaccharides

Another application of lactase enzyme includes the production of galacto-oligosaccharides (GOS). GOS are nondigestible prebiotics and hence play a crucial role in improving human health by modifying intestinal microflora of human and promoting the growth of useful bacteria in the intestine such as *Lactobacillus* and *Bifidobacterium* species. GOS are produced from the transglycosylation activity of lactase during lactose hydrolysis. It can be stored at room temperature for longer period of time due to its acidic conditions. This characteristic of GOS makes it capable of being used for the production of different products without any decomposition. The oligosaccharide content in GOS ranges from 1% to 45% and depends further on the source of enzyme and the total amount of saccharides (Pazur, 1953).

GOS is also present in human milk and is known to help in increasing the natural microflora of small intestine of a breast-fed infant, that is *bifidobacteria*. This bifidogenic activity of GOS plays a vital role in reducing the number of pathogenic bacteria. In view of this characteristic, companies dealing with infant foods are now producing milk- and cereal-based food products containing GOS (Matto & Husain, 2006). Additionally, GOS also functions as soluble fibres and gets fermented by the natural microflora present in large intestine. This fermentation produces end products that are short-chain fatty acids that help in reducing the pH of fecal matter. The GOS is indigestible by the bacteria present in mouth; due to this reason they do not contribute to the development of cariogenicity (Matioli et al., 2003).

5.2.2 Amylase

Amylases are enzymes that degrade starch by catalysing the process of hydrolysis of internal α-1,4-glycosidic bonds of polysaccharides like maltose, glucose, dextrin, and maltotriose, while retaining the α-anomeric configuration in the products (Takata et al., 1992). They can be found in a variety of organisms. These enzymes are known to have multiple applications, and hence, hold a major share in market for the sales of enzymes (Vaidya et al., 2015). For centuries together, amylases derived from microbial and plant sources have been utilized in the brewing industry. Amylases extracted from fungal sources have been employed in the Asian cuisine (Hernández et al., 2006). The growing research interest in amylases obtained from bacterial, fungal, and viral sources in comparison to amylases that are derived from animals and plants sources can be ascribed to the ease in its large-scale production and industrial application (Mukherjee et al., 2009).

Amylases (EC 3.2.1.0) are classified into three subtypes that are α-amylase, ß-amylase, and γ-amylase. The two types of amylases—endoamylases and exoamylases, are categorized on the basis of the method by which the glycosidic bond is attacked (Vaidya et al., 2015). α-Amylases (α-1,4-glucan-glucanohydrolase,

EC 3.2.1.1) are extracellular enzyme (Cherry et al., 2004) that is responsible for degrading α-1,4-glucosidic linkage of starch and associated products and producing oligosaccharides (Baysal et al., 2008). β-Amylase (1,4-α-D-glucan maltohydrolase; glycogenase; saccharogen amylase, EC 3.2.1.2), another form of amylase, works from its non-reducing end and mobilizes the process of hydrolysis of the second α-1,4-glycosidic bond by cleaving two maltose units at a time. At the time of fruit ripening, this enzyme helps in cleaving starch present in the fruit and results in the sweet flavour of ripe fruit. γ-Amylase (EC 3.2.1.3) (Alternative names: Glucan 1,4-α-glucosidase; amyloglucosidase; Exo-1,4-α-glucosidase; glucoamylase; lysosomal α-glucosidase; 1,4-α-D-glucan glucohydrolase), the third sub-type of amylases, is known to cleave α-(1–6)-glycosidic linkage along with the cleavage of the last α-(1–4)-glycosidic linkages at the non-reducing end of amylose and amylopectin to yield glucose. This sub-type has an optimum pH of 3, and hence, is efficacious in acidic conditions (Saini et al., 2017).

5.2.2.1 Structure

Amylase possesses a three-dimensional structure that enables it to bind to substrates. The high activity of specific catalytic groups helps in the cleavage of the glycoside links (Iulek et al., 2000). The human α-amylase is made of 512 amino acids in a single oligosaccharide chain. It comprises calcium and has a molecular weight of 57.6 kDa (Whitcomb & Lowe, 2007). Amylase possesses three domains: A, B, and C. The A domain which is the largest exhibits a classical barrel-shaped $(\beta/\alpha)_8$ super structure. The B domain is cemented to A domain by disulphide bond and is positioned between the A and C domains. The C domain possesses a sheet-like organization and is attached to the A domain by a simple polypeptide chain. The substrate binding site or the active site of the enzyme is positioned in a long cleft which is situated between the carboxyl end of the A and B domains. The calcium ion (Ca^{2+}) is positioned amid the A and B domains. This ion may serve in the process of stabilizing the three-dimensional structure of the enzyme and act as an allosteric activator. The active site of the enzyme comprises 5 sub-sites with the catalytic site located at sub-site 3. Substrate can attach to the first glucose residue present in sub-site 1 or 2, thereby permitting the division to take place between the first and second or second and third glucose residues (Whitcomb & Lowe, 2007).

5.2.2.2 Sources

Amylases are a group of enzymes produced in nature by plants, animals, and microorganisms.

Alpha Amylase

Alpha amylase can be isolated from a variety of sources like plants, animals, and microbes (Pandey et al., 2000). Although alpha amylase has been extracted from a variety of bacteria, fungi, actinomycetes, and yeasts, the enzymes obtained from fungi and bacteria have been found to have major industrial applications. This can be ascribed to their consistency, cost-effectiveness, less time and space requirement for their production, and ease of process optimization and modification (Ellaiah et al.,

2002). The microbial enzyme derived from the microbial sources is capable of meeting the industrial demand and a huge amount of them are commercially available. For centuries, amylases derived from microbes and plants have been used as food additives (Hernández et al., 2006). The bacterial amylases are mostly derived from *Bacillus* sp. like *B. subtilis*, *B. stearothermophilus*, *B. licheniformis*, and *B. amyloliquefaciens*. Most of the fungal sources of amylases belong to terrestrial isolates like *Aspergillus* species (Jamal et al., 2011). The amylases derived from fungi are extensively utilized to prepare oriental foods (Popovic et al., 2009). The alpha amylases have also been isolated from filamentous fungi, which are high-yielding producers of extracellular proteins (Coradin et al., 2011).

5.2.2.3 Applications in Dairy Industry

Effect of Exogenous Amylase on Lactation Performance of Dairy Cows

Many researches have evidently exhibited the resistance of certain exogenous enzymes to the degradation in the rumen, which has the potential to augment the digestibility of feeds and eventually enhance animal performance (Hristov et al., 1998). Various studies have been focussed on the addition of fibrolytic enzymes to ruminant diets as fiber digestion is generally never amplified in the rumen (Beauchemin et al., 2003). Certain in vitro and in vivo evidences have reported that even though starch digestion in the rumen is not usually considered to be limiting, amylase enzymes contribute in enhancing the animal performance (Andreazzi et al., 2018). Intensive lactation augments the intake of feed and passage throughout the rumen. Although this increases the volume of starch passing the rumen without degradation, the capacity of small intestine to digest starch is limited. Researches have reported that supplementing the feed with α-amylase may enhance ruminal starch digestion without an intensive production of volatile fatty acids and increased risk for acidosis (Nozière et al., 2014).

5.2.3 Transglutaminase

All transglutaminases (EC 2.3.2.13), also known as protein-glutamine γ-glutamyltransferase, fall under the class of transferases (Marx et al., 2007; Trespalacios & Pla, 2007). Transglutaminases (TGases) can be found naturally in a variety of living organisms. They are known to participate in several biological functions, which include clotting of the blood and healing of wounds (Jaros & Rohm, 2016). They mobilize the formation of an iso-peptide bond between the group of γ-carboxamides of glutamine residues (donor) and the first order ε-amine groups of different compounds, for example, proteins (acceptors of an acyl residue). Additionally, in the absence of free amine groups, TGase catalyses deamination, where water serves as an acyl acceptor. TGase mobilizes the reactions that bring about substantial modifications in the physical and chemical properties of proteins, which include viscosity, elasticity, thermal stability, and resilience.

For the first time, TGases were derived from tissues of mammals and body fluids to investigate their applications in industry. The first commercially available TGase, extracted from the liver of guinea pig, was not found to be appealing candidate for industrial use ascribing to the limited supply and requirement of Ca2þ for activation which resulted in the high production cost of the enzyme. Likewise, another TGase known as Factor XIII, which was derived from blood plasma, exhibited a major drawback of red pigmentation and the requirement of thrombin for activation. In 1980s, during the screening of microorganisms for TGase activity, microbial TGase, which is independent of Ca2þ and possesses low substrate specificity, was identified from *Streptomyces mobaraensis.* Production of mTGase results from conventional fermentation of the wild-type *S. mobaraensis* strain (GRAS status) with consequent extraction of the protein that is secreted. Thus far, mTGase is the only commercially available TGase in the food industry (Jaros & Rohm, 2016).

5.2.3.1 Structure

mTGase, a monomeric protein with 331 amino acids, has an isoelectric point of 8.9. Its secondary structure comprises 8 β-strands adjoined with 11 α-helices. The enzyme acquires a disk-like shape that has a deep cleft at the edge. At this cleft is situated a single cysteine residue (Cys64). Molar mass of mTGase as evaluated by the technique of mass spectrometry and amino acid sequencing is 37.86 kDa. Usually, mTGase exhibits activity at pH range of 4 and 9. However, the optimum pH of the enzyme lies in the range 6–7. The optimum temperature for mTGase is 50 °C. Although it is immobilized at 70 °C within minutes, it maintains some residual activity at near-freezing temperatures. Another characteristic of mTGase is its stability under high hydrostatic pressure. This characteristic provides the prospects of alteration of proteins, even in situations where they are not available for mTGase under atmospheric pressure (Jaros & Rohm, 2016).

5.2.3.2 Sources

TGases are widely spread in nature (Kashiwagi et al., 2002). They can be derived from various invertebrates and microbial cells (Yu et al., 2008; Griffin et al., 2002) as well as mammalian tissues (Yasueda et al., 1994). TGases can also be found in tissues of various plants such as orchard apple, soy, fodder beet, and topinambour (Falcone et al., 1993). These enzymes have been extracted from microbes belonging to *Streptoverticillium* sp. and *Physarum polycephalum*. TGases are biosynthesized as an extracellular enzyme by *Streptoverticillium* sp., *Streptoverticillium cinnamoneum* subsp. *cinnamoneum* (Duran et al., 1998), *Streptomyces netropsis* (Yu et al., 2008), *Streptoverticillium griseocarneum* (Gerber et al., 1994), *Streptoverticillium ladakanum* (Ho et al., 2000), and *Streptomyces lydicus* (Færgemand & Qvist, 1997).

5.2.3.3 Applications in Dairy Industry

Various researches have reported the commercial availability of TGases and their crucial role in enhancing protein functionality in dairy products. It has been found that these enzymes avert syneresis in yoghurts and result in firmer yet soft texture

(Kieliszek & Misiewicz, 2014). A study reported that TGases at the level of 0.02% improve melting, firmness, flexibility, and organoleptic properties of high fat Mozzarella cheese. However, in the case of Mozzarella with low fat content, 0.05% of TGase provides adequate meltability, flexibility, free oil formation, and organoleptically to the cheese. The cross-linking property of TGase helps in augmenting soft cheese production, reducing syneresis, extending shelf life, and decreasing nutrient bioavailability for deteriorative microorganisms (Mahmood, 2009). It has also been reported that this enzyme can be utilized to partially replace fat in ice-creams, and it is also responsible for increasing overrun, partial amalgamation of fat globules, consistency, melting resistance, firmness, and viscosity in ice cream (Tafes, 2019).

A study reported that up to 0.5% of TGase was more efficacious to enhance the functional properties of yogurt that was made from goat milk. The cross-linking property of TGase was found to be accountable for enhancing the gel consistency and reducing the whey separation substantially in yoghurt (Farnsworth et al., 2006). Another study reported that when stirred yogurt was developed through covalent cross-linking by the combined action of inactivated mTGase and glutathione, no negative effects on the fermentation of yogurt were observed. However, a substantial rise in apparent viscosity as well as protein polymerization was observed in yogurt that was only developed by TGase (Bönisch et al., 2007). The use of TGase in high concentration was found to reduce syneresis and increase the viscosity of yogurt. However, a minor bottleneck was observed with regard to the growth of Lactic Acid Bacteria. This led to reduced production of acid and acetaldehyde in comparison to those reported for the control. Nevertheless, TGase concentration up to 0.3 g/L was reported to be optimum and was recognized as an acceptable substitute to stabilizers to be added in the production of fat-free yogurt (Ozer et al., 2007). Studies identified TGase as an efficacious candidate to enhance the physical properties of yogurt when its constitution was modified by the addition of whey (Şanli et al., 2011).

5.3 Application in Dairy Industry

5.3.1 Lactose-Free Milk

Low levels of lactase production a human beings has been known to be a leading cause of “lactose intolerance”, which is an intestinal symptomatic condition. A lactose-intolerant individual may experience clinical symptoms like nausea, abdominal pain, flatulence, diarrhea, and bloating after consuming food that contains lactose. It has been estimated that about 70% of the world population, including all the age groups, is suffering from lactase deficiency (Xavier et al., 2018). Lactose-intolerant individuals can consume dairy-fermented products that comprise very little or no lactose in them (Saqib et al., 2017). Currently, there is an increase in demand for lactose-free dairy among the lactose-intolerant populations. This can be ascribed to the presence of little or no lactose in these products and their ability to provide vital macro- and micro-nutrients present in milk to individuals suffering from lactose intolerance (Dekker et al., 2019).

The dairy industry employs different processing techniques to reduce the lactose content in the dairy products to produce lactose-free products. Cheese production involves certain techniques which help in reducing the lactose content. For example, Gouda cheese production involves a curd washing step to reduce the lactose content. Although the lactose content in most of the cheeses is already relatively low without ripening, in several other types of cheeses, the lactose content is reduced during the process of ripening with the help of lactic acid bacteria. Generally, aged and hard cheeses like Parmesan, Swiss, or Cheddar cheeses have a very low concentration of lactose. Contrariwise, young and fresh cheeses may consist enough lactose that may be capable of inducing a reaction among individuals with lactose intolerance, depending on the quantity consumed. Butter is another dairy product with low lactose content. The majority of water-soluble constituents of milk inclusive of lactose are eliminated, during the production of butter resulting in the reduction of lactose concentration in butter (Dekker et al., 2019).

Besides reducing the lactose content in dairy products, another solution to produce lactose-free dairy products includes hydrolysing lactose into glucose and galactose using the lactase enzyme. The monosaccharides produced get easily adsorbed in the small intestine and avert the incidence of symptoms associated with lactose intolerance. Currently, neutral lactases and acid lactases are the two kinds of commercially available lactases (Dekker & Daamen, 2011). The former is chiefly employed on an industrial scale to produce lactose-free dairy products, while the latter is provided as a dietary supplement to the consumers for its consumption along with regular dairy products to induce cleavage of lactose in the stomach (Dekker et al., 2019).

5.3.2 Production of Lactose-Free Milk

Researches have reported that lactose dosage of less than 12 g per meal may result in mild symptoms among lactose-intolerant individuals; however, the dairy industry aims to maintain the lactose content as low as possible (Suchy et al., 2010). Currently, there does not exist global agreement on the regulatory requirements for lactose-free claims. In the past, lactose reduction to 0.5% or 0.1% was considered enough by the dairy producers, but, in some countries, lactose content less than 0.01% is considered to be the current requirement to consider milk to be lactose-free. To achieve such low lactose concentration in milk production entails special attention to the milk processing, to the dosage and efficiency of the enzyme used in this process, and to sensitive analytical methods to determine such low amounts of lactose (van Scheppingen et al., 2017).

Presently, there exist two processes that are used in the production of lactose-free milk (Troise et al., 2016; Harju, 2004). These include batch process and aseptic process and both of them use soluble lactase enzyme. Although various studies have suggested the processes that rely on an immobilized enzyme, these processes have not been employed in industrial practice to produce lactose-free milk either in the

past or in present due to issues related to the microbial stability of the end product (Finocchiaro et al., 1980).

5.3.2.1 Batch Process (Pre-Hydrolysis)

The batch process involves the addition of a neutral sample of lactase to a tank containing raw milk and subsequent incubation for approximately 24 h accompanied with continuous slow stirring in order to prevent creaming. In order to prevent microbial growth in the milk, which is not yet sterile, cool conditions with temperature ranging between 4–8 °C are maintained to perform this process. Once the process of incubation is completed, the milk is then pasteurized, homogenized, and packaged.

It is important for the enzyme dosage to be adequate to reach the requisite limit for removing lactose at low temperature of the incubation and during the set time period. It is because of this reason that the enzyme dosage for this process is kept fairly high and enzymes are selected based on their high activity at neutral temperature. The batch incubation is discontinuous as it involves a tank in the factory, delaying the pasteurization of the milk by a day. This may prove to be a setback for certain factories during high productivity. In conditions like these, lactase enzyme having higher specific activity may be helpful in reducing the production time, thereby increasing the total yield of the factory. Additionally, to prevent microbial spoilage of milk that may result due to delay in pasteurization of the milk, it is important for the milk quality to be impeccable.

Generally, the lactose-free milk produced with the batch process is comparatively unresponsive to the side activities of the enzyme. This can be ascribed to the short storage period of the milk at refrigerated conditions and to the process of pasteurization or sterilization after incubation of the enzyme, which is responsible for the inactivation of most of the enzymatic activities. In the past, certain lactase preparations displayed proteolytic side activities; however, these issues seem to be resolved with only rare complaints occurring for the lactose-free milk produced with the batch process (Mittal et al., 1991).

Techniques like chromatography and ultra- and nano-filtration have been employed in combination with lactose hydrolysis to avoid the doubling of the milk sweetness (Harju et al., 2012; Jelen & Tossavainen, 2003). This results in the production of lactose-free milk of outstanding quality, with its taste nearly alike the regular milk.

5.3.2.2 Aseptic Process (Post-Hydrolysis)

The aseptic process involves the sterilization of the milk using the UHT technique and the subsequent injection of the prepared sterile lactase into the milk immediately before packaging (Dahlqvist et al., 1977). This packaged UHT milk is then kept quarantined for almost 3 days at optimum temperature, which provides adequate time for lactose hydrolysis to occur before the milk is distributed to the retailer. The aseptic process is not employed for pasteurized milk for the production of lactose-free milk, due to the absence of quarantine period (Dekker et al., 2019).

Fundamentally, there exist two techniques to obtain sterile lactase enzyme. The first technique involves pre-sterilization of lactase by the enzyme manufacturer and usage of distinct sterile dosing equipment that is required for the sterile injection. The second technique involves the filter-sterilization of the unsterile enzyme immediately before incorporating it to the sterilized milk at the dairy industry (Dekker et al., 2019).

Considering the high incubation time and temperature required in this process, the enzyme dosage can be kept much lower. Process control in this process is not present as the enzyme is vigorous only in the final milk package. The dairy manufacturer should also consider the aspects such as deviation in the storage temperature in un-thermostated warehouses, resulting from the seasonal transitions such as summer to winter, when dosing the enzyme (Dekker et al., 2019).

During the aseptic process, special equipment and consumable costs are essential requirements specifically for the in-factory filtration. Highly skilled operators are employed during the process of lactose injection in order to prevent microbial contamination in the milk. When organized appropriately, the process can function as a completely continuous operation, thus making the aseptic process a desired technique in industries requiring a high yield (Dekker et al., 2019). The aseptic process for the production of lactose-free UHT milk can only be considered to be a fully developed technique after the crucial refinement of the lactase enzyme quality. Researches have reported that the arylsulfatase side activity in the preparation of lactose may result in serious medicinal off-flavours during storage. This has been ascribed to formation of *p*-cresol from sulphonated-cresol, which occurs naturally in the milk (De Swaaf et al., 2006). Currently, arylsulfatase-free lactase enzymes are available commercially. It is important for a lactose-free UHT milk producer to consider utilizing only the supreme quality lactase enzymes for this process in order to avert shelf life-related complication (Dekker et al., 2019).

Lactose hydrolysis in milk has been known to augment the monosaccharides' content, resulting in an efficient Maillard reaction, which may be amplified due to the inadequate proteolysis by proteases. These proteases may be present naturally in the milk or they may originate from the lactase preparation. The amplified reactions lead to the increase in the formation of off-flavours and greater browning of lactose-free milk in comparison to regular milk. When stored at higher temperature, it also results in a reduced nutritional value of the product (Troise et al., 2016; Jansson et al., 2014; Evangelisti et al., 1999). The enhanced maillard reaction may be considered as the critical determinant of the decreased shelf life of lactose-free UHT milk in comparison to regular UHT milk. In the past, various researches have suggested that the batch process for the production of lactose-free UHT milk may result in comparatively higher browning than the milk produced using the aseptic process (Mendoza et al., 2005). However, recent studies have reported the relevance of the storage conditions (temperature) and lactase quality for determining shelf life (Troise et al., 2016). Excellent shelf life was found for lactose-free UHT milk produced with the batch process, and milk browning during storage is, therefore, largely independent of the production process that is used (Dekker et al., 2019).

5.3.3 Other Dairy Products

Besides lactose-free milk, various other lactose-free dairy products are manufactured with the use of enzymatic treatment. Flavoured milk is one such product that is produced using a similar process (McCain et al., 2018). The comparatively stronger production of colour and flavour by the flavoured lactose-free milk has been reported to reduce browning and production of off-flavours due to the maillard reaction. Unlike regular lactose-free milk, the flavoured lactose-free milk requires less addition of extra sugar, due to the lactose treatment; however, in certain flavoured milks like highly sugared chocolate milk, the process of lactose hydrolysis may not be adequate for complete replacement of sugar addition (Li et al., 2015), and thus there may be a requirement to add additional sweeteners. Another lactose-free dairy product is dairy powder, which can be developed from either milk or whey, which is made lactose-free with the batch process. A major setback reported during the production of lactose-free dairy powder is the high monosaccharide content in the treated milk, which causes a reduction in the glass-transition temperature. This phenomenon further results in the contamination of the spray dryer when the drying conditions are not adjusted (Torres et al., 2017). Additionally, packaging of the lactose-free dairy powder should be done cautiously as the high hygroscopic nature of the powder results in its caking during storage. It is because of these challenges that the lactose-free dairy powders are not a part of big markets like regular milk powders. Cheese is yet another dairy product that can be made lactose-free. This is done using lactase enzyme to incubate the cheese milk before renneting. This method is used majorly for cheeses that are young and fresh, as they comprise a considerable quantity of lactose. In the case of ripened cheeses, lactase incubation is not required as lactose gets completely consumed by the lactic acid bacteria. The studies conducted in the past have reported that unlike yoghurt, lactase treatment of cheese milk stimulates the process of acidification during cheese manufacturing. Furthermore, the addition of lactase during the process of ripening enhances the flavour of cheese. It is unclear if these effects are a result of the stimulation of the cheese microbial flora caused either by lactose hydrolysis or residual proteolytic activity occurring during lactase preparations that were available commercially in the past (Marschke et al., 1980). Lactose-free ice cream can also be produced using either lactose-free milk or powders in the ice cream mix (Abbasi & Saeedabadian, 2015). The production of lactose-free ice cream involves the addition of lactase during the ageing period before freezing, once the process of pasteurization and incubation is complete. The lactose hydrolysis increases the monosaccharide concentration which reduces the freezing point of the ice cream. This results in the production of ice cream with a soft texture at the same temperature. Although this may be a desirable attribute in several frozen desserts, it may also cause faster melting. Additionally, the sweetness resulting due to the lactose hydrolysis may help in reducing the addition of extra sugar in the ice creams, and consequently, raising the melting temperature again. In order to avert crystallization of lactose in the ice creams which causes a sensory defect known as “sandiness”, lactase treatment is employed to convert lactose into two monosaccharides—glucose and

galactose, which are highly soluble at high temperature. This treatment is essential when ice cream is produced using whey powder or WPC as the high lactose content may result in the formation of crystals during freezing (Abbasi & Saeedabadian, 2015).

5.4 Lipase

Lipase, considered as triacylglycerol acylhydrolase, EC 3.1.1.3, is a ubiquitous enzyme belonging to the family of hydrolases which reacts with carboxylic ester bonds and is present everywhere, i.e. in plants, animals, and microorganisms. These enzymes are obtained from plant and animal tissues or through the cultivation of microbes. Also, the commercial production of lipase is majorly done with the help of microorganisms such as by genetic engineering through recombinant bacteria and yeasts (Houde et al., 2004; Schmid & Verger, 1998). Naturally, lipase enzymes are useful in hydrolysing fats and oils. They lead to the production of free fatty acids, glycerol, diacylglycerols, and monoacylglycerols from the hydrolysis of triacylglycerols by acting as a catalyst in the reaction. Also, this hydrolysis reaction is reversible in order to allow the enzymes to catalyse the hydrolysis of acylglycerols through free fatty acid and glycerol (Balcao & Malcata, 1998; Macrae & Hammond, 1985) (Fig. 5.1).

Nowadays, due to high catalytic performance, lipase is gaining wide attention in industries such as for flavor development in food industry, in paper manufacturing industry, and more important role in dairy industry. It can help in providing enhanced digestibility, structure, physical properties, and reduced calorie in dairy products (Balcao & Malcata, 1998; Bjorkling et al., 1991).

5.4.1 Characteristics of Lipase

Lipase enzyme is considered as a monomeric protein with molecular weight of 19–60 kDa. Its physical properties are dependent upon chain length of fatty acid,

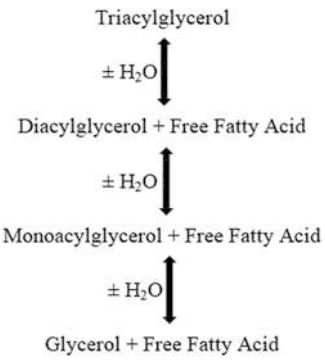

Fig. 5.1 Reaction process of lipase enzyme (Macrae & Hammond, 1985)

position of fatty acid in glycerol backbone, and degree of unsaturation. Also, its activity depends upon the pH of the solution such as lipase is stable at neutral pH and pH 4.0–8.0. For the catalytic activity of enzyme lipase, greater mild conditions and large surface area are required (Aravindan et al., 2007). Among the different lipases produced from plants, animals, and microorganisms, the most vital are microbial lipases. Microbial lipases are gaining greater attention due to their non-toxic nature, convenience, high catalytic activity, greater yield, rapid growth, and usage due to absence of seasonal interference as well as availability of cheaper media for growth (Ray, 2012). Microbial lipases are utilized in different areas such as in cosmetics, detergents, pharmaceuticals, formation of aliphatic acids, leather industry, and in treatment of waste excreted from different industries (Seitz, 1974).

Lipases obtained from microorganisms are broadly classified into two major categories on the basis of their specificity, namely, non-specific and 1,3-specific lipases. Non-specific lipases are those lipases which catalyse the reactions independent of the position esterified in glyceride molecules such as lipases obtained from *Staphylococcus aureus*, *Pseudomonas* spp., *Candida cylindracea*, and *Chromobacterium viscosum*. On the other hand, 1,3-specific lipases are those which react and catalyse only outer, i.e. *sn*-1 and *sn*-3, positions of the glycerol backbone such as lipases obtained from *Rhizopus oryzae*, *Rhizopus delemar*, *Rhizopus niveus*, *Aspergillus niger*, *Mucor javanicus*, *Candida lipolytica*, and *Humicola lanuginose* (Balcao & Malcata, 1998).

Lipases are used as biocatalysts, especially microbial lipases, in various industries such as food industry due to their various functions and properties such as regioselectivity, stereospecificity, able to function in different pH and temperature, substrate specificity, and potential to act upon heterogeneous reactions at the interface between water-soluble and water-insoluble systems (Verma & Kanwar, 2008). On the other hand, bacterial lipases are stable at a temperature range of 30–60 °C and under neutral or alkaline pH except the lipase produced from *P. fluorescens* SIK W1, which is stable under acidic pH of 4.8. Bacterial lipases are also stable in organic solvents (Verma et al., 2012).

5.4.2 Applications of Lipase in Dairy Industry

Lipase has a wide range of applications in dairy industry such as in improving the flavor and modifying the fatty acid chain of dairy products including cheese. The major role of lipases in dairy industry involves the hydrolysis of milk fat. Tissues from animals such as pancreatic glands, i.e. bovine and porcine, and pre-gastric tissues of young ruminants, i.e. lamb and calf for lipase production, are widely used in traditional cheese flavor enhancement process (Ray, 2012).

Recent applications of lipase in dairy industry involve the production of cheese, its flavor enhancement, and lipolysis of cream and butterfat. Lipases are utilized in the manufacturing of enzyme-modified cheese for providing concentrated flavor to cheese. In this process, cheese is incubated with enzymes at high temperature and thus leads to the formation of enzyme-modified cheese which can be used as an

ingredient in various products such as sauces, dressings, dips, snacks, and soups. Addition of lipases in the production of dairy products leads to the release of short chain fatty acids, namely C4 and C6, which provide sharp and tangy flavor to the products along with the release of medium chain fatty acids, namely C12 and C14, which provide soapy taste to the finished products (Hasan et al., 2006). Exogenous lipase enzyme helps in accelerating the cheese ripening process. On the other hand, addition of free lipases results in greater lipolysis which deteriorates the texture as well as flavor of cheese, but encapsulating the lipase helps in maintaining and stabilizing the enzyme-substrate ratio, thus eliminating the problem of excessive lipolysis (Houde et al., 2004). It is also used in the manufacturing of chocolates by production of cocoa butter-equivalent from palm oil with the technique using interesterification. There are many commercial lipase enzymes which are used for the production of different milkfat flavor profiles for milk-based products, such as Snow plum blossom and Palatase 20,000 L (Sarmah et al., 2017).

5.4.3 Other Applications of Lipase

In addition to the dairy industry, lipase also plays a vital role and possesses various applications in other industries such as cosmetics, pharmaceutical, leather, paper, food industry, etc. In food industry, lipase is widely used in the preparation of infant formulas which can provide an alternative of breast milk. The enzyme is also used for the modification of lipid characteristics of oils rich in omega-6 fatty acids such as coconut, sunflower, olive, rice bran, corn oil, and oils rich in omega-3 fatty acids such as linseed and fish oil. In pharmaceutical industries, lipase is used as an ingredient in the formation of products and also utilized in the interesterification of vegetable oil. It helps in enhancement of colour and texture of the dough and used as an emulsifier in many bakery industries. Lipase is also essential in the formation of cosmetic component, i.e. isopropyl myristate, for manufacturing of cosmetic items (Sarmah et al., 2017; Houde et al., 2004).

Lipases are also utilized in detergent industry as an additive in household and laundry detergents in order to vanish the oil stains from the fabrics as these enzymes can withstand harsh pH (10–11) and temperature (30–60 °C) required for washing. It also plays a vital role in paper manufacturing industry by removing the pitch, which is a hydrophobic portion of wood, i.e. waxes and triglycerides, from the pulp required for manufacturing paper (Sharma et al., 2001). In addition to this, microbial lipases such as *Candida rugosa*, *Mucor miehei*, *Pseudomonas fluorescens*, *Rhizopus oryzae*, and *Aspergillus niger* are used for the production of biodiesel. Presently, lipase produced from the microbe *Streptomyces* sp. was considered as most effective for biodiesel production (Chandra et al., 2020).

5.4.4 Introduction of Rennet

Rennet is obtained from the combination of two proteolytic enzymes, namely chymosin and pepsin, which are secreted from the fourth stomach of ruminants including calves, lambs, and kids. It is used in the cheese production from historical times, i.e. 6000 BC. Rennet is majorly available in powder, liquid, and paste forms. The powder and liquid rennet is produced on industrial scale and is obtained from calf abomasa, whereas rennet in paste form is obtained from lamb or kid abomasa. Powder and liquid rennet is most widely utilized in cheese production. Along with rennet, other enzymes produced from different origin are also utilized in manufacturing of cheese (Moschopoulou, 2011). Among the two proteolytic enzymes, chymosin is the most essential protease present in rennet. Chymosin is an aspartic protease which has the properties like pepsin and it cleaves *k*-casein in para-*k*-casein along with a glycomacropeptide (Fazouane-Naimi et al., 2010).

Clotting of milk with the help of rennet takes place in two step reaction; first includes the enzymic hydrolysis reaction and second step involves enzyme-independent protein aggregation reaction. As the concentration of enzyme increases, clotting time of milk decreases because of the greater amount of proteolysis of *k*-casein (Najera et al., 2003).

5.4.5 Different Types of Rennet

Different types of rennet are used in cheese manufacturing which are classified on the basis of their source such as animal rennet, plant rennet, and fungal rennet. Under the classification of animal rennet, calf rennet is widely utilized for cheese production, the reason being its high amount of chymosin. Presently, adult bovine rennet is used as an alternative towards calf rennet due to high pepsin content in adult bovine rennet that results in a product with high pH sensitivity and greater proteolytic activity (Harboe et al., 2010).

Many proteolytic enzymes are derived from plants such as bromelain from pineapple, ficin from *Ficus* spp., and papain from papaya. Other plants include fig, pumpkin, soybean, ash gourd, and milkweed. The enzymes are embedded in the buds, leaves, flowers, seeds, latex, and roots of the plants. But the proteolytic capability of enzymes from vegetable sources is low which makes them unsuitable for cheese production. And if excessive proteolysis occurs, it will lead to bitterness in the finished products. Although for cottage cheese and soft cheese production, extract from berries of *Withania coagulans* was used, it is also not suitable for cheddar cheese manufacturing (Garg & Johri, 1994).

Rennet clotting enzymes are also produced from fungi such as rennet from *Mucor miehei* and *Endothia parasitica*. Enzyme obtained from *Mucro miehei* results in an excellent quality of cheddar cheese, even with high ripening process due to greater stability between 4.0 and 6.0 pH with no loss of its activity and also due to its high heat stability. However, rennet obtained from *Endothia parasitica* is also useful in

cheddar cheese production, but its high proteolytic activity leads to 1.2% yield loss of the cheese (Brown & Ernstrom, 1988).

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Enzymes in Brewing and Wine Industries

6

S. Pati and D. P. Samantaray

Abstract

Enzymes are the bio-catalysts used in breweries and wineries for the biochemical transformation of a substrate to produce finished products. Several enzymes such as amylase, glucanase, protease, glycosidase, pectinase, phytase and many more exogenous enzymes are used in different stages of brewing to improve the production level as well as the quality of beer. In contrast, lipase, lysozyme, pectinase, glucanase, glycosidase, urease, protease, phenoloxidase and ester hydrolase and synthetase are employed in oenology practices for the production of wine with enormous organoleptic properties. This chapter postulates a bird's eye view of different key enzymes used in breweries and wineries, their mechanism of action and their pros and cons.

Keywords

Bio-catalysts · Brewery · Winery · Exogenous · Oenology · Organoleptic

6.1 Background

Beer and wine have concealed a fascinating part of history and our social life. Although the discovery and development of brewing and wine processing signify a cornerstone achievement of humankind, its origin is still mysterious. In ancient civilization, people used to preserve fruits and grains in wooden containers for an extended period to produce beer and wine. The entire process of production is termed as fermentation, which came from theLatin word 'fervere', meaning 'to

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A. Dutt Tripathi et al. (eds.), *Novel Food Grade Enzymes*,
https://doi.org/10.1007/978-981-19-1288-7_6

boil'. The crushed fruits and grains in the wooden container produced bubbles due to microbial action or activities as if they were boiling. During that period, people didn't fully understand that a tiny, eukaryotic fungus was making the entire recipe work (Alba-Lois & Segal-Kischinevzky, 2010). Researchers took ten decades to find out how a diminutive microbe makes the entire fermentation successful and resulted in a potation that became a status symbol in the modern era. Nevertheless, the concept of brewing and wine processing was established prior to thousands of years, which was found in the graves and settlements of early civilizations. Ancient Egyptians made wine 8000 years ago from fruits and labelled it as such by pouring it in fruit-shaped flasks, while beer came to the limelight 7000 years ago from China. Initially, Germany, the United Kingdom, the United States, Belgium, Spain and Italy were the leading countries for beer and wine production. However, in the last five decades, beer and wine production has confronted significant changes as per the demand. Global beer production reached 1940 mhl in 2018, with China being the largest producer as well as a consumer (https://www.statista.com/statistics/270275/worldwide-beer-production). In contrast, as per the data of the International Organization of Vine and Wine 2018, the global wine production has reached 292.3 mhl, with 246 mhl consumption, where, Italy, France and Spain are the highest producers.

Enzymes are the bio-macromolecules that act as catalysts in specific reactions and play a vital role in food science and fermentation technology. Since 6000 BC, enzymes have been recognized as a biochemical substance to be utilized in the processing of various foods, such as cheese preparation, brewing, wine preparation and meat tenderizing to enhance the nutritional, sensorial and functional values of the finished products (Gomaa, 2018; Gurung et al., 2013). Amongst all, enzymes offer a heroic performance in brewing and winery by accelerating the release of digestive sugars and other nutrients used by the yeasts during fermentation (Spier et al., 2016). Brewing refers to the oldest fermentation process where complex starch is bio-transformed to ethanol by the action of yeasts. This traditional process involves a series of complex endogenous and exogenous enzymes that regulate the malting of grains, the mashing of grist and rate of fermentation to produce low-calorie beer with admirable flavour, aroma and texture (Oliver, 2011; Bamforth, 2009). While grapes bio-transformed to wine by yeasts under fermentation. This process is catalysed by a wide array of enzymes that not only convert complex sugar to ethanol but also release several volatile and non-volatile substances to enhance the quality and stability of the wine (Ottone et al., 2020). Traditionally, these enzymes of brewing and winery were produced naturally by yeast or present in the grains or grapes. However, recent trends in fermentation technology pave the way for commercial production of enzymes to enhance the quality and productivity of wine and beer (Claus & Mojsov, 2018). Hence, this chapter offers a comprehensive summary on the sequential processing of brewing and winery using different enzymes as well as their mechanism of action. Moreover, the effect of enzymes on the quality and quantity of the final product and pros and cons have been enlightened.

6.2 Enzymes Used in Breweries

The process of brewing evolved centuries ago as a result of the resourcefulness and artistry of the brewers. Traditionally, lager type is the most common method of brewing, comprising low-temperature fermentation of barley and pure water with an extended maturation period. However, variations have developed in the young beer style, where brewing is not possible without the application of enzymes that hydrolyse complex starch to simple fermentable sugar and then convert it to ethanol and CO_2 by yeast or bacteria. In brewing, the keystones, malted grains and barley are the sources of enzymes, including α- and β-amylase, exo-peptidase, carboxy-peptidase, proteases glucanases and cellulases (Sammartino, 2015). The detailed key role or mode of action of these enzymes is discussed below.

6.2.1 Enzymes Used in Different Stages of Brewing

The competitive brewing industry is repetitively sounding forward to improving the process in terms of quality and manufacturing costs. Brewing that refers to conversion of cereal to ethanol involves two steps: enzymatic hydrolysis of starch to fermentable sugars and conversion of fermentable sugar to ethanol and CO_2. Depending on the brewing process, raw materials and technical preferences, different steps such as malting, mashing, pitching and aging are directly affected by the enzymes, while others are indirectly affected. The detailed process of beer preparation is presented in Fig. 6.1. The extent and achievement of each step depends on the development and stability of native enzymes that ultimately affect the quality and quantity of the final product.

6.2.1.1 Malting

Malting is the initial and prime step of brewing, which stimulates complex carbohydrate and protein-reducing enzymes present in the barley grains. The most prominent endogenous enzymes of malt include α-glucosidase, α-amylase, β-amylase, carboxypeptidase, dextrinase, lipoxygenase, xylanase and glucanase. Besides these, peroxidase, acid or alkali phosphatases, catalase, polyphenol-oxidases and phytase enzymes are also responsible for malting reactions (Spier et al., 2016; Van Oort, 2010). The malting process comprises steeping, germination and drying. In steeping, barley grains are immersed in water for 48–60 h under oxic conditions that initiate germination and biosynthesis of amylase, glucanase, proteases and carboxypeptidase. Amylase acts on the modification of complex starch, whereas β-glucanase hydrolyses β-glucans for malting clarification and proteases act on the complex protein content of the grain (Van Oort, 2010). Then malt is subjected to drying with a temperature between 90 and 140 °C to enhance the flavour and colour of the finished product (Curtis, 2013). The challenge during this step is to maintain the quality of beer as the application of heat can adversely affect the functionality of enzymes.

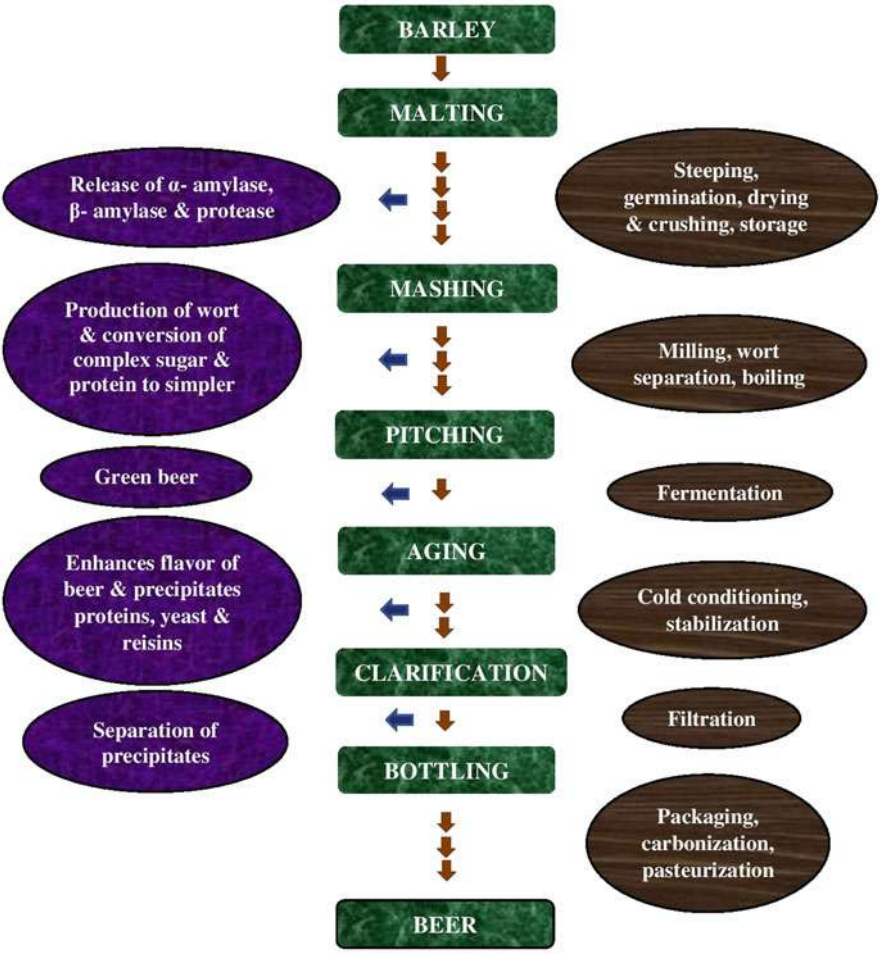

Fig. 6.1 Overview of brewing

6.2.1.2 Mashing

Mashing begins with boiling of malt with water, malt adjuncts and hops at different temperatures such as 50, 62, 72 and 78 °C for proteolysis, gelatinization, saccharification and inactivation of malting enzymes, respectively. Mashing embraces α- and β-amylases and protease, which degrade complex starch to maltose and dextrins, and the undesirable barley endosperm complex proteins to simple proteins, respectively (glutelin and hordein) (Dhillon et al., 2016; Spier et al., 2016). Additionally, exogenous enzymes, glucoamylases and pullulanases are added during mashing that break down the α-1,4 and α-1,6 linkages of starch to produce maltose, dextrin, maltotriose and glucose (Briess, 2013).

6.2.1.3 Pitching

Pitching refers to the addition of different strains of *Saccharomyces* such as *S. cerevisiae*, *S. uvarum* and *S. carlsbergensis* to the fermenter as inoculum. These strains own the MEL gene that synthesize α-galactosidases to cleave oligosaccharide melibiose to glucose and galactose. However, research is still on its way for genetically modified yeasts that can synthesize glucosidase, amylase and glucanase for metabolization of wort and soluble proteins (Stewart et al., 2013; Van Oort, 2010).

6.2.1.4 Aging

The beer obtained after completion of fermentation is called green beer due to its harsh taste and which can be removed by a process called aging. In aging, green beer is collected from a fermentation vat and stored in refrigerated conditions from a few weeks to several months. This process enhances the flavour of beer and precipitates yeasts, proteins and resins. During this period, diacetyl reductase is secreted by yeast, which reduces diacetyl to acetoin to develop an undesirable butterscotch flavour in beer. Thus, exogenous enzyme acetolactate decarboxylases is supplied that directly converts acetolactate to acetoin thereby reducing the undesirable flavour of beer during aging (Spier et al., 2016). In addition, several other enzymes actively involved in brewing are presented in Table 6.1.

6.2.2 Types of Enzymes in Brewing

The brewing process requires a prodigious knowledge of enzymology as it not only improves the quality of beer but also adds innovated attributes to the beer. Thus, enzymes with varied action and properties are used in the brewing industry. Enzymes involved in brewing can be either endogenous or exogenous. Endogenous enzymes are secreted naturally during the process of malting and mashing. Amongst all, the principal endogenous enzymes like amylase, protease, glucanase and peptidase are involved in brewing (Gomaa, 2018; Bamforth, 2009). On the other hand, at the pinnacle of enzyme technology, exogenous enzymes are applied to rectify the issues that evolved during brewing as well as to enhance the shelf life of beer by maintaining quality. Ficin, alpha-acetolactate decarboxylase (ALDC) and papain are commercially available enzymes typically used in brewing (Boulton, 2013). Details of all the enzymes are briefly discussed below.

6.2.2.1 Amylase

During brewing, both α- and β-amylases are involved in the biotransformation of complex starch to simple sugars such as dextrin, maltose, glucose and oligosaccharide (Bamforth, 2017). Endogenous α- and β-amylases are released from the barley, when the outer membrane of the granules is hydrolysed by xylanases and glucanases. During malting and mashing, amylase degrades complex starch, thereby increasing the availability of fermentable sugar. Amylase (α) strikes the internal α-(1–4) glycosidic linkage of α-glucose amylose and amylopectin to produce dextrin, whereas β-amylase cleaves the external α-(1–4) glycosidic bonds of amylose

Table 6.1 Sources and functions of enzymes involved in brewing

Enzymes	Process	Sources	Function
α-Amylase	Malting/ mashing	Endogenous/ exogenous (*Bacillus licheniformis*, *Bacillus subtilis*)	Improve starch hydrolysis and clarification
β-Amylase	Malting/ mashing	Endogenous/ exogenous (*Bacillus licheniformis*)	Improve starch hydrolysis, saccharification, malting and fermentation yield
Protease	Malting/ mashing/ storage	Endogenous/ exogenous (*Aperguillus* sp. and pineapple latex)	Boost fermentation and increase malting, clarification, chilling and storage quality
β-Glucanases	Malting/ mashing/ fermentation	Endogenous/ exogenous (*Trichoderma* sp. and *Orpinomyces* sp.)	Improve malting and clarification, lower viscosity and support clear wort production
Fungal α-amylase	Fermentation	Exogenous (*Aspergillus* sp.)	Increase fermentation yield
Amyloglucosidase	Mashing	Exogenous (*Aspergillus niger*)	Increase glucose content in wort
α-Acetolactate-decarboxylase (ALDC)	Fermentation	Exogenous (recombinant *Bacillus subtilis*)	Reduce fermentation time
Cysteine endopeptidases	Post-fermentation	Exogenous	Improve stability of beer and shelf-life of packaged beer
Glucoamylase	Mashing	Exogenous	Increase glucose content in wort
Phytase	Mashing	Endogenous/ exogenous (bacterial origin)	Reduce initial pH during mashing of lager beers
Pentosanases	Malting	Endogenous	Promotes conversion of pentosans into arabinose and xylose, also improves extraction and filterability of beer

and amylopectin to release maltose. Both the amylases function optimally at pH 5.2–5.5 and temperatures about 62–74 °C (Sammartino, 2015). Moreover, α-amylase is only used endogenously during brewing, while β-amylase is used endo as well as exogenously due to its vital role in saccharification.

6.2.2.2 Protease

Proteases have many reimbursements in brewing like digestion of peptide bonds of proteins to enhance clarification of malting, augmentation of protein solubility, lowering of beer viscosity and favour rapid yeast growth. Temperature labile protease has a key role in the malthouse, functions optimally at 52 °C and can be deactivated at 70–75 °C, however, it can function at a wide range of pH. Though

protease is endogenous, it is advisable to use exogenous protease to maintain the quality of beer. Brewers are acclaimed to use 0.3–1 kg of protease per ton of barley as an overdose of protease can cause foam instability leading to degradation of beer quality (Gomaa, 2018).

6.2.2.3 β-Glucanases

It is the key enzyme of malting and mashing involved in the digestion of the outer membrane of starch that allows other hydrolysing enzymes to act on starch granules. This enzyme not only lowers the viscosity of beer but also helps protease activity in the hydrolysis of a matrix of starch granules to make the kernel soften during germination. Though, β-glucanase are present naturally in barley, in the case of light beer it is supplied externally (0.3–1 kg per ton of wort) for improvement of light quality and texture of the beer (Bamforth, 2017; Lyven, 2016). β-Glucanase hydrolyses the cell wall at an optimum pH of 6 and temperature of 45–50 °C, however, this extremely heat-sensitive enzyme denatures at 60 °C (Bamforth, 2017). Thus, heat-stable microbial β-glucanases of *Bacillus subtilis*, *Aspergillus*, *Penicillium* and *Trichoderma* are used exogenously in the mashing stage of brewing.

6.2.2.4 Exogenous Enzymes

When the enzyme technology is at its zenith, other than endogenous many exogenous enzymes are also supplied to maximize the fine ability of beer during processing, storage and transportation. Some of the prime exogenous enzymes, bromelain, ficin and papain, belong to protease and alpha-acetolactate decarboxylase (ALDC) from lyases is involved in brewing (Boulton, 2013). Ficin, bromelain and papain are the chill-proof enzymes extracted from Pawpaw latex and fig plants and they are responsible for chilling haze by removing polyphenols and polypeptides leading to hydrolyzation of proteins as well as improvement of colloidal stability of beer. This chill-proof enzyme functions in a wide range of pH with temperatures ranging from 60 to 65 °C and supplies directly to the storing vessels at a concentration of 1–2 g/hl of beer (Gomaa, 2018; Boulton, 2013). Besides, ALDC is added to the beer during pilot-scale production for increasing the product yield at less fermentation time, without affecting the quality. ALDC attacks the C–C bond in the α-acetolactate for catalyzing the bioconversion of α-acetolactic acid to acetoin (Hans, 2008; Dulieu et al., 2000). It remains active at 25–40 °C temperature, 5–7 pH and is added directly during the initial period of fermentation at a concentration of 1–5 g/hl of wort and 0.4–1 g/hl of wort in post-fermentation period. Apart from these, many other exogenous enzymes such as pullulanases, phytase and diastase is also used efficiently during malting, mashing and post-fermentation period to enhance the quality of beer.

6.2.3 Effect of Enzymes on Quality of Beer

One of the most momentous advancements in brewing is to study the use and effect of different enzymes for beer production in order to fulfil the demand of consumers

for a beer with an appealing taste and flavour. Therefore, it is the need of the hour to know the effect of enzymes on the quality of beer. Malting is the sole source of endogenous enzymes like α- and β-amylases that are actively involved in the conversion of complex starch to simple fermentable sugar. These enzymes are also responsible for increasing the fermentability by boosting the saccharification, higher product yield and enhancing of thermal stability of mash during brewing, which ultimately removes the bitter taste from beer. The poor activity of β-glucanases can lead to deprived runoff, recovery, spent-grain drainage, filter performance and sedimentation in beer. However, surpassing β-glucanases activity makes the mash thermostable and boosts the fine ability and filterability of beer (Aehle, 2007). Malthouse enzyme protease provides foam stability in brewing. Moreover, the shelf life improvement, maturation and flavour of beer are also greatly affected by enzymatic activity. These enzymes ensure protein solubilization during mashing, together with sugar availability to yeast during fermentation. Enzymes like amylase also decrease the fermentation period during pilot-scale brewing. Furthermore, enzymes in combination with substrate and temperature affect the carbonation, aroma and flavour of the beer. Many commercial enzymes are also cast-off to boost the quality attributes (clarification, texture, colour and flavour) of beer. The enzymes utilized in brewing are assorted in their action and properties and are key factors for the improvement of each step of brewing. Hence, intensive research is highly essential regarding the mode of action and properties of these brewing enzymes.

6.3 Enzymes Used in Wineries

Biotechnological advances facilitate the combined use of fruit juice, yeasts and enzymes to make a surface for wine preparation. Wine is the partial or complete alcoholic fermentation of grape juice by yeasts where sugars are bio-transformed to ethanol and other metabolites. These metabolites, including volatile and non-volatile composites add significant flavour, colour, odour, taste and aroma to wine. Enzymes are either naturally present in the grapes or supplied exogenously to catalyse fermentation process as well as enhance the sensory properties of the wine. In this high-tech era of biotech, endogenous and exogenous enzymes are involved at different stages of wine preparation for smooth management of pre and post-fermentation conditions. Several enzymes such as pectinases, glucanases, glycosidases, xylanases, glucose oxidases, proteases, ureases, peroxidases and proteases are actively involved in the catalyzation of different reactions occurring during wine preparation. The role of these enzymes at various stages are momentarily discussed below.

6.3.1 Enzymes Used in Different Stages of Wine Preparation

Wine production begins with the collection of matured grapes followed by crushing for extraction of juice. Then, the juice is subjected to maceration for must formation, where enzymes start functioning as a part of pre-treatment. The must undergo fermentation by yeasts to obtain raw wine with a precise aroma due to the involvement of specific enzymes. In clarification, pectic enzymes are implied to reduce the viscosity and turbidity of the wine. Finally, suitable enzymes are used in the ageing process to get good quality wine, where pertinent physicochemical properties are incorporated into the wine. The implementation of various enzymes in different steps of wine preparation is presented in Fig. 6.2.

Fig. 6.2 Overview of wine preparation

6.3.1.1 Maceration

Desired enzymes of maceration not only expedite extraction of colour, aroma and antioxidant activity but also regulate ethanol concentration of the wine (Ottone et al., 2020). The endogenous cellulases and hemicellulases catalyse the lysis of grape cells, whereas pectinases lead to the degradation of polysaccharide to accelerate the extraction of juice. These enzymes cause activation, release and solubilization of derivatives of antioxidant activity and colour and fragrance precursors (Garg et al., 2016). Besides this, the addition of glucose oxidase to the must regulate the ethanol concentration of the wine, as it maintains the concentration of glucose in the must by using it as an electron acceptor. However, the by-product H_2O_2 released during maceration oxidized the phenolic components of the wine, resulting in reduced antioxidant activity. Thus, exogenous catalase enzyme is added to the must for conversion of toxic H_2O_2 to non-toxic H_2O.

6.3.1.2 Fermentation

The overall flavour and aroma of the wine are determined by the concentration of volatile components synthesized during bioprocess technology. Wine can be prepared with four types of aromas such as varietal, pre-fermentative, fermentative and post-fermentative. Production of varietal aroma is due to the partial metabolism of grapes depending on the variety, ripening percentage, soil and climate, while pre and post-fermentative aromas are developed during maceration and ageing (Samoticha et al., 2017). Moreover, the fermentative aroma is generated by acids, esters, alcohols and sulfur and carbonylated substrates produced by yeasts during fermentation. Oenology is incomplete without the involvement of yeasts like *Saccharomyces cerevisiae* and *Saccharomyces carlsbergensis.* These yeasts release certain enzymes that enhance the rate of fermentation and product yield and add flavour and aroma to the wine. Invertase is one of the most vital enzymes that causes hydrolysis of saccharose to fructose and glucose (Ribéreau-Gayon et al., 2006), whereas β-1,3-glucanases catalyses the synthesis of mannoprotein in fermentation medium and cell wall hydrolysis. Pectinases and β-glucosidase also found in yeasts catalyse the degradation of pectic components and improve the aroma of the wine respectively (Merín et al., 2014; Villena et al., 2007). Except for these endogenous enzymes, exogenous β-glucanases are also implemented during fermentation for better stability and structure of the wine (Spagna et al., 2002).

6.3.1.3 Clarification and Stabilization

In wine preparation, clarification steps make the way for bottling. The chemical reactions that occur during maceration and fermentation release many insoluble, floating materials that develop a cloudy appearance in the wine. Thus, in clarification and stabilization all the suspended materials are removed by processes like flotation, centrifugation, filtration, pasteurization, refrigeration, maturation and racking to improve the quality of the wine. Pectinase enzymes are added in the initial period of clarification to increase the juice yield, colour and flavour of the wine and to reduce the viscosity and turbidity of must (Martín & Morata de Ambrosini, 2014). It also significantly reduces the filtration time of wine preparation. Though pectinase is

highly indispensable for clarification, they are not synthesized naturally during wine preparation. Thus, commercially synthesized pectinase from microbes and plants is employed during bioprocess technology. On the other hand, protease is added to the medium to circumvent haze formation. Sometimes the reaction medium gets contaminated by *Lactobacillus* sp. and *Pediococcus* sp. causing acidity, mousy taint and buttery flavour in the wine (Bartowsky, 2009; Liburdi et al., 2014). Thus, lysozyme is supplemented, which not only inhibits the growth of these contaminants but also enhances malolactic fermentation (Ottone et al., 2020).

6.3.1.4 Ageing

Ageing potentially improves the quality of the wine, where it is aged for a significant time period till the availability of oxygen for the development of premium flavour, aroma and taste. However, throughout ageing the naturally occurring urea and ethanol react to form ethyl carbamate, which has carcinogenic activity. Therefore, acid urease is added in this period for removal of urea that effectively minimizes ethyl carbamate formation to reduce the toxicity in the wine.

6.3.2 Types of Enzymes in the Winery

Over the past decades enzymes have been established as a processing aid in oenology. The enzymes offer several advantageous bids during pre-fermentation, fermentation, clarification, stabilization and ageing steps that ultimately enhance the juice yield and add colour, odour and flavour to the wine, leading to the generation of extremely fine-quality wine. Pectinase, lipase, lysozyme, glucanases, glycosidases, ester hydrolases and synthetases, phenol oxidases and urease are the potential enzymes widely used in wine preparation (Table 6.2). The above-said enzymes are

Table 6.2 Enzymes of the winery and their functions

Enzymes	Functions
Lysozyme	Prevents the growth of undesirable lactic acid bacteria in the wine
Pectinase	Involved in degradation of pectin components, hydrolysis of cell wall, and enhances the quality, stability, colour and flavour of the wine
Glycosidase	Improves flavour and develops varietal aroma from odourless precursors in the wine
Glucanases	Liberate mannoproteins for the development of varietal flavour and cause lysis of the microbial cell wall to boost the clarification and filtration during wine preparation
Protease	Prevents haziness and reduces bentonite demand of the wine
Urease	Hydrolyses urea to prevent the synthesis of carcinogenic ethyl carbamate produced during the ageing stages of wine preparation
Lipase	Degrades lipids for better extraction of colour during wine preparation
Phenoloxidases	Enhance the stability and sensory attributes of the wine
Esterhydrolases and synthetases	Promote malolactic fermentation and develop fruity flavour in the wine

naturally synthesized during oenological practice; however, in some cases, they are also supplied externally.

6.3.2.1 Pectinase

The grape cell comprises cellulose, hemicellulose, mannan, pectin and xyloglucan linked with proteins. During wine processing, the highly viscous pectin impedes juice extraction, filtration, clarification and diffusion of aromatic components into the must (Claus & Mojsov, 2018). The enzyme pectinase hydrolyses pectin to enhance the juice yield and ease clarification and filtration. Pectinase treatment boosts the absorbance of phenols and anthocyanins by the must, as well as intensifies the colour and clarity of the wine (Mojsov et al., 2011). Polygalacturonase, pectin lyase, pectinesterase and acetylesterase are the pectinase enzymes actively involved in wine preparation. However, pectinase is neither present naturally in grapes nor secreted by the yeasts. Therefore, commercial pectinase solutions composed of 2–5% of active enzymes and additives (preservatives, sugar and salt) are used in wine preparation (Mojsov, 2013). The pectinase available in the market is generally of fungal origin or produced by *Rhodotorula mucilaginosa* and *Cystofilobasidium capitatum*. Nevertheless, high amounts of tannin, alcohol (above 17%) and SO_2 (above 500 mg/l) render the pectinase activity (Van Rensburg & Pretorius, 2000).

6.3.2.2 Lysozyme

Traditionally, SO_2 was added to the medium to prevent microbial contamination in the wine, but an allergic response caused by sulphites pushes the oenologist for an enzymatic solution (König & Fröhlich, 2017; Campos et al., 2016). Thus, lysozyme is used to prevent microbial contamination, which kills the bacteria by cell lysis. Lysozyme stabilizes the wine by preventing malolactic fermentation. As per the International Organization of Vine and Wine, hen's egg lysozyme at a concentration of 250–500 mg/l can be used in wine preparation. Moreover, after completion of bioprocess technology, this enzyme can be removed from the wine by the implementation of fining agents.

6.3.2.3 Glycosidase

Almost 90% of aroma precursors such as phenolic compounds, nerol, terpenes linalool and geraniol are present in grapes' skin in conjugated form as odourless compounds. Glycosidase hydrolyses these precursors to liberate volatile, aromatic terpenes that ultimately activate the organoleptic properties of the wine. These enzymes are naturally found in grapes and promote the liberation of aromatic compounds under optimized condition. However, these grape glycosidases are inactivated at high alcohol and glucose concentrations and at pH 5. Therefore, commercially available glycosidases extracted from the species of *Saccharomyces*, *Pichia*, *Candida* and *Rhodotorula* are also employed in wine preparation (Claus & Mojsov, 2018; Ugliano, 2009).

6.3.2.4 Glucanase

Lactic acid bacteria and some fungus associated with the skin of grapes release viscous polysaccharides, which hamper the wine filtration. Neither flocculants nor filtration can remove these polysaccharides; thus, glucanase is used for reduction of wine viscosity. Both endo andexo-glucanase release mannoproteins to enhance the varietal flavour in the wine. Endo-glucanase is naturally synthesized by *Saccharomyces* species during fermentation, while exo-glucanase is supplemented to the fermentation medium. Moreover, the commercially available exo-glucanase is extracted from fungus like *Taleromyces versatilis* and *Trichoderma* sp., different species of yeast like *Kloeckera*, *Zygosaccharomyces* and *Pichia* and lactic acid bacteria (Claus & Mojsov, 2018).

6.3.2.5 Protease

Proteins present in must or synthesized by starter culture cause allergic reactions in the consumer (Van Sluyter et al., 2015; Rizzi et al., 2016). Though proteins are precipitated after fermentation, the presence of acid, proteolytic and heat-resistant pathogenesis-related (PR) proteins causes undesirable turbidity in the wine. Generally, bentonite is used for the removal of PR proteins, but it can adversely affect the quality and quantity of the wine (Jaeckels et al., 2015). Thus, researchers are now focusing on protease as an attractive enzymatic solution for the removal of these undesirable proteins. Protease can not only enhance the quality and quantity of the wine but also reduce the haziness in the wine. *Saccharomyces* species doesn't show any protease activity; however, some fungus and non-*Saccharomyces* species depict the same. So, nowadays researchers are shifting their attention to selecting or developing protease positive starter culture for wine production.

6.3.2.6 Urease

In wine, fermentation yeast generates urea that is chemically converted to carcinogenic ethyl carbamate (Lonvaud-Funel, 2016). In 1997, urease was established as an enzymatic solution that cleaves urea into CO_2 and NH_3 and prevents ethyl carbamate synthesis. Generally, urease of *Lactobacillus* origin is used at a concentration of 25–50 mg/l in the fermentation medium (Pozo-Bayón et al., 2012).

6.3.2.7 Lipase

Lipids are released during wine fermentation as a result of the autolysis of yeast or grape cells, which cause significant changes in fermentation as well as in the finished product. Hence, lipase is employed in wine preparation to decompose the lipids present in the cell membrane for the improvement of the colour and texture of the wine. It is found in a few wild strains of *Lactobacillus* and yeasts.

6.3.2.8 Esterhydrolases and Synthetases

Esters are either present in grapes or synthesized by yeasts during fermentation, which contributes a significant fruity flavour to the wine. Esterhydrolases and synthetases regulate the concentration of the ester during malolactic fermentation by promoting its synthesis as well as hydrolysis (Ugliano, 2009).

6.3.2.9 Phenoloxidases

Phenoloxidases affect not only the sensory attribute of the wine but also the final phenol concentration of the wine. However, these oxygen-sensitive enzymes on exposure to O_2 cause enzymatic browning of wine. Phenoloxidases include tyrosinase, responsible for implementation of colour to the wine, and laccase, which causes phenol oxidation to enhance the organoleptic properties of the wine (Claus & Mojsov, 2018).

6.3.3 Effect of Enzymes on Quality of the Wine

Enzymes have a very controversial effect on wine preparation as they are highly selective. Several studies have been directed to know the effect of different enzymes on the organoleptic as well as quantitative properties of the wine. The colour extraction of the wine is greatly influenced by xylanase and glycosidase activities. During maceration, the enzymatic treatment enhances the extraction of phenolic compounds while depicting undesirable anthocyanin content and colour parameters. Similarly, glycosidase helps in aroma extraction in the wine; however, an abundance of glucose inhibits its action. Lysozyme causes total inhibition of lactic acid bacteria from wine, but the presence of the residual amount of lysozyme can cause an allergic reaction in the consumer (Liburdi et al., 2014). Furthermore, protease is essential to remove PR proteins from wine, but it is only functional under restricted alcohol concentration, pH and temperature (Espejo, 2020). It can't be ignored that enzymes are highly essential for wine preparation, but the sensitivity of these enzymes influences industry and academia to analyse the pros and cons before further application.

6.4 Pros and Cons of Enzymes Used in Brewing and Winery

Before taking any step forward towards the enzymology of brewing and wine preparation, it is highly recommended to study their pros and cons. The application of enzymes in brewing enhances the maturation of beer, catalyses low-calorie beer production, stabilizes the beer by improving mashing and clarification and reduces the viscosity, leading to fine-quality beer production (Gomaa, 2018). Nevertheless, the cost is the prime barrier in the enzymatic treatment of beer. Besides, the sensitivity of enzymes towards temperature, pH, alcohol and glucose concentration also significantly affects the use of enzymes in brewing. Like in brewing, enzymes also have a significant effect towards the enhancement of the flavour, aroma and taste of the wine. Some enzymes are used to prevent contamination of the wine by toxic microbes and compounds. Enzymes also ease the wine processing for higher yield and lower production cost, leading to a booming profit. On the other hand, it is always advisable to consider the drawbacks of these enzymes for a better future. Sometimes specific activity and side effects of a few enzymes have a detrimental effect on wine quality. Also, the use of commercial enzymes requires a lot of

pre-treatment, which is time-consuming as well as expensive. Intermittently, enzymes undergo unwanted chemical reactions with the components of the fermentation medium and cause an allergic response to the consumer. Hence, a detailed study of individual enzymes is highly essential before their use.

6.5 Conclusion

In the present scenario, breweries and wineries have established themselves as extremely lucrative businesses in the agriculture and alcohol industries. Earlier these two were produced by traditional methods, but in the modern era, the establishment of brewery and winery is not possible without the involvement of enzymes. On account of this, brewers and oenologists amalgamate traditional and advanced enzymological techniques for the development of modern methods for beer and wine production. Amylases, glucanases, proteases and cellulases are the foremost important enzymes being used in beer industries. Though the implementation of enzymes ensures faster and higher beer production, the brewer should screen the appropriate enzyme with eyes open because a slight modification in enzymatic treatment can cause devastating effects on the finished product. On the other hand, enzymes have a flourishing future in the wine industry and a wide array of enzymes are being used for the furtherance of different stages of wine preparation. Pectinases, protease, glucanases, lysozyme and lipase are the enzymes used during the different stages of wine processing for the production of superior-quality wine. These enzymes accomplish every goal to provide chemical, mechanical and thermal stability to wine. Moreover, recent advances in biotechnology pave the way for researchers to articulate a stringent plan of work or phenomenon to overcome various drawbacks of brewery and winery. This chapter intensely discussed several aspects of enzymes used in brewery and winery; however, further study is highly essential in this regard.

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Enzymes in Meat, Fish, and Poultry Products Processing and Preservation-I

7

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Abstract

Enzymes are used in the food industry widespread to modify and enhance the nutritional value, and functional and sensorial characteristics such as color, smell, and flavor of different types of food products.

The technological applications of enzymes in the meat industry are a hot topic nowadays. The most common application of enzymes in red meat products is to increase the meat tenderness. The toughness of beef meat produced from dairy cattle is related to the volume of connective tissue, mainly collagen. Proteolytic enzymes have the potential to tenderize lower-grade meats and increase their market value. Consumers' demand for fresh meat without undesirable excessive fat and salt content leads the manufacturer to consider new possibilities of enzyme application to increase the carcass efficiency by using more effective slaughtering techniques.

The utilization of enzymes to maximize the by-products' efficiency is an undeniable possibility.

The common by-products of the poultry industry are feathers, which can be digested by enzymes and applied for animal feed and also nonfood industries such as films, coating, and packaging due to their high amount of keratins and hydrophobic amino acids. Fat, bone, and mechanical flesh are common by-products of meat processing, which can be digested by enzymatic reactions and used for their special meat properties. Enzymes also can be applied in seafood processing for deskinning and descaling, production of caviar, and recovery of

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A. Dutt Tripathi et al. (eds.), *Novel Food Grade Enzymes*,
https://doi.org/10.1007/978-981-19-1288-7_7

by-products. In the current chapter, the potential application of enzymes in the meat, poultry, and seafood industry will be discussed.

Keywords

Meat · Poultry · Seafood · Enzymes · Preservation

7.1 Application of Enzymes in Red Meat Industry

7.1.1 Role of Enzymes in Meat Tenderization

Meat tenderness, which is determined by meat toughness, depends on many factors such as structure, nature, and the volume of connective tissues mainly collagen and elastin (Lepetit, 2008). Meat tenderness is recognized as one of the main criteria in scoring quality characteristics of meat. There are several methods and equipment to assess the tenderness of meat, such as determination of enzyme activity, scanning electron microscopic (Koohmaraie et al., 1988), analyzing the myofibrillar fragmentation index (Olson & Parrish, 1977), and measuring hydroxyproline (Ashie et al., 2002).

Tenderization is one of the substantial events in postmortem changes which can been affected by decreasing the pH value, temperature, time, and the condition of animal prior to slaughter. During the tenderization, endogenous proteolytic enzymes become active and attack all muscle proteins such as myofibrillar and connective tissues. As the result of proteolytic enzyme activity, the structural and biochemical changes occur, which lead to a significant increase in meat tenderness (Koohmaraie & Geesink, 2006; Herrera-Mendez et al., 2006; Hwang et al., 2003).

7.1.1.1 Endogenous Enzymes

There are several endogenous enzymes which play a role in postmortem tenderization, including calpains, lysosomal cathepsins, and proteasomes. Among these, the calpain system is the primary key of tenderization process. Cathepsins stands in the second level of protein degradation and proteasomes have no significant effect due to unsuitable role of myofibrils as its substrates (Koohmaraie & Geesink, 2006; Kemp et al., 2010).

Calpains

The calpain system, which is activated by calcium and thiol, contains three enzymes three protease enzymes: calpain I (μ), calpain II (m), calpain 3, and calpastatin, which is recognized as calpain inhibitor. Calpain I is linked to myofibrils of muscles, whereas calpain II and calpain 3 are situated in cytosol and sarcomere (near the Z and M line in myofibrillar structure), respectively.

It has been suggested that μ-calpain has an actual role on postmortem proteolysis. Many factors effect on calpain system activity, particularly the calcium ion

concentration; thus, any operation which increases the calcium ion can improve meat tenderness (Moudilou et al., 2010).

Studies revealed that injection of calcium or application of electrical stimulation causes an increase in meat tenderness due to increase in calcium level and decrease in calpastatin activity. The changes in Z line and degradation of myofibrillar proteins such as I troponins, T troponins, desmins, titins, and nebulins are the most common phenomenon during caplain system activity.

Cathepsins and Other Enzymes

Cathepsins contain three classes of enzymes according to the presence of different amino acids in their structure: cysteine, serine, and aspartic peptidase.

The role of cathepsins in tenderness of meat is still uncertain, due to lack of information regarding their release from lysosomes through postmortem storage of meat. It is suggested that small amount of myofibrillar proteins such as actin, myosin, and α-actinin can be affected by cathepsins.

Studies show that other enzymes such as endogenous collagenases, elastases, and proteases can cause the degradation of the structure of collagen and elastin.

7.1.1.2 Exogenous Enzymes

Furthermore, there are another group of enzymes which are derived from plants, animals, and microbial sources and have potential to effect on meat tenderization. The most common exogenous enzymes used for meat tenderization are proteases. The proteases are vital for cell growth as they are capable to catalyze and hydrolysis the peptide bond of proteins and provide amino acids for growth and differentiation.

Proteases have diverse variety due to their structure, protein characteristic, and mode of action. The exogenous proteases are commonly classified by their original source. The exogenous proteases that have been approved by Food and Drug Administration (FDA) and recognized as GRAS substances are as follows: plants (papain, ficin, bromelain), microbes (*Aspergillus niger*; *Bacillus subtilis*; *Rhizopus oryzae*), and animals (trypsin, pepsin).

Plant Enzymes

Papain

Papain is a protease enzyme which contains cysteine and extracted protease from the latex of the papaya plant (*Carica papaya*). This enzyme is a part of defense system of plant and protects the plant against insect. The optimum temperature and pH for enzyme activity is 65 °C and 5–8, respectively.

Commercial forms of papain contain various ratio of papain, papaya peptidase, and chymopapain and lead to different biochemical properties. Papain tenderizes the toughness of meat by acting on amino acids which have aromatic side chain like tyrosine and phenylalanine. Papain can effectively hydrolyze collagen and myofibrillar proteins. Investigations show that injection of papain approximately 30 min before slaughter can cause meat tenderness. As injection of papain to animal may

cause stress symptoms and severe shock, using inactive papain can be an alternative approach.

Ficin

Ficin is extracted from latex of fig fruits (*Ficus glabrata*, *Ficus anthelmintica*). Figs contain ten different proteases and ficin is most popular for its tenderizing effect. Studies revealed that application of ficin in temperatures of 60 and 70 °C has the most influence in tenderization process. It is suggested that ficin has the ability to effect on collagen, elastin, and myofibrillar proteins in addition to increase in water-holding capacity.

Cucumin

Cucumin is another proteolytic enzyme which is achieved from kachri (*Cucumis pubescens*). The researches show that cucumin can be effective on tenderizing buffalo meat, sheep meat, and chicken.

Bromelain

Bromelain is cysteine proteases which originated from pineapple. It has two sources: fruit bromelain and stem bromelain; it has been shown that fruit bromelain is more specific and more effective in comparison to stem bromelain. The optimum temperature for enzyme activity is 50–60 °C; however, the studies showed that the enzyme activity remains at 0 °C and it becomes inactive in temperatures more than 80 °C. The bromelain activity starts by degradation of collagen, and then attacks myosin.

Zingibain

Zingibain is a potent proteolytic enzyme which is obtained from ginger. The studies reported that Zingibain is more effective in meat tenderization when heated. Ginger is a great and economical source of proteases that begin degradation from the I band of each sarcomere and go through M line.

Actinidin

Actinidin is a natural enzyme extracted from kiwi fruit which has been recognized as an agent which can hydrolyze both connective tissue proteins specially collagen and myofibrillar proteins. Studies show that applying actinidin in brine solution can tenderize porcine muscles.

7.1.2 Role of Enzymes in Meat Flavor Development

Flavor has a complex definition which includes several phenomenons such as taste, odor, trigeminal senses, and texture. As well as the mentioned factors, tenderness significantly affects the flavor and the relationship of all these together creates consumer acceptability. Generally, the raw meat is tasteless due to absence of nonvolatile components; however, the flavor precursors are present in the meat

tissue and it is necessary to undergo enzymatic reaction to tastes appears in the meat (Fernandez et al., 2000; Toldra, 1998; Calkins & Hodgen, 2007).

As the results of several enzymes activity such as lipase, protease, glutaminase, and peptidase, biochemical reactions occur and lead to enhance the flavor. These enzymes may be endogenous natural lipases and protease, microbial originated enzymes or those which added to the product throughout the manufacturing procedure. Due to proteases activity mainly cathepsins, trypsin-like peptidases, and *Micrococcaceae* originated enzymes (Fernandez et al., 2000), nitrogenous complexes such as volatile compounds, small peptides, and free amino acids appear. As well as micrococci, other species of lactic acid bacteria, such as *Pediococcus* and *Lactobacillus*, are able to produce intracellular peptidases which contribute to increase the levels of free amino acids. These substances have principal effect on the taste of the product.

Another group of enzymes which takes part in flavor enhancement are lipases. Lipases produced by some microorganisms like lactobacilli or micrococci could effect on the lipids by their lipolytic activity and oxidation, leading to increase in the level of free fatty acids and short chain fatty acids. The substances produced by lipases are responsible to develop the flavor in final product. Although the optimum temperature for maximum activity of microbial lipases is 30–40 °C, some thermophilic microorganisms such as *Pseudomonas*, *Bacillus* sp., *Thermomyces lanuginosus,* and *Aspergillus niger* release lipases which can stand in the higher temperatures (50–65 °C).

Glutaminase also play a role in creating the flavor of sausages by deamidation of glutamine, leading to production of L-glutamic acid and ammonia through hydrolysis of the glutamine amide group. Glutamic acid is an amino acid which is responsible for the flavor enhancement and producing by starter cultures has an important role in flavor in sausage, seasonings such as soya sauce and miso, and pickles. Glutamic acid is the substance which increases "umami" taste in food products. The ammonia, produced by glutaminase, performs as an acid neutralizer. Glutaminase can be obtained from *Bacillus amyloliquefaciens*, *Aspergillus oryzae*, *Debaryomyces,* and *Rhizobium etli*.

7.1.3 Approaches for Utilization of Enzymes as in Meat Tenderizer

There are several traditional approaches to apply tenderizing substances into carcasses. These methods include injection; dipping and spraying the enzymes have both advantages and disadvantages. The main concern regarding utilization of traditional method is incomplete and insufficient distribution of the tenderizing agents into meat pieces. Neglected penetration of enzymes into the meat pieces causes surfaces to become over-tenderized while interior parts of meats remain unchanged (Rhodes & Dransfield, 1973; Lawrie, 1998).

7.2 Application of Enzymes in Seafood Industry

7.2.1 Deskinning

The most important application of enzymes in the seafood industry is to deskinning and descaling. In this procedure the skin is removed with the least damage to the flesh. If the deskinning process done suitable, the edible portion can increase. The main source of enzymes to use in the seafood industry is marine enzymes. Application of enzymes in combination with mechanical methods has been performed for processing of tuna, squid, herring, shrimp shells, Pollock, and skate (Simpson et al., 2012).

7.2.2 Production of Fish Protein Hydrolyzate

Fish protein hydrolyzates are recognized as one of the important by-products of the seafood industry which can be obtained by enzymatic or chemical reactions. The wasted of fish industry such as gills, head, skin, bones, viscera and liver are the main sources for producing fish hydrolyzates which are in the forms of peptides with 2–20 amino acids depends on the type of enzyme, kind of fish and the time of operation.

Fish hydrolyzates also can be obtained by alkali or acid reactions in high temperature and pressure in the traditional method. The major acids utilized in acid hydrolysis are sulfuric acid and hydrochloric acid. One of the disadvantages of acid and alkali hydrolysis is production of great amount of sodium chloride and sodium hydroxide, respectively, which decreases its functionality. Moreover, destruction of tryptophan, a critical amino acid, and production of several toxic compounds are other concerns regarding using acid hydrolysis approach.

On the other hand, enzymatic hydrolysis has several advantages, such as being economical, and required slight conditions of pressure, temperature, and pH. Fish hydrolyzates demonstrate functional properties in food formulation and can be applied as emulsifier or a substance which increase water-holding capacity, protein solubility, gelling activity, and oil-binding capability.

Fish hydrolyzates which are mostly produced by action of alcalase are more constructive compared to poultry by-products.

7.3 Application of Enzymes in Poultry Processing

Poultry industry with various products including chicken, egg, and turkey has a large portion in food sector and human diet. Due to side effects of red meat consumption, nutritional benefits of white meat and ease of preparation of such products for customers and of the poultry industry has been growing fast, as the USA, China, and Brazil have the maximum products, worldwide.

Consequence of such a large development in poultry industry is production of high amount of waste mainly feathers, deboning residue soft meat, blood and also dead birds on arrival. Due to several concern regarding converting the poultry waste to soil fertilizer such as accumulation of fat in soil, excessive levels of oxygen requirement and probable occurrence of pathogens in hatchery, dead and litter compost, utilization of enzymes to use wastes has been recommended. Nowadays, the waste materials of poultry industry are transformed to feather meal, meat and bone meal, blood meal, and fats/oils through rendering process. While the mentioned meals are rich source of protein, their application can be restricted due to their nutritional loss particularly essential amino acids, as well as high amount of lysine, calcium, and phosphorous in meat/bone meat and bone meal.

The significant portion of waste products is feathers which is 8% of the weight of an adult chicken.

Keratin is a protein which constituted approximately 90% of the feathers. Degradation of keratin is a complicated process due to disulfide bonds between polypeptide chains and also hydrophobic interactions and several hydrogen bonds.

Additional to feathers, other parts of the bird such as legs, heads, bones, skin, and viscera contain large amount of various proteins such as collagen, keratin, and elastin. The dead birds which on the arrival are also rich source of proteins. Utilization of poultry by-products helps to avoid environmental pollution; besides, the extracted and hydrolyzed protein can be used as a nutritious part of animal diet.

7.3.1 Utilization of Keratinase for Bioconversion of Feathers

Feathers are a great source of inexpensive protein; however, due to its low nutritional value and low digestibility, its application as a feedstuff has various concerns. Conventional hydrothermal method of degradation for feathers causes losing some essential amino acids, as well as production of non-nutritious amino acids such as lysinoalanine and lanthionine. To overcome the mentioned problems, biotechnological methods via microorganisms have been offered. The keratinase produced by microorganisms such as *Bacillus* spp. has the capacity to hydrolyze keratin and convert it to peptides which can be used as nitrogen fertilizers or animal feed. Controlled enzymatic hydrolysis is an appropriate method for production of bioactive peptides that have the potential to play role as ant antimicrobial, antioxidant and antihypertensive as Fontoura et al. (2014) reported DPP-IV and ACE inhibitor activities of hydrolyzed keratin obtained by feathers of raw chicken.

Another by-product of poultry industry is fat which is significantly located in the skin. Chicken fat contains both ω-3 and ω-6 polyunsaturated fatty acids which can be beneficial to reduce cholesterol. Lee and Foglia (2000) have indicated that *Candida rugosa* and *Geotrichum candidum* are able to produce lipases which act on triglyceride fractions that have been evoked from chicken fat by supercritical CO_2.

7.4 Application of Enzymes for Enhancement of Flavor

In Addition to marine sources for production of protein hydrolyzates, various meat by-products such as bones, bovine by-products, sheep visceral mass and chicken by-products can be treated with different enzymes for this purpose. These protein rich resources, depends on type of applied enzyme and condition have the capacity to enhance the meat flavor when not proper for food use. The main concern regarding using meat by-products as source of protein hydrolyzates is formation of bitter taste.

7.5 Future Prospective

Due to consumer demand for fresh tender meat with minimum fat and salt, and also the manufactures to produce the carcasses with sufficient profits, enzymatic techniques have been utilized to achieve these aims. One of these methods which recently become popular in meat industry is using TGase which is a cold binding is enzymatic technology.

In this method, the cut and timed pieces of meat from one or several animals can be attached together with TGase to form meat products with new shape and size, which can be stored in every temperature.

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Enzymes in Meat, Fish, and Poultry Product Processing and Preservation-II

8

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Abstract

The current chapter provides insights into the applications of enzymes in meat, fish, and poultry products' processing industries. The mechanical and chemical techniques mainly employed to process meat, fish and poultry products are not eco-friendly, consume more energy, and generate by-products. Hence, the substitution of these techniques with enzymes (biocatalysts) could be the best alternative. Besides, the application of enzymes in these industries is gaining momentum since they are eco-friendly, consume less energy, and also aid in obtaining good-quality products with better yield. The enzymes can catalyze the reaction process at mild temperature and pH and are highly specific toward substrate molecules. Enzymes such as proteases, lipases, transglutaminases from plant, animal, and microbial origin are suitable and have been extensively studied to process meat, fish, and poultry products. Currently, novel enzymes are also being developed as a result of technological advancements. The recombinant enzymes being developed through technical advancements in biotechnology for their application in meat, fish, and poultry products processing could be an excellent prospect. This chapter describes the potential enzymes used in the processing and preservation of meat, fish, and poultry products.

Keywords

Meat industry · Seafood industry · Poultry industry · Proteases · Lipases · Transglutaminases

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A. Dutt Tripathi et al. (eds.), *Novel Food Grade Enzymes*,
https://doi.org/10.1007/978-981-19-1288-7_8

8.1 Introduction

Enzymes are termed as "biocatalysts" owing to their natural catalytic nature and are considered to be prime tools in biotechnology and related areas (Fernandes, 2016). Enzymes are mainly protein molecules known to speed up the biochemical reaction rate occurring in the cell (Singh, 2018). Enzymes have been utilized in the processing of food since ancient times, long before their catalytic function was discovered. Homer's epic poem, Iliad, written around the eighth century BC, describes the use of enzyme ficin for milk clotting (Copeland, 2000). Apart from this, the original inhabitants of the Pacific Islands exploited papaya juice for meat tenderization. The colonization of these islands by the British led to the growth of enzyme technology for food processing, especially meat tenderization and also wound healing in European nations (Gomes et al., 2018). At present, enzymes have found their potential application in food, leather, animal feed, biofuel, detergent, etc., industries. In these industries, enzymes have been employed as efficient catalysts (Singh, 2018). The increased demand for enzymes in industries, especially food industries, may be attributed to its high specificity towards the substrate molecule. Besides, enzymes can catalyze the reactions under mild pH and temperature with minimal by-product formation (Shahidi & Kamil, 2001).

Food is one of the necessities of life required for human survival. Despite being an important necessity, the global report on the food crisis (2019 Edition) by the European Commission illustrates that across 53 countries, a population of more than 113 million is experiencing a food shortage, nutritional deficiency, and also improper livelihood assistance (European Commission-GRFC, 2019). Due to the rapid population explosion, extensive utilization of natural resources, disputes across the globe, and variation in climate, there has been an increased demand for food worldwide (Singh, 2018). According to the United Nation Department of Economic and Social Affairs (UNDESA), New York, the population of the world is projected to be increased from 6.9 to 9.1 billion (i.e., 32% rise) by 2050 and this would trigger the hike in food demand up to 70% (UNDESA, 2017; Singh, 2018). Moreover, to mitigate food shortage, in the year 2015, the United Nations (UN) Member States adopted the 2030 Agenda, which mainly aims for the contribution of fisheries and aquaculture in the use of natural resources toward food security and nutrition, and thereby ensure sustainable development in economic, social, and environmental terms (FAO, 2016). A considerable increment in the production of meat and poultry products was also observed to meet the global food demands. According to the Food and Agriculture Organization of the United Nations (FAO), by 2050, the global production of meat is expected to increase to 455 million tons (Chandrasekaran et al., 2015).

At present, combating the current global scenario of the food crisis, elimination of malnutrition and hunger, and ensuring food security have been the most challenging research and development fields. Food processing could play a pivotal role in eradicating the persisting global food shortage (Singh, 2018). Food processing refers to the process where the agricultural raw material is processed into a consumer-ready product. The main objectives of modern food processing include (1) to prepare safe

food (microbiologically and chemically), (2) to deliver products enriched in organoleptic properties and nutrients, and (3) to make convenient food. Generally, chemical treatment methods are implemented to aid production while processing the food for commercial utilization. The substitution of chemicals with enzymes in food processing has resulted in quality products with better yields. Besides, it has also reduced energy consumption and doesn't emit greenhouse gases (Chandrasekaran et al., 2015). The application of enzymes in food industries such as meat, fish, and poultry product processing has been vital in obtaining better-quality products as well as their preservation. The demand for food enzymes in these industries has been growing alongside their increased production across the globe. Moreover, the recombinant enzymes, especially from microorganisms, are also being produced for their application in these industries. In meat and poultry products processing industries, enzymes have been predominantly used to improve the tenderness of beef, pork, sheep, and chicken meat, whereas enzymes in fish-processing industries find a wide array of applications, including the preparation of products, extraction of biomolecules, and preservation (Fernandes, 2016; Suresh et al., 2015). The current chapter primarily focuses on the application of enzymes in the processing of meat, fish, and poultry products, their significance, and also their future scope in the food industry.

8.2 Importance of Enzyme in Food Industry

Due to the rapid population explosion, extensive utilization of natural resources, disputes across the globe, and variation in climate, there has been an increased demand for food worldwide (Singh, 2018). In the food industry, the application of enzymes is promising for the processing of food and also in solving food-related problems (Venugopal et al., 2000; Singh, 2018). At present, enzymes have found their wide application in various food industries, including beverage, dairy, oils and fats, brewing, cereals, confectioneries, bakery, fruits and vegetables, juice, meat, seafood, and poultry (Chandrasekaran et al., 2015). The importance of enzymes in meat, fish, and poultry product processing is described below.

8.2.1 Current Scenario of Meat Production and Role of Enzymes in Meat Processing

Animals such as sheep, goat, pig, cattle, and other livestock are slaughtered, processed, and distributed in the meat industry (Chandrasekaran et al., 2015). According to FAO, in the year 2018, global meat production increased up to 1.2% compared to 2017, which could be easily interpreted in Fig. 8.1 (FAO, 2019). Figure 8.1 depicts the production of meat worldwide from the year 2016 to 2019. Increased production of pork and beef meat was observed in the year 2018, but there was a slight decrease in their production in 2019. However, sheep meat production increased from 2016 until 2019.

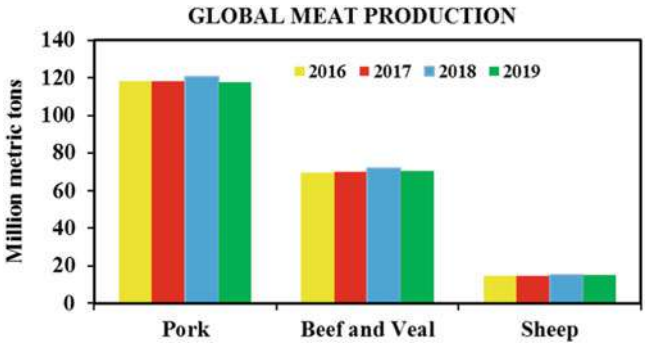

Fig. 8.1 Global meat production from the year 2016 to 2019. (Source: Statista, Production of meat worldwide, 2020)

In the past decades, global consumption of meat has increased due to population explosion and the economic boost in developing nations. Currently, the consumers consider the quality attributes of meat products rather than their price, which signifies that the greater the quality traits of meat products, the greater their importance to meet the increasing consumer demands (Gomes et al., 2018). The most appreciable quality trait of meat judged by consumers is "tenderness". The tenderness is a textural property of food associated with chewiness or cohesiveness. This quality trait of food during mastication manifests little resistance to fragmentation (Ashie et al., 2002; Gomes et al., 2018). The tenderness of the meat is primarily related to the structural integrity of connective tissue proteins and myofibrillar proteins. The key constituent of connective tissue is collagen, and the tenderness of the meat is determined by the amount, type, as well as the degree of intermolecular cross-linking in collagen. Studies have depicted an increased cross-linking in aged animals rather than young animals (Ashie et al., 2002). To improve the tenderness of the meat, the application of enzymes has been found to be desirable in terms of both producing quality products and the cost (Cazarin et al., 2015). There are two underlying mechanisms to obtain the desired tenderness in meat products using enzymes. The first mechanism involves the generation of amino acids or peptide fragments by breaking the covalent bonds of protein molecules. This would result in meat structure alteration. The second mechanism includes the formation of new covalent bonds, which would ultimately change the meat tenderness. The enzyme mainly involved in the new bond formation is transglutaminases (Cazarin et al., 2015). Both the endogenous enzymes (calpains, cathepsins, capsases, and proteosomes) and exogenous enzymes from the plant, animal, and microbial sources (ficin, bromelain, papain, actinidin, cucumin, zinzibain, collagenase, elastase, animal protease, and fungal protease) have been found to play a pivotal role in the tenderization of meat (Ashie et al., 2002; Cazarin et al., 2015; Gomes et al., 2018). Apart from the application of enzymes in meat tenderization, other techniques involve shock wave pressure, calcium chloride injection, mechanical tenderization, electrical stimulation, and elevated temperature storage (Ashie et al., 2002).

8.2.2 Current Scenario of Fish Production and Role of Enzymes in Fish Processing

Over the decades, a tremendous leap has been observed in fish production across the globe. The global fish production in the year 2018 was 178.8 million metric tons, and in the year 2019, it is projected to be 177.9 million metric tons (Statista, 2019; FAO-GLOBEFISH, 2020). Figure 8.2 displays the total global fish production from the year 2016 to 2019. The total global fish production includes aquaculture production, which includes the production of even crustaceans and mollusks (seafood). In the majority of the countries, fresh fish and seafood harvested are mainly sold for local consumption (Suresh et al., 2015). From the total fish and seafood processed, about 20–50% is available for edible purposes, and the rest of it is generated as non-edible by-products or discarded as waste. These by-products are rich in various biomolecules such as oil and lipids, proteins, bioactive peptides, pigments, polymers, vitamins, and minerals (Suresh et al., 2015).

The utilization of enzymes in the processing of fish and seafood has gained momentum due to the advancements in enzyme technology. Enzymes have been employed in fish-processing industries to obtain a diversified range and improved quality products. In fish-processing industries, enzymes have been used for descaling, deskinning, salted fish ripening, production of products such as surimi, fish sauce, and fish protein hydrolyzates, extraction of oil and biomolecules such as chitin, carotenoproteins, and flavor compounds, and removal of exoskeleton from shellfish (Suresh et al., 2015). The enzymes used for fish and seafood processing are mainly derived from microorganisms, plants, and animals (Fernandes, 2016; Suresh et al., 2015). Moreover, research has also been carried out on the enzymes isolated from fish discards and its application in the processing of fish and seafood (Suresh et al., 2015). This approach would help in waste management of fish and seafood by-products, the majority of which are mainly dumped into the environment. The enzymes—both natural and recombinants—derived from microorganisms have been used in fish and seafood processing. Further, the demand for microbial enzymes is more than that for enzymes isolated from plants and animals since the microbial enzymes exhibit better catalytic activity and high yield, and can be genetically

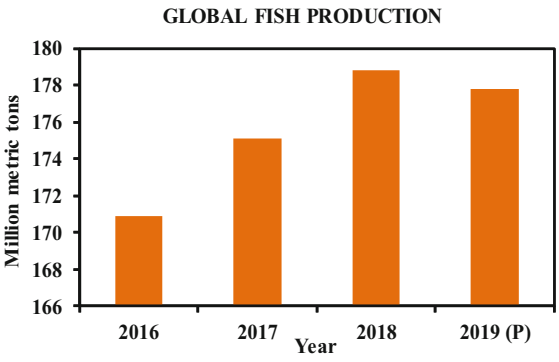

Fig. 8.2 Global fish production from 2016 to 2020. (Source: Statista, 2020; FAO-GLOBEFISH, 2020)

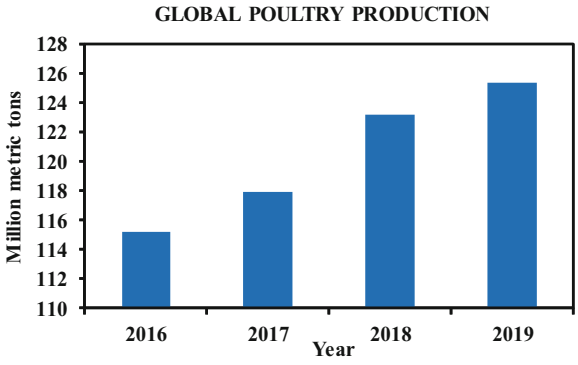

Fig. 8.3 Global poultry production from 2016 to 2019. (Source: Statista, Production of meat worldwide, 2020)

manipulated with ease, regular supply, and rapid microbial growth in low-priced culture media (Suresh et al., 2015). Besides fish and seafood processing, enzymes have also been used in the analysis of processed aquaculture and fish products (Fernandes, 2016).

8.2.3 Current Scenario of Poultry Production and Importance of Enzymes in Poultry Processing

Similar to meat and fish production worldwide, poultry production has also increased over the decades. Figure 8.3 displays the global poultry production from the year 2016 to 2019. From the figure, it is evident that the global poultry production is increasing every year and is higher than global sheep, pork and beef, and veal production. Global poultry production has been soaring to meet the global consumption demands due to overpopulation.

Besides the tenderization of pork, sheep, and beef meat, enzymes have also been used for the tenderization of poultry products. Studies have been carried out on improving the tenderness of poultry meat using exogenous enzymes such as papain, bromelain, proteases, etc., and also endogenous enzymes such as calpains (Bawa et al., 1981; Kang & Warner, 1974; Whipple et al. 1990).

8.3 Enzyme Sources and Types

As described in the previous sections, the enzymes used in meat, fish, and seafood as well as poultry products processing may be from plants, animals, or microorganisms. Apart from exogenous enzymes, the roles of endogenous enzymes are also crucial for the processing of food products. The enzymes from the microbial source are most widely preferred since they can be easily manipulated and microorganisms can be easily cultured. Besides, the trend towards the use of enzymes from marine microorganisms has been increasing from the last few years (Fernandes, 2016). Proteases such as ficin, bromelain, and papain, which are primarily plant-based,

have been widely used in meat tenderization. These proteases possess a sulfhydryl group in their active sites to carry out their catalytic activity. Apart from meat tenderization, these proteases have also been used in the preparation of the fish sauce. Moreover, papain has also been used in the preparation of fish protein hydrolyzates (Fernandes, 2016). Research has also been carried out on the extraction of enzymes from fish-processing discards and their application in the processing of fish products. Enzymes including pepsin, trypsin, gastricin, alkaline proteinase, acidic protease, calpain, lysosomal cathepsins type B, H, L, cathepsin and other lysosomal cathepsins, chymotrypsin, collagenases, chymosin, elastase, lipase, carbohydrases, and alkaline peptidase from fish-processing by-products have been tested for their application in the processing of fish and seafood products (Venugopal, 2016). Most of the enzymes used in meat, fish, and poultry product processing are proteases. Besides proteases, other enzymes (lipases and transglutaminases) play a major role in flavor modification, hydrolysis of fatty acids, and other processing methods. The different enzymes used in the processing of meat, fish, and poultry products, and also their sources are exhibited in Tables 8.1, 8.2 and 8.3, Fig. 8.4.

8.4 Enzymes Involved in Meat Processing and Preservation

8.4.1 Calpains

Calpains are calcium-dependent cysteine proteases mostly found in mammals. Calpain 1 (μ calpain), calpain 2 (m calpain), and calpain 3 are the major calpain proteases of skeletal muscle; this calpain system also consists of an inhibitor of calpain 1 and calpain 2, called calpastatin (Goll et al., 2003). They are activated by micromolar (1–100) and millimolar (0.1–1 mM) concentrations of calcium, respectively (Suzuki et al., 1992). The intracellular calcium concentration is 1 μm or less at most. Therefore, it always exists as a proenzyme until enough calcium is present for activation. Tenderness is an essential aspect of meat quality. The degradation of myofibrillar proteins (troponin T, troponin I, tropomyosin, connectin, C-protein, and desmin) results in the weakening of myofibers. This is a crucial event in the tenderization. μ-Calpain-induced degradation of key myofibrillar proteins is the cause of postmortem proteolysis, and this is the primary source of muscle tenderness (Koohmaraie & Geesink, 2006). This can be achieved by increasing the calcium concentration (Rees et al., 2002) or by lowering the calpastatin activity (Hope-Jones et al., 2010). Electrical stimulation (ES) can also lead to an increased flow of calcium ions into the cytoplasm and may lead to μ-calpain activation (Veeramuthu & Sams, 1999). Calpastatins inhibit calpains and thereby reduce tenderness, but after cooking calpastatin is inactivated, which leads to increased tenderness.

Table 8.1 Proteases used in the processing of meat, fish, and poultry products

Source	Enzyme	Source	Applications
Plants	Ficin	Fig (*Ficus glabrata*, *Ficus laurifolia*, *Ficus anthelmintica*)	Meat tenderization
	Papain	Papaya (*Carica papaya*)	Meat tenderization, preparation of fish sauce, and fish protein hydrolyzates
	Bromelain	Pineapple (*Ananas comosus*)	Meat tenderization and preparation of fish sauce
	Actinidin	Kiwi fruit (*Actinida deliciosa*)	Meat tenderization
	Cucumin	Kachri fruit (*Cucumis pubescens*, *Cucumis trigonas*)	Meat tenderization
	Zinzibain	Ginger (*Zingiber officinale*)	Meat tenderization
Animal	Porcine pancreatin	Pork	Meat tenderization
Microorganisms	Fungal protease	*Aspergillus oryzae*, *Penicilium chrysogenum* Pg222	Meat tenderization, preparation of fish protein hydrolyzates
	Alkaline protease	*Bacillus subtilis*	Preparation of fish protein hydrolyzates
	Thermophilic enzyme E	*Bacillus* strain E.A.1	Meat tenderization
	A1 protease	*Thermus* strain	Meat tenderization
	4-1 A protease	Rt4-1.A	Meat tenderization
	Caldolysin	*Thermus* strain T-35	Meat tenderization
	Elastase	*Bacillus* species EL31410	Meat tenderization
	Collagenase	*Vibrio* B-30	Meat tenderization

Source: Naveena et al. (2004), Venugopal et al. (2000), Cazarin et al. (2015)

8.4.2 Cathepsins

Cathepsins are lysosomal acid proteases found in almost all organisms. There are 13 types of lysosomal cathepsins, 7 of which exist in skeletal muscle (Goll et al., 1983). Cathepsins comprise both exoproteases and endoproteases, and are differentiated from the active site: aspartate (Cathepsin D and E), serine (Cathepsin G), and cysteine (Cathepsin B, H, L, and X) (Sentandreu et al., 2002). They exist as a proenzyme in living tissue. Due to pH fall after cell death, they are released into the cytoplasm or intercellular space due to lysosomal disruption (Duston, 1983). Cathepsins B and L are the major cathepsins involved in muscle proteolysis (Jamdar & Harikumar, 2002). Protease inhibitor cystatin regulates cathepsin B, H, and L in vivo (Turk & Bode, 1991).

Table 8.2 Lipases used in the processing of meat, fish, and poultry products

Source	Source	Applications
Animal	Porcine pancreas	Preparation of ω-3 PUFA concentrates
	Atlantic cod	Production of ω-3-enriched triglycerides, flavor improvement
	Sardine	Production of ω-3-enriched triglycerides, flavor improvement
	Indian mackerel	Production of ω-3-enriched triglycerides, flavor improvement
	Red sea bream	Production of ω-3-enriched triglycerides, flavor improvement
	Salmon	Production of ω-3-enriched triglycerides, flavor improvement
Microorganisms	*Pseudomonas* species	Enrichment of EPA and DHA in sardine oil
	Candida cylindracea	Increasing DHA content, hydrolysis of menhaden oil
	Lactobacillus plantarum	Synthesis of short-chain fatty acid esters

Source: Suresh et al. (2015), Venugopal et al. (2000), Cazarin et al. (2015), Uppada et al. (2017)

Table 8.3 Transglutaminase application in the processing of meat, fish, and poultry products

Source	Source	Applications
Microorganisms	*Streptoverticillum* sp., *Bacillus subtilis*, *Streptomyces* sp.	Fish meat sheet formation, fish meat film and mince formulations, surimi and restructured fishery products, texture modification of finfish, collagen and gelatin bond formation, improve the gel strength of chicken and beef sausages, improve the gel strength of chicken and beef meat

Source: Suresh et al. (2015), Cazarin et al. (2015)

Many research groups ignore cathepsins' contribution to meat tenderization due to various reasons like no large-scale degradation of the actin and myosin in postmortem conditioning (Koohmaraie et al., 1991). To access myofibril proteins and to increase the tenderness, cathepsins must be released from lysosomes (Hopkins & Taylor, 2002). Whipple et al. (1990) stated that there is only a minor association between meat tenderness and cathepsin activity. However, the activities of cathepsins B and L at 8 h postmortem have been found to positively correlate with beef tenderness (O'Halloran et al., 1997). In the myofibrils of rabbit, beef, and chicken, the main myofibrillar proteins (nebulin, tropomyosin, troponin T, I, C, titin) along with actin and myosin are hydrolyzed by Cathepsin L during the postmortem conditioning period (Mikami et al., 1987).

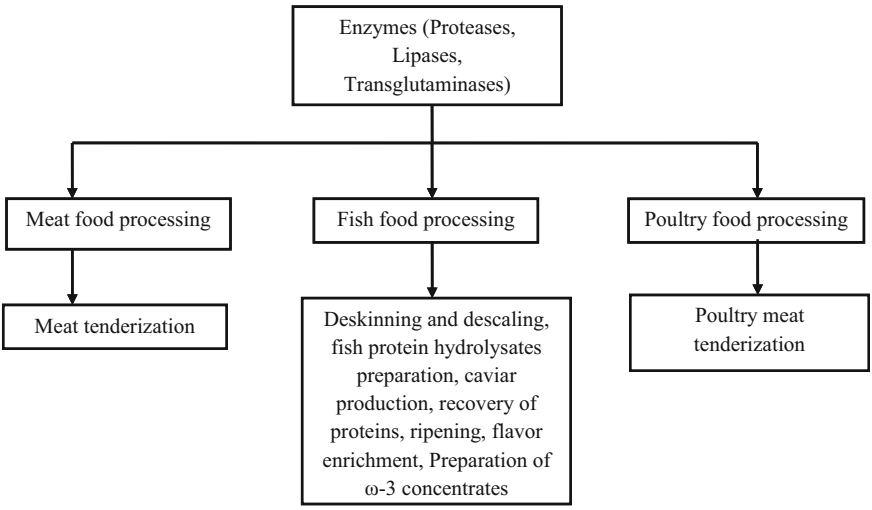

Fig. 8.4 Applications of enzymes in meat-, fish-, and poultry-processing industries

8.4.3 Papain

Papain is the most common enzyme used in meat tenderization. Papain, which belongs to the C1 family of cysteine protease, also called the papain family (Rawlings et al., 2010), is obtained from the latex of the papaya plant (*Carica papaya*), and it plays a major physiological role in protecting the plant from insects (Konno et al., 2004). Papain's commercial importance is primarily due to its good proteolytic activity against a broad variety of protein substrates, and because it is active in a wide range of operating conditions. Smith and Hong-Shum (2003) reported that it has a high optimal temperature (65 °C) and broad pH range (5–8) for its activity. Papain predominantly acts on the muscle structural component, which enhances the meat tenderization (Gracey & Thronton, 1985). It is a highly effective enzyme that is capable of inducing major degradation of collagen and myofibrillar proteins (Ashie et al., 2002), and the maximum tenderizing activity occurs during the cooking process (Tappel et al., 1956). Kang and Rice (1970) reported that papain showed strong activity for a myofibrillar fraction with greater solubilizing activity on the connective tissue. Use of papain leads to over tenderization of meat which resulted in mushy meat, hence the commercial use of papain is limited for meat tenderization (Han et al., 2009).

8.4.4 Ficin

Ficin, the proteolytic enzyme from fig trees (*Ficus* sp.), is a sulfhydryl protease. About ten proteases are present in crude ficin latex (Kramer & Whitaker, 1964). The mostly studied ficins are those from *Ficus glabrata* and *Ficus carica*. It is reported to

hydrolyze and increase the solubility of muscle proteins (El-Gharbawi & Whitaker, 1963). The solubility of meat protein might be increased by ficin by degrading the proteins into units of smaller molecular weight, which, when aggregated, form a three-dimensional network (Ramezani et al., 2006). El-Gharbawi and Whitaker (1963) reported that the optimum pH was around 7 for enzyme activity for collagen and myofibrillar proteins, and about 5.0–5.5 for elastin.

8.4.5 Bromelain

The proteolytic enzymes called bromelain are present in fruits, leaves, and stems of the Bromeliaceae family of which pineapple (*Ananas comosus*) is the most commonly used enzyme in the processing of meat (Doko et al., 1991). The enzyme extracted from the stem is called stem bromelain, and that extracted from the fruit is called fruit bromelain (Vanhoof & Cooreman, 1997). Fruit bromelain is said to have a higher proteolytic activity and specificity compared to stem bromelain (Barrett et al., 2004; Grzonka et al., 2007). This enzyme, like other proteases, results in over-tenderization by degrading myofibrillar proteins and collagen (Melendo et al., 1996). At first, it degrades about 40% of the collagen in the sarcolemma, and then it degrades myosin in the myofibrillar region (Kang & Rice, 1970; Wang et al., 1958). The application of this enzyme is easy and cheap and can be exploited on a household and industrial scale for tenderizing tough meat; it is also a better alternative to chemical tenderizers (Sunantha & Saroat, 2011). Ionescu et al. (2008) investigated the use of bromelain in adult beef with best results at 10 mg/100 g meat, with a tendering time of 24 h at 4 °C, followed by thermal treatment with an increase of 1 °C/ min to 70 °C (when enzyme inactivation occurs). These conditions improved the tenderness of the beef.

8.4.6 Cucumin

Cucumin obtained from kachri (*Cucumis pubescens*) is a protease. Initially, the dried coarsely ground fruits of *C. trigonus* Roxb (locally known as kachri) were traditionally used as a meat tenderizer in some parts of India (Naveena et al., 2004). Cucumin tenderizes meat to a significant degree, and may serve as a basis for commercial meat-tenderizing mixtures (Hujjatullah & Baloch, 1970).

8.4.7 Zingibain

Zingibain protease was isolated from the plant *Zingiber officinale roscoe* (ginger rhizome) by Thompson et al. (1973). This thiol protease shows optimum activity at 60 °C (Naveena et al., 2004). The ginger extracts contain two cysteine proteases with a molecular mass of 29 and 31 kDa, as reported by Su et al. (2009). This enzyme has a greater proteolytic activity when heated (Naveena & Mendiratta, 2001). Its

proteolytic activity produces more tender meat by degrading collagen and actinomycin (Naveena & Mendiratta, 2001; Thompson et al., 1973). Lee et al. (1986) reported that an extensive degradation of myofibrillar proteins is possible with a high concentration of ginger extract: the degradation appears to begin at the I band of each sarcomere and progresses to the M line.

8.4.8 Actinidin

Actinidin or actinidain is a cysteine protease obtained from the kiwi fruit (*Actinidai deliciosa*). Actinidin is stable at a pH range of 7–10 and has optimal activity at 58–62 °C (Yamaguchi et al., 1982). A pH range of 5–7 is also reported by Boyes et al. (1997), which reflects variations in the cultivar and assay used to estimate the proteolytic activity. Both myofibrillar proteins and connective tissue proteins are hydrolyzed by this enzyme (Christensen et al., 2009; Han et al., 2009). But a higher hydrolysis of collagen was reported by Wada et al. (2002).

8.4.9 Aminopeptidases

Aminopeptidases are the major enzymes involved in the characteristic flavor development in meat products. They hydrolyze amino acids from the N terminus of peptides and proteins during meat processing. This generates a large number of free amino acids that produce flavor. In porcine skeletal muscle, aminopeptidase B (BAP), leucyl (LAP), alanyl (AAP), and pyroglutamyl (PGAP) have been localized in the cytosol, and are named according to the preference of specific N terminal amino acid (Flores et al., 1993, 1996, 1997). Except for leucyl aminopeptidase, all are active at acidic pH. In the cytosol of skeletal muscle, around 86% of the total aminopeptidase consists of alanyl aminopeptidase (Lauffart & Mantle, 1998). As reported by Toldra (1992), aminopeptidases show good stability during the processing of dry-cured ham, with activity still recovered toward the end of the process.

8.4.10 Lipases

Lipolysis is the process of degradation of lipids in meat during processing (Toldra & Flores, 1998). Enzymes of fat cells and muscle fibers, as well as bacterial enzymes are involved in lipolysis. However, it is established that in dry fermented sausages, the contribution of bacterial lipase is weak because of many differences in the medium and optimum conditions (Molly et al., 1997). Intense lipolysis occurs in muscle and adipose tissues during the dry-curing of meat; most of the free fatty acid generation occurs during the initial 5 months (Motilva et al., 1993). Important lipolytic enzymes of adipose tissue are lipoprotein lipase, hormone-sensitive lipase, and monoacylglycerol lipase (Belfrage et al., 1984). Lipoprotein lipase, specific for

primary esters, is active at alkaline pH 8.5. Hormone-sensitive lipase hydrolyzes ester bond in triacylglycerols and diacylglycerols. Monoacylglycerol lipase hydrolyzes monoacylglycerols without any positional specificity and is active during salting and post-salting stages. Hormone-sensitive lipase is also called neutral lipase due to its high activity at neutral pH (Imanaka et al., 1984). The muscle lipase, lysosomal acid lipase, active at acidic pH hydrolyzes tri, di, and monoacylglycerol (Imanaka et al., 1984). Lipases can be used to remove fat in meat products (Uppada et al. 2017). Lipase produced from *Lactobacillus* sp. degrades meat in 72 h (Padmapriya et al., 2011).

8.4.11 Fungal Proteases

An exogenous serine protease (EPg222) obtained from *Penicillium chrysogenum* Pg222, an atoxigenic strain, was isolated from dry-cured ham (Núñez et al., 1996). This protease shows optimum activity at 45–55 °C, pH 6, and 0.25 M NaCl (Benito et al., 2002). Benito et al. (2003) reported that EPg222 hydrolyzed key myofibrillar proteins, supporting the tenderization of whole pieces of meat with 5% NaCl. The dry-cured meat product's texture could be improved by fungal proteases. The introduction of EPg222 in dry fermented sausages, ripened with starter cultures, is of great interest to boost sensory characteristics (Benito et al., 2004). An aspartic protease from *Aspergillus oryzae* shows optimal activity at a pH range of 2.5–6, and less than 20% of its activity remains after cooking at 75 °C (Ashie et al., 2002). It does not affect collagen. Its effect on myofibrillar proteins is limited compared to plant protease, with self-limiting hydrolyses at higher concentrations (Cronlund & Woychik, 1986; Ashie et al., 2002).

8.4.12 Elastase

Elastase is the enzyme that breaks down elastin in connective tissue. A new elastase, a protease with high proteolytic activity, was isolated from *Bacillus* sp. EL31410 by Chen and He (2002). It had a marked preference for elastin and collagen, which can contribute to meat hardness, over other myofibrillar proteins at the pH of meat, usually ranging from 5.5 to 6.0, and it has the same tenderization effect as papain on beef meat based on the results of texture, sensory, and structural analyses (Qihe et al., 2006).

8.4.13 Bacterial Protease

Many strains of bacteria play a major role in protein degradation in fermented meat and fish products (Bekhit, 2010). Because of relatively specific activity and low inactivation temperatures, proteases from bacterial sources make them useful for meat tenderization. Alkaline elastase from alkalophilic *Bacillus* sp. strain Ya-B

shows an optimum activity in the pH range of 5.5–6.0 and a temperature range of 10–50 °C (Takagi et al., 1992; Yeh et al., 2002). Compared to plant proteases, bacterial proteases have less hydrolytic activity towards myofibrillar proteins but their ability to degrade collagen was intermediate to that found in bromelain and papain (Yeh et al., 2002).

8.4.14 Animal Proteases

Animal proteases are less used for meat tenderization. Injection of pancreatin on beef semitendinosus tended to improve overall tenderness without affecting the taste (Pietrasik et al., 2010).

8.5 Enzymes in Fish Products Processing and Preservation

Various enzymes from different sources have been exploited for the processing of fish products and their preservation. The main enzymes, which play a key role in the processing of fish and seafood are described below.

8.5.1 Proteases

Proteases find their wide application in the seafood processing industry, especially in deskinning, descaling, salted fish ripening, fish sauce production, squid tenderization, etc. Besides, proteases have also been examined for the extraction of compounds (mainly pigments, oils and fats, and flavors) and the preparation of hydrolyzates from seafood processing wastes (Suresh et al., 2015). Proteases from plant, animal, microbial, and even aquatic sources have been used for the processing of fish.

1. Deskinning and descaling: Deskinning is the procedure involving the removal of fish skin without damaging the flesh. The deskinning process is carried out using rough mechanical procedures,which results in flesh damage and excess waste generation. The replacement of mechanical procedures with enzymes i.e., proteases could improve the edible yield. Commercial proteases such as Protease N and Proleather FG-F have been tested for the deskinning of catfish nuggets (Kim et al., 2014). Descaling of fish involves a harsh mechanical process, which might result in the tearing of fish skin and thereby lower the fillet yield. To avoid this loss, enzymatic descaling using the mixture of proteases is carried out (Fernandes, 2016).
2. Fish protein hydrolyzates: Over more than 50 years, the application of proteases in the fish protein hydrolyzates preparation has been widely studied. The fish protein hydrolyzates are generally prepared by treating fish meat or protein-rich fish processing by-products (fish head, skin, liver, trimmings, bones, and fish

viscera) with proteases at ideal pH and temperature for a few hours, followed by drying. The enzymes preferred for the production of fish protein hydrolyzates include pepsin, trypsin, chymotrypsin, alcalase, etc. The prepared fish protein hydrolyzates are amorphous powder and hygroscopic in nature. The fish protein hydrolyzates contain proteins (80–90%), fats (<5%), minerals (5–8%) and moisture (1–8%) (Venugopal et al., 2000; Venugopal, 2016). Traditionally, the preparation of fish protein hydrolyzates was carried out using either acids or bases. Hydrochloric acid or sulfuric acid (occasionally) was preferred for acid hydrolysis, whereas alkali hydrolysis was carried out using sodium hydroxide. Both acid and alkali hydrolysis are carried out at elevated temperature. Moreover, alkali hydrolysis produces toxic compounds during the reaction process, and acid hydrolysis destroys tryptophan. Besides, fish protein hydrolyzates preparation using low-cost proteolytic enzymes can be carried out at a mild temperature, pressure, and pH, and doesn't produce toxic compounds. Thus, the preparation of fish protein hydrolyzates using proteolytic enzymes is economical (Fernandes, 2016).

Gajanan et al. (2016) prepared protein hydrolyzates from threadfin breams (*Nemipterus japonicas*) a fish frame waste using two different plant proteases, papain and bromelain. The prepared protein hydrolyzates exhibited high Angiotensin-Converting Enzyme (ACE) inhibitory and anti-oxidative activity. The hydrolyzates also showed better functional properties. Similar results were also exhibited by protein hydrolyzates prepared from the muscle of small-spotted catshark (*Scyliorhinus anicula*) using esperase and alcalase (Vázquez et al., 2017).

3. Fish sauce: Fish sauce is a product produced by the fermentation process using endogenous or exogenous enzymes. The underlying process mainly involves the solubilization and digestion of fish proteins by enzymes. The pre-dominant enzymes involved in protein hydrolysis include chymotrypsin and trypsin, alongside cathepsins. When the pH of fish sauce drops from 7 to 5, the action of trypsin and chymotrypsin becomes complicated since they are active at near-neutral pH, whereas cathepsins are active at acidic pH. Fish sauce production using endogenous enzymes is a traditional process, and it's a very time-consuming process. Therefore, to hasten the process, exogenous enzymes such as papain, ficin, and bromelain, and commercial enzymes such as protamex (Protex 51FP) and neutrase have been used (Fernandes, 2016). About 80–90% of the Southeast Asian people consume fish sauce. It is being used as a prime condiment in Southeast Asian cuisines to improve the taste of food. Currently, Thailand is the leading producer of fish sauce in the world (Gowda et al., 2020).
4. Recovery of proteins from fish processing waste: Fish waste is a rich source of proteins such as collagen, myofibrillar proteins, and sarcoplasmic proteins. Proteases derived from the fish wastes (collagenases and pepsin) have been employed for the extraction of collagen from various fish offals, including skins, fins, bones, scales, swim bladders, and heads (Venugopal, 2016).
5. Ripening: Proteases have been known to be involved in the ripening of fermented fishery products as well as salted fish (Venugopal, 2016; Fernandes, 2016). The

exogenous proteases accelerate the development of soft texture and characteristic flavor in fermented fishery products. In Mediterranean countries, salted anchovy (*Engraulis encrasicholus*) is a heavily salted fish comprising around 14–15% of NaCl and has high nutritional value. In many European countries, salting of herring (*Clupea harengus*) is being carried out as a conventional preservation technique (Suresh et al., 2015). The ripening of salted fishes involves a complex biochemical process, wherein the muscle proteins undergo degradation by endogenous enzymes. The main endogenous enzymes involved in the ripening are trypsin, chymotrypsin, and cathepsins (Venugopal, 2016; Fernandes, 2016).

6. Caviar production: Caviar refers to the cured fish roes of salmon, trout, white sturgeon fish, etc. Traditionally caviar was used to refer to riddles and cured roe of sturgeon fish. The most difficult task in the preparation of caviar is the separation of roe from the roe sack. This process is generally termed riddling. Riddling process is generally carried out either manually or mechanically. Recently, the applications of enzymes in the preparation of caviar from various fish species have been examined (Suresh et al., 2015; Venugopal, 2016).
7. Other applications: The proteases have also been used in various other seafood processing operations. Some of these operations include (1) control of curd formation in canned fish, (2) preparation of bioactive peptides, (3) extraction of biopolymers, pigments, and compounds such as chondroitin sulfate and hyaluronic acid, (4) the production of aquafeed and seafood flavoring, (5) reduction of viscosity of stick water, and (6) control of enzymatic browning (Venugopal, 2016).

8.5.2 Lipases

Lipases (triacylglycerol acyl hydrolases EC 3.1.1.3) are the enzymes primarily involved in the hydrolysis of mono, di, and triglycerides and fatty acids. The hydrolysis reaction takes place in the presence of an excess of water, but when there is limited water, lipases promote the synthesis of esters. Lipases also exhibit other esterase type or phospholipase type activities. These activities of lipase have recognized industrial relevance. Lipases differ in their properties and also their origin (plant source, animal source, and microbial source). Lipases have been exploited for isolating oils and fats from seafood by-products. The lipases have also been utilized for ω-3 fatty acids' (eicosapentaenoic acid and docosahexaenoic acid) enrichment of fish oils. Lipases along with other muscle enzymes such as caplains, cathepsins, aminopeptidases, and peptidases have been known to influence the release of flavor compounds (Suresh et al., 2015; Fernandes, 2016; Venugopal, 2016).

8.5.3 Transglutaminase

Transglutaminase (TGAs, protein-glutamine γ-glutamyl transferase EC 2.3.2.13) enzymes primarily catalyze acyl transfer reactions. The reaction includes the

γ-carboxyamide group of peptide-bound glutamine acting as acyl donor and many primary amines acting as acyl acceptors. Simultaneously, many intermolecular and intramolecular covalent bond formation occurs, resulting in the cross-linking of proteins and peptides, and polymerization. In the absence of primary amines, water acts as an acyl acceptor, and the γ-carboxyamide group undergoes deamination to glutamic acid residues. TGase from plant and animal sources are Ca^{2+} dependent enzymes, whereas TGase from the microbial source is Ca^{2+} independent enzyme. Moreover, microbial TGase is active over a wide pH and temperature (0–70 °C). These features of microbial TGase and its cost-effective production make it the most widely preferred enzyme for processing of fish products. TGase has been produced by microorganisms such as *Streptomyces* sp., *Bacillus subtilis*, and *Streptoverticillium* sp. The application of TGases in the fish products processing industry includes preparation of surimi and restructured fishery products, fish meat film formulations, processing of shark fin, modification of texture of finfish, the formation of gelatin and collagen bonds, fish meat mince formulations, drip reduction in thawed fish, freeze-texturization of fishery products, and binding of ingredients with fish meat (Suresh et al., 2015; Venugopal, 2016).

8.5.4 Other Enzymes

Besides proteases, lipases, and transglutaminases, other enzymes have also been exploited for the fish products processing industries. Enzymes such as diamine oxidase, xanthine oxidase, nucleotide phosphorylase, putrescine oxidase, and glutamine dehydrogenase have been used for the evaluation of the quality of seafood and freshness. Restriction endonucleases and Taq polymerase have been used for the identification of fish species and adulteration detection. Furthermore, research works have also been carried out in increasing the shelf-life of seafood products using glucose oxidase enzymes.

8.6 Enzymes in Poultry Products Processing and Preservation

Various exogenous and endogenous enzymes have been exploited for the processing of poultry products. The enzymes used in the processing of pork, sheep, and beef meat are also being used in the processing of poultry meat. Mainly the enzymes are used to improve the tenderness of poultry meat. Papain, ficin, and bromelain enzymes derived from plants have been extensively utilized for the tenderization of poultry meat. The proteolytic enzymes derived from plants are considered to be superior to enzymes derived from bacteria (Ketnawa & Rawdkuen, 2011). The applications of commercial enzymes such as alcalase, neutrase, flavorzyme, protamex, collupulin, bromelain, and alphalase in softening the chicken meat (breast) texture have been studied. Of all the enzymes bromelain and collupulin have shown the marked softening of chicken breast. The exploitation of proteolytic enzymes from *Calotropis procera* latex has also shown to be effective in the

tenderization of poultry meat. Apart from the application of enzymes in poultry meat processing, the addition of enzymes in the improvement of barley and wheat-based feed has also been studied by Perić et al. (2011). They stated that incorporating the enzyme Allzyme SSF into poultry feed had a positive effect on the growth of chicken, and observed increased egg production in layers. Thus, enzymes find extensive application in the poultry industry to prepare poultry feed and poultry meat processing.

8.7 Future Prospects

Nature is a reservoir of several enzymes with promising potential for a wide application in human endeavors, but only a few of them have been exploited for over a century. This scenario's rationale includes that the enzyme might be from an undesirable or unreliable source (from unreachable environments such as deep-sea, or from microorganisms), challenges in production at a commercial scale and downstream processing, and the requirement of harsh conditions for activity. As a result, scientists and technologists continue their attempts to uncover novel enzymes from new origins and experiment with their potential applications, overcoming all obstacles through laboratory research efforts (Chandrasekaran, 2015).

Enzymes are known to play a crucial role in the food industry in producing both traditional and novel products. Enzymes have also found their application in meat, fish, and poultry processing to obtain products with better quality attributes, evaluate the products' quality and freshness, and extend the products' shelf-life. With the increase in the production of meat, fish and poultry products, the demand for enzymes has also increased. The enzymes from plants, animals, and microbial sources have been extensively studied for the processing of meat, fish or seafood, and poultry products. The complete substitution of the chemical processing methods with enzymatic methods would help overcome environmental problems arising due to chemical processing techniques. Further, the application of enzymes in the meat, fish, and poultry products processing industry has excellent prospects since the production of enzymes to be exploited in these industries is cheaper. Their utilization helps to obtain quality products with better yields.

Furthermore, advances in biotechnology, nanotechnology, molecular biology, instrumentation, and bioinformatics have opened up new horizons for designing hybrid catalysts and novel enzymes for diverse food industry applications, including meat, poultry, and fish processing industries. The novel enzymes or recombinant enzymes developed through the modern biotechnological approach would effectively carry out industrial processes at low temperatures, reduce the cost of production and energy consumption, and enhance the products' properties (Chandrasekaran, 2015). Besides, the enzymes derived from seafood processing waste have a promising future for their fish product processing application alongside the recombinant enzymes being developed for enzymatic processing and preservation of meat, fish, and poultry products.

8.8 Conclusion

Currently, the meat, fish, and poultry industries play a significant role in meeting the global food demand. The enzymatic processing of meat, fish, and poultry products processing is still in the early stage, and the introduction of new techniques is resulting in progress. The enzymatic processing technique has the potential to overthrow the chemical and mechanical techniques employed in the processing of meat, fish, and poultry products processing. This would help to overcome the environmental problems caused by chemical and mechanical processing techniques. The most suitable enzymes that could be employed in the processing of meat, fish, and poultry products are discussed in this chapter. The meat and poultry industries mainly utilize enzymes to improve the tenderness of the meat, whereas in fish products processing enzymes have a wide array of applications. Technological advancements have enabled the development of recombinant enzymes from microorganisms for their application in these industries. However, recombinant enzymes are yet to be used in the industries. Thus, the enzymes have a promising future for their application in the processing of meat, fish, and poultry products.

Acknowledgments The authors are grateful to the CSIR-Central Food Technological Research Institute (CSIR-CFTRI), Academy of Scientific and Innovative Research (AcSIR), Ghaziabad, and the Department of Biotechnology (Govt. of India, New Delhi) for the support.

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Enzymes in Functional Food Development

9

Iran Alemzadeh, Asma Sadat Vaziri, Kianoush Khosravi-Darani, and Pierre Monsan

Abstract

Enzymes are ubiquitous biocatalysts, represent a consolidated strategy in various food industries for the transformation of one biological compound to another. In contrast to conventional synthesis, enzymatic synthesis is an important technological advancement as a consequence of sustainability, substrate selectivity, nontoxicity, and low energy input. Therefore, there has been a growing trend in the use of free and immobilized enzymes in various food industrial applications, including the manufacturing of several functional foods and ingredients. Functional foods with health benefits beyond nutrition have gained much attention due to some factors such as interest in health-consciousness and the growing market. A variety of functional foods can be considered to be those fortified, wholes, enriched, or enhanced foods that represent physiological health benefits beyond the essential nutrients provision (e.g., vitamins and minerals) when consumed in adequate amounts on a regular basis. The initial components in functional food supplements naturally consist of fruit, food, beverage, grains, and supplement sectors at a low level. Nutraceuticals or functional food could be produced from the enzymatic reaction utilizable in one of the several areas of food science and technology, that is experiencing fast growth in recent years. Functional food

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A. Dutt Tripathi et al. (eds.), *Novel Food Grade Enzymes*,
https://doi.org/10.1007/978-981-19-1288-7_9

could be enzymatically derived from various reactions: L-asparaginase, an intercellular enzyme, is used in the baking industry which suppresses acrylamide production. Addition of asparaginase is a recommended strategy to decrease the formation of acrylamide, as it catalysis the hydrolysis of asparagine to aspartic acid an amino acid with high nutritive value. Carbohydrate active enzymes such as β-glucanase which is able to proceed on glucose polysaccharides and to further develop the production of β-glucans, the saccharide made up of multiple sugar molecules. β-Glucan may offer a number of health benefits, including lowering cholesterol, improving blood sugar management, and boosting the immune system which is produced by the enzymatic process of beta D-glucose polysaccharide naturally occurring in the cell wall of cereals like oat. Chlorophyllase (Chlase) is widely distributed in the chloroplast, thylakoid membrane, and etioplast of higher plants, such as ferns, mosses, brown and red algae, and diatoms. Chlase catalysis is the degradation of chlorophyll to chlorophyllide (also called Chlide), which is introduced as a food colorant. L-Glutaminase is a catalyst for the production of L-theanine, a free amino acid with many health benefits. L-Theanine synthesis occurs from L-glutamine and ethylamine substrates through the transpeptidation reaction of L-glutaminase, and a demand for this amino acid is expected to grow rapidly in the industry. Lipids such as CLA (conjugated linoleic acid) are in the group of functional food, a result of CLA production from natural oil in the presence of a lipase. CLA is a natural derivative of essential fatty acid, linoleic acid which shows many beneficial effects on the immune system, role in obesity control and anticarcinogenic behavior phytases may find application in food processing to produce functional foods and phytic acid degradation. Phytase is able to decrease the antinutritional effect of phytate (inorganic phosphate complex) adverse impact of inorganic P excretion to the environment while increasing the digestibility of phosphorous (P), calcium, amino acids, and energy. γ-Amino butyric acid (GABA) is an active protein, in rice germ using protease or additives: produced by the decarboxylation of glutamic acid, GABA is catalyzed by glutamate decarboxylase, GABA functions as the main inhibitory neurotransmitter in the central nervous system. Tannase is able to catalyze the production of tea beverages while maintaining the tea's original deep flavor. Engineering of enzymes is a potential strategy in the design of food development according to the specificity and enzyme structural changes. The next future step toward the commercialization of the enzymatic approach in food processing is to enhance their efficiency and mitigate adverse effects on the sensory properties of food products. In this chapter, we mainly highlighted the importance of selected enzymes on food development.

Keywords

Enzymes · Functional food · L-Asparaginase · β-Glucanase · Chlorophyllase · Lipase · L-Glutaminase · Phytase · Protease · Tannase

9.1 Introduction

Enzymes are ubiquitous biocatalysts, represents a consolidated technique in green chemistry for the transformation of one biological compound to another for biotechnological purposes. In contrast to conventional synthesis, enzymatic synthesis is an important technological advancement as a consequence of sustainability, substrate selectivity, nontoxicity, and low energy input. Therefore, there has been a growing trend in the use of free and immobilized enzymes in many industrial applications, including the manufacturing of several functional foods and ingredients. Functional foods with health benefits beyond nutrition have increased rapidly due to factors such as interest in health-consciousness and the growing market. A variety of functional foods can be considered to be those fortified, wholes, enriched, or enhanced foods that represent physiological health benefits beyond the essential nutrients provision (e.g., vitamins and minerals) when consumed in adequate amounts on a regular basis (Alemzadeh et al., 2020). The initial components in functional food supplements naturally consist of fruit, food, beverage, grains, and supplement sectors at low levels (Granato et al., 2020). Nutraceuticals or functional foods involve extraction, purification, concentration, and activation processes in order to be utilizable in one of the several areas of food science and technology, which could be processed by an enzymatic reaction. The discovery, characterization, and stabilization of various enzymes employed in the production of functional foods have been attended increasingly in many researches.

Food and stress are two major factors that have a direct impact on the gut microbiome and are responsible for inflammatory reactions in the human gastrointestinal. Any dysbiosis in the gut microbiota results in several gastrointestinal-associated diseases like diarrhea, irritable bowel syndrome (IBS), and colorectal cancer. The use of functional foods has been increasingly applied as a complementary therapy to rejuvenate and enrich the beneficial gut microorganisms, thereby, promoting the health, alleviate, and relief the symptoms associated with gut-related diseases and side effects of conventional diseases, reduce the risk of cardiovascular diseases, and improve overall well-being. Microbial enzyme engineers or enhance the production of certain naturally occurring dietary substances through fermentation technology (Melini et al., 2019). Functional foods, enriched with prebiotics, synbiotics, and probiotics, as well as other plants- and animal-derived food compounds, are also considered beneficial additives to improve the consumers health and well-being (de Paulo Farias et al., 2019; Vaziri et al., 2019).

Functional food could be enzymatically derived from various reactions; the L-asparaginase enzyme produced by the enzymatic reaction is used in the baking industry, which suppresses acrylamide production. The addition of asparaginase is a recommended approach to decrease the formation of acrylamide since it results in the hydrolysis of asparagine to aspartic acid, an amino acid with high nutritive value (Momeni et al., 2015; Torang & Alemzadeh, 2016).

Lactic acid bacteria strains have been shown to produce glucose, galactose, and oligosaccharides (prebiotics), as a result of lactose hydrolysis and transgalactosidal activities of beta-galactosidase. Another illustration is the production of

prebiotic-based compounds from cereals, a kind of non-starch polysaccharides (NSP), such as β-glucan and arabinoxylan as dietary fiber constituents (You et al., 2016). β-Glucan consists of multiple sugar molecules, which has a significant role in lowering cholesterol level, boosting blood sugar management, and improving the immune system. This essential fiber is synthesized by an enzymatic process through the action of carbohydrase on beta D-glucose polysaccharide, which basically occurs in the cell wall of cereals like barely and oat (Kumar et al., 2020).

Chlorophyllase enzyme (Chlase, chlorophyll-chlorophyllidohydrolase, EC 3.1.1.14) is a hydrophobic enzyme in the chlorophyll hydrolysis to become degraded into chlorophyllide (Childe) and phytol. Chlorophyllide can be further considered as natural water-soluble food colorant alternative to synthetic colorants. Moreover, they possess biological and therapeutic activities, such as anti-inflammatory, antioxidant, antibacterial, wound healing, anticarcinogenic, and deodorizing properties (Hosikian et al., 2010).

Incorporation of lipase enzyme in the esterification of glycerol with CLA and production of enriched fatty acid derivatives with more health benefits and reduction in free fatty acids accumulation is a solution for functional food development. Lipids such as CLA (conjugated linoleic acid) are in the group of functional food, resulting from CLA production from natural oil in the presence of a lipase. CLA is a natural derivative of essential fatty acid, linoleic acid, which shows many beneficial effects on the immune system, obesity control, and anticarcinogenic behavior. Catalytic action of lipase enzyme in the esterification of glycerol with CLA causes the production of enriched fatty acid derivatives with more health benefits and reduction in free fatty acids accumulation. Incorporation of lipase enzyme in the esterification of glycerol with CLA and production of enriched fatty acid derivatives with more health benefits and reduction in free fatty acids accumulation is a solution for functional food development (Kouchak Yazdi & Alemzadeh, 2016a, b; Kouchak Yazdi et al., 2017).

Synthesis of theanine was examined in the presence of the L-glutaminase enzyme produced by *Trichoderma koningii* fungus. Production of L-glutaminase enzyme was first concerned by *T. koningii* using sesamum oil cake under solid-state fermentation (SSF). Then L-theanine production by L-glutamine and ethylamine as substrates and extracted enzyme solution were evaluated (Sakhaei & Alemzadeh, 2017).

Enzyme-catalyzed degradation to enhance the bioavailability of metal ions like iron and zinc in cereal-based foods is an example of enzyme-hydrolysis by phytase. The effects of phytase functionality on amino acid digestibility and energy utilization, and the in vivo functional food specificity have been monitored (Dersjant-Li et al., 2015). Phytases application in functional foods development is recommended for the increment of phytic acid dephosphorylation and degradation. Phytase can increase mineral bioavailability and decrease the anti-nutritional effect of phytate (inorganic phosphate complex) by improving phosphorous (P) digestibility, as well as reducing the negative impact of inorganic P excretion on the environment (Song et al., 2019; Handa et al., 2020).

Proteolytic enzymes are ubiquitous in innumerable biological systems and led to improve functional and biological characteristics of protein byproducts, which can

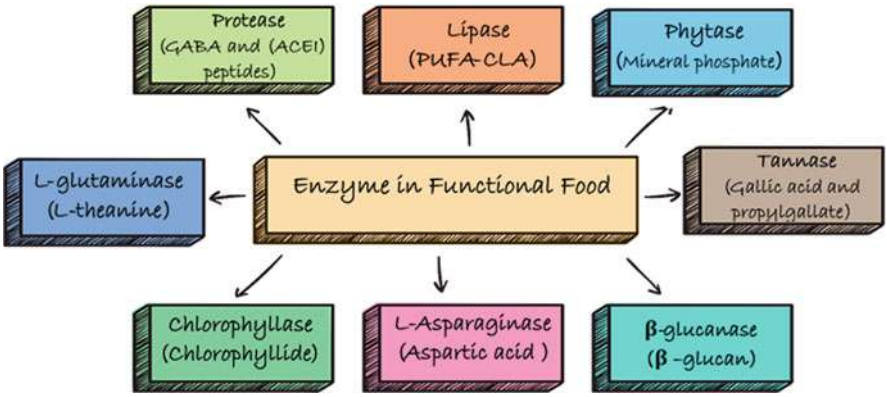

Fig. 9.1 Enzymes accessible in functional food development and the selected bioactive components

be further utilized as protein substrates for food applications (Pokora et al., 2013). Bioactive peptides (BP) are one of the most important bioactive compounds generated from protease hydrolysis. The protein fragments of BPs act as peptide inhibitors of the angiotensin-converting enzyme (ACE), and antimicrobial, anti-aggregating, antithrombotic, anticancer and antihypertensive, and immune-modulatory peptides. The proteolysis of BPs is required for their release and activation since they are hidden and encrypted within the parent proteins, such as casein, lactalbumin, beta-lactoglobulin, and lactoferrin (De Leo et al., 2009). Gamma-aminobutyric acid (GABA) is another potent bioactive protein catalyzed by glutamate decarboxylase. GABA functions as a major inhibitory neurotransmitter in the mammalian brain and central nervous system that strongly effects the behavior and stress management. It is widely distributed in nature and demand for microbial GABA supply has raised to satisfy the needs of GABA-enriched foods (Cui et al., 2020).

Tannase is another group of enzymes that are suitable candidates as biocatalysts in the treatment of phenolic-rich foods. It catalysis the biotransformation of phenolic compounds in beverages to generate a product with enhanced clarity and color appearance, as well as promoting its functional activity. As an example, the process of browning in tea beverages occurs due to the presence of caffeine and catechins in the tea, which results in the formation of insoluble compounds in cold water. Biotransformation by tannase reduces the gallic acid-binding and inhibits the browning effect (Srivastava & Kar, 2009; Cao et al., 2019; Gligor et al., 2019). The enzyme accessible in functional food development and bioactive components is categorized in Fig. 9.1. In this chapter, we describe the functional food components, functional food in health, the role of selected enzymes in functional foods manufacturing, or food improving quality circumstances. Enzymes, such as asparaginase, carbohydrase, chlorophyllase, lipase, L-glutaminase, phytase, protease, and tannase are introduced, and the beneficial health effects are discussed.

9.2 Functional Food Components

Functional foods are enriched or dietary materials that may offer several health benefits beyond basic nutrition. Functional foods for health are an essential factor of an overall healthful lifestyle that consists of a balanced diet from various sources. People should strive to consume a wide variety of foods and food components including the examples listed in Table 9.1. These food components could naturally exist in natural food, such as whole grains, fruits, vegetables, beverages, enriched compounds, and dietary supplements, extracted by chemical approaches or produced by enzymatic processes.

9.2.1 Functional Food in Health

Obesity and malnutrition are major drawbacks associated with diet habits, which are strongly influenced by urbanization, and lifestyle alterations (Tanna & Mishra, 2018). Functional food could inherently include bioactive ingredients or nutraceuticals and could be added from various sources to produce fortified food. The bioactive ingredients are in the group of carbohydrates, enzymes, protein, phytochemicals, vitamins, and lipids (Suleria et al., 2015; Maqsood et al., 2020). These bioactive compounds could be derived from different sources like cereals, seeds, marine-based, microorganisms, plants, animals, and fishery. Since bioactive compounds in natural food products are in low amounts, the bioactive ingredients could be produced by a microbial or enzymatic process in high quantity. The bioactive ingredients have antibiotic, antiparasitic, antiviral, anti-inflammatory, anti-fibrotic, and anticancer activities. Health benefit of the bioactive compounds affects metabolic syndrome (Mets), such as CVD, elevated triglycerides, cholesterol, and blood pressure, besides insulin resistance (Sirtori et al., 2017). Nutraceutical ingredients have specificity such as antimicrobial, antiviral, antioxidant, anti-obesity, anticancer, antidiabetic, anti-inflammatory, nephrotoxic, and arthritis.

9.3 Enzyme Application in Functional Food

Enzymes are catalytic biomolecules responsible for the catalyzing diverse biochemical reactions in several food industries. Enzymatic processes can be a promising alternative to conventional organic synthesis, as they provide eco-friendly, more viable and sustainable conditions, safer products, and use of renewable substrates. Enzymes are therefore possessing a significant role in many biotechnological applications including food quality improvement, as well as food manufacturing operations. Selected enzymes representing these concerns are defined and evaluated respectively.

Table 9.1 Examples of functional food sources selected nutraceuticals, and their biological activity

Functional food sources	Selected nutraceutical	Biological activity	Reference
Coriander fruits (seed and pericarp)	Fatty oil	Antimicrobial, antioxidant, antidiabetic	Sahib et al. (2013)
Non-starch polysaccharides (NPS)	Dietary fiber	Cholesterol reduction	Sirtori et al. (2017)
Pumpkin seeds	Traditional medicine	Antioxidative, hypoglycemic, anticancer	Patel and Rauf (2017)
Yeasts	Various bioactive components	preventing oxidative stress	Rai et al. (2019)
Probiotics	Traditional medicine	Intervention of IBD	Al Mijan and Lim (2018), Grom et al. (2020)
Seaweed	Metabolite	Chronic diseases such as cancer, arthritis, diabetes	Tanna and Mishra (2018)
Mediterranean diet	Various bioactive components	Antidiabetes	Alkhatib et al. (2017)
Plant sterols and stanols	Phytosterols	Plasma cholesterol control	Poli et al. (2018)
Phytochemicals	Various bioactive components	Cancer, CVD, anti-obesity	Gul et al. (2016)
Higher polysaccharide	Dietary fibers	Anti-cardiovascular and cerebrovascular disease	Venkatakrishnan et al. (2020)
Food bioactive compounds, plant foods	Polyphenol, flavonoids	Anticancer	Adefegha (2018)
CLA	PUFAs	Anti-obesity, antidiabetic	Kouchak Yazdi et al. (2017), Yazdi and Alemzadeh (2017)
Cereals	Polyphenols	Anti-chronic, diabetes, anticancer, anti-obesity	Arab et al. (2011)
Marin microorganisms	Bioactive polysaccharides	Anticoagulant, anti-inflammatory, antiviral	Dewapriya and Kim (2014), Suleria et al. (2015)
Date fruit and seed	Phytochemical	Antioxidant, antifungal and antiviral, anticancer	Maqsood et al. (2020)

9.3.1 L-Asparaginase

L-Asparaginase, also known as asparagine amino hydrolase (EC 3.5.1.1), is an intercellular enzyme broadly used in various pharmaceutical and food applications for catalyzing the degradation of L-asparagine into ammonia and L-aspartate. In the medicinal aspect, the asparaginase role is to inhibit cell starvation by reducing asparagines availability (Asselin & Rizzari, 2015). The L-asparaginase has been regarded to be a safe and favorable additive for the mitigation of acrylamide levels during food processing (Baskar et al., 2019).

Acrylamide is a main component in many carbohydrate/starchy-based foods, such as cereals, peanuts, potatoes, vegetables, crips, chips, coffee, biscuits, and lentil, particularly when exposed to high temperatures up to 120 °C. Several food processes including frying, baking and roasting, and process conditions, such as temperature, type of product, humidity, time, and content of reducing sugars and free amino acids result in the formation of acrylamide, and the processing variables influence directly the level of acrylamide content (Zuo et al., 2015). Acrylamide is generated during Millard reactions in the presence of reduced sugars and asparagine. The main consequences of acrylamide formation in food products are the strong color and flavors.

The potential toxicity and adverse effects of this compound on human health were declared (WHO/FAO). L-Asparaginase has remarkable attention in research from past decades, because of its vital role in the metabolism of amino acids, and thus, can be considered for the treatment of different types of cancers and diseases including acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), non-Hodgkin's lymphoma, and food applications (Alam et al., 2019). According to the Global Asparaginase Sales Market Report, the asparaginase market has growing demand. The reported market value for asparaginase was 380 million USD in 2017 and is estimated to reach 420 million USD by 2025 (Global Medical Asparaginase Sales Market Report, 2018). L-Asparaginase is isolated from various plants and animal sources, such as animal cells, algae, chicken liver, serum of rodents, and microorganisms including fungal, yeast, and bacteria. *Bacillus* sp. and *Pseudomonas* sp. are the two main microbial sources of asparaginase. Bacterial sources have gained more attention for industrial application due to their versatile characteristics, easy maintenance, and high efficiency (Shi et al., 2017). There are two types of L-asparaginase; type I and type II. For food and medical applications, type II asparaginase is more favorable. Type II asparaginase shows a high affinity toward L-asparagine and hence it has been implicated for the removal of asparagine before processing the raw ingredients to reduce acrylamide formation (Hendriksen et al., 2016). Although several types of research emphasized the allergic reactions due to the high immunogenicity of the asparaginase and its clinical toxicity, such as headache, dyslipidemia, diarrhea, nausea, mucositis, pancreatitis, liver dysfunction, central nervous system dysfunction, and abdominal pain, type II asparaginase proved to be less immunogenic, and therefore, is more suitable for medical applications (Mitchell et al., 1994; Rizzari et al., 2000; Zuo et al., 2015). Other microorganisms with fewer adverse effects such as eukaryotic microorganisms have

been explored (Patro et al., 2014). Fungal sources are also considered safe asparaginase producers (generally recognized as safe, GRAS). It has been reported that fungic L-asparaginase utilization during food manufacturing leads to acrylamide reduction without any negative impact on the final appearance and nutritional value of the product. This approach mainly occurs from two aspects, interference with Millard reactions and removal of L-asparagine as the main precursor of acrylamide formation (Batool et al., 2016). However, the efficacy of asparaginase formation is not sufficient for the industrial demands (FAO/WHO, 2002; Jha et al., 2012). Two commercial samples of fungal asparaginase in the food industry, PreventAse (DSM) and Acrylaway (Novozymes) are obtained from *Aspergillus niger* and *Aspergillus oryzae*, respectively (Pedreschi et al., 2011; Xu et al., 2016; DSM, 2018). PreventAse presents an optimum pH in the range of 4–5 and temperature at 50 °C, while these values for Acrylaway are 6–7 and 60 °C. The activity of L-asparaginase during food preparation and hydrolysis should be high, thus producing a stable enzyme is of great importance.

The cultivation conditions including media composition, pH, temperature, oxygen levels, and the fermentation technique, namely solid-state fermentation or submerged fermentation, have considerable influence on the production of an ideal enzyme (Brumano et al., 2019). Carbon and nitrogen are the most important substrates in the culture medium used for L-asparaginase production (Cachumba et al., 2016). Glucose is considered the most appropriate carbon source to achieve L-asparaginase (Doriya & Kumar, 2016). L-Asparagine, L-proline, urea, yeast extract, and peptone are some of the important sources of nitrogen for high-yield asparaginase (de Moura Sarquis et al., 2004).

Determination of the optimum pH and temperature of the fermentation medium is also an important step for producing high-yield enzymes. The emergence of engineered enzymes according to the structural and mechanistic methods and direct evolution is needed to enhance the efficiency of enzymes for the synthesis of various products (Bornscheuer et al., 2012). Bakery products, French fries, and roasted coffee are the most widely consumed products that have high acrylamide content.

9.3.2 Carbohydrate Active Enzymes

Glucans are non-starch glucose polymers and one of the most abundant polysaccharides found in the cell wall of fungi, plants, and bran cereal grains (Wu et al., 2018). The glucose moieties link through either or both alpha (α) or beta (β) linkages, so their structures can be either linear or branched, and microfibrillar or amorphous. α-1,3-Glucans sensu strictu (pseudonigerans) is known as the most abundant α-glucans in the cell wall of fungi. Their structure is in the form of microfibrils which make resistance to the cell wall. On the other hand, the structure of β-glucans is a complex of linear or branched. In most cases, the structure is in the form of microfibrils with mostly β-1,3-linear, and β-1,6-branched linkages. One such example is a water-soluble low molecular weight polymers (LMW), which contains β-1,2 besides β-1,6 joined branches and has been isolated from black yeast

Table 9.2 The classification of β-glucans and β-glucanases enzymes from various sources

β-Glucans type	Structure	Description	EC number	References
Bacterial (*Euglena gracilis*)	Linear	β-1,3-glucan	Exo: EC 3.2.1.58 Endo: EC 3.2.1.39	Wu et al. (2018)
Fungal (*Schizophyllum commune*)	Branched	β-1,6-branched, and β-1,3-glucan	Endo: EC 3.2.1.6	Sutivisedsak et al. (2013)
Yeast	Long branched	β-1,6-branched and β-1,3-glucan endo-β-1,3-glucanases	EC 3.2.1.39 EC 3.2.1.6	Sato et al. (2012)
Cereal (barley)	Linear	β-1,3/1,4-glucan	Endo 3.2.1.6	Rodríguez-Mendoza et al. (2019)
Lichen (lichenase, licheniase)	Linear	1,3–1,4-β-glucan	Endo 3.2.1.73	You et al. (2016)

(Aerobasidiumpillulans). β-1,3–1,6-D-glucan represents antitumor, anti-metastatic, and anti-stress effects (Sato et al., 2012). β-Glucans together with chitin are known to be the most important structural constituents of fungal cell walls (Ruiz-Herrera & Ortiz-Castellanos, 2019).

As an example of a β-glucans-producer fungal, *Schizophyllum*, produces schizophyllan, which is a homoglycan natural polysaccharide with a β-1,3 linked backbone and single-1,6-linked glucose side chains at each residue. Its role as a biological response modifier and a stimulator of the immune system provides a variety of applications in the therapeutic and cosmetics industry. This biodegradable polymer has been also used for food preservation by forming oxygen impermeable films (Sutivisedsak et al., 2013).

β-Glucanase is an enzyme classified in the glycosyl hydrolase family that catalyzes the release of glucose from the reducing and nonreducing ends of β-glucan. The glucose released from the catabolic activity of β-glucanase applied in bioethanol and biofuels manufacturing (Lopez-Casado et al., 2008). Selected β-glucanases have an essential role in the pharmaceutical and medical industry by biologically controlling fungal pathogens, synthesizing drug carriers, and producing in vivo immunomodulatory anticancer drugs (You et al., 2016). β-1,3-Glucanases have been used in the food industry, mainly for alcoholic beverages processing. The classification of β-glucans and β-glucanases enzymes is represented in Table 9.2.

The modification of polysaccharides through β-D-glucans hydrolysis results in gluco-oligosaccharides production which provides several biological activities such as immunomodulation (Giese et al., 2012).

Another advantage of exogenous β-glucanase is to incorporate it into wheat and barley-based diets to inhibit the anti-nutritive effect of water-soluble NSP. Benefits of using exogenous enzymes for nutrient digestibility have been reported previously; however, the mechanism of action of these enzymes is not fully recognized. One other application of these exogenous enzymes is to enhance the microbial activity of the poultry intestine (Giese et al., 2012).

β-1,3-Glucanases have been recommended to apply in the food industry to improve the organoleptic properties of the final products. Besides, they have a positive role in the production of feed and animal nutrition (Rodríguez-Mendoza et al., 2019). The sources of glycoside hydrolase β-1,3-glucanase are extended among fungi, bacteria, and plants. The hydrolysis position of structural β-1,3-glucans is on 1,3-β-glucosidic bonds to transfer or hydrolyze glycosides. β-1,3-Glucanases are normally classified into endo-enzymes (EC 3.2.1.39) and exo-enzymes (EC 3.2.1.58). β-1,3-Glucanases are able to act as a protective agent against fungal pathogens and provide immune-modulating and antitumor properties in plants. They are also capable to suppress fungal growth in fermentation media by the convention of β-1,3-glucans into (1→3)-linked β-D-glucan oligosaccharides (Dewi et al., 2016; Wu et al., 2018).

Endoglucanases are generally classified into three categories based on their modes of action: Endo-1,3–1,4-β-glucanase (EC 3.2.1.73), endo-1,3-β-glucanase (laminarinase; EC 3.2.1.39), and endo-1,3(4)-β-glucanase (EC 3.2.1.6). They specifically cleave the blended (1→3) or (1→4) linkages of β-D-glucan. β-Glucanases are important commercial biocatalysts in various industries, from exogenous β-glucanase, which are applicable specifically in reducing the unwanted effects of barley β-glucan in the mashing operation as a step in the brewing industry, and to improve the β-glucan digestibility in poultry feedstuffs, to endoglucanase to convert biomass into bioethanol in and xylanase in bioenergy production (You et al., 2016).

9.3.3 Chlorophyllase

Chlorophyllase enzyme (Chlase, chlorophyll chlorophyllidohydrolase, EC 3.1.1.14) is a hydrophobic enzyme leading to chlorophyll degradation into chlorophyllide (Chlide) and phytol. Chlorophyllase activity is the most important step in the chlorophyll (Chl) breakdown and synthesis (Hörtensteiner & Kräutler, 2011). Another Chl hydrolysis pathway is through pheophytinase, which leads to pheophorbide (Schelbert et al., 2009). The chlorophyll breakdown pathway determines the Chl accumulation and various intermediate catabolites in chloroplasts. Chlorophyll hydrolysis is most evident in fruit ripening, and seasonal changes as leaf senescence and the color of plants convert from green to yellow. Chlorophylls are one of the most important plant pigments that determine the absorption of solar radiation in leaves (Filella et al., 1995). They are a brilliant source of natural green color and can be an alternative to synthetic colorants. Moreover, they possess biological and therapeutic activities, such as anti-

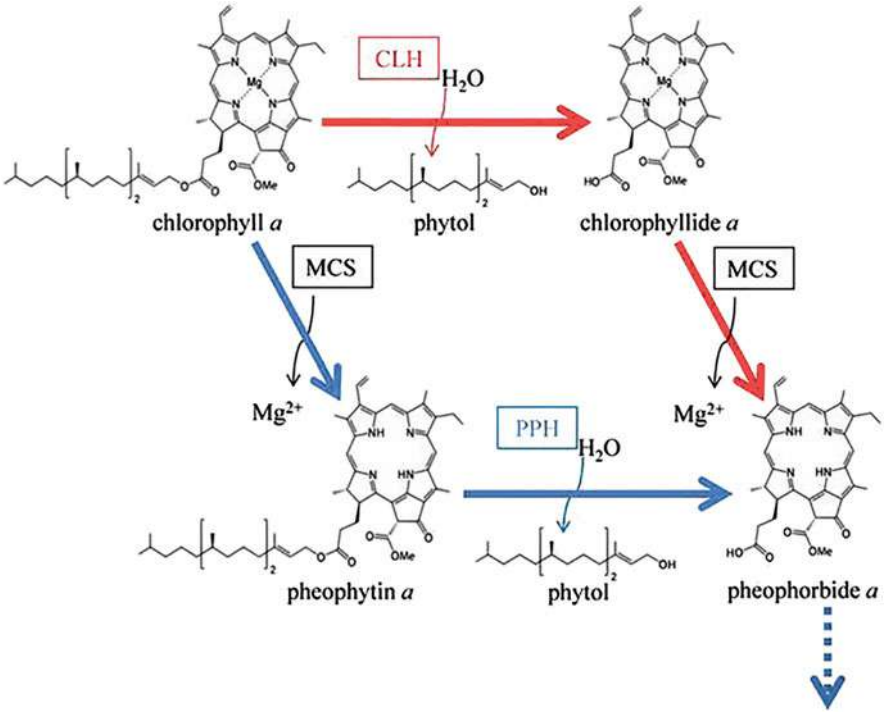

Fig. 9.2 The initial steps of chlorophyll catabolism (CLH and MCS are refer to chlorophyllase and magnesium-dechelating substance, respectively) (Hu et al., 2015)

inflammatory, antioxidant, antibacterial, wound healing, anticarcinogenic, and deodorizing properties (Hosikian et al., 2010).

The activity of chlorophyllase in fruits and vegetables prolongs during storage and processing conditions. Chlorophyllide is obtained through enzymatic conversion of chlorophyll by the cleavage of the phytol group and is used for the preservation of green color in various canned vegetable products. By the aid of magnesium-chelating substrates, the induction of chlorophyll breakdown leads to pheophytins hydrolysis and more pheophorbide formation (Fig. 9.2). A well-known application of chlorophyllide formation is the formation of pheophorbide during olive fermentation. For example, the activity of chlorophyllase in ripe olives of arbequina and picual varieties was studied and demonstrated that the process of oil extraction and propitiating the pheophytization of chlorophylls in the fresh fruit cause a significant reduction in the chlorophyll's pigments (Gandul-Rojas & Mínguez-Mosquera, 1996).

As shown in Fig. 9.2, the catabolism of chlorophyll to synthesize chlorophyllide triggers chlorophyllase. In both decoloration processes and the homeostasis of chlorophylls, chlorophyllase takes part as an effective catalyst in the early steps of chlorophyll breakdown. The role of chlorophyllase in plant development and

survival is vital because it leads to faster degradation of chlorophyll and its intermediates, and further detoxification process (Carle & Schweiggert, 2016).

Experimental evidence in epidemiology has shown the association of green fruits and vegetables with chronic disease prevention, thus, phytochemical foods have established as bioactive dietary compounds. Chlorophyll and its lipophilic derivatives are composed of chlorophyll a and b (fresh fruits and vegetables), metal-free pheophytins and pyropheophytins (thermally processed fruits and vegetables), and Zn-pheophytins and Zn-pyropheophytins (thermally processed green vegetables) are believed to be responsible for such associations, as they are phytochemical compounds. Chlorophyllides, pheophorbides, and sodium copper chlorophyllin (SCC), a commercial-grade derivative, are hydrophilic compounds with the predominant contribution to the dietary chlorophyll role. Antioxidant and antimutagenic activity, mutagen trapping, modulation of xenobiotic metabolism, and induction of apoptosis are attributed to useful biological activities of chlorophyll derivatives which assimilate cancer prevention.

Although the medical evidence of chlorophyll derivatives are mainly focused on commercial-grade SCC, there is significant attention to the cancer-preventive potential of chlorophyll. Further research is required to define the extent to which chlorophyll derivatives are responsible for modulating cancer-risk biomarkers (Ferruzzi & Blakeslee, 2007).

Chlorophyllase catalyzes the hydrolysis of chlorophylls to chlorophyllide and phytol. This catalytic activity of chlorophyllase has potential industrial and agricultural applications. In this context, the application of chlorophyllase could be useful in the removal of green pigments (chlorophyll) from edible oils to improve their oxidative stability. In addition, the products of chlorophyllase, chlorophyllides, and their derivatives (such as pheophytin) have been demonstrated to have antiviral, antioxidant, antimutagenic, and anticarcinogenic activities in vitro. Accordingly, in recent years, trends for purification of natural chlorophyllases or recombinant production of the enzyme has been increased (Sharafi et al., 2017).

9.3.4 L-Glutaminase

L-Glutamine catalysis is the enzymatic reaction of thiamine. Thiamine (γ-glutamyl ethyl amide) is a nonprotein amino acid and is known to be responsible for the sense of relaxation in green tea. L-Thiamine is generally recognized as safe (GRAS), and apart from its umami taste and unique flavor, has several pharmaceutical and physiological effects, such as anticancer, anti-obesity, anti-hypertensive and neuroprotective effects. It may further improve learning ability and mind performance. Thus, thiamine is an essential compound for the human body that should be administrated orally, as it is not synthesized in the body (Haskell et al., 2008; Panesar et al., 2016).

The natural sources of thiamine include plants, animals, and microorganisms. Microbial-derived is preferable sources compared to plant and animal-derived thiamine, mainly due to the low cost of operation, as well as other technical advantages

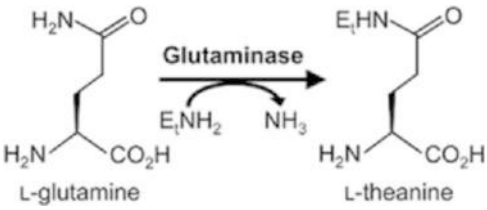

Fig. 9.3 Theanine synthesis reaction catalyzed by L-glutaminase in the presence of ethanolamine ($EtNH_2$) (Sakhaei & Alemzadeh, 2017)

such as higher yields within a limited period of fermentation time. The enzymatic biosynthesis of L-thiamine takes place in the presence of ethylamine and L-glutamine as substrate and catalyst (glutaminase). L-Thiamine production was carried out under solid-state fermentation by the fungal strain *Trichoderma koningii*. The enzymatic synthetic reaction for theanine production is presented in Fig. 9.3.

9.3.5 Lipase

Lipases, also known as triacylglycerol acyl ester hydrolases (EC 3.1.1.3), are capable to catalyze cleavage of carboxyl ester bonds in tri-, di-, and monoacylglycerols. Most lipases are generally recognized as safe (GRAS) and can be employed in the food industry, mainly for diary applications with the aim of ripening acceleration in Italian-type cheeses, and hydrolysis of milk fat to further be used as a flavor enhancer in cheese products (Afarin et al., 2018).

Further applications of lipases in pharmaceutical and fine chemical preparation processes include (but are not limited to) lipase-mediated synthesis of optically pure and optically active polymers, lipase-catalyzed resolution of racemic aqueous mixtures, monoglycerides production (as an emulsifier in the food and cosmetic products), fatty alcohols (as lubricants in hydrophobic waste treatment), and sugar esters as biosurfactants (Tarahomjoo & Alemzadeh, 2003).

Another advantage of lipase incorporation in dairy products is fat and oil-tailoring for enhancing functional and nutritional properties. One such example is employing lipase in conjugated linoleic acid (CLA) large-scale production for the modification of triglycerides and upgrading of low-value oils and fats like human milk fat replacers, cocoa butter substitutes, and enrichment of nutraceutical fats with poly-unsaturated fatty acids (Paiva et al., 2000; Yazdi & Alemzadeh, 2017). CLA is one of the most physiological active fatty acids a natural derivative of linoleic acid, an essential fatty acid, that possesses a conjugated double-bond system for describing octadecadienoic acids mixtures (18:2) (Jiang et al., 1998; Lin, 2006; Gofferjé et al., 2014).

Due to the global rising trend concerning diseases, such as obesity, hyperlipidemia, atherosclerosis, diabetes, cancer, and high blood pressure and the established association of lifestyle on health management, the quality of dietary fat is an important factor that should be considered for prevention and treatment of early stage diseases. In this regard, conjugated fatty acids have gained much attention for their potential health effects on lowering the risk of these diseases, such as

anticarcinogenic activity, immune system enhancement, immune stimulation, lowering atherosclerosis symptoms, and cholesterol reduction (Niezgoda & Gliszczyńska, 2019; Kouchak Yazdi & Alemzadeh, 2016a, b; Afarin et al., 2018).

Natural sources of CLA include dairy products (0.40–0.55% of total dairy lipids) and beef (0.43% of total beef lipids). *Lactobacillus plantarum* is a microbial-derived source of nutritious and valuable components, such as CLA. CLA contains a variety of positional and geometrical conjugated double bonds of linoleic acid (C18:2) isomers, including all possible geometric configurations of trans–cis, cis–trans, cis–cis, and trans–trans isomers (Paiva et al., 2000; Philippaerts et al., 2013).

The 9c, 11t-18:2 and 10t, 12c18:2 isomers of CLA are believed to be responsible for the beneficial physiological effects of CLA. The 9c, 11t-18:2 isomer (rumenic acid), is the main isomer, consists of 90% of the total CLA found in dairy and beef lipids; so, the focus of most research on CLA employment is on cis-9, trans-11 CLA. Moreover, the multitude health-promoting effects of CLA, such as anti-atherogenic activity, are associated with c9, t11 CLA (ANDO, 2004). Other isomers, namely, trans-10, cis-12 (t10, c12CLA), are found in much smaller quantities. c9, t11, and t10, c12 isomers are determined to represent the highest bioactive property (Ogawa et al., 2005). Further investigations on optimizing the experimental design for a safe and selective biological process for CLA preparation to achieve enriched food with high CLA concentration are required (Afarin et al., 2018).

Although the exact mechanism of anticarcinogenic and atherosclerosis risk-reduction benefits is still needed to be studied, clinical evidence demonstrates that the management and prevention of these diseases are achievable through CLA-functional food on regular basis. It has been recommended that the consumption of 3 g/day of CLA is essential to provide essential health factors. It is noteworthy that the attainable CLA from natural resources is approximately 0.3 g/day; therefore, CLA-based dietary supplements and CLA-rich vegetable oils and dairy products should be considered as part of a healthy lifestyle. Main commercial approaches for CLA production consisting of chemical, microbial, and enzymatic methods are represented in Table 9.3. Each technique implements a different protocol and results in a different mixture of CLA isomers. Among them, the alkali isomerization of linoleic acid has been reported to be the most potential and cost-effective method (Yang et al., 2002; Afarin et al., 2018).

According to the potential health benefits demonstrated for CLA, it is a preferable additive for the preparation of functional foods. The synthesis of CLA can be performed either by isomerization of linoleate-rich oils with the aid of an alkaline catalyst or by dehydration of ricinoleic acid-rich oils or their esters using an acid catalyst.

CLA isomers, either in their natural or synthetic forms, have been shown considerable physiological effects on human health. CLA free fatty acid may cause undesirable reactions in the human gut. Thus, it has been recommended to be consumed in the form of triglycerides. One way is to apply sunflower oil for transesterification of lipid, mainly due to its beneficial health effect, via lipase-catalysis. Enzymatic process is an alternative method to catalyze the transesterification reaction biologically to produce triacylglycerols with enriched

Table 9.3 CLA production by various techniques

Method	Catalyst	Substrate	References
Chemical	Alkaline	Linoleic acid	Afarin et al. (2018)
Chemical	Alkaline	Ricinoleic acid	Yang et al. (2002)
Chemical	Metal catalyst ($SnCl_2$)	Methyl linoleate	Philippaerts et al. (2013)
Microbial	*Lactobacillus plantarum* PTCC 1058	Castor oil	Kouchak Yazdi and Alemzadeh (2016a, b)
Microbial	Dairy starter cultures	Linoleic acid	Jiang et al. (1998)
Microbial	*Lactobacillus acidophilus*	Linoleic acid	Ogawa et al. (2005)
Microbial	*Lactobacillus plantarum* A6-1F	Linoleic acid	Zhao et al. (2011)
Microbial	*Lactobacillus reuteri*	Linoleic acid	Lee et al. (2003)
Microbial	*Bifidobacteria* and *Propionibacteria*	Linoleic acid	Hennessy et al. (2012)
Microbial	Lactic acid bacteria	Linoleic acid, castor oil	Ogawa et al. (2005)
Microbial	*Lactobacillus plantarum* JCM 1551	Castor oil	ANDO (2004)
Enzymatic	Crude enzyme from *Lactobacillus delbrueckii* ssp. *bulgaricus* (CCRC14009)	Linoleic acid, oleic acid, linolenic acid	Lin (2006)

residues. Generally, the alteration of fatty acids composition results in promoted nutritional properties of triacylglycerol molecules. Identifying an environmentally friendly and less energy-intensive strategy for oil esterification by using an enzyme as a catalyst should be investigated (Ogawa et al., 2005; Hennessy et al., 2012).

9.3.6 Phytase

Phytic acid, designated as phytate when in salt form, is regarded as a primary reservoir of organic phosphorus in pollen and plant seeds like nuts, cereals, oil seeds, and legumes. It has a high negative charge and is, therefore, able to form ionic complexes with essential cationic minerals (Ca^{2+}, K^{2+}, Fe^{2+}, Zn^{2+}, Mg^{2+}, and Mn^{2+}), proteins, and trace elements (Greiner & Konietzny, 2006). A main anti-nutritional consequence of this interaction is the considerable reduction in dietary minerals bioavailability. Phytase, also known as myo-inositol (1,2,3,4,5,6)-hexakisphosphate phosphohydrolase, act as a dephosphorylating enzyme to transfer phytic acid to more soluble and lower chelating products to inhibit anti-nutritional factors of phytic acid in food products and increase mineral absorption (Kuddus, 2018). Depending on their catalytic mechanism, phytases can be divided into histidine acid phytases, cysteine phytases, β-propeller phytases, or purple acid phytases (Chu et al., 2004) and based on their optimum pH, into acid and alkaline phytases. Dephosphorylation is initiated at the carbon in the myo-inositol ring of phytic acid. There are three different types based on the carbon; 3-phytases (EC 3.1.3.8), 5-phytases

(EC 3.1.3.72), and 6-phytases (EC 3.1.3.26) (Greiner & Konietzny, 2006). Enzymatic dephosphorylation of phytates was originally a strategy to improve the nutritional value of feed by liberating phosphate, especially for mono-stomached animals like fish, broilers, shrimp, pigs, and poultry diets (Konietzny & Greiner, 2004).

Recently, there have been numerous studies showing the potential use of these enzymes for functional food development. One of the major concerns in phytate hydrolysis content during food manufacturing is the selection of an ideal phytase. The activity of added phytase has to be high enough during food preparation. Therefore, a high capability of phytate degradation over a wide range of temperatures and pH is essential (Greiner & Konietzny, 2006). According to previous studies the optimum pH range for acid phytase is 3.5–6.0, and for alkaline phytase is 7.0–8.0. The optimum temperature for high phytate-degrading activity is in the range of 35–80 °C. The optimum temperature and pH of the added enzyme are strongly affected by the source of phytase.

Phytase has been found in plants, microorganisms, and animal tissues. There are already available commercial phytases from different microbial sources, such as *Escherichia coli*, *Peniophora lycii*, *Aspergillus niger*, *Schizosaccharomyces pombe*. Commercial production of phytase from *Aspergillus*, a soil fungus, has several prominent drawbacks, such as fungal enzymes are catalytic efficiency, substrate specificity, and resistance to proteolysis. Bacterial phytases have been distributed in various aerobic and anaerobic species, including *Bacillus* strains, *Pseudomonas* strains, *Raoultella* strains, *Escherichia coli*, *Citrobacter braakii*, *Prevotella* strains, *Selenomonas ruminantium*, *Mitsuokella jalaludinii*, *Megasphaera elsdenii*, *Lactobacillus sanfranciscensis*. The expression of phytase from these bacteria sources is different and it can be regulated by the nature of the culture composition used for growth and the nutrient or energy limitations. The level of inorganic phosphate on the phytase synthesis induction was reported to be effective for all microbial sources, except *Raoultella terrigena* and the rumen bacteria. In some phytase producers, the nature of the carbon source is a significant inducible factor by involving in the catabolism pathway (Konietzny & Greiner, 2004).

Generally, microbial-derived phytases are more favorable economically compared to plant-derived ones, normally due to their thermostability and pH tolerance as well as a broad specific activity. The specific activity of characterized phytasea, is approximately in the range of 10–1000 U/mg at 37 °C and various pH optimums (Greiner & Konietzny, 2006). The highest specific activities are reported for microbial-derived enzymes. In food applications, it is important that the enzymatic properties of phytase to hydrolyze phytate in the gastric conditions be stable under acidic pH and pepsin enzyme. Likewise, for food supplements, an enzyme with acidic pH optimum and highly resistant to digestive enzymes is indisputably attractive.

However, when exposed to pH values above pH = 7.5 or below pH = 4.0, most of the plant enzymes dramatically lose their stability (Greiner & Konietzny, 2006). Besides, the activation of these enzymes is irreversibly affected when exposed to temperatures above 70 °C within a few minutes. Despite the fact that most of the

plant enzymes are inactivated at pH values lower or higher than the pH optima and high temperatures, they are more acceptable among consumers as they are rarely reported as an allergen.

The use of agro-industrial byproducts in the culture media has been suggested as low-cost substrates for the production of phytase (Pires et al., 2019). Corn meal and rice bran were used for the production of phytase from the fungus *A. zeae* B, and the yeast *K. marxianus*. The activity of phytases from agro-industrial products strongly depends on the species of the microorganism. For example, the determination of total phytase activity in the mentioned study revealed tenfold higher values in the yeast extraction compared to those of the fungus extraction (Pires et al., 2019).

Engineered phytases via genetic engineering are a promising type of enzyme that show optimized catalytic features, thermal stability, and broad specific activity for food application. Enhancement of thermal stability by a shift from 80 to 90 and optimum temperature from 55 to 65 for *Escherichia coli* was achieved by using mutagenesis technology. The basis of the phytase synthesis is to mutation of gene site saturation by comparing the amino acid sequences derived from both the available standard program and the homologous proteins (Rodriguez et al., 2000; Garrett et al., 2004).

The incorporation of phytase in the food industry is a growing trend, mainly for two purposes; increasing the mineral bioavailability of a given food by reducing its phytate content and exerting nutritional health benefits in a supplemental product (Konietzny & Greiner, 2004).

Natural phytate-degrading activity, observed in most plants, results in phytate dephosphorylation during food processing such as soaking, germination, and fermentation. This capability of intrinsic phytate-degrading activity in plant seeds exhibits over a wide range of pH values from 3 to 10, and the maximum activity peak occurs at pH = 5–5.5 (Greiner & Konietzny, 2006). Therefore, phytate hydrolysis by a certain phytase at optimum pH and temperature might differ greatly among various plants and microorganisms. Some examples of optimum conditions for maximum phytase activity are represented in Table 9.4.

9.3.7 Protease

Enzymatic hydrolysis for modification of food proteins in functional food applications has been explored for several decades. Proteolytic action of an enzyme results in alteration of molecular conformation of the native protein, and thus, leads to the production of functional and bioactive components as texture enhancers in beverages, infant formula, and pharmaceutical ingredients (Adebiyi et al., 2009). The resultant properties are highly affected by the degree of hydrolysis (DH). Variations in functionality factors such as solubility, stability, gelling behavior, water absorption, emulsion, and foaming capacity are best observed following a low degree of protein hydrolysis (<10%). Low-DH protein hydrolysates are appropriate for enhancing food texture, while high-DH protein hydrolysates are suitable

Table 9.4 The effect of phytase sources on the specific activity and pH and temperature optimum for various applications

Phytase source	Specific activity	pH optimum	Temperature optimum (°C)	Application	References
Yersinia intermedia	3960 U/mg	4.5	55	Feed industry	Huang et al. (2006)
Streptomyces sp. (NCIM 5533)	–	2.5	70	Plant growth	Puppala et al. (2019)
Sporotrichum thermophile	1190 U/mg	5.0	60	Dietary supplement	Maurya et al. (2017)
Aspergillus aculeatus APF1	13.82 U/mg	3.0	50	Biofortified cereals	Saxena et al. (2020)
Enterobacter sp. ACSS	805.98 U/mg	2.5	60	Dephytinizing animal feeds, baking industry	Chanderman et al. (2016)
Pichia anomala	–	4.0	60	Fractionation of allergenic soya proteins	Joshi and Satyanarayana (2017)
Rhizopus oligosporus MTCC 556	31.3 U/gds	5.5	50	Food and feed industry	Suresh and Radha (2016)
Aspergillus niger NCIM 563	160 IU/mL	2.5	50	Organophosphorus pesticides degradation	Shah et al. (2017)
Penicillium oxalicum EUFR-3	12.8 U/g	7.0	40	Food industry	Kaur (2017)
Aspergillus ficuum	5.17 U/gds	6.1	37	Food industry	Tian and Yuan (2016)

for utilization in medical diets, such as hypoallergenic foods or protein supplements (Pokora et al., 2013).

The scientific community has declared the important role of dietary compounds as health-promoting ingredients for food and pharmaceutical applications. Dietary components are mainly considered bioactive substances if their consumption in adequate amounts shows beneficial biological effects on human health. Therefore, the desirable consequences of bioactive compounds incorporation in food production are represented provided that their utilization is in the real sense and without the exertion of side effects (De Leo et al., 2009). Short peptides including 3–20 amino acids are examples of bioactive peptides (BP) that usually encrypted within the primary structure of a larger protein. BPs are encrypted within the parent proteins; so in order to exert their physiologically positive effects on human health, their proteolysis is required.

Biologically active peptides in the protein sequence are defined as "fragments that remain inactive in precursor protein sequences, but when released by the action of proteolytic enzymes, they may interact with selected receptors and regulate the body's physiological functions" (Mohanty et al., 2016). Bovine milk contains approximately 3–3.5% protein of which 80% and 20% are casein and whey proteins, respectively. It exerts a wide range of nutritional, functional and biological activities (Fox, 1989). Many milk proteins possess specific biological properties that make them potential ingredients for health-promoting foods. Many researches have been carried out on bioactive peptides from casein hydrolysates. The list health benefits of biologically activity of milk bioactive peptides are radical scavenging, lipid oxidation inhibition, antiproliferation, stimulate proliferation, antimutagenicity, immunostimulant, immunosuppressant, and antimicrobial (Phelan et al., 2009). These peptides can be liberated by:

1. Gastrointestinal digestion of milk
2. Fermentation of milk with proteolytic starter cultures or
3. Hydrolysis by proteolytic enzymes like pepsin, trypsin, chymotrypsin, etc.

A great variety of peptides are formed during cheese ripening which has been shown to exert biological activities. Furthermore, secondary proteolysis during cheese ripening may lead to the formation of bioactive peptides (Korhonen & Pihlanto, 2006). The occurrence of the bioactive peptides which are naturally formed in cheese depends on the equilibrium between their formation and the degradation of the peptide during cheese ripening. Ripening is a time-consuming process during cheese making. To overcome these problems, some economic and technological methods for acceleration of cheese ripening have been reported (Fox, 1989). Acceleration of ripening may help to increase bioactive peptides. Encapsulation of enzymes in liposome can accelerate cheese ripening (Jahadi et al., 2016; Mohammadi et al., 2015; Zoghi et al., 2018). V_{max} and K_m and stability of the enzyme were increased by encapsulation of the Flavourzyme in liposome (Vafabakhsh et al., 2013; Jahadi et al., 2012, 2015, 2020; Jahadi & Khosravi-Darani, 2017).

Gamma-amino butyric acid (GABA) is another amino acid that consists of four carbons widely found in microorganisms, animals, and plants. GABA has been used in pharmaceuticals and functional foods due to its blood pressure-lowering activity and its role as a neurotransmitter signal in the central nervous system including the mammalian brain (Takahashi et al., 2012). It can also be used for the synthesis of nylon 4 as a monomer (Kim et al., 2007; Park et al., 2013). Its production has been reported to occur by decarboxylation of glutamate, a reaction catalyzed by glutamate decarboxylase (Pham et al., 2016).

The ever-increasing demand for GABA has generated the need for efficient microbial production. Lactic acid bacteria (LAB) are one of the main microbial sources for GABA production, mainly due to their food-grade property and high ability in the direct production of GABA-rich components. The progress toward culture conditions optimization and genetic engineering to achieve physiology-oriented engineering approaches and co-culture methods for the GABA-producing LAB species, the biosynthesis pathway of GABA by LAB, and the glutamate decarboxylase (GAD), as the key enzyme in GABA biosynthesis, is growing rapidly (Cui et al., 2020). The biosynthesis of GABA is known to exhibit superior performance due to high product efficiency, mild reaction conditions, simple process, and low environmental burden (Gavrilescu & Chisti, 2005; Alcalde et al., 2006; Carvalho, 2017). Irreversible α-decarboxylation of L-glutamate is an important pathway in GABA biosynthesis, which is catalyzed by the pyridoxal 5′-phosphate (PLP)-dependent glutamate decarboxylase (GAD) (Xu et al., 2017).

It has been indicated that the enzymatic hydrolysis of collagen provides structural cleavage and better functionality. Enzymatic hydrolysis of bovine collagen and beef bone extract, which were undertaken by Flavourzyme® and Protamex®, respectively, have shown better functional (antioxidant and antimicrobial activity) and physicochemical properties of the final hydrolysates (Vidal et al., 2018).

Peptides have been proved to offer health-promoting benefits for hypertension, a primary risk factor in cardiovascular disease. Short-chain peptides are frequently favored compared to other protein resources like free amino acids combinations, due to their enhanced absorption kinetics (Jahan-Mihan et al., 2011). Thus, the proteolysis process to achieve short-chain peptides is of paramount importance. Angiotensin-converting enzyme inhibitory (ACEI) peptides are among the most recognized peptides. The proteolysis of ACEI peptides is necessary for their bioactivity and bioaccessibility, as they are encrypted within the parent proteins (De Leo et al., 2009).

Protein hydrolysis in peptides occurs both in vitro and in vivo. In vitro degradation takes place during food preparation processes by the addition of isolated or microbial enzymes. The in vivo process occurs within the gut, in the presence of digestive and microbial enzymes of the gut flora. Employing a blend of diverse proteases and a step-by-step procedure should be carried out for consecutive proteolysis (Boutrou et al., 2013).

Recently, dietary supplements based on the protein hydrolysates have already gained much attention in case of being unable to assimilate native proteins due to specific illnesses, such as Crohn's disease or pancreatitis (Posovszky, 2016). The

incorporation of protein hydrolysates in sports nutrition has heightened its significance (Holm et al., 2017). Another consequence of protein hydrolysates is muscle anabolic due to their insulin atrophic effect (Yuan et al., 2017; Rittig et al., 2017). The combination of several effects would couple their nutritional effects with other "bioactivities," an area of study which has received increasing attention nowadays (Tavano et al., 2018).

The enzymatic hydrolysis of soybean protein products has resulted in reducing allergenicity and improving functional properties. The allergenicity of hydrolyzed soybean protein isolates (SPI) by means of alcalase, trypsin, chymotrypsin, bromelain, or papain was investigated. Assessment of chymotrypsin hydrolyzed samples indicated a higher allergenicity potential of digested SPI due to the β-conglycinin protein fragments (Panda et al., 2015).

Allergenicity of food products is regarded as adverse health effects arising from a specific immune response when exposed to a given food reproducibly (Sicherer & Sampson, 2014). Both IgE-mediated and non-IgE-mediated allergenic reactions are included in adverse immune responses (Berin, 2015). In this regard, the selection of a fit protease with desirable specificity and proper hydrolysis procedure can significantly reduce protein allergenicity (Burton et al., 2002). It has been reported that the allergenicity of milk proteins and gluten decreased significantly by proteolysis utilization in food process engineering (Rizzello et al., 2007). Another study confirmed the amplification of food allergenicity by IgE immunoblots measurement when chymotrypsin or bromelain was used for the hydrolysis of soybean protein isolate (SPI) (Panda et al., 2015). A 65% reduction in IgE reactivity was shown for roasted peanut protein hydrolysis using Flavourzyme for 300 min (Berin, 2015). SPI hydrolysis was carried out using pepsin, alcalase, and papain. The results showed the suitability of these enzymes in the destruction of the main soybean allergens. However, the application of papain and Flavourzyme were more beneficial in view of less marked bitter taste production (Meinlschmidt et al., 2016). The effect of enzymatic hydrolysis of SPI by Corollas PP on antihypertensive and antioxidant activities was evaluated (Guan et al., 2018).

It has been proved that the gluten uptake, from various sources like *Triticum* species, rye and barley proteins, and their crossbred varieties, can notably distress genetically susceptible individuals to inflammation of the small intestine, namely celiac and nonceliac diseases (Rizzello et al., 2007).

The effect of proteolytic enzyme modification of egg-yolk protein and white protein during the preparation processes was evaluated and resulted in the production of the bioactive peptide with enhanced properties like antioxidant activity, ACE-inhibitory activity, ferric reducing power, scavenging capacity, water-holding capacity, and chelating capacity (Pokora et al., 2013).

9.3.8 Tannase

Polyphenolic compounds are naturally present in a variety of plant sources, namely tea, nuts, and fruits. These compounds are considered to be health-promoting and

disease-preventing bioactive molecules. They have an important role in decreasing degenerative diseases, such as diabetes, cardiovascular, and cancers by mutagenesis and carcinogenesis-preventive activities (Kuddus, 2018). Therefore, it seems these bioactive compounds are a two-edged sword. However, the bioavailability and bioefficacy of polyphenolic compounds are affected by their capacity to form complexes with macromolecules like proteins, cellulose, pectin, starch, and minerals, generally found in polymeric, glycosidic, and esteric compounds (Arshad et al., 2019). As a result of the formation of complexes with macromolecules, the absorption rate in the small intestine will reduce dramatically (Kuddus, 2018). The higher the molar mass of polyphenolics, the lower the bioavailability activities and fewer nutritional effects (Aharwar & Parihar, 2019).

Hydrolyzable tannins are commonly known as polyphenolic secondary metabolites, in the form of galloyl esters or their derivatives. The attachment of galloyl moieties or their metadepsidic derivatives of tannin to a variety of polyol, catechin, and triterpenoid units is called gallotannins. Through hydrolysis, glucose and gallic acid are produced, which are more beneficial for human health (Chung et al., 1998; Khanbabaee & van Ree, 2001).

Tannin acyl hydrolase, also called tannase (EC 3.1.1.20), is an inducible extracellular enzyme belonging to the esterase superfamily. The molecular weight of tannase is in the range of 31–310 kDa, depending on their source. Generally, fungal tannases have a higher molecular weight range (45–310 kDa) compared to bacterial tannases (31–90 kDa) (Aharwar & Parihar, 2018). The ranges of pH and temperature optima of tannases are 4–8 and 30–70 °C, whereas the stability ones are 3–8 and 20–90 °C, respectively (Selvaraj et al., 2019). Tannase catalysis is the hydrolysis of depside and ester bonds, including gallyol ester of gallic acid and alcohol components of hydrolyzable tannins, like chlorogenic acid, epigallocatechin gallate, epicatechin gallate, and tannic acid. Through hydrolysis, other oligomeric tannins and other useful and absorbable compounds were yielded. A famous example is a gallic acid which is an essential intermediary constituted in the synthesis of trimethoprim antibacterial drug and is applied in the pharmaceutical industry. Propyl gallate is another substrate for the synthesis of antioxidants in the food industry, or even other components applied for clarifying, as well as treating tannery effluents in the juice industry (Aharwar & Parihar, 2018, 2019).

Generally, tannase has extensive applications in the chemical, brewing, cosmetic, beverage, pharmaceutical, and food industries. Tannase is able to degrade plant cell walls, cleaving cell-wall cross-links, and facilitate the absorption of polyphenolic compounds in the small intestine by enzymatically hydrolyzing them into simpler and smaller phenolic compounds (García-Conesa et al., 2001; Shoji et al., 2006; Georgetti et al., 2009). Increasing the antioxidant, anti-inflammatory, immunomodulatory, antiproliferative, and anticarcinogenic activities are the consequences of these modifications (Kuddus, 2018). It is also a well-known commercial enzyme in the elaboration of instantaneous, gallic acid production, and acorn liquor (Aharwar & Parihar, 2018).

Tannase production has been studied extensively. Several microorganisms (fungi, yeast, and bacteria) are used for microbial tannase production; among them, several

fungal genera, including *Aspergilli*, *Penicillium*, *Trichoderma*, and *Rhizopusare* are more typical. Among different microbial sources, fungi, such as *Aspergillus ficuum*, *Aspergillus oryzae*, *Arxula adeninivorans*, and *Aspergillus fumigates*, are the most common sources of the tannase-producer genus in the absence of tannic acid (Cavalcanti et al., 2020). Based on the strain type and culture conditions, submerged fermentation (SmF), or solid-state fermentation (SSF), the enzyme can be inducible or constitutive. Tannase synthesis is induced in the presence of several phenolic compounds such as gallic acid, pyrogallol, tannic acid, and methyl gallate (Aharwar & Parihar, 2018). Agricultural residues have also been used as alternative substrates for economic tannase production (Selvaraj et al., 2019).

However, the synthesis mechanism involved is a controversial issue that should be addressed. SmF has several advantages in sterilization, incubation time, process control, and ease of extracellular recovery (Kumar et al., 2016). In contrast, the advantages of SSF are related to its cost-effective downstream and upstream processes, water-dependent conditions, ease of agro-waste substrates usage, the minimum amount of effluents, and high efficiency, so in most cases, SSF is preferable (Selvaraj et al., 2019). The potential of *Lasiodiplodia plurivora* CAN-10 for tannase production was compared under these two fermentation methods by utilizing *Terminalia catappa* (almond leaves) and *Magnifera indica* (mango leaves) as agro-waste substrates to provide carbon and nitrogen sources (Ire & Nwanguma, 2020). The results of this study revealed that SSF yielded higher tannase production compared to SmF. SmF was used for the production of tannase in the presence of tannic acid (2% w/v) from *Aspergillus* sp. and culture conditions like temperature, inoculums size, moisture content, aeration rate, and substrate consecutively affect the process and the fermentation products (Pandey et al., 1999). More examples of tannase producing sources and the corresponding mechanism that results in the functional enhancements are shown in Table 9.5. More studies are required for the identification of high-yield strains as well as low-cost and efficient processing development (Aharwar & Parihar, 2018). Recombinant DNA technology and genetic engineering for tannase production have been investigated in several new researches. Tannase-encoding genes of *Aspergillus oryzae* were used for producing a wide variety of hydrolytic enzymes, namely proteolytic and amylolytic enzymes. In a study, AotanB (AO09002300047), one of the encoding genes of *A. oryzae*, was overexpressed using a glucoamylase gene promoter, P*glaA142*, and a recombinant enzyme was characterized. Based on the results, the recombinant enzyme produced by *A. oryzae* showed better thermal stability and enzyme activity as compared to *Pichia pastoris*-tannase producer strain (Koseki et al., 2018). In another study, transferring tannase gene into *Aspergillus niger* resulted in better yield of enzyme production and improved characteristics (Liu et al., 2018).

Enzyme stability maintenance during shelf life, handling, transport, and storage at room temperature is an essential property for enzyme commercialization in the food industry. According to the current literature, several techniques such as hydration by spray drying and freeze-drying, immobilization through nanoparticles, with the aid of various additives (adjuvants/carriers), such as carbohydrates, proteins, lipids, gums, and surfactants, as protectors against oxidative denaturation, have been

Table 9.5 Some examples of tannase production from various microorganisms and their related properties (tannase activity, pH, and temperature)

Source	Activity	pH optimal stability	Substrate	A half-life ($t_{1/2}$) temperature	Application	Reference
Aspergillus niger rAntan1	390.4 U/mL	6.0 3.0–8.0	Catechingallate	5.4 h at 60 °C 0.5 h at 70 °C	Green tea extraction	Shao et al. (2020)
Bacillus subtilis PAB2	2868.75 U/mg	5.0 3.0–8.0	Tamarind seed	4.5 h at 60 °C	Bacterial tannase production	Jana et al. (2013)
Penicillium atramentosum	34.7 U/mL 32.8 U/mL	7.5 5.5	Keekar leaves (3% w/v) Amla leaves (2% w/v)		–	Selwal and Selwal (2012)
Pestalotiopsis guepinii	98.6 U/mL	6.9 –	Pomegranate seeds		–	Reges de Sena et al. (2014)
Aspergillus niger Bde14	111.5 U/mL	7.0 –	Propyl gallate	–	–	Liu et al. (2018)
Aspergillus fumigates CAS21	28.5 U/mL (B-cyclodextrin)	5–6 –	2% tannic acid	40–60 °C (in the presence of B-cyclodextrin as an adjuvant)	Sorghum and leather effluent treatment Propyl gallate production	Cavalcanti et al. (2020)
Talaromyces verruculosus	32.18 U/gds	8.0 4–8	Acacia nilotica bark	60 °C	Fruit juice detannification	Aharwar and Parihar (2019)
Bacillus gottheilii M2S2	0.265 U/gds	– –	Triphala (11.532 g)	–	–	Selvaraj et al. (2019)
Aspergillus niger *Aspergillus fumigatus*	21.94 31.89	4.0 4.0	1% tannic acid	30 °C 30 °C	Industrial applications	Cavalcanti et al. (2017)

(continued)

Table 9.5 (continued)

Source	Activity	pH optimal stability	Substrate	A half-life ($t_{1/2}$) temperature	Application	Reference
Klebsiella pneumoniae	39.72	5.52 –	Indian gooseberry leaves	39.72 °C	–	Kumar et al. (2016)
Serratia marcescens	42 U/mL	– –	2% tannic acid	–	Antibacterial activity	Nsayef Muslim et al. (2017)
Bacillus gottheilii M2S2	49.32 U/L	4.74 –	4% tannic acid	–	Clarification of acidic beverages	
Aspergillus niger PN1	148.7 U/g 116.52 88.64	– –	Rice bran and spent coffee ground Coconut residue	–	–	Mansor et al. (2019)
Enterobacter cloacae	4 U/mL	6.0 –	Glucose	50 °C	–	Govindarajan et al. (2019)
Aspergillus UCP1284	12.26 U/g	5.5 –	Cashew bagasse	30 °C	Tannase production	Tatiana et al. (2016)
Aspergillus oryzae	139.22 IU/mL	4.89 –	Pomegranate rind, *Cassia auriculata* flower, black gram husk, and tea dust	34.91 °C	–	Varadharajan et al. (2017)

employed to enhance tannase activity and stability. The properties of the additive compounds have a great influence on the properties of the final enzyme powder, like glass transition temperature, density, particle size, and solubility. Therefore, the adjuvants should be chosen considerably (Cavalcanti et al., 2020). The immobilization method in multi-walled carbon nanotubes for tannase has increased tannase stability (Ong & Annuar, 2018). Apart from the processing method, statistical tools, mainly response surface methodology, have been considered for the optimization of tannase production to reduce the cost and the time (Bhoite & Murthy, 2015).

Five main tannase applications are tea processing, fruit juices clarification and de-bittering, animal feed treatment for nutritional improvement, gallic acid, and propyl gallate production used in food, leather, dye, and chemical industries, and wastewater treatment in agro-industries for decolorization and polyphenolic compound reduction (Aharwar & Parihar, 2018; Cao et al., 2019).

9.4 Conclusion

In this chapter, we have reviewed some of the main enzymes incorporated in food development, influencing the bioavailability and bioactivity of nutraceuticals. Worldwide companies have been established to do high-tech research and development of functional foods that have potentially positive effects on human health beyond basic nutrition. However, scientists should bear in mind that in vitro and in vivo experiments and clinical trials are essential steps that should be considered to guarantee any health claims based on the major factors limiting their bioaccessibility. According to traditional knowledge as well as scientific evidence, enzymes have a dominant role in food biotechnology for the development and commercialization of functional foods. Enzymes utilization in the production of bioactive metabolites and development of bioprocesses has shown an increasing trend. Several bioprocesses which involve enzymes have been established for the development of commercially important nutraceuticals. Yet, many traditional fermented foods have remained unexploited from technological aspects of functional foods and nutraceuticals development. Enzymes, such as L-asparaginase, chlorophyllase, beta-glucanase, L-glutaminae, lipase, and proteases are key enzymes in nutraceutical and functional food production.

Exploring for high-efficient and cost-effective technologies should be concerned in parallel with optimizing the experimental conditions to enhance the yield and physiological stability of these bioactive compounds. Recently, food and agricultural wastes have intensely attended as potential substrates to produce high-value products, namely free and immobilized enzymes. They represent ideal sources of proteins, lipids, and carbohydrates, and therefore, offer remarkable advantages from economic and eco-sustainability aspects. Nevertheless, the large-scale applicability, potential repeatability, and operation costs in real-industrial implementation are some of the challenges that need to be considered more systematically. Another emerging aspect of food enzyme application is the alteration of DNA encoding native enzymes for the development of genetically modified enzymes. Although the

safety of genetic engineering approaches for food processing is a severe concern, there have been promising results for the beneficial employment of genetically modified enzymes in the modern food industry.

More investigations should be done on the development of efficient and sustainable protocols, as well as enhanced properties such as pH and temperature stability, multifunctionality, surface property, catalytic efficiency, eco-friendly and economically efficient to achieve precise and sophisticated food matrixes. In addition, their impact on the resultant food characteristics such as sensory, rheological, physicochemical, and functional properties. All in all, there is a far distance to thoroughly comprehend the relationships between functional chemical compounds, and their dosage, stability, safety, cost, and delivery vehicles. In fact, further considerations should be taken into account for investigating the marketing standpoints of the functional food market based on the current scientific advancements in food science.

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Enzymes as Active Packaging System

10

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Abstract

Standard food packaging is the first step that has a vital role in protecting the product from moisture, water vapor, odors, gases, dust, microorganisms, vibrations, shocks, and pressure force, which preserved the freshness, quantity, and quality of the packaging to reach the customer. Active and smart packaging approaches enhance the communication and protective functions of food packaging to fulfill the demands of consumers for high-quality, safe, and convenient food products. Recently with the technology expansion, novel enzymes with extended applications and specificity have been advanced, and new solicitation areas are still being investigated. This chapter has focused on oxygen-reducing enzymes in food-packaging material, and intelligent packaging technologies are reviewed. The main lines are active packaging materials, enzymes as catalysts in active scavenging systems, active releasing systems, antimicrobial packaging, time–temperature indicators, removal of undesirable food components, oxygen-sensing applications, and the effect of the coating process and composition on the enzyme activity. Also, enzymatic reactions with a brief discussion investigated and explained how enzymes are used in advanced smart and food-packaging systems. Eventually, a comprehensive list of enzymes used in food smart packaging and their application are presented.

Keywords

Enzyme · Intelligent and active packaging · Antimicrobial packaging · Shelf life

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A. Dutt Tripathi et al. (eds.), *Novel Food Grade Enzymes*,
https://doi.org/10.1007/978-981-19-1288-7_10

10.1 Food Packaging

Food packaging is necessary to keep the safety and quality of the foods. Excellent and well-designed packaging protects the product from external and natural agencies that may cause damage to the quantity and quality of the product, tampering resistance, contamination and spoilage, physical and chemical damage. It can maintain the freshness of the food. For making products more applicable and favorable used to reclosable openings, dispensing caps, sprays, and other features. Packaging in a suitable method assists transportation of the packaged goods with easiness and reduces marketing costs (Robertson, 2016). Good packaging has less handling losses and is easy to be handled by one person during loading; therefore, characteristics of packaging cusses that marketing costs to be less (Han, 2003).

10.2 Food Package Properties

According to the degree of protection, food can influence maximum moisture gain or oxygen uptake. Different packaging materials such as metal cans and glass containers can be regarded as nearly impermeable to the passage of odors, gases, and water vapor, although in comparison packaging materials based on the paper can be considered penetrable. Also, packaging materials based on the plastics supply varying degrees of protection, and be contingent mainly on the nature of the polymers utilized in their manufacture.

10.3 Shelf Life

For many foods and drinks in which quality declines with time, it pursues that there will be a limited length of time. Shelf life is when the product becomes undesirable, suitable, and safe to use in specified storage conditions (Labuza, 1982). Shelf life is a usual scuffle for food companies. Manufacturers and producers want foodstuffs to stay fresher to lengthen the time to sell or custom. Therefore, researchers are believed to improve shelf life to give foods and drinks a better fortuity at high quality when consumers are prepared to eat them (Labuza & Schmidl, 1985). Provisions to enhance food product safety and quality, increase, or stabilize food quickly become market demands (Labuza, 1982).

10.4 Active Packaging Systems

Active packaging, smart packaging, and intelligent packaging denote packaging systems utilized with products, such as foods (fruit and vegetables, fish, and seafood products), pharmaceuticals, and several other kinds of products (Ozdemir & Floros, 2004). Active packaging refers to the embedding of special additives into the package to expand or maintain the quality. Active packaging effectively increases

the shelf life of foods by Realini and Marcos (2014). In active packaging, different additives may be used based on the packaging film and packaged food (Lorenzo et al., 2014). Active packaging contains ingredients that can absorb carbon dioxide, releasing oxygen, ethanol emitters, ethylene scavengers antioxidants, and other preservatives (Conte et al., 2007).

Active packaging systems supply various solutions to be controlled by quality attributes that are to be protected. This theme came up, for example, in discussions of decelerating oxidation food products be considered, so the packaging must utilize an active system that includes an oxygen scavenger or antioxidants. Also, if the decay of the product is created by condensation or moisture, the packaging can include a moisture absorber. Eventually, the motivation for the organization of any novel technology is cost.

It should point out that the unique decay mechanism of different foods should be considered previously designing the packaging. The reality, when we have active packaging that it carries out some role other than supplying an inert obstacle to the exterior environment. On the other hand, active packaging is identified as a system in which the product, package, and environment interact in a positive method to expand the shelf life or to obtain some properties (Conte et al., 2007).

The shelf life of packaged food is reliant on abundant features such as the food properties or product-intrinsic (e.g., pH, product color, nutrient content, aroma, texture, water activity, viscosity, biological structure and breathing rate, the existence of antimicrobial compounds) and product-extrinsic or related to the packaging, receptacle, or external environment factors (e.g., visual, and tactile properties of food packaging or dishware, smell, surroundings lighting, temperature and aroma, storage temperature, relative humidity, and the ambient gaseous composition) (Lorenzo et al., 2014).

These features will straight affect the physical, chemical, biochemical, and microbiological deterioration of products and their shelf lives.

Active packaging aims to improve the maintenance of food in the package, and delaying shelf life involves the application of various plans like oxygen removal, temperature control, moisture control, besides chemicals, for example, sugar, salt, carbon dioxide, or natural acids or a blend of these with active packaging.

These developments in active packaging have caused developments in numerous fields, including controlled respiration rate in agricultural products, postponed oxidation in muscle foods, microbial growth, and moisture movement to dried products. Furthermore, active packaging similarly employs choosiness to modify the atmospheric concentration of gaseous combinations interior of the package by coating, lamination, micro-penetration, or polymer blending (Realini & Marcos, 2014).

10.4.1 Types of Food Active Packaging Systems

The active packaging can be utilized in various forms involved as shown in Fig. 10.1.

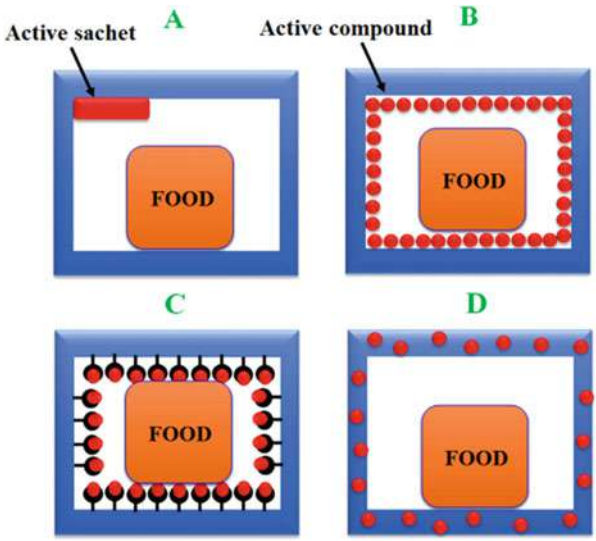

Fig. 10.1 Different types of food active packaging systems (**a**) using the active pouches and pads placed inside the packaging, (**b**) active compound coated onto the polymer, (**c**) blending of the active compound on the packaging surface, and (**d**) straight incorporated into the package structure (Almasi et al., 2020)

10.4.2 Active Compound Release Mechanisms

The direct incorporation of active combinations into the polymer matrix of the packaging system is the most regular kind of active packaging system. In this form of the active packaging system, polymer and the active agent form a composite matrix. In recent years, this type of active packaging system is the most frequently considered for food active packaging systems. There are three types of release mechanisms suggested for directly incorporated food active packaging systems.

1. Diffusion due to release: In this type of release, the active agent diffuses through the macro-porous or micro-porous polymer matrix structure and is moved from the surface of the film into the food. This mechanism is usual for releasing active composites for water-resistant and synthetic polymers. The rate of this kind of release is depended on polymer chemistry, permeability, and porosity.
2. Swelling due to release: low diffusion coefficient causes the incorporated active agent unable to penetrate the polymer matrix. When polymer swell, the diffusion coefficient of the active agent increases; therefore, it penetrates out. Since most foods have moisture and the water is the most popular penetrant, this type of release commonly happens in packing materials moisture-sensitive such as polysaccharides or protein-based films.
3. Disintegration due to release: Deformation of polymer, cleavage, and degradation are the main reasons for this kind of release. Absorption of liquids commonly from aqueous fluids through the polymer matrix influences degradation and deformation rate.

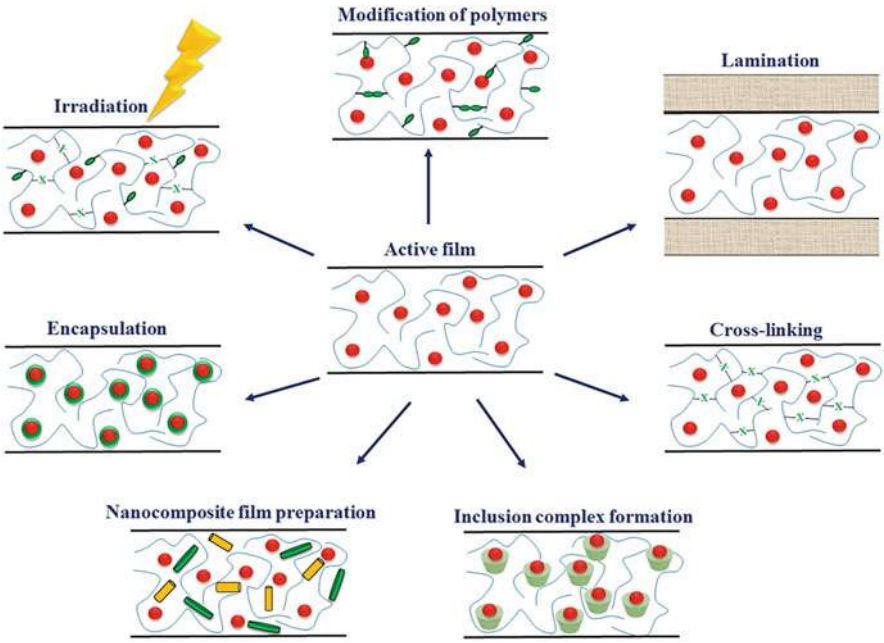

Fig. 10.2 Schematic illustration of different approaches used for release controlling from food active packaging (Almasi et al., 2020)

This kind of release happens in both nonbiodegradable and biodegradable polymers. Several approaches have been suggested to obtain a more controlled diffusion of the active agent from the packaging material. Figure 10.2 summarizes these approaches by a schematic representation.

10.5 Antimicrobial Packing

Antimicrobial packing is a type of active packaging that use some agents which kill or inhibit the growth of microorganisms that may be existing in the packaged product or packaging material itself. For control of unfavorable microorganisms on foodstuffs, antimicrobial materials can be combined and coated inside food-packaging materials (Motta et al., 2004).

For example, antimicrobial polymers provide a good antimicrobial plan for combating pathogens and have already drawn the attention of industrial and academic research (Prazak et al., 2002). Antimicrobial mechanisms demonstrate either active or passive action and polymer material categories, including bound or leaching antimicrobials used. Antimicrobial polymers are used in some industries, such as medical, food, and textile (Motta et al., 2004). Compounding polymers and

their blends with antimicrobial compounds or preservative agents reveal a new approach for designing antimicrobial packing films.

10.6 Enzyme

Enzymes are macromolecular biological catalysts (typically proteins), which significantly are available in all living organisms, which cause reactions to happen naturally, and can be seen in open self-organizing life (Liu et al., 2014). The first feature is its catalytic factor. Enzymes permit living systems to perform reactions that would happen so slowly. Enzymes accelerate (catalyze) chemical reactions; in several cases, enzymes can make a chemical reaction so much faster than it would have been without it (Wierschem et al., 2017).

Enzymes catalyze biochemical reactions with different pathways (Narayanan et al., 2009). A substrate is named chemical species observed in a chemical reaction and reacts with a reagent to create a product. It can also be called for the surface that other chemical reactions are played, or achieved a supporting role in microscopic and spectroscopic techniques. The substrate and enzyme schematic are shown in Fig. 10.3.

Nonenzymatic or chemical reactions are more slower than enzymatic reactions (Brody & Budny, 1995).

The second characteristic of enzymes that makes them significant as diagnostic and tools for research is the specificity they display regarding the reactions they catalyze (Brody & Budny, 1995). Enzymes exhibit absolute specificity, which is, they will catalyze only one particular reaction. Other enzymes will be specific for a specific type of functional group or chemical bond. Overall, there are four different kinds of specificity (Blixt & Tiru, 1976):

Absolute specificity that the enzyme will catalyze only one reaction.
Group specificity in which the enzyme will affect performance only on molecules with particular functional groups, such as phosphate, amino, and methyl groups (Budny, 1989).

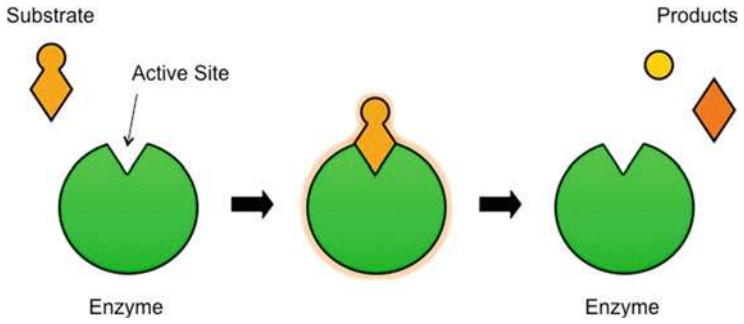

Fig. 10.3 Schematic of enzyme and substrate

Linkage specificity that the enzyme, irrespective of the rest of the molecular structure, will act on a specific type of chemical bond and the best technique to immobilize enzymes or other bioactive composites on polymers. The chemical steps included in covalent bonding are complex and timewasting. Therefore, are not presently practical for joint commercialization for food contact packaging. Enzymes are effective biological catalysts frequently utilized in the food industry to get better performance, quality, and shape of food. Using enzymes as active agents in the food packaging system is not novel technology (Adrian, 1959).

Stereochemical specificity is the enzyme performance with an optical isomer or specific esters. Enzymatic specificity requires two different forms: the first one is the type of chemical reaction and the second one is no chemical reaction, specificity for the reactant, or substrate. Therefore, for each chemical reaction in a biological system, there is a particular enzyme demand for the optimal production of reaction products. It is essential to consider many different biological reactions to pursue numerous different enzymes (Brody & Budny, 1995).

Enzymes, similar to other chemical catalysts, do not change equilibrium conditions. An enzyme enhances a forward reaction similarly, and the reverse reaction means that enzymes speed up the rate a chemical equilibrium is obtained. It considers that the enzyme does not interfere with the equilibrium concentrations ratio for the products to reactants (Nestorson et al., 2008).

In an active package system, the enzyme may be incorporated into the package structure or added straight to the packing to affect a reaction. The enzyme in packaging material should be motionless, and the substrate with a reactant role or a constituent circulated in the site to initiate a reaction. If the enzyme is placed in a static position or immobilized, it may be accomplished by making the enzyme the main part of the packaging system (Klibanov & Dordick, 1989).

Generally, active packaging commonly includes the combination of a chemical into the packaging material. Active packaging providing enzymes contains the incorporation into the packaging material of the specific enzymes in much the same way as the incorporation of a more formal chemical to make the active package. The significant differences are not changing enzyme with reaction and can continue indefinitely; the enzyme is sensitive to changes in some parameters, such as pH, temperature, etc. The range of environmental properties for the performance of the enzyme is a relatively narrow band (Kothapalli et al., 2008). Also, enzymes may be categorized according to the substrates. For example, lipases are used for lipids and proteases for proteins, etc. These are determination for the vast group to break proteins of entities that are lipid under a specific set of conditions or specific to a single protein.

Based on the several researches, the utilized enzymes incorporated in the polymers can act as oxygen scavengers (Labuza & Breene, 1989). One of the most widely investigated enzymes in active packaging is Glucose oxidase plus catalase. When the enzyme is immobilized, the surface carrying the active principle should be directly contacted with the food for activating the redox reaction, and it caused the

application to be limited (Brody et al., 2001). Scavenging reaction happens in the interface of product–packaging.

Glucose oxidase/catalase systems convert glucose into oxygen peroxide and gluconic acid, which is quickly decreased by the catalase enzyme, even though the food may become oxidized. Consequently, the scavenging reaction is suggested to be produced distinctly from the product. The liquid must also contain the reactant (ethanol, glucose). These systems are still being expanded and implemented, but, at present, there is no commercial application (Effront, 1917).

The immobilization can switch the activity of the adopted enzyme. Realty, immobilize the enzyme in the package while maintaining enzyme activities, the chemically modified polymers simplify the covalent bonding with the enzyme. To insert the enzyme into the matrix of polymer, prior chemical purification of the polymer is frequently required. The majority of enzymes are used directly in food processing, and some of them have already been utilized in active packages. For example, Soares and Hotchkiss (1998) presented naringinase in a plastic package. Hydrolysis of naringin reduced the bitterness of contained grapefruit juice (Wu et al., 2019), in Pharmacal biotechnologies industry used from immobilized lactase and cholesterol reductase in a package for liquids. Also, the reduction of lactose in milk has been used lactose content of milk or yogurt during storage. Another example is converting cholesterol reductase into coprostanol (Brody & Budny, 1995). A schematic drawing of the enzyme immobilization process in both immobilization in the dispersion phase and immobilization on the surface of the polymer film is shown in Fig.10.4.

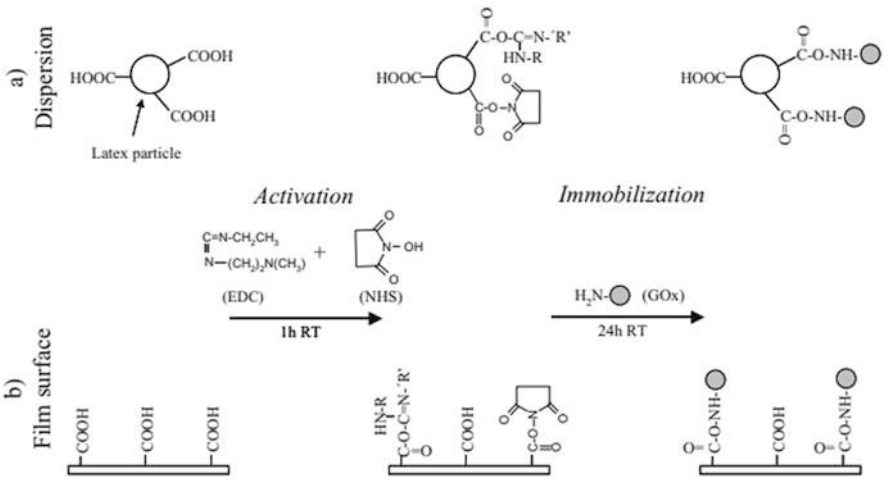

Fig. 10.4 Schematic drawing of the enzyme immobilization process. (**a**) Immobilization in the dispersion phase and (**b**) immobilization on the surface of polymer film formed from a latex dispersion (Nestorson et al., 2008)

10.6.1 Oxygen Scavenger in the Enzyme Packaging System

An oxygen scavenger is a substance that reacts enzymatically or chemically with oxygen, thus safeguarding the packaged food versus oxidative decay and quality changes owing to oxygen (Ahvenainen, 2003). Generally, oxygen scavenging systems follow the oxidation of iron powder or enzymes like glucose oxidase, with oxygen existing in the headspace of the packaging system. These reactions do some activities such as absorbing the great majority of free oxygen in the package, reducing the interaction of oxygen with food, and avoiding deterioration. Usually, oxygen scavengers are placed inside the package with a sachet too permeable to oxygen.

It should be noted that these types of sachets can be utilized for meat products, fresh pasta, and baked kinds of stuff, such as pizza dough, bread, sponge cake and cookies, nuts, coffee, and snack foods like chips. Some security issues are related to utilizing sachet types of oxygen scavenging systems in packaged foods that may ingest sachets by consumers, and the probability of sachet contents leaking into food packaging (Lopez-Rubio et al., 2004). Careful investigation and improvement should be carried out in choosing the suitable sachet material and its seal correctly before commercial application, and suitable alerting must be proclaimed on the package. The combination of catalase and glucose oxidase is a general enzymatic oxygen scavenging system that has been shown in the Fig. 10.5 (Shi et al., 2010). Glucose oxidase in the presence of oxygen reacts with glucose. This enzyme system has been embedded into acrylate matrixes of polymer for the deoxygenation of apple juice (Kothapalli et al., 2008).

10.6.2 Time–Temperature Integrator-Indicators

Time–temperature integrators (TTIs) are indicators that can display food safety and quality changes during distribution, handling, and storage (Taoukis & Labuza,

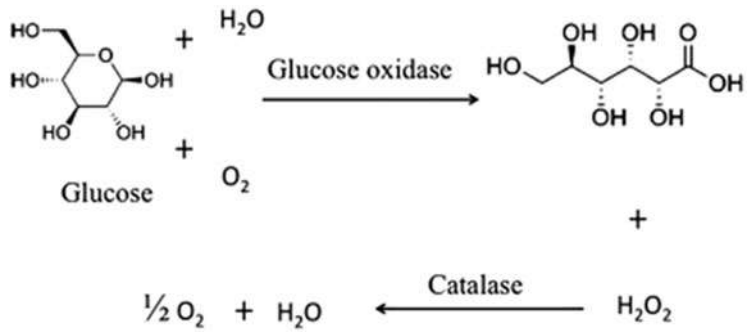

Fig. 10.5 The enzymatic reactions in the oxygen scavenging system using the combination of glucose oxidase and catalase. The mechanism complicated here is adapted from Shi et al. (2010) and Tian et al. (2013)

1989). TTIs are necessary for temperature-sensitive foods to evaluate and control the distribution chain (Giannakourou et al., 2005). TTIs must be low-priced, readable, reliable, and without any poisonous materials. Most of the TTIs are very similar to labels and can be used for food products. Several TTIs have been expanded based on different operating principles. Enzymatic TTIs utilize α-amylase (Guiavarc'h et al., 2004), lipase (Agerhem & Nilsson, 1981), or b-glucosidase (Adams & Langley, 1998). Presently, lipase is an available enzymatic TTI that can be utilized to control quality assurance. Enzymatic TTIs that have been investigated to date have utilized synthetic and then rather expensive chromogenic substrates hydrolyzed to generate a color. Consequently, if an enzyme that uses natural dyes as its substrate were available, a TTI produced using that enzyme would supply various benefits, counting developed visibility, less expensive, and extensive use of dye coding. Laccases are conventionally used for numerous industrial processes counting paper processing, detoxification of environmental pollutants, oxidation of pigments, prevention of wine discoloration, and production of chemicals from lignin (Kim et al., 2012).

10.7 Conclusion

Active packaging technologies suggest novel chances for the industries for protecting their products. Different active packaging systems are currently available based on the chemical and physical structure of the foods. Oxygen, moisture, carbon dioxide, ethylene, and ethanol are considered for the active packaging. The structure of the active packaging and the potentials of the enzymes are mentioned. The importance of this subject has produced many investigations to make more useful systems. In the following, oxygen scavenger in enzyme packaging and time–temperature integrator-indicators were investigated. Finally, investigation shows the improvement of novel indicators and enzymes that are much more precisely designed for the active packaging system.

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Enzymes as a Tool in Food Analysis and Foodborne Pathogen Detection

11

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Abstract

Enzymes are widely and progressively utilized in food processing industries. Enzymes find tremendous application in fruits and vegetable processing, dairy processing, and cereals processing. Nowadays, there is a wide application of enzymes in food analysis. Enzymes serve as an analytical tool for the detection of food-borne pathogen and allergens. These may be utilized as biosensors and are applicable in food industries. This chapter gives detailed information related to application of enzymes in food analysis and detection of food-borne pathogens.

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A. Dutt Tripathi et al. (eds.), *Novel Food Grade Enzymes*,
https://doi.org/10.1007/978-981-19-1288-7_11

Keywords

Enzymes · Food processing · Food analysis · Food-borne pathogen

11.1 Introduction

Enzymes are defined as the biological materials responsible for fastening the reaction rate. Enzymes are the so-called multifaceted biomolecules that are indispensable for biochemical interconversions. They act as a catalyst for all the metabolic processes in the body. The advancement in rDNA technology and protein engineering has enabled us to capitalize on the various functions of enzymes from industrial to biomedical applications. Enzymes are exceptionally effective biocatalysts investigated for wide-reaching catalysis due to a few particular preferences that run from their activity in milder response conditions to their excellent component selectivity and lower ecological and physiological harmfulness (Chapman et al., 2018). Enzymes are initially involved in the dissolvable or immobilized structure for isolation, purification, and production to benefit humanity (Chaplin & Bucke, 1990). The demand for the enzyme increases by 6% yearly, especially in food and feed production (Nampoothiri et al., 2002). Enzymes are widely and progressively utilized in exploration and food processing as significant handling biocatalysts (Ermis, 2017). Enzymes are intricated in a living organism. Enzymes formed in live cells can enhance reaction rates *in vitro*. The acclimatization of enzymes in the food sector is known widely, and intense investigations consistently unravel the global food crisis. This chapter highlights the fundamentals of enzymes, sources of diverse enzymes, and their applications in the food sector.

Microbial enzymes have been catapulted to fame due to their inherent properties of the ease of their production and yield manipulation in laboratory settings. This highlights their significance and the opportunities that pave the way for microbiologists by harnessing the biomolecules' potential. The thoughtful consideration of the enzymes in the food sector has amended the primary methods to afford improved marketplaces with high quality and safety. Previously, this information about enzymes has been used in meat tenderization, baking, brewing, etc. Mainly the enzymes are produced by microbial synthesis. The microbial method for enzyme production has been favored over other methods for quick and inexpensive production.

Furthermore, microbially produced enzymes' harmful content is lesser than other sources. The first commercial application of enzymes was in the production of cheese. Enzymes in the food sector alter traditional and toxic methods, substitute them with sustainable methods, and reduce energy consumption levels. It may be worthwhile to unclose an unexplored trail for enzyme applications, which could be advantageous in the food industry.

Foodborne pathogens influence human health negatively and are comprehended to induce financial losses. Therefore, it is essential to rapidly detect foodborne pathogens and execute steps to assure their inactivation. Immunological, molecular,

and cultural approaches are often exploited to detect foodborne pathogens. High expense, lengthy analysis duration, and the need for technical personnel are some of the drawbacks of these approaches. Biosensors are known as analytical instruments. Biosensors can be used as novel techniques for detecting toxins and pathogens. These biosensors restore various signals (like biochemical signals) to electrical signals that can be measured with the help of a transducer. Various kinds of biosensors are being utilized for the detection of pathogenic bacteria. Biosensors are prompt and cost-effective devices and are employed in multiple areas such as food safety, medicine, pharmacy, measurement of environmental pollution, and military defense. Electrochemical and optical biosensors and piezoelectric immune-sensors are among the most repeatedly used biosensors in detecting foodborne pathogens.

There are many viruses and bacteria, some that cause animal disease, and others that contribute to foodborne human illness and disease that can be tested for the occurrence of foodborne pathogens. Some of the significant subjects of testing include several swine viruses, including swine influenza virus and porcine reproductive and respiratory syndrome virus. Bovine viruses include *bovine viral diarrhea virus, bovine syncytial virus, bovine rotavirus, coronavirus,* and *rhinotracheitis virus.* In addition, the *Hepatitis A virus, flaviviruses, West Nile,* and the *Usutu virus (USUV)* that have recently been on the rise in Europe and some parts of South East Asia are of much importance. The diagnosis and surveillance of bacterial pathogens encompass the full spectrum of animal and foodborne pathogens. These include *Escherichia coli* and bacteria in the *Salmonella, Klebsiella, Clostridium, Listeria, Staphylococcus, Streptococcus, Pseudomonas, Pasteurella, Actinobacillus*, and *Mycoplasma genera.*

Rapid, efficient pathogen detection and fingerprinting are crucial and usually lifesaving to control the foodborne ailment. Presently, detecting and subtyping the pathogens are distinct operations, but merging these two phases via a technique known as "metagenomics analysis," the detection is much prompter and more sensitive as many microorganisms are now employed to produce food enzymes. Enzyme technologies have been thoroughly revolutionized with the recent novel trends in biotechnology applications to fulfill the new and changing requirements of the food industry. There is an extensive tendency toward novelty in enzyme discovery and creating tailored enzymes to make them inclined for industrial food applications.

In the recent past, developments in enzyme informatics tools can simplify designing the enzymatic structure. Also, its computational dynamic simulations can produce the kinetic and energetic course of its mechanism. Enzyme informatics can also recreate an exceptional part in mutant library design and other screening platforms, but its execution of molecular approaches is still time consuming. Recent improvements in the subject will hover around novel frontier in enzyme technologies that could attribute various functional aspects to these enzyme biocatalysts. This study will consolidate the extensive characteristics of food enzymes, their diversified functions in the food industry, the destructive outcomes of foodborne pathogens, and their current detection techniques.

11.2 Enzymes as an Analytical Tool

Human health and healthcare predominately revolve around the quality of food consumed. Appealing enzymes in the food industry can manage the quality food supply problem. Hence, evaluating food quality and ensuring food safety standards are highly important. This crucial factor plays a vital role in evaluating food quality, safety, and the turnaround time to ascertain this. *Enzymes* are biomolecules that inherently possess specificity and sensitivity traits (Fig. 11.1).

Hence enzymes are a better bet than the conventional diagnostic methods that are dreary and overwhelming and require correlative modes to detect food quality and safety. Enzyme-based analytical tools to measure biological functionality are more reliable and consistent. The analytical tools should be high throughput and competent to authenticate and authorize the study of particular aspects of biology, either qualitatively or quantitatively, which is possible by enzymes. Also, a crucial part of developing such tools is to source or amalgamate the desired enzymes with the required activity. Thus, the utilization of enzymes as analytical informers is an inexpensive, simpler, and quicker manner of diagnostics. Microbes have been used from bygone days; yeast is the first reported organism to produce alcoholic beverages using barley in 6000 BC. The microbial enzymes have earned acknowledgment globally for their extensive uses in several trades. Nevertheless, recombinant DNA technology and protein engineering are assembled to confound the demand. It improves consumer goods, expense deduction, natural resources shortage, and environmental safety (Liu et al., 2013; Choi et al., 2015b).

Microbial enzymes play a crucial role (Raveendran et al., 2018). In terms of analytical procedures in the Food and Pharmaceutical industries, enzymes play a vital role. As indicated by food and regulatory agencies, food safety and quality are the censorious criteria recurrent to all food constituents, and these criteria are of substantial lucrative significance. Food safety depends on toxins, dangerous microorganisms, toxins divulged by microbes, insecticides, and pesticides (Terry et al., 2005). Processing of foods with biological enzymes is chronicled inveterate approach. Earlier in 6000 BC, bread, brewing, wine, and cheese making were done

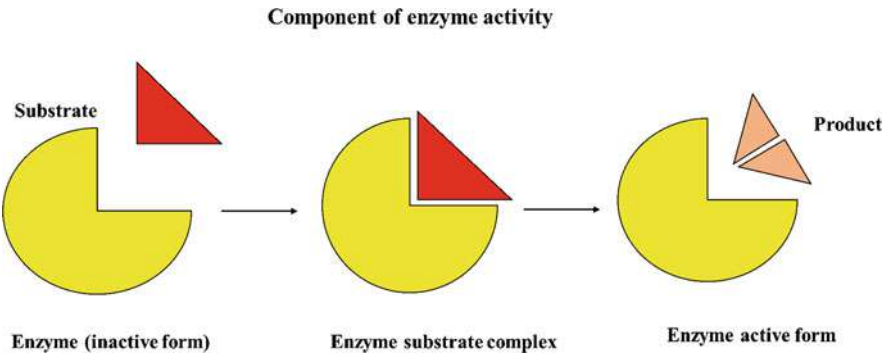

Fig. 11.1 Enzyme activity

with biological agents, but the initial microbial fermentation was from 2000 BC for vinegar production (Vasic-Racki, 2006). In 1930 for juice clarification, pectinases were used. Invertase was used during World War II for the invert sugar syrup production in the sugar industry that developed the use of an immobilized enzyme (Vasic-Racki, 2006). In 1960, the extensive use of enzymes established during the traditional acid hydrolysis was restored by slant based on utilizing amylase and glucoamylases (Panesar et al., 2010).

The pattern for the plan and execution of procedures and creation of products moored in the utilization of enzymes has consistently expanded (Norus, 2006). Enzymes from microbes play a significant part in food ventures since microbial enzymes' stability is higher than plant and animal enzymes. They can be fabricated through fermentation strategies in a lucrative way with less reality prerequisite and high consistency and process adjustment (Gurung et al., 2013). In food analytical strategies, food safety and quality control are essential, and they can be analyzed by numerous analytical procedures like HPLC, GC-MS, and LC-MS (Castillo et al., 2004). In any case, sample preparation strategies are the significant downside of this technique. Progressing diagnostic strategies in the food industry are tedious and rely upon skilled laborers, and these procedures depend on extended division techniques, costly instruments, and exceptionally unadulterated chemicals. In the field of quick screening, there is a requirement for complementary strategies to recognize food safety and quality issues. A more specific and sensitive method for screening food safety and quality issues is enzymatic analysis (Prodromidis & Karayannis, 2002). Enzymes play a crucial role in the analytical process in the food, pharmaceutical, and environmental industry. For governing the compound concentration that performs as an activator, substrate, or inhibition of specific enzymes, enzymes are used as typical analytical devices. For the analysis of modification induced physically and nutritionally and quality criterion of food, the concentration and activity of specific enzymes are used. The list of enzymes and their application, along with the microbial enzymes, are listed in Table 11.1.

11.3 Important Enzymes as Relevant Markers for Food Analysis

Enzymes are extensively used as an important marker, especially in the food and pharmaceutical industry. The broad scope of enzymes has been boon to the food analyst who investigates the different constituents of the food. Food, being more complex with increased macronutrients and very few micronutrients analyzing these food constituents, requires extremely simple and affordable, less time-consuming feasible, and specific techniques. Enzymes are used as markers for food analysis and their lustiness, adaptability, and cost-effective nature (Ashie, 2012).

Table 11.1 Microbial enzymes with their source and application

Microbial enzyme	Microbial source	Application	References
α-Amylase	B. *licheniformis*, *B. subtilis*, *B. stearothermophilus*, *B. megaterium* and *B. circulans*	Liquefaction of starch, fruit juice clarification, improvement of bread quality, brewing, and baking	Lépine et al. (2019), Luang-In et al. (2019)
Glucoamylase	*Aspergillus oryzae*, *Aspergillus niger* and *Rhizopus oryzae*	Glucose fructose syrups, production of beer	Bilal and Iqbal (2020), Karim et al. (2017)
β-galactosidase	*Lactobacillus delbrueckii subsp. bulgaricus and Streptococcus thermophilus*	Reduction of lactose intolerance	Gibson and Wang (1994), Saqib et al. (2017)
Protease	*Photobacterium, Bacillus, Vibrio*	Tenderization of meat, milk coagulation	Aruna et al. (2014), Zhang et al. (2015)
Lipase	*Bacillus sp. Pseudomonas*	Cheese production	Aravindan et al. (2007), Feng et al. (2011)
Esterase	*Bacillus subtilis*	Flavor enhancement, dietary fiber de-esterification	Faulds (2010), Sayali and Satpute (2013)
Pectinase	*Aspergillus niger, A. flavus, and Penicillium chrysogenum*	Fruit juice clarification	Pasha et al. (2013), Abdollahzadeh et al. (2020)
Phospholipase	*Listeria monocytogenes*	Development of cheese flavor	Law (2010), Titball (1993)
Cellulase	*Pseudomonas fluorescens Bacillus subtilis, E. coli, and Serratia marcescens*	Feed of animal, fruit juice clarification	Sukumaran et al. (2005), Islam and Roy (2018)
Xylanase	*Bacillus*, *Cellulomonas*, *Micrococcus*, *Staphylococcus*, *Paenibacillus*, *Arthrobacter*, *Microbacterium*, *Pseudoxanthomonas,* and *Rhodothermus*	Enhancement of beer quality	Camacho et al. (2003), Chakdar et al. (2016)
Glucose oxidase	*A. niger and Penicillium notatum*	Improvement of food flavor	Hanft and Koehler (2006), Nakamatsu et al. (1975)
Catalase	*Staphylococcus*	Hydrogen peroxide removal for production of cheese	Sîrbu (2011)

(continued)

Table 11.1 (continued)

Microbial enzyme	Microbial source	Application	References
α-Acetolactate dehydrogenase	*Enterococcus faecalis, Bacillus licheniformis, and Lactococcus lactis*	Precise beer maturation	Choi et al. (2015a)
Peroxidase	*Bacillus sphaericus, B. subtilis, Pseudomonas sp., Citrobacter sp.*	Flavor development	Regalado et al. (2004)
Asparaginase	*Escherichia coli and Erwinia chrysanthemi*	Shortening of acrylamide formation while baking	Mohan Kumar et al. (2014)

11.4 Enzyme Marker in Pasteurization of Milk

Enzymes play a vital role in detecting milk pasteurization. For increasing the shelf life of milk and milk products, heat treatment is essential. In many countries, an alkaline phosphatase test is used as a standard technique for milk ratification. Non-appearance of alkaline phosphatase in the heat-treated milk shows efficient pasteurization. It is based on the inactivation of the alkaline phosphatase enzyme using heat.

On the other hand, it reduces the pathogenic bacterial population in the milk. Not only for milk, but this rapid detection method can also be used for other dairy products like cheese production. Other primordial enzyme markers for milk include lactoperoxidase and γ-glutamyl transpeptidase (Rankin et al., 2010).

11.5 Enzyme Marker in Blanching

The main aim of blanching is to remove the enzyme polyphenol oxidase. Apart from this, another equitable goal is to decrease microbial growth and increase shelf life. Time taken for blanching is enough to destroy the indicator enzyme. Blanching of frozen foods, are done at different time intervals in order to recognize the relationship between enzyme activity and sensory scores (Williams et al., 1986).

11.6 Novel Analytical Tools

In these decades of higher technological development, the improvement in the food analysis techniques will have greater insight. Analysts and chemists will have greater value in using novel analytical tools. Traditional high-performance liquid chromatography system analyzes the hydrophobic and hydrophilic constituents in reversed phase and the normal phase. However, the conventional method is less susceptible and time consuming. So, to analyze the higher and lower molecular weight analytes,

a novel polymer-based stationary phase is introduced. Capillary electrophoresis that detects the analyte based on the molecular weight and ionic moiety is introduced as an alternative. There is a higher technique named capillary electrochromatography which is better than HPLC and capillary electrophoresis (Huck et al., 2000). The solid-phase extraction can attain a low concentration analyte. Spectroscopic strategies at this phase of the systematic technique either incorporate mass spectrometry (MS) or vibrational spectroscopy individually. Electrospray ionization is used chiefly with mass detectors like time of flight (TOF), ion trap, ion cyclotron, and quadrupoles. Laser desorption ionization methods determine higher molecular weight samples (Feuerstein et al., 2006).

11.7 Enzymes in the Food Industry

Enzymes are used in a variety of ways in the food industry. Enzyme activity aids in the safe digesting of food in various situations. The thermal stability of enzymes has been used extensively to quantify heat treatment; peroxidase activity, for example, is used to determine the sufficiency of blanched vegetables. Lactoperoxidase has also been used to test the effectiveness of the pasteurization process in milk. In some cases, residual enzyme activity affects the flavors or color of products while they are being stored. Lipoxygenase, for example, is responsible for off-flavors in frozen veggies that have not been adequately blanched. The staining of wheat flour and noodles and fruit liquids and pastes is catalyzed by polyphenol oxidase (Nielsen, 2017).

The enzyme is used to test the components of enzyme-substrate foods that are commercially accessible. The glucose content of a complex dietary matrix containing additional monosaccharides can be determined using commonly available enzymes. Enzyme activity measurement concerning food quality is also noteworthy. Milk from mastitic udders, for example, has considerably increased catalase activity. The quantity of bacteria in milk is also connected to catalase activity. Another technique to use enzyme testing to determine food quality is to estimate protein nutritional value by measuring the activity of added proteases on dietary protein samples. Enzymes can track the appearance of breakdown products such as trimethylamine in fish during storage. Enzymes are also used as preparatory tools in food analysis. In fiber analysis, amylases and proteases are used, while enzymatic hydrolysis of thiamine phosphate esters is used in vitamin analysis (Lee et al., 1991). Table 11.2 lists some of the most often used enzymes in the food business.

Food scientists must understand that the environment significantly impacts enzyme activity. At high temperatures or under other situations that are not too far off from ideal, that enzyme can deteriorate and lose its activity. As a result, therapeutic enzymes must be stored and handled with care, usually in the refrigerator or freezer. To effectively carry out enzyme analyses in foods, certain basic enzymology principles must be understood. Following a brief overview of these concepts, instances of enzymatic analysis in food systems are discussed. Under physiological conditions, enzymes are protein facilitators with high specificity and reactivity.

Table 11.2 Application of microbially produced enzyme in industries

Industry	Enzymes	Uses	Microorganisms
Baking	Maltogenic	Enhance shelf life of bread	*Bacillus stearothermophilus,*
	Amylase	Flour adjustment, bread softness	*Aspergillus sp., Bacillus sp.*
	Transglutaminase	Strengthening of dough	*Streptomyces sp., Streptoverticillium sp.*
	Xylanase	Conditioning of dough	*A. niger*
	Glucose oxidase	Strengthening of dough	*A. niger, Penicillium chrysogenum*
	Lipase	Conditioning of dough and its stability	*A. niger*
Dairy	Acid proteinase	Coagulation of milk	*Aspergillus sp.*
	Neutral proteinase	Ripening of cheese, debittering	*B. subtilis, A. oryzae*
	Lactase (beta-galactosidase)	Whey	*E. coli, Kluyveromyces sp.*
	Aminopeptidase	Ripening of cheese	*Lactobacillus sp.*
	Catalase	Cheese making	*A. niger*
	Transglutaminase	Cross-linking of protein	*Streptomyces sp.*
Beverage	Pectinase	Depectinization	*A. oryzae, P. funiculosum*
	Glucose oxidase	For the removal of oxygen	*A. niger*
	Pullulanase	Saccharification of starch	*Bacillus sp., Klebsiella sp.*
	Naringinase	Debittering	*A. niger*
	Alpha-Amylase	Hydrolysis of starch	*Bacillus, Aspergillus*
	Beta-Amylase	Hydrolysis of starch	*Bacillus, Streptomyces, Rhizopus*
	Beta-Glucanase	To inhibit haze formation	*B. subtilis, Aspergillus spp.*
	Cellulase fruit	Liquefaction	*A. niger, Trichoderma atroviride*

Enzymatic analysis determines the condition of a biological system, including meals, by measuring substances with the help of added enzymes or by measuring endogenous enzyme activity. Because enzyme catalysis occurs under relatively favorable circumstances, it is possible to measure relatively unstable molecules that would be difficult to measure using other methods. Furthermore, due to the specificity of enzyme reactions, it is possible to assess the components of complex mixtures without the time and expense of using expensive chromatographic separation procedures.

11.8 Beverage and Dairy Industry

Enzymes regulate the brewing process, ensure consistent, high-quality beer; enhance animal and vegetable proteins' functional and nutritive properties through enzymatic hydrolysis; and increase juice yield while improving color and aroma. Enzyme use

in the food business is divided into several categories, including baking, dairy, juice production, and brewing (Mojsov, 2011). Microbial enzymes are widely used in the baking business, the most prominent application market in the food industry, to increase dough stability, crumb softness and structure, and product shelf life. The usage of enzymes in the dairy industry (Qureshi et al., 2015), which is the next largest application area, is primarily due to the increased use of microbial enzymes in cheese manufacturing (Qureshi et al., 2015). The laboratory must identify appropriate enzymes with the required activity and understand the target analyte and the complexity of the matrix in which the analyte must be measured (Kieliszek & Misiewicz, 2014). Foods are typically very complex matrices, posing a significant technical barrier to the analyst tasked with determining and establishing the levels of various components. As a result, numerous analytical methods, including enzymes, have been developed to meet this issue over time. The usage of enzymes is based on specificity, which allows for the selective, sensitive, and precise measurement of macro- and micronutrients in meals. The development of biosensors with greater robustness and versatility has boosted the use of enzymes as an analytical tool in recent years. Starch is a critical ingredient in baked goods because it aids in the browning and flavoring of the crust. It is found primarily in plants in bread production.

Many enzymes are used for milk processing and cheese production in the dairy industry. Rennet is a coagulant enzyme mixture of chymosin and pepsin used in milk clotting and cheese preparation. They have been extracted from animal sources, mainly from the stomach of young calves (Theron & Divol, 2014). Plant extracts also contain many enzymes, such as leaves of Sodom apple, berries of *Solanum dubium*, *Calotropis procera*, and cardoon extracts, from microbial source *Rhizomucor miehei* and *Rhizomucor pusillus,* contain protease enzyme that helps in clotting. Protease enzyme obtained from *Cryphonectria parasitica* aggregate and transferred milk into curd and liquid part.

11.9 Necessary Enzymes as Relevant Markers for Food Analysis

Enzymes are ubiquitous, and their application has been used from the olden days in human edification. Food processing methods modify the food materials and make them appropriate for consumption (Monteiro et al., 2011). In processing, it has been used in wine, beer brewing, cheese, and bread making from 6000 BC. Based on the enzyme properties, the International Union of Biochemistry approved a class of enzymes used in the food industry, shown in the figure below (Fig. 11.2).

Food enzymes are categorized into two types: food processing and food additives. Most of them are used for food processing purposes, whereas lysozyme and invertase are used as food additives. The main aim of these food processing agents is to increase the edible foods' shelf life and preserve the health and nutritional contents of the food without affecting their taste and aroma. Uses of enzymes in the food industry process carbohydrates, proteins, and fats.

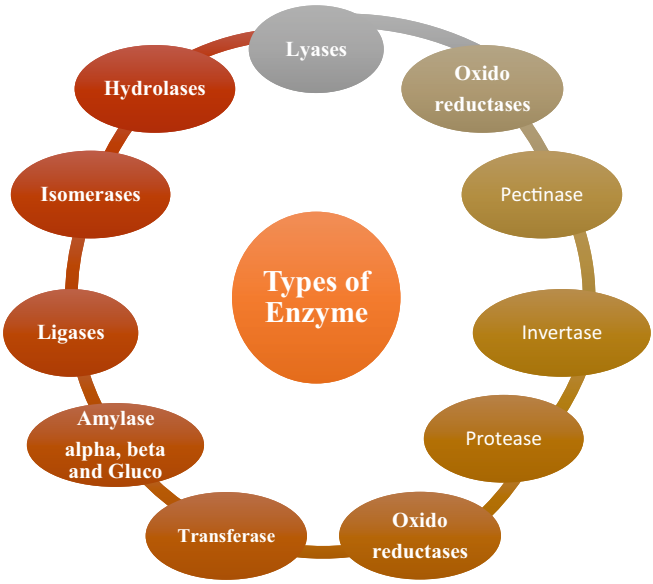

Fig. 11.2 Types of major microbial enzymes used in the food industry

11.9.1 Alpha-Amylase

The most important industrial enzymes account for around 25% of the global enzyme market, which comes from various sources, including plants, animals, and microbes (Kandra, 2003; Reddy et al., 2004). Enzymes catalyze the hydrolysis of internal 1,4-glycosidic bonds in starch to produce low molecular weight compounds like glucose, maltose, and maltotriose (Gupta et al., 2003; Rajagopalan & Krishnan, 2008). The baking sector currently employs a thermostable maltogenic amylase from *Bacillus stearothermophilus*. It is also used to clarify beer and fruit liquids and improve fiber digestion in animal feed (van der Maarel et al., 2002; Gavrilescu & Chisti, 2005; Ghorai et al., 2009).

11.9.2 Beta Amylase

Beta amylase is an enzyme with the systematic name 4-alpha-D-glucan maltohydrolase. It acts on starch, glycogen, other polysaccharides, and oligosaccharides, producing beta-maltose by an inversion, resulting in the sweet flavor of ripe fruit. This enzyme generally presents in bacteria, fungi, and plants. The molecular weight of this enzyme is 223 and 8 kDa and has various applications in the starch, sugar, brewing, and beverages industries. In bacteria, *Clostridium, Bacillus,* and *Pseudomonas* are the major groups of organisms wherein fungi, *Aspergillus*

sp. can produce these enzymes used to manufacture organic acids (Djekrif-Dakhmouche et al., 2006; Hernández et al., 2006).

11.9.3 Glucoamylase

Exoenzymes, also known as scarifying enzymes, are found in a wide range of species. It is used in the food industry for a variety of purposes, including lowering dough staling and increasing bread crust color, converting starch to maltose and other sugars, assisting in the creation of glucose in the fermentation process, and making alcoholic beverages and soy sauce (Blanco et al., 2014). Most glucoamylase enzymes lose their activity at higher temperatures and become stable at lower temperatures. The principal producers of this enzyme are *A. niger* and *A. awamori*. However, *Rhizopus oryzae* is widely used in industrial applications (Karim et al., 2017; Bilal & Iqbal, 2020).

11.9.4 Cellulase

This enzyme is generated mainly by microbes with a molecular weight of 54 kDa (bacteria and fungus). It is a complex enzyme that hydrolyzes 1,4 links in cellulose chains and contains endo-1,4-D-glucanase, exo-1,4-glucanase, and D-glucosidase. Fungi produce more cellulase than bacteria (Gaur & Tiwari, 2015). *Trichoderma reesei, Penicillium*, and *Aspergillus* are the three species. *Bacillus cereus, Bacillus megaterium, Bacillus mycoides*, *Clostridium straminisolvens, Flavobacterium balustinum*, and *Acetobacter xylinum* (Wachinger et al., 1989; Tomme et al., 1995; Gaur & Tiwari, 2015; Sharma et al., 2015; Tabssum et al., 2018). In the food industry, cellulase increases the nutritional characteristics of consumable food products. Macerating enzymes, in addition to the combination of pectinase and hemicellulase (Bhat, 2000), are essential in extracting olive oils to generate superior quality with less waste (Galante et al., 1998). It is also utilized to break up roughage in the dough (Chandrasekaran, 2013).

11.9.5 Pectinase

Pectin is a branching heteropolysaccharide with a high molecular weight. Pectinase has a molecular weight of up to 31 kDa. Fruit juice is made with enzymes that rapidly operate on hard pectin. It splits pectin molecules by making juice less viscous, producing pectin-free starch, refining vegetable fibers, and degumming natural fibers (Christopher & Kumbalwar, 2015). It can be found in higher plants and microbes in their natural state. Pectinase, employed in tea fermentation, eliminates pectin's foam-forming ability and removes the mucilaginous layer from coffee beans in tea and coffee (Oumer & Abate, 2018). Commercial pectinase can be

found in microbial strains such as *Moniliella* SB9, *Penicillium* spp., and *Aspergillus* spp. (Priya & Sashi, 2014).

11.9.6 Invertase

Invertase is a non-pathogenic and non-toxic enzyme also known as beta-fructofuranosidase, and the molecular weight of this enzyme is 205 kDa (Romero Gomez et al., 2000). The primary source of this enzyme is plants and microorganisms. Where *Saccharomyces cerevisiae*, *Candida utilis*, *A. niger* are the primary source for the production of invertase commercially. It is used in alcoholic beverages, lactic acid, and glycerol production. It is also used in the chocolate preparation of infants' food to improve the taste of the food (Christopher & Kumbalwar, 2015).

11.9.7 Protease

Proteases represent one of the major groups of industrial enzymes, with a molecular weight of 126 kDa. It breaks the peptide bonds present in the protein and separates them as amino acids. *Bacillus* and *Aspergillus* spp. is the primary microorganism that produces protease enzymes. It is used for several purposes such as brewing, meat tenderization, baking, hydrolyzed animal proteins, functional meat proteins, etc.

11.9.8 Lipase

Lipase enzymes deconstruct lipids into fatty acids and glycerol. This is something that can be used in the baking sector. It separates milk fat and gives terrific flavors to the cheese. The flavor of the cheeses improves the creamy texture and taste of the products. Excessive lipid lysis leads to unavoidable odors. Most commercial lipases develop flavor in dairy products, meat, vegetables, fruit, baked goods, milk, beer, and others (Nagodawithana & Reed, 1993). The major lipase enzyme producers are *Pseudomonas* sp., *Bacillus* sp., and *Penicillium* sp.

11.10 Enzymes as Biosensors

11.10.1 Enzymatic Biosensors Role in Food Technology

Food quality and safety-related assessment technologies need a necessary improvement since they directly impact the consumers' health. Reported microbial contamination, pesticides occurrence, food adulteration, mycotoxin presence, freshness analysis, presence of claimed antioxidants, health hazards caused by foodborne

microorganisms demonstrated the need for improved and faster technology to ensure the safety and quality of the food products. Conventional techniques for food quality assessments were replaced with fast and easy techniques like biosensors. Biosensors are more extraordinary than other demonstrative instruments due to different food quality and security innovations. Enzymatic biosensors are commonly utilized in the food business (Table 11.3). Different properties of biosensors, especially optical, mechanical, and electrochemical, can be adjusted by utilizing nanomaterials. Biosensors are an appropriate detecting tool for quality control and revealing the contamination in food. It can be a systematic and feasible detecting method in the food chain.

The most important part of the food industry is maintaining food safety and quality control. Biosensors have numerous food safety and quality control applications. Enzyme biosensors are an essential technique as it has a notable usage compared to the conventional method. It is effortless and less time consuming, and it does not involve skilled labor and costly equipment (Santos et al., 2006; Hooda et al., 2018). There are two main biosensors' main constituents: the bioreceptor and the transducer (Viswanathan et al., 2009). Biosensors work on three principles based on the device used to create biosensors, segments of molecular remembrance, and transducer. There are few enzymes for specific foods to act as a biosensor. There are numerous applications in the food industry in which biosensors are involved in quantifying different food constituents to determine their shelf life.

Biosensor technologies have the insightful potential to screen adverse substances in foods on-site and in-situ phases. Biological agents like enzymes usage in biosensors improved the specificity due to their binding affinity to a specific substrate and related catalytic effect. In addition, they lack interference with the compounds present in a complex sample. Due to sensitivity and specificity, enzymatic biosensors are considered an inevitable technology for food quality screening.

Table 11.3 Enzymes utilized in biosensors (Modified from Akyilmaz et al., 2010)

Enzymatic biosensors	Food constituents
Glucose oxidase, glucose dehydrogenase	Glucose
Oxalate oxidase	Oxalate
Tyrosinase	Tyrosine
Malate dehydrogenase	Malate
Galactose oxidase	Galactose
Cholesterol oxidase	Cholesterol
Alcohol oxidase	Alcohol
Peptidase, glutamate oxidase	Aspartame
Tyrosinase	Phenols
Lipoxygenase	Essential fatty acids
Fructose-5-dehydrogenase	Fructose
Glucose oxidase, mutarotase, invertase	Sucrose
Galactose oxidase, peroxidase	Lactose
Glutaminase	Glutamine

This book chapter briefly highlights the recent advancements of enzymatic biosensors' role in food quality analysis.

The food industry participates in the mandated food quality and safety assessment since they are essential for consumer health and nutritional value claims. The need for reliable and straightforward analytical procedures to ensure the quality of the food product from the production stage to consumption has recently been highlighted by an increase in demand for food preservation (Antiochia et al., 2013). The food manufacturing process is divided into primary and secondary processing. During main and secondary processing, toxicants from food additives may infiltrate food items. Toxicants such as drug residues, pesticides, and disease microbes may interact with the chemical compounds in food products during storage, causing food poisoning and rendering them unfit for human consumption. Enzymes are biological molecules that can sense the substrates and catalyze specific biochemical reactions. Like biosensors, devices can identify the specific material or analyte in the food samples. In a biosensor, bioreceptor and the transducer are considered essential components. Bioreceptor is used to screen the biomolecule, and they may be enzymes, antibodies, DNA, or cell. Enzymes were the most used bioreceptor molecules in biosensor applications. Enzymes enhance the reliability of biosensors since their catalytic effects are more specific and sensitive (Monosik et al., 2012). An enzymatic biosensor contains an enzyme to recognize the target analyte and mediate the reaction to produce the chemical signal. This transducer results in a physical signal from the chemical signal and an electronic amplifier. In addition, enzymes are considered promising bioreceptor molecules due to the broader role in the various analytical techniques, the flexibility in terms of transducers types like electrochemical, optical, and thermal modes, required in a low quantity, and the availability of high-quality commercial enzymes. However, enzymes were also associated with certain limitations like size, due to which they were not accessible to the relevant substrates, which in turn diminished the enzymatic activity. Enzymes reported with a limited lifespan may be deactivated by the enzyme-specific components in the sample. Various physicochemical parameters also influenced the enzymatic activity and cost of the commercially available enzymes (Marazuela & Moreno-Bondi, 2002; Borgmann et al., 2012). According to Vargas-Bernal et al.'s studies, enzymes used in the biosensor were classified into six major classes, and the oxidoreductases class was the most used enzyme in biosensor design (Vargas-Bernal et al., 2012). The first enzymatic biosensor was launched in 1962, and glucose oxidase was the first enzyme incorporated in the biosensor (Clark & Lyons, 1962).

Biosensors comprise biological receptors that can recognize a target, such as enzymes, aptamers, cells, antibodies, and so on. Electrochemical, optical, mass, and thermal methods are used in the binding process. Chemical biosensors based on enzymes are based on biological recognition. The enzymes must catalyze a particular biological reaction and be stable under the biosensor's usual working settings to function. The target analyte and matrix complexity in which the analyte must be quantified are used to build biosensors. Combining MBs (magnetic particles) with screen-printed electrodes (SPEs) is the best electrochemical biosensing compound

for food safety (Vasilescu et al., 2016). They are cheaper and can be obtained from various materials with a minimal level of sample volumes (Power et al., 2018). The enzyme-based sensor detects food contamination in various sectors of the food industry as shown in Fig. 11.3.

11.10.2 First-Generation Enzyme Sensors

In 1956, Leland Charles Clark Jr published research on the electrode used to measure oxygen levels in the blood (Clark et al., 1958). Electrochemical sensors and enzyme transducers were described as membranes at a 1962 Academy of Sciences symposium. Clark described the functioning enzyme electrode with glucose oxidase attached to the oxygen sensor later in 1967. Guilbault and Montalvo first described the potentiometric enzyme electrode in 1970. It works by immobilizing the enzyme urease on an ammonium-selective liquid membrane electrode. In 1973, they described a hydrogen peroxide-based glucose and lactate enzyme sensor using a platinum electrode. Klaus Mosbach invented a thermistor (heat-sensitive sensor) in 1974. In 1975, Clark introduced the glucose analyzer from Yellow Springs Instrument Company, which used an amperometric sensor to detect hydrogen peroxide. Lubbers and Opitz described a fiber-optic sensor with an immobilized indication to monitor carbon dioxide or oxygen levels. Later, by immobilizing alcohol oxidase, the optical biosensor concept was expanded to detect alcohol levels.

11.10.3 Second-Generation Enzyme Sensors

With the help of an artificial pancreas, Clemens and his colleagues built an electrochemical glucose biosensor. Miles later marketed it in 1975 as the Bio-stator (Gouda et al., 2002). Bio-stator was not accessible on the market. VIA Medical introduced a new semi-continuous catheter-based blood glucose analyzer. The lactate analyzer LA 640 was introduced by La Roche in 1976 to transport electrons from lactate dehydrogenase to an electrode.

11.10.4 Third-Generation Enzyme Sensors

Advanced biosensors have both immobilized enzymes and mediators in the same electrode. Liedberg used the surface plasmon resonance (SPR) method to track affinity interactions in 1983. In 1984, ferrocene and its derivatives were employed as immobilized mediators with oxidoreductases in the manufacture of low-cost enzyme electrodes based on screen-printed enzyme electrodes, according to Turner and his coworkers.

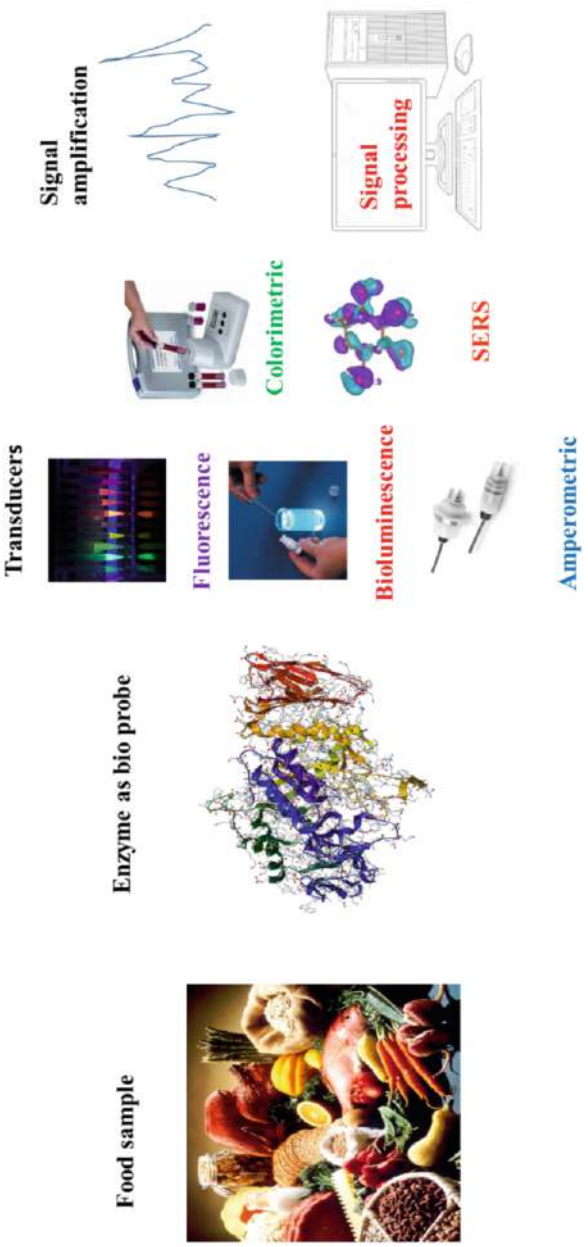

Fig. 11.3 Schematic representation of enzyme-based biosensor

11.11 Types of Biosensor

11.11.1 Electrochemical Biosensors

This biosensor employs an electrochemical transducer, in which metabolic reactions generate electrochemical signals, which are then captured using potentiometric, amperometric, or conductometric systems. It is a chemically distinct electrode with a biological coating covering the conducting substance. These sensors work with electrochemical sensors, which produce an electrochemical signal detected by an electrochemical detector when the electroactive analyte is oxidized or reduced. Voltammetric and potentiometric biosensors are two of the subgroups.

11.11.2 Voltammetric Sensors

Voltammetric sensors are subdivided into amperometric sensors. It measures the current potential features of a specific reaction's reduction or oxidation. Amperometric sensors work on the premise of applying a fixed voltage to an electrochemical cell, which results in a current due to an oxidation or reduction reaction. This current aided in the identification of the reaction's species. Unlike amperometry, which detects current using redox reaction products, this sensor employs glucose, lactate, and sialic acid analyzers. Electrochemical activity is seen in ion channel sensors. The cationic marker of electrostatic attraction or repulsion is improved or suppressed when these analytes are bound to a receptor molecule on an immobilized electrode surface. Voltammetric biosensors such as fumaric, maleic, and adenine nucleotides are employed with these sensors (Fig. 11.4).

The enzyme biosensor is characterized based on the substrate used for the food and drug samples (Amine et al., 2006; Tudorache & Bala, 2007). The hypoxanthine-sensitive sensors are used to detect fish freshness. Similarly, the xanthine oxidase-based biosensors are used to disclose the levels of hypoxanthine and xanthine, mainly involved in meat spoilage. To test the quality of the food products, oxygen-based biosensor has been used. Other than that, enzyme-based biosensors are used in food to maintain the freshness of edible products, detection of glucose level in beverages, analysis of cholesterol in butter, food components of sugars, and the detection of pathogenic organisms (Luong et al., 1991; Mello & Kubota, 2002). Some commercial biosensors used in the food industry are listed in Table 11.4 (Chapman et al., 2018).

11.11.3 Novel Analytical Tools

The nanotechnological interventions for food sensors provide significant benefits in identifying contaminants, especially in the food sector. Chemical nanosensors (chemical nanosensors) and biosensors (nano-biosensors) based on nanomaterials (nanosensors) can be utilized online and incorporated into existing production

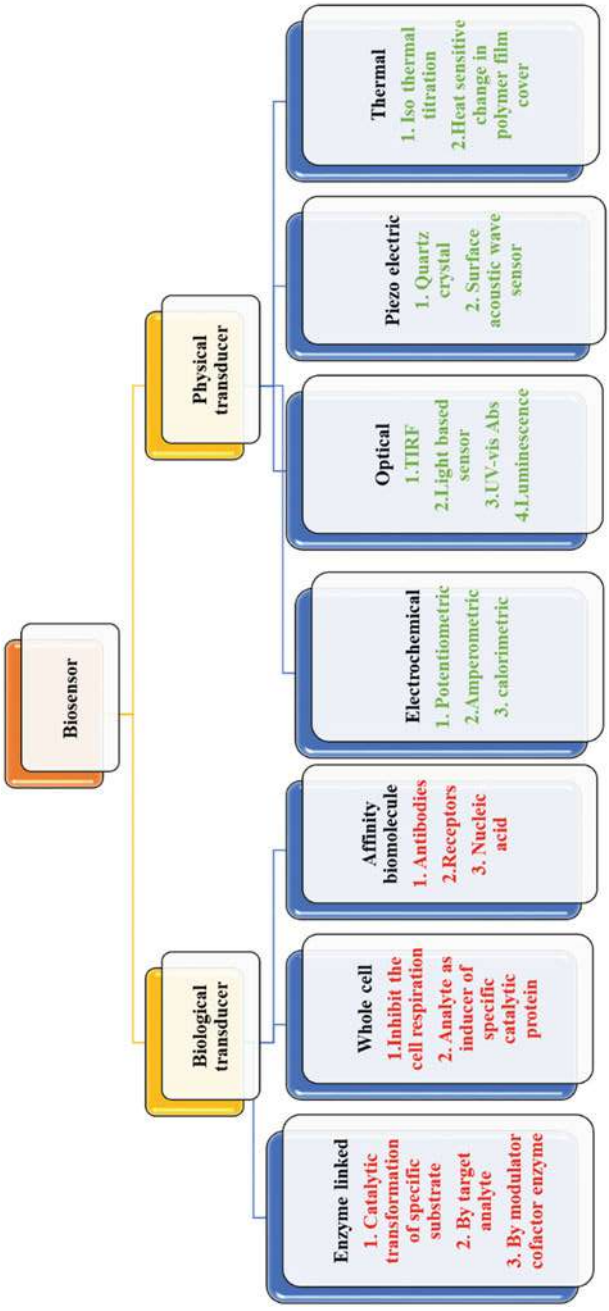

Fig. 11.4 Biosensors and their physical and biological elements.

Table 11.4 Commercial biosensors used in the food industry

S. N.	Company	Biosensor used to detect the materials
1	Affinity sensors	Toxin's detection
2	Ambri Ltd	Detection of *Salmonella*
3	Biacore AB	Detection of mycotoxins
4	Biosensor systems design	Detection of toxins
5	Biosensores S. L.	Detection of toxins
6	Chemel AB	Detection of lactose and saccharose
7	Georgia Research Tech Institute	*Campylobacter* and *Salmonella* detection in pork
8	IVA Co. Ltd	Detection of heavy metals
9	Massachusetts Institute of Technology	E. *coli* O157:H7 detection in lettuce
10	Michigan State University's electrochemical biosensor	*Salmonella* and E. *coli* O157:H7 detection in meat
11	Oriental Electric	Fish deterioration
12	Texas Instruments, Inc.	Detection of allergens
13	Universitat Autonoma de Barcelona in collaboration with CSIC	Detection of atrazine traces

processes and distribution lines or offline as quick, simple, and disposable sensors for food contamination. Food contaminants may be challenging to detect in this environment of chemicals. Nanosensors are an emerging technology that could be used to detect a variety of food pollutants, including mycotoxins and food allergies, due to their unique applications. Nanosensors are thus more cost-effective, quick, and sensitive than instrumental and traditional methods. Recent advancements in nanosensor technology may increase demand for its use in food contamination detection. As the food laboratory faces increased pressure to minimize cost, time, and complexity, the role of nanosensors will become increasingly more crucial. This chapter seeks to provide a broad overview of the potential applications of nanosensors in the detection and analysis of food contaminants. In recent years, the traditional PCR technique has been superseded by a simplified application in POC devices using multienzyme bioreceptors to determine DNA fragments' properties from allergens or adulterants. Surface platform resonance (SPR) characteristics and high conductivity are attributes of nanomaterials used in these sensor systems, such as Au NPs and AgNPs. This will make it easier to incorporate optical and electrochemical sensors. Magnetic nanoparticles, such as Fe_2O_3 NPs, will aid in the separation of analytes and will boost their performance.

However, carbon nanotubes and graphene-based nanomaterials enhance electrochemical signals due to their outstanding electrical conductivity. In 2017, electrochemical affinity biosensing was utilized to test food allergies, adulterants, and gluten. Andrei et al. (2016) used Nanoceria, a nanosensor device with oxidase-like activity, to covert phenolic antioxidants caffeic acid, gallic acid, and quercetin to quinones to measure wine's antioxidant content. He et al. (2018) developed manganese dioxide nanosheets to detect ascorbic acid levels in orange fruit and juice. The

hazardous chemical biphenol A (BPA) is released from the polycarbonate and epoxy resin during the food packaging process. Tyrosinase converts BPA to quinine to detect BPA NPs such as Au, Ni, or Fe_2O_3 (Alkasir et al., 2010).

11.12 Food Packaging

Biosensors play a vital role in detecting foodborne pathogens, and it plays a crucial role in food packaging as a part of smart packaging, which can be used in packaging materials to check their freshness and shelf life. As a part of smart packaging, the freshness indicator, time temperature indicators, integrity indicator, and radio frequency identification are used (Park et al., 2015).

11.13 Enzymes for Detecting Food Adulterants

Food is required for the survival of life. We all eat food to obtain energy for various metabolic functions. Food is required for all living species to grow, function, heal, and maintain life processes. There are many different types of food on the market today, and we all rely on diverse food sources daily, such as vegetables, fruits, grains, pulses, legumes, etc. Small stones in cereals and grains, darkly stained vegetables like cabbage and broccoli, fruits, dark red meat, and other items may have been encountered while shopping for fresh vegetables and other foods. Food adulteration is described as polluting food or food materials by adding a few substances, collectively known as adulterants. Adulterants are substances or products of lower quality that are added to food for economic or technical reasons. The inclusion of these adulterants lowers the nutritional value of food and contaminates it, rendering it unfit for ingestion. These adulterants can be found in various foods that we regularly consume, including dairy, cereals, legumes, grains, meat, vegetables, fruits, oils, and beverages. In underdeveloped countries, contamination or adding to food components is typical. Milk, for example, can be diluted with water to increase its volume, and starch powder is frequently added to increase its solid content.

The urease microbial enzyme from Arthrobacter creatinolyticus was immobilized on a poly (acrylonitrile-methyl methacrylate-sodium vinyl sulfonate) (PAN) membrane to create a potentiometric urea biosensor. Glutaraldehyde was used as a cross-linking agent in the biosensor. The following reaction is catalyzed by urease. The production of ammonium ions produced by the breakdown of urea by urease was used to detect urea in samples. A commercially accessible electrochemical workstation was used to perform potentiometric experiments. Milk samples were spiked with known urea concentrations and evaluated using the biosensor for real-world analysis. The measured potentiometric measurements were in good agreement with the spiking concentrations. The biosensor had a detection limit of 0.3 mM and linearity over the range of 1–100 mM. It was able to maintain appropriate sensitivity for 13 cycles in a row. It could be kept for 70 days at 4 °C.

Adulteration of food items is caused mainly by food firms' marketing strategies, mimicked food goods, a lack of awareness of proper food intake, and gaining profit with fewer inputs.

11.14 Methods of Food Adulteration

Adulteration is the illicit practice of increasing the number of high-quality products by introducing raw and other low-cost materials. This tainted food is highly hazardous and causes various health problems, including nutrition deficiency diseases, kidney disorders, and organ failure, including heart, kidney, and liver failure.

11.14.1 Melamine

Melamine is a commercially manufactured chemical molecule (1,3,5-triazine-2,4,6-triamine). Inflating nitrogen content and proteinaceous substances in food is a significant food adulteration. Melamine's specificity and ability can be determined using two approaches. First, commercial enzyme-linked immunosorbent assay (ELISA) kits were tested using ammeline, ammelide, or cyanuric acid, revealing that 4,6-diamino-1,3,5-triazine structure, or 1,3,5-triazine-4,6-diones were required. Melamine detection limits in infant powder ranged from 0.2 to 3 mg/g per sample (Kim et al., 2010). This melamine produces significant toxicity and kidney stone formation (Hau et al., 2009). Brown et al. (2007) labeled it a hazardous substance because it causes renal failure by forming insoluble melamine cyanurate crystals in the kidneys. Nanosensor to detect melamine in various feed matrices quickly, Au nanoparticles, cadmium telluride (CdTe) quantum dots, SWCNTs, and other sensors are used (Li et al., 2010). Zhou et al. (2011) employed an Au nanoparticle biosensor to detect melamine in various milk and dairy products. Milk powder, infant formula, lactose, whey protein, wheat bran, and wheat gluten are all examples of foods that include it (Mecker et al., 2012). Melamine was also detected using water-dispensable cadmium telluride quantum dots coated with thioglycolic acid.

11.14.2 Meat Adulteration

The demand for ready-to-eat halal foods such as burgers, pizzas, hot dogs, sandwiches, soups, cookies, sweets, and creams has risen recently (Jeddah, 2011; Mascini, 2015). Pork is being used more frequently in Halal meals, including beef meatballs, to maximize profits (Mascini, 2015). It was identified utilizing NPs as a colorimetric nanobiosensor, which changed the color of the meat from pinkish-red to purple with a 525 nm absorption peak. Ali et al. (2011) used hybrid biomaterials with functionalized nanoparticles that are noncovalently or covalently coupled to biomolecules such as peptides, proteins, and DNA to detect pork adulteration in processed meat.

Several adulterants such as food coloring compounds and preservatives have been found with modern technology. Olive oil, milk, honey, and saffron are some of the most commonly reported contaminated foods, which can be discovered using HPLC and infrared spectroscopy (Moore et al., 2012). The adulterants included in butter, pork, and beef meatballs were detected using Raman spectroscopy and FTIR spectroscopy. Adulterants such as sunflower and corn oils commonly replace olive oil and can be detected using FTIR spectroscopy.

11.14.3 Enzymes for Detecting Contaminants

Food adulteration can arise from numerous sources, such as natural toxic compounds, bacterial contamination, pesticides, veterinary drugs, and chemicals used in food dispensation. Identifying this substance is essential for people affected by diseases (Viswanathan et al., 2009). It has been mostly separated into two classes: biological and chemical contaminants. Some critical assays analyze the phytocompound such as sugars, alcohols, amino acids, flavors, and sweeteners. Chromatography and spectroscopy have been used to detect contaminants from the olden days. Currently, many sensors have been developed to detect the presence of these contaminants in food without affecting their taste and flavor.

11.14.4 Biological Contaminants

Toxic and pathogenic biological substances created during food preparation, packing, and storage are biological pollutants. Toxins such as aflatoxins, ochratoxins, and other foodborne microorganisms are the most common pollutants that cause foodborne illnesses. Pathogenic bacteria *include Campylobacter, Salmonella, Listeria monocytogenes, E. coli, Staphylococcus aureus*, and *Bacillus cereus*. Toxicology can be detected using a variety of approaches. The most prevalent mycotoxins generated by Aspergillus spp. are aflatoxin and ochratoxins. It is cancer-causing, teratogenic, mutagenic, and immunosuppressive. A nanosensor has been created to detect this mycotoxin using the immunosensor approach. (Sharma et al., 2010). Using antibody-conjugated MNPs and a magnetic field, Salmonella in milk was detected. Sack et al. (2004) defined Vibrio cholera as an acute intestine infection induced by eating tainted food or water. Cholera toxin, a colorimetric nanobiosensor detected with glycol nanoparticles, is secreted by this bacteria (Schofield et al., 2007).

11.14.5 Chemical Contaminants

Unwanted dangerous chemicals are introduced into foods directly or indirectly from natural sources, pollution, or formation during food processing, distribution, and storage. Pesticides, heavy metals, and melamine are just a few chemical pollutants

Table 11.5 Nanobiosensors used in the detection of chemical and biological contaminants

Nanomaterial	Contaminants	Biosensor
Biological		
Gold nanoparticle	Aflatoxin	Immunoelectrode
Silver core and a gold shell	AF B1	Immuno-dipstick assay
Antigen-modified and antibody-functionalized DCNPs	AF B1 and OTA	Immunosensing probes
Nanostructured zinc oxide	Mycotoxin	ITO glass plate
Magnetic nanoparticles and TiOz nanocrystals	*Salmonella*	Optical nanocrystal probes
Oligonucleotide-functionalized Au nanoparticles	*Escherichia coli*	Piezoelectric biosensor
Glyconanoparticles	Cholera toxin	Colorimetric bioassay
Chemical contaminants		
Gold nanoparticle	Carbofuran	Electroimmunosensor
Antibody competitive nanoparticle	Chloramphenicol	Immunosensor IPR
MTT-stabilized gold nanoparticle	Melamine	Colorimetric sensor
Quantum dots	Melamine	Colorimetric sensor
CNTs	Penicillin	Enzyme-based biosensor

that harm human and animal health. Nanosensors have the potential to be a helpful instrument for detecting chemical pollutants. Table 11.5 shows a variety of nanosensors and nanobiosensors for detecting chemical and biological pollutants.

11.14.6 Biosensors and Bioamines to Quantify the Freshness of Food Products

Chemical compounds produced in surrounding storage due to the decomposition of food components served as a vital signal for food freshness. The concentrations of xanthine, hypoxanthine, and biogenic amines, for example, have altered over time. The breakdown of inosine monophosphate in seafood produces chemicals like xanthine and hypoxanthine, which act as biomarkers for fresh food. Biogenic amines (BA), such as histamine, putrescine, and cadaverine, are also generated by the decarboxylation of amino acids and are used as freshness markers in meat, drinks, and cheese (Calvo-Pérez et al., 2013). Bioamines are essential for numerous physiological activities at low concentrations, but they can cause health problems in consumers. BAs were produced in plants, animals, and microbial populations by pathogenic microbial decarboxylation of specific amino acids, such as histidine, or by the amination and transamination of ketones and aldehydes.

In contrast, others have aromatic structures, such as Phenylethylamine (PEA) and Tyramine (Tyr) (Tyr). Then there are a few BAs that have heterocyclic structures, such as Tryptamine (Tryp) and Histamine (Hist) (His). The presence of BAs in dietary samples was determined using the time-consuming high-performance liquid chromatography (HPLC) method (Hernández et al., 2006). BA's biosensors were

designed as an effective strategy for facilitating quick and exact estimation in the search for a steady approach to accurate discoveries. Many amine oxidase enzyme designs have been used to construct biosensors, to increase precise biorecognition and signal transduction.

11.14.7 Biosensors and Histamine Oxidase Applications

Substances like histamine were biogenic amines. Their presence varies with degrees in several food products, and their increased concentration is considered an indicator of the depleted level of freshness. Histamine intoxication is the most prevalent foodborne infection among humans who consume fish products. If histamine concentration exceeds 500 ppm in fish products induces histamine poisoning (Gonzaga et al., 2009). Bacterial contamination in fish gut and skin induces histidine decarboxylase activity and results in histamine production. Histamine levels were screened by immobilized histidine decarboxylase and horseradish peroxide loaded on the electrodes using the bovine serum albumin and glutaraldehyde complex. Another study used the histamine oxidase enzyme from the bacterial sp. *Arthrobacter crystallopoietes* (HOD) to build a biosensor. HOD biosensor was effectively employed to measure histamine levels in fish and meat products, comparatively yielding a good agreement with the results obtained from HPLC analyses (Rosini et al., 2014).

11.14.8 Biosensors to Quantify D and L Amino Acid

Amino acids have been extensively researched due to their nutritional importance and speed up metabolic processes. D-amino acids (D-AAs) are, on the other hand, primarily found in microbiological, aqueous, soil, and other environmental pollutants. In general, the presence of D-AAs reduces protein digestion in the body, resulting in decreased bioavailability of essential amino acids and nutritional value. D-AAs are also utilized to evaluate the adverse effects of heat and alkaline treatments on dairy products. D-AA biosensors based on the D-amino acid oxidase (DAAO) enzyme were frequently employed to evaluate milk samples (Sarkar et al., 1999; Rosini et al., 2008). The FAD domain is a cofactor expressed in humans, not bacteria, in this peroxisomal enzyme.

11.15 Biosensors and Antioxidants' Concentration

The antioxidant activity of phenolic chemicals found in berries has been shown to help prevent cardiovascular disease and aging and have anti-cancer properties. Since the TYN enzyme converts hydroquinone in foods like red wine to p-quinone, it then reduces to hydroquinone at the electrode and generates a current. The TYR enzyme from Agaricus bisporus is an oxidase enzyme that catalyzes the conversion of

phenolic substances to quinone derivatives, with amperometric techniques detecting the end products. The TYR biosensor can detect the "total phenolic content" and "total antioxidant capacity" of samples with low non-polyphenolic antioxidant levels in general. The reduced lifespan of the Tyr enzyme reduces its stability. The tert-butyl hydroquinone chemical quantities in standard salad dressing items were also measured using the Tyr biosensor.

11.15.1 Biosensors for Heavy Metals

In recent days, heavy metal-induced toxicity among the food has been observed and studied extensively. Heavy metals like Fe, Zn, Cd, Ni, Mn, Cr, Co, Pb, Cu, and Al were leached from the eating utensils as well as cookware made up of iron, old stainless steel, new stainless steel, old and new aluminum, and clay pots lead to metallic contamination of food and water (Ghorai et al., 2009). In practice, the usage of enzyme-based biosensors is used to detect these toxic substances like heavy metals. Since these heavy metals react with the thiol groups in the enzymes like glucose oxidase, urease, glutathione S-transferase, alkaline phosphatase, lactate dehydrogenase, acid phosphatase, and invertase and biosensors, the function is based on enzymatic inhibition. Nevertheless, limited enzymes have an affinity to heavy metals.

11.15.2 Biosensors and Ethanol Concentration

Assessing the ethanol concentration and control is vital in brewing and winemaking and also needs the exact data about the ethanol content in the beverages. Immobilized alcohol oxidase catalyzes the reaction on the ethanol biosensor. Earlier studies on ethanol biosensors used the combination of enzymes of alcohol oxidase (AOx) along with horseradish peroxidase (HRP) and a mediator ferrocene immobilized on an electrode. Later, Shkotova et al. established an amperometric transducer and AOx-based biosensor immobilized on resydrol polymer and used to detect ethanol concentration in alcoholic beverages. Another enzyme, pyrroloquinoline quinone alcohol dehydrogenase (PQQ-ADH), is used in amperometric biosensors to detect ethanol. PQQ-ADH has advantages like oxygen independence and does not require a soluble cofactor. Certain studies employed the bi-enzymatic biosensor to detect the ethanol concentration in alcoholic beverages.

11.15.3 Fructose Dehydrogenase-Based Biosensors

Because fructose is harmless, it is commonly employed in artificial sweeteners. Fructose dehydrogenase (FDH) is a human enzyme that produces fructose in the intestinal mucosa. This enzymatic process is also used to make corn syrup in corn plants (Siepenkoetter et al., 2017). Fructose corn syrup was thought to be a better

substitute for natural sugars. As a result, fructose has been used to manufacture beverages and foods (Antiochia et al., 2013).

On the other hand, overuse of fructose has been associated with high blood pressure, decreased glucose tolerance, insulin resistance, and hepatic steatosis. For many years, biosensors based on FDH have been used to detect fructose content in food and beverages. To immobilize the NDFDH-modified nanoporous gold electrodes, FDH was coupled with a thiol and diazonium-bound carboxylic acid functional group. The fructose level in beverages may be accurately measured using this manufactured electrode.

11.15.4 Xanthine Oxidase-Based Biosensors on Assessing the Fish Freshness

Xanthine is a thoroughly studied fish freshness indicator. Adenosine triphosphate (ATP) concentration in stored fish steadily degraded to generate xanthine as the storage period extended beyond the permissible interval (Venugopal, 2002). On the other hand, manufacturing needs fresh fish for human consumption and fish products. Thus, a few decades ago, investigations began using electrochemical xanthine biosensors as a biomarker to assess the freshness of fish. However, the stability of this biosensor is restricted; thus, Devi et al. devised a xanthine oxidase–chitosan–gold-coated nano-iron modified pencil graphite electrode with a detection limit of 0.1 μM to increase the stability. This biosensor's xanthine detection and storage precision have been increased. Dervisevic et al. developed a CHIT–polypyrrole–AuNP-modified glassy carbon electrode (GCE) with XOD as an electrocatalyst that converts xanthine to uric acid in a follow-up study. With a detection limit of 0.25 μM, this modified amperometric sensor could efficiently detect xanthine.

11.15.5 Lactate Dehydrogenase-Based Biosensors on Quantifying the Lactate Concentration

Lactate concentrations in red wine, white wine, beer, yogurt, curd, and milk samples were used as biomarkers to determine the food quality. The food industry utilized lactate biosensors to detect the desired level of lactate content in fermentation. Lactic acid fermentation is induced by *Lactobacillus* spp. in fermented food products, and the lactate content suggests an enhanced *Lactobacillus* population. Batra et al. created an electrochemical lactate sensor using a PGE modified with graphene oxide (GO) nanoparticles and LDH in 2016. This upgraded LDH–GO–PGE lactate biosensor is currently being utilized to assess lactate levels in fermentation industries (Batra et al., 2016). Lactose-related biosensors were also frequently employed, as lactose intolerance affects roughly 15% of Northern Europeans, 80% of black and Latino persons, and almost 100% of American Indians and Asians. These individuals lack the digestive enzyme galactosidase, which converts lactose to

galactose and simple glucose. Electrochemical biosensors containing co-immobilized-galactosidase and glucose oxidase enzymes were created to assess the trace amount of lactose in lactose-free dairy products.

11.16 Biosensors in Mycotoxins Detection

Fungal species *Aspergillus, Fusarium, and Penicillium* produce specific secondary metabolites that cause food poisoning among humans and animals after ingesting contaminated food products (Turner et al., 2009). Contaminated fruits and cereals induce mycotoxin in certain beverages like beer and wine. Sometimes contamination of livestock animals' feed during the processing also introduced the mycotoxin in food products (Becker-Algeri et al., 2016; Iqbal & Selamat, 2016). Thus, residual mycotoxin presence needs to be evaluated to ensure the food product quality. Mycotoxins were categorized into distinct groups, namely aflatoxins, ochratoxin A (OTA), zearalenone (ZEN), fumonisins, ergopeptine alkaloids, and trichothecenes based on their impact on the economy and associated health risk. Enzymatic electrochemical sensors were developed by "Grupo de Electroanalítica" GEANA (Argentina) using enzyme horseradish peroxidase to assess the CIT in rice.

11.16.1 Epidemiology of Foodborne Illnesses

Foodborne infections that are infectious and toxigenic have been known for more than a century. *Salmonella, Staphylococcus aureus,* and *Clostridium perfringens* were the most common pathogens of concern worldwide by the mid-1900s. Botulism was a fatal disease rarely linked to commercially canned foods, home canning of vegetables, or traditional Arctic marine mammal products.

There was little interest in or research on acute foodborne illness agents during those days. According to what was learned during outbreaks, most outbreaks might have been averted if adequate time and temperature handling and storage protocols had been followed, especially with poultry and beef. *Clostridium, Salmonella*, *and Staphylococcus* issues were mainly resolved after the population was educated. However, additional agents like *Campylobacter, E. coli* O157:H7, *Listeria*, and *Yersinia* species emerged in the 1980s. It took many more years to realize that these disorders might lead to life-threatening complications or even death and that several goods could transmit them. Only a few of these diseases are detectable by existing monitoring systems (Kothary & Babu, 2001; Wang et al., 2010; Fukushima et al., 2011; Lindström et al., 2011). However, more and more international outbreaks are being recognized thanks to DNA typing and next-generation sequencing technology.

11.16.2 Worldwide Status of Foodborne Illnesses

Despite the adoption of new regulatory concepts, know-how, and initiatives on a national and international level, there has been a broad plateau in the number of instances of foodborne disease. The fundamental reason for this is that surveillance of waterborne and foodborne illnesses has been limited in detecting instances other than small clusters of patients. For decades, the traditional system of making outbreak reports available to a central authority in a small number of countries has been the source of our knowledge, but it is far from adequate.

Only significant outbreaks are reported, whereas home infections are never reported. As a result, epidemics in mass catering and restaurants are widely highlighted. Another issue that raises the risk of foodborne disease is the global food market. Changes in lifestyle and activities, more extensive operations, and higher throughput lead to increased profit, increasing the risk of contaminated animal products. Even if the livestock is not ill, larger-scale animal rearing allows for more microbial diseases. Antibiotics are mainly used to aid in the growth of animals. However, widespread use has led to growing antibiotic resistance and inappropriate use for self-treatment of human illnesses, posing a more significant threat to the global population. Other contamination sources, including rats and rodents in warehouses, have been identified as critical connections in the transmission chains of zoonoses.

Newer strains in humans are derived from environmental sources, where intergeneric gene transfer may occur. Larger markets result in a more significant geographic dispersion worldwide, resulting in massive outbreaks with millions of patients if problems arise. This is an example of how tracing a product's origins can be challenging, mainly if the originating company is located in another country. People are introduced to new meals by immigrants, and the public requires a diverse range of products to consume. This can lead to new goods or modifying current ones, risk introducing a new pathogen at each stage (Alvarez-Ordóñez et al., 2013).

11.17 Bacterial Pathogens

11.17.1 Staphylococcus aureus

Because of contamination from humans, cattle, and the environment, several *Staphylococcus* genus species and subspecies are potentially harmful to foods. Both coagulase-negative and coagulase-positive strains can produce enterotoxins that cause human gastroenteritis. *S. aureus* is most commonly associated with food poisoning. Enterotoxins are the most dangerous of the several toxins produced by staphylococci. Enterotoxins are proteins generated by other Staphylococci strains that, if allowed to flourish in foods, form enterotoxin. *S. aureus* is the primary producer of structurally related, toxicologically comparable proteins (Lancette & Bennett, 2001).

Meat and beef products, poultry and egg products, salads such as bacon, fish, rice, potato, and macaroni, baking products such as cream-filled pastries, and cream pies are the most common foods associated with staphylococcal food poisoning. Some of these products are polluted and mismanaged as a result. Chemical, animal, or environmental contaminants can contaminate pre-processed foods. At the same time, foods exposed to temperatures favorable to *S. aureus* growth have a significantly greater capability to form enterotoxin. This is also true of meat and milk products that have been fermented. Secreted enterotoxins are a structurally and functionally related family of virulence factors with critical super-antigenic qualities that disrupt adaptive immunity. Standard enterotoxins (SEA-SEE) and modern enterotoxins are two types of enterotoxins (SEG-SElY).

The pathophysiology of several human diseases, such as toxic shock syndrome (TSS), sepsis-related infections, and pneumonia, is determined mainly by members of these classes. These have emetic properties and are frequently linked to food poisoning outbreaks. The enterotoxins are primarily responsible for the disease's consequences when the patient lacks denaturation resistance. The enterotoxin genes are frequently found on many mobile genetic elements. The prevalence of enterotoxins varies greatly among *S. aureus* isolates, and it is regulated by numerous overlapping regulatory mechanisms (Lancette & Bennett, 2001).

11.17.2 Listeria monocytogenes

Listeria monocytogenes causes occasional outbreaks and ingestion associated with infected food products as another significant cause of death due to foodborne disease. Over the last few decades, *Listeria monocytogenes* have emerged as a foodborne pathogen of considerable importance. Most of our knowledge about foodborne *Listeria* transmission routes has been acquired through the study of worldwide epidemiological evidence. Such outbreaks were related to food from hospitals patients who had consumed spinach, carrots, and radishes.

The use of manure from infected sheep has been blamed for several outbreaks. Most listeriosis infections in pregnant women have resulted in miscarriages, stillbirths, and sporadic or live deliveries of poorly infants. Meningitis, aspiration pneumonia, and sepsis symptoms were seen in many non-pregnant people who showed no evidence of immunosuppression. These outbreaks accounted for around 41% of the overall death rate. Serotype 4b was assigned to some of these isolates, and *L. monocytogenes* isolates from coleslaw packets were known as Serotype 4b.

Over the past few years, whole-genome sequencing has proven to be a valuable tool in *L. monocytogenes* surveillance and outbreak investigations. WGS has allowed rapid and reliable identification of infection clusters, helped trackback to sources of infection, and ultimately removed the origin of infection. Various projects have been initiated to encourage the typing of *L. monocytogenes* based on WGS and many other foodborne pathogens. Nevertheless, there remains a need to create a transition to an international standard. (Camargo et al., 2017; Colagiorgi et al., 2017).

11.17.3 Bacillus cereus

Many extracellular compounds involved in pathogenesis are found in *Bacillus cereus,* and many strains are employed as probiotics in humans. On the other hand, others are highly poisonous and linked to dietary deaths. There are two forms of foodborne sickness caused by these genera: diarrheal and emetic. Enterotoxins produced in the small intestine during pathogen vegetative growth cause the former, while the emetic toxin is pre-formed in the meal before ingestion causes the latter. The disease is spread via food because of the surviving spores in both forms. *B. cereus,* in addition to being an opportunistic pathogen, can cause a variety of systemic and local illnesses, including septicemia and endophthalmitis. The diarrheal disease is typically self-limiting, and the number of *B. cereus* outbreaks is widely underestimated due to the relatively short duration of the disease (typically less than 24 h). The total recovery is swift after the symptoms resolve.

The *B. cereus* community comprises many species whose members are capable of causing foodborne emetic or diarrheal diseases. With the lack of modernity in this pathogen's diagnostic and characterization aspects, tens of thousands of diseases have been documented in the world each year. Still, the issues are far from conquered. Today, methods such as whole-genome sequencing are not widely used to classify *B. cereus* isolates from foodborne diseases. In addition to using state-of-the-art genomic methodologies, it is possible to use WGS techniques to address future microbiological and epidemiological problems raised by *B. cereus* outbreaks in the group. One specific obstacle is that, unlike other pathogens, cases of disease caused by *B. cereus* are usually not reportable, while foodborne diseases are widely documented, regardless of pathology. When mixing this with a usually moderate course of *B. cereus* disease, human isolates of the *B. cereus* are rarely available for techniques such as WGS.

In addition, even if clinical isolates of the *B. cereus* group are obtainable, WGS should not be used to describe the isolate in cases where the disease or infection is mild. The data and methods available from laboratories around the world not just enable further detection of other outbreaks of *B. cereus.*

11.17.4 Clostridium perfringens

Clostridium perfringens produces two diseases transmitted through food: a common foodborne illness and necrotic enteritis (pig-bel). Ingestion of infected foods causes *C. perfringens,* one of the most frequent foodborne infections. While the link between *C. perfringens* and foodborne sickness was first proposed over a century ago, definitive evidence demonstrating that an enterotoxin is linked to the organism's sporulation in diseased intestines did not develop until the 1960s and 1970s. The bacteria have many characteristics that contribute to its capacity to cause foodborne illness: (1) an omnipresent distribution throughout the natural environment, allowing it to contaminate food; (2) the ability to develop rapidly in food, causing high levels

of food poisoning; and (3) the ability to produce an intestinally active enterotoxin, which is responsible for *C. perfringens'* characteristic gastrointestinal symptoms.

C. perfringens-related food poisoning is one of the most common causes of food contamination. The association between *C. perfringens* and foodborne sickness was originally postulated around 100 years ago, it was not until the 1960s and 1970s that conclusive evidence emerged demonstrating that an enterotoxin is linked to the organism's sporulation in the intestines. The bacteria have several characteristics that contribute to its capacity to cause foodborne illness:

1. It has a wide distribution in the natural environment, allowing plenty of opportunities to contaminate food.
2. Its ability to generate heat-resistant spores allows it to survive incomplete food cooking or insufficient sterilization.
3. The ability to produce an intestinally active enterotoxin is responsible for *C. perfringens* food poisoning's typical gastrointestinal symptoms.

However, unlike other foodborne illness agents, it is frequently underreported, owing to the anaerobic conditions required for its development and the sickness's apparent mildness. Meat and poultry-based products, as previously stated, are the most prevalent carriers via which intestinal contents spread after slaughter. The presence of this organism in the spore condition on herbs and spices (as well as meat and poultry) poses a hazard since it can survive the chilling cycle and restart growth in temperature-abused or inadequately cooled items, resulting in the enormous number of cells needed for an outbreak. The organism's specific development requirements are easily met in meat and poultry products.

Temperature exploitation of cooked meals is often linked to outbreaks, showing that the organism's optimum development temperature is relatively high [43–46 °C]. This, together with its ability to form spores, are two remarkable traits contributing to its etiological role in foodborne disease. The sickness is more likely to be purchased in restaurants, hospitals, jails, schools, and caterers (Lindström et al., 2011).

11.17.5 Clostridium botulinum

Botulism comes in a variety of types. Symptoms of foodborne botulism can appear within 12–36 h and can take up to a week or longer after consuming botulinum neurotoxin-containing food. Initial symptoms include nausea and vomiting, though it is unclear whether the neurotoxin or other *Clostridium botulinum* compounds are to blame.

The organism may get established in the intestines of young infants, most likely because the normal intestinal flora has not yet developed sufficiently to prevent pathogen colonization. Chronic constipation, severe exhaustion, and increasing paralysis, as well as other neurological symptoms, may be present in the patient. In some instances, spores of *C. botulinum* have been discovered in specimens from

children who died of sudden infant death syndrome (SIDS) (Health Protection Agency, 2011).

11.17.6 *Salmonella* Species

Serotypes of *Salmonella* can cause localized gastroenteritis in humans, but the severity of Salmonella infections in different hosts is determined by pathogen virulence, host resistance, and immunity. Many serotypes have limited host ranges, such as *Salmonella typhi* and *Salmonella paratyphi* A, linked to human infections, and *Salmonella gallinarum,* linked to poultry infections. Many serotypes have limited host ranges, such as *S. typhi* and *S. paratyphi A,* linked with human infections, and *S. gallinarum,* associated with poultry infections.

Salmonella serotypes Typhimurium and Heidelberg have a broad host range and can colonize and infect various animal hosts. Human infections of *S. typhimurium* are caused primarily by transmission-contaminated foods such as poultry items. Chicken eggs and egg products have been closely connected to *Salmonella enteritidis*. This could be linked to its apparent proclivity for infection and reproductive tissue invasion in sensitive chickens, as well as contamination of internal shell eggs.

Nonetheless, because there are so many cases of salmonellosis from non-poultry and non-food sources, such as domestic mammalian and reptile pets, any attempt to assign quantifiable rates to specific sources is futile. Bacteria may colonize and develop in the small intestine, penetrate and dwell in intestinal tissues, and cause an inflammatory response in the host when people eat a Salmonella infectious dose. *Salmonella* can enter the body through macrophages or other immune cells and go to lymph nodes in the liver, spleen, or mesenteric arteries.

11.17.7 *Shigella* Species

Shigella is spread through fecal–oral, feces, direct touch with people (fingers), food, flies, and inanimate items (fomites). More than two-thirds of all shigellosis cases occur in youngsters aged one to five who appear to use their lips to explore their surroundings. Intake of contaminated water is frequently cited as a method of shigella transmission. When people are crammed together, disease transmission and bacillary dysentery epidemics are intensified. Inadequate sanitation creates an environment for direct fecal–oral contamination, such as in daycare centers, custodial institutions, psychiatric hospitals, nursing homes, and mass displacement and gathering, such as in wartime refugee camps or political unrest.

In addition, those infected with the human immunodeficiency virus (HIV) might develop more severe and chronic types of shigellosis. Secondary attack rates among household contacts could be as high as 40% following primary case exposure. Shigellosis outbreaks appear to follow seasonal patterns in some areas, with

transmission peaking in arid regions like Egypt's hot, dry season. During times of water scarcity, it is primarily ascribed to dirty water use and poor personal cleanliness.

On the other hand, peak events occur in China and Thailand during the rainy season because of water-washed-based transmission after heavy rains. These represent the link between shigellosis and the unclean settings that encourage fecal transmission. Shigellosis is most common in developing countries during the summer months, when raw foods, fresh fruits and vegetables, and recreational facilities are most plentiful. Years later, the proportion of *S. flexneri, S. sunny, S. boydi,* and *S. dysenteriae* cases in developing countries was estimated to be 60% (mainly serotype 2a), 15%, 6%, and 6% (30% of *S. dysenteriae* cases were type 1), respectively; and 16% (predominantly serotype 3a), 77%, 2%, and 1% in developed countries, with nearly half of travelers registering incidents and returning travelers from (Kothary & Babu, 2001).

11.18 The Foodborne Vibrios

11.18.1 Vibrio vulnificus

Vibrio vulnificus is the opportunistic bacteria that cause foodborne illness and devastating infections in humans. Ingestion of raw or undercooked seafood, contamination of an open wound, or a sore contaminated with seawater or seafood drippings are all possible entrance points. *V. vulnificus* produces wound infections by entering existing or parallel wounds, ulcers, and insect bites, in addition to being foodborne. Exposure to seawater or shellfish leaking from a cut is nearly always the cause of these wounds. Puncture wounds from a bite from a marine animal (e.g., crabs, stingrays), stabbing or laceration wounds from the use of shellfish/fish cleaning equipment, lacerations from commercial or recreational use of fishing gear, rocks, or debris in or near seawater, or contamination of an existing wound or insect bite with seawater or shellfish drippings are all reported wound scenarios.

Puncture wounds from a bite from a marine animal (e.g., crabs, stingrays), stabbing or laceration wounds from the use of shellfish/fish cleaning equipment, lacerations from commercial or recreational use of fishing gear, rocks, or debris in or near seawater, or contamination of an existing wound or insect bite with seawater or shellfish drippings are all reported wound scenarios. Because *V. vulnificus* is associated with various seafood species, the health status is unknown. The human sickness produced by this organism and other marine vibrios could become a growing food safety concern. This problem is exacerbated by the fact that demand for seafood is increasing, and it can now only be fulfilled by aquaculture and marine fisheries sources harvesting seafood. These issues highlight the importance of monitoring systems to track the safety of our seafood (Kothary & Babu, 2001).

11.18.2 Vibrio parahaemolyticus

Most foods implicated with *V. parahaemolyticus* infections are seafood or similar foods, such as raw seafood, cooked seafood, fine seafood, and seafood. Tuna, shellfish (oysters, clams, etc.), crab, lobster, and shrimp were among the seafood used. In Japan, a majority of cases are caused by eating raw fish. However, this is not often the case in other countries, where cases are frequently caused by poor preparation, recontamination, or storage. In cases of food poisoning, foods that do not include marine elements may be contaminated by secondary contamination.

In Japan and the United States, *V. parahaemolyticus* food poisoning incidents are only reported during the summer. However, in Thailand, cases are reported all year. The seasonal change in the prevalence of infection may be linked to the epidemic strain's seasonal occurrence in the marine environment. While several reports demonstrate *V. parahaemolyticus* distribution in the aquatic environment in various world regions, epidemiologic research assessing the occurrence of virulent strains in both clinical specimens and the marine environment in an endemic context is rare (Parveen et al., 2008).

11.18.3 Vibrio cholerae

According to epidemiological data, fecally infected water is the predominant mode of cholera transmission. Food has recently been recognized as a life-threatening factor in cholera transmission. Seafood, particularly mussels, oysters, and shrimp, has been implicated when consumed uncooked, undercooked, or prepared with dirty water. While *V. cholerae* can be found in rice, fish, eggs, and milk, environmental studies in Bangladesh have found that most foods are not infected with the bacteria.

Cholera expanded through seaports and river ports during the seventh pandemic, particularly in Africa during the 1970s epidemics. Patients with active disease are regarded to be the predominant source of infection. Asymptomatic carriers may play a role in cholera transmission. However, this is not required. El Tor vibrios appear to live longer in the carrier form than Classical vibrios, but this difference is not epidemiologically significant. It has been demonstrated that *V. cholerae* O1 can survive in the aquatic environment without the help of zooplanktons and that *V. cholerae* is connected with a variety of aquatic flora, including water hyacinth duckweed and green algae. The blue–green algae have been discovered. *V. cholerae* is found in Anabaena variabilis, which serves as a reservoir. It has been demonstrated that *V. cholerae* O1 can survive in the aquatic environment without the help of zooplanktons and that V. cholerae is connected with a variety of aquatic flora, including water hyacinth duckweed and green algae. The blue–green algae have been discovered. *V. cholerae* is found in *Anabaena variabilis*, which serves as a reservoir.

Similarly, the recent isolation of toxigenic *V. cholerae* O1 from coastal waters in the United States and Australia, where cholera cases were unknown, suggests that these species have adapted to the aquatic environment as a natural microhabitat.

Although cholera is a watery illness, new evidence suggests that food, particularly seafood, plays an essential role in cholera transmission. Foods are likely to be fecally contaminated during preparation, primarily if the diseased food handlers work in an unsanitary setting. High moisture content, neutral or alkaline pH, low temperature, high organic content, and lack of other competing bacteria are physicochemical features of foods that promote *V. cholerae* O1 survival and development. Seafood, such as fish, shrimp, crabs, oysters, and clams, have been blamed for cholera epidemics in various nations, including the United States and Australia.

Dirty rice, millet gruel, and vegetables have been linked to epidemics. Cholera can be transmitted through various foods, including fruit (excluding sour fruit), chicken, meat, and dairy products. Food must be made and consumed in a sanitary environment free of feces to prevent foodborne cholera transmission hazards. To reduce foodborne cholera transmission, proper food preparation, storage, and reheating before eating and hand washing with clean water before and after defecation are beneficial health interventions (Sack et al., 2004; Parveen et al., 2008).

11.18.4 Yersinia enterocolitica

Effective health practices preventing foodborne cholera transmission include adequate food preparation, storage, reheating before eating, and hand washing with clean water before and after defecation. According to studies, the potential of *Yersinia enterocolitica* to thrive and develop in chemically contaminated foods under varied storage circumstances has been proven to be better at room temperature and refrigeration than at moderate temperatures. Cooked meals allow *Y. enterocolitica* to survive longer than raw foods, probably due to better nutrient availability and the presence of certain psychrotrophic bacteria, including non-pathogenic *Y. enterocolitica* strains. Bacterial growth in uncooked food can be inhibited. Within 24 h or 10 days, a viable number of *Y. enterocolitica* will increase by more than one million on cooked beef or pork at 25 °C, or 7 °C. Raw beef and pork grow at a slower rate. *Y. enterocolitica* can grow at refrigerator temperatures (soybean curd). Pork, cattle, lamb, chicken, and dairy products, particularly milk, butter, and ice cream, are all potential hosts for *Y. enterocolitica. Y. enterocolitica* can be found in various terrestrial and freshwater habitats, including soil, wetlands, lakes, rivers, wells, and streams, and can persist for long periods in soil, vegetation, streams, reservoirs, wells, and spring water. Infections with *Yersinia pseudotuberculosis* have been observed in various domesticated or caged animals, including horses, goats, deer, and monkeys. In two well-known *Yersinia* outbreaks, infection with human pseudotuberculosis has no known etiology. Untreated drinking water infected with wild animal feces was illegal (Fukushima et al., 2011).

11.18.5 Campylobacter

C. jejuni, C. colli, and *C. lari* have also polluted natural water surfaces. Consuming unchlorinated, contaminated water or eating food prepared with untreated or inadequately treated water might lead to infection.

In every corner of the globe, *Campylobacter* is one of the most common human bacterial causes of diarrhea. Approximately 80% of *Campylobacter* infections are thought to be caused by diet. Infection among young children is hyperendemic in developed countries. Domesticated animals (chickens, pigs, goats, horses, dogs, cats, and birds) are likely to get infected. Ingesting undercooked poultry, pork, and beef, unpasteurized milk, contaminated drinking water, and infected animal excrement can spread this virus to humans.

Campylobacter jejuni and *C. coli* invade domestic fowl with incredible ease. At least 60% of chickens sold in supermarkets are contaminated with *Campylobacter*, and broiler chickens are responsible for 50–70% of human infections in underdeveloped nations. The most common way to contract *Campylobacter jejuni* is by eating undercooked chicken. Red meat, on the other hand, can be contaminated. Infection can also be spread by eating barbecued pork or sausages. Infection can also be spread by eating raw or undercooked fish, shellfish, or mushrooms.

In the recent past, raw and unpasteurized milk was a common source of *Campylobacter* and was responsible for large foodborne outbreaks. However, outbreaks caused by raw milk have dropped significantly in developing nations due to the regular pasteurization of milk and improved public awareness (Acheson & Allos, 2001).

11.18.6 Escherichia coli

Foodborne infections, or infectious diseases spread by food, are a bothersome and sometimes life-threatening concern for millions of individuals. Drinking infected foods or beverages causes foodborne illness. Several different microorganisms, or disease-causing pathogens, can contaminate foods, resulting in various foodborne diseases. More than 250 distinct foodborne diseases have been documented, with the majority of them being infections caused by bacteria, viruses, and parasites.

The epidemiology of foodborne microbial illnesses has improved in the previous 10 years. It is ascribed not only to an increase in the human population's vulnerability to diseases or changes in lifestyle, such as more adventurous eating, fast food, and less time spent preparing food, but also to the introduction of newly known foodborne pathogens. Bowel infections are among the most prevalent infectious disorders in humans (Kaper et al., 2004). The gastrointestinal tract is a specific target for many foodborne viruses.

11.19 Aflatoxins and Aspergillus flavus

11.19.1 Aflatoxins

The aflatoxins are a collection of poisonous chemicals produced by structurally related *Aspergillus flavus, A. parasiticus*, and *A. nomius*. Some strains of these fungi can thrive on specific crops in the field and during storage under favorable temperature and humidity circumstances, resulting in aflatoxins formation.

Approximately 18 aflatoxins: aflatoxins (AF) B1, B2, G1, and G2 are naturally occurring aflatoxins, whereas the other 14 are animal-system metabolic products. Naturally occurring aflatoxins are found in various foods; AFB1 is usually the most prevalent in quantity and toxicity. *A. flavus* is the most frequent corn contaminant in temperate zones around the world, and it produces both AFB1 and AFB2. *A. parasiticus* is the most common peanut contaminant in tropical and subtropical areas, producing all four naturally occurring poisons. Aflatoxin M1 (AFM1) is a poisonous metabolite of AFB1 formed in mammals' livers. Because aflatoxins are very stable compounds, they can be detected in varying levels in finished foods depending on the manufacturing methods or procedures utilized. Aflatoxin levels in foods have been examined concerning various processing methods.

The occurrence of aflatoxins in food and feed is extensively documented in the literature, with the most widespread exposure happening in humidified and hot environments. Aflatoxins (except AFM1) have been discovered in corn and maize products, peanuts and peanut products, cotton, various tree nuts (e.g., Brazil, pistachio, almonds, pecans, and walnuts), copra and its products, edible seeds (e.g., watermelon, sunflower), wheat, sorghum, spices, rice, unrefined vegetable oils, figs, and dried fruits). They were sporadically detected in other materials at very low or negligible rates (Richard et al., 2003).

11.20 *Fusarium* and Fumonisins: Toxigenic *Fusarium* Species

11.20.1 Toxins Produced by *Fusarium* Species

Fusarium is a fungus that causes plant disease and is commonly found in soil. Because they infect plants in the field rather than in grain storage, *Fusarium* species infect cereal grains, known as "field fungus." As of 1996, more than 60 species of *Fusarium* have been identified in raw and cooked foods worldwide, with more than 100 secondary toxigenic metabolites characterized. Variations in mycotoxin-producing potential have been identified between strains within the same species. Detailed assessments of the various presence of the numerous mycotoxins produced by the *Fusarium* species have recently appeared in the literature. The most potent poisons to *Fusarium* include deoxynivalenol (vomitoxin), fumonisins, and zearalenone from human exposure and regulatory concern.

11.20.2 Other Molds and Mycotoxins

Most fungal species found in soil are susceptible to invasion by field crops. Many fungal species can infect growing plants and produce secondary poisonous compounds termed mycotoxins under ideal temperature and humidity conditions. Some fungal species can multiply as plants and grain kernels age, producing poisonous compounds that are not eliminated or destroyed during harvesting. Assume that harvested grains (crops) are not stored properly. In that instance, some fungus on the crops brought in from the field may continue to thrive and create mycotoxins, or they may eventually be replaced by fungi that may multiply under different storage circumstances and produce new mycotoxins. As a result, mycotoxins can infiltrate the food supply throughout preharvest and postharvest periods. Small amounts of these compounds in foods and feeds can be permissible, as long as the amounts are not considered detrimental to human and animal health.

Part of the goal of this chapter is to provide an overview of the significant mycotoxins that are causing significant public health problems at both the national and worldwide levels. Aflatoxin A, deoxynivalenol, fumonisins, patulin, and zearalenone are the most commonly regulated mycotoxins (Zinedine et al., 2007).

11.21 Foodborne Viruses

11.21.1 Caliciviruses

Caliciviruses are an enteric virus family that infects a variety of marine and terrestrial organisms. While there are three genogroups of human caliciviruses, there are two distinct physical and biological forms of human viruses: classic human caliciviruses and Norwalk-like viruses. These viruses are known as small round standardized viruses (SRSVs) in the literature. While classic calicivirus strains have been shown to induce gastroenteritis in adults, classical caliciviruses are primarily associated with self-limiting gastroenteritis in newborns and babies. Traditional caliciviruses account for just a small percentage of instances of infantile gastroenteritis. As a result, traditional caliciviruses are generally considered less medical and foodborne concern than the NLV.

Multiple genotypes are occasionally discovered during outbreak investigations. This common group infection goes undiscovered until an infected person contaminates a typical food or water source or fosters rapid human-to-human transmission through intimate contact with people in a closed or institutional setting. Contact with dirty linens, vomitus, excrement, aerosols, or fomites can direct person-to-person transmission in these conditions. NL disease outbreaks have been reported at banquets, geriatric institutions, mental units, emergency rooms, cafeterias, recreational lakes, swimming pools, dormitories, campgrounds, hotels, schools, restaurants, and cruise and navy ships (Scharff, 2012).

Although mussels are well known for spreading NL illness, other foods have also played a role in the disease's transmission. Viruses are commonly transmitted

through raw fruits and salads. Surface contamination of fruits and vegetables can occur due to irrigation or fertilization, harvesters or transporters, or food preparation contamination. Sick food handlers have contributed significantly to the spread of NL illness by contaminating foods with unwashed hands or exposing objects to unsanitary surfaces. One illness outbreak in Newfoundland was caused by mixing potato salad in a sink where a restaurant worker had vomited the day before. According to epidemiological studies, many NL disease outbreaks are caused by a general lack of awareness among some food handlers due to inadequate sanitation and hygiene training.

11.21.2 Hepatitis

Hepatitis A and E are the only hepatitis viruses spread through the feces–oral route; the others are primarily transmitted through parenteral routes. Hepatitis A virus (HAV) is transmitted chiefly through fecal–oral, direct person-to-person contact, and contaminated food and drink. For example, HAV is sexually transmitted, particularly among homosexuals, and has been documented on multiple occasions via blood products, particularly factor VIII. The hepatitis E virus is spread through contaminated food and infected water. Except for vertical transmission, there is no known parenteral transmission of the hepatitis E virus.

11.21.3 Hepatitis A Virus

Virus-related disease burden varies substantially around the globe and is directly influenced by socioeconomic growth and sanitation rates. The disease is more prevalent in small and high endemic countries; in the former, infection and disease are uncommon due to low virus circulation in the population due to adequate cleanliness and sanitation. In countries with high endemicity, on the other hand, the virus circulates widely. As a result, infection is common in young children, often asymptomatic and unnoticed, contributing to long-term safety. This is true in areas of intermediate endemicity, or countries with the highest disease rates, where developed and developing people coexist (Mesquita et al., 2011).

11.21.4 Hepatitis E Virus

The development of hepatitis A and B viral serological tests revealed that neither virus was responsible for most hepatitis cases. This resulted in the coining of the term non-A, non-B hepatitis, which had two epidemiologically distinct variants: a parenteral type with transmission routes similar to parenterally transmitted hepatitis B virus infection and an enterically distributed non-A, non-B hepatitis with transmission routes similar to hepatitis A virus infection.

On the Asian continent, enterically non-A transmitted non-B hepatitis was responsible for enormous epidemics of watery hepatitis. Serological investigations revealed that the etiologic culprit was a new virus unrelated to hepatitis A. The infection was significantly more widespread than the geographic location of the significant waterborne epidemics suggested. In addition to outbreaks, the virus, commonly known as the hepatitis E virus (HEV), caused sporadic instances of hepatitis among indigenous populations and imported cases from travelers in developing countries.

The virus is present at lower concentrations in the blood, up to 100,000 particles per mL, and the viraemic stage is mainly associated with the time when the virus is expelled in the feces. It can now explain why the number of blood-borne hepatitis A infections is increasing. It has not been identified whether the virus secreted in saliva has a function in transmitting the hepatitis A virus. No evidence is found that sharing dining utensils, cigarettes, or kissing can spread hepatitis A infection.

The fecal–oral route is the most common hepatitis A virus transmission, with person-to-person transmission being the most fundamental mode of virus transmission. The findings of infection rates among patient household contacts and children in the surroundings of daycare centers exemplified this point.

The virus is hardy, and it can survive in water and food for up to 10 months. As a result, foodborne outbreaks (common-source epidemics) are becoming more widespread. Food handler contamination—generally a food handler in the disease's asymptomatic pre-icteric stage—can cause foodborne outbreaks. It will mostly show up in those who do not wash their hands properly and handle raw foods like salads, sandwiches, and cold meats. Foods may be polluted, particularly shellfish from water near sewage outlets or vegetables fertilized with untreated human night soil. Drinking or bathing in filthy fecal water can potentially result in severe waterborne outbreaks.

There is no doubt that improvements in clean water and sanitation and personal and public hygiene have considerably impacted the global epidemiology of hepatitis A infection. Endemic transmission has ceased in several highly developed countries, such as Scandinavia, Germany, Switzerland, and Japan. Imports or travelers returning from underdeveloped nations account for most illnesses in these countries. HAV antibodies are present in less than 10% of blood donors in these countries. In most other affluent countries, endemic viral transmission is also growing, resulting in increased vulnerability in adults and higher morbidity and mortality (Scharff, 2012).

11.22 New Technologies for Microbial Detection

Some of the new techniques for microbial detection are ELISA, Lateral Flow Immunoassay (LFIA), colloidal gold label-paper-based dipstick, and quantum dots labels. ELISA has better sensitivity and lesser time consuming compared to culture-based technique. The LFIA technique is a simple, rapid, feasible, and potent foodborne pathogen detector. Other novel techniques to detect the antibodies are

optical strategies—gold nanoparticles combined with Raman spectrum molecules, label-free method for pathogen detection, and electrochemical strategies—high sensitivity, feasible, and fast compared to optical strategies (Jyoti et al., 2011).

11.23 Microbial Biofilms and Food Safety

Biofilm is formed by surplus microbial communities based on food fabricating conditions and the colonizing species. Combined biofilms possess higher resistance to the anti-biofilm compounds like disinfectants and biocides (Giaouris et al., 2015). Biofilms are made of extracellular matrices, and those matrices are formed of polysaccharides. These matrices can attach to hard surfaces like equipment in food industries. Overall, biofilm formation helps the microorganism associated with the food industry resists chemical protection and physical and mechanical resistance (Wingender et al., 2016). The most crucial part of biofilm in the food industry is that most microbes that form biofilms in the food industry are human pathogens, and they get attached to the surfaces of food industry equipment (Colagiorgi et al., 2017).

11.24 Biofilm in the Food Industry and Health Defects

Foodborne illnesses depend on the biofilm formation on the surface of the food industry. The occurrence of biofilm changes the type of the food industry, such as pipes, water, animal carcasses, dispensing tubes, and packing materials (Camargo et al., 2017). The five critical foodborne pathogens that can form biofilm are *Bacillus cereus, Escherichia coli, Listeria monocytogenes, Salmonella enterica, and Staphylococcus aureus*.

11.25 Techniques for Controlling the Formation of Biofilm in the Food Industry

Biofilm detection is incompatible with the classic culture-based approach. It takes time, and few bacteria are viable but not culturable (VBNC) because they have limited metabolic activity. The biofilm produced by VBNC-PCR amplification is used to detect it. Biofilms can also be detected via metagenomics and metatranscriptomics. The previous approach of biofilm detection is being replaced with an alternate method for improved efficiency due to the time consuming and higher reactive equipment.

Furthermore, these old technologies are unsuitable for use in the food business. As a result, online monitoring is employed. Electrochemical detection and vibrational detection are two different approaches. Controlling biofilm in the food business can be done in various ways. These approaches include cleaning and disinfection, clean-in-place, chemical-based controls, ultrasonication, enzymatic disruption, phage, and hurdle technology.

11.26 Food Chain—Safety and Quality

As the distance between the producer and the consumer has grown due to globalization, one of the significant issues is supplying quality food without contamination. Food traceability is an essential criterion in ensuring food safety and quality. Globalization has posed a challenge not only to food safety but also to food quality. Consumers want to know everything there is to know about retailing history. Traceability is crucial in this case. Traceability is based on unit identification, product tracking, and data storage principles. The importance of food in the country cannot be overstated. Food production is a global phenomenon that influences social, economic, and environmental factors. Assume that any food-related difficulties will impact the country's socioeconomic condition. It has an impact on the environment because of increased transportation carbon labeling. Food providers have recently relied on two techniques to assure global food safety: managing the food chain by adhering to regulations and standards and regulating logistics through traceability. FAO and WHO have a significant role in food safety, FDA legislation, and International Standardization Organizations (ISO) participating in the international food trade. Analyzing the risk Hazard Analysis and Critical Control Points (HACCP) is essential for maintaining food safety standards. After HACCP, good manufacturing procedures are implemented. Many rules and regulations were in place to assure food quality and safety across the food supply chain (Aung & Chang, 2014).

11.27 Methodologies for Improved Quality Control Assessment of Food Products

In these decades of higher technological development, the improvement in the food analysis techniques will have greater insight. Analysts and chemists will have greater value in using novel analytical tools. Traditional tools are more time consuming and require skilled labor, so the demand for novel analytical tools has increased. Traditional high-performance liquid chromatography system analyzes the hydrophobic and hydrophilic constituents in reversed phase and the normal phase. However, the conventional method is less susceptible and time consuming. So, to analyze the higher and lower molecular weight analytes, novel polymer-based stationary phase is introduced. Capillary electrophoresis that discrete the analyte based on the molecular weight and ionic moiety is introduced as an alternative. There is a higher technique named capillary electrochromatography which is better than HPLC and capillary electrophoresis (Huck et al., 2000). Spectroscopic strategies at this phase of the systematic technique either incorporate mass spectrometry (MS) or vibrational spectroscopy individually. Laser desorption ionization determines higher molecular weight samples (Feuerstein et al., 2006). Vibrational spectroscopy is used for detection.

11.28 New Developments in Food Quality Assurance and Safety Control

The essential aspects of food are maintaining food safety and food quality. There were numerous techniques to detect food safety primarily. We rely upon the traditional method, which is time consuming and high cost. Then after several findings in the 1960s, an electronic nose was introduced, which will mimic the human olfactory system with its sensors. There were many factors for accepting and rejecting the food and one among the important factor is the aroma, the smell of the food. Aroma plays a crucial role in food. Aroma acts as a biomarker to test food contamination. Few biosensors are listed in Table 11.6.

The food industry is benefiting from significant progress in developing enzymatic biosensors with various transduction systems that can be used in food safety, quality, and process control; studies are primarily focused on determining the composition and contamination of raw materials and processed foods. The food industry is benefiting from significant progress in developing enzymatic biosensors with various transduction systems that can be used in food safety, quality, and process control; studies are primarily focused on determining the composition and contamination of raw materials and processed foods.

11.29 Conclusion

Intense research is ongoing to find a solution to the global food crisis. Enzyme assimilation is well known in the food processing industry. This chapter addresses the fundamentals of enzymes and the sources of various enzymes and their applications in the food industry. The intrinsic features of microbial enzymes have rocketed them to renown due to their simplicity of synthesis and yield manipulation in laboratory settings. Enzymes in food processing convert physiochemical processes to environmentally friendly processes, improve product biodegradability, and reduce energy usage. The food processing industry could benefit from a new approach for enzyme uses. Foodborne infections also harm human health and create

Table 11.6 E-nose in accordance with food (Rayappan et al., 2017)

Food	E-nose
Meat	NST 3210 emission analyzer, along with the ANN prediction algorithm, Heracles II electronic nose, Artificial Olfactory System (AOS) ISE Nose 2000, Total Volatile Basic Nitrogen (TVB-N), Cyranose-320
Milk	BH-114: Bloodhound Sensors Ltd., Applied Sensor 3320
Fish and seafood	Colorimetric array of sensors
Fruits and vegetables	FOX 4000
Adulterants	Pen 2

financial losses. As a result, rapid diagnosis of foodborne pathogens and the application of countermeasures are critical. Preventing foodborne illness requires quick detection of the pathogen. Enzymes can be used as biosensors to detect food adulteration and contamination for faster food product screening. The dietary substrate determines the enzyme biosensor's characteristics. Molecular diagnostics and serological methods are two new technologies for food sector monitoring.

While there are various methods for detecting food safety, the most common is the time consuming and expensive method. The electronic nose stimulates the olfactory system of humans using sensors, which is a breakthrough. Many aspects influence whether or not a person accepts or rejects food. One of the most crucial factors is the aroma of the dish. In food, the aroma is significant. The aroma as a biomarker can detect the contamination in food. With the expanding dimensions of the food industry, several critical procedures such as food preservation, monitoring, adulteration, and foodborne diseases are critical to the industry's overall performance. Furthermore, enzymes perform multiple roles in all processes, one of which is the constant monitoring of food and food products.

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Enzymes in Flavor Development and Food Preservation

12

Fataneh Hashempour-Baltork and Parastou Farshi

Abstract

Flavors and fragrances have extensive application in the pharmaceutical, cosmetic, feed, chemical, and food sectors. Most of the flavor compounds on the market are formed from animal and plant sources, through chemical synthesis or extraction; however, a prompt alteration for the bio-production and use of flavor compounds with (micro) biological origin—bioflavors—is occurring. Enzymatic flavor production is commonly preferred over fermentation due to their high yields of production. On the other hand, it is undeniable that food is important for mankind and there is not any way of living without eating. Although the need to feed has not changed during the years, the way of food preservation has seen a lot of changes in today's global market which is highly competitive, and it is desirable to use the cheapest method of food preservation, such as food additives which can be a good choice. Among food additives, enzymatic process for longitude shelf life is one of the suitable choices. Using of bioprocesses involving enzymes in food processing can decrease the antinutritive factors and toxins of the by-products, besides solving the environmental issues, and increasing their consumer acceptance by enhancing their nutritive value. Although, it is important to select the proper fermentative processing technologies.

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A. Dutt Tripathi et al. (eds.), *Novel Food Grade Enzymes*,
https://doi.org/10.1007/978-981-19-1288-7_12

Keywords

Enzyme · Flavor · Food preservation · Shelf life · Fermentation · Bioflavor · Ecofriendly

12.1 Enzyme in Flavor Development

12.1.1 Introduction

In the modern food industry, different types of enzymes have been applied for the synthesis of important food additives, food properties, and development of food production processes. Flavor and fragrances have found wide application in the food, feed, cosmetic, chemical and pharmaceutical sectors. Most of the flavor compounds in the market are formed from animal and plant sources, which takes place through chemical synthesis or extraction; however, there is a quick alteration for the bio-production and use of flavor compounds with (micro) biological origin, namely, bioflavors. Chemical synthesis usually results in an unfavorable and environmentally unfriendly production process of racemic mixture compounds. Moreover, the consumer has established a "chemophobia"-approach for synthetic or chemical compounds, specifically for food and products being used in the home. The definition of natural flavors by EC Flavor Directive (88/388/EEC), is "flavoring ingredients or formulations which are achieved by suitable enzymatic, microbiological, or physical processes from plant or animal origin materials" (Vandamme & Soetaert, 2002). Until now, animal and plant sources were considered to be essential sources of bioflavors; however, the amount of these compounds is usually very low in these sources or they are only present in exotic (plant) species, which results in their expensive formulation and isolation (Carocho et al., 2015). However, nowadays biotechnology is considered as a preferred method with the advantage of "natural" labeling that can draw consumers' attention. De novo microbial production (fermentation) and enzymatic synthesis are two biotechnological ways to obtain bioflavors. Enzymatic flavor production is commonly preferred over fermentation due to their high yields of production (Asunción Longo & Sanromán, 2005; Vandamme & Soetaert, 2002).

Enzyme immobilization and coenzyme regeneration techniques can lead to an effective and exclusive biocatalytic processes for flavor synthesis (Kragl et al., 1996). The benzaldehyde with almond and cherry taste can be made from the cyanogenic glycoside amygdalin, which is present in almond meal and cherry kernels, by mandelonitrile lyase and b-glucosidase enzymes (Fig. 12.1). An industrial example for this is L-menthol production which is the major ingredient present in peppermint oil (Fig. 12.2) (Menzel & Schreier, 2007).

Recently, synthesis of flavor compounds by biotechnological processes has gained growing importance in the food industry. By considering the growing market, and the growing public concern for the total wholesomeness and chemical safety of food ingredients, researches on bioprocesses and inventing new products are also

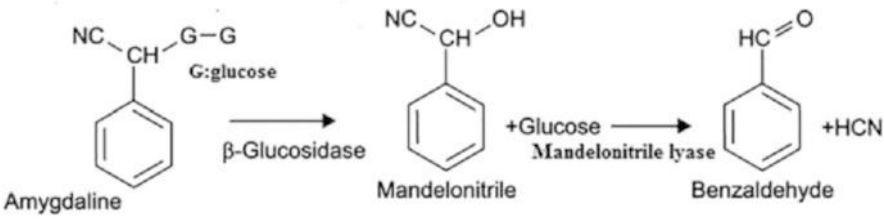

Fig. 12.1 Enzymatic route to benzaldehyde

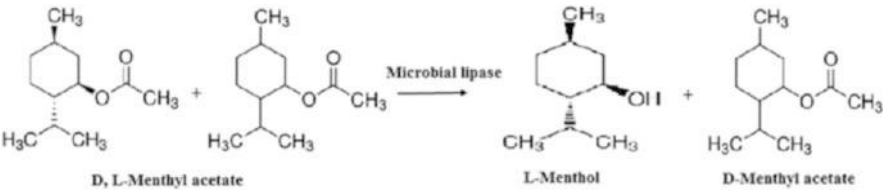

Fig. 12.2 Stereoselective bioconversion of DL-methyl acetate into L-menthol

increasing (Basset, 1990). In 2017, the total global flavors and fragrances market got to US$28.2 billion, which means an increase of 4.6% rather than the previous year, and it is estimated to increase at 4.9% average annual rate to get to approximately US$36 billion in 2022 (Consultants, 2018). A wide range of options are provided by enzymes, for food flavor production. The specificity of the enzymes and whether they are being used through whole-cell or cell-free systems support the production of chemicals that are hard to synthesize; also their stereoselectivity is considered as a principal advantage for the food industry where a particular optical conformation can affect flavor properties. Application of enzymes as food additives can produce or liberate flavor from precursors, as well as correcting off-flavors produced by specific naturally occurring compounds, or those that are produced during processing (Bigelis, 1992). Besides intentional addition of enzymes to the food, they can also be inherent to the food or may originate from microbial sources, or may come from contamination.

In this chapter, several ways that enzymes can have effect on flavor are reviewed, showing examples of actual research, their potential applications, as well as examples of the fundamentals of biosynthesis and the market potential of some products. In some instances, processes and reactions known for two decades are stated and they are updated with recent developments, such as reaction in organic media or whole-cell biocatalysts.

12.1.2 Enzymatic Synthesis of Flavor Compounds

12.1.2.1 Lyases

Alliinases

Alliinases [S-alk(en)yl-L-cysteine sulfoxide lyase; alliin lyase; EC 4.4.1.4] remove S-alk(en)yl-L-cysteine sulfoxides, and in this way they produce pyruvate, ammonia, and thiosulfinates, (Fig. 12.3). Pyridoxal-5′-phosphate (PALP) is needed by this enzyme as a cofactor.

The strong aroma and flavor of the most of the species of the Amaryllidaceae family are because of the activity of this enzyme. Among other lyases, this enzyme is detached from the substrate in the intact tissue, and the reaction occurs upon breakdown of cells. The first alliinase to be explained was from garlic bulbs (Tishel & Mazelis, 1966). Until now alliinases have been purified from onion, Welsh onion, Chinese chive, garlic, and leek (Whitaker et al., 2002). Despite that they are from the same genus, their specific alliinases are different in physicochemical properties. It is many years that garlic and onion are being used by several cultures for different purposes. In spite of having therapeutic and nutritional importance in diets, they are being consumed around the world due to their unique flavor and their properties, such as enhancing the flavors of other foods. Diallylthiosulfinate (allicin) is the major sulfur compound in garlic that provides its distinctive aroma. This thiosulfinate results from the elimination reaction by alliinase which is done on S-2-propenyl-L-cysteine sulfoxide (alliin), via the intermediate 2-propenesulfenic acid. There are three flavor precursors in onions, such as S-methyl-L-cysteine sulfoxide, S-propyl-L-cysteine sulfoxide, and S-1-propenyl-L-cysteine sulfoxide. Different thiosulfinates made by the activity of alliinase on the mentioned substrates impart distinctive onion flavors, based on their concentrations (Masuda et al., 2012). Other *Allium spp.* have diverse combinations of these substrates in addition to S-2-propenyl-L-cysteine sulfoxide; this compound is not present in onion (Masuda et al., 2012). It is stated that the lachrymatory factor in onion (syn-propanethial-S-oxide) is originated from S-1-propenyl-L-cysteine sulfoxide (Jayathilaka et al., 2014).

Alliinases do the primary reaction which is responsible of producing distinctive aromas and flavors in vegetables of the *Allium* genus. The maintenance of full enzymatic flavor is the principal challenge of food processors who deals with

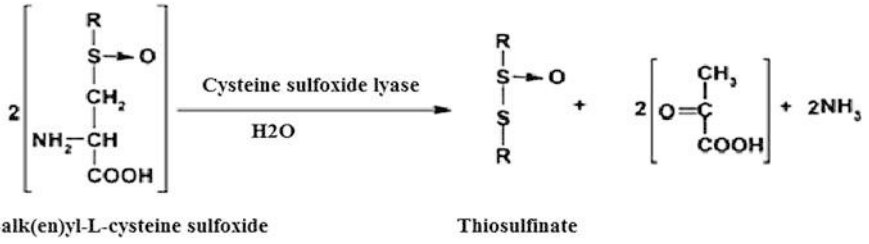

Fig. 12.3 Alliinase general reaction

onion and garlic. Some preharvest and postharvest practices, such as environmental factors influencing the flavor strength, including growing temperature, water supply, and sulfur content of soil, are monitored in alliaceous foods to enhance their flavor potential (Kamenetsky, 2007). Flavor potential of onions goes up with storage of its bulb for a few months (Petropoulos et al., 2017). Nearly, half of the S-1-propenyl-L-cysteine sulfoxide is bound as a peptide, L-glutamyl-S-allyl-L-cysteine sulfoxide, in nature. It has been stated that the substrate is liberated by the activity of glutamyl transferase. The most part of the flavor potential of the onion is lost during dehydration, which is due to the loss of the substrate more than the enzyme. In the other word 30% of the alliinase substrate is changed to cyclo alliin during the primary phases of dehydration.

Cyclo alliin and the S-alkyl-L-cysteines are not substrates for alliinase. The altered onion powder includes only one-third of the onion aroma of fresh onion juice (Ramírez & Whitaker, 1999). Meticulous consideration of the speed of drying is critical throughout the design of the dehydration process to diminish the substrate loss; process with longer time normally leads to better yields. The activity of alliinase can also lead to the development of unnecessary pigments in alliaceous vegetables, such as pink pigments in onions and leeks, and green pigment in garlics (Whitaker et al., 2002). Despite the ability of alliinase to catalyze the preliminary reaction and thiopropanal-S-oxide formation (in onion), the formation of pigment is dependent on the concentration of carbonyl and may differ from pink to red based on the composition.

Cystine Lyases in Plants

Cystine lyases (cystathionine L-homocysteine lyase; EC 4.4.1.8) is able to cleave L-cystine through generating pyruvate, ammonia, and thiocysteine or cysteine persulfide (Fig. 12.4). The substrate for this enzyme is not exactly determined. Cysteine lyase and cystathionase are both found in fungi and bacteria. However, cystine lyase does not consider cystathionine as a substrate, in plants. The activity of this enzyme was first identified in plants by Tishel and Mazelis (1966). They studied the capability of homogenates of cabbage leaves for degrading L-cystine (not L-cystathionine), to pyruvate. Also it is stated that this enzyme needs pyridoxal-5′ phosphate (PALP) as a cofactor.

Cystine lyases have importance for food quality. These enzymes catalyze the preliminary reaction in important vegetables of the Brassica genus, producing distinctive aromas and flavors. Cruciferous vegetables, such as cabbage, cauliflower, Brussels sprouts, and broccoli, have specific sulfurous flavors and aromas which

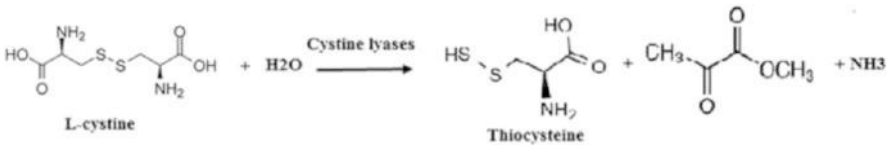

Fig. 12.4 General reaction of cysteine lyase

results from the activity of the mentioned enzymes and subsequently tissue softening happens in these vegetables. Cystine lyase was identified to be the key enzyme in broccoli, and its activity results in off-aroma decay (Barrett et al., 2000).

In a study, addition of the purified cystine to blanched broccoli was observed and the enzyme-added broccoli could produce the aroma characteristic of the unblanched broccoli. Chin and Lindsay (1993) determined the sulfur compounds produced in damaged tissues of cabbage as dimethyl disulfide (DMDS), dimethyl trisulfide (DMTS), and methanethiol (MT). Although these compounds are specific to brassica vegetables, they are unfavorable for the consumer. These unpleasant sulfurous odors make people to avoid consuming them, especially in the use of modified atmospheres. The mechanisms of the formation of these compounds in cabbage, which results from the activity of cystine or cystine sulfoxide lyase, were clarified (Chin & Lindsay, 1994a). Researchers could not find significant differences between the DMDS formation in anaerobic and aerobic circumstances; however, DMTS was only identified in anaerobic conditions. Different treatments, such as soaking the vegetable in caraway (Carum carvi) seed extract, ascorbic acid (500 ppm), phosphoric acid (0.1 M), sodium hydroxide (0.01 M), and tertiary butylhydroquinone (TBHQ), have been done to decrease the levels of these undesirable volatiles in broccoli stored in modified atmosphere condition (Chin & Lindsay, 1994b). All of the mentioned treatments could decrease the intensity of sulfurous aromas, in which alkaline treatment had the highest effect that could suppress the formation of DMDS. Obenland and Aung (1996) discovered that the formation of MT and DMDS in an intact broccoli flowerets can be nearly removed by infiltration of hydroxylamine which is an inhibitor of cystine lyase, and can be increased 2.8 times by infiltration of S-methyl cysteine sulfoxide before the anaerobiosis.

12.1.2.2 Isomerase

Xylose (Glucose) Isomerase

High-Fructose Corn Syrup (HFCS) is a combination of fructose and glucose made from cornstarch (Parker et al., 2010). HFCSs have extensive use in food industry and the most substantial application is their addition to soft drinks as a sweetener where they substitute cane and/or beet sugar. HFCs have 10–20% lower price than sucrose, in the same levels, and have less calorie due to the lower absorption of fructose. Glucose and fructose in HFCS have better solubility rather than sucrose and this accounts for their higher advantage to sucrose. Therefore, they have lesser affinity for crystallization in a broad range of food products. This feature has made them suitable compounds for application in different products, such as meat products, ice cream, canned products, confectionery, bakery, jam and jellies, sauces, and pickles (Parker et al., 2010).

The process for producing HFCSs includes three continuous enzymatic steps. Starch which is primarily originated from corn is used as the raw material in this process. Starch is consisted of amylose (linear) and amylopectin (branched) which are both polymers of D-glucose. The glucose moieties are linked by 1,4-glycosidic

bonds in amylose and the main chain of amylopectin. However, the branches are linked by 1,6-glycosidic bonds.

The starch which is produced from corn is exposed to liquefaction, in the first step of enzymatic process, by application of a thermostable bacterial amylase (EC 3.2.1.1) from *Bacillus spp,* such as *B. stearothermophilus* and *B. licheniformis*. In this step, enzyme is able to produce a dextrin solution with a dextrose equivalent (DE) of 10 by cleaving the 1,4 linkages in an endo fashion.

In the second step, other enzyme named as glucoamylase (amyloglucosidase; EC 3.2.1.3) releases single glucose moieties from the nonreducing end by hydrolyzing the dextrins in an exo fashion.

Glucoamylase shows a higher activity for the 1,4-glycosidic bonds rather than the 1,6-branch bonds. Moreover, in some situations a debranching enzyme, such as pullulanase (EC 3.2.1.41), is also used and the produced glucose solution has a DE of 95.

In the next step of enzymatic process, other enzyme named as glucose isomerase catalyzes the conversion of glucose into fructose until a concentration of 42% (molar fraction based on total sugars). This reaction is exclusive because it is taking place in a column reactor using immobilized form of the enzyme. Glucose isomerase was one of the earliest enzymes used for this purpose on an industrial scale. This process is done by encapsulating the enzyme in a packed-bed column reactor with 1- to 2-mm particles (Ruiz-Matute et al., 2010). The diameter and height of the columns may be 1.5 m in and 4–5 m, respectively. This system has a high yield which produces 500,000 kg fructose (dry weight) per day by 4000 kg enzyme. High-glucose syrup with DE ¼ 95, resulted from previous glucoamylase reaction with 45% solids and pH adjusted to 7.5, is continuously pumped through the column. Several factors have effect on the temperature and flow rate of the system. The flow rate should be suitable to provide enough residence time for production of 42% fructose. Low flow rates give rise to the risk of microbial contamination, also extremely high rates do not provide enough conversion and result in channeling through the support material. Temperatures below 55–60 °C increase the syrup's viscosity and increase the risk of microbial contamination. However, higher temperatures decrease the stability of the enzyme and results in the production of unfavorable by-products via chemical reactions such as Maillard reactions.

Practically, temperatures of 55–60 °C are being used. Regulation of the glucose stream's flow rate can compensate for the enzyme's inactivation to maintain the required conversion. These processes can continue for several weeks, under the mentioned circumstances until the activity of the enzyme has become too low. After getting to that point, the columns are disassembled and packed again with a batch of fresh biocatalyst. The final HFCS is consisted of sugars, such as oligosaccharides (up to 4%), fructose (42%), and glucose (54%) (Parker et al., 2010; Ruiz-Matute et al., 2010).

12.1.2.3 Oxidoreductases

Oxidative and reductive processes play a significant role in foods. They affect many aspects of food stuffs, such as taste, shelf life, texture, nutritional value, and

appearance. Both nonenzymatic and enzymatic redox processes are included. Sometimes processes lead to detrimental effects, such as textural problems, off-flavor, and decreased shelf life. In some cases, also, they provide positive effects to the final aroma, a better texture, a more desired appearance, or an improved shelf life. It is very important to control redox behavior throughout all stages of processing and storage, in food systems. Redox reactions in foods were controlled primarily by addition of antioxidants or chemicals, designing air-tight packaging materials, cautiously choosing raw materials, or by adjusting process situations, until now. However, there is not much paid attention to redox reactions in foods by the addition of oxidoreductases or by modifying the profile or changing the content of oxidoreductases in raw materials of foods by genetic implements. Apparently, the main reason for this little attention for using oxidoreductases in foods is that it is still not possible to produce most of the enzymes, cost-effectively. Moreover, general concerns about the application of recombinant enzymes in foodstuffs are indicating their commercial introduction. Various oxidoreductases are included in the in situ or in vivo biogenesis of appropriate aroma compounds, in vitro production of flavors and top notes, and in the endogenous development of off-flavors. The present applications of oxidoreductases for controlling the taste of food products are provided below**:**

Lipoxygenases

Lipoxygenase (LOX), which was previously known as carotene oxidase or lipoxidase, is a dioxygenase containing iron, which catalyzes the oxidation of polyunsaturated fatty acids comprising of cis,cis-1,4-pentadiene groups (arachidonic, linolenic, and linoleic acids) to the equivalent conjugated cis-, trans-dienoic monohydroperoxides. Moreover, LOX has a broad range of substrates which are phenolic compounds (Markus et al., 1991); also it is able to oxidize other substrates rather than the actual substrate. This process is identified as co-oxidation and involves compounds, such as polyphenols and carotenoids. LOX is one of the endogenous enzymes that plays a significant role in off-flavor formation and flavor generation and in almost all food products it is originated from raw materials of plant. Based on the type of food product and final concentration, a flavor compound can be a desirable aroma component or an off-flavor at higher concentrations. For instance, alcohols and C6-aldehydes derived from oxidation of (poly)unsaturated fatty acids, which are catalyzed by the LOX, mostly have a positive impact on the aroma profile (such as in wines and juices), but in other products, such as beverages (e.g., beer), does not have desirable effect on flavor. Similarly, endogenous LOX is known to have impact on the bread flavor due to generating carbonyl compounds in dough systems (Pico et al., 2015).

The C6 compounds' formation involves the consecutive action of four enzymes, including two redox enzymes, such as a LOX, and an acylhydrolase, a yeast-derived alcohol dehydrogenase, and a hydroperoxide lyase (Whitaker et al., 2002).

Normally, the formation of off-flavor is inhibited by the means of LOX isoenzyme-deficient crop variants, by genetic tools or by screening or controlling the oxygen levels during processing, in addition to eliminating the putrid oxidation

products subsequently (Mandal, 2012). Physical techniques, such as adsorption, or enzymatically altering the undesired alcohols or aldehydes to their equivalent carboxylic acid which are less flavorful, can be good ways to deodorize the off-flavors. This can be done by aldehyde dehydrogenase/oxidase or alcohol oxidase. The application of redox enzymes for this purpose has been proved for different food products, such as fish oil, cream, noodles, soybean products, cooked rice, and margarine (Raghuvanshi & Bisht, 2010; Antrim & Taylor, 1990).

Additionally, LOX is mostly applied to dairy products and beverages to modify their flavor profile for in vitro production of numerous natural top note flavors. Examples of these applications can be the conversion of polyunsaturated fatty acids to delta-decalactone (butter flavor) (Tunick, 2007) or to several short to medium-chain aldehydes/alcohols (Schwab et al., 2008; Scrimgeour et al., 2005). A well-known flavoring derived from fatty acid (FA), amdaldehydes/alcohols, comprises the above-mentioned C5 and C6 compounds, (E3, Z5, Z8)-undecatetraene (seaweed), 1-octen-3-one (field mushroom), (E3, E5)-undecatriene (balsamic), (E2, E6)-nonadienal (cucumber), and (Z5)-octadien-3-one (geranium leaves).

The regioselectivity and unsaturation degree of the LOX determines the formation of various hydroperoxy compounds, from which consequently the mentioned compounds can be derived by enzymatic reactions. Linolenic acid is being used as a substrate for (Z3)-hexenol; however, for most of the other alcohols/aldehydes, higher unsaturated fatty acids are needed. (Z3)-hexenol production has lately been industrialized by means of plant homogenates, such as green peppers and alfalfa sprouts, which are pretty rich in hydroperoxide lyase enzyme which is needed to cleave the hydroperoxide fatty acid to more little fragments.

Reductive enzymatic power of baker's yeast was responsible to transform (Z3)-hexenal to (Z3)-hexenol (Ricard, 1991). After the linoleic acid hydroperoxide formation, a unique strategy is needed to be followed for the production of lactones. It includes the fermentative oxidation of the hydroperoxide intermediate by the yeast *Pichia etchellsii*, and the consequent cyclization of 5-hydroxydecanoic acid to the equivalent S(-)-decalactone (Laane et al., 1997).

Moreover, there are relatively extensive spectrum of phenolic compounds that LOXs accept as substrates. Conversions of coniferyl benzoate and isoeugenol from Siam resin into vanillin is within the interest of flavor industry. Presently, the industrialization of these biotransformations is hindered due to the fact that isoeugenol is not easily accessible and that coniferyl benzoate is hard to work within a reactor (Priefert et al., 2001). Other reactions of LOX which are well known involve the co-oxidation reaction of carotenoids to ionone. Also it is stated that LOX and alpha-amylase combination can generate a toasted or cookie-like flavor in starchy materials (Desjardins et al., 1997).

Alcohol Oxidases and Dehydrogenases

Generally, aldehydes are considered as stronger flavor compounds rather than their alcoholic equivalents. Thus, alcohol oxidases are the enzymes which are of interest for the in vitro production of flavoring compounds in beverages. Enzymes, such as methanol oxidase from *Candida, Pichia,* and *Hansenula,* are good examples of

alcohol oxidases that are used for the formation of natural acetaldehyde from ethanol (Lopez-Gallego et al., 2007). This enzyme is produced throughout growth on methanol. Phase cells are collected and incubated with ethanol, at the final stage of the logarithmic growth. Therefore, 1.5% natural acetaldehyde concentrations can be reached, that can be further concentrated to the preferred application level. The substrate specificity of the alcohol oxidase is different, in various yeasts. Hence, this method can also be used to transform other alcohols, to their equivalent aldehyde. Therefore, dehydrogenases can be used as substitutes to alcohol oxidases. However, there is a limitation to use these dehydrogenases, because they need the costly cofactor $NAD(P)^+$instead of oxygen which is cheap, as an electron acceptor. Although there are several designed complex NAD(P) cofactor stimulating systems and significant cost reductions, it is obvious that oxidases are preferred in industrial applications to their dehydrogenase equivalents. The application of dehydrogenases for food purposes is limited to whole-cell transformations. There is a special kind of alcohol oxidase, namely, vanillyl alcohol oxidase (VAO) which is derived from *Penicillium simplicissimum*. Recently, it has been indicated that this enzyme, which contains flavin and is relatively stable, has an extremely wide substrate specificity and can convert para-substituted phenols into flavoring compounds or flavor precursors (Ewing et al., 2018). In addition to the natural coniferyl alcohol and vanillin, allylphenols and various vinylphenols, such as para-vinylguaiacol, can be generated from cheap raw materials and oxygen (as an electron acceptor). VAO can also be used for the production of building blocks of flavor. VAO can also be applied to a natural mixture of phenolic compounds to enrich foods or flavor preparations with a variety of aldehydic compounds and vinylic/allylic.

Sulfhydryl Oxidases

Formation of disulfide bonds from (protein) thiols is catalyzed by sulfhydryl oxidase. This enzyme is a glycoprotein which contains Cu/Fe and has been detected in goat, pig, human rabbit, bovine, and rat milks (Thorpe et al., 2002). The enzyme is able to oxidize the cysteine and sulfhydryl groups of glutathione, and milk proteins using molecular oxygen as electron acceptor, and transform them to their equivalent disulfides (Faccio et al., 2011). SOX of bovine milk might be added to UHT milk for reducing the cooked flavors. The enzyme has been immobilized on porous glass, and its efficacy in improving the cooked flavor has been showed on a pilot scale through the immobilized enzyme columns.

Peroxidases

Peroxidase (POX) happens extensively in nature and is the name which is generally applied to a group of both nonspecific and specific enzymes which use hydrogen peroxide as an electron acceptor. POXs, particularly the enzymes containing heme, are able to catalyze a majority of various reactions, including N-demethylation, oxidation, hydroxylation, and sulfoxidation; therefore, there is a special interest for these enzymes in the production of specific flavoring top notes. Demethylation of methyl N-methylanthranilate (ex Citrus) to mono-methylanthranilate is an example of the application of these enzymes (Van Haandel et al., 2000). The mentioned

produced compound is the main top note flavor in Concord grapes. Horseradish, microperoxidase, and soybean were found to be appropriate catalysts for this reaction. Moreover, POX increases the fresh flavor profile when it is used in tomato paste (Wilding & Woolner, 1997).

Polyphenol Oxidases

Polyphenol oxidase (PPO) enzymes are a group, including various enzymes with different activities. Tyrosinase, a monophenol monooxygenase, is able to transform a phenol into a catechol group. The other enzymes of this group, catechol oxidase or 1,2-diphenol oxidase (EC 1.10.3.1), converts catechol into an **O**-quinone, and laccase is able to produce **p-**quinone from 1,4-diphenol. Generally, the first two activities are related, since catechol is much more oxidized rather than a phenol. Also most of the enzymes, that are able to catalyze the oxidation of 1,4-diphenol, can act on 1,2-diphenols. In addition, guaiacols which are polyphenolic compounds impart the bitter taste in several food products. PPOs are able to oxidize these compounds; therefore, they can be used to decrease bitterness. There are several studies about the application of these enzymes for this purpose, such as debittering of cacao beans (Takemori et al., 1992), Adzuki beans (Okazawa et al., 1993), and coffee beans (Small & Asquith, 1989). The other example of their application is the use of laccase for debittering of olives (Charoenprasert & Mitchell, 2012).

12.1.2.4 Hydrolayses

Lipase and Esterases

Lipases and esterases are lipolytic enzymes that are able to catalyze the hydrolysis of triglycerides which are primarily different in the activities on substrates. These enzymes are different in substrates which can be in emulsion or solution forms. Lipases show extremely greater activity on emulsified substrates and prefer long-chain fatty acids; however, esterases indicate their activity on aqueous solutions of short acyl chain esters (Arpigny & Jaeger, 1999). Ester synthesis is generally catalyzed by enzymes, such as esterases and lipases, which can be expressed in many food microorganisms. These enzymes are serine hydrolases and are able of hydrolyzing or synthesizing esters based on the environmental conditions. Microbial lipases are commercially important due to their better stability, availability, and affordability rather than lipases from other sources (Molimard & Spinnler, 1996). Generally there are following common types of lipases according to (Waldmann & Parhofer, 2019): milk lipases; lipoprotein lipase; pancreatic lipases; hepatic lipases; hormone-sensitive botanical lipases; and microbial lipases, including *Candida* and *Torulopsis* yeast species, *Geotrichum*, *Aspergillus, Rhizopus, Penicillium,* and *Mucor* mold species, *Staphylococcus*, *Pseudomonas*, and *Achromobacter* bacterial species. Esters synthesized by lipase play a significant role in food industry as flavor and aroma constituents. Synthesizing esters by lipase can be conducted in vessels in batch or continuous condition and esters achieved from these sprouts have more purity and low cost, rather than other chemical methods which require special conditions and lead to redundant by-products production. However, low conversion

rate of these systems is their main drawback (Aravindan et al., 2007). Commercial lipases are mostly used in dairy products, meat industry, baked foods, fruit, vegetables, beer, and wine (Nagodawithana & Reed, 1993). Immobilized lipase on silica and microemulsion-based organogels are extensively being applied for synthesis of ester (Ghosh et al., 1996; Sharma et al., 2001). Lipases are typically highly specific. For instance, in a study Larios et al. (2004) in which the synthesis of esters in n-hexane was considered, it was found that lipase (fraction B) from *Candida antarctica* indicates substrate specificity. This specificity involves both acids and alcohols. It was shown that it has activity on unsaturated fatty acids and short-chain fatty acids with linear and branched chain structures, and 2-phenylethyl, n-butyl, isopentyl, and geraniol as alcohols. Generally enantiomeric selectivity of enzymes is among their important properties for food aroma synthesizing. As an example, only (S)-form of 2-methylbutanoic acid methyl ester has the fruity flavor which is the main flavor compound of apple and strawberry. It is found that lipases from *Aspergillus niger, Rhizomucor miehei,* and *Aspergillus javanicus* have the selectivity for this flavor compound (Kwon et al., 2000). Moreover, in a medium without solvent, lipase from *Candida rugosa* was selected for L-menthol esterification with long-chain unsaturated fatty acids to regulate the strong flavor related to it, whereas it was acted weakly on D-menthol (Shimada et al., 1999). However, some lipases indicate only restrained enantioselectivity, like *Candida antarctica* lipase (fraction B) selectivity for chiral short-chain carboxylic acids (Larios et al., 2004). Generally, lower water activity medium is preferred for lipase activity, so solvents with high hydrophobic activity are more favorable due to their insolubility in water layer around the enzyme surface which is important for protection of their active stereoconfiguration (Huang et al., 1998; Langrand et al., 1990). Additionally, enzymatic synthesis of esters can take place in systems without solvent. In these systems, reactant itself (i.e., an alcohol) acts as a solvent. This method is desirable in food industry, but because of the heterogeneity of the reaction, there are limitations, such as mass transfer and consequently low reaction rate in these systems. Researchers could find a new thermostable esterase derived from a marine mud metagenomic library, with potential use in industry, which is named as EST4. It is reported that this enzyme can efficiently produce several short-chain flavor esters under high concentrations of substrate and in media which is nonaqueous (Gao et al., 2016).

Researchers found that thermo-tolerant strain, such as *Bacillus licheniformis* (cloned in E. coli), has good ability to produce thermophilic lipolytic enzymes. These enzymes can tolerate high temperatures and organic solvents, which indicates their promising feature to use them for ester synthesis (Alvarez-Macarie & Baratti, 2000; Dominguez et al., 2004; Fuciños et al., 2005). In a study by Chang et al. (2001) the capability of mutant and wild-type lipases from *Staphylococcus epidermidis* to catalyze the formation of flavor esters was investigated in aqueous phase. This lipase was shown to have strong specificity for geranyl esters, unsaturated esters, and medium-chain esters. Seitz has stated that *Debaryomyces klockeri* and *Candida mycoderma* yeasts can produce lipases that have the best activity at pH 4.5 (Seitz, 1974). In another study it is shown that lipases from *Penicillium, Aspergillus*, and *Paecilomyces*, which are responsible for the fatty acid profile of the

cocoa bean, show different activities and specificities which are useful when they are applied to milk fat (Hansen et al., 1973).

Applications of lipolytic enzymes require good interdependence between the total substrate composition and the enzyme activity. For instance, the activity of milk lipase was as a result of the effect of the both stimulatory and inhibitory activity of several milk components on the enzyme (Hansen et al., 1973). In a study by Baianu et al. (2003), the free lipase derived from *M. miehei* could conduct the direct esterification of geraniol and citronellol with short-chain fatty acids, which had high yields in n-hexane. According to Sánchez et al. (2002), flavor esters, such as hexyl esters, produced by lipases are green note flavor compounds that are widely used as flavor and fragrance in different industries, including food industry. Recently, there is an increasing requirement for natural flavors, including "green note" characterized by hexanol derivatives. For instance, hexyl butyrate is of particular significance as it characterizes a model of flavor ester. Shieh and Chang (2001) have investigated the capability of immobilized lipase from *R. miehei* to catalyze the transesterification of tributyrin and hexanol. They determined that decreasing the amounts of tributyrin as a co-substrate would decrease the cost for the production of the hexyl butyrate. However, for transesterification of hexanol with triacetin with the same lipase, results indicated that substrate molar ratio and temperature of the reaction were the factors with the paramount importance and the added water content had less impact on the reaction.

In dairy industry lipases and esterases are regularly used to enhance the buttery flavor of the final product. Lipases are mostly used to hydrolyze milk fat. By modifying fatty acid chain lengths, lipases can enhance flavor of different kinds of cheeses. Short-chain fatty acids, specifically butanoic acid, play an essential role in dairy products' flavor and different commercial enzymes which have different specificities towards short-chain fatty acids are considered as flavoring tools (Saerens et al., 2008). There are several studies about the cheese ripening acceleration and fat, cream, and butter lipolysis by lipases (Ghosh et al., 1996; Sharma et al., 2001). Lipase from pregastric glands, such as bovine and porcine, and pregastric tissues of ruminants were initially used for enhancing cheese flavor.

Various kinds of cheeses can be produced by using a specific kind of microbial source or mixture of them. To produce enzyme-modified cheese (EMC) usually cheese is incubated with enzymes at an increased temperature and in this way a concentrated flavor is produced which can be used in sauces, snacks, soups, and dips. In these cheeses the concentration of fat is tenfold higher rather than normal cheeses (Ghosh et al., 1996; Sharma et al., 2001). Indigenous or exogenous milk enzymes can contribute to lipolysis. Exogenous enzymes can be either microbial lipases and/or mammalian pregastric esterase lipases. This process is an essential factor in flavor development of cheese. From different reviews on lipolysis in cheese, it can be derived that lipolysis results in the FFAs formation which are considered as major flavor compounds of cheeses and can be transformed to other flavor compounds, such as acetoacetate, aldehydes, β-keto acids, lactones, methyl ketones, esters, and secondary alcohols which are more effective and can directly affect flavor in different kinds of cheeses (Collins et al., 2003). Lactic acid bacteria (LAB) are

used in cheese fermentation as starter adjuncts, starter cultures, or as secondary microbial flora (Beresford et al., 2001; Crow et al., 2001). Moreover, Custry et al. (1987) reported that methyl ketones are main group of compounds contributing to cheese flavor and are originated from the FAs released by lipolysis reaction. A secondary flora is needed for enzymatically oxidization of these FAs into methyl ketones via β-oxidation. Additionally, the oxy fatty acids in milk fat can also be the source of methyl ketones.

Development of cheese flavor is a complex process in which enzymes from different sources are responsible for the degradation of proteins, carbohydrates, and milk fat. Straight-chain carbons of C2 to C18 are the main FFAs in cheese which have quite high flavor perception thresholds, leading to different flavor notes, such as pungent, goaty, rancid, soapy, waxy, or cheesy notes (Collins et al., 2003). However, the flavor perception thresholds of esters which are resulted from FFAs with carbon chain of less than C10 is much lower, revealing a fruity flavor notes which are pleasant for some people. Esters are also able to mask effect of off-flavors, such as pungent and sharp off-flavors. On the other hand, high levels of ethyl esters of short-chain FFAs can cause a fruity flavor defect in cheeses, such as Cheddar (Bills et al., 1965; McGugan et al., 1975).

Esterases of LAB can hydrolyze aromatic esters, thioesters, and alkyl esters, as well as hydrolysis of b-naphthyl or p-nitrophenyl esters of FAs, partially hydrolyzed milk fat, and synthetic glycerides. It has been stated that esterases of Lb. casei, Lc. Lactis, and Lb. helveticus are able to hydrolyze ethyl esters of FAs of C2 to C6, acetate esters of hexanol, propanol, and butanol, as well as aromatic esters, such as phenyl acetate and phenyl thioacetate. Researchers could increase ester production by lowering water activity (0.8 5 and 0.9) and increasing the levels of substrates (up to 1000 ppm of FFA and alcohol) in model system of cheese containing esterases from several LAB. According to studies, each esterase results in hydrolysis of a different flavor-active ester (Fenster et al., 2000, 2003a, 2003b). *Candida lipolytica* was used in the manufacture of Blue cheese (Price et al., 2014). Moschopoulou (2011) successfully indicated the flavor of Pecorino cheese by using lipolytic enzymes derived from microorganisms obtained from the abomasum of lambs. Rhizopus lipase has been applied to development of artificial butter flavors and in yogurt manufacture (Singh & Banerjee, 2017).

Generally, it is known that esterification of FFAs with ethanol results in ester formation and these esters impart aromatic molecules in foods, specifically in cheeses. Ethyl esters or methyl esters derived from short-chain fatty acids mostly carry significant fruity flavors, whereas thioesters from thiols have sulfur and cabbage aromas (Collins et al., 2003; Fox & Wallace, 1997).

It is known that LAB have little contribution to lipolysis, but additional cultures, such as molds usually have high activities in fat conversion in the case of surface-ripened cheeses (Molimard & Spinnler, 1996). The derived flavors from the fat conversion are predominantly important in Camembert and Roquefort (soft cheeses).

Esterases of LAB are also known as alcohol acyltransferases due to their ability in transferring fatty acyl groups from a glyceride to either water, which is called hydrolysis, or alcohols, which is called alcoholysis, in aqueous systems. Moreover,

esters synthesizing in aqueous systems can be done through alcoholysis from other alcohols and glycerides. For instance, it is found that *St. thermophilus* is able to synthesize aromatic esters (2-phenyl ethyl hexanoate and 2-phenyl ethyl octanoate) from an aromatic alcohol (2-phenyl ethanol) and two glycerides [monooctanoin (mono-caprylin, C8) and dihexanoin (dicaproin, C6)](Liu et al., 2003a). Generally, di- and monoglyceride substrates are preferred for alcoholysis by LAB esterases which have the same specificity in hydrolysis (Liu et al., 2003a).

In cheese medium ester synthesizing can also done by selecting specific starters that have high esterase or transferase activity, by changing the mono-, di-, and triglyceride composition of the cheese fat glycerides, or by controlling ethanol availability. For instance, in cheese made with mesophilic starters, by increasing the ethanol level, ethyl hexanoate and ethyl butanoate concentrations are significantly increased (Crow et al., 2002). In other study by spiking 1 M ethanol in Cheddar-type cheese curd, during an incubation of 2 months, ethyl butanoate and ethyl hexanoate are synthesized by the recombinant esterase from *Lb. rhamnosus*. Availability of ethanol is possibly an essential factor in controlling ester formation in cheese. Also primed cheeses with prehydrolyzed milk fat contain more esterases and this is because of the presence of more di- and monoglycerides and it is found that without addition of ethanol there is no ester formation in these cheeses. Moreover, higher ester levels in cheese curd result in fruity aroma. In addition, some commercial fungal lipases also found to synthesize esters from glycerides and ethanol in cheese medium; also it is shown that they also have similar mechanism to LAB (Liu et al., 2003b). Moreover, yeasts and molds have effect on ester formation in cheese by producing the alcohols needed for ester synthesizing in cheese.

Salvadori (1961) could double the flavor of Pecorino cheese prepared with ewe's milk by addition of lipases taken from bacteria which were isolated from the abomasum of lambs. In addition, it is found that esterase from *Mucor miehei* also shows lipolytic activity in cheese similar to that achieved by pregastric oral esterases (Richardson & Nelson, 1968).

In a study by comparing flavor characteristics of Romano cheese and Fontina cheese containing *Mucor miehei* esterase to that of cheeses prepared with pregastric esterase, it was found that by using fungal esterase and pregastric calf esterase at the same lipolytic activity in the Fontina cheese, the desired flavor develops in both cheeses. However, based on lipolytic activity, in Romano cheese it was necessary to add a five time greater of fungal esterase, rather than pregastric kid esterase. According to Baillargeon (1990), lipase from *Geotrichum candidum* which is a dairy yeast has selectivity towards unsaturated fatty acids with cis-9 double bonds, such as oleic, linoleic, and linolenic acids. Also, *Yarrowia lipolytica* (a dairy yeast) could majorly release oleic and palmitic, from milk fat (Suzzi et al., 2001). It was also previously found that *G. candidum and Y. lipolytica* can selectively release unsaturated long-chain fatty acids from milk fat, in the cheese environment (Das et al., 2005). However, these FAs have not favorable flavor and they impart a soapy flavor to cheese, but they can be converted to conjugated linoleic acid (CLA) by *Propionibacteria* which are usually used in Swiss cheese production (Jiang et al., 1998). However, in another study researchers used three strains of

Propionibacterium freudenreichii ssp. Shermanii as adjunct strains which are able to convert linoleic acid to CLA in laboratory media, with strains of *Yarrowia lipolytica, Geotrichum candidum,* and *Lactobacillus fermentum* (to produce ethanol from lactose) for preparation of washed-curd, dry-salted cheese, but the results showed the failure of the *Propionibacteria* strains to form CLA from linoleic acid, in the cheese media (Das et al., 2005). CLA introduction in dairy products been also done by lipase immobilization method (Baianu et al., 2003).

Based on different cheese varieties and production conditions, nonstarter LAB and the derived products can be varied. Psychrotrophic bacteria produce heat-stable lipases which can adsorb onto the milk fat globules and resist pasteurization. They may contribute to lipolysis in cheese made from milk prior to pasteurization (Cousins et al., 1977). *Penicillium sp.* (a Psychrotrophic bacteria) can produce extracellular lipases which are predominantly responsible for lipolysis in mold-ripened cheeses (Cousins et al., 1977; Smit et al., 2005). Facultative hetero-fermentative lactobacilli, such as *Lb. casei, Lb. paracasei and Lb. plantarum*, which are dominant in cheese varieties as nonstarter LAB, are weakly lipolytic. However, obligatory homo-fermentative lactobacilli, such as *Lb. delbrueckii subsp. lactis, Lb. helveticus,* and *Lb. delbrueckii subsp. bulgaricus*, which are used as starters, also produce esterases (El Soda et al., 1986). According to Bhowmik and Marth (1990) *Pediococcus* and *Micrococcus* are also weakly lipolytic. The effect of FFA on the flavor of hard Italian cheese varieties is more than Blue mold-ripened cheeses, which is probably due to neutralization by increasing the pH during ripening, and also the principal effect of methyl ketones on the flavor of Blue mold cheeses. Actually alkan-2-ones (2-methyl ketones) is the dominant flavor of Blue mold cheeses which is derived from FFA. β-oxidation, which is the pathway of alkan-2-onesformation, involves the liberation of FAs by lipolysis, and their oxidation to β-keto acids. After decarboxylation of them, alkan-2-ones with one less C atom are formed. This compound can be reduced (which is reversible in aerobic conditions) and leads to the formation of corresponding secondary alcohols (alkan-2-ols).

Intramolecular esterification of hydroxy fatty acids results in the formation of lactones. For instance, γ- and δ-lactones, which are strongly flavored, stable, and the main lactones present in cheese, are formed from δ- or γ-hydroxy fatty acids. According to Urbach (1993) in full-fat cheeses, δ-decalactone increases to a maximum concentration (in 14 weeks) and then its concentration decreases; however, in low-fat cheeses, the δ-decalactone level remains constant during ripening. However, Cheddar cheese flavor is improved by decreasing the δ-decalactone level, indicating that δ-decalactone plays an insignificant role in flavor of Cheddar cheese (Dimos et al., 1996). In a study, lipase from a *Sporidiobolus pararoseus* strain was derived and it was found that the specificity of the enzyme for triglyceride was comparable to pregastric esterase. Using this microbial lipase during the process of mozzarella cheese imparted a strong flavor to the cheese. Hence, the lipase taken from *S. pararoseus* can be used instead of pregastric esterase in dairy industry (Mase et al., 2010). In a study six lipases/esterases, related to buttery flavor production, were compared. It was shown that *Candida cylindracea* lipase has the most

specificity to butanoic acid and production of buttery flavor in cream. Moreover, this enzyme led to the highest concentration of free fatty acids (FFAs) (Saerens et al., 2008).

Based on literatures milk lipases have insignificant importance in cheeses prepared with pasteurized milk, and milk bacterial lipases, which are resistant to thermal condition, can act better in cheese production from pasteurized milk. Moreover, both bacterial and milk lipases have importance in milk fat hydrolysis, whereas bacterial lipases and esterases (from Streptococcus cremoris) have significant role in cheeses made from pasteurized milk (Chambers et al., 2010).

Using pregastric esterase in pasteurized milk for Cheddar cheese production results in more FFA and flavor improvement; however, enzyme's activity is lower than its activity in raw milk. Also it was shown that the flavor of Cheddar cheese is associated to a balance between acetate and FFAs and the ratio of 1.0 to 0.55 of acetate: FFA results in the most favorable flavor. Microbial lipases increase FFAs which affect the flavor of cheese (Collins et al., 2003). Kosikowski (1975) also studied the prospective application of microbial lipases in continuous cheese processing, and suggested good flavor development in Cheddar cheese from different fungal lipases and in Blue cheese from *Aspergillus sp* lipase. However, organoleptic properties of Cheddar cheeses are increased in the presence of gastric lipase in their production (Wolf et al., 2009). Moreover, there are studies about the effect of lipase application in Feta (Karami et al., 2009) and Samsoe cheese (Jensen, 1970) in their flavor development.

Lane and Hammer (1936) were the first people suggesting that homogenization of cows' raw milk or cream which is used in Blue cheese production can activate the milk lipase in it; therefore, they concluded that homogenization has a great impact in cheese flavor development and concentrations of volatile acids are two to fourfold greater in cheese produced by homogenized milk. Studies showed that *P. roqueforti* lipase acts specifically on triglycerides with smaller molecular weight. Secondary alcohols and methyl ketones are fundamental compounds for the Blue cheese flavor characteristics and it is known that they are derived from FFAs which are abundant in Blue cheese (Tunick, 2007). Other researchers found out that methyl-n-amyl ketone (2-heptanone) has a significant role in Blue cheese flavor characteristics, which is suggested to be derived through the P. roqueforti activity on caprylic acid. The methyl ketones' formation and their metabolism by fungi in mold-ripened cheeses act through different enzymatic mechanisms, such as release of FFAs from triglycerides of milk fat by lipases (Walker & Mills, 2014). Dwivedi and KINSELLA (1974) studied the continuous addition of lipolyzed milk fat and gradual release of FFAs, in Blue cheese through the action of milk lipase. As a result, a product was formed which was suitable for application in salad appetizers, dressings. Its amount was 7- to 12-fold more than the ketone amount in a decent Blue cheese, and its flavor efficacy was 4 times higher. Also these researchers reported the production of methyl ketone by *P. roqueforti* from lipolyzed milk fat and suggested that higher concentrations of FA had an impermanent inhibitory effect on the FAs metabolization by enzyme.

Researchers, who studied the effect of pregastric esterases from lamb, kid, and calf on bovine milk fat substrate, showed that these lipases have preference for releasing short-chain FFAs. Each esterase had specificity to release butyric and other short-chain FFAs and they were different in the release ratio of short-chain to long-chain FFAs producing specific flavor characteristic while using cheese. It was found that kid esterase has the highest specificity for butyric and other short-chain FFAs.

Studies on Provolone cheeses showed a direct relationship between FFAs (such as butyric acid) and flavor quality and acceptance in Provolone and Romano cheeses (Christensen, 1964; Collins et al., 2003). According to application of both lamb pregastric esterase with lamb gastric extracts, results in more suitable "Provolone-like" flavors, showing that addition of gastric lipase makes Provolone cheeses organoleptically more acceptable. Overall, the extensive use of lipolytic enzymes and commercial rennet paste in Italian cheese manufacture indicates the importance of controlled lipolysis in this industry (Richardson & Nelson, 1968). It is found that gastric lipase also has application for accelerating the cheese ripening and its flavor development in different cheeses, such as Provolone, Ras, and Cheddar cheeses. Addition of calf lipase increases the release of FAs (C4–C6) rate and accelerates the development of flavor. The drawback of this method is that the lipase remains active after ripening and can lead to development of an intense rancid flavor. By addition of the mixture of fungal protease and lipase, relatively high-soluble proteins and FFAs will develop in various cheeses which show better development of flavor in 3 months of ripening. Also the amount of enzyme addition is of high importance and high level of it results in unfavorable characteristics and decrease in the yield (Jahadi et al., 2016).

Literatures indicated that using liposome technology for cheese ripening decreases the losses in yield and bitterness (Jahadi et al., 2015; Vafabakhsh et al., 2013). In cheese made from unpasteurized milk, the lipase which is inherent in the cheese has significant effect in lipolytic action (Jahadi et al., 2020). *P. roqueforti* and *P. camemberti* in Camembert and Blue-veined cheeses are considered as lipolytic cultures and are responsible for lipases production, that accounts for lipolysis. Moreover, lipases are often added to Italian cheese, such as Romano, Provolone, and Paramesan to increase their flavor (Ghosh et al., 1996). Through ripening, there is a constant rise in the concentration of released FAs and total soluble nitrogen (Jahadi et al., 2012). Lipases are able to liberate the FAs from triglycerides, and in this way they can trigger cheese flavor development (Maia et al., 1999; Mohammadi et al., 2015).

In Gouda and Cheddar cheeses, esters, such as ethyl butyrate, have high contribution to their flavor. Moreover, extra esters in Cheddar can impart fruity defect (Caspia et al., 2006). Furthermore, there are compounds in Cheddar and Camembert cheese, resulting from degradation of phenylalanine (such as phenylacetaldehyde, 2-phenylethanol, and the ester phenylethyl acetate), playing role in its floral rose-like aroma and desirable floral note (Carunchia Whetstine et al., 2005).

There is an increasing interest for lipolytic enzymes in baking industry. Findings suggested (phospho) lipases application as alternatives for producing emulsifying lipids due to the enzymes ability to degrade polar wheat lipids to produce

emulsifying lipids in situ (Collar et al., 2000; Kirk et al., 2002). Lipase was mainly used to increase the flavor in bakery products through esterification (releasing short-chain fatty acids). In addition to flavor development, it also extended the shelf life of most of the bakery products. Also softness and texture can be developed by lipase catalyzation (Laboret & Perraud, 1999). Lipase from *A. oryzae* was also used in baking industry, as processing aid (Greenough et al., 1996). Hydrolytic enzymes, such as lipase, were discovered to be effective in decreasing the initial firmness and enhancing the specific volume of breads (Laboret & Perraud, 1999). According to Sánchez et al. (2002), yeasts with LIP A (bacterial lipase gene) result in enzyme with high productivity which can be used in bread as an additive. Butter flavor increases, by hydrolyzing the butterfat with an appropriate lipase in baked foods.

Recent studies have investigated the possibility of application of various enzyme systems for milk fat modification to incorporate them into bakery products. Generally, lipases of animal origin were found to be most suitable for milk fat modification and including milk fat that is modified by these enzymes in bakery products led to the formation of better flavors in them rather than milk fats modified by other enzymes. *Penicillium roqueforti, Geotrichum candidum,* and *Acromobacter lipolyticurn* lipases were mostly inappropriate due to the liberation of a large concentrations of medium- or long-chain fatty acids and production of musty or soapy flavors. Also lamb and kid esterases were not suitable due to the production of excessive quantities of short-chain FAs imparting rancid flavors in bread formulations (Arnold et al., 1975).

Vanillin (4-hydroxy-3-methoxybenzaldehyde), which is a generally desirable flavor, mostly is present in Vanilla Planifolia beans. This flavor is extensively used in foods (Priefert et al., 2001). Ferulic acid is precursor of vanillin and feruloyl esterase has been recognized as the critical enzyme in the biosynthesis of it. This enzyme can be produced in microbial cultures of several fungi grown on various pretreated cereal brans (Mathew & Abraham, 2005). The metabolism of ferulic acid in some microorganisms has also been investigated (Falconnier et al., 1994; Narbad & Gasson, 1998).

Carboxyl esterases (carboxyl ester hydrolases) are enzymes extensively used in different industries (Ewis et al., 2004). These enzymes prefer to catalyze the hydrolysis of esters of short-chain FAs, but they also are able to catalyze the synthesis of ester and transesterification reactions (Bornscheuer, 2002). Amid these esters, flavor acetates from primary alcohols have a great application due to their characteristic flavor and fragrance (Romero et al., 2005). Isoamyl acetate is also one of the most significant flavor compounds used in the food industries and is produced using lipases. In a research, the ability of type II esterase enzyme to catalyze the esterification of isoamyl alcohol to isoamyl acetate which is less toxic compound was studied. This compound is one of the most substantial flavor compounds used in the food industries due to its distinctive banana flavor (Krishna et al., 2001). This ester is widely used as a flavoring compound in several foods and drinks, such as artificial coffee, honey, butterscotch, and alcoholic beverages. Production of isoamyl acetate is typically doable through Fischer esterification

mechanism (Welsh et al., 1989) and all of the enzymatic synthesis reactions of this compound were taken place using lipases (Krishna et al., 2001). Also there are applications of lipases in ice cream and single cell protein in which C. rugosa lipase has significant role in their flavor characteristics (Sánchez et al., 2002).

Tomato flavor, which is composed of a complex mixture of volatiles containing multiple acetate esters, is resulted from esterases activity. It is believed that red-fruited species of the tomato clade collects a quite low content of acetate esters rather than the green-fruited species and the difference is related to the insertion of a retrotransposon adjacent to the most enzymatically active member of family of esterases, which results in higher expression of the esterase and decrease in the levels of multiple esters that have negative impact on human preferences for tomato (Goulet et al., 2012).

Studies on the flavor and odor characteristics of Suanyu (a traditional Chinese fermented freshwater fish), in the presence of the esterase activity of Saccharomyces cerevisiae 31, Lactobacillus plantarum 120, and Staphylococcus xylosus 135, indicated that all strains displayed esterase activities in both intracellular and extracellular fractions against p-NP-butyrate, p-NP-octanoate, and p-nitrophenyl (p-NP)-acetate. Moreover, it was found out that a low Aw (water activity), a low pH, and also a high temperature would be more suitable for Suanyu processing, which results in more flavor compounds which are favorable for consumers (Gao et al., 2017).

Also in wine industry high levels of 4-vinylguaiacol and 4-vinylphenol were identified from grape juice primarily treated with two enzymes. Firstly, cinnamoyl esterase activity releases cinnamic acids from the related tartaric acid esters. Secondly, decarboxylase activity by the yeasts transforms cinnamic acids into 4-vinyl phenol and 4-vinyl guaiacol (Dugelay et al., 1993).

In oil industry *Cooney and Emerson strains of Mucor miehei* esterase has some functions. This enzyme have impact on natural fats such as lard oil, beef tallow, and vegetable oils and some synthetic substrates, such as sorbitol esters of FAs. Also fatty acid profiles which are resulted from the hydrolysis of beef tallow and soy oil at pH 8.0 with both pancreatic lipase and M. miehei esterase, are comparable (Moskowitz et al., 1977).

Protease

Proteases are enzymes able to break down the peptide bonds of proteins; they are categorized as alkaline, neutral, and acid proteases. Animals, plants, and microorganisms can be potential sources for these enzymes, in different conditions, such as high salt concentrations. Wouters et al. (2016) explained that the flavor and odor of the protein constituents have significant impact in food industry. In fact, peptides and amino acids, besides other molecules, such as salts or sugars, define the taste of foods. The human gustatory system is able to detect 5 basic flavors, such as sweetness, bitterness, saltiness, sourness, and savoriness or umami. Sweetness, bitterness, and savoriness are the major tastes associated with peptides (Iwaniak et al., 2016). Bitterness needs a particular care, due to its cause for product rejection. The peptides' flavor can change based on their amino acid sequence. Studies have

indicated a correlation between the chain length of the peptides and their bitterness. Their bitterness increases when they are made by up to 8 amino acid groups. Furthermore, this is affected by its general hydrophobicity and the particular amino acid positioned at the N- and C-terminus. For instance, the terminus position of tyrosine or phenylalanine can determine the bitter taste (Iwaniak et al., 2016; Maehashi & Huang, 2009). Therefore, selecting the right protease to accomplish the hydrolysis of protein extract can reduce the unpleasant flavor in the final product and also produce peptides with suitable tastes. For instance, during cheese production and ripening, a continuous proteolysis is normally considered to be a required condition for producing right flavor of cheeses (particularly Camembert or Brie) (Singh et al., 2016). Moreover, proteolysis occurs during the main biochemical modifications in cheese making, considering that in most circumstances, the first step is an enzymatic coagulation. In the next steps, during the cheese ripening, a progressive transformation of flavor and texture occurs. In fact, proteolysis is not taking place only by the activity of external added enzymes but also it can be done by enzymes of microorganisms. In Blue-veined cheeses, Blue molds generate a typical odor and flavor through the conversions catalyzed by their enzymes. The same happens at the moldy surfaces of cheeses, such as Camembert and Brie (Seratlić et al., 2011).

Glutaminase

L-Glutamic acid is a verified flavor-enhancing amino acid which is extensively used as a fundamental seasoning in food service, home cooking, and processed food industries around the world. On the other hand, L-glutamic acid is originated from proteins present in raw materials in fermented foods. L-Glutamic acid can also be produced by the hydrolysis of L-glutamine in the protein hydrolysate; however, in the absence of glutaminase, most of the released L-glutamine is transformed to pyroglutamic acid which is tasteless (Fig. 12.5).

Soy sauce is a product known for its unique and strong umami taste, which is created through its hydrolysis and fermentation. Umami taste induced by monosodium L-glutamate has been widely accepted as one of the basic kinds of tastes which is also named as savory, broth-like, or meaty taste. In fact, this taste is linked to the taste characteristics and the chemical structure of L-glutamyl oligopeptides. The presence of glutamic acid in di- or tripeptides is strongly correlated with umami taste, because of the fact that the anionic L-glutamyl oligopeptides can be present as umami taste (Zhang et al., 2017). Glutaminase derived from *Aspergillus oryzae* is often used for the soy sauce fermentation, and

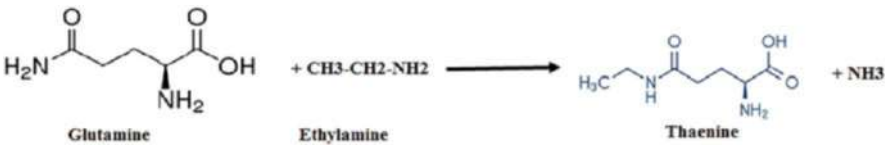

Fig. 12.5 Formation of pyroglutamic acid in the absence of glutaminase (tasteless)

high salt concentrations in the soy sauce fermentation process can inhibit its activity. Thus, salt-tolerant type of this enzyme have been studied for application in soy sauce fermentation. Glutaminase enzymes from *Micrococcus luteus, Bacillus subtilis,* and Aspergillus oryzae have been widely studied (Masuo et al., 2005). The glutaminase of *B. subtilis* is commercially available. Zhuang and others (Zhuang et al., 2016) indicated the significance of peptides' effect on the umami taste of soy sauce. They showed that the amino acid sequences of the peptides (Glu-Ala-Gly-Ile-Gln, Ala-Leu-Pro-Glu-Glu-Val, Ala-Gln-Ala-Leu-Gln-Ala-Gln-Ala, Glu-Gln-Gln-Gln-Gln, and Leu-Pro-Glu-Glu-Val), with the presence of glutamic acid/glutamine persistent, are responsible for the umami taste in soy sauce.

Theanine (γ-glutamylethylamide) is an amino acid derived from a nonprotein source that initially was isolated from green tea leaves (Sakato, 1949). This amino acid is considered to be a distinctive amino acid in nature due to its limited occurrence to the *Camellia genus*, especially the tea-producing plant, *C. sinensis* (Nathan et al., 2006). Theanine is acknowledged for its unique umami taste. Therefore, teas containing high theanine are normally considered to have higher quality. Theanine can be formed from ethylamine and glutamate using sugar fermentation reaction of baker's yeast as an ATP regenerating system and bacterial glutamine synthetase reaction (Nandakumar et al., 2003). There are some beneficial effects for theanine, including improvement of learning ability, memory, and relaxation, strengthening the immune system vascular diseases, prevention of cancer, and weight gain (Kimura et al., 2007; Owen et al., 2008). In a mixture of γ-glutamyl donor and γ-glutamyl acceptor, glutaminase from *Pseudomonas nitroreducens* has been found to catalyze a γ-glutamyl transfer reaction. This enzymatic method has been industrialized to produce theanine using glutaminase enzyme from *P. nitroreducens*. For the enzymatic synthesis of theanine, GGT (γ-glutamyl transpeptidase) catalyzes the hydrolysis of γ-glutamyl compounds and transmissions of γ-glutamyl moiety from γ-glutamyl compounds, such as glutathione, to amino acids and peptides. Also GGT was responsible for synthesizing other physiologically significant γ-glutamyl compounds, including γ-glutamyl taurine and γ-Glutamyl-L-3,4-dihydroxyphenylalanine (γ-glutamyl DOPA). Additionally, bitter taste of some amino acids can be decreased by the mean of γ-glutamylization by GGT (Suzuki et al., 2002).

Thermolysin

Synthetic sweeteners, involving anticariogenic and noncaloric sweeteners, have been suggested to be a substitute to natural, calorie-containing sweeteners to decrease the risk of cardiovascular diseases, diabetes, and obesity. For instance, saccharin, aspartame, acesulfame potassium, and sucralose are examples of sugar substitutes regularly used alone or with other natural sweeteners as food additives, in several foods and soft drinks. Aspartame (α-aspartyl-phenylalanine methyl ester), which is a peptide-type artificial sweetener, has been broadly used in processed and pet foods, and beverages and drinks, such as tea, coffee, and sports drinks. The sweetness degree of this sweetener is around 200 times higher than that of sucrose. Aspartame thermolysin is a neutral protease from *Bacillus thermoproteolyticus* and

it is revealed that its enzymatic activity enhances by increasing NaCl concentration. The reverse reaction of hydrolysis of peptide bonds in the mixture of N-carbobenzoxy-L-aspartic acid and DL-phenylalanine methyl ester can be catalyzed by this enzyme (Inouye et al., 2007; Ogino et al., 2010). At the end, the N-carbobenzoxy-protecting group is detached chemically, and unreacted D-phenylalanine methyl ester is altered to DL-phenylalanine methyl ester which is its racemic form.

β-Galactosidase

D-glucoside glucohydrolase, also called lactase or β-Galactosidase, is able to catalyze the hydrolysis of the galactosyl moiety from the nonreducing end of several galactosyl-oligosaccharides. This enzyme is usually used in the dairy industry for hydrolyzing lactose, Gal-(β1,4)-Glc, which is the main saccharide naturally present in milk (Dutra Rosolen et al., 2015). Moreover, this reaction (hydrolyzing lactose to galactose and glucose by β-galactosidase) inhibits the lactose crystallization in condensed and frozen milk products besides strengthening the sweetness. Moreover, β-galactosidase is capable of catalyzing transgalactosylation, in which a galactose moiety is transmitted to the saccharides, emerging different galacto-oligosaccharides (GOs). GOs have some advantageous effects, such as decreasing blood serum cholesterol level, suppressing the colon cancer progress, and improving the mineral absorption (Vulevic et al., 2013). The initial application of glycosidases was in wines which was used to liberate flavor compounds from glycosidic precursors. The first reason for this is that significant flavor compounds in wines from *Vitis vinifera* cultivars are present as flavorless glycoconjugates in grapes, and the second reason is that the glucose inhibitory effect of -glucosidases restricts their use to the media containing low levels of glucose (<1 g/L), such as wine. However, due to the presence of flavor glycoconjugates in various fruits, enzyme applications have also been reported for fruit juices.

A. Wine Flavor Enhancement: During grape juice fermentation, there is only a slight decrease in the amount of glycosidic flavor precursors. This can be described by the low levels of glycosidase activities of enological yeast strains and grapes (Hernandez-Orte et al., 2008). Therefore, there is a special interest for the use of enzymes, such as pectinases and hemicellulases, which are commercially available fungal enzymes (mainly derived from *Aspergillus spp*.) that also have glycosidase activities. However, recent regulations do not accept including the enzymes in the wine. The hydrolysis of monoterpenyl glycosides in muscat cultivars releases odorous alcohols that can be identified by sensory analysis and can be analyzed by GC/MS. Generally, the addition of glycosidase during wine making increases in the concentration of monoterpenes, shikimate-derived compound, and C13-norisoprenoids (Hernandez-Orte et al., 2008).

B. Fruit Juices: There are few studies about the use of glucosidases in fruit juices which is due to the inhibition of them by glucose of the "key enzyme" glucosidase in enzyme derived from fungi. According to a study curing passion and mango fruit juices with a glucosidase derived from *Candida cacaoi* resulted in a rise in monoterpene concentrations (Chanprasartsuk & Prakitchaiwattana, 2015). Nevertheless,

this increase was pretty low rather than acid hydrolysis. Moreover, immobilization of the enzyme on sodium alginate beads, released less terpenols rather than the free enzyme. The addition of immobilized *C. molischiana*-derived glucosidase (onto Duolite A-568 resin) to apple juices, mango, strawberry, apricot, and peach, caused a rise in the concentration of monoterpene alcohols, such as 2-phenylethanoal, geraniol, terpineol, benzyl alcohol, and linalool (Pogorzelski & Wilkowska, 2007). However, there were no differences in the flavor liberations using immobilized and free glucosidase from *C. molischiana*. The immobilized enzyme was stated to be much more stable in fruit juice settings. In passion fruit juice, an immobilized (onto cellulose or acrylic beads) endo glucosidase derived from *A. niger* increased the concentration of volatile compounds, such as benzyl alcohol, benzaldehyde, and linalool. Generally, an aroma improvement was determined in the treated juices (Pogorzelski & Wilkowska, 2007).

Although by application of glucosidase, the levels of volatile compounds in fruit juices have increased, there were two significant parameters restricting the effectiveness of the process: firstly, it is known that there are lots of flavor compounds in fruit juice, such as diglycosides, that in general, fungal glucosidases are not able to hydrolyze them; secondly, the examined fungal glucosidases are prevented by glucose, which might then constraint their action in fruit juices. There is a report about the process that the amount of glycosides alters in a sweet wine, with 30 g/L glucose, in the presence of fungal glycosidases. It is shown that the monoterpene diglycosides' level decreases remarkably in the enzyme-treated wine, which can be described by the activity of exoglycosidases. The produced monoterpenyl-D glucosides were not hydrolyzed and consequently they were accumulated in the wine. This clearly shows that the fungal glucosidase activity is inhibited by glucose, which significantly limits the release of volatiles as it is approved by their analysis. In contrary, the hydrolysis of monoglucosides was very efficient though a dry wine was made using the same conditions, such as the same enzyme dosage and grape juice (Pogorzelski & Wilkowska, 2007).

Naringinase(α-L-Rhamnosidase)

Naringinase (EC 3.2.1.40) is mostly used for the naringin' breakdown which is the main bitter flavanone glycoside present in citrus fruits (Puri, 2012). Through the activity of α-rhamnosidase and β-glucosidase, naringin is converted to a naringenin and rhamnose. The sources for naringinase are mostly fungal isolates, such as *Circinella, Eurotium, Aspergillus niger, Rhizopus, Trichoderma, Fusarium,* and *Penicillium,* and bacteria, such as *Pseudomonas paucimobilis, Bacillus sp., Thermomicrobium roseum, Bacteriodes distasonis,* and *Burkholderia cenocepacia* (Puri, 2012). Naringinase derived from fungi has more application rather than the bacterial ones due to increased yield. Naringinase, as a debittering enzyme, has a principle role in food processing when it is added to fruit juices, in both immobilized and free forms. There are several food additives, such as sweeteners and biopolymers, which can be produced by naringinase or rhamnosidase. Moreover, there is another application for naringinase in addition to arabinosidase and β-glucosidase to develop the aroma of wine (Custodio et al., 1996). It is also stated

that naringinase can be used in preparation of tomato pulp and prunin, and treatment of kinnow peel waste (Puri, 2012).

Citrus juice is considered as one of the most commonly used juices in the world. However, mostly the taste of it is too bitter and this bitterness is mainly because of the presence of the naringin in the citrus fruits. Naringinase (α-L-rhamnosidase) is used to diminish the bitterness by breaking naringin into rhamnose and prunin and it is regularly used in the industrial production process of citrus juice (Puri et al., 2005). β-glucosidase, which is responsible for hydrolyzing the prunin to naringenin (a flavorless flavanone) and glucose, is usually used with naringinase, for a successful debittering (Table 12.1).

12.2 Preservation

12.2.1 Introduction

It is undeniable that food is important for mankind and there is not any way of living without eating. Therefore, it is highly important for the health of everyone to eat food. Although the need to feed did not change during the years, the way of eating has seen a lot of changes. Nowadays, food additives are needed to make various foods that are meeting the progressively challenging market and legal demands (Saltmarsh & Saltmarsh, 2013). Foods can be made in different facilities and then can be transported to local markets that are inside the same country or even in distant ones (Atkins & Bowler, 2016). Food transportation in suitable conditions needs special equipment and considerations, such as controlled packaging, refrigeration, or using some additives to prevent food spoilage and diminish food alterations. In today's global market which is highly competitive, it is desirable to use the cheapest method of food preservation and food additives can be a good choice.

12.2.2 Lysozyme

Antimicrobials with animal origin are compounds, such as proteins and enzymes that are isolated from animals. Currently, the only certified natural antimicrobial with animal origin that is used in US and the EU is lysozyme (E-1105). This enzyme is derived from eggs (Carocho et al., 2015), and it has high antimicrobial activity against gram-negative bacteria. The reason for this higher activity is that gram-negative bacteria are mostly (90%) composed of peptidoglycan, and lysozyme's antimicrobial activity is dependent on the hydrolysis of the β-1,4 linkage site of the peptidoglycan in the bacterial walls. This enzyme has only a moderate effect against gram-positive bacteria that have much less peptidoglycan in their wall, and apparently it has no activity against fungi or yeasts (Barbiroli et al., 2012). It is reported that the principal commercial use of this natural antimicrobial is in the cheese industry, that is used for prevention of "late blowing" of cheese; also there are studies about its application in eggs, beef (200 mg/90 mg), and milk (2 mg/mL in

Table 12.1 Enzyme role related to food flavor

Enzyme role	Enzyme	Bound volatiles	Flavor like	Ref
	B-glucosidase	Benzaldehyde, benzyl alcohol, 2-henylethanol, CI3-norisoprenoid, monoterpenes, CI3-nonsoprenoids, Marmelactones, hexanol, 2-henylethanol, linalool, dienediols, nerol	Papaya, apple, apricot, peach, yellow plum	Eriksson (2012)
	Endo-glucosidase	Terpenes (linalool)	**F**ruit**y**	Martinez Ortega (2015)
	ß-glucosidase	Vanillin	Vanilla	Esparan (2015); Lim (2012)
Enhance or produce flavor	Tannase	Terpenes (linalool)	**F**ruit**y**	Beniwal et al. (2013)
	Pectinase	2-phenyl ethanol, benzyl alcohol, benzoic acid	Tomato	de Andrade Júnior and Andrade (2015)
	Anthocyanase	Terpenes (linalool)	**F**ruit**y**	Yang et al. (2020)
	Glucosidase	Norisoprenoids from lutein, violaxanthin, neoxanthin	Grape	Williams et al. (1992)
	Lactoperoxidase, xanthine oxidase, proteinase, lipase	Complex (methyl esters, ketones, aldehydes, and FFA)	Milk and milk products	Campbell and Drake (2013)
	Sulfhydryl oxidases	Oxidase the volatile thiol compounds	Removal of cooked flavor in UHT milk	Faccio et al. (2011)
	Limonoate dehydrogenase	Oxidize limonin (a bitter compound of grapefruit and orange) to nonbitter limonoate A-ring lactone	Debittering	Kola et al. (2010)
	Diacetyl reductase	Reduce diacetyl (responsible of unpleasant butter-like note) to 2,3 butylene glycol	Removal of diacetyl from beer	Krogerus and Gibson (2013)
Elimination of off-flavors	Naringinase	Hydrolyzes the naringin (responsible for the bitter taste) to prunin and rhamnose	Debitterize kumquat, grapefruit juice or	Kiefl et al. (2017); Zeng and Huang (2011)

(continued)

Table 12.1 (continued)

Enzyme role	Enzyme	Bound volatiles	Flavor like	Ref
			grapefruit concentrate	
	Laccase	Significant reduction of the concentrations of guaiacol and 2,6-dibromophenol	Elimination of musty, earthy, or like roots	Schroeder et al. (2008)
	(poly)phenol oxidases	Oxidation of poly phenols	Debittering of coffee, cacao, and olives	Kongor et al. (2016)
	Lipoxygenase	Hexanal, nonanal, nonenal, and 1-octen-3-ol	Beany-grassy flavor in soy bean	Iassonova et al. (2009)
Production of off-flavor	Lipoxygenase	2-methoxy-3-isopropyl-(5 or 6)-methyl pyrazine, hexanal, (E,E)-2,4-nonadienal, and (E,E)-2,4-decadienal	Off-flavor in pea milk	Zhang et al. (2020)
	Lipoxygenase	1-octen-3-one, hexanal, (E,E)-2,4-nonadienal, and (E,E)-2,4-decadienal	Off-flavor in soy milk	Zhang et al. (2020)

25 mL milk) (Sung et al., 2011). Lysozyme has also been assessed for making edible coatings and biofilms (Barbiroli et al., 2012) and its synergistic effects were studied in corporation with other natural antimicrobials (Bayarri et al., 2014).

12.2.3 Oxidoreductase

Redox enzymes have applications for extending the shelf life of food products. These enzymes include those that able to remove oxygen or reactive oxygen species, such as H2O2 and superoxide anion, and those that are capable of generating antimicrobial agents. Therefore, the stability of foods can considerably be improved with regard to their appearance, taste, and microbial spoilage.

12.2.3.1 Lactoperoxidases

Lactoperoxidase which belongs to the superfamily of peroxidase-cyclooxygenase enzymes is the most important enzyme present in the bovine milk in concentrations of approximately 30 mg/L. This enzyme shows its antimicrobial activity through the lactoperoxidase system, including lactoperoxidase, hydrogen peroxide, and thiocyanate, which are referred to as the lactoperoxidase system (LPS). The intermediate compounds formed through the oxidation of thiocyanate have antimicrobial activity (Silva et al., 2014). The main application of lactoperoxidase enzyme is to preserve raw milk, specifically in places that it is not readily available to refrigerate it. By

addition of thiocyanate to the milk, the LP system will start showing its antimicrobial activity. It is necessary to add thiocyanate, because of the low amount of thiocyanate in the milk which is not enough to initiate the antimicrobial activity. The antimicrobial activity of the LP system is activated by production of thiocyanate (SCN) oxidation products, primarily hypothiocyanate ions (OSCN), that are able to attack the sulfhydryl groups of major metabolic enzymes of the microorganisms. Moreover, the LP system does not affect the mammalian cells. Usually barely 10–20 ppm of lactoperoxidase is necessary for an efficient system. Also the required amount of thiocyanate and H2O2 is pretty low which are 10–25 ppm and 10–15 ppm, respectively. H2O2 is also bactericidal, but it shows its antimicrobial activity at higher concentrations (300–900 ppm) (Martin et al., 2014). Hence, lactoperoxidase (LPO) is usually used in combination with enzymes that generate H2O2. Moreover, in the LP system the amounts of cofactors and also the oxidation products are toxicologically harmless. LP system has applications in dental products, veterinarian products such as antidiarrheal, milk replacers, and antimastitis, and food products such as functional foods, cheese, fish, meat, poultry products, and liquid milk. There are claimed applications of this system alone or in combination with other systems for pickled foods, fish products, Lactobacillus fermented milk products, and white mold cheeses (Laane & Bruggeman, 2002). Moreover, there is an interest for its effect on yogurt. By addition of LPO to yogurt, the production of unnecessary acid of LAB in it is stifled (Masud et al., 2010). The mentioned applications are becoming accessible now that isolating the lactoperoxidase of milk is doable with high purity on an industrial scale. This system is commercially available now, at considerably low costs. Latest applications recommended for lactoperoxidase are preservation of fruit juices, and also using as coating of foodstuffs (Cissé et al., 2015).

12.2.3.2 Xanthine Oxidases

Xanthine oxidase (XO, EC 1.2.3.2) is broadly spread in microorganisms, plants, and animals. It is able to catalyze the oxidation of hypoxanthine to xanthine and consequently conversion of xanthine to uric acid. Moreover, XO is capable of oxidizing a wide range of pteridines, purines, and aldehydes, with simultaneous reduction of O2 to H2O2. Under definite circumstances, XO also is able to produce the highly reactive superoxide anion. Bovine milk is a good source of XO containing 35 mg. L of it. (Lin et al., 2015). XO also produces superoxide anion during oxidation of its substrates and in this way it involves the oxidative deterioration dairy products (Zarepour et al., 2010). This enzyme can also make a bactericidal or bacteriostatic agent in milk by producing H2O2 which is used in the lactoperoxidase system of milk (Whitaker et al., 2002).

12.2.3.3 Superoxide Dismutases and Catalases

Superoxide dismutase (SOD; EC 1.15.1.1) and catalase (EC 1.11.1.6) are the enzymes in milk that are capable of removing reactive oxygen species formed by other chemical or biochemical processes (Koh & Kim, 2001). Application of SOD is for catalyzing the superoxide anion (produced by XO) reduction, to O2 and H2O2. Consequently, catalase is able to convert H2O2 to oxygen and water. So application

of exogenous SOD, together with catalase, is a very efficient antioxidant in dairy products (Koh & Kim, 2001). Also it was shown that SOD can be a protection against free radical damage in beer (G Yao et al., 1987). Clearly, the commercial application of SOD as an antioxidant depends on the cost, especially rather than chemical antioxidants. However, it is known that SOD has not commercial application as an antioxidant in food systems.

Catalase have application for cold sterilization of milk in areas without refrigeration and also it is used for the treatment of cheese milk in developed countries (Czyzewska & Trusek, 2018). Sweet potato, *Aspergillus niger*, liver, and beef are good sources of catalase. There is also growing interest in using immobilized catalase reactors for milk pasteurization or glucose oxidase–catalase reactions (McSweeney, 2016). In addition to the application of SOD and catalase for elimination of reactive oxygen species, there are other enzymes, such as ascorbic acid oxidase, alcohol oxidase, glucose oxidase, and D-amino acid oxidase, that can be used to remove them but in lower amounts. The drawback of these enzymes is that they produce H2O2, which is a potent oxidant. Addition of catalase can be a way for removing H2O2, but then oxygen is produced again. Lately, new enzymes (such as laccase from PPOs) have been stated to use for deoxygenation of juices and beer (Nunes & Kunamneni, 2018). These enzymes remove oxygen more efficiently and do not produce H2O2 which is a benefit of using them; therefore, it is not needed to combine them with catalase. Catalase is usually used in combination with glucose oxidases for food preservation. Ough (1975) used a catalase with glucose oxidase for removing the oxygen from wine prior to bottling and assessed the acetaldehydes formation. Results indicated that proper treatment of the wine with the enzyme makes the amount and color of acetaldehyde stable (Röcker et al., 2016). Catalase is also used in milk processing industry to remove peroxide of the milk; it is used in bakery industry for removing glucose from egg white. Also in food wrappers this enzyme is used to inhibit and control oxidation and perishability of food (Röcker et al., 2016). However, this enzyme has limitations for using in cheese industry (Sindhu Raveendran et al., 2018).

12.2.3.4 Glucose Oxidase

Glucose oxidase is usually used in baking industry for making better dough due to its oxidizing effects. Moreover, this enzyme increases the aroma, flavor, and stability of food products by eliminating oxygen and glucose from egg white and diabetic drinks. Glucose oxidase modifies the flavor, texture, color, and shelf life of food products and inhibits decay and spoilage. Glucose oxidase is used to extend storage life of food products during packaging, by eliminating oxygen (Hanft & Koehler, 2006).

12.3 Conclusion and Future Prospect

Recently, there is a developing attention for food research by the international scientific community, and the principal areas of their focus include the increased production of food products, modification of foods with improved nutritive value, flavor development, increasing shelf life, strengthening food protection with procedures to preserve food from food pathogens, packaging technology and materials, and quality control. Food industry researchers are directing serious researches about the improvement of modified foods with additives (Chandrasekaran, 2012). The World Health Organization (WHO) food safety unit has stated the role of microorganisms and derived enzymes from them for getting safe, secure, and nutritive foods. Therefore, this can be applied to food processing industries. It is important to determine and select the proper fermentative processing technologies and then to improve, and apply them in the food industries. These technologies can be used for preparation and storage of food, in addition to transforming the vast amount of waste materials, resulting in the environmental damage.

Using of bioprocesses involving enzymes in food processing can decrease the antinutritive factors and toxins of the by-products, besides solving the environmental issues and increasing their consumer acceptance by enhancing their nutritive value. Thus, food industries are changing their functions toward green processes. However, widespread research and control on the stability of bioprocessed products, food-borne illness, development of fermentation process, and risk of food contamination are needed (Chandrasekaran, 2012). Currently, industry is considering the enzymatic catalyzes as a way to produce high amount of its products in environmentally friendly green processes, also as a tool to make additional value to the final product and increase the economical production. Therefore, the increased need for enzymes and enzymatic processes is making a new movement in enzyme technology, supported by improvements in chemical sciences biotechnology.

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Enzymes from Genetically Modified Organisms and Their Current Applications in Food Development and Food Chain

13

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Abstract

The fulfillment of nutrition needs is in high demand due to the increase in population, especially in developing countries with a reduction in arable land. Though the use of natural enzymes from plants, animals, and microbes is high, the demand and modern food processing conditions require improved enzymes both qualitative and quantitatively. Current recombinant DNA technologies such as altering genetic code and introduction of a gene allow genetic modification in an organism, which gives desired products. Some of the genetically modified foods are on the market with rigorous safety assessment and many of them are under development. Genetically modified enzymes that ameliorate the processing of carbohydrates, protein, and lipid are in pipeline to use in various food industries. This chapter emphasizes the enzymes derived from genetically modified organisms; the methods used to generate genetically modified organisms and the use of these enzymes in food industries for the application of various food development and food chain. The chapter will also highlight people's thoughts and safety concerns about the use of enzymes from genetically modified organisms.

Keywords

Enzymes · Recombinant DNA technology · GM foods · Applications

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A. Dutt Tripathi et al. (eds.), *Novel Food Grade Enzymes*,
https://doi.org/10.1007/978-981-19-1288-7_13

13.1 Introduction

Enzymes are proteins (also referred to as biocatalysts) that can be extracted from cells, which catalyze the biochemical reactions in living organisms. They can be used to catalyze a good range of commercially important processes in paper and pulp, textile, chemical and pharmaceutical, agriculture, and food industries. Among food, enzymes represent a major share in the enzyme industry. For many years, enzymes are utilized in food processes, like for the clarification and filtering of wines and beers, for baking, the assembly of cheeses, and far more (Robinson, 2015). Global population is accelerating and is estimated to reach ten billion by 2057. The use of industrial enzymes is also estimated to increase in correlation with the population from 5.8 billion USD in 2020 to 20 billion USD in 2057 (Fig. 13.1). The preferred source of enzymes are plant and animal, which constitutes around 15% and the remaining 85% of industrial enzymes are from microorganisms such as fungi and bacteria. The economic and technical ease put microorganisms forefront of enzyme synthesis.

The development of recombinant technology brought considerable improvements in the enzyme industries by generating Genetically Modified Organisms (GMOs). In today's market, around 50% of the enzymes are derived from these GMOs, which are intended to increase their yield, purity, specificity, and stability (Petersen, 2005; Poulsen & Bucholz, 2003). As per FAO (Food and Agriculture Organization of the United Nations). GMOs are "the organisms those not occur by natural reproduction or natural recombination." GMOs are the major source of enzyme production in food industries that requires a comprehensive understanding of the integration between enzymology, molecular biology, and bioinformatics. The enzymes from GMOs especially from the microorganisms have several safety concerns such as contamination with toxins, allergens, and other impurities (de Santis et al., 2018; Srivastava, 2019). Thus, before getting into the marketing, enzymes from GMOs need approval from regulatory bodies such as the Food and Drug Administration (FDA; USA) and European Food Safety Authority (EFSA; Europe) with rigorous assessment in addition to regular ethical concerns. This chapter deals with the enzymes from GMOs; applications in food industries and regulatory concerns for their use.

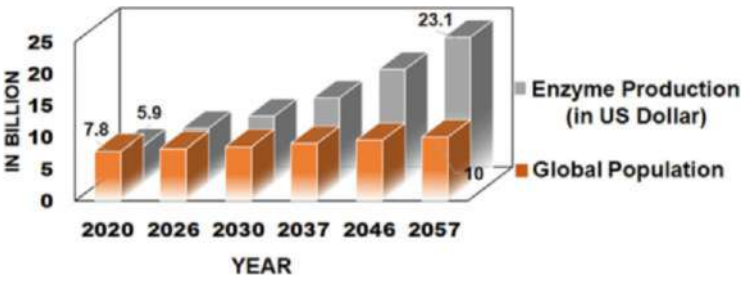

Fig. 13.1 Projected global population and enzyme production

13.2 Use of Genetically Modified Organisms for Enzyme Synthesis

The biological variants form very often due to natural recombination and mutation by changes in the surrounding microenvironment. More than a century, we also exploiting the genetic materials that are naturally present in animals, plants, and microbes. Initially, the geographic distribution of genetic variants occurred unknowingly from one part of the world to another with distinct climate conditions. For example, Asian soybean to America and American potato throughout the world. The history of genetic engineering begins with Charles Darwin's notions of species variation and selection. The evolution of biotechnology leads to the development of biological techniques that help to overcome unbeatable physiological barriers and to exchange genetic materials among all living organisms. In late 1950s and 1960s, the scientists discovered the double helix structure, central dogma, and genetic code which led to the development of DNA recombination technology in 1973 by Cohen et al. The development in the DNA recombination technology showed that the genetically engineered DNA molecules can be transferred among same species and in different species.

13.2.1 Genetically Modified Organisms

According to World Health Organization (WHO), GMOs are the organisms (i.e., plants, animals, or microorganisms) in which the genetic material (Deoxyribonucleic acid) has been altered in a way that does not occur naturally by mating and/or natural recombination. The genetic modification occurs in two ways; either mutation or recombination, which may be spontaneous/natural or induced. The evolution of biotechnology leads to the development of biological techniques that help to overcome unbeatable physiological barriers and to exchange genetic materials among all living organisms. The technology is often called modern biotechnology, recombinant DNA technology, or genetic engineering.

Initially, the first-generation GMOs were developed for improving the quality and yield by increasing their resistance to certain diseases and unfavorable environments. In 1983, the first genetically modified plants antibiotic-resistant tobacco and petunias were produced by three independent research teams (Bevan & Chilton, 1982; Fraley, 1983; Herrera-Estrella et al., 1983). In 1990, China first commercialized genetically modified tobacco and in 1994 USA marketed the genetically modified tomato which is approved by the Food and Drug Administration (FDA). Many transgenic crops, such as Canola with modified oil composition, cotton and soybeans resistant to herbicides, etc. have received FDA approvals. Genetically modified potatoes, eggplants, strawberries, carrots, and many more are available in the market (Bawa & Anilakumar, 2013). The second-generation GMOs were aimed to transfer genetic information from one organism to the other to improve specific product quality and quantity.

13.2.1.1 GM Plants

The Food and Agriculture Organization (FAO)/International Atomic Energy Agency (IAEA) listed more than 3000 plants that have been developed using genetic modification with physical and chemical mutagenic agents. These GM foods are either used for the human consumption or used as feed for livestock and poultry.

The first commercialized food crop is papaya, which was genetically modified for its resistance against papaya ringspot virus. Later, some fruit ripening characteristics have been improved using genetic modification. Polyphenol oxidase is responsible for the production of melanin in apples and potatoes, which cause a brown tint when exposed to air. The restriction of polyphenol oxidase synthesis below 10% by genetic modification allowed the apple and potato remain same after slicing. The herbicide-tolerant varieties of alfalfa, soybean, sugar beet, sweet corn (maize), and cotton have been developed using genetic engineering. GM Golden rice, which contains beta-carotene (precursor of vitamin A) along with enhanced iron content has been approved in various countries. At present, more than 170 million hectares are under cultivation of GM crops worldwide (James, 2006).

13.2.1.2 GM Animals

Though GM animals are in the use of biomedical and pharmaceutical applications to produce certain recombinant proteins and to study selective gene functions, the generation of GM animals for food from a variety of target species traits is still on bench side due to the inefficacy of the production and ethical issues. The major causes to attain genetically modified organisms are; to increase animal health, (Tong et al., 2011; Wheeler et al., 2001), to make them disease resistant (Lyall et al., 2011; Richt et al., 2007), to improve growth rate (Devlin et al., 2009), to increase meat quantity (Lai et al., 2006), to increase milk production and alter composition (Wu et al., 2012), and to increase wool production (Damak et al., 1996). The fast-growing Atlantic salmon is the only GM animal that has been approved for food by health Canada and USA-FDA, in which the growth hormone gene from Chinook salmon was transferred into the Atlantic salmon genome. The use of this fast-growing GM fish for the food in future is still uncertain.

13.2.1.3 GM Microorganisms

Microorganisms, produce endogenous enzymes responsible for several food products like yoghurt, cheese, beer, wine, vinegar, and many more. Humans used these microorganisms even without knowing they are responsible for the fermentation process in various food products (Jay et al., 2005; Zhang et al., 2017). During the eighteenth century, the discovery of the microorganisms responsible for a certain process in food industries began to research efficient ways to use microorganisms (Barnett, 2003). The discovery of DNA followed by recombinant technology allowed genetic alteration to optimize the use of microorganisms in effective way for food production. These genetic modifications either improve the production of existing proteins or increase their potential or produce a new protein with different characteristic features. The advancement in molecular biology led to widespread use of GM microorganisms in food industries and is recognized as an environmentally

friendly and cost-effective method for food production. The yeast *Saccharomyces cerevisiae* was the first GM microorganism used for enzymes synthesis in the dairy and brewing industries. The trypsin and chymosin from the GM microorganisms served as an alternate for animal sources such as pigs and cattle. Replacement of certain plant-based products by GM microorganisms could meet the demand with reduced land usage and waste generation. The GM yeast-based production of vanillin, an alternate for vanilla extract; GM bacteria that produce riboflavin are certain examples of the use of GM microbes in food industries (Brochado et al., 2010; Schwechheimer et al., 2016). Other food ingredients known to be produced by microbes are includes vitamins, amino acids, nutritional proteins, oligosaccharides, flavoring agents, and sweeteners (Adrio & Demain, 2010).

13.2.2 Enzyme Synthesis from GM Microbes

According to legal authorities such as FDA, the European Food Safety Authority (EFSA), the enzymes are considered nutritional additives. The major industrial enzyme market relies on the food and beverage industry, which is expected to reach 2.3 billion USD in 2021 (Raveendran et al., 2018). Although the majority of the organisms including plants and animals produce enzymes, microbial sources become more valuable. Table 13.1 shows various industrial enzymes used in the food and animal feed industries.

13.2.2.1 Advantages of Using Microbes for Enzyme Synthesis

(a) Many MO produce enzymes and release into the medium which simplifies the extraction method during the purification. It is easy to extract intracellular enzymes as well with the disruption of microbial cells compared to the extraction process from animal and plant sources (Robinson, 2015).
(b) Specific microbial strains can be used for the yield of well-characterized enzymes with specific properties (Chang et al., 2016).
(c) Cost-effective production due to shorter time and simple facility requirements. The whole production can be achieved in one location where animals and plants need to be transported to extraction facilities (Raveendran et al., 2018).
(d) High yield can be obtained for microbial enzymes compared to animal and plant sources where the availability depends on a specific period, which could limit the continuous production (Singh et al., 2016).
(e) Microbial enzymes usually have a higher activity and stability (Vieille & Zeikus, 2001).
(f) Genetically modified microbial strains can be easily produced in order to obtain higher yields, optimized enzyme products, and better characteristics. Modifications of animals and plants are difficult and ethically more sensitive (Lievens et al., 2015).

The wild-type microbes are unstable and may not produce desired quality enzyme, especially in fermenter conditions. Sometimes the feedback repression occurs that

Table 13.1 Industrial enzymes used in food and animal feed industries

Industry	Enzyme	Function
Dairy	Lactase (beta galactosidase) Aminopeptidase catalase Proteinase (acid and neutral) Lipase Transglutaminase	Lactose reduction in milk, coagulation, faster cheese ripening, debittering, flavored cheese, protein crosslinking
Baking	Amylase Transglutaminase Lipase Glucose oxidase Xylanase	Bread softening, enhance lifespan, dough conditioning, dough stability, and strengthening
Beverage	Pectinase Cellulase Amylase (Alpha and Beta) Beta Glucanase Protease Glucose oxidase Pullulanase Naringinase Limoninase Aminopeptidases	Pectin digestion, starch hydrolysis fruit liquefaction, protein breakdown, oxygen removal, debittering, restrict mist formation
Animal feed	Beta glucanase Phytase Xylanase	Enhance digestion

Fig. 13.2 Major steps involved in the enzyme production from genetically modified microbes

ultimately affect the enzyme production. Considering the advantages of using microbial strain for the food enzyme production, the genetic modifications can be easily done in the microbial strains which improve the industrial enzyme production. GM microbes could overcome these drawbacks and enhance the production of quality enzymes. The GM microbes mainly certain bacterial and fungal strains have been optimized for increased enzyme production with a limited synthesis of unwanted secondary metabolites (Deckers et al., 2020). The GM enzymes are mostly expressed in well-established standard microbial model systems like *Escherichia coli, Bacillus,* lactic acid bacteria, filamentous fungi, and yeast. These microbes have advantageous characteristics over other microbes, which resulted in the use of a number of biotechnological applications. The major steps involved in industrial enzyme production are given in Fig. 13.2. Few strategies that are required to produce genetically modified enzymes are;

1. Codon optimization for the heterologous expression and to limit mRNA folding for optimal translation (Rosano & Ceccarelli, 2014).
2. In-depth understanding of enzyme sequence, corresponding amino acids, structure, and related activity. This will allow one to modify the sequence by mutation-like site-directed mutagenesis to alter or increase the enzyme activity, specificity, and structural stability (Hua et al., 2018).
3. Developing an efficient screening protocol for identifying effective variants (Packer & Liu, 2015).
4. Knowledge about fusion and truncation of target sequence to produce an enzyme with multiple functions and to eliminate the unwanted sequences those are not important for the function (Dediu, 2015).

13.2.2.2 Selection of Host Organism

In order to obtain a successful enzyme synthesis, it is important to select a host organism having suitable characteristics for the production and expression of the specific enzyme and that is relatively cheap to culture. The type of expression vectors, the transformation methods, and the selection markers used to select the transformed strains need to be determined are also important for obtaining genetically modified organisms for use in food industries (Olempska-Beer et al., 2006).

Many expression systems are available for the recombination of bacteria resulting in the easy integration of modifications. Bacteria are not able to perform posttranslational modifications resulting in only simple proteins being produced. In the case of fungal strains, correct folding and posttranslational modifications were done by the subcellular organization and they secrete a limited amount of proteins into the medium (Demain & Vaishnav, 2009). The commonly used bacterial host strains are *Escherichia coli, Bacillus subtilis, Bacillus licheniformis, and Bacillus amyloliquefaciens*. Availability of molecular techniques for the construction of an expression system in *E. coli* results in the high-yield enzyme production in the cytoplasm and additional purification steps are required. However, the Bacillus species secrete higher concentrations of the enzymes into the medium (Puetz & Wurm, 2019). *Aspergillus niger, Aspergillus oryzae, and Trichoderma reesei* are commonly used filamentous fungi. A. oryzae used for the production of fermented food, which is also known to produce mycotoxin aflatoxin. The mutant strain was constructed to overcome this issue, in which the production of these products was reduced (Meyer, 2008).

13.2.2.3 Gene Transfer Methods

Expression Vector System

Once a suitable host has been selected, the selected host strain is modified by using the expression vectors. A vector is a DNA molecule that is a vehicle to transfer foreign genetic material into another cell. Plasmids, viral vectors, cosmids, and artificial chromosomes are the different types of vectors. Plasmids are called expression vectors those express transgene in the target cell, and they generally have a promoter sequence that drives the expression of the transgene. A typical expression

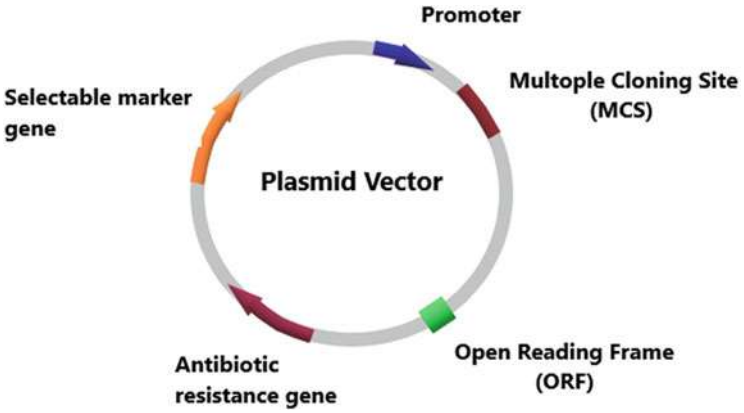

Fig. 13.3 Basic structure of expression vector used in recombinant enzyme synthesis

vector is shown in Fig. 13.3. An expression vector contains, at the least, an origin of replication (ORI), a Multiple Cloning Site (MCS), in which the gene of interest will be integrated, and a selection marker for the selection of the recombinant strain (Fig. 13.1). Promoter and terminator regions are also present to control the expression of the gene of interest and the selection marker. In order to avoid an increase in the metabolic load on the host organism and to avoid reduced plasmid stability, the size of the vector is kept as small as possible (Rieder et al., 2019).

It is important to select the recombinant strain and the selection is based on the selectable marker. A selectable marker is usually a gene that shows resistance to an antibiotic that would otherwise kill the normal cells which lack these genes under culture conditions with a specific antibiotic. Kanamycin (kanR), ampicillin (ampR), and tetracycline (tetR) resistance genes are commonly used in the bacterial expression vector. Antibiotic resistance markers are associated with a public health concern, which is replaced by auxotrophic strains, where it requires specific nutrients for growth. Some auxotrophic markers are amdS, URA3, which are usually used as the selectable marker in fungal strains (Hjort, 2007; Olempska-Beer et al., 2006).

Preparation of GM Microbes

The common method employed in the preparation of GM microbes is to cut open the plasmid DNA; inserting the target DNA molecule (named molecular cloning) and transform it into host microbes, which can efficiently express the target DNA. Dissecting each part, (1) the plasmid expression vector has been chosen based on the host organism; (2) the target DNA has been amplified from the target genome which has restriction enzyme site overhanging on both the ends that matching to the sites present in the multiple cloning site of the plasmid; (3) restriction digestion of the plasmid vector and amplified target DNA molecule using restriction enzymes (which can form either blunt or sticky ends); (4) Purify the restriction digested DNA and plasmid; (5) Ligate the plasmid and target DNA using ligase; and (6) transform the recombined plasmid to the host organism. The recombinant DNA molecule can

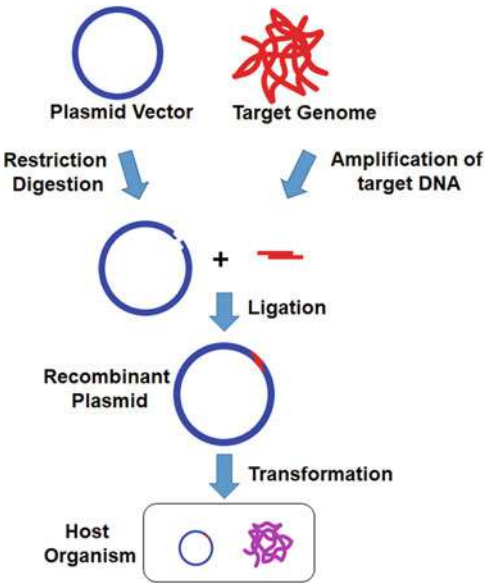

Fig. 13.4 General recombinant technology involved in the recombinant enzyme synthesis

be transformed into cells of higher organisms (Fig. 13.4). Transformation of the target DNA is done by transfection in which the introduction of nucleic acids into cells by nonviral methods. Calcium phosphate, DEAE-dextran, or other substances are nonviral methods of transformation. Physical methods used are electroporation which cellular membranes of an organism are disrupted by using an electrical pulse, direct microinjection into a cell, and biolistic particle disruption into cells using gene guns. The plasmids are the most commonly used DNA carrier molecules and the viral DNA molecule also can be used to stably express the target enzyme. Plasmids are isolated from bacterial cells and specific sequences of DNA molecule are altered in vitro by insertion or deletion. Antibiotic resistance, ß-galactosidase, luciferase, etc. act as gene reporters for the screening of transformed cells.

Other Gene Editing Options

Several molecular tools are used to insert desired DNA sequence directly into the genome or delete unwanted DNA bases from the genome. For example, the insertion of functional genes such as active promoter sequence activates the production of the desired enzyme and the insertion of transporter sequence can improve the transport of enzymes. The source of DNA can be either endogenous or exogenous from closely related/distinct organisms. Currently, the use of synthetic DNA allows fast development of GM microbes rather than isolating and amplifying from the other organisms. Recent techniques allow to insert DNA sequences to specific loci, which can be confirmed by genome sequencing. Today, the discovery of CRISPR (Clustered Regularly Interspaced Palindromic Repeats) facilitates insert or delete DNA sequence at precise position using specifically designed guide RNA sequences that can recognize specific DNA code.

13.2.3 Enzyme Extraction Process

The extraction and purification of enzymes from GM microbes is much easier compared to plant and animal sources. Depending on the enzyme of interest and the host-microbe, the growth environment, such as pH, temperature, gas exchange rate, and substrate can be optimized during the extraction process while using GM microbes in comparison with wild-type organisms. Current genetic engineering techniques enabled the large-scale production of enzymes for use in food and other industrial applications. The level of downstream processing mainly depends on the application of the finished product (Headon & Walsh, 1994; Wheelwright, 1989). The overall enzyme extraction and purification process is illustrated in Fig. 13.5. The enzyme extraction process at the industrial level involved two basics bioreactor-based fermentation methods, which are (1) submerged and (2) solid-state fermentation.

1. **Submerged Fermentation**
   Submerged fermentation (SF) is the most preferred method for industrial-level enzyme production, where the selected GM microbes (bacteria or Fungi) are cultured in the liquid nutrient medium at a higher concentration of oxygen. In this process, the microbes release the enzymes to break down the complex carbohydrates, proteins, and lipids in the medium. A large fermenter with a high capacity of around 1000 cubic meters is used to produce a large quantity of enzymes. Simple batch reactors are used, where the reactor is filled with selected microbe and nutrients and allowed for fermentation. After fermentation, the contents are emptied for further enzyme purification. On the other way, the batch-fed process is a continuous process, where the sterile nutrient mix is added

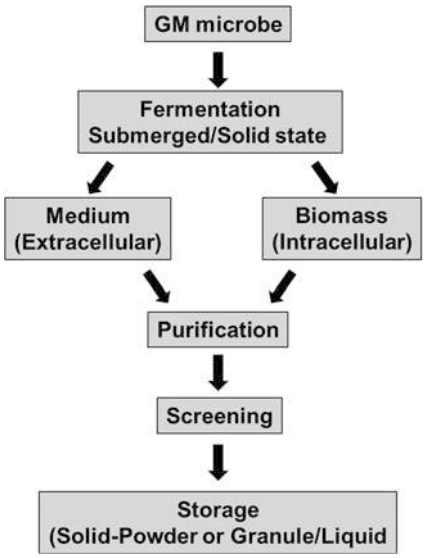

Fig. 13.5 Enzyme extraction and purification process from GM microbe

to the fermenter at the same level of fermented broth leaving for further enzyme purification.

2. **Solid-State Fermentation**
   Solid-state fermentation (SSF) is another method that involves the culture of the GM microbe on a solid substrate, such as pulp, corn, wheat or rice bran, and grains. The advantages of SSF over SF are; a high concentration of enzyme production with less affluent in a simple fermenter. In SSF, the factors such as particle size and moisture play a major role in enzyme production. For example, smaller substrate particles make larger surface area microbial growth, whereas larger particles provide efficient aeration. In SSF the water level should be optimized for enzyme production, as moisture level greatly influences the microbial activity.

The enzyme produced during fermentation should be further processed to purify the enzyme from the biomass and other byproducts. First, the solid waste and the biomass are removed from the medium and the solution is further concentrated using filtration, evaporation, and crystallization. Chromatography method such as ion-exchange chromatography is employed to remove impurities. If the enzyme is localized intracellular, the biomass has to be collected and processed further for enzyme purification. Depending on the nature and use of enzymes, the enzymes can be further stored in lyophilized powder form, liquid form if crude, or granule form. Some enzymes are immobilized on the surface of inner granules.

13.3 GMO-Derived Enzymes in Food Industries

From the ancient period onwards a wide variety of enzymes from a natural source has been used for the production of numerous food products. Natural sources for obtaining enzymes are animals, plants, and microorganism. There are about 3000 known enzymes produced by all animals, green plants, fungi, and bacteria that catalyze about 4000 biochemical reactions (Patel et al., 2017). Among these sources, microorganisms have been involved in the food industry for various applications like the production of alcoholic beverages, ethyl alcohol, organic products, dairy products, and drugs including antibiotics through fermentation (Verma et al., 2018). For example, the usage of chymosin for cheese production and pectinases for fruit and beverage processing (Chandrasekaran, 2015). In modern food processing methods extreme conditions, such as high temperature, high pressure, extreme pH, salinity, etc., are required to improve the commercialization of enzymes and their products (Li et al., 2012). This condition may affect natural enzymes. So, most of the enzymes produced by microbes are no longer native enzymes, but instead engineered versions. Genetic modifications through genetic engineering and recombinant DNA technology creates Genetically Modified Organisms (GMOs) that have specific modifications introduced into genetic material which enable the production of new protein or food ingredient, enhance the production, and modify the characteristics of an existing enzyme for a new application (Maghari &

Table 13.2 Common GM Microorganisms involved in food enzymes production

Microorganism	Species
Bacteria	Escherichia Coli Bacillus megaterium Bacillus brevis Lactobacillus lactis Pichia pastoris
Yeast	Saccharomyces cerevisiae Hansenulapolymorpha, Schizosaccharomycespombe Yarrowialipolytica
Filamentous fungi	Aspergillus oryzae Aspergillus Niger Trichoderma reesei Penicilliumpurpurogenum Rhizopusoryzae

Ardekani, 2011). Thus, the quality and productivity of enzymes can be improved. Enzymes obtained from genetically modified organisms are intended to improve or alter the enzymological properties or to increase the purity and yield of enzymes. Although there are challenges and safety concerns for using recombinant proteins in food processing, genetically modified enzymes are promising because of their potential benefits for the food industry, consumers, and the environment (Zhang et al., 2017).

Food enzymes are commonly used to perform a wide variety of applications in food production, which include; lactase that reduce lactose in food and dairy products, amylases that strengthen dough in bakery, mannase that helps in coffee production, proteases that hydrolyze protiens, chymosin that used to make cheese, glucoamylase, and transglucosidase those reduces starch into simple sugar and phospholipase that refine vegetable oil. Considering the safety and synthesis of other metabolites, selected bacterial, yeast, and fungal species are commonly involved in the recombinant enzyme synthesis (Table 13.2). The list of major enzymes derived from GM microbes and their function are given in Table 13.3. The main industrial enzymes can be classified into three groups: carbohydrases, proteases, and lipases (Castro et al., 2018). Microorganisms secrete a variety of enzymes that plays an important role in the food manufacturing industries (Hanlon & Sewalt, 2020). With the growing research on genetically modified organisms, production, and development of these enzymes are also getting better.

Carbohydrases: Carbohydrases belonging to glycosidase family are a set of enzymes that catalyzes hydrolysis of complex carbohydrates (polysaccharides) into simple sugars (monosaccharides). These enzymes are used in the food industry for baking, brewing, sweetener production, etc. Carbohydrases include amylases, cellulases, glucanases, pectinases, xylanases, invertase, galactosidase, and others (Castro et al., 2018).

Amylase: Amylase catalyzes the hydrolysis of glucosidic linkages of large polysaccharides (starches) into oligosaccharides. These enzymes are used in the

Table 13.3 List of major GM food enzymes and their functions

Food enzymes	Functions
Amylase	Breaks down starches and sugars found in grains, fruits, and vegetables
Galactosidase	Breaks down the polysaccharides and oligosaccharides in foods, such as legumes and cruciferous vegetables
Beta-glucanase	Breaks down plant cell walls (cellulose) beta-linked glucose polymers associated with fibers, grains, and cereals
Cellulase	Breaks down food fiber (cellulose) found in fruits and vegetables
Glucoamylase	Breaks down off long chain carbohydrates or starches (corn, potatoes, wheat and rice) into sugar
Invertase	Breaks down simple sugar (fructose and fruit sugar)
Lactase	Breaks down lactose found in milk
Lipase	Breaks down fats and lipids
Pectinase	Breaks down pectin, a polysaccharide found in plant cells in many fruits and vegetables
Peptidase	Breaks down proteins into amino acids
Phytase	Breaks down carbohydrates; helpful in the breakdown of phytic acids in the leaves of plants, grains, seeds
Protease	Breaks down protein found in meats, fish, poultry, grains, nuts, gluten, milk proteins (caseins)
Xylanase	Breaks down plant nutrients from vegetables with high fibercontent (fibrous veggies, grains, and legumes)

production of maltodextrin, modified starches, glucose and fructose syrups, production of bread, cakes, and pastries (De Souza & Magalhaes, 2010). These enzymes degrade starch in the flour used as dough of bread into smaller dextrins which enhance the rate of subsequent fermentation by the yeast and reduce the viscosity of dough which in turn improves the volume and texture of the product. Through the action of enzyme additional sugar is produced in the dough, which improves the quality of the bread, such as taste, crust color, and toasting qualities (van der Maarel et al., 2002).

The conditions during the development of fermentation processes are very important such as thermostability, pH profile, pH stability, and Ca-independency. Industrial source of α-amylase is the bacteria *Bacillus licheniformis*. Usually, the α-amylase produced by wild-type *Bacillus licheniformis* is thermostable but acid-sensitive. Genetically modifying *Bacillus licheniformis* through directed evolution by replacing active site domain His residues with Arg and Asp residues produces α-amylases having higher activity at pH 4.5 than the wild type enzyme which enhances the properties of an enzyme such as starch liquefaction (production of nutritive sweeteners from starch), saccharification and fermentation processes where the pH of the starch slurry is usually <6.0 (Liu et al., 2014). Genetically modifying *Rhizopus oryzae* using site-saturation mutagenesis of His 286 produces α-amylases having higher optimum temperature (60 °C) and lower optimum pH (4.0–4.5) than

the wild-type enzyme which makes the enzyme more suitable for maltose syrup production (Li et al., 2018).

Glucosidase: These enzymes are involved in the breaking down of starch and glycogen into their monomers. It is used as a flavor enzyme to enhance the flavor of wine, tea, and fruit juice. β-glucosidases play a critical role in plant biomass deconstruction. β-Glucosidases cause flavor liberation by catalyzing the breakdown of β-1, 4-glycosidic linkages and producing free fermentable glucose used for the production of glucose syrups from fruits and other plant tissues. In modern industrial conditions, wild-type fungal β-glucosidases show weak activity at high glucose concentrations limiting enzymatic hydrolysis of plant biomass. Genetic modification of *Trichoderma harzianum* by site-directed mutagenesis efficiently improved the functional properties of a β-glucosidase where the enzyme displayed high glucose tolerance levels by the substitution of two amino acids that act as gatekeepers, changing active-site accessibility and preventing product inhibition. The efficiency of the engineered enzyme was enhanced by the yield of glucose and ethanol (Santos et al., 2019). Genetically modifying *Aspergillus aculeatus* using site-saturation mutagenesis produces β-glucosidase having enhanced hydrolytic efficiency, especially to cellobiose, and improves saccharification of alkaline-pretreated bagasse (Baba et al., 2016).

Xylanses: These enzymes are involved in breaking down the hemicellulose in the plant cell wall, specifically hydrolyzes *xylan* and *arabinoxylan*. It is used in the application of the food industry for bread making, the production of corn starch, clarification of fruit juice and wine, and alcoholic fermentation. In bread-making process, xylanases are added to the dough for stabilizing the dough by hydrolyzing polysaccharides in the wheat and making it more flexible, and enhancing the gluten strength. Treating with xylanase increases moisture retention and shelf life of the dough (Ahmad et al., 2014). It is also used in fruit juice processing where it breaks down the polysaccharide plant cell walls to intensify the juice extraction efficiency and thus extracting more nutrients and aroma compounds. Also, it helps in juice clarification by decreasing cloudiness (Bajaj & Singh, 2010).

Even though xylanases have potential applications there is a need for xylanases that can endure relevant processing conditions and industrial settings. A variant of this enzyme generated using site-directed mutagenesis exhibited higher specific activity and was utilized in the production of xylooligosaccharides from wheat straw under thermal and alkaline conditions (50–65 °C, pH 7–10) (Faryar et al., 2015). Endo-b-1,4-xylanases with modification in secondary binding sites also showed increased specificity toward water-insoluble, but not water-soluble, wheat arabinoxylan, and its application in bread making resulted in increased loaf volumes (Leys et al., 2016).

Glucanase: The glucanase enzymes help in the breakdown of polysaccharide glucan by hydrolyzing the β-glycosidic bonds between glucose molecules in glucans. This enzyme is mainly employed in the brewing industry where it hydrolyses barley gums. It helps in beer brewing by lowering the viscosity of warts, improves clarification by breaking the turbidity system by hydrolyzing the beer haze, and aid in production of clear wart. β-glucanase is usually found

endogenously in barley itself and is called endo-b1, 3-1, 4-glucanases having an optimum pH and temperature values of 6.0 and 45–50 °C; and denaturizes at 60 °C. So, industrial source β-glucanase from microbial organisms is used for the standard production of light beers. Industrial sources for glucanases are *Bacillus amyloliquefaciens* and fungi of the group In the brewing industry β-glucanase hydrolyzes barley gums. Glucanases are also used during wine-making processes where it hydrolyses *Botrytis* and yeast glucans and thus enhance the clarification and wine filterability. β-1, 3-1, 4-glucanases used in malt extract production in the brewing industry are obtained from *Bacillus* species. Genetically modified forms of β-1, 3-1, and 4-glucanases were produced by replacing Lys with Ser and these modified glucanases showed higher efficiency under industrial operating conditions with improved optimal temperature and thermostability and halostability following replacement of hydrophobic Lys 48 with Ala and Leu (Lee et al., 2017).

Invertases: Carbohydrases that hydrolyze sucrose and polysaccharides into fructose and glucose as final products. They are used as a catalytic agent in obtaining an artificial sweetener and so it is used in food industry for confectionery, syrups, condensed milk, infant milk, and beverages (Kotwal & Shankar, 2009). When sucrose is present in higher concentrations invertases are able to synthesize fructo-oligosaccharides through fructotransferase. Though the natural sources of invertases are plants, bees, and microorganisms, the most prominent organisms used for invertase production are the filamentous fungi belonging to the *Aspergillus* genus and yeast such as *Saccharomyces cerevisiae* and *Candida utilis* (Veana et al., 2018). From *Aspergillus* genus *A.niger, A.terreus, A.japonicus, A.versicolor A.tubingensis, A.fumigatus, A.oryzae, A.aculeatinus,* and *A.homomorphus* are the main source.

The most important characteristic required for the invertase application in the industry are pH and thermostabilities (50–75 °C). Fungal invertases have these features, i.e., they have these optimal values and stabilities but low-specific activity. This limitation had been overcome using gene modification in the host organism. Invertase produced by genetically modified *Saccharomyces cerevisiae* where the enzyme was modified by the substitution of hydrophilic residues in the active site region or peripheral loops with hydrophobic amino acids have optimal pH and stability ranges for sucrose breakdown into glucose and fructose (Sainz-Polo et al., 2013) Genetically modified invertase with high transfructosylating activity is used for simple and efficient production of prebiotic fructo-oligosaccharides.

β-galactosidase: commonly known as lactase, is an enzyme that hydrolyzes lactose. This enzyme has wide applications in food processing industries. It helps in the digestion of lactose present in dairy products and converts it to glucose and galactose. This enzyme holds importance in food industries as it manufactures lactose-hydrolyzed products which act as a "predigester" for people with milk intolerance problems, milk destined for cheese and yogurt making, for the production of sweeteners and hydrolyzed whey syrups (Dekker et al., 2019). Lactases are used in the production of ice cream and sweetened flavoured and condensed milks. The sources of *β*-galactosidases are microbial, plants, and animal origin. Among these sources, microbial lactase shows higher productivity, and it is cost-–effective. Bacteria, fungi, and yeasts are the microbial sources. On a commercial and industrial

scale, the most commonly used sources of β-galactosidase are *Aspergillus* and *Kluyveromyces* (Saqib et al., 2017). The enzyme source is selected depending on the required reaction conditions. Bacterial β-galactosidases work with optimal pH between 2.5–5.4 and are mainly used for acidic whey hydrolysis whereas yeast β-galactosidase shows maximum activity at pH 6.0–7.0 which is more suitable for the hydrolysis of milk and sweet whey. The temperature at which lactose-free milk product is processed is relatively low. The lactase derived from the dairy yeast *Saccharomyces lactis* has optimal process conditions (35–40 °C, pH 6.6–6.8) that are close to the natural temperature and pH of the milk (O'connell & Walsh, 2006). It shows considerable activity at lower temperatures, down to about 4 °C, and slows the growth of spoilage bacteria thus it is the most widely used commercial lactase source. Another source for lactase is fungal lactase derived from *Aspergillus niger*. Its optimal process conditions are around 50 °C and pH 3.5–4.5 so its application is limited to acid (or acidified) whey or lactose.

To satisfy the growing demand for lactase the development of genetic engineering technology makes the large-scale industrial production of lactase possible through the introduction or modification of genes to promote characteristics such as higher enzyme activity or higher production. Modification of lactase produced by microbes through directed evolution had high activity in industrial type conditions for milk processing, that is, substrate lactose, buffer pH 6.75, and 8 °C. The production of recombinant strains using the *lac*A gene from *Aspergillus niger* and *Saccharomyces cerevisiae* can produce a high quantity of lactase (Domingues et al., 2010).

Proteases/peptidases: The major use of proteases in the food industry is the hydrolysis of protein matrices to enhance flavor, texture, or functional properties in dairy, meat, and fish products. Commonly used food proteases are derived from animals which include trypsin, chymotrypsin, chymosin/rennet, pepsin, etc., and papain, bromelain, and ficin from plants and from microorganisms microbial acid protease, proteinase A, alkaline proteases, flavourzyme, etc. (Mazorra-Manzano et al., 2018). Microbial proteases had vital roles in the production of traditional fermented foods.

Trypsin: Trypsin belongs to the serine protease superfamily that cleaves on the carboxyl end (C-terminal) arginine (Arg) and lysine (Lys) on peptide chains. Trypsin is used as a baking enzyme to improve the workability of dough, in the extraction of flavorings from vegetable or animal proteins, to control aroma formation in cheese and milk products, to improve the texture of fish products, to tenderize meat, in the production of hypoallergic food, i.e., proteases can break down specific allergic components present in cow's milk into nonallergenic peptides and diminishes the risk of babies developing milk allergies (Bu et al., 2013).

Usually, trypsin for food uses has been obtained from purified extracts of bovine or porcine pancreatic tissue but the trypsin from mammal sources has several limitations, including high costs, security, and ethical issues. So microbial trypsin is employed for easy preparation and low costs. But there have been a few strains, including those from the genera of *Fusarium*, *Streptomyces*, and *Trichoderma* reported. The reported microbial trypsins show poor specific activity and operational

stability. Thus, trypsin is derived from genetically modified microorganisms for example *F.venenatum* containing the gene for trypsin from *F.oxysporum*, which is used as a food processing aid. The genetic modifications improve serine protease enzyme yields. Studies also showed that trypsin produced by recombinant *Streptomyces griseus* showed higher enzyme activity.

Chymosin (rennin): is an aspartyl proteinase. It is found in the fourth stomach of the unweaned calf. It leads to limited or partial proteolysis of K-casein in milk resulting in clotting. The enzyme is used in the food industry as a processing aid for the manufacture of cheese and curd and other milk coagulants. As an industrial enzyme it is widely used in cheesemaking and it was mainly extracted from dried calf stomachs. But the cheesemaking industry has expanded beyond the supply of available calf stomachs and in order to meet the increasing global demand expression of chymosin has been achieved in many species such as *Escherichia coli*, *Bacillus* species, *Aspergillus* species and yeast (Wei et al., 2016). Thus 80% of fermentation chymosin is produced as recombinant proteins in microorganisms with protein produced in *Aspergillus* and yeast the most widely used.

Expression of yak chymosin in gene-recombinant *Pichiapastoris* is the method used for large-scale production of chymosin (Ersoz & Inan, 2019). Some *Lactococcus* spp. also help in the production of chymosin at an industrial scale. Some other common microorganisms that are involved directly or indirectly in the production of chymosin are *E. coli*, *Kluyveromyces lactis, P. pastoris, Aspergillus awamori.*

Aminopeptidases: Enzymes that hydrolyzes the peptide bond of N-terminal amino acids in peptides and proteins. Cleavages by the peptidases will change the flavors of the proteins in a food matrix. Therefore, aminopeptidases are used in the production of cheese, beverages, flavorings, meats, and milk products (Nandan & Nampoothiri, 2020). They are specifically meant to enhance and optimize aroma and flavor. The addition of aminopeptidases can accelerate the maturing of cheese. Various recombinant aminopeptidases have been produced from *Lactobacillus rhamnosus*, *Lactococcus lactis,* and *Aspergillus sojae*.

The activity and stability of proteases depend on the amino acid composition and their respective position. Protein engineering modifies the protease structure in such a way that enhances the activity, specificity, and stability of protease. Bacillus spp. are the main producers of alkaline proteases with high thermal and pH stabilities. The cold activity at 10 °C and alkali-resistant properties of a *Bacillus alcalophilus* alkaline protease were enhanced through directed evolution using error-prone PCR for use in cold-temperature food processing (Liu et al., 2014). Similarly, an alkaline serine protease from mesophilic *Bacillus pumilus* was engineered to have increased hydrolytic efficiency at 15 °C without compromising thermostability (Zhao & Feng, 2018). Acid protease from a mutant *A. oryzae* strain was produced using solid-state fermentation with potato pulp powder, with enhanced glycine releasing activity (Murthy & Kusumoto, 2015). A truncated neutral protease from *A. oryzae* had optimum pH of 8.0 and an optimum temperature of 55 °C, and its enzymological characterization suggested that it was efficient in antihypertensive peptide production, debittering, and food oil processing (Ke et al., 2012).

Lipases: Lipases are the enzymes that catalyses the hydrolysis of long-chain triglycerides (fats and oils). These are used for the following applications: dairy, baking, cocoa butter substitutes, human milk fat substitutes, egg processing, and edible oil production. In baking and dairy applications, the addition of lipases enhances and accelerates the development of aromatic notes (Goncalves et al., 2019). In vegetal oil processing, lipases allow a significant increase in oil yield and enhance the appearance of end product, i.e., improves their texture and softness. Also, lipases are used to enhance the emulsification properties of egg yolk lipids. These enzymes help in developing new functional ingredients and functional foods, such as cocoa butter equivalents or human milk fat equivalents. Lipases help in the breakdown of milk fats and provide characteristic flavors to cheeses. The flavor is due to the release of free fatty acids produced during the hydrolysis of milk fats. Microbial lipases are produced by fungal, yeast, and bacterial species. *Candida sp., Aspergillus sp., Rhizomucor sp., Rhizopus sp., Humicola sp., Yarrowia lipolytica,* and *Pseudomonas sp.,* etc. are microbial sources for lipase (Tan et al., 2003).

Lipases are used widely for different applications in the food industry and thus it is necessary to meet the increasing demand and enzymes capable of tolerating industrial conditions. Lipase secretion by *Rhizopus oryzae* was improved by rational design of the N-glycosylation sites especially for the use in the edible oil and fat industries (Yu et al., 2017). Genetically modified *Candida rugosa* by site-directed mutagenesis produces lipases having high catalytic efficiency for producing fatty acid methyl esters and diglycerides as food emulsifiers (Chang et al., 2014).

13.4 Regulatory Management of GMO-Derived Enzymes

The Generally Recognized as Safe (GRAS) is well-known process that gives general supporting materials such as methodology involved and safety of the enzymes used in food industries from genetically modified organisms. Section 201(s) of the Act [21 U.S.C. 321(s)] exempts the use(s) of a substance that is GRAS from the definition "food additive." In general, the enzyme preparations should confirm the purity and specifications according to "Enzyme Preparations" of the 6th (2008) or the current edition of the Food Chemicals Codex (FCC). According to the USA, if an enzyme is produced to use in meat and poultry products, FDA has to approve it in consultation with the Food Safety and Inspection Service (FSIS) of the U.-S. Department of Agriculture. The Food Allergen Labeling and Consumer Protection Act of 2004 (FALCPA) is used to prevent food allergens contamination from enzyme. Genetically modified food enzymes, which are produced from genetically modified organisms fall under the regulation, (Regulation (EC) No 1829/2003) according to these regulation food enzymes produced from a part or whole of a genetically modified organism (GMO) but it does not contain GMO. Processing aid of the enzymes is not included in this regulation because food produced with GMO are not included in this regulation. Genetically modified microorganisms (GMM) which are producing food enzymes and are available in commercialized preparations need authorization according to the regulations (EC) No. 1332/2008 and (EC) No.

1829/2003. The European regulation (EC) No. 1829/2003 is only regulation for the material which is produced from genetically modified sources is used in enzyme preparation or food production. Food enzymes fall under two categories either an ingredient or processing aid. The risk assessment performed by the European Food Safety Agency (EFSA) for food additives requires European regulation (EC) No 1129/2011.

There are many other European regulations for the safety evaluation for use of food enzymes in terms of food additives, enzymes, flavorings they are (1) (EC) No. 1332/2008: Food Enzymes (2) (EC) No. 1334/2008: Food Flavourings (3) (EC) No. 1331/2008: Common Authorization Procedure (4) (EC) No. 1333/2008: Food Additives (The European Parliament and the Council of the European Union, 2008). Some specific food enzymes had already been given authorization for use according to regulation (EU) No. 231/2012 (The European Parliament and the Council of the European Union 2008), which allows the specifications according to regulations (EC) No. 1333/2008 and (EC) No. 1493/1999 lysozyme and invertase are authorized for use in food additives. Lysozyme, Urease, and β-glucanase are authorized for use in wine production according to regulation (Deckers et al., 2020).

13.4.1 Safety Concern for GMO-Derived Enzymes

Deriving food enzymes from Genetically Modified organisms have raised concerns regarding the chance of contaminating the food with bacterial toxins or mycotoxins, or some uncharacterized extraneous substances that may act as allergens (de Santis et al., 2018). Since the organisms itself is genetically modified there is also a chance for modifying their allergenic properties which increases the safety concerns (Olempska-Beer et al., 2006) There are situations in which industrial workers exposed to environments using genetically modified enzymes have developed type I sensitization (Budnik et al., 2017). The regulation of enzymes including those produced by genetic engineering is a major concern in the food industry. So before bringing genetically modified food enzymes into the market, it is necessary to obtain approval from various regulatory bodies such as the US Food and Drug Administration, the Association of Manufacturers and Formulators of Enzyme Products, the European Food Safety Authority, and these processes vary in different countries. Also, ethical and religious concerns are raised for genetically modified enzymes, such as the raw materials used in the fermentation of microorganisms for the production of enzymes should be halal (Ermis, 2017).

Genetically modified food enzymes are typically better suited for specific industrial applications than their native counterparts, and research on their enzymological properties in comparison to wild-type enzymes enables us to better understand how to optimize structure-function relationships. Currently, novel genetically modified food enzymes are mostly used for applications in food processing involving carbohydrates, followed by lipids. Although there are challenges for safe use, genetic modification strategies for the development of novel food enzymes are highly promising for the future, and we expect that more genetically modified

enzymes will be introduced in various aspects of food processing. The production of enzymes by the process of genetic engineering emphasizes on economic production, enhanced enzyme purity, and naturally responsive production processes. Genetically modified microbes are used to produce a wide variety of products, ranging from synthetic insulin to vitamin C and enzymes like chymosin involved in food application. Appropriate purification, concentration, and formulation of enzymes with appropriate substances are crucial for further use in food industries (Hanlon & Sewalt, 2020).

13.4.2 Pros and Cons of GM Food Enzymes

13.4.2.1 Improvement in Food Processing

Increased scarcity of arable lands with increased population forced to use recombinant DNA technology in food industries. Several platforms are working together to make and deliver GM food enzymes as risk-free safe product (Fig. 13.6). The achievements in molecular biology in the past several decades have led to a widespread use of GM microbes in the production of food substances including enzymes. Currently, these genetically modified enzymes are employed in several food industries to improve in food processing. Manipulation of regulatory genes, restrict feedback inhibition, increase the shelf life, overcome the rate-limiting steps, reduce the process cost and production time, environment friendly are some of the

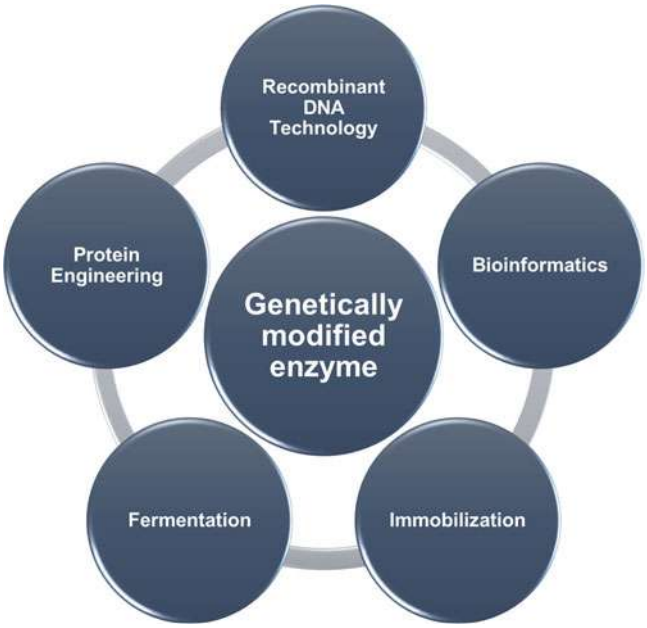

Fig. 13.6 Improved technologies currently involved in genetically modified enzyme production

major improvements that GM microbes could provide. For example, the use of enzyme Amino peptidases to modify by debituminize proteins which helps in the large scale production of food products such as milk, cheese meat, beverages and flavoring (Molinaro et al., 2005; Raksakulthai & Haard, 2003; Izawa et al., 1997). Pectinolytic enzymes are also widely used in the food industry for large-scale production of wine and juice production (Semenova et al., 2006).

13.4.2.2 Health and Ecological Risks Associated with GM Food Enzymes

Smaller recombinant enzymes are easily over-expressed in *E.coli,* but the larger proteins and the proteins that require posttranslation modifications cannot be expressed. Major health-related risks associated with GM foods enzymes are allergenicity, genetic hazards, and toxicity. These problems are associated with the change in pleiotropic effects when a new gene is inserted into the organism which may also cause the deletion of natural genes in the modified organism (Bawa & Anilakumar, 2013). Ecological risks associated with the food enzymes are resistant to antibiotics and in this genetic modification, antibiotics are frequently used as selection markers to distinguish whether the genes got transformed into bacteria. Thus genetically modify an organism have the risk of transferring the genes of antibiotics resistance.

13.5 Conclusion and Future Aspects

It has been more than 15 years, since the use of genetically modified enzymes in the food industry. With the development of genetic engineering and recombinant DNA technology, the production of enzymes enhances the natural responsive production and purity of enzymes. It also offers safe handling and cost-effective production of enzymes in the commercial market. The prime factor to be considered before commercialization of a food enzyme is the host organism that expresses an enzyme. A microbe used in enzyme production should be well characterized, which should not make any adverse effects by synthesizing byproducts like toxins. The safety and hazardous risks of the enzymes from genetically modified organisms have to be evaluated similarly to other enzymes those in use.

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Current Applications of Enzymes in GM (Genetically Modified) Food Development and Food Chain

14

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Abstract

New applications are introduced for food enzymes with regard to their impact on the improvement of organoleptic attributes and quality of foods other than their cost-effectiveness compared to chemical alternatives. Along with technological advances, novel and specified enzymes have been widely used in food industries. The enzymes may be extracted from plants and animals or be produced through biotechnological processes. In this regard, microbial fermentation is preferred compared to the other sources to some extent because it is a convenient process, needs lower cost, and is conducted consistently in the laboratory. Moreover, the fermentation process can be optimized by the use of genetically modified (GM) microorganisms or the addition of recombinant enzymes through which the production yield will be increased significantly. This chapter overviews the use of GM microorganisms in order to produce food enzymes. Advantages of the biological systems including recombinant DNA and metabolic engineering for the production of food enzymes are also discussed. Due to the importance of changes that occurred in the structure of GM enzymes, their halal status is further studied. In addition, the main regulations set by countries and the safety evaluations by risk assessment agencies on GM enzymes are addressed.

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A. Dutt Tripathi et al. (eds.), *Novel Food Grade Enzymes*,
https://doi.org/10.1007/978-981-19-1288-7_14

Keywords

GM enzymes · Halal status · Recent developments · Regulation · Safety concern

14.1 Introduction

It was predicted that the market volume of industrial enzymes will have a significant growth in the early future years (Robinson, 2015). The utilization of enzymes especially for the production of food and beverages represents a wide industrial enzyme market. For industrial use, it is possible to extract enzymes from plant and animal sources or obtained by fermentation using Wild-Type (WT) or Genetically Modified (GM) strains of bacteria or fungi for which both have been manipulated to enhance protein production and limit the production of unfavorable secondary metabolites. In addition to genetic modifications of the desired strain, it is also possible to modify the enzyme itself for the purpose of obtaining an improved yield and improve the characteristics of enzymes (Singh et al., 2016). On the other hand, by genetic modification of producing microorganisms, the enzyme can be modified to have better performance and improved properties.

14.2 Food Enzymes Production by Genetically Modified Microorganisms

Foods that contain, or manufactured from genetically modified organisms (GMOs) are defined as GM foods (Robinson, 2015). The utilization of GM for the industrial production of food enzymes (FE) is beneficial because it allows suitable production sources to be combined with the production of the desired enzymes. For example, in the case of the native enzyme producer strains that are not comfortable for industrial applications, such as the enzymes producing pathogenic strains, the coding genes can be extracted and introduced into other organisms that are more appropriate according to food safety for the production of enzymes. Genetic modifications are also used to inhibition the production of unwanted secondary metabolites such as toxins, by deletion of their native genes to prevent their expression. To build an effective recombinant strain, various points must be taken into account. First, it is crucial to select a host organism which has the proper properties for the expression and production of the desired enzyme and has a relatively inexpensive culture. Then, vital parameters are used to catch the optimal GM for industrial use including the expression vector, the methods employed for transformation, and the selection markers to choose the transformed strains must be specified (Liu & Kokare, 2017; Deckers et al., 2020).

14.2.1 Selection of Host Organism

The reasonable host strain is selected on a case-by-case analysis. The most used host are discussed below.

14.2.1.1 Bacteria

A simple and inexpensive host organisms are bacteria, with many expression systems suitable for recombination, leading to an easy combination of modifications. However, because of protein folding issues, only simple proteins are made in bacteria. In addition, bacteria are unable to make posttranslational modifications (Deckers et al., 2020).

Commonly used bacterial host strains are including *Escherichia coli*, *Bacillus subtilis*, *B. licheniformis*, and *B. amyloliquefaciens*. For *E. coli*, many molecular toolboxes are identified to ease the construction of expression systems available for high-yield enzyme production. However, proteins are produced in the cytosol, leading to the requirement for extra purification steps. Nevertheless, various species of Bacillus can secrete higher concentrations of the enzymes such as amylase and proteases into the medium (Zhang et al., 2019; Trono, 2019).

14.2.1.2 Fungi

Fungal host strains contain a subcellular organization, which lets proteins be folded properly and perform posttranslational modifications. Moreover, fungi secrete few amounts of their own secondary metabolites into the medium, easing the subsequent purificationsteps. Any other way, filamentous fungi generally produce low numbers of non-fungal recombinant proteins (Deckers et al., 2020).

Filamentous fungi that commonly used for the production of recombinant proteins are *Aspergillus niger*, *A. oryzae*, and *Trichoderma reesei*. *A. oryzae*, for instance, has been utilized for decades to produce fermented foods, but the production of the mycotoxin, aflatoxin is also known. To solve this problem, the mutant strain BECh2 was built, in which the cluster of the aflatoxin gene and the cyclopiazonic acid coding gene was removed and the production of Kojic acid was declined (Fernandes, 2010; Deckers et al., 2020).

14.2.2 Construction of Expression Vectors and Transformation Processes

Expression vectors are used for introducing modifications indifferent host strains. The expression vector usually includes at least One Origin of Replication (ORI), a Multiple Cloning Site (MCS), where the gene is integrated, and a marker for selecting the modified strain (Fig. 14.1). Besides, the promoter and terminator regions are available to control the expression of the desired gene and selector marker. To prevent an increase in metabolic expressions in the host organism and a decrease in plasmid stability, small size is considered for the vector (Patel et al., 2017).

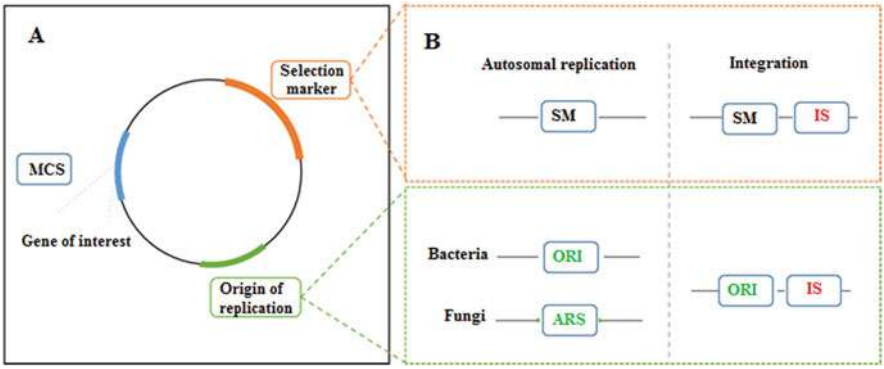

Fig. 14.1 Schematic representation of the expression vector. (**a**) Expression vectors include the origin of replication (ORI), the multiple cloning site (MCS) in which the desired gene is integrated, and a Selection Marker (SM). (**b**) An episomal plasmid and an integrated plasmid, containing a flanked integration site can be formed. ARS is an independent replication sequence and IS represents the integration site (Patel et al., 2017)

Bacterial expression vectors can either be used in the form of integration into the host genome (integrative plasmid) or they can be extra-chromosomal, utilizing independent replicating plasmids (episomal vector). Yeast and fungal expression vectors are usually integrative. In addition, shuttle vectors are commonly used for eukaryotes. These vectors can inter both prokaryotes and eukaryotes, and contain selective markers that work in both MB groups. To insert the host genome, the integrative plasmid consists of two Integration Sites (IS) that flanks via the Multiple Cloning Site (MCS), allowing the cleavage of the vector and then consolidation by recombination in the host genome containing homologous ordering to the IS. The integration usually takes place at a unique locus. In such a case, an extension homologous to a specific desired site needed to be added to the expression cassette (Patel et al., 2017).

Episomal plasmids contain an origin of replication (ORI) that is homologous via the ORI of the host type. Autonomous replication in eukaryotes utilizes an Autonomous Replication Sequence (ARS), which is the same as the ORI found in prokaryotes. Different methods of transformation are employed to introduce the vector DNA into the host organism. For bacterial host strains, this method contains conjugation that needs cell-to-cell contact, electroporation that is done by using competent cells, or vector incubation by constructing protoplasts. Protoplast fusion is mainly utilized for gene transfer into eukaryotes (Deckers et al., 2020).

14.2.3 Induction of Gene Expression

In order to the purpose of catch a high enzyme yield, an effective expression of the interested gene is required. One of the most common strategies used to upgrade the expression level is done by mix integrations (Patel et al., 2017). For this purpose,

various procedures are used, such as the use of high quantities of DNA in the transformation process; high antibiotic concentration pressure that putting to use; or simultaneous or sequential integration of multiple expression vectors that involve different markers for the selection of transforms or via applying copies of two preferred genes in a bidirectional vector (Deckers et al., 2020).

Other important factor affecting gene expression is the promoter strength. The expression is usually conducted by a group of inducers and suppressors that distinguish constitutive promoters from tunable promoters. Constitutive promoters are independent of the transcription factors that brought about environmental agents and regulate the transcription from basal genes. Tunable promoters, as opposed to constitutive promoters, are induced or suppressed by the biotic or abiotic factors in the environment (Fitz et al., 2018). For example, amyL and amyMare, respectively used as promoters for *B. stearothermophilus* maltogenic amylase and *B. licheniformis* α-amylase genes (Deckers et al., 2020), and TAKA amylase promoter is usually utilized in *A. oryzae* (Vieille & Zeikus, 2001). The last tunable promoter is activated via the transcription induceramyR (amylolytic gene expression) and is suppressed via the regulator creA (carbon catabolite suppressor) (Nedovic et al., 2011). Another promoter that is commonly used by *T. reesei* or some other fungi, is the promoter of cellobiohydrolase I gene (cbh1), and activated by cellulose (Olempska-Beer et al., 2006; Trono, 2019).

A simple solve for tandem repetition of one-directional expression cassettes is to apply bidirectional promoters (BDPs). These primers lead to the bidirectional gene transcription from one or more than one gene (Hui et al., 2006). Combination expression of the desired gene and the selective marker is also available (Rieder et al., 2019) while in order to use industrially, larger groups of accessed promoters are required. These promoters control variable expression rates as well as combining various regulatory profiles that are required in each expression direction (Yan & Wu, 2017).

14.2.4 New Technologies to Introduce Genetic Modifications

Recently, new technologies become involved in genetic engineering. The example of these technologies is "Clustered Regularly Interspaced Short Palindromic Repeat" and "CRISPR-associated protein 9" (CRISPR/Cas9), that derived from archaeal and bacterial cells (Meyer, 2008). The Cas9 endonuclease makes Double-Stranded Breaks (DSBs) in the desired DNA. By the application of endogenous repair routes, the genomic DNA can either be conveyed via offering transformation or by the insertion of a particular donor sequence. This is done through the Non-Homologous End-Joining (NHEJ) DNA repair processes or by the Homologous Repair (HR) technique (Song et al., 2019; Deckers et al., 2020). Opposed to eukaryotes, the NHEJ DNA repair system is not present in bacterial cells. A, these bacteria are not able to remedy the CRISPR/Cas9-mediated leakages, leading to the demise of wild-type strains. Therefore, the CRISPR/Cas9 system can request an election of recombinant types as a substitute for antimicrobial coding marker genes (Deckers

et al., 2020). Using this method, the production of extracellular pullulanase has been induced in a strain of *B. subtilis*. Because proteases can debase other enzymes, like pullulanase, the genes involved in protease generation have been removed (Dijck et al., 2003). The genes responsible for the production of unfavorable products during the fermentation such as foam, or involved in spore formation may also be disrupted to construct a more suitable strain for food enzyme production (Clyne & Kelly, 1997). CRISPR/Cas can also be requested for the transformation of eukaryotes. For instance, a strain of *Penicillium subrubescens* has been constructed in which the ku70 gene was deleted. This gene has participated in the non-homologous end-joining (NHEJ) DNA rebuild system. With this deletion, the homologous repair (HR) system is preferred, and for this reason, the subjunction of a wanted DNA sequence into the leakage site is performed (Zou et al., 2012).

14.3 Recombinant Food Enzymes

The origin of enzymes usage in industries of food processing dates back to 1874, when Christian Hansen obtained rennin (chymosin) from calves' stomach and used it for cheese production (Petersen et al., 2006). Chymosin is now made up of microorganisms that involve the bovine prochymosin gene, which is imported by recombinant deoxyribonucleic acid (rDNA) technology. The bovine chymosin, expressed in *E. coli* K-12, is the first recombinant enzyme approved by the Food and Drug Administration (FDA) for use in the food industry (Flamm, 1991).

Different enzymes that are utilized in food staffs are developed in recombinant microorganisms. Enzyme producers use new genetic technologies to obtain enzymes through desired properties. These kinds of enzymes most of the time are produced by microorganisms that are fastidious in growing on laboratory or industrial scales. By selecting suspicious host microorganisms, it is possible to produce recombinant strains and produce enzymes that are significantly free of unwanted enzymes or other microbial metabolites. Advanced enhancement of food processing resulted in a request for a wide range of food processing enzymes via properties reasonable for food processing conditions (Beilen & Li, 2002). For instance, usually applied sweeteners like glucose or fructose syrups are usually made from corn starch utilizing hydrolyzing enzymes. In the first stage of starch hydrolysis, the starch is recognized by using α-amylase for 2–5 min via heating at 105 °C and next put at 90–100 °C for around 1–2 h. Advances in rDNA technology have made it catchable to engineer α-amylases by improving the stability of heat and high compatibility with other parameters of the liquefaction stage. These improvements have been made by understanding the α-amylase amino acid sequences via the DNA sequence of the α-amylase genes. Another enzyme recently used in food processing has also been cached using rDNA technology. These enzymes are well-known commercially as enzyme ministration. An enzyme preparation usually contains the interested enzyme and varied additional substances, such as preservatives, diluents, and stabilizers. These extra substances are usually well-known ingredients that are accepted for utilization in food staffs (Olempska-Beer et al., 2006).

There are simultaneous techniques that are possible to applied to reproduce recombinant enzymes, such as rational design, directed evolution, and semi-rational design, based on the amount of information about the conformational structure and task of the enzyme, the enzyme sequences, and the alterations to be made to catch the expected result (Rajamanickam et al., 2017; Yang et al., 2013; Vogl et al., 2018).

The basis of rational design is the replacement, insertion, or removing of a unique DNA coding the fragment for an amino acid in relation to a specific activity that needs to be developed. The knowledge about the structural conformation of the protein and its chemical interactions is vital. Most of the time, site-directed mutagenesis is beneficial for changes of a specific amino acid by another to gain the requested activity (Rajamanickam et al., 2017; Vogl et al., 2018; Song et al., 2019). For instance, a thermostable pullulanase from *B. naganoensis* was made from a rational design. This enzyme is applied to hydrolyze amylopectin and make amylose in starch (Donohoue et al., 2018).

Directed evolution is consisting of two steps. First, is genetic diversity which is produced by random mutagenesis, and is an imitation of evolution. This can be done by error-prone PCR, mutagenesis via chemicals, or UV irradiation. In the second step, a thorough screening and detection process, which is usually performed by agar-plate method, to select the modified enzymes that have the desired characteristics. For this case, it is not necessary to have knowledge about the enzyme's structure and activities (Rajamanickam et al., 2017; Vogl et al., 2018).

The semi-rational design combines both the above techniques. In order to this reason, information is catchable for the enzyme's structure and tasks. Plus, there is no understanding about the frequency to be made to gain the desired results. Therefore, in the first step, amino acids undergo mutations by a specific fragment (in relation to the desired properties). Then a screening and evaluation step is completed, like the evaluation of directing (Song et al., 2019; Rajamanickam et al., 2017).

14.4 Application of Metabolic Engineering and Synthetic Biology in Food Enzyme Production

Metabolic engineering is used to optimize cellular procedures, native to a particular organism, to make a combination of interest from a desirably inexpensive and simple substrate. Utilizes different collections of data, a set of compositions and conditions are applied to maximize the generalization of the desired compound and to prevent chemicals and conditions that inhibit the update rate and other specific critical activities available to reach these goals; metabolic fluxes modifications show the main option. In summary, metabolic engineering is known as the directorial advancement of cellular properties by modifying specific biochemical reactions or the introduction new reactions using recombinant DNA technology. Some common strategies used in metabolic engineering are overexpression of enzyme-encoding genes that limit the rate of the biosynthetic pathway, block rival metabolic pathways,

heterologous gene expression, and enzyme engineering (Stephanopoulos, 2012; Kim et al., 2020).

Synthetic biology point to design genetic composition libraries including promoters, codons, terminal sequences, transcriptional factors, assembling devices, genetic circuits, and even a perforated organism; in addition to obtain quantitative information to create models that can demonstrate the behavior of biological systems (Cameron et al., 2014).

While synthetic biology provides champers and information on various biological processes, metabolic engineering tries to utilize all of this information to become optimal for the biological production of the desired compound. It is possible to note that some instances of synthetic biology can be arranged as examples of genetic engineering. However, these two areas are depending on the development of real methods, and tools for DNA transformation. These fields of research seek to achieve basic needs such as arbitrary changes in DNA sequences, production of specific mutations, the recombination between a variety of genetic circuits or biosynthetic pathways, the deletions of genes, and placement of DNA fragments into the organism genome or in a plasmid. Although PCR and its related methods are one of the superior equipment for making some necessary changes (specifically applied for extracting parts of a particular area) they are ineffective for other purposes (Boyle & Silver, 2012). Some examples of employing metabolic engineering processes to evolve in the production of food enzymes are discussed here.

By replacement of the bacterial genes with yeast-preferred genes, Wang et al. (2011) performed mutation and synthesis of tyrosine ammonia lysine (TAL), which elevated translation and vigorously increased p-coumaric acid and resveratrol biosynthesis. They showed that a low-affinity, high-powerful bacterial araE transporter could increase resveratrol yield, without direct resveratrol transfer. Yeast cells containing the araE gene generated up to 2.44-fold more resveratrol than control cells. They offered the transformed yeast cells for industrial applications in fresh grape juice production.

Microbial Cell Factories (MCFs) have appeared as evolving technology for the combination of previous single techniques and complicated multistep procedures into a unit self-replicating microorganism (Dhamankar & Prather, 2011). The usual way to create an MCF is to create a different or artificial de novo biosynthetic pathway in a chassis organism to achieve a new composition. The goal product could be native to a microorganism that is fastidiously cultured/engineered. The product also can obtain from a complex organism such as a plant that industrial production by inexpensive, or from a manner non-native to all microorganisms as a compound from an artificial metabolism. The MCF has also developed a convenient route to generate new derivatives of the desired compound. For example, phenylpropanoids, such as resveratrol, are natural secondary metabolites produced by plants that have been shown to have therapeutic effects and are commercially valuable. Most bioavailable derivatives of resveratrol have also been obtained from an MCF. A new biosynthetic route for resveratrol constitution in *E. coli* was designed using different enzymes obtained from bacteria and plants (Choi et al., 2010), and afterward evolved by adding a glycosyltransferase (from Bacillus), which led to the generation of

resveratrol glucoside derivatives like resveratrol 3-O-glucoside and res-veratrol 4′-O-glucoside) in an *E. coli* MCF (Choi et al., 2014). It is with reckless enzymes that a new combination of enzymes can give rise to new de novo biosynthetic routes that lead to the acquisition of new chemicals by using MCFs. So long as there are different published examples containing the yield of isobutanol (Atsumi et al., 2008a, 2008b), styrene modification (Pugh et al., 2011), production of 3-hydroxybutyric acid (Tseng et al., 2009), or native silk protein (Xia et al., 2010). Most enzymes in nature are able to react on a spectrum of substrates in order to maximize evolutionary compatibility and this development may be engineered (Khersonsky & Tawfik, 2010).

Rational design is the purification of a target DNA sequence that codes a protein to desired mutations because of improve their catalytic task, consistency, or some other representative like linking domain specificity. Devises such as Gibson assembly, the BioBricks or Golden Gate can make different genetic systems to shape genetic circuits, expression cassettes, or equipment (Casini et al., 2015). Finally, the utilization of Lambda-red, CRISPR-Cas9, and other new combination systems lead to imbed varied structures into expression vectors or in vital loci of the genome of the desired organism. It is possible to use some of these techniques to introduce genes of a partial or complete metabolic route for the synthesis of a special compound, and they can also be applied to delete genes from an organism that are antagonistic to the desired synthesis. Moreover, these methods let point mutations be performed that reduce the function or expression of native proteins to alter the metabolic flux. However, in order to the generation of all of these changes, it is necessary to have specific information about the enzymes captive in the pathway that contains these actions and information about the organism in which the modifications happen. (Esvelt & Wang, 2013; Liu et al., 2015). Thus, the genome sequence of organisms, the characteristics of proteins, and the metabolic studies make very beneficial equipment and information (Fig. 14.2).

Additional techniques and procedures have been crucial to reach other goals in the progression of both areas. Some instances include BioBricks, Gibson Assembly, Gap-repair, Lambda-red, MAGE, Recombinase techniques (integrases), and CRISPR-Cas9 (Stephanopoulos, 2012). Simultaneous sequencing of defined and non-defined organisms (metagenomics), allows for gaining more completed information. For example, the list of enzymes and processes has been developed, and some biological phenomena (e.g., infections by viruses or phages) have been reached (Goodwin et al., 2017).

14.5 Halal Status of Recombinant Enzymes

Halal term is a familiar concept in Muslim countries which have been interested in non-Muslim populations in recent decades. Today, it is globally accepted that a halal certificate refers to both religiously permitted and safe/qualified commodities. Therefore, the halal logo facilitates international trade (Ab Talib, 2017). However, it makes some difficulties for non-Muslim countries' exporters such as European

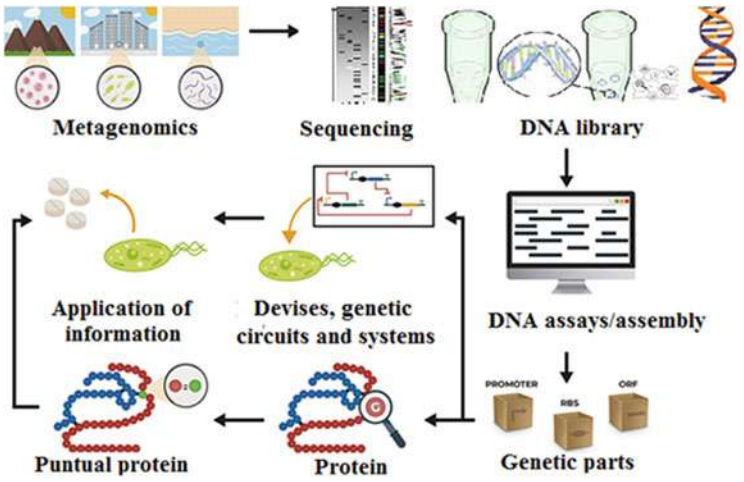

Fig. 14.2 Various steps for the progression of synthetic biology and metabolic engineering procedures

because of their lower knowledge about halal (Tieman, 2017) As a global rule, foods and their ingredients should be free of compounds that originated/are made from haram sources or exclude alcohol. On the other hand, the animals should be slaughtered in a way that is accepted by Islam (named as Dhabihah or Zabiha) (Alzeer & Abou Hadeed, 2020).

Halal commitment is a critical issue in the whole food chain at the international level. In 2001 a Japanese company was condemned due to the production of non-halal monosodium glutamate as a flavor enhancer to achieve better economical productivity. The process was included using BactoSoytoneas a culture media prepared by the enzyme derived from porcine that is not acceptable in the halal concept. This violation was further conceded by the manufacturers that were given the halal certificate by Indonesian authorities for their products. The event obliged other enzyme manufacturers to be approved by the halal certification bodies in the world (Fischer, 2011). There are two types of certificating for halal foods including 1) site registration to evaluate the ability of suppliers in fulfilling the requirements and 2) certifying the imported products (Hanzaee & Ramezani, 2011). Unfortunately, the halal certifiers grow slowly compared to other scientific associations in the world due to the disagreements on the halal concept (Halim & Salleh, 2012). On the one hand, the term Istahala has different understanding and interpretations among communities. There is not a consensus on the final products produced from the non-halal origin when they underwent chemical transformation up to microstructures or molecular level. On the other hand, there is a doubt about the synthetic genes designed in the laboratory which are modeled from haram sources such as pork (Riaz & Chaudry, 2019).

Enzymes are food components that are traded worldwide and their halal assurance is a concern among Muslims. Food enzymes are produced from different

sources of plants, animals, and microbes. Several points are considered in their eligibilities by Islamic traders. The enzymes should be extracted from halal sources and produced in media containing halal raw materials. Furthermore, fermentation media should contain the approved organisms other than halal ingredients if a microbial source is used for enzyme production. It will be important when genetically modified (GM) microorganisms activate for enzyme synthesis. Based on the World Health Organization's definition, "genetically modified organisms can be defined as organisms (i.e., plants, animals, or microorganisms) in which the genetic material (DNA) has been altered in a way that does not occur naturally by mating and/or natural recombination" (WHO, 2014). The source of genes and method of preparation determines the fate of GM products. For example, accordance of GM foods with religious remarks is the most important factor for Malaysians in preparation of their food basket (Oz et al., 2017). It is also a deterministic factor in Islamic countries such as Iran through which the GM foods and components such as enzymes first checked whether they are halal followed by safety evaluation. In this way, the genome of host cells that is manipulated to encode the enzyme should be originated from a halal source (Ermis, 2017). Some steps are generally monitored in halal control points of enzymes extraction and production including the source (animal or microbial), cleanliness of the production utilities, the agents released into the environment, acceptable processing-aid, substrate or raw materials, and packing process. However, up to 0.5% of alcohol may be acceptable when an optimum activity is required (Riaz & Chaudry, 2019).

Use of animal-based enzymes is limited in some cases such as restrictions on the dietary intake of vegans other than halal uncertainties (Nugraha et al., 2015). In comparison, the enzymes extracted from conventional plants are not doubtful (Khattak et al., 2011) and there is no concern about plant-derived genes inserted in transgenic microbes (Riaz & Chaudry, 2019). However, microbial sources are used extensively for enzyme production because of their cost-effectiveness and ease of process control for optimization. Furthermore, it eliminates the concerns of animal origin and slaughtering methods to meet halal criteria in enzyme production (Riaz & Chaudry, 2019). Microbial enzymes are commonly produced by submerged fermentation (Khattak et al., 2011). Several recombinant food enzymes have been engineered to promote resistance of the wild type against harsh conditions of temperature/pH and better reactivity of the GM enzyme with substrates in the environment. *Pichia pastoris* (which was recognized as safe by US Food and Drug Administration) (Espinoza-Molina et al., 2016) and *E. coli* are two species commonly used as a host cells in this regard (Espinoza-Molina et al., 2016; Zhang et al., 2019). In addition, GM *Streptococcus thermophiles*, as a common starter culture of yogurt, capable of antioxidant enzymes' expression was evaluated by Carmen et al. in vitro and positive results were observed in the suppression of oxidative stress in the colon cancer model (del Carmen et al., 2017). The bacteria was manipulated by insertion of the gene responsible for antioxidant enzyme' production isolated from *Lactobacillus casei*BL23 as an anticancer and anti-inflammatory probiotic bacterium (del Carmen et al., 2014; Jacouton et al., 2017) that is considered as a halal source. Moreover, transfer of the encoding gene of

chymosin from camel (Aboulnaga, 2019) and bovine (Ulusu et al., 2016) to *E. coli* and from calve to *E. coli*, *Kluyveromyces marxianus* and *A. niger* (Fernandez-Salguero et al., 2003) in coagulation of milk protein are other examples. At the end of fermentation, the enzyme will be in accordance with halal merits if the aforesaid factor is met in the processing. AbdLatip and Nordin in 2018 listed endemic psychrophilic bacteria of South Pole as a halal source able to produce enzymes resistant against the cold condition. In their opinion, bioengineering of these microorganisms toward specific characteristics would be of interest in the future because their low-temperature resistance limits the side reactions and lowers the required activation energy (Abd Latip & Hadry Nordin, 2018).

Compared to foods, therapeutic agents may be exempted from halal regulations, occasionally. For example, Alzeer and Hadeed in 2020 listed the orally administered capsule of "Nutrizym 22" containing the pancreatic enzymes for pancreatitis treatment as a highly critical pharmaceutical in view of halal because of its porcine origin (Alzeer & Abou Hadeed, 2020). It is expected that there would be less restrictions on therapeutic compounds when there are no halal alternatives.

A big deal in halal assurance is that labeling regulations in some countries suffer from adequate liabilities. In this case, there is no obligation on listing the ingredients of GM foods that might be originated from non-halal sources (Alhariri, 2020). With regard to the enzymes, it is due to the fact that they may decompose during the process, and no residue is left in the final products. Moreover, some regulatory bodies believe that the labeling (if the product does not include significant changes in the composition or make an adverse impact on the consumers) may threaten the products' sales because of consumers' misconception about health risks arising from GM ingredients (Uzogara, 2000).

14.6 Regulation on GM Enzymes

GM foods were firstly released to the market in 1994 in the United States by introducing a GM tomato containing the gene expressing an enzyme responsible for modulation of the ripening process (Ujj, 2016). Since then, the countries have had different strategies in accepting or withdrawal of such products. In general, European countries have more restrictions on GM foods than America, and no GM products are approved and marketed without thorough risk assessment or clear labeling. Although, the products processed by GM enzymes or the animals fed by GM feeds may be considered lenient (Yuen-Ting Wong & Wai-Kit Chan, 2016). For example, the bakery products prepared by GM amylase do not require labeling in the EU (European Commission, 2003a). In 2019, a member of the European Commission explained the EU regulation on GM foods. Other than the risk assessment, market surveillance, and labeling, the regulation includes risk communication of the results of safety evaluation performed by the European Food Safety Authority (EFSA) which is responsible for risk assessment in the EU. The regulation was implemented by the authorities for the engineered targeted gene not conventional breeding with a long history of safety in humans, animals, and the environment.

However, like many other populations, people are not interested in GM foods in Europe and the most quantities of authorized GM products are used for animal feeding (Bruetschy, 2019). The list of authorized GM cops by the EU is available on the European Commission website (European Commission, 2020a) and EU regulation on genetically modified food enzymes is addressed in the directives of EC No. 1332/2008 and EC No. 1829/2003 (European Commission, 2003b, 2008). As mentioned, safety evaluations of GM food enzymes in Europe are done by EFSA and the results are freely published to be available within the EU and for other countries. The approved enzyme by EFSA can be commercialized under the rule of the two above directives. The EU directive on GM modified food and feed, which is in accordance with the Cartagena Protocol on Biosafety, clearly mentioned that the processing aids are out of the scope of the directive and considered differently (European Commission, 2003b). Therefore, if the food enzyme is used as a processing aid so that irreversibly denatures, degrades, or removed before consumption, is not covered by the directive. On the contrary, if the food enzyme is an ingredient or additive in the food, it will fall under the scope of the directive of GM food and feed that should be mandatorily listed on the labeling. Importantly, there is an exception about the enzymes are or possibly contain allergenic or intolerant compounds that should be mentioned on the labeling every time (European Commission, 2014).

The United States has the biggest share in the production of GM foods in the world (Oz et al., 2017). It was estimated that the arable land in the United States is 1.4 times more than that Europe but the GM crops are cultured approximately 600 times in the United States (Ujj, 2016).

Three governmental bodies in the US are involved in the safety evaluation of GM foods from farm to fork that in turn included the Department of Agriculture (USDA), Environmental Protection Agency (US EPA), and Food and Drug Administration (US FDA). The first one engages in the protection of GM plants in the farm from pests and diseases and also protects the environment against possible risks arisen by GM crops. The second body is responsible for the safety of both environment and humans faced with the GM traits. The last administration is responsible for the safety of GM foods and feeds taken by humans and animals (Schiemann et al., 2019). The US regulatory bodies focus on the GM characteristics rather than the method of product's synthesis. The USDA classified the GM seeds to regulated and deregulated articles which are those that require direct- and exempted from overseeing, respectively. It is based on the known safety level of the seeds. A joint collaboration of USDA and US EPA is required for the safety evaluation of GM edible crops containing pesticides. In this way, the producer should examine and present the amount of pesticides existed in the final food to US EPA. The producer is allowed by US EPA to conduct a field trial of the edible pest-resistant GM crops on a farm of 0.5 hectares or larger under US EPA consultation. The USDA studies source of the gene producing the toxin, its characteristics, the environmental impact, and its impact on nontarget organisms while the US EPA is responsible for toxicological, allergenicity, and gastrointestinal fate studies on the toxin in the GM corps (Craig et al., 2012). Other than plant-sourced biotechnological products, the safety

investigation of GM microorganisms and their byproducts such as enzymes and other chemicals is led by US EPA (Wozniak et al., 2012). After approval, the product can be commercialized preferably under the consultation of the US FDA which oversights the safety of GM foods through which the producer is the holder of safety responsibility (Craig et al., 2012). List of FDA-approved GM crops is freely available on its website for the readers (US FDA, 2019).

Less restrictions exist in the United States on foods containing GM ingredients that are overseen by the US FDA. The administration has stated that GM foods are as safe as or better than their non-bioengineered counterparts to some extent (US FDA, 2020a). The agency supposed that GM food does not impose new/different risks to the consumer compared to its counterpart. Therefore, no need for pre-market approval is required unless there would be evidence of safety concerns of the bioengineered product other than those expected in the wild type. The US FDA believes that an initial assessment of the food additives was undertaken and further evaluation on the GM counterparts will not be necessary if the new event and trait introduce no side-effect in humans (Yuen-Ting Wong & Wai-Kit Chan, 2016) It is against the public perception about this kind of foods. Scott et al. in 2016 reported that nearly 64% of Americans did not agree with GM foods and most of them would not be affected by further informing strategies conducted on risks and benefits (Scott et al., 2016).

Commercialization of GM foods in the US can be accompanied by FDA consultation but is not compulsory to the industries. The US FDA persists in pre-market examinations conducted by the producers to find out whether the GM foods containing the gene extracted from the allergenic source makes an immune response in humans. It would be well feasible for the known allergenic foods such as Brazil-nut but will be a big deal for unknown sources (Jacobson, 2000).

Nonetheless, no obligation is assigned for labeling GM foods in the United States because the US FDA believes that the bioengineered food is not majorly different from the wild type and labeling may make the additional cost to the consumer (Marchant & Cardineau, 2013). However, some states in the New England region enacted local rules on GM labeling. For example, Vermont State in 2016 and Maine in 2013 (northeast of the US) were obliged listing the GM ingredients when they are added more than 0.9% to foods or Connecticut State (northeast of the US) in 2013 mandated GM infant formula labeling (Oz et al., 2017).

China is a competitor of genetic modification in the world with a strict rule for approval by using widespread research. Initiation of GM foods' investigation in China was later than the European Union and the United States. However, the current president of China in 2013 emphasized the both issues of safety and innovation in bioengineering so that the marketed GM products should be approved by strict regulations and the country does maintain its superior position in GM commercialization (Feng & Yang, 2019). China is one of the most consumers of GM crops especially grains in the world. The Chinese government tried to speed up the commercialization of GM foods in the country but it did not succeed as expected because of consumers' concern (Yuen-Ting Wong & Wai-Kit Chan, 2016). Direct responsible for agricultural genetic modification in China is the Ministry of

Agriculture and the duties are delegated by the ministry to other bodies. They are included in Inter-Ministerial Joint Meeting for integration and coordination of GM issues, National Biosafety Committee for safety evaluation of GM products, the Standardization Committee to amend the safety standards, testing institutions responsible for characterization and safety examination of GM products, and other peer governmental departments responsible for a comprehensive inspection of final products (Jian-ping et al., 2015). There are several steps of evaluation of GM foods before their commercialization in China. The first one is biosafety assessment which covers laboratory to field investigations. The committee in charge of biosafety evaluation categorized the products into 1 to 4 risk groups based on their threat to the life cycle (Yuen-Ting Wong & Wai-Kit Chan, 2016). In this regard, no or negligible risk, low risk, moderate risk, and high risk refer to classes 1 to 4 respectively (European Commission, 2020b). Obviously, a restricted regulation is set for classes 3 and 4. China makes an obligation on GM foods' labeling through which the GM ingredients or those additives degraded under processing (such as an enzyme) should be named on the label (Yuen-Ting Wong & Wai-Kit Chan, 2016). To the contrary, Feng and Yang stated that adequate specification has not been made in Chinese law about labeling GM food (Feng & Yang, 2019). Safety regulation on GM foods in China undertakes three issues of risk assessment (back to the early twenty-first century for GM agricultural foods), labeling of GM foods to alarm the consumers about the content, and risk communication (Sun, 2019). However, the results of a survey showed that the Chinese population tended to lower its intake of GM foods in 2013 compared to 2002. It may be due to their increased knowledge about these types of foods. The authors believed that the oriented advertisement of Chinese media (that are managed by the government) against GM foods might be an important reason. However, the increased significant rate of neutral citizens on GM foods in 10 years along with strict regulations and fast-growing research on GM sources in China are effective factors on GM food development in the country (Zheng et al., 2017).

14.7 Safety Concerns of Recombinant Genes and Enzymes and the Allergenicity Assessments for Development of GM Foods

Genetic engineering is a polar issue in the world so there are several advocates and critics for and against the technology, respectively. The advocates are interested in the promising events evolved in new products by the activity of recombinant genes compared to native ones. Today, other technologies are served to increase the functionality of recombinant proteins. For example, recombinant dextransucrase and dextranase were protected by encapsulation techniques to be more efficient under intended use. The enzymes were coated with edible polymers of alginate and pectin (Sharma et al., 2019). Such polymers which are nontoxic, biodegradable, well-defined film-former, and resistant to higher temperatures and gastrointestinal conditions (Moslemi et al., 2018; Cheraghali et al., 2018; Sharma et al., 2019),

protected the core in favor of non-digestible oligosaccharides' higher production from carbohydrates under harsh condition (Sharma et al., 2019). In comparison, the critics of genetic engineering mainly worry about unknown risks that may be observed in humans, animals, and the environment in the future.

Bacteria, yeast, and fungus are utilized as donors or hosts for genetic modification. Trono in 2019 listed a large number of bioengineered food enzymes which are used in different industries of dairy, meat, cereal, beverage, sweetener, and emulsifier (Trono, 2019). Some approved genetically modified food enzymes are listed as GRAS and are available at GRAS Notice of the US FDA (US FDA, 2020b). It means that those GRAS enzymes can be used in the market without achieving any safety approval by regulatory bodies. Safety considerations of GM enzymes in the US are mainly assessed by the suppliers in collaboration with US FDA.

The new traits improve technical feasibility, such as high-or low-temperature stability, salt tolerance, specificity, and optimized activity under a developed range of pH. For example, bioengineered α-amylase produced by *B. licheniformis* showed high acid resistance by replacing histidine with aspartic and glutamic acid for starch saccharification or high affinity of thermostable lipase to short-chain fatty acids in dairy triglyceride help flavor enhancement. Mesophilic *B. pumilus* was modified to produce a thermostable protease with increased hydrolytic activity at 15 °C (Zhang et al., 2019). However, some indications of negative symptoms from genetically modified foods and enzymes arose. For example, allergenicity symptoms were observed after consumption of StarLink maize engineered by insertion of pesticide gene from *B. thuringinesis* which resulted in an unintended impact on humans. Moreover, the bioengineered soybean by using the gene extracted from Brazil-nut in favor of methionine overexpression induced an allergenicity response (Dadgarnejad et al., 2017). Another example is the observed allergenicity of cooked and uncooked transgene pea following the insertion of the kidney bean gene (Yavari et al., 2016). It might be due to the intrastructure changes in the new protein that made it resistant to high temperature and digestion. Budnik et al. performed IgE diagnostic test in the blood of 813 workers exposed to genetically modified enzymes used in the production of foods, drugs, and chemicals from 2007 to 2013. The results boded that 23% of the all had high level of IgE, most of them sensitized to α-amylase and the least frequency was reported for lipase while the highest blood IgE concentration was diagnosed in those exposed to phytase, xylanase, and glucanase (Budnik et al., 2017).

Importantly, new organisms may produce some enzymes involved in unintended biological mechanisms and produce unintended or harmful chemicals. In addition, the new gene may interfere with the gene integrity and silent or activate the gene toward unwanted metabolic pathways. They may also lead to acquired resistance of pests on the farm by time and propagation of the resistant genes to other violent species. There is a concern regarding the use of antibiotic-resistant genes as a marker in the examination of transgene products by which they remain active in the presence of antibiotics if well affected by the bioengineering process. Researchers worry about the transfer of antibiotic coding genes to pathogenic bacteria and resident flora of human and animal digestive tract (Dadgarnejad et al., 2017). In comparison,

some explanations are presented against the concern. The fans of genetic engineering believe that a chain of reactions should be occurred to transfer the target gene into an unintended organism. They are included in excision of the gene, its resistance within the digestive tract, its insertion into the second organism, its compatibility and stable joining to the host, and active expression within the unwanted cell. In addition, the specialists stated that the normal flora within the digestive tract already has the gene encoding antibiotics and no additional worries would be expected if the known genes are used for this purpose (Dadgarnejad et al., 2017).

It is believed that glycoprotein provokes the immune response directed by immunoglobulin E and glycosylation process is a key factor in vital systems toward the formation of allergenic proteins. Allergenicity of transgenic protein is evaluated by several methods. At first, bioinformatics tests that examine homology of the new protein and the known allergens are conducted (structural information of the allergens is available at databases). Then, the serological tests consisting of immunoglobulin E binding assay are done when more than 35% similarity in a sequence of 80 amino acids in the new protein with the allergens is detected by bioinformatics assay. Pepsin resistance test together with quantity evaluation which is important in risk assessment studies is another way of safety evaluation. In this way, a quantified protein is examined because less allergenic foods may be more harmful than the others due to their high frequency of consumption. Moreover, the heat stability and in vivo trials referred to as animal studies are common examinations. Although, in vivo trials may not be reliable enough because it is species-specific and could not be strictly extrapolated to humans in some cases (Yavari et al., 2016).

A guidance has been presented by EFSA about allergenicity assessment of new proteins stimulated a non-IgE mediated response like what is observed in coeliac patients. It includes finding out the source of protein and the population exposure followed by amino acid sequencing by which its similarity to the known allergens is determined. Performing in silico tests and examining the binding possibility of the protein residues to the receptors overexpressed in coeliac disease (HLA-DQ2- and HLA-DQ8) to evoke immunogenicity is a further step. Finally, in vitro digestibility test is conducted (Naegeli et al., 2017).

Food enzymes that are commonly used by the industry are mainly produced by microorganisms and their full characterization especially on the transgene organisms is done by EFSA. The first step is collecting the taxonomic information up to strain level for both bacteria and fungi. The whole-genome sequencing of chromosomes and plasmid is further required. Third, an antimicrobial resistance test is done on the producing microorganism to avoid propagation of the resistance gene among other viable organisms. If the microbe comprises antimicrobial-resistant gene, the enzyme must be free of genetic materials of the producing organism (Silano et al., 2019). EFSA developed a Qualified Presumption of Safety (QPS) list containing the microorganisms subjected to and passed the safety evaluation for food enzyme production. Filamentous fungi are excluded from the list because of the possible production of toxic secondary metabolites, occasionally (Koutsoumanis et al., 2020). The QPS document also includes a safety assessment approach for enzymes producing microorganisms for additional minor evaluation, if required. Food enzymes

produced by the microorganisms not-listed in the QPS or those genetically modified in the QPS list should be free of viable cells to make sure of safety. In addition, no genetic materials of GM microbes and non-GM microbes carrying antibiotic-resistant genes should be found in the products. Toxicity evaluation of food enzymes by EFSA is done by in vitro genotoxicity assay and micronucleus test via cell culture method and in vivo 90-days trials in rodents via the oral route. Allergenicity assay is done by in vitro gastric simulation test which is a marginal way because it is not reliable enough and should be used in combination with other methods. Food enzymes within a group are assessed separately because those with similar functionality may have different structures leading to different clinical reactions in humans (European Food Safety Authority, 2020). The applicants are required to present a full description of genetic modification including the changed gene, the new trait, and the altered mechanisms in the GM organism (Silano et al., 2019).

Comprehensive data have been released by EFSA about the enzymes produced by genetically modified microorganisms. Estimation of oral toxicity in the form of margin of exposure (MOE) by EFSA is based on the maximum recommended dosage of the enzyme in the industry and European food consumption database which may be overestimated in some cases. Acceptable MOE in Europe with limited variety in the population would be 300 and more compared to the varied geographical countries which may accept MOE 1000 and higher. Therefore, based on the reference points or reference doses, deciding about the safety of foods is hold on the risk managers of each country depending on their local dietary intake (Jamali et al., 2020; Moslemi et al., 2020). EFSA has evaluated the toxicity of GM food enzymes by the reference point of NOAEL (no observed adverse effect level). Some examples of GM food enzymes safety evaluations are as follows. They mainly limited to carbohydrase that are of the most common enzymes in the food industry.

α-amylase produced by GM *Pseudomonas fluorescens* (BASF Enzymes LLC Company) intended for glucose syrup production was free of viable cell and genetic materials. The enzyme was subjected to toxicity assessment within 90 days of trial in rats and a reference point (NOAEL) of 887 mg/kg body weight per day were detected. It was not genotoxic and no similarity to the known allergens was detected. The EFSA scientists believed that induction of adverse reaction by the enzyme might not be avoidable although it was considered rare. Notwithstanding, the GM α-amylase contained antibacterial that will pose the consumers at risk of antibacterial resistance. Therefore, the GM α-amylase was ignored for human dietary consumption (Silano et al., 2020).

α-amylase produced by GM *B. licheniformis* (Novozymes Company) intended for glucose syrup production was introduced as safe by EFSA and no genotoxicity and allergenicity were detected. Toxicological evaluation was not emphasized for the suggested industrial use as more than 99% of the enzyme will be removed at the end of syrup production (Silano et al., 2018g).

α-amylase produced by GM *T. reesei* (Danisco US Inc) for production of distilled alcohol and brewing purposes was studied and no genotoxicity was detected. The enzyme was not similar to the allergenic compounds. Nonetheless, a NOAEL of 230 mg/kg body weight per day was reported which led to MOE of 135 by

calculating the dietary exposure of 1.701 mg/kg body weight per day in Europe. The low MOE might be due to overestimation of dietary exposure as the scientists presumed its intake from all brewing products and extrapolated the data of restricted time to longtime exposure. As a conclusion, they reported that the enzyme can be used but a low concern would not be avoidable under the defined condition of oral consumption if no residue remained in the foods (Silano et al., 2019a).

α-amylase from GM *A. niger* supplied by Novozymes Company was not genotoxic and MOE for bakeries that contain the GM enzyme accounted for 370 in Europe. Two similar segments in amino acid sequences were observed in the allergenicity test which was matched to respiratory, not oral allergens and resulted in a safe status of the GM enzyme for oral intake (Silano et al., 2018).

The enzyme glucan 1,4-α-glucosidase produced by two different GM organisms of *A. niger* and *T. reesei* for conversion of starch to glucose syrup, baking, and brewing purposes was not genotoxic. NOAEL of 1244 and 1149 mg/kg body weight per day was detected for the enzyme produced by recombinant *A. niger* and *T. reesei*, respectively. But, one segment of 80 amino acid in the enzyme of both origins showed more than 35% similarity with a respiratory allergen. Although, the EFSA panel did deny the low safety concern with regard to the oral intake of the enzyme, particularly in those susceptible to the known respiratory allergen, but the enzyme was finally considered safe for oral intake. It is due to the fact that more than 99% of the enzyme is removed in glucose syrup and variable dietary exposure would be expected for dough and beer at different countries (Silano et al., 2018a, 2020a). Same results were observed for glucoamylase produced by GM *A. niger* for conversion of starch to glucose syrup and brewing purposes. A NOAEL of 1360 mg/kg body weight per day and MOE of 375 were determined for European countries. Similarity of the enzyme with the known respiratory allergen was neglected according to the fact mentioned above for glucan 1,4-α-glucosidase. Therefore, GM glucoamylase was considered as safe for dietary consumption (Silano et al., 2018b).

Three domains of GM maltogenic amylase produced by recombinant *B. subtilis* (produced by Novozymes A/S) showed more than 35% similarity with the known respiratory allergens but the GM enzyme was reported as safe by EFSA for bakery, brewing and starch processing because no evidence was found about their allergenicity under dietary exposure. No genotoxicity and acceptable MOE for Europe was also reported (Silano et al., 2018c). In agreement, the same results and conclusion on genotoxicity (negative), oral toxicity (acceptable risk), and allergenicity (three similar domains with the known allergens) were further made by EFSA for maltogenic amylase derived from GM *B. licheniformis* (Danisco US Inc) (Silano et al., 2020b).

Xylanase is a commonly used enzyme in the cereal industry for brewing and starch/gluten production. Its counterpart produced by *T. reesei* (AB Enzymes GmbH) showed no genotoxicity, high MOE in Europe (more than 43,000), and no allergenicity. GM xylanase is removed at the end of grain processing but a trace amount of the enzyme delivered from brewing products might pose the consumer at low risk. However, the EFSA considered it safe under the defined oral condition of

use (Silano et al., 2020c). The enzyme endo-1,4-β-xylanase from GM *B. subtilis* of two different strains of LMG S-27588 and LMG S-24584 (produced by Puratos N. V.) for baking purposes showed no genotoxicity, allergenicity, and high MOE (1363 and 2176 for European countries, respectively). In comparison, a recombinant DNA segment was detected in the food enzyme. Although the last finding was against EFSA requirements, the EFSA panel considered the GM enzyme safe for commercialization under the defined condition of use. It might be due to the amount of MOE far from the danger cut-off point and the no antimicrobial reaction found in the purity test of the GM enzyme (Silano et al., 2018h,d). The enzyme endo-1,4-b-xylanase produced by *T. reesei* strain DP-Nzd22 (DuPont company) was also certified as safe for commercialization by EFSA (Silano et al., 2018e). To the contrary, endo-1,4-β-xylanase from GM *A. niger* was not accepted to be released because of no adequate data achieved by the Ames test and the EFSA panel could not conclude about its genotoxicity (Silano et al., 2017).

Pullulanase produced by GM *B. subtilis* (Novozymes Company) and *B. licheniformis* (Danisco Company) for glucose syrup production from starch and brewing purposes showed no oral toxicity within the exposure range of European and no homology with the known allergens was found. Therefore, the GM enzyme has been considered safe for human consumption under the defined condition (Silano et al., 2017a, 2019b).

β-galactosidase synthesized by GM *E. coli* (supplied by Clasado Ingredients Ltd.) was withdrawn for commercialization despite passing the toxicological and allergenicity experiments due to the inclusion of genetic materials in the food enzyme and no adequate data on their viable cells' containment (Silano et al., 2020d).

Xylose isomerase produced by GM *Streptomyces rubiginosus* supplied by Danisco Company for immobilized isomerization of the sugar into fructose was studied and no genotoxicity and allergenicity were detected in vitro. The GM strain has had antibiotic- resistant gene but no viable cell and DNA fragments were detected in the GM enzyme. Provided that the enzyme will be removed after the process or not entered into the product through immobilization, little risk of dietary exposure and oral toxicity is expected. Thus, the GM enzyme has acquired EFSA acceptance for commercialization in food (Silano et al., 2020e).

Lysophospholipase expressed by GM *A. niger* (produced by Novozymes A/S) usable for starch processing and oil degumming showed no genotoxicity and NOAEL of 1356 mg/kg body weight per day was determined. Nonetheless, no similar sequence of amino acids with the allergens was detected. The scientists reported that there may be a low safety concern regarding the frequent dietary exposure to the enzyme, but the enzyme removal after processing declines any health concern in the final product (Silano et al., 2020f).

Triacylglycerol lipase produced by GM *Ogataea polymorpha* (Danisco US Inc.) showed no genotoxicity, MOE of more than 1280 for Europe, and no similar amino acid segment with the known allergens. No viable cells and DNA residue were observed in the GM enzyme and it was considered safe for use in the food industry by EFSA (Silano et al., 2020g).

The approved acetolactate decarboxylase from GM *B. licheniformis* (produced by Novozymes A/S) for brewing purposes had no genotoxicity, allergenicity, and very high MOE (more than 300,000) (Silano et al., 2018f).

14.8 Conclusion

Enzymes found in nature have been beneficial for the generation of fermented foods for thousands of years. The enzymes producing microorganisms that obtained from natural sources date back to the late-nineteenth century. Furthermore, the progress in molecular genetics and cell biology over the past four decades have altered enzyme generation processes. It is possible to choose and extract enzyme-encoding genes and show them in host microorganisms that are well compatible with large-scale industrial fermentation. In order to utilize effective promoters and introducing variant copies of the enzyme coding genes, enzyme performance can be significantly increased. Also, it became possible to adopt enzyme characteristics to food processing conditions by using recombinant DNA technology. The evolution of metabolic engineering in relation to systems biology in recent years and use them for designation of biological systems promises to produce more sufficient food enzymes in the early future. Currently, USA, EU, and China are the main players in GM foods worldwide. The GM enzymes approval is based on the results of widespread risk assessments. Safety evaluation includes homology assessment of the GM enzymes to the known allergens, immunology evaluation, pepsin resistance, heat resistance, and in vivo trials. With regard to labeling of the GM foods, the US FDA is more lenient than the two others because it believes that the approved GM products do not represent an additional risk to the consumers compared to the traditional foods. However, all GM enzymes should be listed on the labels of food packages in China. Upon the fate of the enzymes under processing, the EU exempts the GM enzymes added as processing aid from labeling while those additives should be mandatorily listed on the labels. Besides the all-positive points addressed about the GM enzymes; the improvements have introduced a new controversial issue in Islamic countries. As a rule, halal food enzymes should be derived from halal sources, produced in environments containing halal ingredients, and exhausted from alcohol up to the acceptable level in the final product. In addition, the gene responsible for encoding recombinant enzymes should be extracted from halal origin.

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Enzyme Immobilization and Its Application Strategies in Food Products

15

Nafiseh Sadat Naghavi, Nazanin Sanei, and Martin Koller

Abstract

Enzymes are the dominating class of biocatalysts, which are extensively employed in food industries. Immobilization of enzymes on inactive and not soluble supports practically increases their efficiency owning to their high stability and multiple reuses, while it can negatively impact enzyme activity. The characteristics of immobilized enzymes are based on the procedure of immobilization and achieved beneficial properties, such as biocompatibility, chemical and thermal stability, the impossibility to dissolve (leak) in the reaction liquids, reconstitution, recyclability, and cost efficiency. Various immobilized enzymatic systems, like proteases, amino acylase, glucose isomerase, β-galactosidase, aspartase, lipases, or glucosidase have been shown to be techno-economically used in food industries on a multi-ton's scale per year. This chapter provides a general survey of the benefits and applications of the immobilization enzymes with a major focus on the food industries.

Keywords

Food enzymes · Immobilization · Reuse · Cost efficiency

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A. Dutt Tripathi et al. (eds.), *Novel Food Grade Enzymes*,
https://doi.org/10.1007/978-981-19-1288-7_15

15.1 Introduction

Immobilization is a process used for transformation of biocatalysts (cells and enzymes) or bioactive components from a soluble phase to an insoluble state by using an insoluble carrier or by encapsulating them within a matrix material (Van de Velde et al., 2002; Chen et al., 2016). As a consequence, the properties of the native catalysts may change significantly, and in order to be able to explain the observed reaction behavior the laws of heterogeneous kinetics have to be applied. The immobilization of enzymes has become a major process in manufacture, medication, and biotechnology over the past decades. Scientists have developed many processes based on methods from physical adsorption and covalent binding to encapsulation or entrapment into polymers (Ispas et al., 2009; Alkan et al., 2009; Koszelewski et al., 2010; Klein et al., 2011; Defaei et al., 2018). Already in 2010, the market volume of immobilized enzymes amounted to almost 6×10^9 US-$ with a currently clear upwards trend, with food/beverage production and pharmaceutical applications constituting the major fields of application, each of both occupying about 21% of the entire market for immobilized enzymes (DiCosimo et al., 2013).

In contrast to free, dissolved enzymes, immobilized enzymes are of higher stability and more resilient against inhibition by heavy metals and other chemicals, non-optimal conditions of pH-value, temperature, or salinity in the environment, and turbulent flow regimes. Importantly, the high variety of reported immobilized enzyme systems provides a range of beneficial aspects, such as convenient recovery of enzymes and products, which reduces the number of process steps, recyclability of enzymes, continuous operation mode of enzymatic reactions, high overall productivity, possibility of sudden termination of reaction on demand, contribution to sophisticated development of enhanced bioreactor designs ("immobilized enzyme reactors"), and decreased environmental pollution and economic costs (Sheldon, 2007a; Defaei et al., 2018). Drawbacks of using immobilized enzymes encompass lower activity and reaction rates compared to free enzymes, susceptibility to putrefaction ("fouling"), additional cost for supports (carriers), or the need for disposing spent (exhausted) immobilized enzymes (Basso & Serban, 2019).

To date, various types of matrices and supports have been utilized to immobilize enzymes. Polysaccharides, glass beads, nano-metals, petro-plastics, and biopolymers are the most frequently used matrices. To immobilize enzymes, the binding strength between support and enzyme depends on the type of chemical or physical reaction, which heavily depends on the nature of the support surface, and the interaction between individual enzyme molecules (Kahraman et al., 2007; Asgher et al., 2014; Rana et al., 2014; Reshmi et al., 2006; Dey et al., 2002; Defaei et al., 2018). Especially the immobilization of enzymes on nanosized supports, such as nanofibers, nanobeads, carbon nanotubes (CNTs), and other nanoparticles for different reactions driven by biocatalysts, is nowadays emerging as an innovative research area (Sheldon, 2007a; Klein et al., 2011; Reshmi et al., 2006; Defaei et al., 2018).

Also enzymes of importance for food technology have been immobilized on various matrices. Some examples are given in Fig. 15.1. The immobilized food

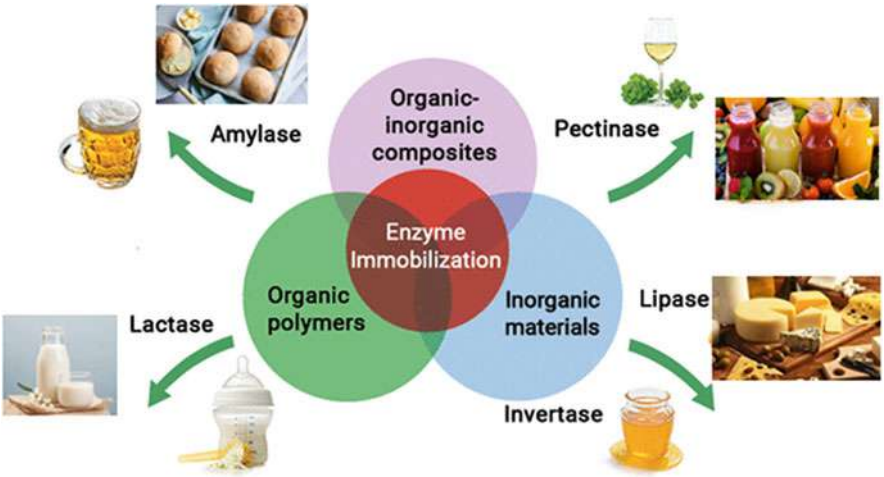

Fig. 15.1 Examples for the applications of different supports for immobilization enzymes used in food processing

enzyme that was the first to be industrially utilized already in the 1970s was amino acylase (EC 3.5.1.14), a hydrolase which was implemented for the production of racemic mixtures of D- and L-amino acids from N-acyl-amino acids as substrates. Industrially, this reaction took and still takes place in columns carrying the immobilized enzyme, while the substrate solution is washed thoroughly. Two other effectually immobilized enzymes were the glycosidase invertase (EC 3.2.1.26), an approved food additive (E 1103) which is used in fructose-rich corn syrup production, and lipases (EC 3.1.1.x), a versatile group of amphiphilic that are applied in the hydrolysis and transesterification of oily products, which, beyond food industry, is also a novel route toward biodiesel production from waste lipids. The advantages of immobilized enzymes in food industries are increased productivity, reduction of product recovery cost, and ultimately increased yields for diverse food products in the future (DiCosimo et al., 2013; Homaei et al., 2013).

15.2 Methods of Enzyme Immobilization

A number of methods can be used to immobilize a given enzyme; these methods encompass simple reversible physical adsorption, formation of ionic bonds between enzymes and supports, and generation of firm covalent linkages (Fig. 15.2). Such immobilization methods are categorized into reversible (adsorption, ionic binding, disulfide formation, affinity binding) and irreversible (covalent binding, cross-linking, physical entrapment [matrix entrapment and encapsulation]) methods. Apart from this classification, immobilization methods can also be classified according to the type of chemical reaction involved in binding as support binding and entrapment methods (Homaei et al., 2013).

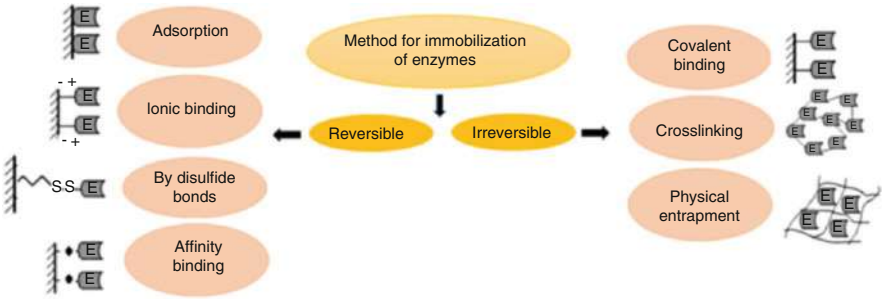

Fig. 15.2 The categories for classification of enzyme immobilization methods

15.2.1 Irreversible Methods

In irreversible enzyme immobilization, the biocatalyst that binds to the matrix cannot be detached without causing an effect on the biological enzyme activity or the structural properties of the support. The most usual methods of irreversible enzyme immobilization encompass covalent binding, cross-linking, and entrapment or encapsulation.

15.2.1.1 Immobilization via Formation of Covalent Binding

This method is widely utilized for enzyme immobilization. Based on this procedure, covalent bonds are created between the chemical groups of the enzyme molecule and the chemical groups present on the support. This method leads to formation of a stable bond between enzyme and support, resulting in the prevention of detachment of enzyme from the support when in use. This covalent bond is typically formed between the side-chain amino acids of biocatalysts, such as aspartic acid, arginine, histidine, and the functional groups, like imidazole, indolyl, phenolic, and hydroxyl present on the support. This method is used when the complete absence of enzyme in the product is desirable. However, it leads to chemical modification of enzymes and, as a result, the activity of the enzyme can get reduced (Homaei et al., 2013). As an option to increase activity of enzymes immobilized via covalent linking, linear spacers are often used to combine enzyme and support, giving the enzyme enough space for mobility and reaction (De Maio et al., 2003). An important example in this context is the carboxyl- and amine-reactive linker 1-ethyl-3-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), which can link hydrophobic polymeric supports with enzymes (Mohan et al., 2015).

15.2.1.2 Cross-Linking

Crosslinking resorts to covalent linking between the enzyme and active molecules, and is used to generate biocatalytically active polymeric particles, which can conveniently be used for given reactions. This mechanism is also known as "copolymerization". Enzymes bind to each other with the aid of bi-functional reagents, such as glutaraldehyde, glutardialdehyde, glyoxal, diisocyanates, hexamethylene

diisocyanate, and toluene diisocyanate, which build bridges between individual enzyme molecules. The major drawback of this procedure is that the multifunctional reagents used for cross-linking the enzyme may reconstruct or change the structure of the enzyme, which again causes the loss of catalytic activity (Albayrak & Yang, 2002).

15.2.1.3 Physical Entrapment

This method involves the physical trapping of biocatalysts into a film, gel, fiber, coating, or microencapsulation (Costa et al., 2005). In this procedure, the enzyme or reactive molecule can be mixed with a polymer to attach to it, resulting in creation of a lattice structure that encapsulates/entraps the enzyme. The advantages of this immobilization method include generation of a vast biocatalytically active surface area generated by the substrate and the enzyme, in only a low volume. The most important disadvantages of this method are the probable inactivation of the enzyme during microencapsulation, enzyme leakage into the environment, and the need for typically high concentrations of the enzyme. The most frequently used supports that are used for encapsulation of enzymes are polymers, such as cellulose, collagen, polyacrylamide gel, gelatin, alginate, starch, silicone, and rubber (Nisha et al., 2012).

15.2.2 Reversible Methods

15.2.2.1 Adsorption

The adsorption of enzymes on supports, such as activated charcoal, alumina, and ion exchange resins is among the simplest techniques used to limit enzyme mobility (Brady & Jordaan, 2009). Depending on the nature of amino acids present on the surface of enzymes and the chemical nature of the support, the enzyme is fixed by non-covalent binding trough ionic and hydrophobic interactions, or by formation of hydrogen bonds. This method can be achieved by mixing an aqueous enzyme solution with a matrix for a defined time, followed by a washing step to remove the remaining free enzyme from the immobilization matrix. This method of immobilization is simple and has little effect on enzyme activity and can be repeatedly applied by adding fresh enzyme solution ("recharging" of the support with biocatalyst). However, in this method, shortcomings, like fast enzyme desorption from the support, or loss of activity by changing pH-value, temperature, solvent, and ionic strength of the surrounding environment, can hardly be avoided (Costa et al., 2005).

15.2.2.2 Ionic Bond Formation

Immobilization by generation of ionic bonds is primarily based on the formation of ionic bonds between the enzyme molecules or reactive molecules and a solid matrix that has a charged ionic surface. The main advantage of ionic binding in comparison to physical binding is the strength of generated bonds that is stronger in case of ionic bonds than the power responsible for formation of covalent bonds (Torres et al., 2002). Such non-covalent immobilization assays can be reversed/deactivated by

alterations in the temperature, solvent polarity, and ionic strength conditions (Nisha et al., 2012).

15.2.2.3 Immobilization via Disulfide Bridges

This method involves the configuration of disulfide (-S-S-) bridges between the enzyme and the support. The major benefit of this method is the ability to recover the bonds created between the activated solid surface and the thiol groups on the enzyme, because the leakage of bounded protein leads to the release of high amounts of low-molecular-weight thiol from disulfide bridges. The feasibility of reusing the polymeric matrix after enzyme deactivation may facilitate the actual large-scale immobilization of enzymes in industrial procedures, where their use is currently not economical because of the high cost of support materials (Ovsejevi et al., 2013).

15.2.2.4 Affinity Binding

Affinity immobilization combines the properties of the enzyme to maintain under varied physiological conditions. This can be done via two routes of support pre-junction by an affinity ligand for desired enzyme, or enzyme attachment to the organism that has an affinity toward the support. The benefits of this procedures are that the enzyme is not exposed to any unusual chemical conditions, minimal conformational changes occur during immobilization, and the high activity of immobilized enzyme is maintained (Sardar et al., 2000).

15.3 Classification of Different Support Materials for Immobilization of Food Enzymes

The reaction occurring between the enzyme and a carrier generates an immobilized enzyme with particular structural, biochemical, physical, and kinetic characteristics. Carriers can be divided into different groups based on their appearance or their chemical components. The support can be a synthetic or biological organic polymer, or an inorganic solid. The support must display certain features, like extended surface-to-volume ratio, high permeability (mass transference), acceptable functional groups for enzyme binding under non-denaturing conditions, hydrophilic moieties, insolubility in water, chemical and thermal stability, mechanical strength, high recalcitrance, applicable particle shape, resistance to microbial attack, regenerability, biological safety, and low or acceptable price (Sheldon, 2007b; Garcia-Galan et al., 2011). Furthermore, multi-enzyme biocatalysis on only one support, especially processes resorting to multi-enzyme cascades, is an emerging approach to generate high-value chemicals on an industrial scale (Xu et al., 2020).

15.3.1 Classification of Different Support Materials Based on the Chemical Structure

15.3.1.1 Inorganic Solid Supports

Different inorganic solids, such as zeolites, alumina, silica, and mesoporous silicas, are possible to use for enzyme immobilization. Silica-based supports are the best applicable matrices for immobilization of enzymes in the industrial manufacturing of enzymatically produced products, in addition to research purposes (Vianello et al., 2006; Pierre, 2004; Hudson et al., 2008). In general, the high surface area provided via silica gel matrixes is nonpareil. Moreover, silica gel can be facilely treated to obtain the desired shape, pore size, and microchannels to allow reaction between substrates and ligands. Furthermore, silica gel is mechanically stable and chemically inactive; it is therefore environmentally benign for manufacturing and industrial procedures (Blanco et al., 2004). The simplest and cheapest methods for enzyme immobilizing, namely, attachment on silica, are realized by simple adsorption. For instance, this method is utilized for generation of enzyme formulations in detergent powders that release the enzyme into the washing liquid during the washing process (Deere et al., 2002).

Polymers

A recent method of enzyme immobilization is based on covalent linkage of such enzymes to polymers that undergo significant structural conformation changes in response to even minor environmental changes in terms of pH-value, temperature, and ionic strength (Klouda & Mikos, 2008). A studied sample is poly (N-isopropyl acrylamide) (poly NIPAM), a thermo- and bio-compatible polymer (Klis et al., 2009). Aqueous poly NIPAM has its critical solution temperature (CST) at around 32 °C. Above the CST, it becomes dissolvable because of release of water molecules from the polymer fibers. Thus, the bio-conversion can take place under states that maintain the enzyme solubility, thereby minimizing diffusional restriction. Subsequently, an increase in temperature above the CST leads to detachment of the immobilized enzyme, thus the enzyme is recovered and can be reused (Virtanen & Tenhu, 2000; Virtanen et al., 2000; Lozinsky et al., 2003). Polyurethane has recently been proposed as an entrapping polymer that retains the bioactivity of biocatalysts for long times. The usage of this polymer resulted in a remaining crude oil degradation capacity of 44.31% by a microbial consortium after more than 6 months (Kazemzadeh et al., 2020).

Polymers that possess electrical conductivity have already been successfully synthesized and utilized in different areas, including biotechnology. Recently, a new class of polymers has been proposed as novel electro-active conjugated polymers. This kind of supports exhibit interesting electrical and optical characteristics previously reported only for inorganic systems. Electronically directing polymers are different from all the familiar inorganic crystalline semiconductors, such as silicon. They are molecular in nature and long chains are absent in them. Immobilization of enzymes and biosensor construction are two applications of these polymers (Cirpan et al., 2003). Lots of theoretical models

have also been associated with the electrochemical entrapment of enzymes to evaluate the polymer thickness, enzyme configuration, and the level of enzyme loading in the biosensor design (Gerard et al., 2002).

Nanomaterials

The headway of nanotechnology in the 1990s was preceded by the quick evolvement of nanobiotechnology including the construction of nanobiocatalysts. In the early methods applied in nanobiocatalysis, enzymes were immobilized on various nanostructured materials utilizing conventional procedures, like simple adsorption and covalent linkage. This method attracted attention for immobilizing enzymes on a wide range of nanostructured matrices, like porous nanomaterials, electroconductive nanofibers, and magnetic nanoparticles (MNPs). This large area provides better enzyme loading, which in turn improves the enzyme mobility in comparison to immobilized enzyme systems on conventional matrices. One of the special advantages of nanostructured materials is that the pore size in nanopores, nanofibers, or nanotubes can be controlled at nanometer scale (Homaei et al., 2013).

Recently, nanobiocatalytic methods have evolved from simple strategies for immobilization of enzymes (Homaei et al., 2013). By rapid advancement in nanotechnology, MNPs are presently extremely interesting. The physico-chemical characteristics of MNPs can widely differ from the properties of the bulk material from which the nanoparticle is made; this has attracted attention in these materials also for enzyme immobilization. For instance, magnetizing a particle in a particular direction by magnetic anisotropy is usually done on the surface of a particle (Schellenberger et al., 2002). Nanoparticles constructed by ultra-small superparamagnetic iron oxide (Kooi et al., 2003; Keller et al., 2004), cross-linked iron oxide (CLIO), and mono-crystalline iron oxide (Krause et al., 2004) all were fabricated as imaging elements in magnetic resonance imaging (MRI). Magnetic particles are used for enzyme immobilization for the purpose of increasing the stability of the biocatalyst, to maintain the stability of the catalyst, and, importantly, they can conveniently be separated from the interaction environment and recovered by applying an external magnetic field. (Bilal & Iqbal, 2019). MNPs perform best at typical sizes ranging between 10 and 20 nm, where superparamagnetism emerges (Netto et al., 2013). Such magnetic particles have been suggested for biotechnological applications (Kluchova et al., 2009; Defaei et al., 2018) or for developing analytical systems, like biosensors (Bilal & Iqbal, 2019; Kouassi et al., 2005).

Nanostructured metal oxides (NMOs) recently became of interest in the area of enzyme immobilization because these materials have the best structural revising and high bioactivity, which leads to elevated sensing properties (Antony et al., 2016). As a part of NMOs, MNPs have been widely utilized in enzyme immobilization because of their advantageous properties, such as their size, magnitude, higher safety levels, better reusability, wide surface, and large capacity of enzyme loading. Because they have an inactive surface that limits direct binding to enzymes, protective molecules must be coated on MNPs to supply dynamic functional groups for immobilization of enzymes (Amirbandeh & Taheri-Kafrani, 2016; Mehnati-Najafabadi et al., 2018). With all these benefits in mind, MNPs are highly inhibited in acidic and oxidative

conditions. Therefore, coating of the outer protective surface is so vital to sustain the consistency of MNPs (Landarani-Isfahani et al., 2015). In a recent study, Defaei et al. (2018) immobilized the hydrolase α-amylase (EC 3.2.1.1) onto naringin-functionalized MNPs by ionic reactions. The MNPs were covered with naringin, which is a biocompatible flavonoid. The appearance, structure, and features of functionalized MNPs and the immobilization status of the nanocomposite were determined by analytical instruments, like thermogravimetry (TGA), vibrating sample magnetometer (VSM), Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy with energy dispersive X-ray (SEM-EDX), and transmission electron microscopy (TEM). In addition, the optimum conditions of temperature, pH-value, interaction time, and enzyme tendency for better immobilization were evaluated. The results evaluated the optimum conditions for α-amylase immobilization on the synthesized nanocarrier at pH 6.5 and a temperature of 55 °C. Reuse experiments showed high maintenance of immobilized α-amylase activity even after 10 reaction repeats. Furthermore, the storage consistency of enzyme immobilization was repaired by immobilization compared to that of the free enzyme and it retained 60% of its original activity even after 6 weeks of storage at 4 °C. Improving the catalytic properties of enzymes through immobilization has made this nanobiocatalyst a feasible tool in bio-industrial systems.

Another field of application of MNPs as supports for immobilized enzymes is the pharma sector; here, it was shown that the enzyme penicillin G acylase (PGA; EC 3.5.1.11) can be immobilized to functionalized MNPs (epoxy-activated magnetic cellulose beads) due to the cavity and affinity forces in the matrix of activated cellulose, and applied for hydrolytic removal of the side chain of penicillin G molecules, generating 6-aminopenicillanic acid as product. Improved biocatalytic activity and stability of the enzyme were reported for this process in comparison to the use of free enzyme (Luo & Zhang, 2010). In recent years, this process was improved by various research groups globally, such as by Liu and colleagues, who covalently immobilized PGA on hydroxy- and aldehyde-functionalized magnetic Fe_2O_3/Fe_3O_4 nanoparticles (Liu et al., 2020), or Zhaoyu et al., who used novel di-functional magnetic "nanoflowers," equipped with epoxy groups and hydrophilic catechol as well as with phthaloquinone groups enabling the covalent coupling of penicillin G acylase (Zhaoyu et al., 2020).

15.3.1.2 Organic Solid Supports

Different biological polymers, especially hardly water-soluble or de facto water-insoluble polysaccharides, like agarose, cellulose, starch, and carrageenans, have been heavily utilized as matrices for enzyme immobilization. These polymers create highly inactive aqueous gels, with the property of high gel formation even at low concentrations (Van de Velde et al., 2002).

The best described and most widely applied synthetic polymers used to carry the immobilized enzymes are acrylic resins, like sepabeads or eupergit. These macroporous polymers are copolymerized by using N,N′-methylene-bi-(methacrylamide), glycidyl ether, methacrylamide, allyl methacrylate, and glycidyl methacrylate. They are very hydrophilic and highly chemically and mechanically

stable in the entire pH-range of 0 to 14 and are not inactivated even at a sudden and tremendous pH-change. A basic disadvantage of hydrophilic resins are diffusion limitations that have been shown in kinetically monitored procedures. Immobilization by covalent binding to acrylic resins has been successfully utilized for a variety of enzymes in industrial operations (Katchalski-Katzir & Kraemer, 2000; Boller et al., 2002).

Enzymes may also be immobilized in biological or artificial hydro- or cryo-gels in an insoluble environment. For example, poly (vinyl alcohol) (PVA) crucibles made by melting ice are frequently used for whole-cell immobilization (Tripathi et al., 2010). Also, because of their minor size, free enzymes can spread from the gel matrix and get dissolved in an aqueous medium. To trap free enzymes, the enzyme size should be enlarged by mechanisms, such as cross-linking. Another option to increase the biocatalytic enzyme size is to design a composite material with a polyelectrolyte. Because of their ampholytic nature, proteins are released either as polycations or polyanions, based on the environmental pH-value. Therefore, the typically can form complexes with polyelectrolytes of opposite charges (Homaei et al., 2013).

15.4 Multi-Enzyme Immobilization

Multi-enzyme immobilization is a technique that co-establishes more than one enzyme on appropriate supports/carriers, or joins enzymes using a linking agent (cross-linker) with no carriers (Ren et al., 2019). Enzymes will be adjacent to each other, and the total material-transport restriction will be decreased during co-immobilization, which has been shown to boost the activity of enzymes by matrix canaling and raising the consistency and ability to reuse them. As support materials can mainly influence the characteristics of enzymes, selection of support has been mentioned as a hot subject in the majority of reports on enzyme immobilization techniques. To date, different matrices, like CNTs, graphene, metal-organic frameworks (MOFs), DNA nanocomposites, silica, and polymers, have been used for immobilization of multi-enzyme, which can efficiently shelter enzyme activity from biological challenges, such as heavy metals or high temperature (Sheldon & Woodley, 2018; Ren et al., 2019). There are three major types for co-immobilization of enzymes, such as random and positional co-immobilization, and compartmentalization (Hwang & Lee, 2019).

Biocatalytic transformation by a cascade of enzymes ("multi-enzyme catalysis") is an emerging technology to manufacture various industrially valuable compounds; briefly, it mimics the cascadic catalytic steps of pathways in living cells. In this context, positional multi-enzyme immobilization has been demonstrated as a viable tool for effective immobilization of co-enzymes, which can trigger the rates of coenzyme cascade reactions by fine-tuning the sequence of immobilized enzymes, and which, first of all, improves channeling of the substrate flow, and avoids the formation of unwanted side products, thus leading to higher overall yields. Moreover, such positional multi-enzyme immobilization approaches result in high

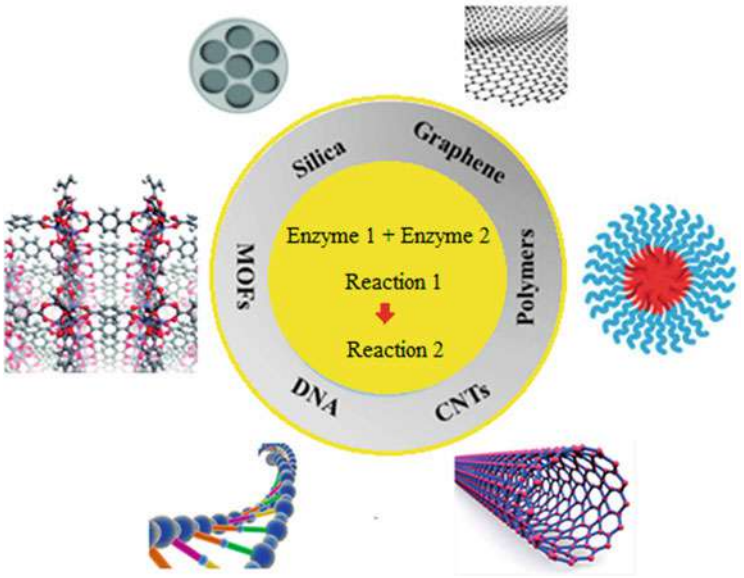

Fig. 15.3 Schematic illustration for multi-enzymes co-immobilization using supports, such as polymers, graphene, silica, MOFs, DNA, and CNTs

stability and reusability of involved enzymes. Polymers, DNA nanostructures, graphene, or CNTs are the most frequently used supports for positional multi-enzyme immobilization grace to their expedient capability to manage the relative positions of enzymes by usual reactions (Xu et al., 2020).

As an imitation of the organization of natural enzymes in cellular environments, compartmentalization can divide enzymes by varied features and ratios spatially, which can inhibit enzymes proteolysis, biological decomposition, or exposure to toxic agents (Marguet et al., 2013). As an example, a compartmentalized co-enzyme operation was created on the base of inorganic nanocrystal–protein complexes via a simple precipitation technique that showed increased overall catalytic performance in comparison to the use of free enzymes. For this purpose, the versatile enzyme horseradish peroxidase (HRP; EC 1.11.1.7), a well-known oxidoreductase, was combined with $CuSO_4$ in aqueous environment in order to form HRP-incorporated complexes; subsequently, glucose oxidase (GOx; EC 1.1.3.4) was attached on the surface of the complexes via the cooperative reaction between Cu^{2+} and protein amino acids (Li et al., 2014). Different organic and inorganic bases can be applied for immobilization of multi-enzymes as described in Fig. 15.3.

DNA nanotechnology has turned out to be a feasible method to construct complex bio-molecular nanocomposites because of the ability of programmed DNA hybridization. For immobilization of multi-enzymes, it is important to decrease the mass transfer consistency by managing the relative location and directions of a variety of enzymes in a limited space. Therefore, DNA nanotechnology has been used as an effective instrument for the multi-enzyme immobilization in

which notably addressable DNA nanocomposites can ease the suitable self-aggregation of varied enzymes and optimize the substrate penetration (Xu et al., 2020).

15.5 Kinetics of Enzyme Immobilization

Compared to measurements of the kinetics of enzymes in absolute solution, the interactions of immobilized enzymes have only scarcely been shown under spatially sole substrate and uniform conditions, such as pH-value. In addition, immobilization can change the innate kinetics of the enzyme by altering the structure of the enzyme and surrounding microenvironment. To diminish the effect of immobilization on the enzyme catalytic activity, the molecular configuration of the enzyme should be considered (Gonzalez-Saiz & Pizarro, 2001).

Immobilization usually enhances enzyme maintenance at the expense of lower catalytic function (Sheldon, 2007a; Garcia-Galan et al., 2011). Nevertheless, the appropriate choice of enzyme immobilization techniques may reduce or increase the enzyme activity (Rodrigues et al., 2013, 2011; Mateo et al., 2007). For example, enzyme immobilization on highly active supports can improve the multipoint covalent linkage and prevent the enzyme from being inactive after immobilization (Pedroche et al., 2007; Mohan et al., 2015). Similarly, the selection of enzyme loading and immobilization matrix may prefer the partition of H^+ or OH^- ions, and change the local pH-value in the support. The changed pH-value may cause the immobilized enzyme to work under conditions close to the pH optimum (Pedroche et al., 2007). The conformation of the enzyme molecule across the immobilization process may also affect its function in the presence of detergents. MCA immobilization of lipases can enhance their activity because the enzyme is immobilized in an active combination (Fernández-Lorente et al., 2006).

Omitting standard metrics for enzymatic activity assessment, such as K_m, V_{max}, and k_{cat}, or extracting separated function metrics from data sets other than interaction progress curves can be prevented using the available literature to lead to rational choose of immobilization schemes (Herzog et al., 2005; Koh & Pishko, 2005). To enhance an acceptable comparison between the studies about kinetics, minimal reporting standards (STRENDA) have been reported that contain features of model election and error diagnosis (Tipton et al., 2014; Gardossi et al., 2010).

15.6 The Usage of Enzyme Immobilization for Production of Different Food Products

Unlike pharmaceutical industries and some chemical industries, the food industry requires the production of vast quantities of commercial products. For this purpose, the cost of the biocatalyst should be lowered, thus, using immobilized enzymes show acceptable operational consistency that allows lots of repetitive production cycles to be carried out. In the food sector, continuous fermentation processes are preferred to

batch processes, especially when large quantities of material are manufactured. Examples of broad-scale usages of immobilized enzymes for production of food products are explained in the subsequent paragraphs of the chapter at hand.

15.6.1 D-Glucose/Xylose Isomerase for Production of High Fructose Corn Syrup

The use of immobilized D-glucose/xylose isomerase (EC 5.3.1.5) in the preparation of highly fructose-rich corn syrup (HFCS) shows a largely applied commercial procedure encompassing an enzyme immobilization step, with a high quantity of enzyme that is used, and expedient product yield (Crabb & Shetty, 1999). Generally, the enzyme, which belongs to the top three industrially used enzymes (others being proteases and amylases), catalyzes the interconversion of D-glucose to D-fructose and D-xylose to D-xylulose, respectively (Gaikwad et al., 1992). HFCS is predominantly used in fructose production that is applied as a sweetener for beverages and foods, or utilized directly as a food and beverage ingredient. While D-xylose is the native substrate for D-glucose/xylose isomerase function, the enzyme has a wide substrate spectrum, and in its industrial application it produces D-fructose from D-glucose in an efficient manner (Bhosale et al., 1996).

Today, HFCS production processes are performed in fixed bed reactors that are ordered in parallel and operated continuously (Nedwin et al., 2014). D-glucose syrup that is stemming from corn is converted into a mixture that is generally named as HFCS-42. This syrup contains approximately 42%, 50%, 6%, and 2% D-fructose, D-glucose, maltose, and maltotriose, respectively, as well as low quantities of other sugars. Higher concentrations of fructose that are obtained by chromatographic enrichment of the 42% syrup to a final 90% D-fructose (HFCS-90) content is used in soft drinks. The two commercially available immobilized D-glucose/xylose isomerase preparations, most commonly used for these processes, are on the basis of inexpensive inorganic supports, like bentonite clay and diatomaceous earth with cross-linking the enzyme by glutaraldehyde. The resulting composite is dewatered and then dried in a fluidized bed dryer. The obtained immobilized D-glucose/xylose isomerase preparations are very consistent, with a half-life of more than 1 year and are used in a packed bed reactor at 60 °C (Basso & Serban, 2019).

15.6.2 Epimerase for Production of Allulose

D-allulose (D-psicose or "pseudofructose") is a low-calorie monosaccharide sweetener recommended due to its sweeting similarity to dextrose, and about 70% of the sweetness of sucrose. D-allulose is the C3-epimer of D-fructose. The difference between allulose and fructose is that allulose is hardly metabolized in the human body and has almost zero calories (only 0.007 kcal/g)). Globally leading food and beverage manufacturers are the main target markets for allulose that replace dextrose, fructose or HFCS in their products, which is important to decrease calories in

parallel with maintenance of the properties obtained by the sugar ingredient, such as browning, bulking, texture, and sweetness. Allulose is also supposed to display potential antihyperglycemic effects, and was shown to prevent postprandial hyperglycemia in humans. More than 0.2 g per day human allulose intake is estimated when consuming naturally found materials, such as processed cane and beet molasses, coffee treated via steam, wheat products, and HFCS. In 2012, allulose was labeled as generally recognized as safe (GRAS) by the FDA and approved as sweetener in food products (not yet approved in, e.g., the European Union!), so it is commercially utilized as food additive in some parts of the world. Moreover, ketose-3-epimerase (EC 5.1.3.31), an isomerase which is found in various microorganisms, can interconvert fructose to allulose and vice versa (Basso & Serban, 2019).

15.6.3 β-Galactosidase for Production of Tagatose

Another emerging sweetener is the ketohexose tagatose, which can be isolated from animal origins. Tagatose is a kind of sweetener with 92% fructose sweetness, but contains only 38% of fructose calories. Its catabolic route is different from sucrose; therefore, it has insignificant effect on insulin and blood glucose levels. Moreover, tagatose is considered a "tooth-friendly" compound for dental care products. Tagatose can be gained from lactose with only 16% of the sweetening of the later. The disaccharide lactose (β-D-galactopyranosyl-(1 → 4)-D-glucose) is a natural sugar found in milk and normally makes up 2–8% of its total mass. The process of tagatose production utilizing the immobilized glucosidase β-galactosidase (3.2.1.23) has been reported in 2014. This process suggests preparation and valorization of lactose present in whey at a concentration of 18 wt.-%. It is possible to obtain tagatose by lactose hydrolysis using immobilized β-galactosidase to produce the monomers glucose and galactose. Then glucose is removed from the mixture by deglycosylation using baker's yeast; now, tagatose is obtained by epimerization of galactose with aerated $Ca(OH)_2$ (Basso & Serban, 2019). There have also been reports on the utilization of immobilized L-arabinose isomerase (EC 5.3.1.4) to obtain tagatose in stirred tank reactors or continuous flow systems (Lim et al., 2008; Oh, 2007).

15.6.4 Other Sweeteners Produced by Immobilized Enzymes

Another sweetener for food and beverage industry, also accessible by means of biocatalysis, is the canonic amino acid L-aspartate (2-aminobutanedioic acid). Aspartate production is performed by amination of fumaric acid; this reaction is catalyzed by the lyase enzyme aspartase (aspartate ammonia-lyase, EC 4.3.1.1). Already in 1973, Tosa et al. reported the application of aspartase from *Escherichia coli* for aspartate production from ammonium fumarate; these authors immobilized the enzyme by different methods: ionic binding on cellulose derivatives or sephadex,

physical adsorption to silica gel or calcium phosphate gel, covalent binding to cellulose azide or diazonium derivatives of cellulose, or entrapping in polyacrylamide gel lattice. The authors obtained the most active immobilized aspartase by the entrapping process; regarding optimum pH-value, temperature, and ion concentration, no differences were observed between the immobilized and free enzyme in terms of kinetic constants and heat stability. Excellent conversion yields were reported for this process when operated in continuous mode using columns packed with the immobilized aspartase (Tosa et al., 1973). Later, the same group of authors immobilized *E. coli* entrapped in a polyacrylamide gel lattice as whole-cell biocatalyst for continuous aspartate production in a packed column reactor (Tosa et al., 1974). Currently, aspartate is produced on an annual scale of 10^4 tons by cross-linked whole cells or by the isolated enzyme immobilized by different methods (reviewed by DiCosimo et al., 2013).

Moreover, aspartame is another sweetener produced as a dipeptide of L-aspartate and L-phenylalanine. For this purpose, in 1981, Oyama et al. immobilized the hydrolase enzyme thermolysin (EC 3.4.24.4) by different methods: physical adsorption to Amberlite XAD-7 and XAD-8, ionic binding to the ionic ion exchange resin Amberlite IRA-94, adsorption on glass beads, and covalent linking to a hydrophilic ethylenediamine-derivatized polymer gel by glutaraldehyde. The immobilized enzyme assays prepared via these different ways were tested for formation of aspartame by reaction of the precursors N-(benzyloxycarbonyl)-L-aspartic acid with L-phenylalanine methyl ester by incubating the mixture of the substrates in the solvent ethyl acetate, which normally would denature the enzyme. Substrates moved from the organic phase to the aqueous phase in the support, where the reaction took place, and the product (aspartame) diffused back to the organic phase, from which it could be recovered. Among the different tested immobilization strategies, physical adsorption to Amberlite XAD-7 and XAD-8 resulted in the best yields (Oyama et al., 1981). Later, this immobilization technique for thermolysin was successfully used by Miyanaga et al. for continuous production of the aspartame precursor N-(benzyloxycarbony1)-L-aspartyl-L-phenylalanine methyl ester from N-(benzyloxycarbony1)-L-aspartic acid and L-phenylalanine methyl ester in a mixed organic solvent system consisting of *tert*-amyl alcohol and ethyl acetate in a column reactor. Here, excellent conversion yields of 99% were achieved under optimized conditions (Miyanaga et al., 1995).

15.6.5 Enzyme Immobilization for Debittering of Citrus Fruit Juices

The polyphenol naringin, a flavonoid, is responsible for the bitter taste of citrus fruits. There is increased interest in fruit juice industry by using highly efficient immobilized enzymes for debittering of citrus fruit juices (Puri et al., 2008). Already in 1979, Olson and co-workers reported the immobilization of commercially available naringinase (mixture of the hydrolases α-rhamnosidase, EC 3.2.1.40, and β-glucosidase, EC 3.2.1.21; hydrolyzes naringin to naringenin, glucose, and rhamnose) in a reactor system consisting of polysulfone hollow fibers; immobilization

took place by ultrafiltration of the enzymes into the sponge region of the hollow fibers. After 210 min of continuous operation, 50% of naringin contained in grapefruit juice was hydrolyzed at 25 °C and a flow rate of 300 mL/min (Olson et al., 1979). Other approaches to immobilize naringinase encompass entrapping in alginate, which resulted in 60% debittering of kinnow juice after 3 h when using a total enzyme activity of 30 U (Puri et al., 1996), immobilization on electrospun cellulose acetate nanofibers (Huang et al., 2017), on chitin (Tsen, 1984) or chitosan microspheres (Bodakowska-Boczniewicz & Garncarek, 2019) by linking with glutaraldehyde, or adsorption of the enzyme on mesoporous molecular sieves via glutaraldehyde for naringin hydrolysis in white grapefruit juice (Lei et al., 2011). A rather bizarre protocol for naringinase immobilization was developed by Puri and colleagues, who attached the enzyme on chicken egg white beads obtained by cross-linking the protein with glutaraldehyde; debittering of Kinnow juice achieved an efficiency of 68% (Puri et al., 2001). In addition, Busto et al. immobilized thermophilic *Aspergillus niger* naringinase by entrapping it into a PVA hydrogel matrix, which was cryostructured in liquid nitrogen, to generate beads biocatalytically active for naringin hydrolysis. Authors reported high stability of the beads; after storage at 4 °C for 2 months, they retained 75% of initial activity (Busto et al., 2007).

15.6.6 Lipases for Production of Vitamin C Esters and Cocoa Butter Analogs

One of the main water-soluble natural antioxidants is L-ascorbic acid (vitamin C). L-ascorbic acid and its derivatives act as free radical scavengers, reacting with oxygen, and destroying it. Moreover, hydrophobic long-chain fatty acid ester derivatives of L-ascorbic acid are used as antioxidants in fat-rich food because of their higher ability to dissolve in fats in comparison to the typical hydrophilic compound vitamin C, which is insoluble in oils (Burham et al., 2009). In this context, ascorbic palmitate and stearate are currently prepared by reaction between ascorbic acid with sulfuric acid, followed by re-esterification with the corresponding fatty acid; finally, a purification step by re-crystallization is carried out (Ferreira-Dias et al., 2013). In a biocatalytic approach, immobilization of *Candida antarctica* B lipase (CalB) was utilized for generation of ascorbyl esters. The biocatalytic conversion can reach a yield of approximately 95%, depending on process temperature, the level of removal of the side product water, and fatty acid chain length. In spite of the fact that enzymatic synthesis suggests some benefits to the current chemical procedures, such as interaction in the lower temperatures than chemical reactions temperatures, higher material purity, and decreased downstream processing expenditure, many of the manufactures of ascorbyl esters still carry out this synthesis by chemical processes, because of the long interaction time needed by the enzymatic procedure and the high price of the immobilized enzymes in contrast to the chemical catalysts (Villeneuve, 2007).

The source of vegetable oils, such as palm, rapeseed, canola, and sunflower, specifies the physical characteristics of fats and oils present in food products,

because each oil has a different arrangement and type of saturated mono- and polyunsaturated fatty acids in the 1, 2, and 3 locations of triacylglycerides. To obtain the suitable melting properties of fats and oils, especially in the generation of margarine and baking fat, chemical hydrogenation, fractioning, and esterification have been applied. The enzymatic transesterification of food oils and fats is one of the benefits because of the option to better monitor the product composition compared to chemically transesterified products due to the removal of the hydrogenated trans fats that have important health challenges (Marangoni & Rousseau, 1998; Asif, 2011).

Enzymatic transesterification was first investigated to produce an equivalent of cocoa that used the sn-1,3 specificity of different fungal lipases. Cocoa butter homologs are semisolid oils that commonly have a melting temperature of 37 °C. They are obtained from more cost-effective origins than cocoa, like palm, sunflower, or rapeseed oil. A variety of commercial processes have been developed to produce the equivalent of cocoa butter with elevated amount of the demanded triglycerides, 1(3)-palmitoyl-3(1)-stearoyl-2-monooleine, and 1,3-distearoyl-2-monooleine, required for chocolate production. Most systems are made by using fungal lipases immobilized by surface adsorption or encapsulation in liposomes (Basso & Serban, 2019).

15.6.7 β-Galactosidase for Lactose Hydrolysis

Bovine milk contains 4.3–4.5 wt.-% lactose that exposes 38–40% of the whole milk solids. Lactose in milk and milk products is not hydrolyzed in the stomach or in the initial part of the small intestine; it enters to other parts of the intestine and gets hydrolyzed into the monosaccharides D-galactose and D-glucose by the glycosidase β-galactosidase (lactase, EC 3.2.1.23) excreted by the intestinal microflora. About 65% of the entire human population (up to 90% in some Asian countries) are unable to secrete sufficient quantities of β-galactosidase, causing many health disorders. Elimination of lactose from milk and milk products makes them suitable for consumption by people with lactose intolerance (hypolactasia), so the dairy industry has demonstrated great interest to develop advanced lactose hydrolysis processes based on β-galactosidase. Because the sweetening potential of lactose, glucose, and galactose is 20, 70, and 58%, respectively, of sucrose, lactose-hydrolyzed milk is sweeter than pristine milk (Panesar et al., 2010).

The simplest but most expensive solution for this problem is to add free β-galactosidase to whole milk. Enzyme activity is stopped after complete substrate hydrolysis, typically combined with pasteurization. Another procedure is the usage of immobilized β-galactosidase for processing of skimmed milk; after completion of hydrolysis, the fat fraction is added again to the hydrolyzed milk to reassemble its nutritious components. This technique, of course, displays the benefits of recycling and reusing the immobilized enzyme in contrast to adding free enzyme, and the final product is free from additional ingredients, like enzymes or components or the enzyme formulation that can constitute putative allergens.

The techno-economic evaluation of lactose removal by immobilized β-galactosidase from the fungus *Aspergillus niger* dates back as far as to 1990; that time, Axelsson and Zacchi calculated the cost for a tank reactor operated in batch mode, free and immobilized β-galactosidase, a continuously operated stirred tank reactor (CSTR), and for a plug-flow tubular reactor (PFTR). For all cases, the mass transfer behavior and enzyme deactivation were considered. As outcome, the authors concluded that enzyme immobilization indeed is economically more feasible when compared with application of free enzymes, although the high cost for enzyme immobilization itself still constitute an obstacle. The lowest cost of 0.48 Swedish crowns (SEK) per kg lactose to be hydrolyzed, calculated for a half-life time of 80 days, were calculated for immobilized enzyme in the PFTR; however, calculated costs for using immobilized β-galactosidase in a batch reactor were only insignificantly higher with 0.66 SEK/kg lactose. In any case, these two modes of using immobilized enzyme were considerably lower in costs than for the case of free enzyme in a batch reactor (2.10 SEK/kg lactose) (Axelsson & Zacchi, 1990).

In 2003, Roy and Gupta used the commercially available β-galactosidase preparation Lactozym™ (Novozymes, Denmark) from the yeast *Kluyveromyces fragilis*. This preparation is GRAS for hydrolysis of whey to produce lactose-poor milk. The authors immobilized Lactozym™ on cellulose beads via covalent epichlorohydrin coupling. In a column serving as fluidized bed reactor, whey lactose was hydrolyzed by >90% within 5 h, while the same enzyme, when applied in continuous batch mode, took 48 h for the same hydrolysis outcome. It was possible to reuse the immobilized enzyme three times without decrease in the biocatalytic performance of the fluidized bed reactor column. In the same fluidized bed reactor, also lactose in whole milk was converted to glucose and galactose up to 60% within 5 h (Roy & Gupta, 2003).

A more recent example for the use of an immobilized β-galactosidase is the enzyme isolated from the yeast *Saccharomyces lactis*. This enzyme was immobilized by entrapment in cellulose triacetate fibers. The entrapped enzyme was reused for 50 times with the reduction of enzyme activity less than 9% in a rotary horizontal column reactor; 10 tons milk were processed per day via this process on industrial scale (reviewed by Basso & Serban, 2019). Moreover, *Aspergillus oryzae* β-galactosidase was immobilized by covalently binding the enzyme to an ion exchange resin based on polyphenolic formaldehyde (Hirohara et al., 1981; reviewed by Basso & Serban, 2019).

15.7 The Usage of Immobilized Enzymes in Transforming Food Waste

A notable amount (approximately 40%) of all types of food are disposed as waste (Godfray et al., 2010), and these losses not only lead to environmental pollution but also affect the entire food chain. This amount varies between different geographic regions, and one should differentiate between food waste *sensu stricto* and agricultural waste. These waste streams are responsible for a major global challenge both in

causing environmental pollution and ethical concern considering the huge number of people starving worldwide. With the global population expected to increase to 9.8 billion until 2050, suitable technological solutions should be developed to solve this problem. Some technical proposals are represented at the food processing level. Liquid food processing waste contains numerous organic carbonaceous compounds; therefore, it has high biological oxygen demand (BOD) that causes problems for direct disposal of them to wastewater removal plants. Here, the disposal of about one million liters of lactose-rich whey per day only on the Northern Italian region constitutes a prime example (Koller et al., 2016). Hence, the lipid, carbohydrate, and protein contents of food and agricultural waste liquids are leading to high BOD; however, at the same time, they have the potential to be converted to valuable products, thus upgrading waste liquids into potential recoverable sources. Examples of such conversions include oxidation, hydrolysis, acylation, and phosphorylation of carbohydrates as well as glycosylation and deamination of amino acids, and esterification and hydrogenation of lipids. In particular, esterification processes are widely used for production of different value-added food and agricultural products. Waste oils and animal waste lipids from the slaughtering and rendering industry can be transesterified with alcohol to generate biofuels (Koller et al., 2018). Esterified sugars can be applied as surfactants, and esterified starch may be used as biodegradable plastics and adhesives. Esterification of flavonoids was reported to increase their life time, health, and acceptance characteristics (Walle, 2009). Traditional processes to these transformations require significant amounts of chemical catalysts and energy resources that have limited reactivity, and lead to formation of by-products, especially when done in complex matrices, like food waste liquids (Alissandratos & Halling, 2012; Fang et al., 2002).

15.7.1 Carbohydrate Wastes

Food processing waste streams which are carbohydrate-rich can easily be converted by the enzymatic valorization catalyzed by hydrolases and isomerases into more valuable products, like sweeteners and prebiotics. In fact, some of the best accepted, well-known procedures in food and agricultural systems begin with the use of carbohydrate substrates. In this context, immobilized thermophilic enzymes ("thermozymes"), which have been studied for the production of high fructose corn syrup, could be progressed and used for valorization of food waste liquids that are carbohydrate rich (Andler & Goddard, 2018). Emtiazi et al. (2001) used immobilized cellulase enzymes from *Aspergillus terreus* to decrease chemical oxygen demand (COD) by cellulose removal (40–80%) from pulp manufacturing waste.

15.7.2 Lipid Wastes

According to the significant impact of environmental and economic waste stream valorization, waste oil can be converted to value-added products, such as biodiesel,

surfactants, and lubricants, by the use of enzymes. Lipases, like most other enzymes, can be mined from different microbial sources with different performance properties. For instance, lipases produced by *Thermomyces lanuginosus* and *Candida antarctica* were used for lipid hydrolysis and esterification, respectively, yielding valuable products, such as biofuel, from waste cooking oil. More than 90% conversion was obtained after 10 h of hydrolysis and 10 h of esterification reactions. Noteworthy, after 5 catalysis repeats, the lipase from *C. antarctica* retained its activity, while the lipase from *T. lanuginosus* lost some of its activity after each use (Vescovi et al., 2016). In another study, *Rhizomucor miehei* lipase and *C. antarctica* lipases were immobilized on silica particles that were epoxy-functionalized and used to enhance the performance of biofuel generation from waste cooking oil. A 91.5% conversion rate was achieved during 10 h (Babaki et al., 2017).

Other examples for immobilization of *C. antarctica* lipase for conversion of lipid substrates to value-added products encompass the use of support materials as diverse as core–shell MNPs for conversion of waste cooking oil to biodiesel (Mehrasbi et al., 2017), immobilization by adsorption on poly (styrene) nanoparticles (Miletić et al., 2010), covalent attachment on chitosan-based hydrogels (Silva et al., 2012), or adsorption to green coconut fibers (Brígida et al., 2007).

15.7.3 Proteinaceous Wastes

Proteinaceous food waste may stem from different origins like dairy products (whey retentate), grains (Zhi et al., 2017), oilseeds (Doshi et al., 2014), soybeans (Laskar et al., 2018), eggs (Hong et al., 2019), or even poultry feathers (Pernicova et al., 2019). Proteases are used for hydrolyzing proteins from waste streams and converting them to biological peptides or useful chemicals, such as the monomers that build up polymers. Here, enzymatic pathways are more favorable than chemical reactions, which are not easily controllable, as observed for the degradation of tryptophan via acid hydrolysis (Kumar et al., 2015). Immobilized trypsin has been used for hydrolysis of dairy waste, such as whey protein (retentate fraction remaining after ultrafiltration of full whey), as an alternative to well-established acidic hydrolysis (Koller et al., 2019). Immobilization of bovine pancreas trypsin on porous polymethacrylate with a pore size of 2.1 μm has led to 9.68% hydrolysis. The hydrolysis degree had reached ~6% under the same conditions when using free trypsin, which indicates the need for optimization of the immobilization process. Most significantly, the peptide analysis differed between the immobilized and free trypsin that showed the effect of immobilization methods on enzyme selectivity for hydrolysis of amino acid sequences (Mao et al., 2017). Trypsin has also been immobilized on reusable matrices containing spent grain and lignocellulose for hydrolysis of whey protein (Tavano, 2013; Bassan et al., 2016).

15.8 Commercialization of Immobilized Enzymes for Usage in Food Industries

For successful commercialization of procedures based on immobilized enzymes, the overall process cost is the decisive factor. Some of the major factors determining the cost of immobilization, such as the used matrix (support), the enzyme itself, the chemicals used in the immobilization procedure, and the special equipment that may be needed should be considered. Current requests for immobilized enzymes make up only a small portion of the whole enzyme market (DiCosimo et al., 2013). Different uses of immobilized enzymes may be developed in food manufacturing, medical applications, and food research studies (Homaei et al., 2013). Other examples for commercial usages of immobilized enzymes are synthesis of organic materials on laboratory scale as well as analytical and pharmaceutical usage. Moreover, apart from the food sector, the application of immobilized enzymes for removal of eco-pollutants from aqueous environment might be an important future field of research and development, as it was suggested for the removal of endocrine-disrupting compounds from wastewater by covalently immobilizing HRP on filtration membranes (Mohan et al., 2015; Rathner et al., 2017). For each commercial application, the appropriate selection of a particular immobilized enzyme or strategy of immobilization should be based on the advantages and disadvantages of the performance of free and immobilized enzymes, respectively. This shows that the establishment and use of immobilized enzymes needs profound understanding of affecting functional and economic factors, in-depth kinetic studies, plus the knowledge about the current market requirements and trends (DiCosimo et al., 2013).

15.9 Conclusions

Enzymes usage in soluble form for food processing is well established. Although a high variety of enzymes have already been immobilized and used in different food manufacturing industries, only few procedures have become practical and economical, and succeeded in getting established on the long term. Numerous recent concepts have been attempted or are being used in this field. The future of such applications and operations will depend mainly on their cost and also political decisions. Despite the fact that not all established transformations involved in food processing and food production can to date be replaced by biocatalytic techniques resorting to immobilized enzymes, and although many immobilization processes holding promise in lab-scale experiments are not yet scalable to industrial dimensions, the outlook for immobilized enzymes in food industry is indeed promising considering current food industry trends to become more efficient and sustainable, combined with the rapid progression of immobilized enzyme techniques. In any case, due to the growing human population on Earth and a remarkable decrease in limited natural food resources, the future use of immobilized enzymes may elevate significantly in order to produce higher amounts of food products, and even to unlock alternative food sources for enhanced global food security.

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Prospects and Challenges in Food-Grade Enzymes Industrial Production

16

Musliu Olushola Sunmonu and Mayowa Saheed Sanusi

Abstract

Food-grade enzymes are biocatalyst in industrial production of food and they have numerous advantages over conventional chemical processes due to their ability to achieve efficient and sustainable processes and products. It has found extensive application in bakery, beverages, meat, cheese, alcoholic and juice industries. Commercial food-grade enzymes have been preferred to be sourced from microorganisms (bacteria, fungi, yeast, mold) rather than from plants and animals because of their high production yield and controlled conditions. The relevance, applications and challenges of some novel technologies; enzymes screening, enzymes immobilization, solid-state fermentation, recombinant DNA, protein engineering and bioinformatics as techniques for producing food-grade enzymes were discussed. The sources and the industrial applications of some selected food-grade enzymes, such as amylase, proteases, lipase, rennet, cellulase, lactase, invertase, glucose oxidase, glucose isomerase and catalase in the food industry, and food-grade enzymes safety issues, were also discussed.

Keywords

Industrial application · Food-grade enzymes · Food industry · Novel technologies · Safety

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A. Dutt Tripathi et al. (eds.), *Novel Food Grade Enzymes*,
https://doi.org/10.1007/978-981-19-1288-7_16

16.1 Introduction

The emergence of the food-grade enzyme for industrial production can be tagged as green revolution approach due to its catalytic biological nature in transforming food into various value-added products in different food industries, such as in beverages and juices, baking, development of functional foods, meat, wine, dairy, fats and oils. The food-grade enzyme has been projected as a potential replacement for chemical additives or harsh physical conditions (temperature, pressure, pH) used in industrial production, therefore guarantees sustainable production for safe and nutritious food supply. Also, with an increase in the awareness on the need to consume a healthier diet which has increased consumers' preference for new and convenient food products manufactured from natural food additives has further increased the use of food-grade enzymes. Presently, food-grade enzymes are being used in the food industry to increase diversity, quality and varieties of food produced. The food-grade enzymes can be generated from the plants, animals and microbial sources. However, out of the entire sources used for enzymes generation, microbial is the most preferred choice in the food industry. Enzymes from microbial sources can be produced in control conditions at a faster rate with minimal harmful by-products and in larger quantities. Large-scale Industrial production has explored the use of *Saccharomyces cerevisiae*, *Aspergillus oryzae*, Fungi *Aspergillus niger* and *Bacillus subtilis* bacteria as sources for microbial enzymes production. Some of the commercially important food-grade enzymes produced from this sources are proteases, rennet, pectinases, invertases, cellulases, lipases, amylases, catalase, lactase, raffinase, pullulanase, glucose oxidase, etc. Most of naturally occurring enzymes from plants, animals or microbial sources are not often suitable for economical production processes and sustainability. Therefore, there is a need to adopt novel technological approaches that can be used to improve the industrial production of food-grade enzymes. Uzuner and Cekmecelioglu (2019) reported that industrial enzymes market is competitive with minimal profit margin and the enzymes are currently manufactured by few companies in the United States, Denmark and Germany with approximately twenty (20) enzymes being commercially produced at present. Hydrolase represents 75% of food-grade enzymes in the market. Carbohydrase (i.e. tannase, phytases, amylases, pectinases, cellulases, β-galactosidases), lipase and proteases are family of hydrolase usually employed as food-grade additives or processing aids in the food industry. This chapter provides an overview of the prospect and challenges of a novel application of techniques for improving the industrial production of food-grade enzymes through screening, enzyme immobilization, solid-state fermentation, recombinant DNA technology, protein engineering, and use of bioinformatics tool in enzyme engineering. The potential application of some selected food-grade enzymes used for industrial production and the safety of food-grade enzyme were discussed.

16.2 Food-Grade Enzymes Screening

Screening technique is used for isolating enzymes by judiciously selecting plant, animal and the species and strains of microorganism or cells. Maximum volumetric productivity is used as an objective function for selecting strains or original cultures with the capacity to produce the preferred enzyme of high productivity rate (Ramos et al., 2011). Also, screening is used in finding alternative strains with improved possibility of cloning proposed enzyme. The process of isolating enzymes from extremophile source using screening techniques is useful in stabilizing, showing high activity and turnover numbers, and specificity of enzymes when operated under severe environmental conditions for industrial application (Synowiecki et al., 2006). For instance, there is a rising interest in the sourcing of enzymes from isolations of bacteria from the marine environment, such as proteases from several genera esterase from *Vibrio fischeri*, inulinase from *Cryptococcus aureus*, amylolytic enzymes, glucosidases and invertase/inulinase from *Thermotoga neapolitana DSM 4359* (Dipasquale et al., 2009; Rastogi & Bhatia, 2019). In the meat and dairy industry, cryophiles are needed potential microbes for screening. Since the industry operates under room-temperature lactase, protease and pectinase can be obtained for the production of lactose-free milk, milk coagulation and cheese maturing, fruit juice or oligosaccharide production, respectively (Nakagawa et al., 2004, 2006; Wang et al., 2008; Mao et al., 2010). In the screening technique, the major challenge is to cultivate the screened and identified microbes in laboratory conditions most especially the extremophile. However, if the extremophiles are expressed in mesophiles using genetic engineering, they can be easily cultured in the laboratory with easier protein extraction and purification (Rastogi & Bhatia, 2019).

16.3 Food-Grade Enzyme Immobilization

Food-grade enzyme immobilization simply means confining enzymes in a given portion of space with the use of microencapsulation or by the use of solid matrix, such as cross-linking, ion exchange, adsorption and covalent binding, so that the enzyme can be physically separated from the reaction medium. The prospect of the industrial application of food-grade enzyme immobilization is that it permits the reuse of the enzymes and also extends its usefulness to be incorporated in the food products or food processing to improve productivity. Among the food-grade supports used of immobilization are alginate, carrageenan, agar, glass beads, polyurethane foam, polyacrylamide, metal surfaces and vermiculite (Ramos et al., 2011). Ramos et al. (2011) reported that for protease enzymes, vermiculite which is less-expensive support using adsorption had been used for immobilization. It is a must to consider food-grade support for any food-grade enzymes immobilization. According to Sumantha et al. (2006), immobilization of microorganism during the production of the enzyme has been reported to produce higher enzyme yields than non-immobilized cells of bacteria or mycelia. Some of the immobilized enzymes that had been applied in the food industry are immobilized glucose isomerase for

manufacturing high-fructose corn syrup, immobilized aminoacylase for manufacturing amino acids, immobilized lactase for producing tagatose, immobilized invertase for producing inverted sugar, immobilized lipases for producing trans-free oils (cocoa butter equivalents, and for modification of triacylglycerols), immobilized β-fructofuranosidase for producing fructooligosaccharides and immobilized isomaltulose synthase for producing isomaltulose, respectively (Rastogi & Bhatia, 2019). From an economic perspective, it is advisable to immobilize expensive enzymes to have a less-expensive process (Fernandes, 2010). The challenges associated with immobilization are that if the procedure of immobilization is not properly done, it might lead to the loss of enzyme activity which might be as a result of mechanical and chemical stress (Rastogi & Bhatia, 2019).

16.4 Solid-State Fermentation

Fermentation is a technique used for the synthesis of food-grade enzymes. The conventional fermentation method of synthesizing enzymes is through submerged fermentation. The type of substrate used in the submerge fermentation is a determinant in obtaining either high or low enzyme activities. For instance, complex substrate suspended or dissolved in an aqueous medium is expected to give enzymes with high activities as compared with a simple substrate in submerged fermentation. Aside from the type of substrate, the continuous operation has been reported to favour enzyme yield as compared with the batch operation process which is simple to handle and also versatile (Ramos et al., 2011). Optimization of submerge fermentation parameters through the use of advance experimental design, such as response surface methodology, artificial neural network and radial basis function, had been reported as tools that can be used to obtain optimum parameters combination aimed at given maximum volumetric productivity of the enzymes during fermentation (Sumantha et al., 2006). However, solid-state fermentation is gaining greater interest in recent years than the conventional submerged fermentation. The reason is that in solid-state fermentation, cultures are grown of moist solid rather than in aqueous liquid as in submerge fermentation. Also, the culture media are not usually complex; the substrate media for solid-state fermentation can be enriched with nutrient. The enzyme of interest in solid-state fermentation is not diluted, thus paves the way for easy purification after synthesis. There is also low effluent generation, catabolic repression hardly affects the synthesis of enzymes and higher inoculum and relatively low humidity content used in solid-state fermentation reduces extraneous microbial contamination (Ramos et al., 2011). The wide use of agricultural waste as a substrate in solid-state fermentation is another advantage of this technique over submerge fermentation as it aids in reducing the cost associated with enzyme synthesis (Aguilar et al., 2008). Sumantha et al. (2006) reported that the use of wheat bran as a substrate for solid-state fermentation gives a higher yield of proteases than in submerge fermentation using *Aspergillus spp.* and *Mucor pusillus*.

The use of two or more substrates has been reported as a way of increasing the yield of the enzymes in solid-state fermentation (Aguilar et al., 2008).

16.5 Recombinant DNA/Genetic Engineering

According to the Food Drug Administration (FDA) in the USA, Escherichia coli K-12 also known as bovine chymosin is the first recombinant enzyme. The recombinant deoxyribonucleic acid (rDNA) techniques or genetic engineering has now been used to produce chymosin by transforming microorganisms with bovine prochymosin gene. At least there are thirty-six (36) enzymes which are genetically modified microorganisms (GMOs) among the one hundred and sixty (160) enzymes produced for use in the food industry based on Association of Manufacturers and Formulators of Enzyme Products (AMFEP) enzymes lists. To achieve the synthesis of efficient enzymes that is free of undesirable enzymes or metabolites of microbes using rDNA, appropriate selection of host microorganisms as recombinant strains with potential DNA sequences that can code for the defined enzyme amino acid sequence is needed. For instance, engineering microbial host stains were applied to some fungi strain to manipulate or eliminate their capacity to release metabolites that are toxic to the formulation of the synthesis enzyme (Olempska-Beer et al., 2006). The application of rDNA has immensely increased the availability of food-grade enzymes with distinct properties to meet the food process and food matrices requirement. Also, it has assisted in synthesizing enzymes that have desirable sensitivity and specificity at a low cost of production.

Rastogi and Bhatia (2019) stated that the application of rDNA can be implemented in batch or continuous operations using cheaper raw material, therefore lessens the criteria for enzyme extraction and purification. The use of food-grade enzymes from genetic engineering has minimized the cost connected with upstream product processing and also increases productivity (Panesar, 2010; Rastogi & Bhatia, 2019). α-amylases, proteases, lipases and pullulanases used in food applications, such as in hydrolysis of starch, are produced by using improve strains (Ramos et al., 2011; Rastogi & Bhatia, 2019). Despite this entire prospect, the main challenge of genetically improved strain is that is not yet certified safe for consumption by the FDA and European Union due to the rise in public consciousness regarding the non-consumption of genetic modified processed foods. Also, the lack of standard in its production and purification procedures has caused a restriction in its application food technology.

16.6 Food-Grade Enzymes by Protein Engineering

The improvement of the food-grade enzyme in terms of stability and tolerance to severe temperature, pH and organic solvents can be achieved through protein engineering where the catalytic rate is improved and amino acids are modified for enhancing substrate specificity. Rational protein design and direct evolution are two

approaches used by protein engineering either exclusively or alongside each other (Jackel et al., 2008; Tiwari et al., 2012; Rastogi & Bhatia, 2019). According to Singhania et al. (2010), previous knowledge of the function and structure of the protein to introduce the desirable traits using sited mutagenesis is required for rational protein design approach, while for direct evolution, information of the protein is not compulsory and random mutagenesis is performed by error-prone PCR or chemical mediation. In order to forecast the overall structure of enzyme using rational protein design, multiple sequence alignment technique, homology modelling and protein treading methods are used (Gulati & Poluri, 2016). Rational design is easier to improve properties during screening due to an infinite number of protein variants generated (Singhania et al., 2010). Quax et al. (1991) used rational design to generate mutant glucose isomerase from *Actinoplanes missouriensis* with enzyme activity that is thermal and pH stable as against the parental enzyme. Double mutant isomerase (G138P, G247D) generated using rational design showed 2.5-fold increases in half-life and 45% specificity increment as compared with the wild type (Zhu et al., 1999). A similar approach was also reported by Lin et al. (2008) for generating amylase mutants from the strain of TS-23 *Bacillus sp.*to enhance the thermal stability of the enzyme. However, some of the biocatalysts generated by direct evolution are glucoamylases, cellulases, α-amylases, lipases and proteinases (Singhania et al., 2010).

16.7 Application of Bioinformatics Technique in Enzyme Engineering

In order to analyse the information in enzymes data within a limited time either online or off the network, the novel application of bioinformatics technique in enzyme engineering is the recent trend useful in performing sophisticated computations analysis (Alderson et al., 2012). The enormous of structural data and sequencing coming out of nuclear magnetic resonance spectroscopy, X-ray crystallography, mass spectrometry and NGS next-generation sequencing (NGS) for enzyme informatics are now better understood by the application of bioinformatics technique (Rastogi & Bhatia, 2019).

16.8 Application of Food-Grade Enzymes for Industrial Production

The use of food-grade enzymes for industrial production has widened their application in the development of different food products. The application of some selected food-grade enzymes for industrial production of food are as follows.

16.8.1 Application of Food-Grade Amylase Enzymes

α-amylase and β-amylase have been used over years for the industrial production of maltose and glucose by applying the enzyme on starch. The application of α-amylase has paved way for the production of glucose syrup and has replaced the chemical hydrolysis of starch in most starch manufacturing companies (Reddy et al., 2003). The sources used for commercial production of α-amylase are some selected strains of bacteria (*Bacillus* spp.: *B. amyloliquefaciens*, *B. licheniformis*, *B. subtilis*, *B. cereus*, *B. stearothermophilus*, *B. licheniformis; halophilic bacteria: Chromohalobacter sp.*, *Halobacillus sp.*, *Haloarcula hispanica*, *Halomonas meridiana;* fungi: *Aspergillus* species and few species of *Penicillium*: *P. brunneum*, *P. fellutanum*) (Konsoula & Liakopoulou-Kyriakides, 2007; Erdal & Taskin, 2010). α-amylase had been used in the baking industry to play an important role in enhancing the quantity, aroma and taste of baked products. Jegannathan and Nielsen (2013) reported that the addition of amylase and lipase enzymes in bread helps in extending its shelf life and reduces crystallization. Antistaling in bread and improved softness can also be achieved by the addition of α-amylase (Gupta et al., 2008). However, more research still needs to be done on the application of α-amylase in the baking industry as a small overdose of α-amylase can result in sticky bread (Singh et al., 2019). For the fermentation of raw starch into ethanol, a combination of α-amylase produced from *Streptococcus bovis* and glucoamylase from *Rhizopus oryzae* has been reported to improve the ethanol production (Singh e*t al.*, 2019).

16.8.2 Application of Food-Grade Protease Enzymes

Protease enzymes can be the source from plant, animal, humans and microorganisms (bacteria, fungi and virus) and are known to hydrolyse peptide bones of proteins. Protease enzymes produced from microorganisms' source are the most preferred for industrial production due to ease of genetic manipulation and extensive diversity, respectively. They are also known to favour low manufacturing cost, extensive chemical and physical characteristics, lack of seasonal variation, large production in industrial fermentators and quick culture development (Singh e*t al.*, 2019). Protease enzymes of a wide variety have been exploited for industrial production in food and pharmaceutical industries because of their specificity in action (Kalpana et al., 2008). Almost 60% of the total food-grade enzymes in the market fall under the category of protease enzymes (Mala et al., 1998). Alkaline and neutral proteases produced for commercial purpose are usually generated from *Bacillus* genus. Powdered bacteria proteases derived from *Bacillus* can be stable and remain 95% active for a year. In the food industry, proteases can be applied in cheese production, baked products making, soy hydrolysates production and meat tenderization. Proteases from *A. oryzae* can improve bread colour and texture when mixed with flour dough. More so it also aids in the reduction of mixing time. Heredia-Sandoval et al. (2016) stated that the gluten content of bakery products can be decreased and degraded

using proteases. In the dairy industry during cheese manufacturing, vegetable rennet, animal rennet, genetically engineered chymosin and microbial milk coagulants are milk coagulating enzymes used. Paracasein and micropeptides are generated from the hydrolysis of peptide bone using protease enzymes in cheese making. However, chymosin is good for generating casein. In beer production, application of protease and amylase enzymes have been used as an efficient alternative way of achieving malting process, thus saves energy and agricultural land that would have been used if the conventional malting that involves drying was to be used (Jegannathan & Nielsen, 2013). Microbial proteases have been reported to hydrolyse proteinaceous in beer that causes haze formation, while production of wort can be achieved by the use of *B. subtilis* proteases to solubilize proteins from barley adjuncts (Singh e*t al.*, 2019). For commercial purpose, improved tenderness of meat which is the organoleptic quality of meat as desired by the consumers and for easy marketability can be achieved by using thermophile protease derived from *Bacillus* strain, caldolysin from *Thermus* strain, collagenase from *Clostridium histolyticum*, and aspartic protease from *A. oryzae* (Bekhit et al., 2014). For industrial production of aspartame which is known as nutrasweet (zero-calorie sweetener), enzymatic synthesis of aspartame has been preferred over chemical method due to their ability to maintain stereospecificity. Proteases have been reported to be used to synthesize aspartame by catalysing the reverse reaction in two amino acids, thus maintain stereospecificity and reduce the cost of production (Singh e*t al.*, 2019).

16.8.3 Application of Food-Grade Lipase Enzymes

Lipases are important food-grade enzymes due to their biocatalysts activities in breaking down fats and oils, esterification, hydrolysis, interesterification, acidolysis, alcoholysis and aminolysis. Lipases can be sourced from the plant (barley, corn and cotton), animal (adipose tissues, blood, gastric juices, intestinal juices and pancreatic secretion), yeast (*Candida* and *Torulopsis*), filamentous fungi (*Geotrichum*, *Humicola* and *Rhizopus*), bacterial species (*B. subtilis*, *P. fragi*, *B. megaterium*, *S. aureus*, *Burkholderia cepacia*, *P. aeruginosa*, P. pseudoalcaligenes) and fungi species (*Helvinal anuginosa*, *Rhizopus delemar*, *Eurotium herbariorum*, *A. niger*, *M. circinelloides and Penicillium citrinum*) (Hasan et al., 2006; Sachan & Singh, 2015; Singh e*t al.*, 2019). However, the microbial source is mostly used for commercial production of lipase due to their ability to reduce the cost of production, greater widespread ability in animal and plant lipases, and greater stability (Singh e*t al.*, 2019). Hasan et al. (2006) and Treichel et al. (2009) noted that lipase enzymes have found application in food processing, pharmaceuticals, agrochemical, detergents, paper and cosmetic industries. The first commercial lipase from recombinant technique was isolated from *Thermomyces lanuginosus* which is a fungus and since then many other recombinant lipases have been produced for commercial purpose (Singh e*t al.*, 2019).

Lipases have been applied extensively in the dairy industries for hydrolysis of milk fat, lipolysis of cream, producing cheese products and in enhancing the flavour

of cheeses in order to accelerate cheese ripening (Aravindan et al., 2007). In the bakery industry, lipolytic enzymes have been used to produce emulsifying liquids in situ by degrading wheat lipids instead of using traditional emulsifiers. Also, the application of lipolytic enzymes helped in the release of short-chain fatty acids through esterification, thereby aids in improving the flavour of baked products flavour. Apple wine has been produced by the Japanese company Tanabe Seiyaku with improved alcoholic content and aroma by isolating lipase enzyme from *R. delemar* or a *Candida* species during fermentation (Singh e*t al.*, 2019). Lipases have also been used to improve the quality and texture of mayonnaise, whipping and dressing products. In the meat and fish processing, lipases are used to enhance and remove fat. Ramarethinam et al. (2002) reported that volatile flavours and the aroma of black tea were increased using lipase secreted by *Rhizomucor miehei*. Lastly, in the fat and oil industry, cheap oils have been converted into nutritional-rich oils, oleic acids oils, low calorie triacylglycerols and polyunsaturated fatty acid using microbial lipases (Gupta et al., 2003). Besides commercial production in fats and oils modification, low energy consumption and high level of specificity can be achieved using lipase enzyme modification when compared with chemical modification.

16.8.4 Application of Food-Grade Rennet Enzymes

Rennet is enzyme made up of chymosin, lipase and pepsin and it is usually referred to as complex enzymes. Rennet can be generated from the plant, animal and microbial sources. Rennet has been extensively applied in the commercial production of different types of cheese. However, rennet from the microbial source is mostly used for cheese production worldwide. For instance, *R. miehei*, *Irpex lactis*, *A. oryzae and R. pusillus* are the good source of microorganism that can be used to generate rennet for cheese production (Neelakantan et al., 1999; Singh e*t al.*, 2019). Also, microbial rennet has served as a replacement for mammalian rennet used in cheese production due to consumers' demand of non-animal derivative. Unlimited availability, low cost of production, bulk production and no risk of disease transmission are some of the advantages of microbial rennet over animal rennet (Singh e*t al.*, 2019). The application of rennet enzyme is one of the most important uses of enzymes in the food industry.

16.8.5 Application of Food-Grade Catalase Enzymes

Catalase enzymes also known as hydroperoxidases and is popularly known commercially for converting hydrogen peroxidase to water and oxygen. According to Singh et al. (2019) one molecule of catalase can transform millions of hydrogen peroxide to water and oxygen per second. Catalase can be found in all living organism but mostly source from microorganisms and bovine liver (Chelikani et al., 2004; Tukel & Alptekin, 2004). Plant leaves with high antioxidant properties are *M. sylvestris* L., black gram (*Vigna mungo*) seeds, cotton, sunflower and

pumpkin (Eising et al., 1990; Kandukuri et al., 2012). Catalase found industrial application in the removal of hydrogen peroxide used in sterilizing, oxidizing and bleaching agents (Ertaş et al., 2000). The combination of catalase and glucose oxidase has been used for the preservation of some selected foods. The enzyme can also be used in egg processing but has limited usage in cheese production.

16.8.6 Application of Food-Grade Lactase Enzymes

This enzyme is very important in the dairy industry, most especially in the production of low-lactose or lactose-free food, such as flavoured milk drinks, ice cream, yoghurt and frozen dessert. Lactase can be the source and isolate from animal organs, plants, yeasts, bacteria or molds. For industrial production of lactase, it is usually source from *A. niger*, *A. oryzae* and *Kluyveromyces lactis* due to their safety of use history and the numerous safety tests they undergo before use. The source used for generating the lactase and the method of preparation determines its properties, such as the temperature and pH. For optimal performance of immobilized lactases, the procedure of immobilization and the type of carrier are important. Acidic pH of 2.5 to 4.5 is favourable for fungi lactase production, while neural region pH is favourable for bacterial and yeast lactase with ranges of 6.5–7.5 and 6–7, respectively. The prospects in the utilization of lactase, hydrolase and β-galactosidase have been extensive research due to the discovery of enzymes immobilization techniques (Mehaia & Cheryan, 1987). The application of lactase in milk has assisted in preventing diarrhea and severe tissue dehydration which affect lactose intolerance people. It has also been reported to increase digestibility, sweetness and scoop and creaminess of ice cream, yoghurt and frozen dessert. Increase in sweetness and sugar addition level reduction has also been ascribed as another advantage of lactase-treated milk in the production of flavoured milk drinks.

16.8.7 Application of Food-Grade Cellulase Enzymes

Cellulases are enzymes used for extracting constituents from soy protein, essential oils, aromatic products, green tea and sweet potato in the food industry. Dillon et al. (2004) described cellulase enzymes as enzymes that break the glucosidic bond of cellulose microfibrils and release oligosaccharides, cellobiose and glucose. Cellulases can be produced from filamentous fungi (*Aspergillus*, *Chaetomium*, *Fusarium*, *Penicillium*, *Phoma* and *Trichoderma*), anaerobic bacteria (*Acetovibrio*, *Bacteroides*, *Butyrivibrio*, *Caldocellum*, *Clostridium*, *Eubacterium*, *Erwinia*, *Pseudonocardia*, *Ruminococcus* and *Thermoanaerobacter*) and aerobic bacteria (*Acidothermus*, *Bacillus*, *Cellvibrio*, *Pseudomonas*, *Streptomyces*, *Staphylococcus*, and *Xanthomonas*) (Soares et al., 2012; Singh e*t al.*, 2019). Among all the sources of producing cellulolytic enzymes, *Aspergillus* has been reported to be the most outstanding (Singh e*t al.*, 2019). Combination of cellulases, hemicellulase and

pectinase is currently being used in the food industry to extract and clarify fruits juice. Cellulase can also be used to degrade solid phase, thus enhances liquefaction.

16.8.8 Application of Food-Grade Invertase Enzymes

This enzyme has a great commercial significance in the production of inverted sugar and is known for its sweetness effect. Invertase can catalyse sucrose to glucose and fructose through hydrolysis. Temperature and pH within the range of 40–60 °C and 3–5, respectively, are more favourable for more active invertase. At the industrial level, invertase is mostly isolated from yeast, such as *S. cerevisiae*, and there is ongoing research for the use of high yielding filamentous fungi for production of invertase. Examples of the filamentous fungi are *Rhizopus* sp. And *Aspergillus casiellus*. The filamentous fungi *Rhizopus sp.* was cultivated in the medium of wheat bran to obtain invertase (Soares et al., 2012); *Aspergillus casiellus* was inoculated in a medium of soybean meal and the invertase was isolated after 72 h, while in yeast, invertase (S-b D-fructofuranosidase) is isolated from *S. cerevisiae* (Singh e*t al.*, 2019).

16.8.9 Application of Food-Grade Glucose Isomerase Enzymes

In the food industry glucose isomerase has found extensive application in the production of fructose-rich corn syrup. The enzyme is also known as d-xylose ketol isomerase which aids in catalysing reversible isomerase from d-glucose and d-xylose into d-xylulose and d-fructose, respectively. Protein engineering technique is being explored to generate thermostable glucose isomerase enzymes (Hartley et al., 2000). Glucose isomerase has also been used for bioconversion of hemicellulose into ethanol (Singh e*t al.*, 2019). For commercialization of glucose isomerase, the application of biotechnology in isolating mutants of promising prospect is important.

16.8.10 Application of Food-Grade Glucose Oxidase Enzymes

Glucose oxidase is majorly used in the food industry to eliminate harmful oxygen in food product containing probiotic microorganisms. The enzyme allows catalysing oxidation–reductions reaction through the prosthetic group flavin adenine dinucleotide (FAD). High level of glucose oxidase can be isolated from filamentous fungi, such as *Aspergillus versicolor* and *Rhizopus stolonifera* (Guimarães et al., 2006). Singh e*t al.* (2019) suggested that the use of glucose oxidase through biotechnology can be used to improve the stability of probiotic bacteria in yoghurt without adding chemical additives.

16.9 Safety of Food-Grade Enzyme

Industrial food-grade enzymes are generally considered safe and are known as food additives, while some considered them as food processing aids. The safety regulation on the use of food-grade industrial enzymes differs among countries, premarket approval of the enzymes and the information provided by the manufacturer. The prominent occupational food safety risks that have been associated with the use of industrial food enzymes are mainly allergic reaction (in the respiratory tract), irritation risks, residual microbiological activity, chemical toxicity and oral toxicity among consumers (Spök, 2006; Ramos et al., 2011; Singh et al., 2019). Ramos et al. (2011) stated that enzymes are sensitizers and can cause allergic reaction in the respiratory tract (asthma) upon inhalation and also irritation upon contact with the skin. However, aside from the occupational risk, the final consumers of food products containing food-grade enzymes rarely report allergic reaction because low levels of the enzymes are usually used in food formulation which would have eventually be deactivated upon processing and before reaching the consumers. The future challenges that might be associated with the safety concern of food-grade enzymes are toxic substance arising from the by-product of contaminants present in the preparation of the enzyme. Adequate attention must be paid towards fermentation broth contaminate or environmental conditions of some phylogenetical strains used in enzyme production. Although with the advent of genetic engineering technique, toxicity problems can easily be eliminated. Food industry and national or European regulatory bodies must develop strategies to harmonize and enforce legislation on the available food enzymes that are categorized as a novel or those with unusual properties and their food safety assessment practice to eradicate occupational risks that may arise from their use. One of the current issues related to food enzymes legislation is that in the USA, Japan and Canada food-grade enzymes are considered as food additives, while Australia regards food enzymes as food processing aids. WHO/FAO, joint committee of food additives, does not differentiate food-grade enzymes either as a food processing aids or food additives (Ramos et al., 2011). However, EU food legislation considered more than 160 food-grade enzymes as food processing aids, while invertase and lysozyme enzymes are the only two food-grade enzymes considered as food additives. National legislation of France, Hungary, Denmark and Poland do subject food-grade enzymes that are referred to as food processing aids to authorization before use, while in the UK the authorization is voluntary (Ramos et al., 2011). Therefore, it is imperative for legislation of each country worldwide to define the ranges of the permitted enzymes and their applications.

16.10 Conclusions

For the future purpose, there is need to explore the benefits of novel approaches, such as recombinant DNA technology, screening, protein engineering, enzyme immobilization, solid-state fermentation and use of bioinformatics tool in enzyme

engineering in generating food-grade enzymes, and to adapt the enzymes for sustainable and economical industrial production processes. Understanding the novel approaches can aid the industrial production of food-grade enzymes which can be useful in improving food product quality and consistency, preventing potential harmful by-products in the food, reducing raw material dependence during processing, replacing chemical additives, reducing process time, increasing shelf life and creating more opportunities for enhancing the aromatic, structural and textural properties of food. In addition, application of food-grade enzymes for industrial processes should be able to lead to the development of new or improve healthy food products and food properties within the food processing industry; bakery, dairy, beverages, juice, alcoholic, meat, fats and oils, vegetables and other industries. Proper selection of enzymes source, enzymes isolating method and purification technique that is safe and non-toxic must be carefully selected as they influence the cost of production of food-grade enzymes and yield. Solid-state fermentation produces higher enzymes than submerged fermentation at a lower cost. National legislation of countries should make provision for permitting and regulating products from food-grade enzymes and their applications. More research should be channel towards the application of enzymes technologies in food processing to broaden our knowledge and future use because consumers are now demanding for food products or additives that are free of chemicals.

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Nanotechnology and Food Grade Enzymes 17

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Abstract

Public concerns about food safety and its health, on the one hand, and the rapid advancement in emerging technologies and the green ones, on the other hand, have led to less interest in chemical interactions occurring in the food industry. Enzymes are proteins and consequently sensitive to environmental effects such as pH and temperature. Additionally, they are less likely to be reused and the cost of their recovery is exorbitant. Therefore, their application as natural catalysts has been limited. Having tackled the problems associated with free enzymes, enzyme immobilization made it possible to reuse and store enzymes. Then, nanotechnology implemented this technique to end in nanobiocatalysis which merges nanotechnology and biotechnology and stabilizes enzymes accordingly. Nano carriers improve enzymes' activity and efficiency, stability, and storage ability as they possess unique features such as nanoscale size, high surface/volume ratio, and variety in composition and structure. Nanobiosensors are another breakthrough in bio and nanotechnologies which can substitute chemical and electrochemical

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A. Dutt Tripathi et al. (eds.), *Novel Food Grade Enzymes*,
https://doi.org/10.1007/978-981-19-1288-7_17

sensors for analysis, quality control, and food safety measurement. Recently, nanomaterials-based biosensors have acted as the focal point in food contamination detection, namely pesticides, antibiotics, and heavy metals, due to their variety, highly specialized activity, and high precision. In this chapter, we investigate nanotechnology and food enzymes, probable risks, and legal aspects of nanomaterial application in food bioprocess.

Keywords

Nanotechnology · Enzyme · Nanobiocatalysis · Nanobiosensor · Food analysis · Food safety

17.1 Introduction

The prefix "nano" comes from the Greek word for dwarf which means "one-billionth" and the nanometer is equal to one-billionth of a meter that is extremely small to see even by a conventional light microscope (Ravichandran, 2010; Sundarraj, 2019). The late Norio Taniguchi initially applied the prefix "nanotechnology" in 1974 and Richard Feynman first gave its concepts in 1959 (Handford et al., 2014; He & Hwang, 2016).

Nanomaterials are tiny particles at least ranging from 1 to 100 nm in size in one dimension, bio-persistent or insoluble in nature, synthesized through numerous ways. Nanotechnology is a branch of science that manipulates the materials at nanoscale which are synthesized in various routes, and are used in different fields including electronics, medicine, agriculture, and food industries (He & Hwang, 2016; Ghasemi et al., 2017).

This reduction in size results in highly impressive features and functions. Nanomaterials are able to be applied in different forms such as nanotubes, nano rods, nanoparticles, nano sheets, and nanofibers in various technologies and industries of food, electronics, catalysts, energy, and agriculture (Jafarizadeh-Malmiri et al., 2019; Handford et al., 2014). Comparing nanomaterials and materials in bulk form, it can be concluded that changing the particle sizes of materials into nanoscale increases the ratio of surface area to volume, leading to unique physical, mechanical, chemical, optical, and biological properties such as solubility, absorption of the cells, delivery properties, and the residence time in the cells of body. On the other hand, it affects the bioavailability, safety, nutritional value, and efficiency properties (McClements & Xiao, 2017). This factor plays a critical role in creating unique features, so engineered nanomaterials have raised more attention in the industries of agro-food, medicine, sewage water treatments, and others (Haroon & Ghazanfar, 2016).

Recently, the industries of food and agricultural have had significant investments in nanotechnology which enhance the quality of product and reduce total prices (Yu et al., 2018a). Firstly, Pasteur introduced nanotechnology by using it in pasteurization process in food industries to omit the bacteria of spoilage (1000 nm) which

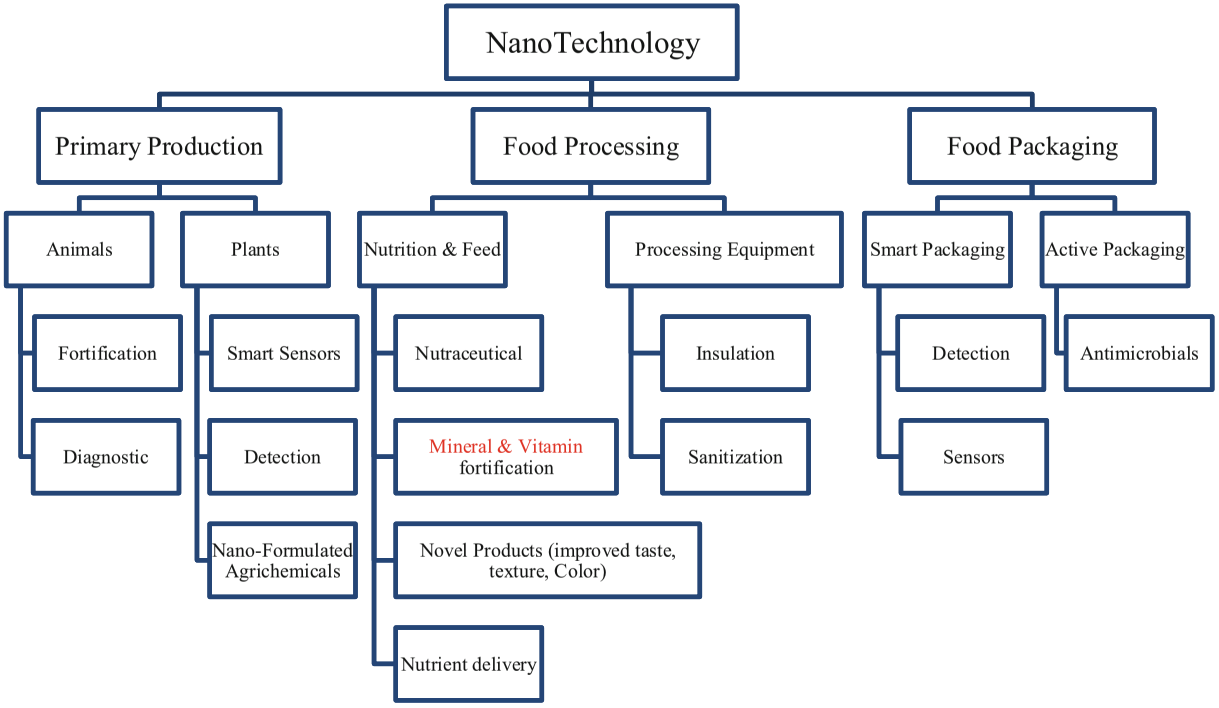
made the first step to transform the food processing and improve food quality. Watson and Crick first built a model for the structure of DNA at almost 2.5 nm which enabled the applications in biomedicine, biotechnology, production processes, and agriculture (Hansen et al., 2013). Nanotechnology has immense potential in the postharvest food processing. The main applications of nanotechnology in the food and agriculture structures are food processing and security improvements, production, packaging of food which enhances its functionality, food bioavailability, nutrition and flavor, pathogen detection, protection of the environment, nutrients absorption of plants, and the cost-effectiveness of storage and distribution. It also modifies the particle size, size distribution, surface charge, possible cluster formation, and delivery methods (Rashidi & Khosravi-Darani, 2011; Ghasemi et al., 2018). Study in nanotechnology has high potential to advantage humanity in food-related industries (Fig. 17.1).

Generally the potential of applying nanotechnology in food industries has been in nanocarriers (such as nanoparticles, nanoemulsion, nanocomposites, nanobiocomposites, and nanolaminates), nanosensors and nanobiosensors (for the control of safety and quality of the food), processing (such as nanofiltration, nanoscale enzymatic reactor, heat and mass transfer, nanofabrication and nanocapsules for modification of absorption), delivery, packaging, formulation, DNA recombinant technology, evaluation, etc. (Ghasemi et al., 2018; Jafarizadeh-Malmiri et al., 2019), but the main nanotechnology applications in food industries are food nanostructured components and food nano sensing to increase the safety and quality of food. Improvements in the production of inorganic nanoparticle have permitted the preparation of effective nanosensors to identify pathogens or pesticides rapidly in food (Neethirajan & Jayas, 2011; Nile et al., 2020).

Our main goal in this chapter was to provide a brief overview of the use of nanotechnology and nanobiotechnology, namely enzymes, in/on nanocarriers on the food industry improvements especially food packaging. Also, the potential applications and future perspectives of nanobiotechnology on food safety were discussed. Also this chapter overviews the information usually accessible about nanomaterials' risk assessments, marketing concerns, safety and toxicity aspects, and regulatory aspects.

17.2 Overview of Nanotechnology and Nanobiotechnology Applications in Food Industry

Request for extra innovative improvements and technologies in valuable tools and materials for the life study are supported by the technologies of nanotechnology and biotechnology which are among the promising technologies in the twenty-first century (McClements & Xiao, 2017). Nanotechnology is a technology that uses materials and devices possessing at least one dimension sized in nanoscale, which plays an essential role in the food and agriculture parts, contributes to improve the crop, progresses the food quality and safety, and supports the health of humans through innovative and novel approaches (Jafarizadeh-Malmiri et al., 2019).



accessible about nanomaterials' risk assessments, marketing concerns, safety and toxicity aspects, and regulatory aspects.

Fig. 17.1 The nanotechnology applications in food areas

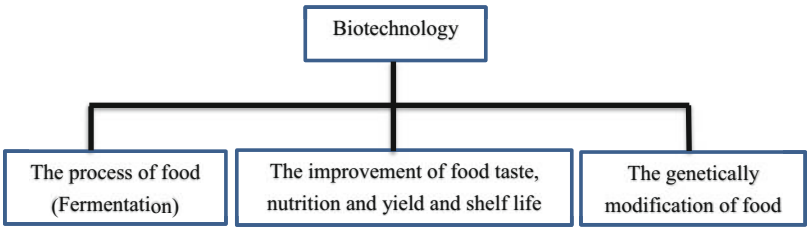


Fig. 17.2 The applications of biotechnology in food industries

Generally, biotechnology contains physiological processes of biological agents such as microorganisms, enzymes, and living cells. Actually, the techniques and knowledge of biology in biotechnology have been used to manage the cellular, genetic, and molecular processes for developing services and products in different fields such as food and agriculture and medicine (Faramarzi et al., 2020; Jafarizadeh-Malmiri et al., 2019). Biotechnology is helpful in the processing of foods (the processes involved enzymes and fermentation) and in removing malnutrition, hunger, and illnesses from all the countries (Haroon & Ghazanfar, 2016). The most important biotechnology applications in industries of food are categorized into three groups which are shown in Fig. 17.2.

In food industries biotechnology is applied to food processing as shown in Fig. 17.1 by using microorganisms and their enzymes to improve characteristics including the aroma and taste, texture, nutritional values, and shelf life in foods which is known as fermentation (Haroon & Ghazanfar, 2016; Ruane & Sonnino, 2011). In other words, in the process of fermentation the microorganisms break and catabolize the organic compounds under aerobic and anaerobic circumstances for manufacturing the ultimate products. Also biotechnological tools can be applied for modifying food genetically, which means that genetically engineered microorganisms are applied in order to produce organic acids, vitamins, sweeteners, amino acids, nutritional supplements, and edible oils. This process can be developed from the insertion of functional gene on DNA into a host bacteria (such as lactic acid bacteria) (Haroon & Ghazanfar, 2016; Jafarizadeh-Malmiri et al., 2019).

Nanobiotechnology has obtained more attention nowadays because of its varied applications in the industries of food. Progress in nanobiotechnology leads to a better viewpoint in enzyme technology and has more importance toward commercialization of enzymes, as well as a further development in catalytic performance (Jafarizadeh-Malmiri et al., 2019).

Nanobiotechnology is the integration of nano-engineering and molecular biology which combines the design of novel materials in nanoscale and devices with the wonderful characteristics for cells, biological molecules, and enzymes to find the solution for the most important difficulties in biology (Ghouri et al., 2020; Jafarizadeh-Malmiri et al., 2019).

The investigation in the field of nanobiotechnology in the food industries mainly contains adding biosensors, antimicrobial, antioxidants, and further nanomaterials in the form of nanoparticles, edible nanocomposite films, and nanobiosensor at

packaging. In recent years, Nanobiotechnology has been a center of attention in food packaging. Innovative food packaging has significant results on preserving safety and quality of foods, during storage and transportation to customers. Actually, packaging can be able to extend the shelf life of foods by controlling disagreeable conditions and factors such as microorganisms, moisture, enzyme activities, and chemical contaminants. Many studies have been done on the application of nanobiotechnology in packaging, which are described in Table 17.1.

As shown in Table 17.1, the merging of the materials for food packaging and active ingredients is a novel way for surface microbial contamination control in foods. Several nanomaterials such as silver exhibit antimicrobial influences. The potential perspectives of nanocomposites for the applications of packaging for foods based on nano and bio ingredients, including biodegradable and edible nanocomposite films, have achieved consideration because the coatings on food products have significant effects in preservation, distribution process, and selling various foods (Morais et al., 2014).

Nanosensors can identify particular indicators of the metabolism of pathogens or can inform the customers of the light, temperature of product, or changes of humidity in the environment during storing. This can omit the need for expiration date in various occurrences and causes the consumers know the precise state of spoilage in food and develop the products' shelf life (Jafarizadeh-Malmiri et al., 2019). The sensor which is applied for the biological organisms is known as biosensor. Biosensors are used for the detection of biomolecule such as several microorganisms and pathogens, glucose, and urea and as a complementary tool to classical analytical methods such as high-performance liquid chromatography which is required for food analyses. It is clear that the applications of nanomaterial in biosensors prepare chances to produce a novel aspect for biosensor technologies for applying them in food analysis (Jafarizadeh-Malmiri et al., 2019; Sundarraj, 2019).

Nanobiosensors gained their specificity from the reaction of biological binding, which includes enzyme/substrate/cofactor, antigen/antibody, nucleic acid hybridization, chemical interactions, and ligand/receptor in combination with different transducers. Nowadays enzymes are applied as nanobiosensors much more than the others (Thakur & Ragavan, 2013).

Today, enzymes can improve texture, nutritional value, and appearance and may produce desirable aromas and flavors in a wide range of applications such as processing of the cheese and starch, bakery, and producing the various fruit juices and further drinks. Food enzymes sometimes are originated from plants or animals (such as amylase, an enzyme for starch digestion), but most of them come from a range of useful microorganisms (Fathi et al., 2018).

Enzymes are biological catalysts which can lower activation energy due to accelerating the chemical reactions (Jafarizadeh-Malmiri et al., 2019). Due to their excellent properties such as specificity, selectivity, and high activity, enzymes have various applications in food productions such as yogurt, bread, cheese, and fermented beverages (Sheldon, 2007; Jafarizadeh-Malmiri et al., 2019). But use of native enzymes in food have following limitations which are effective keys in enzymatic production and their commercialization (James et al., 1996):

Table 17.1 Nanobiotechnology applications in food industries

Method of nanobiotechnology	Type of nanomaterial	Matrix	Application	Reference
Nanoparticles	Silver	Fresh-cut melon, poultry meat, asparagus, orange juice, beef meat exudates	Delays the growth of aerobic psychrotrophics, yeasts, and molds; effective as an antimicrobial material in killing *Staphylococcus aureus* and *Escherichia coli*	An et al. (2008), Fernández et al. (2009), Fernández et al. (2010), Fernandez et al. (2010), Emamifar et al. (2010)
	Titanium oxide	Chinese Strawberry, jujube	Decreasing browning, slow-senescence, down ripening, and decay	Li et al. (2009)
	Zinc oxide	Liquid egg albumen, orange juice	Decreased *Lactobacillus plantarum*, yeast, Salmonella, and mold counts with no changes in the parameters of quality	Emamifar et al. (2010), Emamifar et al. (2012), Jin and Gurtler (2011)
	Silver oxide	Apple slice	Delays microbial spoilage	Zhou et al. (2011)
Edible nanocomposite film	Silica in situ improved the biodegradable films of PVA/chitosan (CS)	Cherries	Significant reduction in the permeability of moisture and oxygen by 10.2% and 25.6%, respectively, and extending the preservation time of cherries	Yu et al. (2018b)
	Edible film with chitosan	Ready-to-eat (RTE) roast beef	Chitosan exhibited antimicrobial activity against *Listeria monocytogenes*	Beverlya et al. (2008)
Nanosensors	Cadmium telluride quantum dot nanoparticle (CdTe QD)	2,4-D (herbicide)	The pesticide of 2,4dichlorophenoxyacetic acid was detected	Vinayaka et al. (2009)
	Hetero-structured silicon/gold nanorod	Suspension of salmonella bacterial in 1% BSA solution	Au/Si nanorods based biosensor could detect Salmonella	Park et al. (2007)

- High prices
- The need of various enzymes
- Low stability of the enzyme
- High residual enzyme remained in the product and difficulties for their recovery and reuse
- Incompatibility of the continuous process
- Low activity
- Higher labor costs

Therefore, immobilization appeared as a new and powerful method to overcome these limitations and achieve enzyme separation, recovery, lifetime cycle rate, and good stability. By enzyme immobilization, the enzyme mobility artificially becomes restricted that leads to change of its properties, structure, and activity. On the other hand, this process is based on absorption of enzymes onto nanocarriers known as nanobiocatalysis (Jafarizadeh-Malmiri et al., 2019). The methods and conditions for immobilizing the enzymes are explained in Chap. 14 completely. Application of this nanotechnology as a novel method can improve the material properties such as remote addressability, multifunctionality, multi-compartmentalization, and stability (Cushen et al., 2012). So use of carriers for enzymes in nanoscale can improve the enzyme characteristics such as maximum surface area per unit mass, diffusional limitation, the loading of enzyme, the activity of enzyme, the possibility of magnetic separation, resistance to proteolytic digestion, and better rate of electron transfer which causes more applications for nanobiocatalyst in food industries (Faramarzi et al., 2020).

The nanoencapsulated enzymes, antigens, antibodies, glycol-proteins, and polysaccharides can be recommended as effective nanobiosensors in food packaging, which can detect numerous chemical or microbial spoilage of foods and eliminate pathogenic microorganisms, thus reducing diseases in people as a consequence of food consumption (Bajpai et al., 2018; He & Hwang, 2016). Immobilization of enzymes in/on the nanocarriers is a critical and essential step for the design of nanobiosensors. Nanobiosensors were advanced for being sensitive, small, portable, very exact, quantifiable, reproducible, stable, and trustworthy (Kumar & Neelam, 2016). So in this chapter the use of enzymes as nanobiosensors was discussed completely.

17.3 Application of Nanotechnology with Food Enzymes

Recent advances in biotechnology have shown increasing attention to the use of modern technologies for the development and growth of green and sustainable biotechnology using enzymes. Immobilization of suitable enzymes along with the use of biodegradable has gained currency as reduced chemical consumption has received much attention for many attractive applications, including biocatalysts and biosensors. The main challenge of using bioprocesses with enzyme catalysts is high operating costs due to low stability and inability to reuse enzymes on large-scale

industrial processes. Enzyme immobilization has been growing rapidly in recent years. Immobilization of enzymes means that they are physically restricted to a specific area by contact with the support, while trying to keep their catalytic activity at a maximum level. This technology is usually able to protect enzymes against adverse chemical and environmental conditions such as changes in temperature and pH level. Importantly, immobilized enzymes can be recovered and reused on a large-scale continuous industrial scale. However, immobilization may lead to a series of structural changes that may lead to serious changes in the properties, activity, and performance of enzymes relative to their natural state. To solve these problems, the process of immobilization of enzymes in various methods and various carriers is optimized to consider the best option with the highest efficiency for the intended purpose (Jafari, 2019; Netto et al., 2013).

Nanotechnology as a new and advanced technology offers more efficient methods than previous conventional methods to overcome technological barriers in many areas, especially for food production (Jafari et al., 2015). Many researches have been conducted on the use of nanotechnology in food for the production, preservation, packaging, and delivery of biologically sensitive food and pharmaceutical compounds. Nanoscale food, compared to micro type, leads to increased solubility, more bioavailability and more accurate and controllable release. Nanomaterials (NMs) have been highlighted in technological advances due to their adjustable physical, chemical, and biological properties with better performance than their bulky types. NMs are classified based on their origin, composition, size, and shape. The ability to predict the unique characteristics of NMs increases the value of each classification for their industrial applications. In the following section, more attention will be paid to the different nanostructures and their applications for food enzyme immobilization (Shahiri Tabarestani & Jafari, 2019).

17.3.1 Enzyme Immobilization in/on Nanocarriers

The protein and sensitive nature of enzymes justifies the need to select appropriate immobilization methods to maintain their structure and activity. Significant advances in the recognition of enzymes and their immobilization have led to the use of NMs in various structures and compounds as a support to immobilize enzymes. Enzyme carriers, in general, should have a large surface area, functional groups, as well as biodegradability. NMs have a much higher surface-to-volume ratio than conventional carriers and can provide more and better enzymatic immobilization (Jia et al., 2014; Saallah et al., 2016).

Enzyme immobilization methods are generally divided into three main categories: entrapment or encapsulation, binding to a carrier or matrix, and cross-linking. Immobilization may be reversible or irreversible, involving direct adsorption to the surface. Different bonds on the surface of enzymes lead to various interactions, such as hydrophobic, electrostatic, and van der Waals forces and hydrogen bonds. Types of irreversible immobilization include immobilizing the enzyme by covalent bonding to the carrier surface, entrapment in the matrix, or

encapsulating the enzyme. In this case, it prevents the enzyme from being washed during the reaction, but these supports cannot be reused after finishing the work and reducing the activity of the enzyme and should be discarded. In contrast, reversible immobilization allows recovery of enzymes after inactivation of the enzyme, but some of the enzyme may be lost during washing. Cross-linking agents (such as glutaraldehyde) are commonly used in cross-linking method. These agents have very low molecular weight relative to the enzyme, so in this method the enzyme remains almost unchanged and is also called a carrier-free biocatalyst with 100% activity. It is undeniable that in order to achieve optimal immobilization, a proper understanding of support and enzyme properties is required. On the one hand, changes in the three-dimensional structure of enzymes and the amphiphilic nature of proteins (hydrophobic inner part and hydrophilic outer part), the functional groups of supports, and the type of bonds they can form should be carefully considered (Netto et al., 2013).

Advanced developments in nanotechnology have created different modes for enzyme immobility in different nanomaterials (Husain, 2010). The combination of enzymes with nanomaterials as nanobiocatalysts (NBs) provides a better option than a variety of chemical catalysts that can be referred to green technology. Enzyme immobilization has been used by various nanostructures such as nanofibers, nanotubes, and nanoparticles. These supports are preferred because of their large specific surface areas (great surface area-to-volume ratios) which can provide extreme immobilization enzymes (Agustian et al., 2016; Beig Mohammadi et al., 2016). However, some nanostructured materials also have their own disadvantages. For example, mesoporous silica typically binds enzyme molecules to its inner surface, which restricts the diffusion of the substrate to the enzyme and reduces activity. Nanoparticles and nanotubes also dramatically reduce mass transfer limitations, while their dispersing and recycling is more challenging. Conversely, electrospun nanofibers have a high ability to solve these problems and can be favorable supports for enzyme immobilization (Beig Mohammadi et al., 2016).

17.3.1.1 Nanofibers

Nanofiber membranes with special properties such as continuous, porous, and nano-open structure allow easy access of the substrate to the active sites of enzyme molecules. Therefore, compared to other nanostructures, substrate can more easily diffuse in this continuous porous space and perform the necessary reactions. In general, nanocarriers for enzyme immobilization can be solid or liquid, spherical or non-spherical, fibers, tubes, and network characteristics (Wen et al., 2017). During the last few years, materials based on one-dimensional nanostructures (nanofibers and nanotubes) have created a subject of substantial interest due to their unique properties. Nanoelectrospun fibers, for instance, have achieved more attention compared to other thicker fibers made of the equal material, and their applications in different industries are relatively new. Immobilization of enzymes and encapsulation of nutraceutical compounds or probiotics in/on nanofibers for maintaining the quality and effectivity of foods have gained due to the specific properties of nanofibers. In addition, nanofibers produced by electrospinning method have many

advantages over their counterparts by traditional methods, such as drawing, template synthesis, self-assembly, phase separation, and melt-blowing techniques (Neo et al., 2018).

Nanofibers are continuous filaments with diameters ranging from a few nanometers to 1 μm. In general, nanofibers are produced by electrospinning process with different apparatus programs. Due to its unique properties (high surface-to-volume or surface-to-mass ratio, high porosity, and easy to use for fabricating various structures), nanofibers are potentially useful for different fields in food industries such as enzyme immobilization, nutrient delivery, food packaging, food indicator, and biosensing (Ghosal et al., 2018).

Electrospun nanofibers are formed by various materials such as synthetic and natural polymers alone or in combination with repulsion of electrostatic charges and elongation of the solution. Electrospun technique began in 1897 and it was revitalized during the 1960s by Taylor. Finally, in the 1990s, with the advent of nanotechnology, much attention was received. In recent years, this technology has been developed due to valuable features such as high efficiency, good mechanical properties, low-diameter distributions, and usability for many natural or synthetic polymers or their combinations (Neo et al., 2018).

The electrospinning mechanism is considered as the combined effects of electrostatic repulsions by the charges collected on the exterior layer of the polymer solution and the Coulombic forces exerted by an external electric field. A remarkable degree of sufficient molecular interaction and cohesion to resist electrical tension leads to the formation of a fiber, and this process is referred to as electrospinning. Generally electrospinning does not alter the structure of systems, but reduction of material to the nano size may lead to change some of their properties (Ghorani et al., 2017; Alehosseini et al., 2017).

The desired polymer is prepared to produce nanofibers in a suitable solvent. Solvents are evaporated using high-pressure evaporative electric fields. Finally, ultrathin polymer structures as continuous fiber structures are collected dry by the collector plate. This mechanism is visible in Fig. 17.3.

- All ingredients must be nontoxic.
- Their preparation is cost-effective and affordable.
- They must be resistant during production (heating, freezing, drying, and mechanical stresses), storage, and transportation.
- No adverse effects on organoleptic properties of food products must be observed (appearance, texture, aroma, and taste of the mouthfeeling).
- This not only must not reduce the bioavailability of enzymes and nutrients but also it must have the ability to increase them (McClements et al., 2009).

Enzymes are actually biocatalysts that have many applications in the commercial and industrial regions of food processing, bioreactors, and biosensors. Commercial and recyclable use of biocatalysts depends on the degree of efficiency of the immobilization technique and the residual activity of the enzymes. Methods of enzyme immobilization are divided into different categories: carrier-free.

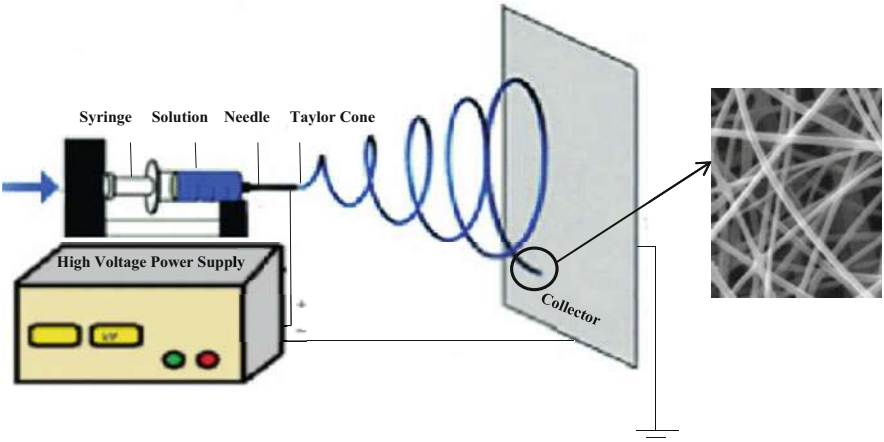


Fig. 17.3 Schematic of the electrospinning process and nanofibers production

Enzyme immobilization on these nanofibers can enhance and retain their activity and binding of enzymes to the nanofibers as well as enzyme separation for recycling. Enzyme encapsulation in nanofibers can also be used as an efficient method to preserve the third structure and activity of the enzyme. The main limitation for immobilized enzymes on nanoelectrospun fibers is the nature of a liquid system for dissolving enzymes and water-insoluble polymers that can remain resistant in a variety of environments and are easily separated. Fiber materials are generally the most desirable supports due to their inherent properties such as good mechanical strength, high specific surface area, and inter-fiber porosity (Gabrielczyk et al., 2018; Rojas-Mercado et al., 2018).

Enzymes can be immobilized after electrospinning by covalent or noncovalent bonding on the fibers, or encapsulated coelectrospinning methods within polymer fibers. Encapsulation of ficin and papain enzymes in nanofiber polyvinyl alcohol (PVA) electrospun and glutaraldehyde (GA) as cross-linking agents were studied. The results showed phycin and papain remained stable 92% (after nine consecutive use periods) and 40% (after four consecutive use periods) of their initial activity, respectively (Rojas-Mercado et al., 2018).

Phospholipase A1 was successfully immobilized on modified surface (MS) chitosan:polyethylene oxide (CS:PEO; 90:10) nanofibrous mat (NFM) with atmospheric plasma (AP) at different times (2, 6 and 10 min). Scanning electron microscopy (SEM) images showed that the membrane structures maintained the nanofiber porous structures and uniformity before and after AP treatments. PLA1 was successfully immobilized by covalent bonding with functional groups of chitosan NFM. The results showed 80% of initial activity of immobilized PLA1 on APSM CS: PEO NFM was still maintained after ten consecutive use periods (Beig Mohammadi et al., 2016).

17.3.1.2 Nanoparticles

Nanoparticles are generally unique and discrete particles with three dimensions with a size of 100 nm or less and have very different properties from bulk materials. Magnetic nanoparticles are composed of various elements, especially iron, nickel, cobalt, and their various chemical compounds. In the food industry, due to the existing safety limitations, nontoxicity of good biocompatibility, nanoparticles commonly used include iron oxides such as Fe_3O_4 magnetic nanoparticles.

Like other nanoscale materials, they perform better than bulk magnetic materials in sizes of 10–20 nm. Magnetic nanoparticles are very efficient as they can be easily separated and recovered using a magnetic field during the process. Since surface energies play an important role, surface interactions between magnetic particles and proteins must be carefully considered. Bates noted that the adsorption capacity of proteins is highly correlated with the activation of chemical bonds on the particle and the protein surface. The absorption capacity of proteins is highly related to the activation of chemical bonds on the particle and the surface of the protein. Nanoporous materials have a lower enzyme load as support but no restriction on substrate diffusion. In contrast, porous materials have a lower restriction of substrate diffusion while showing a high enzymatic load (Netto et al., 2013). Immobilization of α-amylase on carboxylated magnetic nanoparticles, lipase on magnetic Fe3O4 chitosan to synthesize ascorbyl palmitate, invertase on silica-modified magnetic nanoparticles in order to hydrolyze the potato starch to glucose and fructose are successful examples of the application of nanoparticle-immobilized enzymes in the food industry (Cao et al., 2016; Wang et al., 2015; Bayramoglu et al., 2017).

17.3.1.3 Nanosheets

The carbon family is one of the most widely used materials to immobilize enzymes. For this reason, different methods of producing carbon-derived nanomaterials have been studied. Among these materials, graphene is used in the immobilization of enzymes due to its remarkable optical, thermal, electronic, and mechanical properties. It is composed of carbon atoms with a dense two-dimensional structure similar to a honeycomb network. Graphene, in addition to successful drug and gene delivery, has been able to immobilize enzymes due to maintaining the natural protein structure and activity of enzymes (Singh et al., 2014).

Immobilization of Laccase obtained from *Aspergillus oryzae* (for ethanol production, clarification of wine and tea) and α-galactosidase (for hydrolysis of raffinose oligosaccharides (RFOs) cause of flatulence in soybeans) on graphene nanosheets are significant examples of the application of nanosheets in food industries (Singh et al., 2014; Skoronski et al., 2017).

17.3.1.4 Nanotubes

In recent years, carbon nanotubes (CNTs) have been considered for enzyme immobilization due to their special surface properties along with their desirable structural and mechanical properties as well as their very specific surface. The main types of CNT, including single-walled (SWCNTs) and multiwalled (MWCNTs), are used to immobilize enzymes which provide higher enzyme loading and lower mass transfer

resistance. MWCNTs are generally composed of multiple layers of graphite that surrounded a central pipe, which are less expensive and more stable in harsh thermal and mechanical conditions. Both covalent and noncovalent methods could be carried out for binding enzymes to nanotubes (Homaei & Samari, 2017).

Mukhopadhyay et al. (2015) immobilized pectate lyase by entrapment in SWCNTs. The results showed enzymatic behavior and its stability boosted at different temperatures, namely 4 °C and 80 °C and repeated freeze-thaw cycles. In other study enzyme activity of Laccase immobilized on MWCNTs maintained was more stable than the free one after nine cycles and storage time (Tavares et al., 2015).

17.3.2 Application of Nanobiocatalyst in Food Industry

17.3.2.1 Food Products

Different immobilized enzymes have been used by different nanostructured supports in different food industry processes, some of which are discussed in this section (Table 17.2).

Mosafa et al. (2013) immobilized papain onto silica-coated magnetic nanoparticles for applying in pomegranate juice clarification. The immobilized papain, compared with the free papain, improved storage stability and exhibited enhanced enzyme activity, good reusability, and well tolerance to different pH and temperature of the medium. Both the immobilized and free enzymes were influenced for the pomegranate juice clarification (Mosafa et al., 2013).

Sojitra et al. (2016) prepared nanobiocatalyst of magnetic tri-enzyme by co-immobilizing three enzymes simultaneously, pectinase, cellulase, and α-amylase, onto nanoparticle of amino-functionalized magnetic for clarification of juice. The results showed that up to 75% of residual activity of the immobilized enzymes was retained even after reusing eight cycles consecutively. Finally, the clarification of grapes, pineapple, and apple juices using magnetic tri-enzyme was obtained, with 46, 53, and 41% reduction in turbidity, respectively, during 150 min treatment (Sojitra et al., 2016).

Shahrestani et al. (2016) successfully immobilized xylanase onto 1,3,5-triazinefunctionalized silica encapsulated magnetic nanoparticles (MNPs) for clarification of fruit juice. The results showed that the immobilized xylanase on functionalized MNPs (Xy-MNP) had excellent catalytic activity at 65 °C and pH 6.5. Also, Xy-MNPs had a significant influence on juice clarity and showed quite impressive stability so that up to 55% of the initial activity of the immobilized enzymes were retained even after 10 reaction cycles consecutively in the enrichment of the juices (Shahrestani et al., 2016).

Patel et al., (2018) immobilized Smt3-D-psicose 3-epimerase onto nanoparticles of the functionalized iron oxide magnetic for the clarification of wash from the pomace of kinnow and apple fruits. The results showed that the immobilized enzyme had excellent storage stability by missing only 20% activity during 60 days of storage at 4 °C. Up to 90% of initial activity for the immobilized enzyme retained even after reacting 10 cycles of catalyzing D-fructose epimerization consecutively.

Table 17.2 Application of nanobiocatalyst in food industry

Product	Types of nanocarriers	Type of enzyme	Results	References
Soybean oil	Superparamagnetic Fe3O4 nanoparticles	Lipase	Immobilizing lipase improved storage stability, exhibited enhanced enzyme activity until 70% initial activity, 90% soybean oil transesterification	Xie and Ma (2010)
Pomegranate juice	Silica-coated magnetic nanoparticles	Papain	Immobilizing papain improved storage stability and exhibited enhanced enzyme activity, good reusability, and well tolerance to different pH and temperature of the medium. Immobilized enzymes could be influenced for the pomegranate juice clarification	Mosafa et al. (2013)
α-Amylase	Magnetic nanoparticles		Up to 70% of the initial activity of the immobilized enzymes was remained. Simply recovered	Baskar et al. (2015)
Fruit juice	1,3,5-triazinefunctionalized silica encapsulated	Xylanase	Up to 55% of the initial activity of the immobilized enzymes were retained even after 10 reaction cycles consecutively	Shahrestani et al. (2016)
Grapes, pineapple, and apple juices	Nanoparticle of amino-functionalized magnetic	Pectinase, cellulase and α-amylase	Up to 75% of residual activity of the immobilized enzymes were retained even after reusing eight cycles	Sojitra et al. (2016)
Wash from the pomace of kinnow and apple fruits	Functionalized iron oxide magnetic nanoparticles	Smt3-D-psicose 3 epimerase	Excellent storage stability by missing only 20% activity during 60 days up to 90% of initial activity for the immobilized enzyme	Patel et al. (2018)

(continued)

Table 17.2 (continued)

Product	Types of nanocarriers	Type of enzyme	Results	References
			retained even after reacting 10 cycles of catalyzing D-fructose epimerization consecutively	
Soybean oil	Atmospheric plasma surface modified chitosan:polyethylene oxide (90:10) nanofibers	PLA_1	78.50% Immobilization efficiency retained 80% of its initial activity after 10 time of recycling decreased phospholipids to 8.6 mg/kg after 6 h	Beig Mohammadi et al. (2020)
Pineapple juice	Polyethylene glycol grafted magnetic nanoparticles	Pectinase	Up to 59% turbidity reduction occurred in treated pineapple juice Up to 55% of the initial activity of the immobilized enzymes was retained after 10 repeated uses	Kharazmi et al. (2020)

Immobilizing made significant improvements in storage stability of the enzyme, its recycling efficiency, and thermal tolerance (Patel et al., 2018).

Kharazmi et al. (2020) immobilized pectinase covalently onto nanoparticles of polyethylene glycol grafted magnetic via trichlorotriazine for fruit juice clarification. The immobilized pectinase exhibited improved operational stability, enhanced catalytic activity, and simple reusability. Up to 59% turbidity reduction occurred in treated pineapple juice with immobilized pectinase. In addition, it exhibited high operational stability of immobilized pectinase and the enhanced reusability, and up to 55 and 94% of the initial activity of the immobilized enzymes was retained after 10 repeated uses for clarification of the pineapple juice and 125 days storage at 25 °C, respectively. It can be suggested for juice and food processing industries (Kharazmi et al., 2020).

Lipase was immobilized onto superparamagnetic Fe3O4 nanoparticles by covalent bonding for soybean oil transesterification. The results demonstrated the residual activity to be almost 70% of its initial activity after four cycles (Xie & Ma, 2010).

The immobilization of PLA_1 on APSM CS:PEO NM by Beig Mohammadi et al. (2020) for soybean oil degumming was carried out. The result showed the immobilized PLA_1 can be reused 10 times without a significant loss in the enzyme activity. In addition, high enzyme loading, effective catalytic activity, and easy

recovery reusability were observed. The residual phosphorus content by immobilized PLA_1 decreased to less than 10 mg/kg after 6 h, which is acceptable according to the standard for the physical refining of soybean oil (Beig Mohammadi et al., 2020).

The magnetic nanoparticles were used as a carrier for immobilized fungal α-amylase. This nanobiocatalyst is used for the production of glucose from starch. The results showed the potato starch had more affinity toward immobilized α-amylase than free type. In addition, the α-amylase simply recovered after each progression (Baskar et al., 2015).

17.3.2.2 Application of Nanotechnology with Food Enzymes in Food Packaging

Materials used in food packaging should improve mechanical, barrier, and antimicrobial properties, as well as imbedded nanosensors to monitor packaged food during transportation and storage. Nanofibers amplify mechanical property to protect foods with brittle texture; adding green tea and oregano extracts to edible films prepare antioxidant activities for packaging materials. Monitoring of changes of food quality during storage could be possible by smart packaging, such as application of multiple pH dyes in fibers used as packaging materials. Nanofibers, as a product of nanotechnology, as well as antimicrobial nanoparticles with controlled release properties, and bioactive components could have significant role in food industries. The structured polymeric films containing nanofibers make great surface area and mass transfer rates, they could be used to detect pathogens in food.

There have been many efforts to innovate nanostructure functional food system, in order to control food safety by nanosensors and nanobiosensors; make tools for objective transfer of nutrients for their nanotherapy; extend controlled release system of nanoencapsulated materials; and form enzymatic reactor in nano size for extension fortification of food with valuable ingredients such as essential fatty acids.

Bionanocomposites, such as polymer nanocomposites, with hybrid nanostructure can improve gas barrier and thermal and mechanical features. Biodegradable types could be environmentally friendly, because of reducing the need to use plastics. These materials need not only inorganic particles to create biopolymer structure but also some additives such as surfactants.

Nanotechnology-derived food products are significantly expected to be reachable for people, because of development of some new innovations such as electrospun nanofibers as a food packaging material or to encapsulate valuable components (Dwivedi et al., 2018).

Nanoparticles, nanoemulsions, nanocomposites, and nanostructured materials are used for food processing, delivery, formulation, and packaging, which are very important for food safety and biosecurity. Application of nanosensors in food packaging has many advantages, and there are some examples including:

- Palladium, platinum, and gold based nanosensors to detect any sort of color changes in food, any gases produced by spoilage, any changes in light, heat,

humidity, and chemicals into electrical signals, and toxins such as aflatoxin in milk.
- Single-walled carbon nanotubes and DNA to monitor soil condition needed to crop growth and detect pesticides on fruit and vegetable surface.
- Carbon black and polyaniline to detect carcinogens and pathogens in food.
- Array biosensors, electronic noses, nano-test strips, and nanocantilevers to monitor color changes resulting from food spoilage.
- Nano-smart dust in order to detect any type of environmental pollution.
- Nanobarcodes to detect agricultural products quality.
- Nanobiosensors in order to detect viruses and bacteria.
- Biomimetic sensors (protein and biomimetic membranes) and smart biosensors to determine mycotoxins and some toxins, act as pseudo cell surfaces to assist pathogens detection and removal.
- Surface plasmon-coupled emission biosensors (containing gold) to detect pathogens.
- Cerium oxide immunosensors and chitosan-based nanocomposites to detect some toxins like ochratoxin A.
- Carbon nanotubes and silicon nanowire transistors to detect staphylococcal enterotoxin B and cholera toxin.
- iSTrip of time-temperature indicator/integrator to detect the spoilage of food based on the history of temperature.
- Abuse indicators to detect desired temperature has been achieved or not.
- Partial temperature history indicator, when the temperature exceeds a certain pre-determined value.
- Full-temperature history indicator to register continuous temperature changes considering time and detect frozen food temperature changes.
- Reflective interferometry in order to detect *E. coli* contamination in packaged foods (Pradhan et al., 2015).

To keep food quality and safety during storage and transport, food packaging plays a key role, by extending the shelf life. Therefore, the type of food packaging materials is very important, as well as being biodegradable and using natural polymer instead of petroleum-based plastics. Some biodegradable polymers used in food packaging are:

- Polysaccharide-based polymers, such as alginate, cellulose, chitin, chitosan, gellan, pectin, starch, and xanthan.
- Protein-based polymers, such as collagen, gelatin, whey protein, soy protein, and zein.
- Aliphatic polymers, such as polylactic acid and polyhydroxybutyrate.

Today, in order to control microbial contamination on surface of foods, active components are to be combined with edible films. The application of biopolymer needs using some additives to make better their mechanical and thermal properties as well as retarding oxidation, controlling of respiration rate, migration of moisture, and

microbial growth. In active packaging, shelf life of foods would be prolonged by using antioxidant and antimicrobial agents in formulation of packaging materials. Organic acids, bacteriocins, and enzymes are some examples of active components used for the formulation of packaging with antimicrobial property.

Because of some specific features of bionanomaterials, like improvement of sustainability and also biological activity, they might be used to encapsulate and deliver some micronutrients. Chitosan, a biopolymer having particular properties, which has been extracted from shells of shrimp and crab, is nontoxic and biodegradable and possesses film forming and antimicrobial properties. Nanoparticles of chitosan have more surface area-to-volume ratio and more effectiveness compared to bulk ones, which make them appropriate for formulation of food packaging materials.

Metal nanomaterials, such as silver and gold, have excellent antimicrobial activity and can show inactivation of microorganisms. As silver nanoparticles could absorb and break down ethylene, they are able to prolong the senescence of fruits and vegetables in packages with films containing them. Also, it is shown that they have good effectiveness for strains such as *E. coli,* which are resistant to silver (Jafarizadeh-Malmiri et al., 2019).

In order to detect *E. coli* O157:H7AQ19 -the pathogen separates by immunomagnetic method- after hydrolyzing of p-nitrophenyl phosphate by alkaline phosphatase the absorbance of p-nitrophenol will be red. Another example of enzyme application for meat freshness monitoring is using yeast sensor (Trichosporon cutaneum), in which signals of flow-injection analysis will show polyamines and amino acids producing by meat, and increasing of bacteria count during its storage.

Most sensors for quality control of meat packaging, which have been introduced by researchers, are not commercialized and should be developed for releasing to market. The application of antimicrobial ingredients in coating in smart packaging by nanomaterials can be a promising impact on food packaging. Of course, fast monitoring methods are needed to check a lot of food packages during storage and shipping for a long period of time (Fatima Mustafa & Andreescu, 2018).

Biocatalysts can have many advantages as an aid in bioprocessing and bioactive packaging, because these components or any reaction adjusted by enzymes help bioactive materials to bring a substrate to react. In fact, an enzyme is a specific catalyst that can increase product quality by producing useful ingredients or decomposing unpleasant ones. Immobilized enzymes not only can be simply recovered, and used again, but also have a low cost for environment, used materials, if the suitable method for immobilizing of enzyme be used. By using nanomaterials for applying immobilized enzymes in food packaging, increasing the ratio of surface area to volume, production scaling up, and mechanical resistance will happen (Wong, 2016).

The intelligent packaging using nanosensor will have a lot of advantages in food industries. Very small chips will be put in the food packages, which act as an electronic bar code, in which food would be monitored in all steps including process,

storage, and even consumption. Developing of wireless nanosensor networks is necessary to cover limitations of nanosensors.

In future, intelligent food packaging using nanotechnology could be a necessity for food safety and quality control by precise and fast methods. These new packaging tools need innovation and advancement of nanosensors as well as lowering the cost (Fuertes et al., 2016).

Time temperature integrators (TTIs) are one of the newest commercialized methods for monitoring fresh foods, which need cold chain during distribution and handling. TTIs functions are based on some reactions, such as enzymatic, microbial, and photochemical, which are dependent on temperature, and reaction speed increases by temperature enhancement.

In a kind of TTIs, lipase shows color changes which are dependent on pH changes. As the spoilage of meat usually is made by microbial growth and enzymatic autolysis, TTIs using enzymatic biological reaction are very effective tool as a freshness indicator and fresh and frozen meat spoilage trend. TTIs can simply be applied into packaging material formulation for frozen fish, fruits and vegetables, and dairy products. Available commercialized TTIs demonstrate changes of color which are simultaneously dependent on temperature and time.

As in meat spoilage, most bacteria use glucose; predicting of meat shelf life can be possible by glucose measuring. The color of some indicators which work on chemical reaction will change.

In rigor mortis, glucose changes to lactic acid and protons in muscles, by anaerobic fermentation. This happens in cold packages of meat as well as freeze-thawed meat products. Lactic acid will simply be detected by pH indicator dyes. In order to control the quality and freshness of food products, in commercialized glucose sensors, glucose oxidases is immobilized on the electrodes surface and ferrocene acts as a mediator.

Electrochemical biosensors are used to monitor food quality in smart packaging. Two main electrochemical biosensors are biocatalytic devices and affinity sensors. The first one redox enzymes, whole cells, or tissue slices would produce electrical signal, and in the second type, antibodies, antibody fragments, or aptamers are targets. In another kind of electrochemical biosensors, enzymes are used to recognize analytes. These are very specific, not expensive, and could simply be suited to materials of packaging (Park et al., 2015).

In active packaging, silver nanoparticles are investigated by many researchers due to their antimicrobial property against foodborne pathogens, and inhibition of fungi and some viruses. Because of silver bactericide effect, binding to bacteria DNA, proteins, and enzymes will happen. Nanoparticles of silver can destroy outer and cytoplasmic membranes of bacteria by inhibition of their respiratory chain enzymes and be effective against *E. coli,* when used in agar film formulation.

The antibacterial activity of nano titanium dioxide particles (nano-TiO2) is also known, although it be limited by exploring UV irradiation. DNA damage through hydroxyl radicals and attack to outer and inner membrane of bacteria are two proposed mechanisms for their bactericide effects. It is shown that plastic films

coated by TiO2 nanoparticles could stop *Penicillium expansum* spoilage in apple, tomato, and lemons, because of their photocatalytic activities when exposed to light.

Also, it is observed the effectiveness of lactase or cholesterol reductase to increase food quality, when are enclosed by packaging materials, as well as adsorption into nanoclays incorporation by polymers (Sharma et al., 2017).

Some sensors utilize enzymes like laccase or tyrosinase for biocatalytic conversion of phenolic antioxidants to their quinones, which will be measured by electrochemical reduction at a low applied potential.

Antimicrobial packaging by inhibition of microbial growth will extend shelf life of food products. In this packaging, active agent, such as organic acids, enzymes will be coated on inner layer or enclosed by packaging material. Silver nanoparticles have been used in biodegradable film, and their antimicrobial activity against *E. coli* and *Staphylococcus aureus* and increasing cherry tomatoes packages shelf life was shown. It is suggested to study on the toxicity of AgNPs, and their potential migration from packaging into food, before commercialization (Mustafa & Andreescu, 2020).

17.3.3 Application of Immobilized Enzyme on/in Nanocarriers as Biosensors in Food Analysis and Safety Aspects

Biosensing is a way for detection, in which combination of biological components and physicochemical methods are used. By increasing of food adulteration, the application of nanobiosensors in safety analysis has got an important potential as high sensitive and selective detection methods. Better identify of target, fast recognition by enhancement of signal production, boost of selectivity and sensitivity, and reduction of time analysis are some of the advantages of biosensors. Various nanomaterials have had effectiveness as biosensors, which can be classified into metallic nanoparticles, metal compound nanomaterials, carbon materials, non-metallic nanomaterials, nanostructures, and composite nanomaterials. Traditional and time-consuming methods to detect microorganisms, as well as increasing demands for quick tests, without culture, led to find the other selective and sensitive techniques. Initial screening of food bacteria could be one of the nanobiosensing advantages. Trace quantity of pathogens' DNA could be detected by nanomaterials and amplification methods such as polymerase chain reaction (PCR), or recognize by antibodies and or aptamer (antibodies as a part of a sensing). Inappropriate storage might make contamination of animal feed and agricultural products with mycotoxins or bacteria toxins; therefore, the detection of toxins is very important in point of food safety. Detection by nanomaterials in biosensing technique could be particularly way as well as fast, susceptible, compared with ordinary methods. For example, aflatoxin B1 has been detected by nanoparticles based biosensors, using antibody-functionalized mesoporous carbon (MSC). Also, fluorescent nitrogen-doped carbon dots on gold nanoparticles (AuNPs) have been used to detect ochratoxin A, an aptamer-based assay method, which is highly selective for other toxins, such as deoxynivalenol, fumonisin B1, and zearalenone. Gold nanoparticles doped

ferrosoferric oxide nanoparticles, cadmium telluride quantum dots-glucose oxidase, and silver nanoclusters are some of metal compound nanomaterials used for toxins detections. Application of nano-extraction using mass spectrometry to extract aflatoxins from liquid samples by an absorbant (polydopamine-coated on nanoparticle) has been reported, as well as to quantify, that was coupled with HPLC-MS/MS.

Pesticides are another harmful components, if accumulate in humans and animals. Therefore, to control the Maximum Residue Levels, using suitable determination methods are necessary. Inhibition of acetylcholine esterase by pesticide is a fast and widely used method, in which organophosphorus compounds and carbamates specifically are inhibited by the enzyme. By nanobiosensing assay, thiocholine generation by acetylcholine esterase catalysis would cause the accumulation of gold nanoparticles, and better their recovery. Another method is direct detection by organophosphorus hydrolase, in which ordered mesoporous carbons are used to immobilize cell surface-displayed enzyme, and could detect paraoxon, parathion, and methyl parathion. Some pesticides would be detected by nanobiosensors based on their electrochemical activities, such as malathion and lindane. The most prevalent method to detect pesticides in food is an immune test by nanobiosensors. Prevention of photochemical property of nanomaterial by pesticides would be the principle of antibodies reorganization, for example, blocking of gold nanoclusters/grapheme hybrid's electrochemiluminescence by pentachlorophenol.

The uncontrolled usage of some antibiotics in animal feed to raise the growth would result in the increase of antibiotic residues in their meat and milk. Therefore, some recognizing assays based on aptamer, antibody, and liposome are used to detect antibiotic residues in food, by using some nanomaterials like glucose oxidase and nanoparticles of gold.

Detection of heavy metals, as hazardous elements, can be done by recognizing some biomolecules such as nucleotides (with colorimetric assay for Hg2+ using gold nanoparticles), DNAzyme (for lead ions with DNA-stabilized AgNCs), amino acid (cadmium and leads ions with graphene-enhanced electrochemical signals), and antibodies (for cadmium biosensing based on coreshell of bimetallic nanoparticles of gold and silver enhanced Raman scattering).

Existence of some components could be recognized by biosensors, like melamine, as a food adulteration (to increase protein content milk), by aptamer of triplex molecular beacon and fluorescent nanocluster of silver or nitrite, as a hazardous additive, by electrochemistry of myoglobin on a reduced glucose oxidase-multiwalled carbon nanotubes-platinum nanoparticles.

Biosensor is an apparatus or tool which contains a biological sensing material composed of a chemical or physical transducer, to convert chemical or biological signals into electrical ones (Fig. 17.4).

The potential of a biosensor will be increased by application of nanomaterials such as carbon nanotubes, nanowires, magnetic nanoparticle, and nanorod. These materials increase sensitivity and decrease limits of detection.

However, formal methods for detecting foodborne microorganisms, like enzyme-linked immunosorbent assay, polymerase chain reaction, and the application of fast, reliable, specified, and low-cost analysis assays, such as electrochemical biosensors,

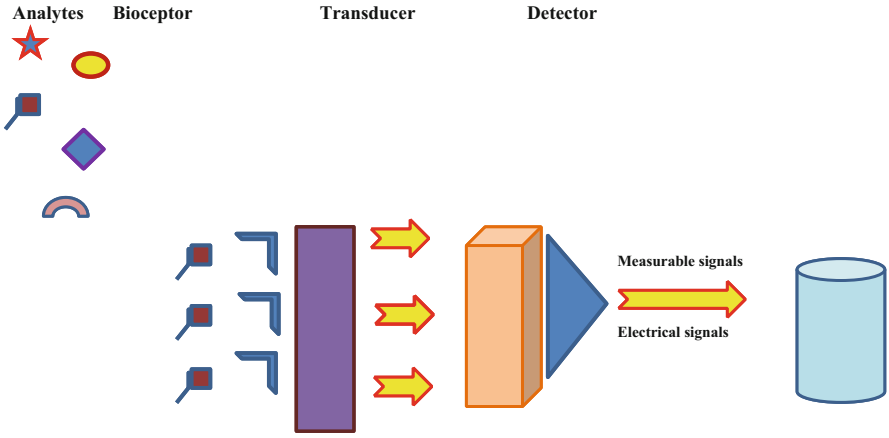


Fig. 17.4 A schematic biosensor

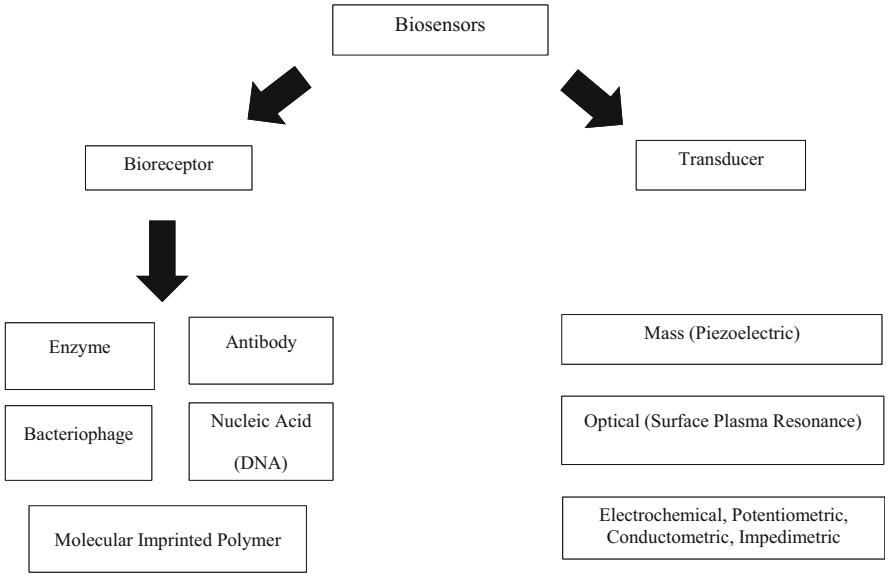


Fig. 17.5 Biosensor classifications and methods

have their own importance. E. coli and Salmonella are examples which have been detected by amperometric and electrochemical DNA biosensors. Classifications and methods of biosensors can be seen in Fig. 17.5.

Transducers, the main part of biosensors, cause physical responses, which may include heat absorption (calorimetric biosensor); variation of charge distribution (potentiometric biosensor); motion of electrons made by a redox reaction (amperometric biosensor); or total effects of reactants (piezo-electric biosensor).

Three creations of biosensors are included: ordinary one, an electrical reply of converter resulted by product reaction; the next one set by particular mediator among convertor and reaction for better reply; in the newest one reaction makes reply without involving product or mediator, straightly.

The main features of a biosensor are having high particularly for the reason of analyses, stability in ordinary storage and a lots of experiments; precise and reproducible reactions with no affected by physical variables such as temperature; no toxic affect; low price, small size, and user-friendly application.

Raghu et al. (2014) immobilized acetylcholinesterase on carbon paste electrode by silica sol–gel method, which worked as an electrode to monitor Malathion and Acephate in foods. The detection and quantification of these two pesticides were done directly, without extraction and pre-concentration as well as less quantity of enzyme. They demonstrated that this immobilized enzyme can work as a biosensor with high sensitivity and low cost.

Biosensors are made of three parts: biological recognition segment, transducer, and a signal processing part. In biosensors, in which enzymes are immobilized on the surface of transducers, enzyme work as a bridge with analyte. These biosensors have many usages in different applications, such as monitoring of food safety. Good sensitivity and specificity and the least preparation of samples are some of their advantages.

One of the biosensor groups which is the greatest one and are produced commercially is electrochemical enzyme-based ones. The use of nanocomponents in biosensors would improve their sensitivity, stability, and detection limit.

Electrochemical enzyme-based biosensors have had successfully and commercially application as a bioanalytic system with a good selectivity and stability, much better than previous ones. These biosensors in nanoscale have more efficiency in being sensitive, selective, and stable as well as detecting limit and response time, comparing to macroscale ones, because nanomaterials increase sensitive surface of the transducer and effectiveness of enzyme immobilization by increasing the ratio of surface to volume. Nanomaterials have good electrical conductivity, magnetic properties, and catalytic activity, which could also improve biosensor properties. These materials have been classified into two groups: organic and inorganic; metals, quantum dots, and zeolites are the examples of the former group, and carbon nanotubes, graphene and graphene oxide, and calixarenes for the latter one.

Inorganic nanomaterials accelerate transfer of electron and catalyze electrochemical reactions, while the organic ones amplify electrochemical signals to increase the level of biocompatibility. These biosensors could have application in food industries, because of their good biocompatibility, which would make them stronger with more specific usage.

Biosensors can be used to recognize a variety of biomolecular complexes, such as antibody, antigen, living cells, and enzyme-substrate interactions. Their nanoscale can detect molecules like glucose because of being small, transportable, having linearity of response, as well as more selectivity, sensitivity, and stability.

In fact, a biosensor is composed of three important parts: a bioreceptor molecules (to classify target analyte), a biotransducer component (to convert a chemical or

biochemical signal into an electrical signal), and finally an electronic system including amplifier, processor, or recorders. The biosensors could be classified into electrochemical, potentiometric, calorimetric, amperometric, resonant, ion-sensitive, and optical. Nanobiosensors are included to optical, bio, chemical, physical, and sol-gel types. Nanosensors matrix would protect food from effects of enzyme or dye toxicity. Of course, their usage in food industries depends on the detection type. For example, chemical or bio detection for optical nanosensors, in which their color changes by reaction with chemical or biological reagents. In biological nanosensors, antibody/antigen; DNA; enzyme interactions would happen. While, chemical composition or molecular concentration may occur in chemical type, or pressure, force, mass, and displacement in physical ones.

Knowing the mechanism of nanobiosensors is very important, for example, by spraying a nano bioluminescent on a food, visual glow could indicate microbial contamination, or released nano particles, such as nano-silver used in active packaging will react with microorganisms. In amperometric nanosensors, organophosphorus pesticides could be detected by mechanism of dual enzyme electrode system.

Technology of biosensors effects on many sections, including pharmacology, medical care, and food industry, in which safety has an important role, as well as easy, quick, and precise control of nutrients and pollutants.

In fact, enzymatic biosensors are electrochemical ones for food analysis, in which substrates would be detected either from their transformed products obtaining by enzymatic catalysis or by the detection of enzyme inhibitors.

To detect xanthine in fish, immobilized xanthine oxidase (MWCNTs) on multiwalled carbon nanotubes and a copolymer to act as a biosensor were used. It was shown that MWCNTs could have increased sensitivity more than biosensor when was applied to immobilize alone. By immobilization of glutamate dehydrogenase in MWCNTs, detection limit of monosodium glutamate was improved.

Nanotechnology has an important role in the agri-food industries for very distinctive properties of nanomaterials. Recently, they are used to detect food adulterants, pathogens, and toxins. Enzymatic nanobiosensor is made of an enzyme immobilized on the surface of a transducer.

Enzymes in nanobiosensor adjust the sensitivity and stability of them; therefore, more substrate specificity makes more enzyme turnover number and thermostability, the main necessity for a biosensor. Bioconjugation is a biosensing method with cost-effectiveness, based on the nature of interaction between nanomaterials and the enzyme surface functional groups. In nanobioconjugation technique, physical (adsorption, entrapment), covalent (covalent binding, cross-linking), and bioaffinity types are used to plan a strong nanobiosensor. Weak bioconjugation could have a little activity of biosensor, following the change of the enzyme active site conformation. Therefore, by more understanding functionality of a biomolecule at interface, better choice to plan and improve a bioconjugation would happen.

Nanobiosensors have been used to detect the pathogens in foods, and the optical assays like fluorescence and surface plasmon resonance are the most usable techniques. It was demonstrated that by using covalently co-immobilized enzymes choline oxidase and peroxidase on zinc oxide nanorod films, detection limit would

be improved, as well as xanthine, when xanthine oxidase immobilized on nanocomposite.

Another example of developing nanobiosensor is detection of glucose in fruit juice by immobilizing pyranose oxidase on gold nanoparticles, which was considered as a quick and sensitive method. The other technique to detect this analyte is immobilization of glucose oxidase on nanoclusters. Fructose has been detected in honey by fructose dehydrogenase which was immobilized on carbon nanotubes. To detect Escherichia coli, immobilization of b-galactosidase on carbon nanotubes has an effect on the lowering boundary of detection. It is supposed that enzymatic nanobiosensors get a perfect and important part of food quality control.

17.4 Risk Assessment of Nanomaterials in Food Industries

17.4.1 Legal Aspects

It is true that universities, companies, and governments have become intensely interested in the application of nanotechnology and nanomaterials in the food industry. However deceiving this area might seem, it is of utmost importance to consider its regulatory issues if this is supposed to be successful. In other words, population acceptance is highly dependent on regulations that assure nanomaterials' safety. According to the latest guidance of EFSA (European Food Safety Authority) any new food which contains nanomaterials is considered a novel system that must be fully tested and authorized to be consumed by society. EFSA is also responsible for verifying the latest approaches which are useful for measuring their safety. It must not be forgotten that for all kinds of nanoengineered foods, it is still vital to follow all regulations regarding conventional, non-nanotechnological food components.

Additionally, it has been proven that size reduction process carried out in nanotechnology can induce specific changes in features and biokinetics of food matrices which consequently leads to toxicological effects compared with their conventional counterparts. So, their safety must not be assumed identical to their conventional non-nanomaterial peers, which means for a specific nanomaterial, specific considerations and data must be provided individually.

Detailed characterization, impurity recognition, and physicochemical knowledge also provide pivotal insight into safety assessment. Material composition and purity, empirical formula of a complete matrix, particle size, particle shape, structure, surface chemical composition, surface area, surface charge, appearance, melting point, boiling point, density, porosity, dustiness, pH, and the final form of the material (if it is powder, dispersion, etc.) are among physicochemical properties that must be taken into account while testing the nanomaterials' safety.

It is also probable that changes in production process may add new impurities or modify physicochemical and morphological properties of the food matrix, and hence alter its safety.

Direct and indirect exposure to nanomaterials is another influencing factor when analyzing a nanomaterial safety. Feed additives, inhalation, pesticides, dermal exposure, and migration or transfer of nanomaterials from the packaging agent into food are some areas which must be closely examined to assure a safe component being accepted in food chain.

17.4.2 Toxicity

As a result of the extremely wide application of nanomaterials in food industry, their safety has become a complicated matter to deal with as there has always been a gap in data provided for emerging nanoparticles' safety. These days, nanomaterials are being used from the farm up to the moment that the food is consumed by people at homes. Nanoscale pesticides, fertilizers, functional ingredients namely food additives, flavor enhancers, or packaging enzymes, and nano sensors are part of this hugely implemented technology. Therefore, precise investigation of its properties, potential threats, and daily safe intake dose is mandatory.

Silver, silica, iron, and titanium dioxide are considered as most consumed nanoparticles in food industry, especially in beverages. For instance, Ag NPs are used as an antimicrobial agent in food products. However, consumption of Ag NPs in edible products has caused some concerns as various experiments declared Ag NPs toxicity for cells as they can change the normal functionality of mitochondria, produce reactive oxygen particles, and boost membrane permeability.

Silicon dioxide (SiO2) nanoparticles, which are widely used in powdered products as an anticaking agent to improve their flow properties, have been proven to be risky when accumulated in liver to a certain extent. Silica nanoparticles which are used as anticaking agents can be cytotoxic in human lung cells when subjected to exposure (Athinarayanan et al., 2014). On the contrary, Yun et al. reported no toxicity of these nanoparticles when consumed orally by rats during 13 weeks. Therefore, more studies are needed to avoid any ambiguity concerning the nanoparticles safety.

Titanium dioxide NPs are widely consumed in cosmetics and medicine; in food products titanium dioxide nanoparticles act as white coloring agent. Higashisaka et al. (2015) claimed that titanium dioxide shows no toxicity in human and animal cells. Iron oxide nanoparticles, which are among interdisciplinary substances, showed to be noncytotoxic when used at concentrations below 100 mg/mL. However, massive consumption may end in accumulation in a targeted organ, possibly leading to iron storage, which can be hazardous toxic. Hence, their consumption is safe provided that they are used in a limited amount below 100 mg/mL (Buyukhatipoglu & Clyne, 2011).

17.5 Future Trends

Enzyme immobilization is a matter of concern in both academia and industrial sections. As a consequence, combination of nanotechnology and biotechnology boosts actions which result in immobilized enzymes' success. In order to increase the use of nanobiocatalysts, the following factors must be taken into account: (1) the ability to be separated and recycled easily and reusing high activity enzymes, (2) minimizing the structural changes of enzymes during stabilization and decreasing the inactive sites of nanobiocatalysts to reduce costs, (3) increasing the biocompatibility and stability of nanobiocatalysts in alimentation and pharmaceutical applications in various temperatures and pHs, and (4) simultaneous use of multiple enzymes with high compatibility in sequential reactions. Rapid advancement of nanotechnology can create opportunities to produce nanobiocatalysts to be used in food, medicine, pharmaceutical, and biological applications.

17.6 Conclusion

The latest attentions to nanomaterials and their wide applications have made it possible to immobilize enzymes, maintain their enzymatic activity, reuse, and finally recycle them. Various nanostructures such as nanofibers, nanoparticles, nanotubes, nanosheets, and others have been proven to act as efficient media for enzyme stabilization. If produced under good manufacturing practice and according to health regulations, these particles are preferred in comparison with their chemical counterparts. Enzymatic biocatalysts have become the center of attention as sensors in order to ensure food quality and safety. They are used as indicators of pesticides' residues, pathogenic microorganism, and their toxic metabolites. Nanotechnology and stabilization on nanostructures have been widely used in the production process of these biosensors.

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Clean Energy Production Technologies
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Thermochemical and Catalytic Conversion Technologies for Future Biorefineries

Volume 1

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Clean Energy Production Technologies

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The consumption of fossil fuels has been continuously increasing around the globe and simultaneously becoming the primary cause of global warming as well as environmental pollution. Due to limited life span of fossil fuels and limited alternate energy options, energy crises is important concern faced by the world. Amidst these complex environmental and economic scenarios, renewable energy alternates such as biodiesel, hydrogen, wind, solar and bioenergy sources, which can produce energy with zero carbon residue are emerging as excellent clean energy source. For maximizing the efficiency and productivity of clean fuels via green & renewable methods, it's crucial to understand the configuration, sustainability and techno-economic feasibility of these promising energy alternates. The book series presents a comprehensive coverage combining the domains of exploring clean sources of energy and ensuring its production in an economical as well as ecologically feasible fashion. Series involves renowned experts and academicians as volume-editors and authors, from all the regions of the world. Series brings forth latest research, approaches and perspectives on clean energy production from both developed and developing parts of world under one umbrella. It is curated and developed by authoritative institutions and experts to serves global readership on this theme.

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Thermochemical and Catalytic Conversion Technologies for Future Biorefineries

Volume 1

Springer

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ISSN 2662-6861 ISSN 2662-687X (electronic)
Clean Energy Production Technologies
ISBN 978-981-19-4311-9 ISBN 978-981-19-4312-6 (eBook)
https://doi.org/10.1007/978-981-19-4312-6

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Dedicated to my beloved mother

Preface

The rise in global energy needs and fast depletion of the existing conventional fossil fuels force the stakeholders to search for alternatives. Biomass such as food crops, lignocellulosic, algal, and hybrid energy crops can be converted to biofuel and different value-added chemicals, thus acting as a potential substitute for petroleum-based biorefineries. Also, this strategy helps to overcome the pollution caused by the management of this waste generated worldwide. The widespread availability and structural composition of biomass motivated the scientific community to look at it as a potential substrate for the oil and chemical industry. Thus, leading to the development of biomass-based biorefinery. However, the conversion of biomass to high-value products is not as easy as it seems. It involves several stages such as pretreatment, hydrolysis, fermentation, and recovery. The pretreatment and hydrolysis of biomass involve several thermochemical and advanced catalytic technologies. This book will attempt to provide an account of knowledge on biomass available for biomass-based biorefineries. It focuses on understanding the recalcitrance of biomass and how it limits the overall conversion efficiency. It also gives an insight into different conventional approaches available for pretreatment and hydrolysis of the biomass. The chapter deals with highlights on how enzymes can be a powerhouse and play pioneering roles in biomass valorization. The book will also throw light on technical aspects of thermochemical conversion strategies such as pyrolysis, gasification, and organosolv methods. Further the generation of value-added materials such as high-quality bio-oil, biochars, and biobased chemicals are also included in the book. These high-value compounds can be put to widespread application in biofuel, biocatalyst, waste bioremediation (heavy metal removal), air purification, and effluent treatment applications. The book will also provide literature on the limitations of already existing technologies and prospects of each technology. This book is of interest to teachers, researchers, bioenergy scientists, capacity builders, and policymakers. Also, the book serves as an additional reading

material for undergraduate and graduate students of energy studies, chemical engineering, biotechnology, and environmental sciences. National and international energy scientists and policymakers will also find this book to be a useful read.

Ajmer, Kishangarh, Rajasthan, India Pradeep Verma

Acknowledgment

First, I would like to convey my gratitude to the series editor Dr. Neha Srivastava and Prof. P.K. Mishra considering the submission of this book entitled *Thermochemical and Catalytic Conversion Technologies for Future Biorefineries-Vol I* under the book series *Clean Energy Production Technologies*. I am thankful to Springer Nature for accepting my proposal to act as editor for this book volume. This volume of the book series is only possible because of the support from all the researchers and academicians who contributed to the book; therefore, the editor is thankful for their contribution. I would also like to thank my Ph.D. scholar, Dr. Bikash Kumar, currently working as a Post-doctoral Researcher at IIT Guwahati for providing me with all the necessary technical support and editorial assistance during the entire stage of book development. I am also thankful to the Central University of Rajasthan (CURAJ), Ajmer, India, for providing infrastructural support and a suitable teaching and research environment. The teaching and research experience at CURAJ has provided the necessary understanding of the needs of academicians, students, and researchers in a book that was greatly helpful during the development of the book.

I am always thankful to God and my parents for their blessings. I also express my deep sense of gratitude to my wife Savita and my son Mohak and daughter Netra for their support during the development of the book and in life.

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Chapter 1
Scope and Characteristics of the Biomass Sources Suitable for Biorefinery Applications

Azeem Asghar, Muhammad Afzal, Rizwan Haider, Muhammad Sajjad Ahmad, and Muhammad Aamer Mehmood

Abstract Biomass-derived biorefineries seem a promising approach for the complete valorization of biomass into bioenergy and a range of bioproducts. However, the product quality and bioprocessing strategy highly depend on the nature, composition, and quality of the biomass feedstock. The source and cultivation conditions do not only affect the quality and composition of the biomass but also affect the cost of biomass. Therefore, the choice of cultivation conditions and selection of biomass suitable for its subsequent use is critically important. Accordingly, residual biomass from agricultural or industrial activities or biomass production on marginal lands using wastewater offers an opportunity to produce low-cost biomass without creating any competition with food or land for food. However, it is important to consider that how these conditions affect the nature and composition of biomass with reference to its downstream processing. This book chapter covers the desired characteristics of the biomass to consider it as a potential feedstock for biorefinery while achieving environmental and economic sustainability.

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P. Verma (ed.), *Thermochemical and Catalytic Conversion Technologies for Future Biorefineries*, Clean Energy Production Technologies,
https://doi.org/10.1007/978-981-19-4312-6_1

Keywords Feedstock characteristics · Environmental sustainability · Bioenergy · Biorefinery

Abbreviation

GHG Greenhouse gases emissions

1.1 Introduction

Urbanization, industrialization, and transportation depend on fossil resources to meet their energy needs (Tey et al. 2021), but their depletion and negative impacts on the environment face challenges in terms of both social and economic impacts (Liu et al. 2021). A strong global dependence and huge consumption of petroleum derivatives are causing political and environmental concerns due to greenhouse gases emissions (GHG) like nitrogen oxides, methane, and carbon dioxide (NO_X, CH_4, CO_2). Accordingly, there has been a rush towards finding alternative resources to fuel the current and rising future needs of liquid fuels. It is believed that biomass is one of the most promising sources, due to its renewable and sustainable nature and is considered a third fuel source used to generate bioenergy, chemical, and industrial products.

Lignocellulosic biomass (the second-generation feedstock) has shown maximum accessibility in subtropical, and tropical macroclimates worldwide with an annual production of 1.3 billion tons, whereas only 3% of it is being used to produce biofuels, bioenergy, and biochemicals (Baruah et al. 2018). Biomass-derived biorefineries are believed to be an encouraging path to develop a new bio-industry by using the plants' biomass for gaining the food, non-toxic and pure feed, and materials for the chemical industries, energy, and other valuable products (Sanna et al. 2016). The selection of the raw material for biorefinery primarily is influenced by the origin and nature of the feedstock. Cultivation conditions affect the composition of biomass which subsequently determines the process parameters for the downstream processing of the biomass. Instead of using freshwater and agricultural lands, biomass for biorefineries can be produced using non-arable lands and urban wastewater. Likewise, integration of the biomass production landscapes with the phytoremediation using floating gardens or constructed wetlands could be a promising green approach to produce biomass as a feedstock for the biorefinery (Zhang et al. 2020). This chapter is focused on the impact of biomass source, its composition, and nature for its utilization as a biorefinery feedstock.

Biorefinery is a multidisciplinary approach that involves the transformation of biomass through sustainable processing to produce biofuels and value-added chemicals with a zero or minimum waste generation, where each by-product of the process is believed to be recycled to ensure environmental sustainability and

cost-effectiveness. The biorefinery approach is a range of combined techniques targeting to complete suitable conversion of biomass into their value-added products such as hydrogen, carbohydrates, and proteins, with the simultaneous production of biofuel, bioenergy, and biochemical (Bhardwaj et al. 2021; Carvalho et al. 2014; Kumar and Verma 2021; Verma 2020). Biorefineries need prime conversion efficiency of lignocellulosic biomass, within efficient production of energy (Hirani et al. 2018). The European vision of biorefinery 2030 document entitled supply of the tools and data to enable policymakers to draw a framework for the development of sustainable research based, with a network of refinery playing a vital role (Płaza and Wandzich 2016). Several biorefinery systems are believed to be developed in the future by taking the advantage of modern flexible technologies for efficient biotransformation of biomass into myriads of green products (Panwar et al. 2011). With respect to the technology employed, biorefineries can be categorized as: (i) pyrolysis-based refinery, (ii) fermentation-based refinery, (iii) hydrothermal-based refinery, and (iv) bio syngas-based refinery. All of these are used to produce materials, biofuels, and chemicals from the biomass.

1.2 Low-Cost Sources of Biomass

Production of biomass on agricultural land in huge quantities is a big challenge at present due to scarcity of the fertile soils so marginal lands can be employed for this purpose and instead of freshwater, the bioenergy crops can be cultivated using urban wastewater (Asghar et al. 2021) which offers additional benefits of phytoremediation and mitigating atmospheric carbon (Kurade et al. 2021). Wastewater is often rich in nutrients, where no additional sources (e.g., fertilizers) are needed to achieve higher biomass productivity. Besides, it does not only reduce the cost of cultivation but also does not cause any competition with the freshwater sources and agricultural lands. Another possibility is the integration of biomass production facilities with the wastewater treatment plants. For instance, para grass was cultivated on industrial and urban wastewater using a floating garden system, and it was found that overall biomass productivity was better when compared to the para grass biomass produced on the soil (Asghar et al. 2021; Singh et al. 2022; Agrawal and Verma 2021). Similarly, salt-affected soil was used to produce biomass in huge quantities without any fertilizers, fungicides, or pesticides with low cost and ensure environmental sustainability. The biomass of *Brachiaria mutica* (Asghar et al. 2021), *Typha domingensis*, *Phragmites australis,* and *Leptochloa fusca* (Afzal et al. 2019b) has been cultivated on wastewater and *Parthenium hysterophorus, Pennesetum benthiumo* (Ahmad et al. 2019), *Calotropis procera* (Ahmad et al. 2021), *Urochloa mutica* (Ahmad et al. 2017), and *Cymbopogon schoenanthus* (Mehmood et al. 2017b) have been cultivated on salt-affected soils. The biomass productivities of these grasses on poor soils have shown a promising potential of these soils to produce low-cost biomass (Fig. 1.1).

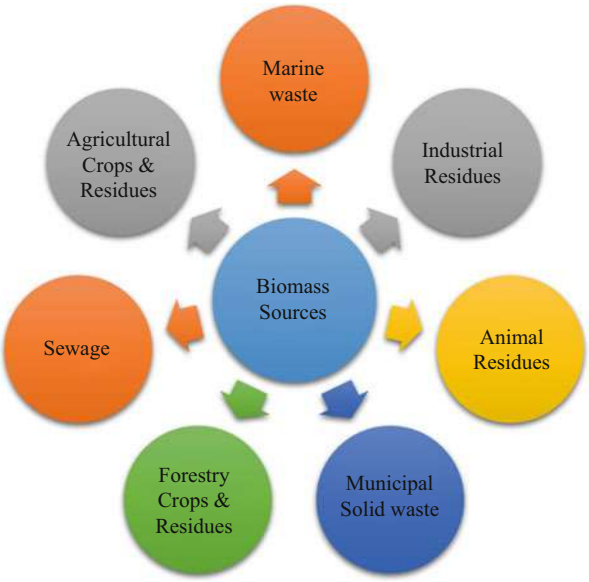

Fig. 1.1 Global sources of biomass sources for bioenergy production

Table 1.1 Different lignocellulosic biomass and their composition

Biomass	Extractives (%)	Hemicellulose (%)	Lignin (%)	Cellulose (%)	References
Pearl millet	4.26	23.16	15.75	48.93	Laouge and Merdun (2020)
Calotropis procera	32.5	33	17.91	16.57	Ahmad et al. (2021)
Ricinus communis	16.40	22.40	20.20	38.42	Kaur et al. (2018)
Sagwan sawdust	11.3	12.9	24.7	51.1	Gupta and Mondal (2019)
Rice husk		17.16	26.26	33.10	Ashraf et al. (2019)
Sunflower disc		14.12	27.87	31.46	Ashraf et al. (2019)

Biomass is either classified according to its composition/characteristics (Table 1.1) or the source (Table 1.2). Low-cost biomass can be obtained through agricultural practices, residues from forestry, industrial wastes, aquaculture, or municipal waste (Poveda-Giraldo et al. 2021). It is estimated that global plant biomass production is around 200×10^9 metric tons where 90% of the biomass is lignocellulosic in nature (Saini et al. 2015). Lignocellulosic biomass mainly comprises three components including cellulose (35–50%), hemicellulose (20–35%), and lignin (14–26%) with a small percentage of extractives. Macromolecules gather to form 3-D polymorphic structures which represent their function to deliver skeleton material with stiffness to the cell wall of plants (Madanayake et al. 2017). The main part of lignocellulosic material is hemicellulose, cellulose converted into

Table 1.2 Sources and products of biomass feedstocks

First generation	Second generation	Third generation	Fourth generation
Rich sugar biomass	Lignocellulosic biomass	Aquatic biomass	Rich solar-efficient aquatic biomass
Sources			
Sugar beet, sugarcane, starch, vegetable oils	Non-food crops wheat straw, maize stovers, corn, wood, solid waste	Algae, microalgae, cyanobacteria	Genetically engineered Plants and algal materials
Products			
Biogases, bio-oils, and biofuels	Biochemicals, electricity, power, heat, biogases, bio-oils, and biofuels	Fertilizers, biochemicals, electricity, power, heat, biogases, bio-oils, and biofuels	Biogases, bio-oils and biofuels, fertilizers, biochemicals, electricity, power, and heat

monosaccharides unit by a process of depolymerization such as xylose, glucose along mannose, galactose, sugar acids (galacturonic and glucuronic acid), and arabinose. In biomass, the matrix of hydrogen and covalent bond connect the lignin and hemicellulose with cellulose and also observed that lignin due to its strong chemical bonding nature also plays a role as binding agent (Kassaye et al. 2017).

1.3 Cost-Effective Cultivation Practices and Impact of Cultivation Condition on the Biomass Characteristics

Wastewater and marginal lands have a great economic impact in terms of cost used to produce biomass because these both resources it does not use agricultural land and have no negative effect but in addition, it makes more profitable because the use of non-agriculture land and eliminate the toxic, heavy metal from wastewater which directly improve the bioenergy production. Biomass cultivation in wastewater bodies used a novel approach with a very low cost called a floating wetland system (Afzal et al. 2019a; Asghar et al. 2021; Goswami et al. 2021a). Marginal lands and wastewater which are not used for agriculture purposes can be used to produce cost-effective biomass without posing any negative impact on the environment. Municipal wastewater and industrial wastewater are serious global environmental problems, so a new phytoremediation treatment of wastewater is employed for the cultivation of biomass using a floating gardening system. So, this approach is a *three-in-one* approach offering wastewater treatment, fixation of atmospheric carbon, and carbon-neutral biomass production. The potential of poor soils for the cost-effective production of biomass is shown in Table 1.3.

Table 1.3 Low-cost biomass sources for bioenergy

Crop	Nature, adapted climate	Biomass Yield (Tons ha^{-1} y^{-1})	Potential characteristics	Energy potential	References
Eichhornia crassipes	Perennial aquatic plant native to tropical desert to the subtropical or warm temperate desert to rainforest zones	28,000	Fast-growing, perennial, aquatic weed	124–127	Ruan et al. (2016)
Phalaris arundinacea	*P. arundinacea* now has a world-wide distribution. It is regarded as native to both North America and Eurasia	35–45	Extensive single species stands along the margins of lakes and streams and in wet open areas		Alhumade et al. (2019)
Bamboo (*Bambusa balcooa*)	Perennial, adapted to moderately acidic loamy soils, warm humid environment	40–50	One of the fastest-growing plants, alcoholic and phenolic compounds	41.85 GJ ha^{-1}	Mehmood et al. (2017a)
Cardoon (Cynara *cardunculus)*	Perennial, adapted to high temperature and low rainfall	7.4–14.6	Artichoke oil, feedstock for the first biorefinery and biodiesel	138 GJ ha^{-1}	Francaviglia et al. (2016)
Eucalyptus (*Eucalyptus obliqua*)	Perennial, adapted to temperate, tropical, and subtropical poor soils	20	Source of essential oils, phenolic compounds, medicinal compounds, fast growing	233–245 GJ ha^{-1}	Wadhwani et al. (2017)
Giant reed (*Arundo donax* L.)	Perennial, adapted to Mediterranean environment	36	Resistant to drought, used in dissolving pulp, durable yields, impressive bioenergy feedstock	2281 CH_4 kg^{-1}	Nassi o Di Nasso et al. (2013)
Poplar (*Populus tremula*)	Circumpolar subarctic and cool temperate	6–15.8	Biofuels, carbon mitigation potential, and fast growing	92 TW h^{-1}	Saha and Eckelman (2015)
Reed canary grass (*Phalaris arundinacea* L.)	Perennial, adapted to temperate regions, wet-soils, colder climatic, flood plains	15	Drought-tolerant, phytoremediation, sources of drugs	97 GJ ha^{-1}	Lord (2015)

Switchgrass (*Panicum virgatum* L.)	Perennial, adaptive to versatile growth conditions, C4-pathway	1–22	Ethanol, butanol, biogas production, thermal energy, phytoremediation, drought, and flooding tolerant	60 GJ ha^{-1}	Nassi o Di Nasso et al. (2015)
Virginia mallow (*Sida hermaphrodita*)	Perennial, native to sandy or rocky river shores, temperate	9–20	Source of food and nectar for honeybee rearing, the potential for bioenergy production	219.5 GJ ha^{-1}	Ţîţei (2017)
Wolffia arrhiza	Smallest vascular plant on earth rich in water-loving species		*Wolffia arrhiza* is a species of flowering plant	171 kJ ha^{-1}	Ahmad et al. (2018)

1.4 Correlation of the Biomass Characteristics with the Biomass Valorization Techniques

Nature and biomass composition affects the down-streaming processing which offers various benefits along with the challenges. Valorization analysis of hemicellulose and cellulose shows that it has a high capacity to produce biochemicals, biofuels, paper, pulp, and animal feed. For example, to achieve the attractive target agro-industrial residues such as rice husk (Singh et al. 2014), pruning waste (Martín et al. 2010), sugarcane bagasse (de Araujo Guilherme et al. 2019), fruit peels (Gordon et al. 2011), coffee cut-stem (Aristizábal-Marulanda et al. 2020), and wood materials have been excessively employed for the production of biofuels (Poveda-Giraldo et al. 2021). Various biomasses including para grass (Asghar et al. 2021), *Wolffia arrhiza* (Ahmad et al. 2018), *Calotropis procera* (Ahmad et al. 2021), Chinese liquor-industry waste (Ye et al. 2018), and microalgae biomass (Agrawal et al. 2020; Goswami et al. 2021b, c, 2022; Mehariya et al. 2021; Shahid et al. 2019) have been produced using industrial wastewater and/or municipal wastewater. While the modification in the biochemical composition in response to wastewater cultivation showed positive impact on the biomass with respect to its usage as a bioenergy feedstock.

The basic conversion processes include pyrolysis, gasification, torrefaction, and fermentation where either biochemical or thermal reactions are to progress (Akhtar et al. 2018). There are other less common, and more experimentally or exclusive thermal processes that may lead to cost-effectiveness, such as the hydrothermal upgrading process (Smith et al. 2018). The biomass with higher lignin content may be more suitable to produce biochar, while biomass with higher moisture and less lignin content may be more suitable for fermentation. Similarly, the biomass which contains some chemicals that have allelopathic impact can be processed using a two-stage biorefinery approach, where residual biomass can be used for bioenergy production through pyrolysis or fermentation after extracting the high-value biochemicals for applications such as medicine, preservatives, fragrance, cosmetics, and nutraceuticals.

1.5 Conclusion and Recommendations

Biorefinery is an environmentally friendly approach for the biotransformation of biomass with minimum waste and efficient resource recovery. The fate of biomass feedstock depends on its biochemical composition, source, cost-effectiveness, ease of transportation, and mechanism of processing. While cultivation conditions and seasons sometimes can be easily employed to modify the biochemical composition of the biomass for biorefinery applications. In the future, integration of the biorefinery with the urban wastewater treatment and phytoremediation systems could be cost-effective and could result in multifaceted opportunities. However,

the impact of cultivation conditions, involvement of machine learning, modeling, and simulation-based studies should be conducted to refine the methodologies for achieving commercial robustness.

Competing Interests All the authors declare that they have no competing interests.

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Chapter 2
Recalcitrance of Lignocellulosic Biomass and Pretreatment Technologies: A Comprehensive Insight

Rohit Rai, Vikash Kumar, and Prodyut Dhar

Abstract Plant biomass is an excellent lignocellulosic source that can produce renewable and environment-friendly biofuels. However, the natural physiochemical structure of plant lignocellulose has strong recalcitrance and heterogeneity, which results in low yields of biofuels, limiting its effective valorization in biorefineries. This rigidity of lignocellulose presents economic and technical challenges in biomass conversion processes. Various pretreatment methods are used separately and in combination to resolve this. Pretreatment methods change the structure and chemical composition of the plant biomass, which makes it more accessible to the conversion systems for biofuel production. This chapter will discuss the physical and chemical basis of lignocellulose recalcitrance and the biomass components contributing to it. This chapter will also explain the role of pretreatment strategies in biorefineries and their influence on the structure and composition of lignocellulosic biomass. The fundamental understanding of biomass recalcitrance and the role of pretreatment methods can aid in the efficient utilization of lignocellulosic biomass in biorefineries and the development of future pretreatment methodologies.

Keywords Lignocellulose · Recalcitrance · Pretreatment · Biorefinery

Abbreviations

[EMIMAc]	1-Ethyl-3-methylimidazolium acetate
ASA	Accessible surface area
BET	Brunauer-Emmett-Teller

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P. Verma (ed.), *Thermochemical and Catalytic Conversion Technologies for Future Biorefineries*, Clean Energy Production Technologies,
https://doi.org/10.1007/978-981-19-4312-6_2

CSC	Cellulose synthase complex
CESA	Cellulose synthase A
CSL	Cellulose synthase-like family
DES	Deep eutectic solvents
DP	Degree of polymerization
G	Guaiacyl
Glu	Glucopyranosyl
GT	Glycosyltransferases
H	*p*-hydroxyphenyl
IPCC	Intergovernmental Panel on Climate Change
LCB	Lignocellulosic biomass
LCC	Lignin-carbohydrate complexes
NMMO	*N*-methylmorpholine *N*-oxide
S	Syringyl
T_g	Glass transition temperature
TSA	Total surface area
UDP	Uridine diphosphate
UTP	Uridine triphosphate
X	Xylopyranosyl

2.1 Introduction

The rapid industrialization and population growth this century have created a high demand for energy and materials, which is essential for the proper functioning of day-to-day life. They hold a huge economic significance and their scarcity can have a high impact on the stability of the global economy, industries, technological advancement, and diplomacy around the world. Conventional fuels and materials are petroleum-based, whose reserves are depleting rapidly in several nations. It also has huge drawbacks due to the environmental harms associated with it such as global warming, water pollution, and climate change (Foston and Ragauskas 2012). According to the Intergovernmental Panel on Climate Change (IPCC) report 2021 (IPCC report 9 Aug 2021), global warming is increasing rapidly and can cross 1.5 °C of global warming in coming decades, if the quick, large-scale reduction in greenhouse gas emissions is not done. Another highlight of the report states that climate change is happening globally and affecting almost every region on the planet severely. The change in the water cycle, rainfall and flooding patterns, and sea-level rise in the recent decades is prominent proof of this. Most of the harmful environmental emissions originate from conventional petroleum-based sources. These limitations have incited the search for alternative fuel and material resources in recent decades that have the potential to replace conventional resources (Foston and Ragauskas 2012). In that context, alternative fuels and materials from plant biomass are becoming an upcoming choice as it is the most abundant biomass source

in the world. It also has exciting features such as renewability, low cost, sustainability, and environment-friendliness that suggest its potential in becoming the leading source of fuels and materials in the coming future. However, utilization of plant biomass to obtain bioproducts is not as simple as it contains an inherent resistance to various types of treatments (chemical, physical, and biological) which is called biomass recalcitrance. The biorefinery approach has been utilized in recent years to solve this complication by using plant biomass to obtain products like biogas, biofuels, pulp and paper, building block chemicals, adhesives, and surfactants. Biorefineries are facilities that have a role in changing biomass into several value-added products and are usually characterized by the feedstock used; for example, forest-based biorefineries, corn-based biorefineries, and algae-based biorefineries. Some challenges that are present in using the biorefinery approach are variation in biomass composition, production of fermentation inhibitors, and quality control. In addition, the effective performance of pretreatment and conversion strategies available during industrial scale-up are for a wide variety of biomass are the major hurdles (Agrawal et al. 2020; Goswami et al. 2021; Kamm 2014; Ng et al. 2017).

This chapter discusses the structure, properties, and synthesis of various chemical components of plant biomass (lignocellulosic) and its recalcitrance concerning the contribution of various physical and chemical components present. It also highlights the role of pretreatment in biorefineries and the change in physical properties and chemical composition of lignocellulosic biomass on the application of various pretreatment processes.

2.2 Lignocellulosic Biomass (LCB)

The plant biomass is composed of lignocellulose that has lignin (5–30%), hemicellulose (20–35%), and cellulose (35–50%) as its components. The collective term for cellulose and hemicellulose is holocellulose which are polysaccharides with high molecular weight (325–490 kDa) (Bjurhager et al. 2012) whereas, lignin is made up of polymerized phenylpropanoic acids into a three-dimensional (3D) structure. Lignocellulose components are present in the form of a heteromatrix in which the individual components are linked extensively. The complex arrangement of cellulose, hemicellulose, and lignin in the cell wall is unique. Cellulose arranges itself in a fibrous crystalline structure and is present in the core of the lignocellulosic complex as presented in Fig. 2.1(a). Hemicellulose is present between both macro- and microfibrils of cellulose whereas lignin is present in the outer part of the complex and has a structural and protective role (Bajpai 2016). The other constituents present in the LCB are extractives (11.4%), pectin (10–35%), ash (8.52%), and proteins (10%) (Alvarez-Barreto et al. n.d.; Hernández-Beltrán et al. 2019).

The ratio of lignocellulosic components (cellulose, hemicellulose, lignin) in plants varies across different species, and even in the same plant, it may vary depending on the stage of growth and age. The various forms of LCB present are herbaceous (lettuce, ferns), agricultural (rice, wheat), softwoods (balsa, spruce), and

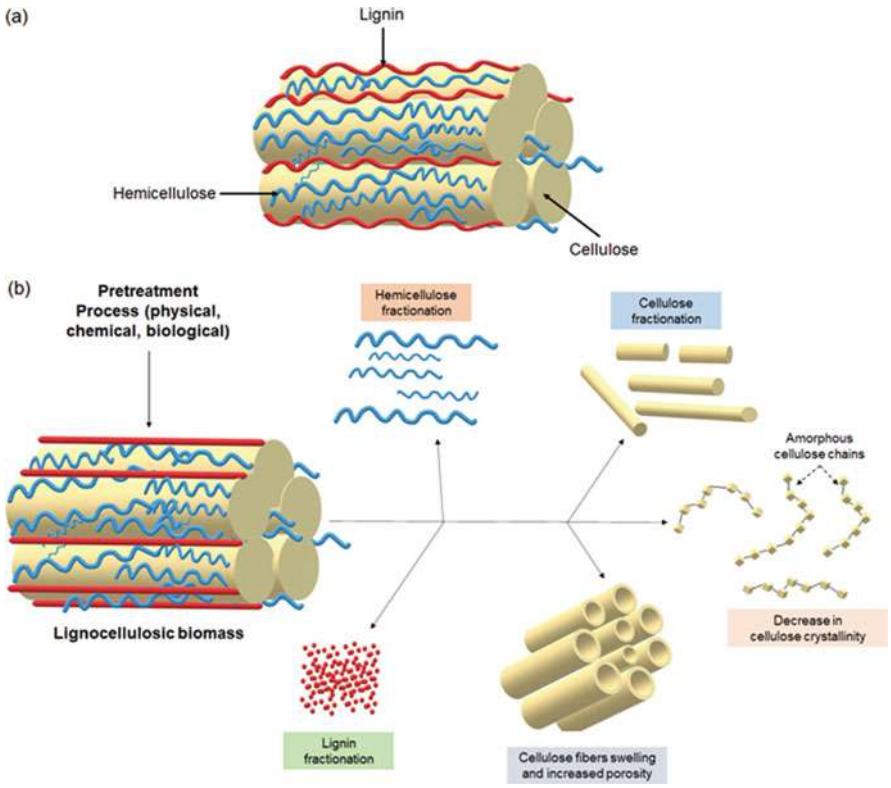

Fig. 2.1 (**a**) Schematic representation of lignocellulose complex containing cellulose, hemicellulose, and lignin (**b**) schematic representation of changes induced after pretreatment of lignocellulosic biomass

hardwoods (birch, hibiscus) (Bajpai 2016). The woody biomass of hardwoods and softwoods has a different lignocellulosic composition. Hardwoods have a higher amount of cellulose and hemicellulose with fewer amounts of lignin (18–25%) whereas softwood has a comparatively higher amount of lignin (25–35%). The lignin in softwood is mainly composed of coniferyl alcohol and is termed "guaiacyl lignin." In contrast, hardwood lignin consists of coniferyl and sinapyl alcohols which is termed as "syringyl-guaiacyl lignin." The ratio of these two lignin types of lignin mentioned varies in different lignin in a ratio of 4:1–1:2 (Demirbaş 2005; Rowell et al. 2005). A significant difference in non-woody (e.g., agricultural) and woody biomass (softwood and hardwood) is their chemical composition and physical properties. Woody biomass is structurally stronger, denser, and physically larger than the non-woody plants. Woody plants also have higher lignin contents than other groups. Generally, LCB biorefineries utilize agricultural biomass and their by-products because it is a cheap waste substrate that could be used in biorefineries for yielding high value-added products (Kumar et al. 2020; Kumar and Verma 2021; Zhu and Pan 2010). The diversity in the lignocellulosic components in biomass and

their interactions significantly affects the selection and optimization of appropriate pretreatment methods which in turn affects the cost and economical aspects of the bioproducts made out of it (Demirbaş 2005; McMillan 1994).

2.2.1 Chemical Interactions in Lignocellulosic Biomass

The components of lignocellulose are bound to each other with several linkages which helps in designing the complex structure of LCB. There are five types of bonds found in the lignocellulose structure that are (i) hydrogen (*–H*) bonds, (ii) carbon-carbon (*C–C*) bonds, (iii) ester bonds, (iv) ether bonds, and (v) acetal bonds. These linkages have a major role in forming two types of bonding interactions, i.e., bonding within a component (intramolecular bonding) and bonding between the components (intermolecular bonding). The intermolecular bonds formed during lignin biosynthesis between the lignin and the carbohydrates (cellulose and hemicellulose) and the combined structure of lignin and carbohydrates are called lignin-carbohydrate complexes (LCC). During its formation, lignin substitutes most of the water in the cell wall and results in making a tough solid matrix by forming covalent interactions with the carbohydrates present. To obtain value-added products from LCB, its components need to be separated so that the individual components of biomass become more accessible to treatments. The LCC makes biomass processing in biorefineries challenging as it inhibits the fractionation and isolation of individual components (Harmsen et al. 2010; Tarasov et al. 2018). The type of linkages that are present in LCC are (a) benzyl ether bonds, (b) glycosidic or phenyl glycosidic bonds, (c) benzyl ester bonds, and (d) acetal or hemiacetal bonds. Benzyl ether bonds α-carbon of mannosyl or glucosyl residues of the carbohydrates with the arylpropane unit in lignin whereas glycosidic bonds have a function to link side-chain hydroxyl moieties of the lignin with the carbohydrates. In the case of phenyl glycoside, the lignin which is linked by glycosidic bonds has a phenolic hydroxyl group. Benzyl ester bonds link carbohydrates (mostly xylan) with lignin by utilizing uronic acid of sugar and hydroxyl (−OH) group (at α or γ carbon) of lignin. Acetal bonds are formed by the hydroxyl group of carbohydrates and the carbonyl group of phenylpropane of lignin molecules. These linkages in LCC almost combine all the lignin present in the wood with cellulose and hemicellulose and are important for valorization aspects (Tarasov et al. 2018; Zhao et al. 2020). The intramolecular bonding between individual components of lignocellulose will be discussed later in the chapter.

2.3 Components of LCB: Cellulose, Hemicellulose, and Lignin Derivatives

2.3.1 Cellulose: Structure, Synthesis, and Properties

Cellulose holds its position as the most abundant natural polymer worldwide and has an annual global production of approximately 1.5×10^{12} tons (Klemm et al. 2005). Rapid research in the previous decades in the field of cellulose has enabled its utilization at a large scale in industries. The properties of cellulose such as biodegradability and renewability are attractive features and its properties can be further modified by physical and chemical modifications. This broadens its potential applications in industries such as textiles, coating materials, optical films, and functional materials (Heinze and Liebert 2012; Mohanty et al. 2000). Cellulose is present abundantly in plant species such as Kapok (*Ceiba pentandra*) (70–75%), Jute (*Corchorus*) (60–65%), Hemp (*Cannabis sativa ssp. sativa*) (70–75%), and Bamboo (*Bambusa vulgaris*) (40–55%). Cotton (*Gossypium*) plants are the most common cellulose source and contains a high amount of cellulose (~90 wt%) (Hon 2017). The structure and properties of cellulose influences its utilization for industrial applications. Since cellulose is the core component of the lignocellulose complex, its structural understanding and property evaluation is important to develop and select appropriate pretreatment techniques for the lignocellulose utilization in biorefineries.

2.3.1.1 Cellulose Structure

Cellulose structure is composed of D-glucopyranose moieties in a $^4C_1$ chair type configuration which is the minimum energy conformation. The individual glucose rings are joined with *β-(1,4)* glycosidic linkage in which the cellulose chain turns alternatively by 180 degrees as presented in Fig. 2.2(a) (Krässig 1993; Rao et al. 1967). The hydroxyl groups are found at the *C2, C3*, and *C6* position on the glucopyranose ring in the cellulose chain which can participate in various chemical reactions as shown in Fig. 2.2(b). The ends of the cellulose chain are different from each other. One end of the chain is non-reducing in which the anomeric carbon participates in the formation of glycosidic bonds and contains a pendant hydroxyl group whereas the other end is reducing in which the D-glucopyranose unit contains an aldehyde group (Krässig 1993). The hydrogen bonding in and between the cellulose chains and its pattern plays a significant part in the determination of cellulose chain properties such as reactivity, solubility, and crystallinity (Kondo 1997). Both intramolecular and intermolecular hydrogen bonds are present in cellulose chains that are formed by the interaction of hydroxyl groups and glucose ring oxygen atoms, and glycosidic linkages in the cellulose chain. Intramolecular hydrogen bonds are the reason for cellulose chains rigidity and high viscosity of the solutions formed. On the other hand, the intermolecular hydrogen bonds provide strong linkage among chains of cellulose. The hydrogen bonding results in the

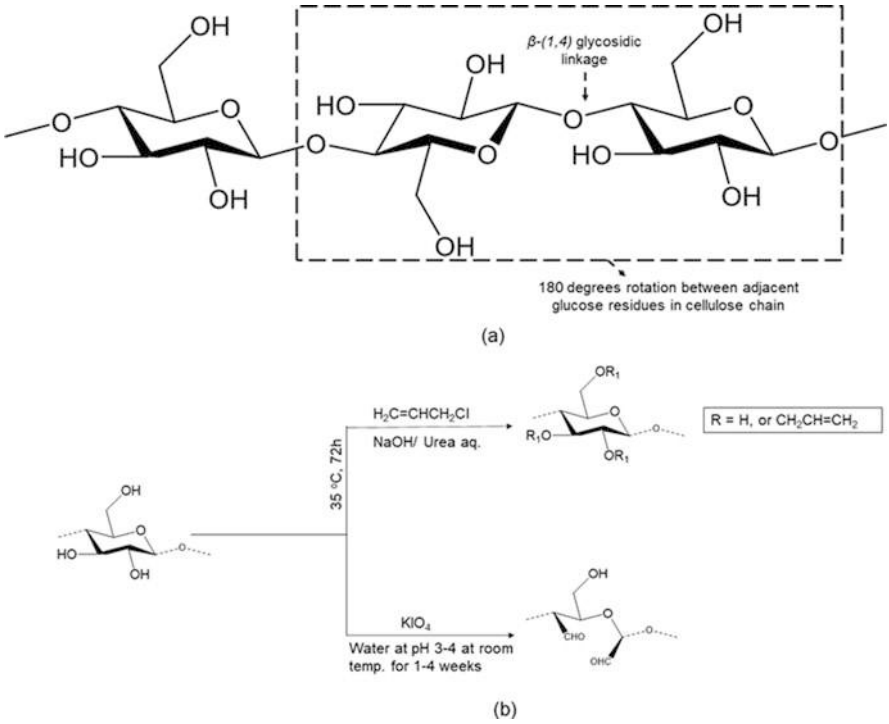

Fig. 2.2 (**a**) Structure of cellulose chain containing glucopyranose units linked with *β-(1,4)* bonds (**b**) participation of hydroxyl groups of at *C2, C3*, and *C6* position of glucose in cellulose chain into various chemical reactions

creation of three-dimensional crystal structures of cellulose chains (Gardner and Blackwell 1974; Krässig 1993). The six different crystalline cellulose types are cellulose I, II, III_I, III_{II}, IV_I, IV_{II}, also called cellulose polymorphs (Gardiner and Sarko 1985; Wada et al. 2001; Zugenmaier 2001). In natural systems, only cellulose I polymorph is present and the other polymorphic forms are derived from it by chemical treatment. The native "cellulose *I*" is further categorized into two different allomorphic forms, namely *Iα* and *Iβ* and their proportions differ from species to species in plants. In LCB, the *Iβ* form is present abundantly. Cellulose *Iα* has a triclinic *P1* unit cell, and each unit cell has a single chain. The dimensions and angles of the unit cell of cellulose *Iα* are $a = 6.72$ Å, $b = 5.96$ Å, $c = 10.40$ Å, $\alpha = 118.08^{o}$, $\beta = 114.80^{\circ}$, and $\gamma = 80.38^{\circ}$ (Poma et al. 2016). On the other hand (Moon et al. 2011), I*β* allomorph of cellulose I have a monoclinic $P2_1$ unit cell, and each unit cell has two cellulose chains. The dimensions and angles of the unit cell are $a = 7.78$ Å, $b = 8.20$ Å, $c = 10.38$ Å, $\alpha = \beta = 90^{\circ}$, and $\gamma = 95.5^{\circ}$ (Poma et al. 2016). The average degree of polymerization (DP) of cellulose chains in plants is between 1000 and 30,000 (Gardner and Blackwell 1974; Krässig 1993; Poma et al. 2016).

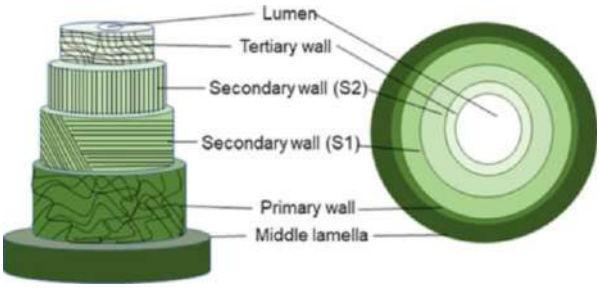

Fig. 2.3 Schematic representation of different layers presents in plant cell wall and arrangement of cellulose fibers in these layers

In crystalline forms of cellulose, the pattern of chain alignment is close and ordered which is linked side by side with hydrogen bonds. Amorphous cellulose is shortly ordered and linked by isotropic intermolecular hydrogen bonding. The amorphous regions of cellulose chains; however, help in forming turns between adjacent chains. The amorphous regions also display random orientations and low density (de Souza Lima and Borsali 2004). The cellulose chains in the cell wall are present as fibrils that have their diameter in the nanometer (nm) range. These fibers arrange themselves in bundles to form microfibrils and microfibrils during which the magnitude of dimensions increase from nm to micrometers (μm) (Krässig 1993). During growth, plant cells first form the primary cell wall which is thinner than secondary and tertiary cell walls. The primary and tertiary walls have a disordered arrangement of cellulose nanofibrils whereas cellulose microfibrils are arranged parallelly in secondary wall sublayers as presented in Fig. 2.3 (Rytioja et al. 2014). The morphologies and dimensions of cellulose in cell walls differ from species to species. For example, spruce cellulose fibers are untwisted and cotton fibers are twisted whereas straight and round fibers are found in bast plants. However, these microfibrils have the same internal structure across the various plant species (Ioelovich 2008).

2.3.1.2 Chemical Properties of Cellulose

Pure cellulose is insoluble in the water because of its crystalline structure, hydrogen bonding, and high molecular weight. Due to this, special procedures and mediums are used to dissolve cellulose. The solvents that are used for the dissolution of cellulose are segregated into two categories, namely (i) derivatizing and (ii) non-derivatizing solutions. Derivatizing solutions (e.g., CF_3COOH, HCOOH) form covalent linkages of low stability with the polymer whereas non-derivatizing solvents (e.g., dimethylsulfoxide, ammonia) only interact physically with the polymers. An important process used for cellulose dissolution at the industrial level is with the mixture of copper hydroxide and aqueous ammonia (Schweizer's reagent), followed by precipitation in dilute sulfuric acid which is the part of fiber production in the viscose process. An alternate strategy for dissolution of cellulose is by dissolving it physically with *N*-methylmorpholine-*N*-oxide monohydrate

(derivatizing) which is used in the Lyocell process (Heinze and Liebert 2001). Most of the solvents that are used for cellulose dissolution are developed and processed at the laboratory scale. Some of the semi-industrial scale solvents utilize the applications of ionic liquids such as salts of 1-alkyl-3-methylimidazolium [(1-Ethyl-3-methylimidazolium acetate) (EMIMAc)] (Ebner et al. 2008; Liebert 2008; Swatloski et al. 2002). Ionic liquid for cellulose dissolution came into existence in the year 2002 and are proved to be a good solvent for cellulose because the anions in the ionic liquids are always accompanied by equal numbers of cations to maintain the electroneutrality. Different ionic liquid display difference in solubility and spinning characteristics for cellulose. For example, EMIMAc dissolved cellulose display a poor spinnability whereas *N*-methylmorpholine *N*-oxide (NMMO) cellulose solutions shows high draw ratios during spinning (Hauru et al. 2016).

Even before the nature of cellulose was understood completely, glucan cellulose was utilized as chemical modification precursor for cellulose. The hydroxyl groups of the cellulose participate in the chemical reactions and several industrially important compounds are obtained from cellulose modifications. For example, cellulose in the partially nitrated form is sold commercially with the trade name of "Celluloid" which acts like plastic. Another important chemically modified cellulose product is cellulose acetate which is highly utilized in industries and research. The chemical modifications of cellulose include acylation, sulfation, amino modification, carbonylation, silylation, and oxidation (Gerhartz 1988; Habibi 2014). However, the mechanisms of these chemical modifications are beyond the scope of this chapter and are mentioned to give an idea about varieties of chemical modifications of cellulose available.

2.3.1.3 Mechanism of Cellulose Synthesis in Plants

The synthesis of cellulose in plants is performed by cellulose synthase (CSC) enzymes. It is present as a rosette-like complex in the cell membrane of plants. The catalytic part of the CSCs is called CESA protein (cellulose synthase A) (Chen et al. 2018). CESA subunits arrange themselves to form a rosette-type structure which is composed of 36 CESA subunits. These 36 CESA are organized into six subunits, and each subunit contains six CESA units. CESA is present as a complex in the cell membrane that can synthesize cellulose (Endler and Persson 2011; Turner and Kumar 2018). The *CESA* genes are responsible for the synthesis of cellulose in plants. Some *CESA* genes are necessary for primary cell wall formation (*CESA1, CESA3, CESA6*), whereas some are needed for the construction of secondary cell wall (*CESA4, CESA7, CESA8*). During cellulose synthesis in plants, glucose subunits are polymerized by CESA to form cellulose. The glucose subunits are utilized as uridine diphosphate glucose (UDP-glucose). The enzyme UDP-glucose phosphorylase forms UDP-glucose by utilizing uridine triphosphate (UTP) and glucose-1-phosphate (Guerriero et al. 2010). Sucrose synthase (Susy) enzyme has also been assumed to connect cellulose synthesis by converting sucrose and UTP into UDP-glucose. During the initialization of cellulose synthesis, the enzyme has two

UDP-glucose units in the substrate-binding site, and after that, polymerization begins. During polymerization, glucose subunits are introduced to the non-reducing end of the chain, and the chain is released in the extracellular part simultaneously. This exported cellulose chain then assembles and crystallizes to form cellulose microfibrils (Brown Jr and Saxena 2000). The system for plant cellulose synthesis is based on the mechanism of *Arabidopsis thaliana,* which is used as a model for plant species (McFarlane et al. 2014).

2.3.2 Hemicellulose: Structure, Properties, and Synthesis

Hemicellulose is the most abundant polysaccharide after cellulose in the plant's system. They are also the most abundant heteropolysaccharides found in nature. The hemicellulose family is diverse in context to their origin and relative proportion to cellulose in the plant cell wall. On a molecular level, hemicellulose displays a high variation in the degree of branching and molecular weight. However, their DP is comparatively less than cellulose (Timell 1967). In-plant species, the composition of hemicellulose differs in terms of the types of sugar and their respective amounts. Even in the same plant, several forms and amounts of hemicellulose may exist in different macroscopic structures (root, core, branches). The difference in hemicellulose composition and the amount is also observed in different cell wall layers and within the wood structure (heartwood, latewood, and earlywood). Hemicellulose composition in the hardwoods is quite similar in species such as birch and aspen and is composed mainly of xylan (high amount) and mannan (low amount). In the case of softwood, the major monomer is glucomannan, and arabinogalactans and xylans are present in the smaller amounts. In recent decades, there has been a huge development towards complete characterization of the hemicellulose, providing a comprehensive understanding of hemicellulose structure which was a great challenge in the last century (Albertsson et al. 2011).

2.3.2.1 Structure of Hemicellulose

The hemicellulose is a complex heteropolysaccharide composed of a single or combination of pentoses and hexoses. The pentoses involve monosaccharides such as xylose and arabinose whereas hexoses include D-galactose, D-glucose, D-mannose, D-glucuronic acid, etc. (Aspinall 1959; Aspinall 1962). Fucose and rhamnose sugars are also found in some hemicelluloses. The composition of sugars varies in plant species based on the tissue of origin and geographical location. The representative structure of hemicellulose is considered as *β-(1,4)* linked xylopyranose subunits that are present in various plant parts including stem, root, and leaves. The complexity of the hemicellulose structure is enhanced further by branching in the form of side groups at the *C2* and *C3* positions of the sugar. Examples of these side groups are

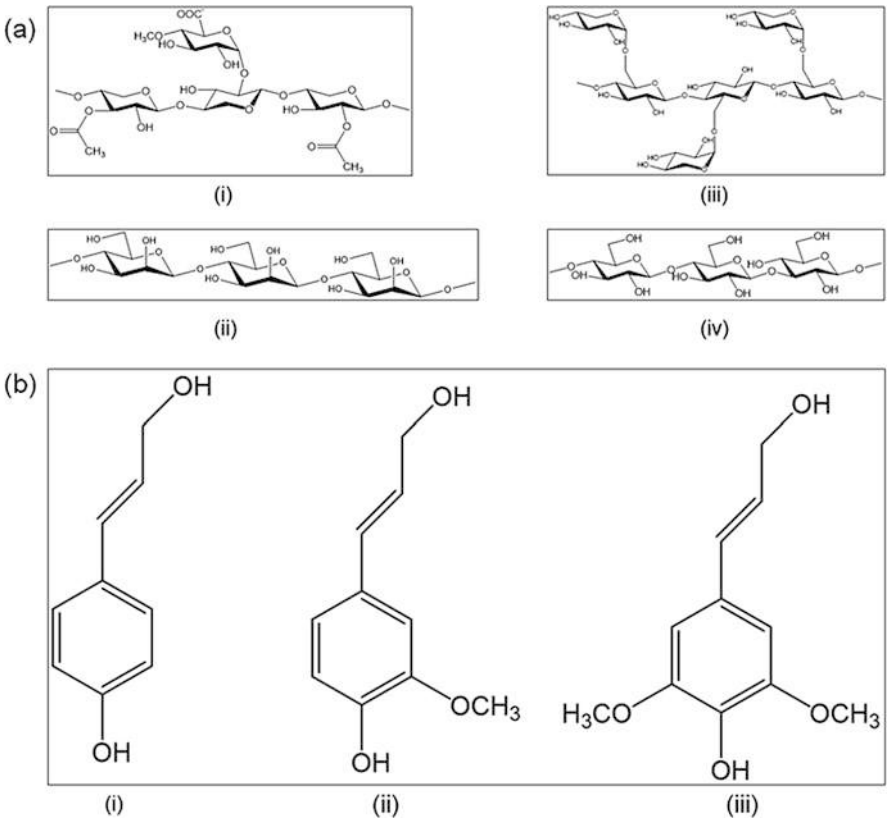

Fig. 2.4 (**a**) Structure of different types of hemicellulose present in plant cell wall (i) xylan, (ii) mannan, (iii) xyloglucan, (iv) *β*-glucans (**b**) structure of lignin monomers (i) *p*-coumaryl alcohol, (ii) coniferyl alcohol, (iii) sinapyl alcohol

glucuronic acid, rhamnose, arabinofuranose, and phenolic acids. The phenolics like coumaric acids have antimicrobial, antioxidant, and anti-inflammatory properties (Adams and Castagne 1951; Aspinall 1962; Ghali et al. 1974).

Due to the different kinds of sugars involved in the hemicellulose structure, they are categorized into four types that are (i) xylans (ii) mannoglycans or mannans, (iii) xyloglucans, (iv) *β*-glucans (Scheller and Ulvskov 2010) as shown in Fig. 2.4(a). The first group, xylans are the most common and abundant form of hemicellulose. It is composed of xylopyranosyl subunits linked with *β-(1,4)* bonds. It is substituted by branches containing arabinosyl, glucuronosyl, and acetyl residues based on the extraction method and biomass source (Hauru et al. 2016). The xylan found in straw has L-arabinofuranose in its side chains. Some plants also contain the acidic xylans in which the acidic group is provided by the *4-O*-methyl-D-glucoronic acid. The complex forms of xylans contain xylopyranose, galactopyranose, furanose, and uronic acids as their side groups. The xylans that contain simple chains of

xylopyranosyl subunits linked with *β-(1,3)* and *β-(1,4)* or both the bonding are called homoxylans whereas the xylans that contain a large variety of sugars are called heteroxylans (Ebringerová and Heinze 2000; Scheller and Ulvskov 2010; Teleman et al. 2000).

Mannans are the second group of hemicellulose and are abundantly present in the softwood hemicellulose. The mannans are of four types: (a) mannan, (b) galactomannans, (c) glucomannan, (d) (galacto)glucomannans. Mannans are composed of mannose sugar linked with *β-(1,4)* bonding whereas the galactomannans are composed of D-mannopyranosyl subunits with *β-(1,4)* bonding. At *C6* position, the D-galactopyranosyl with short branching are linked to mannan units in galactomannans. The glucomannans contain a D-mannopyranosyl subunit liked with *β-(1,4)* linkages. Some glucomannans have D-galactopyranosyl components as their side groups and therefore and are called (galacto)glucomannans. In softwoods, the mannans are usually acetylated at *C2* and *C3* positions. Mannans are utilized commercially in food industries as thickeners and emulsifiers (González 1978; Scheller and Ulvskov 2010).

The third group, xyloglucans are formed of D-glucopyranose subunits linked with *β-(1,4)* linkages and are present within primary cell walls of angiosperms, grasses, and fruit plants (Fry 1989; Yapo and Koffi 2008). The xylopyranosyl (X) subunits combine with glucopyranosyl (Glu) at *C6* position with *α*-linkages, based on which xyloglucans are further categorized into two groups: (a) XXXGlu group and (b) XXGluGlu group. XXXGlu groups have four glucopyranosyl repetitive subunits, of which three contain xylopyranosyl units. In the case of XXGluGlu, two of the four repetitive glucopyranosyl subunits are xylosylated followed by two non-substituted units (Cui and Wang 2009).

The last group of hemicellulose is *β*-glucans which are linear hemicelluloses containing *β-D*-glycopyranosyl subunits similar to the structure of cellulose. The main difference between cellulose and *β* -glucans is that the cellulose only contains *β-(1,4)* linkages whereas *β*-glucans contains both *β-(1,3)* and *β-(1,4)* bonds. Two types of building blocks are found in the *β*-glucans: (a) cellotriosyl and (b) cellotetraosyl. The cellotrisyl units are composed of two continuous *β-(1,4)* bonds and a *β-(1,3)* linkage is present in between them. On the other hand, the cellotetraosyl has three repetitive *β-(1,4)* bonds followed by a *β-(1,3)* bond. The relative abundance of cellotriosyl and cellotetraosyl varies from one plant to another and depends on the growing environment. For example, barley is found to be rich in *β*-glucans whereas wheat hemicellulose is majorly composed of arabinoxylan (Cui and Wang 2009; Izydorczyk et al. 1998). The structural aspects of hemicellulose are crucial to understanding as it has a significant effect on the properties of this polysaccharide which eventually affects the properties of LCB.

2.3.2.2 Properties of Hemicellulose

The physical, chemical, and functional properties of hemicellulose depend on the DP, molecular mass, macroscopic structure, and branching (Belgacem and Gandini

2011). The hemicellulose is different from cellulose as it contains both pentose and hexose sugars and displays a lower DP. Hemicellulose is non-crystalline and is more accessible to utilization compared to cellulose (Dumitriu 2004). However, like cellulose, hemicellulose is also insoluble in water and there are several methods used for their solubilization and recovery. Arabinoxylan or *β-(1,4)* xylan (unsubstituted) forms insoluble complexes by aggregation which is stabilized with intermolecular hydrogen bonding. It forms a left-handed helix having three folds which appear as an extended twisted ribbon-like structure in solid-state. The formation of aggregated complexes could be the most probable reason for their low solubility in water (Belgacem and Gandini 2011). Xylans from hardwoods can also be solubilized in alkaline solutions which is a high yield method. The products obtained out of this solubilization are similar to native hemicellulose, except all the acetyl side chains, *p*-coumaryl, and feruloyl moieties are saponified (Puls 1997). Similar to cellulose, hemicellulose could also be extracted using ionic liquids which is an upcoming strategy for dissolving individual components of the lignocellulosic components. Ionic liquids such as 1-butyl-3-methylimidazolium chloride (BmimCl), and 1-butyl-3-methylimidazolium bromide (BmimBr) can be used to dissolve hemicellulose (Yuan et al. 2019).

In hardwood, xylans are present abundantly whereas in softwood mannans are most common. Both these groups contain hydroxyl groups with them which allows them to participate in several chemical reactions with multiple chemical agents. These reactions are performed for the enhancement of the properties such as hydrophobicity, thermal stability, and water resistance. The chemical reactions and modifications such as acetylation, esterification, etherification, fluorination, benzylation, and cross-linking could be done with hemicellulose which widens its scope of application as a polymer. For example, hemicellulose has the ability to react with acids, chlorides, and acid anhydrides. The substitution of the hydroxyl group in this reaction will affect the properties of hemicellulose such as thermoplastic behavior and waterproofing. The degree of substitution with reaction with acyl chlorides is affected by reaction conditions such as reaction temperature, time, and the molar ratio of the substrate. Another example of hemicellulose participating in reaction would be esterification which is generally performed with a fatty acid chloride. This modification has shown an increase in the water-resistance properties due to the presence of long-chain fatty acids on the surface and can be used for coating applications (Hu et al. 2020).

2.3.2.3 Hemicellulose Synthesis in Plants

From the previous sections, we know that hemicelluloses are composed of a variety of sugars. The biosynthesis of these individual sugar constituents varies depending on the type of sugars which is being synthesized. In the hemicellulose, the mannans, xylans, xyloglucans, and mixed-linkage glucan biosynthesis are generally considered. Similar to all the polysaccharides that are present in the plant cell wall, hemicelluloses are also synthesized by the activated nucleotide sugars such as

guanosine diphosphate (GDP)-glucose, uridine diphosphate (UDP)-galactose, and GDP-mannose. The enzyme cellulose synthase-like family (CSL) are glycosyltransferases that majorly participate in hemicellulose synthesis. The proteins from this family such as CSLA, CSLC, and CSLH are found in the Golgi of the plant cell and have a huge role in the synthesis of mannan, xyloglucan, and mixed-linkage glucans, respectively. They synthesize the hemicellulose components in Golgi and export them to the cytoplasmic side for deposition into the cell wall. The enzyme β-mannan synthase (ManS) is an example of a CSL enzyme that is responsible for the heteromannan synthesis and uses GDP-mannose as a substrate (Pauly et al. 2013). Although CSL proteins have a huge role in hemicellulose synthesis in plants. For backbone synthesis in xylan, these proteins do not participate. The proteins belonging to glycosyltransferases (GT) GT43 and GT47 (present in Golgi) are responsible for xylan backbone synthesis. These proteins are type II membrane proteins with a single membrane anchor at *N*-terminal which is different from CSL family multiple transmembrane segments. The processes involved in hemicellulose biosynthesis are backbone formation, modifications by hydrolases, acetylation, reducing end modification, and formation of side chains of the participating constituents (Scheller and Ulvskov 2010).

2.3.3 Lignin: Structure, Properties, and Synthesis

Lignin comes after cellulose and hemicellulose polysaccharides in terms of polymer abundance in nature. Lignin is a complex aromatic phenylpropanoid polymer that is associated with the cellulose and hemicellulose in the plant cell wall (Boerjan et al. 2003). It helps in cementing these polysaccharides in the cell wall chemically and physically. Lignin has a structural and protective role in the plants which makes them rigid and inaccessible to biotic and abiotic stress conditions (Kirk 2018). It also aids in plant growth and development by enhancing water conduction through xylem vessels. In the industrial valorization of the LCB, lignin has been always considered a non-desired product such as Klason lignin. Klason lignin is an insoluble residue that is obtained after the acid hydrolysis of plant biomass. However, the lignin wasted in the biorefineries can be utilized which will strengthen the economic value of lignocellulosic biomass-based industries. Lignin has been utilized as emulsifying agents, binding agents, reinforcing materials, and composites. The recent research in the direction of lignin extraction, depolymerization, and subsequent valorization has shown excellent results which suggests that it contains a huge potential from an industrial and commercial perspective and is yet to be unleashed (Ponnusamy et al. 2019; Whetten and Sederoff 1995).

2.3.3.1 Structure of Lignin

The phenylpropane units that are involved in the lignin formation are called monolignols. The three types of monolignols that constitute lignin are (i) *p*-hydroxyphenyl (*H*) (ii) guaiacyl (*G*), and (iii) syringyl (*S*) structures. These monolignols combine through radical-based polymerization to produce a three-dimensional lignin structure. The building blocks of lignin are *p*-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol as presented in Fig. 2.4(b). Lignin is a heterogeneous material and does not have a regular structure like cellulose and the exact chemical structure of lignin is not completely defined (Hatakeyama and Hatakeyama 2009). The monolignols in lignin are bonded with carbon-carbon *(C-C)* and ether linkages. Trifunctionally linked adjacent units present branching sites that generate the network structure of lignin. The types of linkages that are present between the monolignols in the lignin are aryl glycerol-*β*–aryl ether *(β–O–4)*, non-ring benzyl-*O*-aryl ether *(α–O–4)*, coumaran *(β–5)*, 1,2-diaryl propane *(β–1)*, diaryl ether *(4–O–5)*, and *β–β* (Lebo Jr et al. 2002). In gymnosperms, the lignin majorly consists of the *G* subunits with a small number of *H* units whereas in angiosperms dicot lignin majorly consists of *G* and *S* units. Softwood contains a high amount of *H* units which are abundant in grasses. The variation in lignin composition not only varies with taxa, species, growth conditions, and environment but also differs from cell to cell. It can be dissimilar even at the level of cell wall layers which was revealed by infrared, Raman, and ultraviolet spectroscopy. The concentration of lignin in the plant cell is uniform in the secondary cell wall; however, a high rise in the concentration of lignin is seen at the primary cell wall area and middle lamella periphery. In typical wood cells, the pattern of lignin distribution is like this with a high amount in the interfiber area and low concentration in the bulk of the cell wall. In the primary cell wall and middle lamella, the lignin is present as spherical entities, however, in the secondary cell wall, lamellae formation occurs that is based on microfibril orientation. In the lignocellulose, lignin is the most complex and least characterized component. Comprehensive studies on the structure and distribution of lignin in plant cell walls can potentially unravel the new findings which will further improve the understanding of this complex polymer (Boerjan et al. 2003; Vanholme et al. 2010).

2.3.3.2 Properties of Lignin

The general properties of native lignin are its high thermal stability, high carbon content, biodegradability, stiffness, and antioxidant activity. Lignin in the wood is present in an amorphous form in the cell wall and does not present an absorption peak in the visible light spectrum (Huang et al. 2019; Melro et al. 2018). The color in lignin derived from heartwood is a result of impurities such as flavonoids and tannins. Kraft and sulfite pulps are also brownish because of the presence of chromophoric moieties. Lignin shows UV absorption range which is the reason for

the apparent color of lignin (Huang et al. 2019). Using techniques such as light scattering and vapor pressure osmometry, the milled softwood lignin molecular weight is calculated as 20 kDa. However, more variation to this molecular weight can happen depending on the method of estimation and wood source. For the solubility properties of lignin, like cellulose and hemicellulose, lignin is also an insoluble polymer. The lignin is soluble in solvents such as acetone, pyridine, and dioxane (Lebo Jr et al. 2002). The organic solvents are traditionally used for lignin extraction (e.g., methanol, acetic acid); however, it displays low solubility. An alternative to the organic-based solvents could be the use of ionic liquids that have demonstrated intriguing properties such as non-toxicity and high thermal stability. These solvents are also called "green solvents." Some solvents that have been utilized for lignin dissolution are s 1,3-dimethylimidazolium methylsulfate, [Mmim][$MeSO_4$] and 1-butyl-3-methylimidazolium methylsulfate, [Bmim][$MeSO_4$]. Another category of "green solvents" includes deep eutectic solvents (DES) which are formed by mixing a hydrogen bond donor and a hydrogen bond acceptor. Some of the DES that can be used for lignin dissolution is choline chloride and formic acid (2:1) and propionic acid and urea (1:2) (Melro et al. 2018). Lignin acts as a thermoplastic polymer and has a range of glass transition temperatures (T_g) which depends on the isolation method, heat treatment, and sorbed water. For example, the T_g of dioxane lignin was found to be 440 K. The process of delignification is enhanced by the thermal softening of lignin in chemical pulping. It also improves the strength of bonds of fibers in paper-making process (Atack and Mi 1978; Hatakeyama 1972).

Lignin can participate in a variety of chemical reactions such as reduction, oxidation, and hydrolysis. It is also susceptible to enzymes. The hydroxyl, carbonyl, and benzylic hydroxyl groups in the phenolic structure of lignin enable it to participate in chemical reactions. The abundance of these functional groups varies depending on the wood species, morphological location, and isolation methods (Andersson and Samuelson 1985; Schuerch 1975). These chemical reactions result in reactive intermediates formation such as phenoxy anion and phenoxy radical. These radicals are significant in processes such as lignin isolation, enzymatic degradation, and light-induced discoloration. Lignin also demonstrates electrophilic substitution which aids in its modification process. The reactions that generally participate in lignin modification are nitration, ozonation, chlorination, etc. A clear characterization of the lignin properties is an important step towards lignocellulose valorization in biorefineries. The process involved in the treatment and extraction of individual components in lignocellulose can be strategized efficiently for the complete characterization of lignin properties (Melro et al. 2018).

2.3.3.3 Lignin Biosynthesis in Plants

Lignin biosynthesis in the plant systems begins with the formation of monolignols that are synthesized by the general phenylpropanoid pathway. In this, phenylalanine acts as a common precursor of monolignols throughout the plant systems. However,

tyrosine also acts as a precursor in grasses. The phenylalanine first deaminates to form cinnamic acid. This reaction is performed by the enzyme ammonia-lyase which is one of the most studied enzymes in plant systems. The cinnamic acid obtained from this is then hydroxylated to *p*-coumaric acid by enzyme cinnamate 4-hydroxylase which is a monooxygenase. After the synthesis of *p*-coumaric acid, the lignin biosynthesis divides into two branches (Whetten and Sederoff 1995). The first branch sequentially forms caffeic acid, ferulic acid, and 5-hydroxyferulic acid that participates in the formation of *G* and *S* monolignols along with the intermediates from the other branch. The synthesis of caffeic acid by *p*-coumaric acid occurs through hydroxylation with the enzyme coumarate 3-hydroxylase. The second branch progresses through the conversion of *p*-coumaric acid to *p*-coumaroyl-CoA by enzyme coumaroyl-coenzyme *A 3*-hydroxylase. After the formation of *p*-coumaroyl-CoA, this pathway branches further in which one leads to the formation of caffeoyl CoA and feruloyl CoA whereas the other leads to the formation of *p*-coumaroyl alcohol. The *p*-coumaroyl alcohol participates in the formation of *H* monolignols whereas caffeoyl CoA and feruloyl CoA help in the formation of *G* and *S* monolignols (Mamedes-Rodrigues et al. 2018). Figure 2.5 shows a schematic representation of *p*-hydroxyphenyl (*H*), guaiacyl (*G*), and syringyl (*S*) subunits biosynthesis pathway in plant cell along with the enzymes involved in each step.

After the formation of monolignols, these are exported to the cell wall, where their oxidation and polymerization occurs. The accumulation of monolignol *4-O-β-D*-glucosides in the cambial tissues in both gymnosperms and angiosperms is the basis of the hypothesis that these are the transport or storage forms of monolignols (Steeves et al. 2001). The completion of monolignols transfer to the cell wall is followed by the process of dehydrogenation and polymerization. The process of dehydrogenation helps in the formation of monolignol radicals and is performed individually and in combination by the enzymes (Kumar and Verma 2020a, b; Bhardwaj et al. 2021) such as laccases (Agrawal et al. 2019; Agrawal and Verma 2020), peroxidases, coniferyl alcohol oxidase, and polyphenol oxidases (Christensen et al. 2000; Onnerud et al. 2002). The formation of monolignol radicals then proceeds into the polymerization process by the radical coupling process. The radicals because of their electron delocalization are relatively stable and present in a coupled form. The extension of three-dimensional lignin polymer occurs by the cross-coupling reaction. However, these reactions quench the radicals; therefore, two coupling molecules require new radicals for every extension. These radicals in the synthesizing lignin polymer are produced with the transfer of radicals from monolignols or their intermediates. For example, in the sinapyl alcohol coupling reactions, *p*-coumarates function as intermediates. Due to their rapid oxidation by peroxidase, they can supply the radicals to sinapyl alcohol which is comparatively stable radical (Hatfield et al. 1997; Takahama and Oniki 1994). Oxidation of *p*-coumarates, therefore, occurs only when all sinapyl alcohols are polymerized. The radical coupling process completes the process of lignification in the cell walls of plants. The lignin is an intriguing component of LCB because of its aromatic nature and complexity. It also has a very significant role in providing LCB a resistant nature

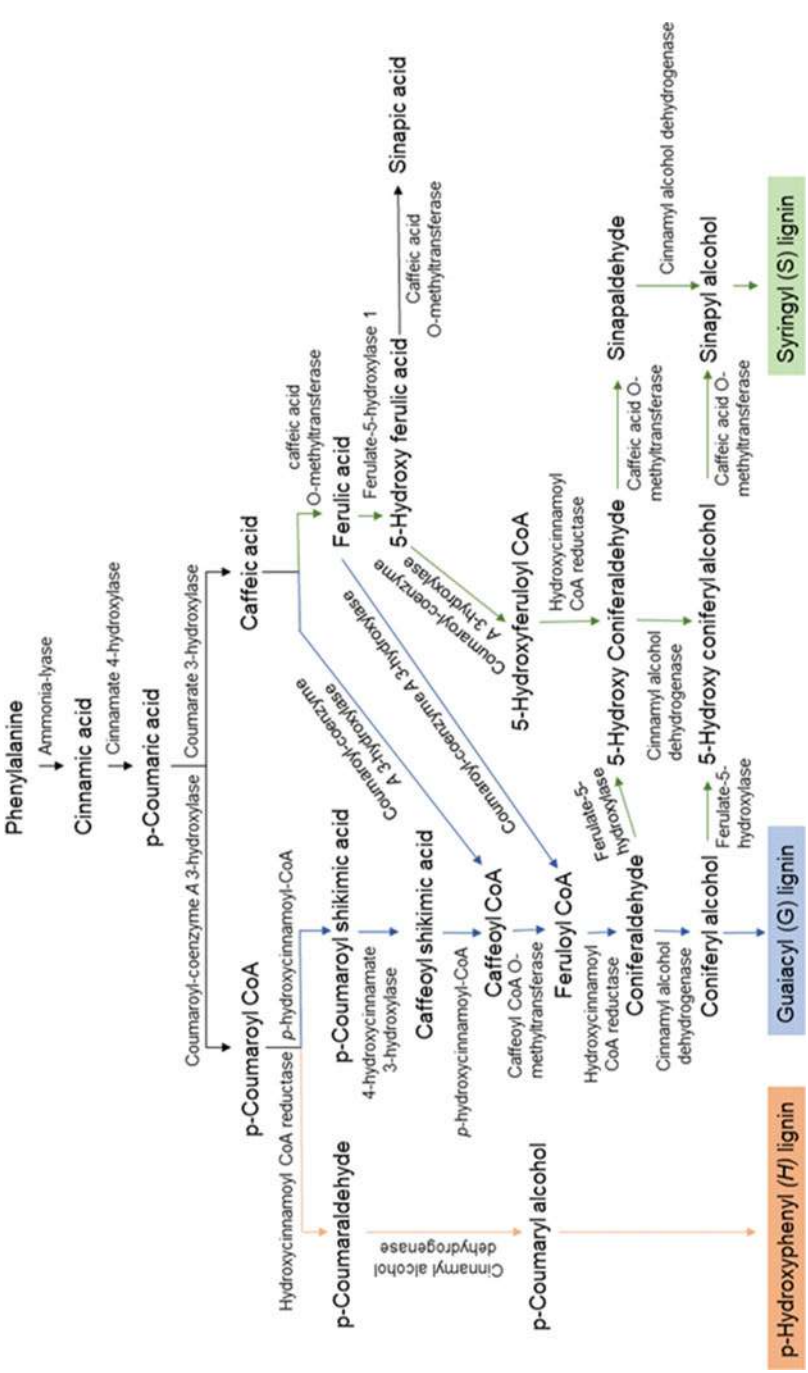

Fig. 2.5 Biosynthetic pathway of *p*-hydroxyphenyl (*H*), guaiacyl (*G*), and syringyl (*S*) subunits of lignin in plants cell

towards the environmental agents (Boerjan et al. 2003). In the further sections, this resistance or recalcitrance of LCB will be discussed in detailed and the contribution of individual components will also be highlighted.

2.4 Recalcitrance of Lignocellulose Biomass (LCB)

Since plants are present in an environment where they interact with a large number of biotic and abiotic agents, they have been evolved to form a complex structure. This structure is difficult to deconstruct by the pathogens and protects the plants. This resistance of plant biomass is called biomass recalcitrance (Himmel et al. 2007; Ritter 2008). Due to this, the processing of LCB in refineries is difficult as it restricts the depolymerization of its components to their respective monomers. The recalcitrance of LCB possesses difficulty in bioproducts production. The components of the plant cell wall that were discussed previously have a huge contribution to the recalcitrance of LCB. LCB is composed of lignin, glycosylated proteins, and cross-linked networks of polysaccharides (Ritter 2008). The main factors that contribute to the lignocellulose recalcitrance are (i) physical properties of the plant cell (ii) chemical composition (iii) and their mixed interactions as displayed in Fig. 2.6 (Zhao et al. 2012a).

2.4.1 Effect of Physical Properties of LCB on Recalcitrance

2.4.1.1 Cellulose Crystallinity

The cellulose in LCB is present as crystalline and amorphous forms. The crystalline portion of cellulose exists in the form of microfibrils and dozens of these microfibrils combine to produce a paracrystalline assembly. This structure has a high number of hydrogen bonds and is more resistant to degradation by enzymes when compared to amorphous portions of cellulose (Laureano-Perez et al. 2005). The crystallinity of cellulose reduces with the enzymatic digestion of the cellulose. Since cellulose

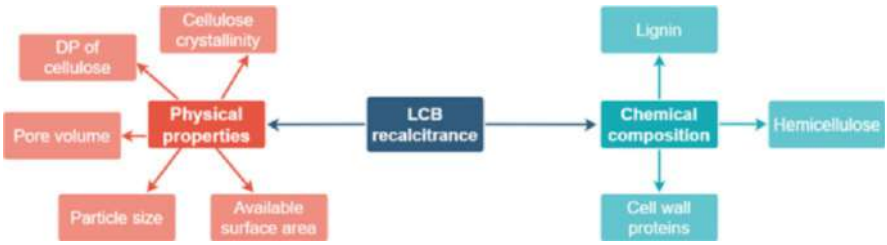

Fig. 2.6 Schematic representation of physical properties and chemical composition-based factors contributing to lignocellulose recalcitrance

crystallinity differs from species to species; therefore, the recalcitrance present by LCB from various biomass sources could be a result of it (Zhao et al. 2012a). However, the conversion of native cellulose to its polymorphic forms can reduce the extent of hydrogen bonding, making it more suitable for digestion (Chundawat et al. 2011). The amorphous portion of cellulose is more suitable for hydrolysis as it is more disordered and has lesser hydrogen bonds than crystalline cellulose (Zhang and Lynd 2004). The quantitative parameter to determine the cellulose crystallinity is called Crystallinity Index (C.I %) which is obtained using X-ray diffraction (Zhao et al. 2008). In LCB, hemicellulose and lignin are present that can interfere with the crystallinity index results of the cellulose and can result in an inaccurate result. To resolve this, biomass crystallinity is measured using methods such as infrared (IR) spectroscopy, Raman spectroscopy, and solid-state 13C NMR (Park et al. 2010). Although crystallinity is considered an important factor towards LCB recalcitrance, some studies also show that cellulose hydrolysis does not depend on the crystallinity of the cellulose in LCB. A possible reason for this could be that the use of pure cellulose for determining the effect of crystallinity on cellulose digestion does not give an idea about the heterogenous biomass (Chandra et al. 2007). The other structural properties that affect the LCB recalcitrance are DP of cellulose, pore volume, particle size, and surface area (Zhao et al. 2012a).

2.4.1.2 Degree of Polymerization (DP)

The number of monomers per unit of a polymer chain is called the degree of polymerization (DP). DP of cellulose defines the number of glucose molecules per unit of the cellulose chain. The process of enzymatic hydrolysis is depolymerization of the cellulose chains by an individual or synergistic action of enzymes such as endo-*1-4-β*-glucanase and *β*-glucosidase. It has been observed that hydrolysis of cellulose with shorter chains is much easier than longer chains (Duff and Murray 1996; Sánchez 2009). Similar to crystallinity, DP is also not an independent factor and affects the overall properties of the LCB. For example, the beating of LCB reduces the fiber DP but increases the swelling behavior. The studies on the effect of DP on biomass recalcitrance have proved that it is contributing factor and its variation can influence the LCB digestion process (Nazhad et al. 1995).

2.4.1.3 Effect of Pore Volume, Particle Size, and Surface Area

The enzyme absorption on the biomass substrate is necessary for its degradation by enzymatic systems. This makes the surface area of biomass which is accessible to the digestion system, an important factor in LCB recalcitrance. The overall accessible surface area (ASA) depends on the physical properties of LCB such as porosity, particle size, and pore volume. For example, an increase in the pore volume or decrease in the particle size can lead to an increase in ASA (Zhao et al. 2012a). Several studies have proven the effect of ASA on LCB recalcitrance; however, there

is a limit to reduction in particle size after which the digestion does not improve. This particle size limit was found to be 350–590 μm in LCB (Chang et al. 1997; Moniruzzaman et al. 1997; Wen et al. 2004). The other factors that can influence the ASA and eventually LCB recalcitrance are pore volume and porosity. The total surface area (TSA) of LCB is a function of two components: (i) the external surface area of LCB which depends on the length and width of the section and (ii) internal surface area which is majorly affected by the number of substrate pores, cracks, and the size of the lumen. For cellulose, the internal surface area is higher by one or two orders than the external surface area (Chandra et al. 2007; Chang et al. 1981). It has been proven by several studies (Huang et al. 2010) that the internal surface area is the most important factor towards LCB recalcitrance. For cellulose as a substrate, the enzymatic systems access it mainly through the pores, and about 90% of enzymatic digestion is controlled by the ASA of the pores (Wang et al. 2012). The increase in TSA usually increases the ASA of biomass. However, the increase in the pore areas will increase the TSA but the pore has to be of a size that can entrap the enzyme systems performing hydrolysis (Tanaka et al. 1988; Zhang and Lynd 2004). The untreated biomass samples usually have the micropores and macroporous type of pore structures and no pores less than 100 nm diameter is present in them. However, after pretreatment nanopores between 50 and 100 nm are observed in the biomass (Meng et al. 2015). From multiple studies, the rate-limiting pore size limit for LCB was estimated to be 5.1 nm below which the enzyme systems cannot access the pore's internal surface area (Zhao et al. 2012a). The reason for this is the size of cellulase compositions obtained from an organism such as *Trichoderma reesei* lies in this size range (~5.1 nm). The methods to measure TSA and ASA are based on the quantity of cellulase absorbed on a substrate. BET (Brunauer-Emmett-Teller) is a classical method to measure the TSA by utilizing nitrogen absorption. The shortcomings of BET are changes on biomass surface characteristics due to drying and use of nitrogen for estimation that ring in difference than enzymes used for hydrolysis (Mansfield et al. 1999; Zhang and Lynd 2004). The other methods that can be used to calculate interior surface area of the biomass are mercury porosimetry, X-ray scattering, DSC (differential scanning calorimetry), and NMR (nuclear magnetic resonance) (Beecher et al. 2009).

2.4.2 Contribution of Chemical Components of LCB on Recalcitrance

2.4.2.1 Lignin

Lignin has been widely accepted as the most important factor that is responsible for the recalcitrance of LCB. Conventionally, lignin present in the LCB is removed by applying a variety of methods which has been observed to enhance biomass hydrolysis. From the structural aspect, lignin is present as a physical barrier that restricts the access of enzymes to the cellulose and hemicellulose present in the biomass

(Chang and Holtzapple 2000; Zhao et al. 2012a). The LCC formed by lignin and carbohydrate complexes also limits the accessibility to the LCB components (Chabannes et al. 2001). It is observed that woody biomass due to its high lignin content requires a comparatively harsh treatment process for its valorization than the grass-based biomass. The crucial factors that determine the effect of lignin on LCB recalcitrance are the variety of monolignols present and their abundance, and type and degree of cross-linking among polysaccharides and lignin in the cell wall (Laureano-Perez et al. 2005; Zhu et al. 2010). The structural aspects of lignin have a crucial function in determining the extent of biomass hydrolysis (Chandra et al. 2007). Some studies (Rollin et al. 2011) also suggest that removal of lignin does not necessarily imply that after this LCB would be more accessible to the enzymatic digestion system. Delignification certainly increases the rate of biomass hydrolysis; however, its removal does not ensure enhanced access to the cellulose substrate. An intriguing observation about the lignin in LCB is that it can absorb cellulase enzymes irreversibly. However, this ability to absorb cellulase depends on the biomass source and composition of lignin (Pan 2008; Sutcliffe 1986). Enzyme cellulase binds the lignin with electrostatic and hydrophobic interactions and the binding is spontaneous which was observed from thermodynamic studies. The time required for adsorption of cellulase molecules to lignin was estimated to be 3 h during studies which is much higher than the time required for cellulase adsorption to the pure cellulose (~60 min). The free energy change (ΔG°) of cellulase adsorption on lignin is 29.9 to −37.1 kJ/mol which shows that the binding is a result of physical adsorption. The effect of temperature is not significant in the case of cellulase adsorption to lignin as it is to cellulose; however, the ionic strength of the salts can decrease the cellulase binding to lignin. The adsorption of cellulase enzyme onto the lignin is in the range of 4.87–15 mg/g of lignin which was calculated by Langmuir and Freundlich isotherms (Nakagame et al. 2011). Due to the complex nature of LCB, it is difficult to determine whether these interactions have a significant role in the non-productive binding of the enzyme or not. Apart from lignin, hemicellulose and proteins are also present in the cell wall which also contributes to the LCB recalcitrance.

2.4.2.2 Hemicellulose

Hemicellulose as a component of LCB also contributes to the recalcitrance by acting as a physical barrier. Removal of hemicellulose seems to improve the digestibility of LCB (Zhao et al. 2012a). Xylan, which is present abundantly in hardwoods, can affect the recovery of sugars after hydrolysis and the amount of enzyme required (Bura et al. 2009). The addition of enzyme hemicellulases during enzymatic digestion has also been observed to enhance the hydrolysis of cellulose. This is due to the action of hemicellulases (xylanase) on the hemicellulose which degrades to form xylose, arabinose, and the low molecular weight lignin fragments that are associated with them (Mansfield et al. 1997; Yoshida et al. 2008). However, compared to lignin, the effect of hemicellulose on biomass recalcitrance is not that significant as it is less complex and easier to remove by both the pretreatment and hydrolysis system

(Zhu 2011). Cellulose and hemicellulose are present as holocellulose in the LCB. In recent years, the studies related to the properties and valorization of holocelluloses have increased. For example, Park et al. (2017) in their work studied the effect of hemicellulose content on holocellulose fibril properties. It was observed that with the decrease in the hemicellulose content, crystallinity index, and specific surface area of holocellulose nanofibrils increased, whereas the filtration time and diameter decreased. However, increase in hemicellulose content showed a rise in elastic modulus and tensile strength of nanopaper sheets formed from holocellulose nanofibrils. In another study by Huang et al. (2020), water-soluble ammonium polyphosphate and methyl trimethoxysilane modified bamboo residue were used to obtain the holocellulose nanofibrils. These nanofibrils were further used to prepare the aerogels using the freeze-drying process. The formed aerogels were flexible, soft, and has a 3D network-like structure. The formed aerogels also have good flame retardant and hydrophobic properties. The thermal conductivity of this aerogel was 0.039 W/m K which shows its applications for preparing thermally insulative materials.

The removal of hemicellulose by the enzymatic or chemical process can reduce the biomass recalcitrance and enhance the digestibility of cellulose. The backbone of hemicellulose in LCB is present in a highly acetylated form. These acetyl groups amount to 1–6% of LCB which varies in different plant species. The acetyl moieties are considered to contribute significantly to biomass recalcitrance (Grohmann et al. 1989; Peng et al. 2011). The attachment of acetyl moieties to the cellulose decreases its enzymatic hydrolysis. From several studies, it was observed that deacetylation of LCB can increase its hydrolysis rate. This is because acetyl moieties can interfere with the recognition sites of enzymes which can reduce the rate of hydrolysis. It may also affect the hydrogen bonds-based binding of cellulose with cellulase. Acetyl moieties on cellulose can also increase the chain diameter, which provides the steric hindrance to the enzymatic systems (Pan et al. 2006; Zhu 2005).

2.4.2.3 Proteins in the Cell Wall

The proteins present in LCB also have an effect on its enzymatic hydrolysis. The functional class of proteins present in the plant cell wall are: (i) proteins acting on carbohydrates, (ii) oxidoreductase proteins, (iii) proteases, (iv) proteins with interaction domains, (v) proteins possibly involved in signaling, (vi) structural proteins, (vii) proteins related to lipid metabolism, (viii) miscellaneous proteins. Some examples of these proteins are glucoside hydrolases, peroxidases, and expansins. The type and abundance of cell wall proteins vary with biomass source and cell types in the plant (Albenne et al. 2013; Cassab 1998). They either contribute to the LCB hydrolysis or discourage it. The endogenous enzymes in the plant cell wall such as hemicellulase (endoxylanases), cellulase (endoglucanase), and enzymes that play a role in debranching of the polysaccharides (ferulic acid esterase) have a positive effect on biomass hydrolysis (Lagaert et al. 2009). The proteins that have a negative effect on biomass hydrolysis inhibit the activity of the biomass-degrading enzymes

present in the cell wall. Some examples of these enzymes are xylanase inhibitors and polygalacturonase inhibiting proteins. However, the effects of these proteins can be overlooked because these proteins are active in freshly harvested biomass and during processing and storage of biomass, these proteins undergo denaturation and degradation (Han and Chen 2007; Zhao et al. 2012a).

2.4.3 *Combined Effect of Physical Properties and Chemical Compositions on Recalcitrance*

In the previous sections, the role of physical properties and chemical composition on the LCB recalcitrance was discussed. The chemical structures have their own set of interactions which enhances the recalcitrance. However, combined interactions of these physical properties and chemical compositions play a huge role and are interrelated. The removal of a chemical component would certainly affect the physical properties of the cell wall, whereas varying the physical properties would reflect upon the chemical compositions of LCB. For example, the removal of lignin and hemicellulose increases the porosity of biomass which has a direct effect on the ASA for enzymes (Mansfield et al. 1999). Similarly, a decrease in DP and crystallinity of cellulose results into particle size reduction and increased specific surface area. The factors that affect the accessibility to biomass can be categorized into two factors: (i) Direct and (ii) Indirect factors. In direct factors, enzymatic digestion system interacts with the ASA of LCB, which makes it one of the most important factors for enzyme hydrolysis (Jeoh et al. 2007). Indirect factors are related to the structure of the biomass. Examples of these factors are particle size, pore size and volume, DP and crystallinity of cellulose, and chemical composition of LCB. However, in some cases, the indirect factors can also affect the enzyme hydrolysis process directly. An example for this would be the cellulase adsorption by the lignin which reduces the effective enzyme concentration (Zhao et al. 2012a). It is clear from the discussion that recalcitrance in LCB is not the product of a single structural or compositional factor. The interactions between chemical components result in the formation of rigid and compact LCB structures which makes its utilization difficult in the natural form. A solution to counter this rigidity is a process called pretreatment. Pretreatment is the most significant process in the lignocellulose biorefineries, and there has been a lot of research on this process to utilize the biomass effectively. Pretreatment is a key that opens the full potential locked in the LCB for its successful valorization in biorefineries which will be discussed in detail in the next section.

2.5 Pretreatment: Introduction and Its Role

The LCB as biomass is resistant to breakdown by biological and chemical agents due to its recalcitrance, as discussed in the previous section. Pretreatment processes are required to remove this recalcitrance so that LCB can be utilized optimally in biorefineries. It influences the recalcitrance by processes such as hemicellulose degradation, lignin sheath removal, reduction in DP, and crystallinity of cellulose present in the fibers. The pretreatment process enhances the accessibility to chemicals and enzymes towards the digestion of LCB. Different biomass sources display the variation in the physical properties and composition which requires the selection of different pretreatment processes. Different pretreatment strategies induce different effect on LCB and are utilized according to their special features (Baruah et al. 2018). The goals associated with the selected pretreatment processes are (i) minimum degradation of sugars capable of fermentation, mainly the ones obtained from hemicelluloses, (ii) production of biomass that is able to give a high sugar yield on enzymatic hydrolysis, (iii) minimization of inhibitor formation such as phenolic compounds from lignin degradation, acetic acid from hemicellulose, hydroxymethylfurfural (HMF), furfurals, and other acids such as levulinic acids, formic acid from sugars as shown in Fig. 2.7, (iv) removal and recovery and of lignin for successful valorization, (v) low cost and less energy requirements, (vi) hemicelluloses removal from cellulose, (vii) removal of acetyl groups that interfere with lignocellulose recognition by enzymatic systems, (viii) reduction in particle size and increase in porosity of the biomass, (ix) enhancement of pore size of the components for efficient hydrolysis, and (x) minimization of downstream processes involved in the product recovery, which otherwise will make the bioproduction costly (Chiaramonti et al. 2012; Ramos et al. 2019; Zhao et al. 2012b). These goals are interrelated in nature meaning that changing one of these can affect the others. For example, removal of hemicellulose in LCB can also improve the pore size of the biomass which will enhance the hydrolysis yield. The increase in the porosity also affects the pore size which is a limiting factor for biomass hydrolysis. The selection of a particular pretreatment process for biomass depends on the type/source of biomass origin and the product required (Chiaramonti et al. 2012). The assessment of a particular pretreatment for a biomass can be done by parameters such as (i) the accessibility of water-washed or unwashed biomass to hydrolyzing systems, (ii) the total recoverable amount of carbohydrates released (monomeric and oligomeric units present in solid or liquid phase), (iii) the fermentation ability of hydrolysates obtained after pretreatment which can be characterized by product yields and microbial growth during fermentation, (iv) the formation of useful by-products for the synthesis of other value-added products besides the main intended product (Ramos et al. 2019). Pretreatment is one of the most important processes in biorefineries and needs strategic planning as it determines the economic viability of the process. The energy balance, chemical consumption, production of enzymatic and fermentation inhibitors, and environmental effects are some other factors that are considered before selecting the appropriate pretreatment process

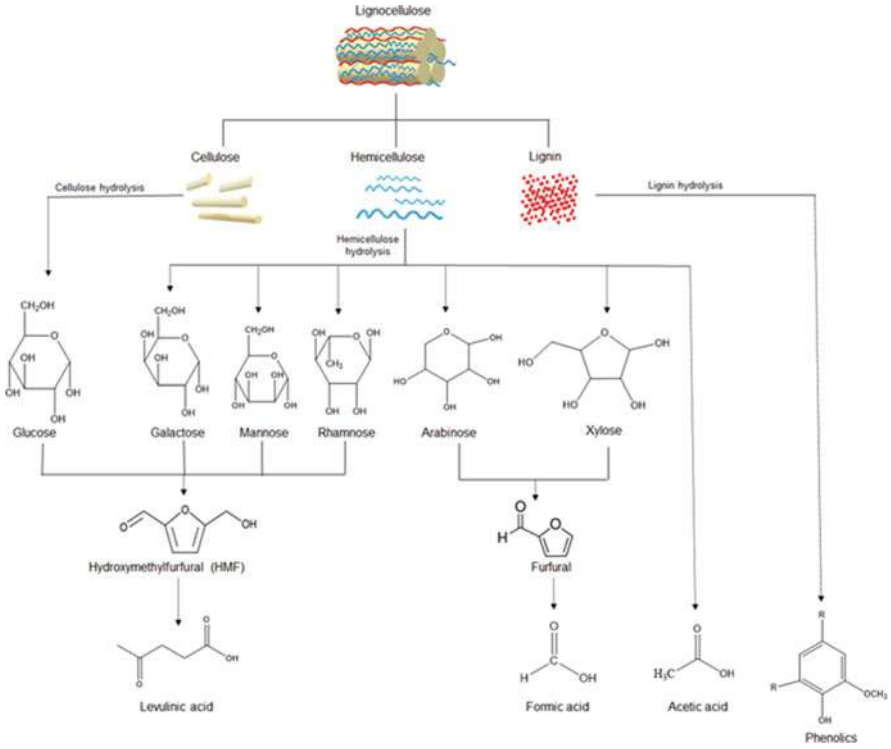

Fig. 2.7 Schematic representation of by-products and inhibitors produced from lignocellulosic biomass valorization and the respective products that can be obtained with them

(Kapanji et al. 2019; Ng et al. 2019). The selection of the pretreatment also influences the upstream and downstream systems that are applied during the processing of LCB. For example, in upstream processes, the choice of the pretreatment process can influence the harvesting and storing conditions of biomass and the chemical and techniques utilized for it. Similarly, the pretreatment process applied can also influence the enzyme loading needed for hydrolyzing the substrate, downstream processing utilized for recovery and isolation of the product, and wastewater generated from the process that can be recycled (Bensah and Mensah 2013; Harmsen et al. 2010). The types of pretreatments utilized in biorefineries are physical, chemical, physicochemical, biological, and combined pretreatments. Physical pretreatment processes, as the name suggests, use the physical and mechanical processes to bring down the recalcitrance of LCB. It reduces the particles size and increases ASA for hydrolysis. These methods are generally used in combination with other pretreatment methods. Some examples of physical pretreatments are milling, radiation pretreatment, extrusion, freezing, and pyrolysis (Zhao et al. 2012b). Chemical pretreatment uses the chemicals for pretreatment of LCB under defined conditions. The mechanism of chemical pretreatment varies with the type of chemical utilized. Some examples of chemical pretreatment are acid pretreatment,

alkaline pretreatment, sulfite pretreatment, oxidative pretreatment, ozonolysis, organosolv pretreatment, and ionic liquid-based pretreatment (Bhardwaj et al. 2020a; Kumar et al. 2018; Soltanian et al. 2020). Physiochemical pretreatment methods use physical and chemical methods in combination to increase the efficiency of the pretreatment. Some examples are ammonia fiber explosion (AFEX), steam explosion, liquid hot water pretreatment, wet oxidation, and ultrasonication (Chiaramonti et al. 2012). Biological pretreatment processes use the application of biological agents such as microorganisms or enzymes to reduce the recalcitrance possessed by LCB. Some examples of this pretreatment are fungal, bacterial, enzymatic, and microbial consortium-based pretreatment (Kumar et al. 2018; Bhardwaj and Verma 2020; Bhardwaj et al. 2020b; Soltanian et al. 2020). The last category in pretreatment processes would be the combined pretreatment. The different pretreatment strategies have their own shortcomings and to reduce this, two or more pretreatments could be applied to the biomass. This will help in increasing the efficiency of the pretreatment by improving sugar production, less time consumption, and less production of inhibitors. It also helps in improving the economic viability and product yield. Some examples are combined CO_2 and steam explosion pretreatment, combined biological and dilute acid pretreatments, combined alkali and ionic-liquid pretreatment, and microwave-assisted alkali pretreatment (Kumari and Singh 2018). This information about the main products that are generated in different pretreatment processes from the individual biomass component can supplement in choosing the right pretreatment method for a particular product. For example, in cellulose, acid hydrolysis generates glucose; pulping, bleaching, and enzymatic hydrolysis yield cellulose nanocrystals whereas pulping, bleaching, and mechanical processing produces cellulose nanofibers. For hemicellulose, acid hydrolysis generates monosaccharides that constitute xylose arabinose, etc., autohydrolysis and mild acid hydrolysis generate oligosaccharides whereas partial acid hydrolysis and functionalization produce hydrocolloids. For lignin, oxidation forms vanillin, acid or alkaline hydrolysis produces phenolic acids, and sulfite pulping produces lignosulfonates (Ramos et al. 2019).

Based on the discussion above, it can be concluded that pretreatment is a crucial step in LCB valorization in biorefineries. Some of the examples of pretreatment used for lignocellulosic biomass are presented in Table 2.1. along with the methodology and sugar yield obtained. In recent years, the development of a low-cost and efficient pretreatment process has been researched thoroughly and the pretreatment strategies available at present allow excellent performance in biomass utilization. However, these strategies still have some obstacles that are needed to be solved such as high energy and chemical demands, waste production, efficient recovery of LCB components, and production of inhibitors. The role of pretreatment in biorefineries is huge and contributes largely to the cost associated and therefore requires careful selection. Selecting the right pretreatment process is not an easy task and requires the understanding of economic and sustainability aspects, biomass used, products required, and optimum conditions for operation.

Table 2.1 Examples of different pretreatments processes applied to lignocellulosic biomass along with their methodology and respective yield

Substrate	Pretreatment	Type of pretreatment	Methodology	Yield	References
Rice hulls	Acid hydrolysis	Chemical pretreatment	H_2SO_4 (0.3 %), solid: liquid ratio 5: 95, 152 °C, 33 min	Glucose 47.2 g/100 g glucan; xylose 86.3 g/100 xylan	de Moraes Rocha et al. (2011)
Corn stover	Supercritical CO_2 explosion	Physicochemical pretreatment	5g raw material, 165 °C, 34.5 MPa, 60 min	Total sugar 37 g/100 g raw material	Huisheng et al. (2013)
Switchgrass	Ammonia fiber explosion	Physicochemical pretreatment	Ratio: 1.5:1 ammonia: biomass loading (w/w), 140 °C, 20 min	Glucose 69.3 g/100 g glucan, xylose 67.7 g/ 100 g xylan	Garlock et al. (2012)
Sorghum bagasse	Alkaline hydrolysis	Chemical pretreatment	Ammonium hydroxide (28% v/v), 130 °C, 1 h	Glucose 42 g/100 g raw material	Chen et al. (2012)
Sugarcane bagasse	Liquid hot water	Physicochemical pretreatment	High pressure CO_2, solid: liquid ratio: 1:12, 115 °C, 60 min	Glucose 30.43 g/L, xylose 9.82 g/L	Gurgel et al. (2014)
Rice straw	Ionic liquids	Chemical pretreatment	1-ethyl-3-methylimidazolium acetate, 5% solid, 120 °C, 5 h	Glucose 450 g/kg rice straw	Poornejad et al. (2014)
Sitka spruce	Organosolv	Chemical pretreatment	Ethanol: water 60:40 (H_2SO_4, 1%), solid: liquid ratio 1:10, 180 °C, 60 min	Glucose 38.6 g/100 g biomass, Ethylglycosides 12.5 g/100 g raw material	Bouxin et al. (2014)
Mandarin peel	Steam explosion	Physicochemical pretreatment	Hot popping: 150 °C, pressure: 15 kgf/cm	Glucose 45.1 g/100 g raw material; fructose 18.4 g/100 g raw material	Choi et al. (2013)
Japanese cedar wood	*Ceriporiopsis subvermispora*	Biological pretreatment	8 weeks at 28 °C, 70% moisture content	35% and 5% conversion of holocellulose to methane with and without pretreatment, respectively	Wagner et al. (2018)

2.6 Pretreatment-Induced Changes on LCB

LCB in its natural form shows recalcitrance towards enzymatic and chemical hydrolysis which is the basis behind the use of pretreatment in biorefineries. The pretreatment processes applied to LCB change its chemical composition and physical properties. Generally, pretreatment disrupts the physical barriers of the cell wall, removes lignin and hemicelluloses, alters the cellulose crystallinity, helps in swelling of pores, and increases porosity as shown in Fig. 2.1(b). The pretreated biomass due to these changes becomes different from the natural LCB which could be observed from variation in physical properties of kraft pulp before and after pretreatment as shown in Table 2.2. (Popescu et al. 2009).

Physical pretreatment changes the crystallinity, particle size, and surface area whereas chemical pretreatment generally focuses on swelling of cellulose and lignin removal (Mussatto 2016). Considering physical pretreatments, the ball milling process performs the size reduction (~78%), decreases cellulose crystallinity (from 74.2% to 4.9%), and DP, with ASA of~ 2.13–2.26 m^2/g (Yeh et al. 2010; Zhu 2011). Another physical pretreatment based on radiation-based, significant hemicellulose (xylan increase 13.9–16.3%) and lignin removal (26.3–6.8%) is observed. In terms of physical properties, it enhances the ASA and decreases the cellulose crystallinity (27.3–24.6%) and DP (1920–170). The ball milling process does not degrade the components of biomass; however, physical pretreatment affects the chemical composition of the LCB (Shin and Sung 2008; Zheng et al. 2009). Ultrasound-based physical pretreatment modifies the structure by cavitation and breaks LCC complexes. For the chemical pretreatments, dilute acid shows significant removal of Kalson lignin and partial degradation of hemicellulose (xylan 8.1–5.5%) which almost disrupts the structure completely. It removes almost all the acetyl groups too (up to 90%), decreases crystallinity (up to 4.4%), and DP (up to 84.7%) of cellulose. In concentrated acid hydrolysis, swelling of cellulose, partial hydrolysis of hemicellulose, and coalescence and condensation of lignin are observed. In alkali pretreatment, significant removal of lignin (>50%) with partial degradation of hemicellulose disrupts the physical structure of LCB. It also removes the acetyl groups completely, increases ASA significantly, with notable and some decrease in the cellulose crystallinity and DP, respectively. In supercritical CO_2 pretreatment, partial hydrolysis of hemicellulose, and an increase in ASA and pore volume are

Table 2.2 The change in physical properties of unbleached and bleached kraft pulp (Popescu et al. 2009).

Properties	Unbleached kraft pulp properties	Bleached kraft pulp fibers
Viscosity (L/g)	1.10	0.728
Density (bulk) (m^3/kg)	0.0019	0.0018
Water retention value (kg/kg)	1.59	1.43
Elastic modulus (N/mm^2)	2420	2129
Tensile strength (kN/m)	1.92	1.74
Kappa number	26.8	3.2

observed. In oxidative delignification, significant removal of the lignin (~50%) is seen with almost complete removal of hemicellulose. It also removes acetyl groups partially, increases ASA significantly, decreases DP of cellulose. Ionic liquid pretreatment partially removes the lignin (15.6–36.3%) (Sathitsuksanoh et al. 2014; Zhao et al. 2012b). It significantly increases ASA, completely removes acetyl groups, converts crystalline cellulose to amorphous, and shows a decrease in cellulose DP (1198–812). Organosolv pretreatment (organic acid-based) shows significant lignin removal (60–90%) and almost completely degrades hemicellulose. It shows a significant increase in ASA with some decrease in cellulose DP. Physiochemical pretreatments such as stream explosion almost completely remove hemicellulose (80–100%) and partially removes lignin (26–50%), however, cause significant changes in redistribution and structure. For physical properties, it significantly removes the acetyl groups and increases ASA significantly. In liquid hot water pretreatment, partial lignin removal (35–60%) happens with significant changes in structure and redistribution of the components with significant and almost complete degradation of hemicellulose (90%). For biological pretreatments, in fungal degradation, partial removal of lignin (13–41%) and good recovery of hemicellulose (81%) is observed with some cellulose degradation. It enhances the ASA and reduces the cellulose DP to some extent. For enzymatic pretreatment, lignin and hemicellulose degrading enzymes such as laccases, peroxidases, and xylanase comes into play that potentially degrades the lignin and hemicellulose in the LCB and increase ASA for its access to the chemical and enzymatic hydrolysis system (Zhao et al. 2012b). Figure 2.8 shows the different physical, chemical, physiochemical, and biological pretreatment methods available for LCB and the changes in biomass induced by them.

Wheat straw as a lignocellulosic substrate when undergoing thermal pretreatment, several changes are observed in the LCB composition. At 180 °C temperature, an increase in the cellulose content is observed, from 34.5% to 47%. At this temperature, a reduction in the hemicellulose content was observed. The reason could be the generation of hydronium ions which could have degraded the materials, generating simple compounds from complex LCB components. Significant removal of lignin was also observed at this temperature. The results also show an increase in crystallinity from 39.7% to 48.5%. The reason for the increase in crystallinity could be a decrease in hemicellulose, lignin, and amorphous cellulose content in the biomass. The fracture of lignin and hemicellulose structure also changes the chemical structure of the biomass, resulting in the change in chemical bonding which was found by Fourier-transform infrared spectroscopy spectra (FTIR) (Rajput and Visvanathan 2018). Bagasse which is obtained from the hybrid of wild and commercial sugarcanes (energy cane bagasse) when undergoes the pretreatment with ionic liquids such as [EMIM][OAc] shows a significant removal of lignin (~32%) with slight xylan and glucan degradation (14% and 8.8%, respectively). The treatment also improved the enzymatic digestibility of the energy cane bagasse. After pretreatment, a disordered and loose structure of biomass was observed from scanning electron microscope (SEM) observations. The crystallinity of cellulose in the biomass also decreased from 56.28% to 24.52% (Qiu et al. 2012). Rice straw

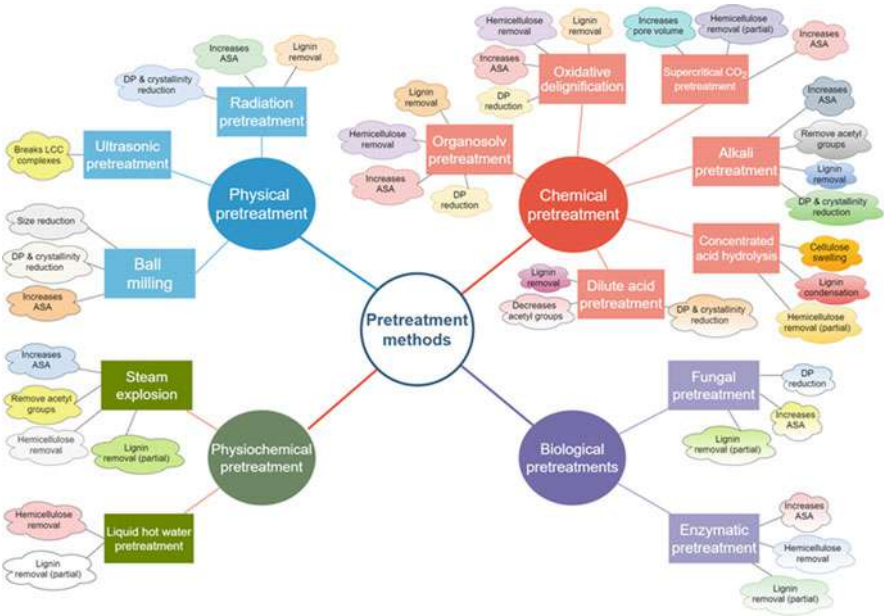

Fig. 2.8 Schematic representation of changes induced after pretreatment of lignocellulosic biomass

when pretreated with alkali shows a significant change in the biomass composition. At 12% NaOH pretreatment for 1 h, the glucan content in the biomass increased from 34.99% to 64.96% whereas the xylan content decreased from 21.55% to 9.07%. Lignin content and extractives also decreased from 24.28% to 4.96% and 16.05% to 14.85%, respectively, during these conditions. The effect of pretreatment in changing the physical properties and chemical composition of the biomass is quite evident and is proven with several studies on different substrates (Kumar and Verma 2019; Harun and Geok 2016). Each pretreatment applies a unique set of changes to the biomass. The innovative pretreatment methods that allow better accessibility to biomass and minimize the waste is a topic of interest from a research perspective and is expected to grow more in the future to make a zero-waste biorefinery. The biorefinery-based production is although classified into various types depending on the feedstocks utilized, pretreatment processes involved, and types of intermediates generated. However, the biorefineries can be divided into different phases such as phases I, II, and III. Phase I biorefineries use single feedstock and produce a single product (e.g., pulp and paper, biodiesel) whereas phase II biorefineries utilize a single substrate to generate multiple products (e.g., bioethanol production). Phase III biorefineries use various feedstocks to produce multiple type of products (LCB biorefinery, whole crop biorefinery). The innovation that has been introduced in the LCB biorefinery in recent years is also starting to gain industrial attention. This has led to a variety of LCB biorefinery products adapted by industries (e.g., succinic

acid, ethanol, and lactic acid) which projects new prospects for this area in the coming future with unlimited possibilities (Takkellapati et al. 2018).

2.7 Conclusion

The rapid consumption of natural petroleum-based reserves to obtain fuels and materials is leading to their quick depletion and huge environmental pollution. It is high time to search for alternative natural substrates to obtain these products which will reduce the dependence on petroleum reserves and pollution caused by them. In recent decades, lignocellulosic biomass from wood has been identified as a suitable substrate for this purpose which can replace the conventionally used petroleum-based raw materials. However, the inherent resistance or recalcitrance of the biomass protects it from biological and chemical agents and suppresses its utilization at full potential. To counter this, pretreatment processes are used in biorefineries that make the LCB more susceptible to the degradation process and facilitate its optimal utilization in biorefineries. The pretreatment processes are of different types that change the physical properties and chemical composition of biomass depending on the biomass used and the pretreatment process and conditions. The ongoing research is towards the development of innovative pretreatment methods with high efficiency for pretreatment that can increase the economic value of LCB-based biorefineries and industries.

Acknowledgment R.R is thankful for the junior research fellowship from Department of Biotechnology, Govt. of India (DBTHRDPMU/JRF/BET-20/I/2020/AL/07). P.D. is grateful for the Ramalingaswami fellowship and the research grant obtained from the Department of Biotechnology, Govt. of India (BT/HRD/35/02/2006). The authors are grateful to School of Biochemical engineering, Indian Institute of Technology (IIT-BHU), Varanasi for their kind support which made this work possible.

Competing Interests All the authors declare that they have no competing interests.

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Chapter 3
Understanding Biomass Recalcitrance: Conventional Physical, Chemical, and Biological Pretreatment Methods for Overcoming Biomass Recalcitrance

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Abstract Pretreatment of lignocellulosic waste is one of the costliest phases in transforming cellulosic material into fermentable sugars. It represents one-third of the overall process cost, and about 90% of the dry weight constitutes cellulose, hemicellulose, lignin, and pectin. Hydrogen bonds and some covalent bonds bind the carbohydrate polymers to lignin firmly. The existence of lignin in lignocelluloses barricades the plant cell against the breakdown action by fungi and bacteria. The purpose of the pretreatment procedure is to disrupt the crystalline phase of cellulose and disintegrate the lignin structure, improving the porosity of the lignocellulosic material.

It further provides acids and enzymes access to hydrolyze cellulose by expanding the porosity of the lignocellulosic material so that it readily attacks to break down the cellulose. Pretreatment is therefore done: (i) to facilitate hydrolysis for the formation of sugars, (ii) to keep away the decaying or waste of carbohydrates, (iii) to prevent the creation of by-products that hinder the hydrolytic activities and fermentation that follow, and (iv) make it economical. Numerous pretreatment protocols are employed for treating biomass to overcome the problem faced during pretreatment. In this

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P. Verma (ed.), *Thermochemical and Catalytic Conversion Technologies for Future Biorefineries*, Clean Energy Production Technologies,
https://doi.org/10.1007/978-981-19-4312-6_3

study, we have dealt with various pretreatment adopted against lignocellulosic biomass, which is a measure of their potential as feedstock for biofuels.

Keywords Lignocellulosic waste · Pretreatment · Cellulose · Lignin

Abbreviations

AFEX	Ammonia fiber explosion
HMF	5-hydroxymethylfurfural
HPSE	High-pressure steam explosion
OLP	Oxidative lime pretreatment

3.1 Introduction

Rapid population expansion, drop-in biodiversity, and natural resource switches in pollution and climate (Correa et al. 2019) have prompted academics and policymakers to focus on sustainable energy policies to reduce greenhouse gas emissions (Andrée et al. 2017). Furthermore, the depletion of fossil deposits is occurring faster due to an increased demand for fossil fuels due to global industrialization and motorization (Perin and Jones 2019). Fossil fuels are still the world's principal energy source, responsible for more than 88% of primary energy consumption (Li et al. 2019). Their burning releases greenhouse gases, mainly CO_2 (Alalwan et al. 2019; Goswami et al. 2020). As a result, substituting fossil fuels with renewable energy sources allow us to fix these problems by reducing global temperature rise (Scaramuzzino et al. 2019; Goswami et al. 2021). Renewable energy is the proportion of renewable energy to total primary energy consumption, which comprises the equivalent of direct energy from hydroelectric sources (excluding storage through pumping), geothermal, solar, wind, tidal, and wave energy other factors. Power is provided by solid biofuels, biodiesel, different liquid biofuels, biogas, and the renewable component of municipal garbage.

On the other hand, biofuels are fuels generated directly or indirectly from biomass (OECD 2019; Goswami et al. 2022a, b). Biomass is the largest and most important renewable energy source today, after coal, oil, and natural gas (Azevedo et al. 2019; Kumar and Verma 2020a). It can generate various energy sources because the carbon dioxide released during combustion is equivalent to the amount absorbed by the plant throughout photosynthesis, so biomass can be termed carbon neutral (Ibarra-Gonzalez and Rong 2019). Lignocellulosic biomass, the world's most cost-effective and highly renewable natural resource (Hernández-Beltrán et al. 2019; Agrawal and Verma 2020; Goswami et al. 2022c) is the most significant potential feedstock for bioenergy production.

3.2 Pretreatment and Hydrolysis of Biomasses: Overview

Pretreatment or fractionation is a valuable technique for changing the structure of lignocellulosic biomass (Fig. 3.1) and making the holocellulose (cellulose + hemicellulose) bioavailable for bioconversion (Rajendran et al. 2018; Bhardwaj et al. 2021). Pretreatment's primary goal is to break down the cell wall's physical barriers, allowing it to depolymerize and lower cellulose crystallinity (Karimi and Taherzadeh 2016; Kumar et al. 2020). Depending on the type of biomass, pretreatment has been recognized as a crucial stage in the bioconversion of lignocellulosic biomass for utilization in biorefineries (Mupondwa et al. 2017). Pretreatment must be cost-effective, as operational and capital expenditures could account for more than 40% of total processing costs (Bhutto et al. 2017). There have been various studies on biomass pretreatment methods, which can be divided into four categories: (1) mechanical processes; (2) chemical processes; (3) physicochemical processes; and (4) biological processes, which employ microbial consortia or enzymatic means (Fig. 3.2).

3.2.1 Physical/Mechanical Pretreatment

Physical approaches can improve lignocellulosic biomass's pore size and surface area while reducing cellulose crystallinity and polymerization. Milling, sonication, mechanical extrusion, and irradiation are physical pretreatment.

Mechanical pretreatment minimizes the size of the particles by milling, chipping, or grinding (Kumari and Singh 2018). Pretreatment with mechanical equipment is a well-known way of enhancing biogas production, but due to its high energy requirements, it is still a costly method (Veluchamy and Kalamdhad 2017).

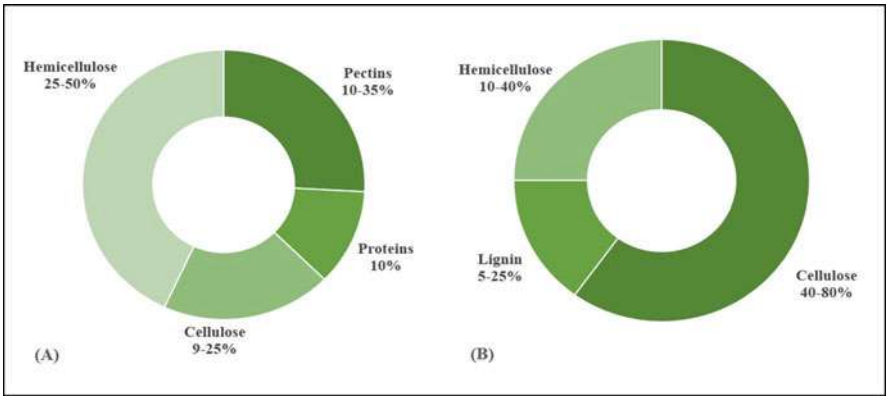

Fig. 3.1 (**a**) The primary cell wall, (**b**) secondary cell wall of the plant's cell wall

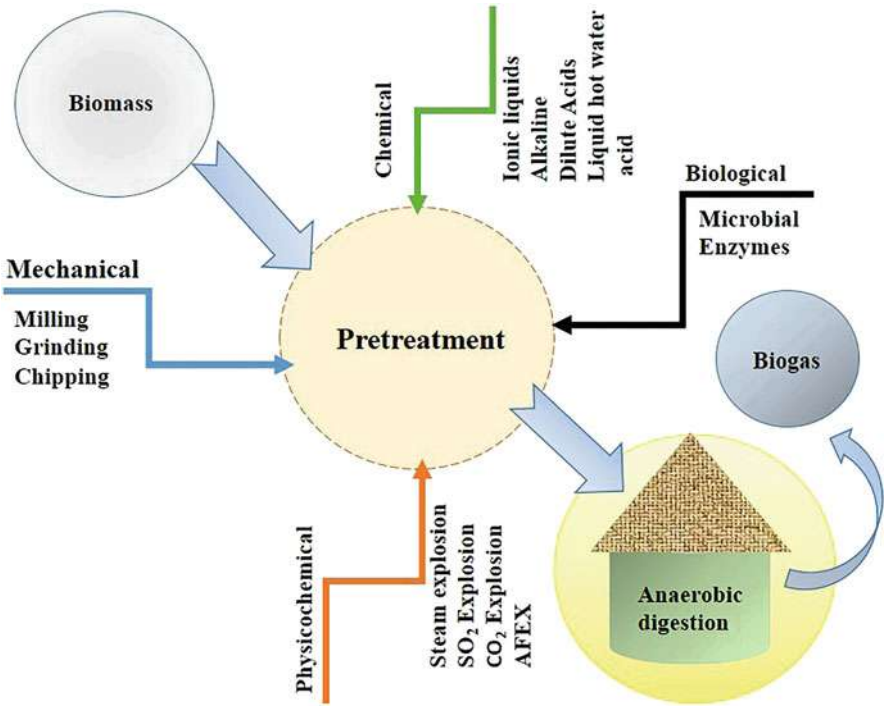

Fig. 3.2 Bioavailability of lignocellulosic biomass after pretreatment

To improve lignocellulosic biomass's accessible surface area and porosity, it must first be reduced in size, lowering cellulose crystallinity and improving the accuracy of the following processing phase and the overall production line (Rodriguez et al. 2017).

Mechanical pretreatment benefits from not producing any secondary inhibitory compounds, implying that they might be used for methane synthesis or other bioprocesses (Dahunsi 2019a). Mechanical processing of six distinct lignocelluloses produced structural material degradation and enhanced methane output by 22%. However, excessive lignocellulosic biomass size reduction can impair methane generation efficiency (Kumari and Singh 2018). Operational variables such as particle size, mechanical pretreatment processing time, and mechanics all affect the performance of the operation in terms of cost energy. According to the literature, there is no standard particle size acceptable for the methanation process, and it varies depending on the type of substrate and the biomethanation procedure. Dumas et al. (2015) found no correlation in wheat straw biogas production with particle sizes ranging from 0.7 to 0.2 mm, and that methane output did not go up at 0.048 mm. The impacts of various particle sizes of wheat straw, rice straw, Mirabilis leaves, cauliflower leaves, *Ipomoea fistulosa* leaves, dhub grass, and other agricultural residues were investigated at 0.088, 0.40, 1.0, and 6.0 (Sharma et al. 1988). They also stated that the biogas yield difference between 0.4 and 0.088 mm was not

statistically significant. De la Rubia et al. (2011) found that particle size 1.4–2.0 mm produced the maximum methane yield in sunflower oil cake, 17% greater than particle size 0.355–0.55 mm. Compared to both straws' particle size of 0.75 mm, the particle size of 0.30 mm of wheat and rice straw did not exhibit any substantial improvement in methane yield, according to Chandra et al. (2015). Both particle sizes, however, increased as compared to the untreated particle size of 1.5 mm.

Tsapekos et al. (2015) studied six different mechanical pretreatments that produced particle sizes ranging from 0 to >20 cm. They found that mechanical pretreatment that made particle sizes greater than 20 cm, which accounted for 33% and 41% of the overall biomass, did not yield over untreated biomass. The proportion of biomass with more than >20 cm particle sizes was only around 22% of the total biomass; the methane output was significantly higher and enhanced by 25%. Herrmann et al. (2012) revealed a substantial connection between particle length and methane yield when evaluating different particle lengths of similar agricultural material. Two particle sizes, 2 mm and > 20–100 mm, on various substrates, including softwood, hardwood, and cotton, were studied by Krause et al. (2018) and discovered that lowering particle size enhanced methane output.

On the other hand, particle reduction processing time is essential for energy and the efficiency of lignocellulosic biomass bioconversion (Bhutto et al. 2017). Rodriguez et al. (2017) evaluated 30 and 60 min of mechanical pretreatment and found that 60 min resulted in a 21% increase in methane output, while 30 min did not. The milling equipment's speed must be addressed in order to reduce the cost of energy utilized in mechanical pretreatment (Tsapekos et al. 2017) investigated the effect of different milling speeds, ranging from 200 to 1200 rpm, on CH_4 production through meadow grass and found that the milling rates studied made no difference. Treatments resulted in a 27% enhancement in CH_4 output compared to untreated material.

There was also a drop in methane yield when particle sizes were greater or lower than the range of 2–5 mm (Chandra et al. 2015; Menardo et al. 2012; Dell'Omo and La Froscia 2018). According to Menardo et al. (2012), increasing the particle size of wheat straw to 2 mm increased CH_4 production by 83%. Similarly, at particle sizes of 5 mm and 20 mm, barley straw increased methane output by 54% and 41%, respectively (Menardo et al. 2012). Because of the differences in particle size, milling time, and milling speed, the optimal mechanical pretreatment technique appears to depend on the type of lignocellulosic material, and no general strategy can be advised.

Waste liquid streams from pulp and paper factories are attracting a lot of attention in Northern Europe. On steam-pretreated birch wood, Lamb et al. (2019) employed ultrasonication, a Fenton-like reaction, or a combination of the two to see if a favorable influence on biomethane generation could be obtained. A Fenton reaction uses iron ions and hydrogen peroxide to oxidize organic pollutants like inhibitors and poisonous compounds that can harm microorganisms. Birchwood was steam processed for 10 min at 210 °C in the study. After that, the STEX material was ultrasonicated for 2 h at a pH of 4, with or without the addition of hydrogen peroxide and $FeCl_3$. The findings imply that a strong ultrasonication combined with a

Fenton-like treatment negatively influenced biogas generation, but a softer treatment increased production. They led to a shorter residence time to get near the maximum BMP, which has a significant impact on reactor size (or biogas capacity), which is the most expensive part of a biogas plant.

3.2.1.1 Milling

Milling can make lignocelluloses more accessible to cellulases based on the intrinsic cellulose ultrastructure and crystallinity degree. Cellulases are enzymes that catalyze the breakdown of cellulose; however, for the enzymes to operate optimally, substrate supply must be increased. Before introducing lignocellulosic materials to enzymatic hydrolysis, they should be milled and size reduced.

Colloid mills, dissolvers, and fibrillation are suitable for wet materials, while hammer mills, extruders, cryogenic mills, and roller mills are suitable for dry materials. Ball milling can be used for both wet and dry materials. Hammer milling is the best pretreatment method for wastepaper. By lowering the crystallinity and size of the material, milling promotes enzyme degradation. Particle size reduction of near 0.2 mm can be achieved through milling and grinding. Particle size reduction is possible to a degree; then, particle size reduction does not influence the pretreatment procedure.

Small particle sizes corn Stover of 53–75 m is more productive than corn stover with large particle sizes, 475–710 m. Because of the variation in particle size, productivity has a considerable impact on the pretreatment process. (Bondy et al. 1998; Taherzadeh and Karimi 2008; Walpot 1986) found that ball milling reduces the crystal index from 4.9% to 74.2%, making it pretty appropriate for straw saccharification under mild hydrolytic circumstances with more fermentable sugars yield.

Grinding and milling procedures are not the only types of physical preparation. Falls et al. (2019) investigated the impact of "shock treatment." For example, the method depended upon the effects of an immediate shock wave on material when treated with enzymatic hydrolysis. They investigated various lignocellulosic materials in the study, including bagasse, corn stover, poplar wood, sorghum, and switchgrass. One of the objectives was to examine the digestion of oxidative lime pretreatment (OLP) substrates by glucan enzyme after ball milling and shock treatment. All substrates responded better to shock treatment than ball milling at a hydrolysis time of 24 h. Ball milling outperformed shock treatment when the residence duration was increased. There was a significant improvement in digestibility compared to solely OLP substrates, leading to increased glucose production (Galbe and Wallberg 2019).

Milling is a technique that can be employed in conjunction with enzymatic hydrolysis to improve hydrolysis results. It is possible to carry out mechanical action, mass transfer, and enzymatic hydrolysis in parallel when two techniques are coupled. Compared to biomass pretreatment without milling technology, small

enzyme feeding is required in a bill mill reactor. Numerous ball beads contribute a critical part in cellulose hydrolysis, and a 100% hydrolysis rate can be obtained.

3.2.1.2 Irradiation

Microwave irradiation is a commonly used irradiation technology for plant biomass processing. This pretreatment approach has several advantages, including the convenience of use, higher heating capacity, quick processing time, minimum inhibitor generation, and lower energy use. In 1984, a group of researchers from Kyoto University in Japan reported the first case of microwave irradiation in a closed container. In the presence of water, they microwaved sugarcane bagasse, rice straw, and rice hulls. Glass jars of 50 mL, 2450 MHz energy, and 2.4 kW microwave irradiation were utilized for microwave therapy (Azuma et al. 1985).

Traditional pretreatment procedures used high pressures and temperatures. In response to the extreme heat, chemical connections between lignocellulosic materials break down, boosting substrate availability for enzymes. In traditional healing processes, lignocellulosic material is heated to temperatures between 160 and 250 °C using an injection of high-pressure steam or indirect heat. However, to avoid temperature gradients, lignocellulosic material must be crushed into small particles.

Microwave is a suitable alternative for avoiding significant temperature gradients since it evenly distributes heat and prevents lignocellulosic material breakdown into humic acid and furfural. For effective degradation, microwave irradiation is paired with a mild alkali agent. Alkali and irradiation combination pretreatment of switchgrass resulted in 70–90% (Hu and Wen 2008). Microwave irradiation is done at a high temperature, requiring using sealed containers to attain that temperature.

Microwave demonstrates three properties: penetration, reflection, and absorption. Penetration, reflection, and absorption are three characteristics of the microwave. Microwaves penetrate through glass and plastic, acquiring energy from water and biomass, whereas metals reflect them. Microwave reactors can be classified into two sorts based on these characteristics: those that allow microwaves to flow through and those that reflect them. The first type of microwave reactor is glass or plastic, whereas the second type is steel.

Microwaves can enter the reactor through quartz windows, which are positioned in the reactor. For microwave irradiation pretreatment, a closed, sealable, pressure-resistant glass tube container with a Teflon gasket can be utilized at a high temperature of 200 °C. The temperature of the microwave is controlled and maintained using sensors. Due to their features like thermostability, corrosion resistance, and zero absorbance, Teflon-coated sensors are an ideal choice.

Some scientists utilize Teflon vessels in microwave ovens because of their beneficial features (Komolwanich et al. 2014; Azuma et al. 1984). The size of a ship might range from 100 mL to several hundred milliliters. On the top of Chen and Cheng's microwave (Chen and Cheng 2011), a 650 mL vessel with a 318 mm length, a connected nitrogen bottle, gauges, and thermometers are installed.

Aside from glass jars and stainless-steel tanks with temperature and pressure sensors, a microwave input control system and a mechanical stirrer are also used.

3.2.1.3 Expansion and Extrusion

When materials travel through a die with a defined cross-section, they emerge with a fixed, definite profile. Sugar is extracted from biomass using the extrusion method. Mechanical extrusion pretreatment has several advantages: adaptability to changes, no degradation products, a regulated environment, and high throughput. Extruders are divided into two types: single screw and twin screw.

3.2.2 Physicochemical Pretreatment

Physicochemical approaches are utilized to dissolve lignocellulosic parts of the structure based on moisture levels, allowing the lignocellulosic content to be conveniently available for the hydrolysis step while preventing inhibitor development. Although these approaches are more difficult to apply, their significant impact on lignocellulose substrate pretreatment assures high production in the following bioprocesses (Theuretzbacher et al. 2015; Kumar et al. 2020). Physical pretreatment, in general, necessitates more expenditure of energy, making it a costly procedure that is not very economical on an industrial level. As a result, the focus of many previous studies is on process optimization and the criteria for lowering the energy, time, and costs of this treatment.

3.2.2.1 Explosion

High-pressure Steam Explosion (HPSE)

This approach involves treating lignocellulosic biomass with high-pressure saturated steam and then rapidly lowering the pressure, resulting in explosive decompression of the biomass. The temperature of the steam explosion is set to 160–260 °C, and the pressure is set to 0.69–4.83 MPa for a few seconds to minutes, after which the biomass of lignocellulosic plants is exposed and held at atmospheric pressure, triggering hemicellulose hydrolysis and ending the process with explosive decompression. The opportunity for cellulose degradation grows as cellulose breaks and lignin converts due to the high temperatures. Acid and many other acids generated throughout the steam explosion pretreatment played a part in the breakdown of hemicellulose. Because of turbulent material flow and rapid material flashing to air pressure, lignocellulosic material fragmentation occurs (Jørgensen et al. 2007; Li et al. 2007). H_2SO_4 or CO_2 is utilized in steam explosion pretreatment to reduce time and temperature and inhibit product generation while increasing hydrolysis

efficiency, leading to 100% hemicellulose removal. For softwoods, steam explosion pretreatment is ineffective; nevertheless, adding an acid catalyst throughout the procedure is required for the hydrolysis of the substrate through the enzyme. Biomass can be treated by steam at a specific temperature without the need for extensive dilution. At the end of the process, a sudden pressure release quenches the entire process and decreases the temperature. Rapid heat expansion opens the particle structure of biomass, which is employed to stop the reaction: moisture content, residence duration, size of the chip, and temperature all impact the steam explosion. Optimal hemicellulose hydrolysis and solubilization can be done in two ways: Low temperature and lengthy residence time vs high temperature and short residence time. Steam explosion pretreatment has an intense energy need. However, mechanical pretreatment requires 70% more energy than steam explosion pretreatment to achieve a similar reduction in particle size. So far, steam explosion pretreatment with the inclusion of a catalyst has been tried, and due to its cost-effectiveness, it has the best chance of being scaled up at a commercial level. Steam explosion pretreatment is being employed on a small scale at the Iogen demonstration plant in Canada. Steam explosion pretreatment is an effective technique for hardwood and agricultural wastes.

Ammonia Fiber Explosion (AFEX)

The AFEX technique is a physicochemical pretreatment technique. This low-temperature technique uses concentrated ammonia (0.3–2 kg ammonia/kg dry weight) as a catalyst. In a high-pressure reactor, ammonia is introduced to biomass; pressure is swiftly removed after 5–45 min of cooking. This operation is usually carried out at a temperature of roughly 90 °C. Because of its volatility, ammonia can be collected and reused. AFEX works on the same idea as a steam explosion. Apparatus such as a reactor, thermocouple well, pressure gauge, pressure relief valve, needle valve, sample cylinder, temperature monitor, and vent are used in AFEX procedures. AFEX pretreatment of several blades of grass and herbaceous crops appears to boost the fermentation rate. AFEX technology is used to treat alfalfa, wheat chaff, and wheat straw. AFEX technique cannot remove hemicellulose or lignin. Hence, only a tiny amount of material is solubilized. During AFEX pretreatment, hemicellulose is breakdown into oligomeric sugars, and deacetylation occurs, resulting in hemicellulose insolubility. Ninety percent of cellulose and hemicellulose hydrolysis was achieved after AFEX pretreatment of Bermuda grass and bagasse. The efficiency of AFEX pretreatment diminishes as the lignin amount of biomass, such as newsprint, woods, nutshells, and aspen chips, increases. The maximal hydrolysis yield for newspaper and aspen chips after AFEX pretreatment was 40–50%. As a result, AFEX pretreatment is not recommended for the processing of biomass with high lignin content.

Carbon Dioxide Explosion

The treatment of supercritical CO_2 explosions is classified as physicochemical pretreatment. Scientists attempted to generate a technique that would be less expensive than ammonia fiber explosion and work at temperatures lower than stream explosion temperatures. Supercritical carbon dioxide, which acts as a solvent, is used in this method. At ambient temperature, supercritical fluids are compressed above their critical point. Carbonic acid is formed when CO_2 is dissolved in water, with unique properties that make it less corrosive. Carbon dioxide molecules enter tiny pores in lignocellulosic biomass throughout the process because of their small size. The CO_2 pretreatment was carried out at a low temperature, which helped prevent sugar degradation by acid. When CO_2 pressure is released, the cellulosic structure is broken, making the substrate more accessible to cellulolytic enzymes for hydrolysis (Haghighi et al. 2013; Kumar et al. 2009; Kumar et al. 2009; Kumar and Sharma 2017). Dale and Moreira (1982) pretreated alfalfa with carbon dioxide and detected a 75% theoretical release of glucose. Zheng et al. (1998) conducted studies on recycled paper and sugarcane bags to compare the effects of ammonia explosion, steam pretreatment, and CO_2 pretreatment. CO_2 explosion prevention is more economical than AFEX, according to the findings.

3.2.2.2 Mechanical/Alkaline Pretreatment

Individually applied mechanical techniques are rarely appropriate (Souza-Corrêa et al. 2013). Grinding-related mechanical pretreatment has the merit of being environmentally friendly, as it does not require the use of chemicals like alkalis or acids. However, because it is an energy-intensive operation, it is best utilized with different pretreatment procedures to save energy and reduce operational expenses. Lignin creates a physical and chemical hindrance to enzyme exposure to cellulose, and mechanical therapies cannot remove it (Miura et al. 2012; Shi et al. 2015). Various kinds of ball milling and compression milling (dry/wet, centrifugal, jet, and vibratory) have been utilized in a range of research studies, either alone or in combination with other procedures (acid, enzyme, and irradiation) to make material handling easier in future processing (Licari et al. 2016; Rahmati et al. 2020).

3.2.3 Chemical Pretreatment

To chemically pretreat lignocellulosic biomass, acids, alkalis, and organic solvents are utilized. One of the most promising is (Gupta and Verma 2015; Kumar et al. 2020), which can be highly effective in breaking down more complex-structured feedstocks (Pellera and Gidarakos 2018; Bhardwaj et al. 2020a). It also improves carbohydrate bioavailability by expelling lignin and/or decreasing cellulose

crystallinity and degree of polymerization (Behera et al. 2014; Kumar and Verma 2020b). Chemical pretreatment is gaining popularity since it is usually less expensive and results in a faster and more effective breakdown of complex chemical molecules.

3.2.3.1 Liquid Hot Water

This treatment process is also referred to as "hot compressed water." This type of pretreatment uses high temperatures (160–220 °C) and pressures (up to 5 MPa) to keep water in a liquid state. Chemicals and catalysts, on the other hand, aren't employed in the fluid hot water pretreatment procedure (Kumar and Sharma 2017). For around 15 min, liquid water is in contact with lignocellulosic biomass in this process. There is no need for quick leisure or extension in this pretreatment procedure because pressure prevents evaporation. This approach has proven to be extremely productive on sugarcane bagasse, wheat and rye straw, corncobs, and corn Stover. So many researchers utilize different terminology to describe this pretreatment process, such as solvolysis, aqueous fractionation, and hydro thermolysis (Kumar and Sharma 2017; Sparling et al. 2011; Zhuang et al. 2016). Depending on the direction of the biomass flow, pretreatment of liquid hot water can be done in three different manners, with the water flowing straight into the reactor. The first approach includes bringing the biomass slurry and water to a high temperature and keeping it there at pretreatment conditions for a certain period, and then cooling it down. Countercurrent pretreatment is the second approach, which involves pumping hot water against the current. The flow-through pretreatment method, which includes running hot water over lignocellulosic biomass that serves as a fixed bed, is the third alternative.

Abdullah et al. (2014) showed the impact of liquid hot water pretreatment on the varied hydrolysis rates of cellulose and hemicellulose. The optimization of different parameters was examined in two steps of the process. The first phase, hydrolyzing hemicellulose, was carried out with reduced severity, but the second step, cellulose depolymerization, was done with great seriousness. The disadvantage of using liquid hot water for pretreatment is that it uses a lot of energy. The benefit of this method is not using chemicals or catalysts and inhibitors (Sparling et al. 2011).

3.2.3.2 Acid (Weak and Strong Acid Hydrolysis)

In this treatment, acids are applied to pretreat lignocellulosic biomass. Acid pretreatment releases inhibitory compounds, making it a less desirable pretreatment option. After acid pretreatment, inhibitory compounds such as furfurals, aldehydes, 5-hydroxymethylfurfural, and phenolic acids are generated in high numbers. Based on the type of final use, acid therapies are divided into two categories. The first treatment type is short (1–5 min) but uses a high temperature of >180 °C, while the second treatment method is long (30–90 min) but uses a low-temperature 120 °C.

Because of the acid treatment, additional processes of biomass hydrolysis can be skipped although washing is required before the fermentation of sugars to eliminate the acid (Kumar and Sharma 2017; Sassner et al. 2008). Acid pretreatment necessitates the use of reactors that are resistant to corrosive, hazardous, and toxic acids; as a result, acid pretreatment is quite costly. Rectors have been produced in various ways, including flow-through, percolation, shrinking-bed, countercurrent rector, batch, and plug flow. The ability to recover concentrated acid at the end of the treatment is crucial for improving the economic viability of acid pretreatment. They concentrated on lignocellulosic biomass treatment.

Sulfuric Acid

The most often used acid is sulfuric acid. Acid pretreatment can improve the hydrolysis process for producing fermentable sugars from lignocellulosic biomass. Poplar, switchgrass, spruce, and corn stover are all commonly pretreated with sulfuric acid. The sugar content of Bermuda grass and rye was reduced by 19.71% and 22.93%, respectively, after acid pretreatment. Rice straw was processed with aqueous ammonia and dilute sulfuric acid in two phases in a percolation reactor. When using ammonia, a 96.9% reducing sugar yield was attained; however, when using diluted acid, a 90.8% yield was achieved. *Eulaliopsis binata* released 21.02% of sugars, 3.22% lignin, and 3.34% acetic acid, as well as inhibitors in trace levels when exposed to dilute sulfuric acid (Kim et al. 2011; Tang et al. 2013).

Because it is less expensive and more effective, pretreatment with a sulfuric acid concentration of 4 wt% is preferred. The dilute H_2SO_4 responsible for biomass breakdown causes xylose to be broken down further into furfural. At high temperatures, dilute H_2SO_4 hydrolysis is most efficient (Mosier et al. 2005).

Hemicellulose must be eliminated to improve glucose output from cellulose, and diluting H_2SO_4 is an essential instrument for this. For cost-effective biomass conversion, a high xylan-to-xylose ratio is mandatory. Xylan makes about one-third of the total carbohydrate in most lignocellulosic materials.

The first is characterized by intense temperature and constant flow method for modest solid loadings, whereas the second is attributed to the standard batch procedure for substantial solid loadings. The first type's temperature and solid loadings are >160 °C and 5–10%, respectively, whereas the second type's temperature and solid loadings are around 160 °C and 10–40%, respectively (Brennan et al. 1986; Esteghlalian et al. 1997).

In addition to sulfuric and hydrochloric acids, other acids such as oxalic and maleic acids are applied to pretreat lignocellulosic biomass. Oxalic and maleic acids have a higher pK_a value and solution pH than H_2SO_4. Since dicarboxylic acids have two pK_a values, they are more efficient at hydrolyzing biomass as sulfuric and hydrochloric acids.

Lower yeast toxicity, no odor, a more comprehensive pH and temperature range for hydrolysis, and no inhibition of glycolysis are other advantages. The khyd/kdeg ratio of maleic acid is high, favoring cellulose hydrolysis over glucose breakdown.

(Lee and Jefferies 2011), the impacts of oxalic, sulfuric, and maleic acid preprocessing on feedstock were investigated when the severity factor was combined (CSF). Maleic acid produces a more significant xylose and glucose amount that are higher than oxalic acid levels.

Phosphoric Acid

Another study by Geddes et al. (2010) showed the production of sugar monomers by sugarcane bagasse and found that pretreatment with 1% (w/w) H_3PO_4 at 160 °C for 10 min yielded greater monomers, which this pretreatment method was reportedly proper because it successfully hydrolyzes hemicellulose in the substrate. As a result, cellulose is more easily digestible by enzymes.

3.2.3.3 Alkaline Hydrolysis

The hemicelluloses ratio hydrolyzes under low pH, but the cellulose and lignin parts do not. Instead, at a higher pH, lignin is solubilized, which is the basis for several pulping processes used to produce high-quality journal papers. Depending on the degree of the pretreatment, the amount of solid material that dissolves varies substantially. Hemicelluloses and cellulose can be transformed to oligo- or monosaccharides when a high acid level is applied at high temperatures. In considerably more acidic circumstances, carbohydrates break down to different compounds such as furfural, 5-hydroxymethylfurfural (HMF), levulinic acid, and so on. Temperatures of 140–200 °C are typical, with a minute to hours of residence time (Galbe and Wallberg 2019). Some bases, in addition to acids, are utilized in biomass processing. The amount of lignin in the sample had a significant impact on the alkaline treatment's outcome. Although it takes days and hours, treatment with alkali necessitates minimal pressure, temperature, and environmental conditions than other pretreatment processes. Sugar decomposition is reduced in alkali treatment compared to acid treatment, and caustic salt removal and recovery are also possible and straightforward. Ammonium, sodium, calcium, and potassium hydroxides are all alkali pretreatment agents; nevertheless, the much more commonly utilized substance is sodium hydroxide alkali pretreatment agent, while the least is calcium hydroxide but the most influential of all alkali pretreatment agents.

Calcium can be bounced back in insoluble calcium carbonate by neutralizing it with CO_2. Limekiln technology can be used to renew calcium hydroxide. A temperature controller, a tank, a CO_2 scrubber, a water jacket, a water and air manifold, a pump, a tray, a frame, a temperature sensor, and a heating element are required for alkali pretreatment. Making a lime slurry utilizing water is the initial step in pretreatment. The next stage is to spray the slurries lime over the biomass and then keep it for hours or days, depending on the circumstances. Increasing temperature lowered contact time, according to Elshafei et al. (1991), Kim and Holtzapple (2006), Lee et al. (1994), and MacDonald et al. (1983). The crystallinity index

rises as lignin and hemicellulose are eliminated during lime pretreatment. The structural properties that emerge from lime pretreatment influence the hydrolysis of pretreated biomass. Chang and Holtzapple (2000) discovered a connection between three structural aspects: lignin, acetyl content and crystallinity, and enzymatic digestibility. They concluded that (1) significant delignification is adequate to give high digestibility regardless of crystallinity or acetyl concentration. (2) Hydrolysis is aided by the removal of parallel barriers such as dignification and deacetylation. (3) While crystallinity has little impact on the final sugar yield, it is involved in the initial step of the hydrolysis process. Based on these principles, the lignin amount should be reduced to 10%, and it is necessary to terminate all acetyl groups through a particular pretreatment method. Consequently, alkaline pretreatment is critical for enzyme exposure to cellulose. Lignin elimination can significantly improve enzyme function by improving enzyme access to cellulose and hemicellulose while removing nonproductive adsorption sites.

Sodium Hydroxide

In another work, Harun and Geok (2016) used NaOH to pretreat rice straws. At 55 °C, the straw was prepared for 1 h and 3 h, respectively. All samples were treated with a 2–12% (w/v) NaOH solution and a 1:20 (w/v) rice straw to NaOH solution ratio. One of the outcomes was that using 1 h of 12% NaOH enhanced the glucan concentration by 85.6% (compared to untreated straw). In this setting, the delignification of the straw was likewise at its maximum, at 79.6%. Straw was treated for 3 h with 2% NaOH although it gave the highest effective total carbohydrate content, at 79.2% (Galbe and Wallberg 2019).

Hydrogen Peroxide

H_2O_2 is possible to combine the alkaline reagent and hydrogen peroxide. In a broad range of lignocellulosic materials, H_2O_2 enhances enzymatic accessibility, resulting in higher enzymatic hydrolysis yields (Dutra et al. 2018). Lignocellulosic biomass is bleached with the bleaching chemical H_2O_2. Although many free radicals are created during pretreatment, the biomass does not leave residues since it dissolves into oxygen and water with few secondary products. The oxidative fragmentation of lignin side chains leads to lignin removal from the lignocellulosic matrix (Song et al. 2013).

In terms of delignification and enzymatic digestibility, Yuan et al. (2018) discovered that alkaline hydrogen peroxide beats single alkali reagents. However, keeping a constant pH throughout the procedure for avoiding hemicellulose excision, which is an exceptionally essential criterion for process efficiency (Zhuang et al. 2016), limits this technique. Delignification using alkaline hydrogen peroxide enhances lignin depolymerization and requires gentle temperature and pressure settings (Su et al. 2015), lowering inhibitor generation (Rabelo et al. 2014).

Ayeni et al. (2019) evaluated the operating settings for alkaline hydrogen peroxide pretreatment of sugarcane bagasse. They discovered that 0.3% hydrogen peroxide concentration and 100 °C for 4.6 h were the optimal pretreatment conditions. Siciliano et al. (2016) found that treating olive mill residues anaerobically with a low oxidant concentration at room temperature increased the anaerobic treatability by up to 0.328 L/g of COD (chemical oxygen demand) removed. Katukuri et al. (2017) also discovered that treating *Miscanthus floridulus* substrate with 0.8% H_2O_2 boosted methane yield by 49% over untreated *Miscanthus floridulus* substrate.

Alencar et al. (2017), on the other hand, investigated the H_2O_2 recycling technology. They looked at the ability of recycled H_2O_2 in five different reuse cycles and discovered that each one was less efficient than the last.

Before anaerobic digestion, Dahunsi (2019b) investigated the impact of acid or alkaline H_2O_2on pineapple skip preprocessing. The temperature was adjusted around 75–115 °C, the residence period was varied between 6 and 46 min, and the H_2SO_4 concentration was 0–2% (v/v) according to response surface methods. Alkaline H_2O_2 was used in a similar technique. The study's primary findings were that enhanced alkali pretreatment resulted in lignin depletion and a biogas output that was 67% more than the treatment by acid (Galbe and Wallberg 2019).

Wet Oxidation

In this pretreatment process, biomass is treated at high temperatures (>120 °C) for half an hour at 0.5–2 MPa pressure using oxygen/air and water or hydrogen peroxide Haghighi et al. 2013; Varga et al. 2003). This kind of pretreatment is also employed for wastewater treatment and soil remediation. For pretreatment of lignin-rich biomass, this approach has proven to be quite effective. The efficiency of the wet oxidation preprocessing method is influenced by elements, for example, reaction time, oxygen pressure, and temperature. Water behaves like acid at high temperatures, inducing a hydrolysis reaction as the concentration of hydrogen ions grows with temperature, lowering the pH value. In wet oxidation pretreatment, pentose monomers are generated due to hemicellulose breakdown, and lignin is oxidized, but cellulose is unaffected. Some reports of alkaline peroxide or sodium carbonate are added to the mix. The presence of these chemical agents aids in lowering temperature reactions and reducing inhibitory compound production. Attempts to improve hemicellulose breakdown at high temperatures result in inhibitory chemicals such as furfural and furfuraldehyde. However, the number of inhibitors produced by wet oxidation pretreatment is far lower as compared to that produced by liquid hot water pretreatment or the steam explosion approach. Because of two factors, this technique has very little chance of being used on a large scale. The volatile nature of oxygen is one factor, while overprice of hydrogen peroxide utilized during the procedure is another (Bajpai 2016).

Ozonation

Pretreating lignocellulosic biomass with ozone is an ideal approach to reducing lignin. The application of ozone pretreatment to biomass improves its in vitro digestibility. This preparation does not produce inhibitors, a significant benefit because conventional chemical pretreatment leaves hazardous residues. Ozone (O_3) is used as an oxidizer in ozone pretreatment that breaks lignin. O_3 gas is a powerful oxidant that breaks down lignin and creates soluble compounds with lower molecular weight. Wheat straw, bagasse, cotton straw, green hay, poplar sawdust, peanut, and pine can all be prepared with ozone by the breakdown of lignin and hemicellulose; however, all of them are the only materials that can be O3 pretreated (Ben-Ghedalia and Miron 1981; Kumar et al. 2009; Quesada et al. 1999).

A catalytic destroyer of O_3, a trap made of iodine for measuring catalyst potential, an O_2 cylinder, an O_3 generator, a three-way valve, an O_3 UV spectrophotometer, a valve for controlling pressure, and a gas used in the process are all included in an ozonolytic apparatus. Because lignin oxidation decreases as biomass moisture content rises, moisture amount substantially influences lignin oxidation via ozone exposure.

At lower water concentrations, ozone mass transfer is reduced, which impacts its reactivity with biomass. Blocking pores by water film causes a more extended ozone residence period (Mamleeva et al. 2009). Organic acids production lowers the water pH during ozonolysis. Alkaline environments trigger delignification because lignins linked to carbohydrates are removed (Chen et al. 1984; Travaini et al. 2015).

The formation of inhibitory chemicals is linked to biomass delignification. Delignification produces several aromatic and polyaromatic chemicals (Travaini et al. 2013). In a study, Bule et al. (2013) discovered structural alterations in lignin; NMR research demonstrated different lignin subunits have aromatic opening and degradation of -O-4 moieties.

In a spectrum, the concentration of aromatic carbon signals dropped. Changes in methoxy groups were discovered, indicating that the ester-linked structure was disintegrating. Batch reactors, Drechsel trap reactors, fixed bed reactors, rotatory bed reactors, and multilayer fixed bed reactors are the reactor models applied for O_3 biomass processing. Most experts employed plug flow reactors (Li et al. 2015).

Heiske et al. (2013) investigated the properties of single-layered and multi-layered bed reactors to increase the transformation of wheat straw to biogas. A single-layered reactor produced straw with a lignin content of 16.2%, while a multi-layered reactor produced straw with a lignin content of 7.2% at the bottom layer. In ozone-pretreated wheat straw, Kádár et al. (2015) discovered that wax breakdown drives the formation of fatty acid molecules. When corn stover was processed with ozonolysis, Williams (2006) found that over 49% of the lignin was broken down.

3.2.3.4 Solvent Extraction

Scientists have been interested in the application of ionic liquids to pretreat lignocellulose over the term. Ionic liquids containing cations or anions are a new type of solvents with great thermal stability and polarity, as well as a lower melting point and vapor pressure (Behera et al. 2014; Zavrel et al. 2009). Significant organic cations and modest inorganic anions make up ionic liquids: temperature, cations and anions, and pretreatment time all impact the interaction of ionic solvents with biomass.

Ionic liquids conflict with lignocellulosic materials for h-bonding, disrupting networks.1-ethyl-3-methylimidazolium diethyl phosphate-acetate, 1-butyl-3-methylimidazolium-acetate, cholinium amino acids, cholinium acetate, 1-allyl-3 methylimidazolium chloride, and chloride are ionic liquids used to treat rice husk, water hyacinth, rice straw, kenaf powder, poplar wood, wheat straw, and pine (Kumar and Sharma 2017). Imidazolium salts are one of the commonly applied ionic liquids. Dadi et al. (2006) found that using 1-butyl-3-methylimidazolium chloride as a pretreatment doubled the output and frequency of hydrolysis.

Liu and Chen (2006) employed 1-butyl-3-methylimidazolium chloride, referred to as (Bmim-Cl), to pretreat rice straw and observed that it modified the wheat straw structure by Bmim-Cl, resulting in a considerable improvement in the hydrolysis process. Polymerization and crystallinity were reduced, thanks to Bmim-Cl. Research by Kuo and Lee (2009) found a twofold increase in hydrolysis yield from sugarcane bagasse compared to untreated bagasse. In a study by Li et al. (2010), 1-ethyl-3-methylimidazolium-acetate was used to pretreat switchgrass at a temperature of 160 °C for 3 h to eliminate lignin. Tan et al. (2011) observed that 1-butyl-3-methylimidazolium chloride pretreatment of palm trees resulted in 62.9% lignin reduction, enzymatic digestion was improved, while cellulose crystallinity was minimized. After ionic liquid pretreatment, minor changes in biomass composition occurred, but considerable alterations in biomass structure were found. Ionic liquid pretreatment is less desirable than alternative treatments due to its chemical and thermal durability, least hazardous processing circumstances, the low vapor pressure of solvents, and the ability to establish a liquid condition throughout a broad range of temperatures.

Ionic liquids are simple to recycle and don't degrade. Cellulase unfurls and inactivates due to the incompatibility between cellulose with ionic liquids, which is a downside of using ionic liquid pretreatment. Since cellulose dissolves at less temperatures and viscosities, viscosity is an essential factor in terms of average energy consumption, ionic liquids should be addressed. Mäki-Arvela et al. (2010) proposed that high temperatures result in different side reactions and unfavorable effects, such as ionic liquid stability deterioration.

3.2.4 Biologicals Pretreatments

Pretreatment with biologicals is a viable alternative to chemical pretreatment, but it has a slower reaction rate which is inconvenient for business (Wyman et al. 2018).

3.2.4.1 Fungi

Various microorganisms, including white, brown, and soft rot fungus, are used to pretreat lignocellulose. White-rot fungi are the most well-known and influential delignification microorganisms (Raveendran et al. 2016; Agrawal and Verma 2021). Although brown rot fungi contribute to lignin degradation, white-rot fungi are the most prominent and influential delignification microorganisms. Auxiliary enzymes such as hydrolytic enzymes, cellobiose dehydrogenase, aryl alcohol oxidase, glyoxal oxidase, copper oxidase, and cellobiose dehydrogenase are released by most fungi that degrade lignocellulose, causing the concurrently or specific destruction of cellulose and hemicellulose, as well as lignin (Rudakiya and Gupte 2017; Alam et al. 2021; Verma 2022; Bhardwaj et al. 2020b).

These enzymes help break down lignin and polysaccharides by producing free radicals and intermediates. The effects of biological pretreatment with laccase, manganese peroxidase, and versatile peroxidase at different incubation times—0, 6, and 24 h—were investigated by Schroyen et al. (2014). The presence of phenolic compounds, which are methane inhibitors, was not detected in the pretreatment. The laccase enzyme increased biomethane synthesis by 25% after 1 day of the process of incubating bacteria, but prearrangement with peroxidase enzymes increased the biomethane output by 17% while decreasing the process of incubating bacteria incubation time from 1 day to 6 h.

To improve methane generation, Liu et al. (2017) used *Ceriporiopsis subvermispora* to evaluate fungal pretreatment of two forest wastes, namely hazel and acacia branches, and two agricultural leftovers, namely barley straw and sugarcane bagasse. Compared to untreated hazel, the fungus' manganese peroxidase and laccase increased methane emissions by 100%. While fungal pretreatment of lignocellulosic biomass is favorable to the environment and the economy, they are time-consuming. According to Liu et al. (2017), a unique bioreactor should develop aerobic and aseptic conditions for fungal pretreatment.

According to Van Kuijk et al. (2015), selecting substrate-specific and effective strains and recovery duration and carbohydrate losses can be reduced by changing the cultural conditions. Biogas generation from lignocellulosic biomass can be boosted via inoculation with *Neocallimastix frontalis*, an anaerobic fungus isolated from the rumen, according to Dollhofer et al. (2018).

3.2.4.2 Bacteria

Microbial pretreatment surpasses enzymatic pretreatment in the anaerobic digestion process due to their greater variety and tolerance to external parameters such as temperature and pH. Rouches et al. (2018) isolated bacterial strains from soil (*Bordetella muralis* VKVVG5), silverfish stomach (*Citrobacter werkmanii* VKVVG4), and millipede (*Paenibacillus s*p. VKVVG1) and used them to speed up the hydrolysis of water hyacinth.

Water hyacinth was pretreated for 4 days with *Citrobacter werkmanii* VKVVG4 at a rate of 109 CFU/mL, which enhanced the solvability by 33.3%. Others, on either hand, proposed using anaerobic lignocellulose degrading consortiums. According to Kong et al. (2018), in mesophilic and thermophilic settings, anaerobic consortia TC-5 decomposed wheat straw by 45.7% and increased methane output by 22.2% and 36.6%, respectively.

During 24 h of exposure, rumen fluid was proven to enhance the productivity of rapeseed stems and leaves by 47% (Baba et al. 2017). Yuan et al. (2016) pretreated cotton stalks with the thermophilic microbial consortia MC1.

The findings confirmed that the amounts of soluble chemical oxygen and volatile organic compounds rise rapidly in the early phases of the pretreatment, peaking the highest methane CH_4 output at 128 mL/g over 6 days of MC1 pretreatment. The CH_4 emission was not distinguishable between 122 mL/g and 122 mL/g. Sawdust inoculated with a microbial consortia LCDC obtained from decaying sawdust produced 72.6% more methane than control, according to Ali et al. (2017). They found a significant decline in lignocellulose contents during 10 days of treatment (Hernández-Beltrán et al. 2019).

3.3 Conclusions

The most prevalent materials for biofuel production are lignocellulosic substrates, and this digestion procedure aids in waste disposal while also providing one of the most important sources of renewable bioenergy. Because of their low bioactivity, the structure of lignocellulosic residues continues to pose technological challenges, and proper implementation of digesting technology requires the pretreatment of these resistant substrates. However, as it can be observed, the key constraints are energy expenses, operating expenses, and the generation of anti-proliferative chemicals, all of which substantially impact the downstream bioprocess for producing biofuel or additional products with enhanced value.

A mix of diverse pretreatment may provide a solution that can be tailored to the substrates and downstream bioprocesses to yield bioenergy and certain other products. Biorefineries that produce two or more bioproducts on the same system are inclusive biorefineries, which could be a potential approach for establishing the techno-economic viability of lignocellulose substrate use.

Competing Interests All the authors declare that they have no competing interests.

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Chapter 4
The Pioneering Role of Enzymes in the Valorization of Waste: An Insight into the Mechanism of Action

Anupama Binoy, Revathy Sahadevan, Suchi Chaturvedi, and Sushabhan Sadhukhan

Abstract Finite petro-based reserves and a surge in environmental pollution demands the valorization of waste into revenue streams like biofuels and other industrial commodities. Enzymatic technology provides an eco-friendly platform for the same with higher product yields. Enzymes act as a catalyst in the reaction, and the matter of value addition in this technology is its requirement in low quantity and reusability. They have been included in the valorization of lignocellulosic (woody, agro, and food) waste, treatment of wastewater, and degradation of non-biodegradable hazardous waste. Microbial flora has enormously experimented as well as explored in the conversion of this waste into valuable products. In addition to that, protein engineering and metabolic engineering have been seen as new biotechnological trends in the same field. In this chapter, we will focus on different classes of hydrolytic enzymes based on the structural composition of different types of biomass with special attention to their catalytic activity. The mechanistic action of these enzymes will also be discussed in lieu of their use at various stages in the

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P. Verma (ed.), *Thermochemical and Catalytic Conversion Technologies for Future Biorefineries*, Clean Energy Production Technologies,
https://doi.org/10.1007/978-981-19-4312-6_4

transformation of waste to value-added substances. We will also shed light on the future advancement through the biotechnological revolution in the field of enzyme technology.

Keywords Hydrolytic enzymes · Valorization of waste · Lignocellulosic waste · Wastewater · Biorefinery

Abbreviations

AAO	Aryl alcohol oxidase
BOD	Biological oxygen demand
CAZy	Carbohydrate-active enzyme
CLEAs	Cross-linked enzyme aggregates
COD	Chemical oxygen demand
DNA	Deoxyribonucleic acid
DyP	Dye-degrading peroxidases
EG	Ethylene glycol
FAD	Flavin Adenine Dinucleotide
GH	Glycoside hydrolases
GHG	Greenhouse gas emission
GLOX	Glyoxal oxidase
GOS	Galacto-oligosaccharides
LiP	Lignin peroxidase
MnP	Manganese peroxidase
MSW	Municipality solid waste
P2O	Pyranose 2-oxidase
PAH	Polycyclic aromatic hydrocarbons
PCR	Polymerase chain reaction
PE	Pectin esterase
PE	Polyethylene
PET	Poly ethylene terephthalate
PG	Polygalacturonase
PGL	Polygalacturonate
PL	Pectin lyase
PMG	Polymethygalacturonase
PMSF	Phenyl methane sulfonyl fluoride
POPs	Persistent environment pollutants
PP	Polypropylene
PS	Polystyrene
PUs	Polyurethane
PVC	Polyvinyl chloride
RNA	Ribonucleic acid
TPA	Terephthalate
VP	Versatile peroxidases;
XYNII	Endo-1,4-ß-xylanase II

4.1 Introduction

The global surge in population and simultaneous automation have resulted not only in depletion of petro fuels reserve but have also piled up different forms of waste globally. In the global waste management outlook, GWMO 2015 stated that the global waste piling accounts for nearly 7–10 billion tonnes which include households, commercial, industrial, and construction-based waste (Wilson and Velis 2015; Agrawal and Verma 2022). Moreover, the major issues in front of humankind have been climate change, greenhouse gas emissions (GHG), depleting resources, and increasing pollution for a long. Waste management has existed in our society for a long in the form of landfills, incineration, composting, etc. but they are least suitable for organic waste due to problems associated with them like the generation of toxic gases such as methane and toxic leachates to the underground water bodies, etc. This calls upon the urgency to frame an economy based on renewable resources fulfilling the shooting energy demands (Kuo 2019; Goswami et al. 2022; Agrawal and Verma 2021) and also builds up sustainable methods to convert our waste into valuable revenue streams. Adapting to a sustainable way can be envisioned by valorizing waste into biofuels (different forms of bioenergy to replace fossil fuels), biomaterials, and other value-added bio-ingredients. This process is commonly associated with the concept of biorefineries where the waste biomass is upgraded and transformed into a spectrum of invaluable and marketable commodities. Waste can be of different forms. The most common and important problem faced during the valorization of waste is the complexity of the composition of waste. Organic or biomass waste is solid or liquid waste that can be found in many forms like agro and food waste, forestry residues, waste generated from food processing industries, etc. The basic units of the biomass waste are rich in protein, sugar, and fat, which indeed make them an ideal feedstock for enzymatic valorization. Enzymes are introduced at various steps in the process of valorization. For instance, the lignocellulosic mass from the agro-waste is subjected to pretreatment by exposing them to delignifying enzymes which removes the protective lignin component. Thus, enabling the hydrolyzing enzymes to easily solubilize the polymers like cellulose, and hemicellulose into their monosaccharides and oligosaccharides. These monomer units are either subjected to fermentation using microbes for the production of biofuels or enzymatic modification like oxidation/phosphorylation for valorizing into valued products (Andler and Goddard 2018; Bhardwaj and Verma 2021). Enzymes are molecular catalysts triggering biochemical reactions. They catalyze the reaction with high substrate and product selectivity at optimum temperature and pressure. Reusing enzymes for several reactions further makes the procedure cost-effective. All this opens up an opportunity for introducing greener processing strategies that are more sustainable for the ecosystem (Kennedy et al. 2006). Enzymes are ubiquitous in all forms of life performing different purposes and therefore can be extracted from them through purification and characterization (Yada 2015; Bhardwaj et al. 2021a; Kumar and Verma 2020a). Systems biology has encompassed a role in understanding the molecular basis of different enzymes. Through various bioinformatics tools and

algorithms, system biology enables us to form a link between different biological components in an organism thereby allowing us to modify and decipher newer enzyme candidates involved in waste valorization as well (Bhatt et al. 2019). Here in this chapter, we will be focusing on different enzymes which participate in hydrolysis of biomass component. As enzymes are very specific to the composition of its substrate, we will also shed light on the composition of different types of biomasses. To better understand the action of hydrolyzing enzymes and their application, it is necessary to take a glimpse at the different characteristics of enzymes and the different factors which affect the activity of enzymes. This chapter will also highlight the advanced enzyme technology and trending opinion on increasing the efficacy of enzymes for valorization of waste.

4.2 Enzymes and Their Characteristics

Enzymes are biological catalysts known to increase the rate of biochemical processes happening inside a living organism (Blanco and Blanco 2017). They were first introduced by Frederick W. Kuhne as molecules of higher molecular weight that help in the fermentation of sugar to alcohol. Enzymes are mostly composed of proteins except for some RNA which exhibit enzymatic characteristics in certain biological processes. Enzymes are associated with cofactors (inorganic metal ions like Cu^{2+}, Zn^{2+}, Mn^{2+}) and/or coenzymes (organic or organometallic complexes) for their activation and function (Renneberg et al. 2017). An enzyme without the cofactor or coenzyme is known as an apoenzyme and the complete active form is referred to as the holoenzyme (Litwack 2018). The accurate structural conformation of enzymes plays a crucial role in determining their activity and any parameters, physical or chemical, that alter the native confirmation will affect the efficiency of their catalytic activity. Based on the type of catalytic reactions, enzymes are categorized into six groups. *Oxidoreductases* catalyze oxidations or reduction reactions. *Transferases* help in the transferring of functional groups between two molecules. *Hydrolases* catalyze hydrolysis (lysis in the presence of water). *Lyases* are involved in the removal or addition of groups to form or reduce double bonds. *Isomerases* catalyze the internal arrangement of atoms in a molecule to form isomers and finally, *lipases* catalyze condensation reactions to form bonds between carbon–sulfur, carbon–carbon, or carbon–nitrogen (Blanco and Blanco 2017; Kumar and Verma 2020b).

4.2.1 Enzyme–Substrate Interaction

An enzyme facilitates a suitable environment for the substrates to form products at an enhanced rate. The substrate undergoes non-covalent interaction with the enzyme in a specific site called the active or substrate-binding site of the enzyme to form a

substrate–enzyme complex. This enzyme–substrate complex later transforms into a product and releases the enzyme back. The catalytic action of enzymes is favored at optimal pH, temperature and ionic strength, etc. and any alterations will affect the catalytic activity of the enzymes. For example, a temperature rise will cause an alteration in the enzyme's native conformation (denaturation) which will reduce their catalytic activity. Small molecules can also affect the activity of enzymes and are known as inhibitors. The important difference between competitive and noncompetitive inhibitors is their preference for the binding site on enzymes. Competitive inhibitors compete for the active site on the enzyme whereas noncompetitive inhibitors engage with sites other than that of the active site. Both the inhibitors eventually bring conformational changes in the structure of the enzyme and facilitate inhibition of its activity. The active site of an enzyme has a unique and specific sequence of amino acids which in turns increases substrate specificity and selectivity. Enzyme specificity is first explained by the "Lock and Key model" introduced by Emil Fischer in 1894. The assumption of the rigid structure of enzymes was falsified later in 1958 through the "Induced Fit Model" proposed by Daniel Koshland where the concept of the transition state of the enzyme–substrate complex was introduced. According to this model, the conformation of the enzyme's active site as well as in some cases conformation of the substrate itself will undergo small changes during the formation of the enzyme–substrate complex (Blanco and Blanco 2017).

4.2.2 Enzyme Thermodynamics and Kinetics

An enzyme, just like any catalyst increases the reaction rate by lowering the activation energy of the reaction (Fig. 4.1).

The kinetics of the enzyme–substrate is explained by Leonor Michaelis and Maud Menten in 1913. Michaelis-Menten theory is based on the following reaction between enzyme (E) and substrate (S) to yield the product (P) through the formation of the enzyme–substrate complex (ES).

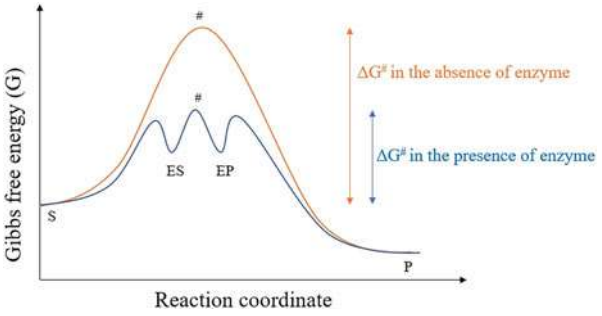

Fig. 4.1 Energy profile diagram of an enzyme-catalyzed and uncatalyzed reaction. Where S represents a substrate, ES is the enzyme–substrate complex, EP is the complex of enzyme and product just before their degradation to product, P, and # represents the transition state

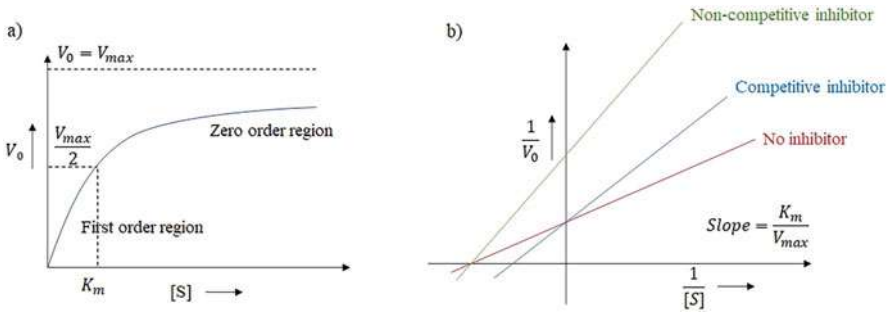

Fig. 4.2 The graphical representation of (**a**) Michaelis-Menten equation; (**b**) Lineweaver-Burk plot in the absence of inhibitor and the presence of competitive and non-competitive inhibitors

$$\mathrm{E} + \mathrm{S} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} [\mathrm{ES}] \xrightarrow{k_2} \mathrm{P} + \mathrm{E} \tag{4.1}$$

Michaelis and Menten derived an equation for the rate of the catalytic action of enzymes based on the assumption that the formation of ES and the reverse reaction to E and S is faster than its conversion to P and E and then applied the steady-state approximation for ES complex. The Michaelis-Menten equation is given as,

$$V_0 = \frac{V_m[S]}{[S] + K_m} \tag{4.2}$$

where V_0 is the rate at which ES converts into a product, V_m is the maximum reaction rate at the saturated concentration of the substrate, [S] is the concentration of substrate, and K_m is Michaelis constant, it is the concentration of the substrate needed to acquire $V_m/2$. The graphical representation of this equation, V_o *vs* [S] plot is given in Fig 4.2a. This equation is applicable to single enzyme–substrate interaction at constant enzyme concentration. At a low concentration of substrate, the enzymatic reaction follows first-order kinetics where the rate of reaction is proportional to substrate concentration. At a high concentration of substrate, the reaction follows zero-order kinetics, which means the rate of reaction is independent of substrate concentration.

Enzyme activity in the presence of inhibitors is well understood from the Lineweaver-Burk plot which is a graphical representation of the reciprocal of the Michaelis-Menten equation, as shown in Fig 4.2b.

$$\frac{1}{V_0} = \frac{1}{V_{\max}} + \left(\frac{K_m}{V_{\max}}\right)\frac{1}{[S]} \tag{4.3}$$

4.3 Chemical and Structural Factors Guiding Enzyme Hydrolysis

Biomass waste is biodegradable organic matter produced directly or indirectly by the energy utilized from the sun through the process of photosynthesis. The source of waste is very diverse and varied in terms of its composition. They are mainly composed of constituents like lignocellulosic biomass, starch, chitin, triglycerides, proteins, etc. (Tuck et al. 2012; Kumar and Verma 2020b). Due to the high fixed carbon content in them, these feedstocks have been in past and are even now used to generate energy and heat by burning them which could pose threat to the environment by the release of toxic air pollutants such as PAH (polycyclic aromatic hydrocarbons), particulate matters, POPs (persistent organic pollutant) (Sivertsen 2006). Policymakers have directed the industries to focus on the sustainable valorization of worldwide generated biomass so that such a resourceful matter is not just dumped as waste or burned into ashes. Currently, they are utilized as major feedstock in biorefineries for biofuels production, biogas, and biofertilizers.

4.3.1 Composition of Lignocellulosic Biomass

Lignocellulosic biomass is categorized based on the source of its generation. It could be organic matter from woody terrestrial forest residues, herbaceous residues from agriculture (corn cobs, sugarcane bagasse, rice and wheat husk, fruits and vegetable residues generated from fields and market), green waste from Municipality Solid Waste (MSW), animal and human sources, aquatic organic mass, and organic mass generated by anthropogenic ways as well. The sustainable utilization of biomass offers a huge advantage as they are widely available worldwide, reduces the overall cost of fuels by introducing them as an alternative source, and finally, contributes to the reduction of greenhouse gas emissions. Moreover, the overall production and process cycle of sustainable alternatives exhibit a zero-carbon dioxide balance. Lignocellulosic biomass is structurally composed of cellulose, hemicellulose, protein, lipids, etc. They also contain some active ingredients like antioxidants, polyphenols, lignin, pigments/carotenoids, etc. These constituents are arranged in layers to form the complete lignocellulosic biomass structure with lignin being the outermost layer, hemicellulose occupying the middle space, and cellulose placed at the core of the mass (Fig. 4.3).

Interestingly, cellulose is the major substrate in the biorefineries for biofuels and chemical commodities production. The percentage of these components varies based on the type and source of lignocellulosic biomass. Almost 15% of the total lignocellulosic mass comprises protein also. The lignin component renders a high resistance to cellulose access by the hydrolytic enzymes. Various pretreatment methods have been adopted to remove the lignin part to give more access to hydrolytic enzymes to degrade the high polysaccharide component of cellulose and

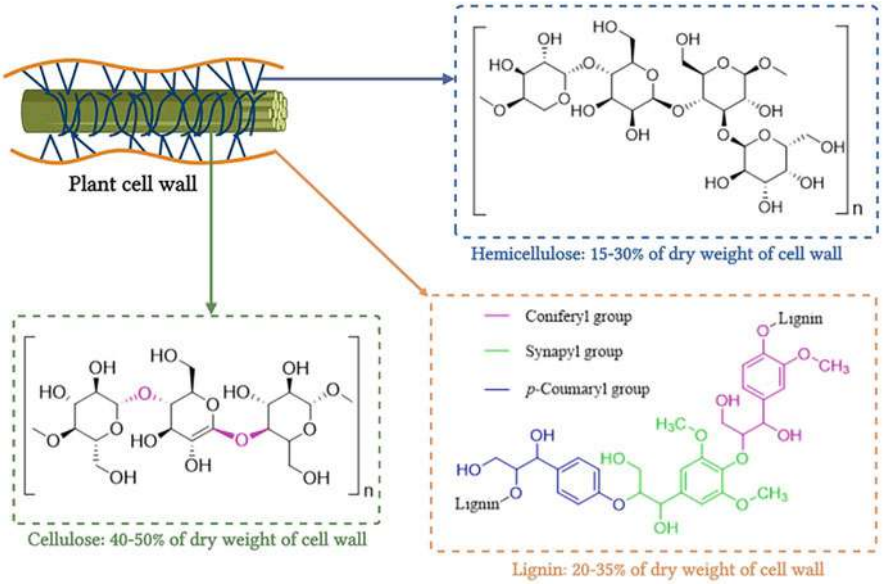

Fig. 4.3 Structural representation of lignocellulosic biomass: The plant cell wall is composed of a lignocellulosic structure where lignin forms the outermost covering, followed by hemicellulose and the most internal part is made up of cellulose components

hemicellulose. Depending upon the chemistry of the components present in the lignocellulosic biomass, enzymes are selected to degrade it.

4.3.1.1 Cellulose

Cellulose is an important cell wall component of the plant. It is a high molecular weight polysaccharide composed of D-glucose units linked with each other through *β*-1,4-glycosidic bonds forming the basic repeating unit called cellobiose, 4-*O*-*β*-D-glucopyranosyl-D-glucopyranose (structural basis of cellulose) (Fig. 4.4a). A cellulose primary chain is composed of almost 500–1400 monomer units. These primary chains are further arranged in a parallel array to form the higher structural unit called microfibril. Several microfibril units form cellulose fibril, the higher structural unit (Robak and Balcerek 2018; Bhardwaj et al. 2021b). The extensive intramolecular (O_6–O_2 and O_3–O_5) and intermolecular (O_3–O_6) hydrogen bonds and Van der Waals forces in cellulose structures give its crystalline nature, high tensile strength, and recalcitrance to hydrolysis. The amorphous cellulose corresponds to regions where the above-mentioned molecular bonds are disrupted giving twists and torsions to the structure resulting in interspersed disordered regions in cellulose. The structural or crystalline region of cellulose is highly packed not allowing even a single water

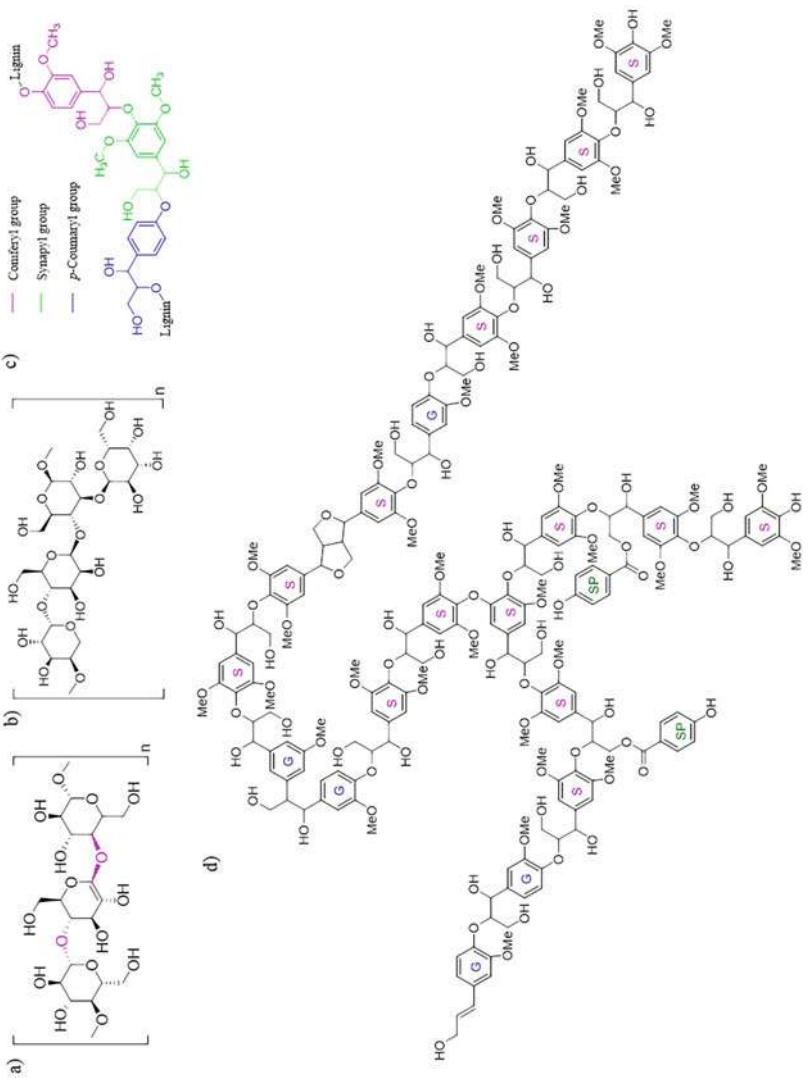

Fig. 4.4 Chemical structures of components present in lignocellulosic plant's cell wall: (**a**) basic skeletal structure of cellulose: Glucose units combined by *β*-(1,4)-glycosidic linkages; (**b**) basic skeletal structure of hemicellulose having *β*-(1,4)-xylose-*β*-(1,4)-agarose-*β*-(1,4)-mannose-*β*-(1,4)-glucose-*α*-(1,3)-galactose motifs; (**c**) basic skeletal structure of lignin component; and (**d**) Structure of lignin in poplar plant. Where S is the syringyl group, G is the guaiacyl, and SP is the synapyl basic groups

molecule to enter, but the distorted amorphous region is very easily accessible to enzyme hydrolysis (Betts 1991).

4.3.1.2 Hemicellulose

Hemicellulose stands second to cellulose in terms of its abundance on the planet. In comparison to cellulose, hemicellulose is highly branched with almost 500–3000 sugar units as monomers. Hemicellulose is a heteropolymer comprising of side chains with xylans as pentoses, mannans, and glucomannans as hexoses or arabinogalactans and galactans as galactose units. Apart from monosaccharides, they also accompany typical uronic acids like D-glucuronic, 4-*O*-methylglucuronic, and D-galacturonic acids (Fig. 4.4b).

Xylan: Xylans are water-soluble polysaccharides made up of repeating units of *β*-D-xylopyranose linked by *β*-(1,4)-glycosidic bonds. This primary chain also comprises other carbohydrates such as xylose, mannose, arabinose, rhamnose, or 4-*O*-methylglucuronic acid.

Mannan: Mannans are the most important constituent of hemicellulose which helps the hemicellulose to bind to the cellulose counterpart. They are widely found as a component in the angiosperm cell wall. *β*-D-mannopyranosyl units are formed by *β*-1,4 linkage along with a small ratio of galactans in the linear chains. There are four different types of mannans present: galactomannans, galacto-glucomannans, glucomannans, and linear-mannans.

Galactans: Galactans are made of galactose as repeating units linked through *α*-1, 3, and *β*-1,6 linkages to form 4-*α*-D-galactopyranosyl and 3-*β*-D-galactopyranosyl attached in alternate fashion. They are long polymeric chains not commonly found in all forms of plants. They are majorly found in some algae, seeds, buds, or flowers (Li et al. 2013).

4.3.1.3 Lignin

Unlike cellulose, lignin has an irregular three-dimensional structure with no specific repeating units. Lignin acts as the protective and cementing cover in plants which helps arrange the fibers together to enhance the compactness of the wood thereby making it more resistive. It helps in gluing hemicellulose with cellulose, and it resists the access of enzymes to cellulose by acting as a physical barrier. It is an amorphous organic compound comprising phenylpropanoid units with three different types of *p*-hydroxycinnamyl alcohol: coniferyl alcohol, sinapyl alcohol, *p*-coumaryl alcohol (Fig 4.4c, d). Overall lignification is species specific and is obtained by several cross-linking reactions between the radicals formed by oxidation and resonance delocalization in phenylpropanoid monomeric units. Lignin is synthesized in the plants via shikimic acid pathway. The structural integration of different cellulosic components makes it very recalcitrant to hydrolysis by enzymes. The cellulose and hemicellulose are attached through hydrogen bonds, meanwhile, lignin forms five different types of

lignin carbohydrate bonds to bind to hemicellulose: γ-esters esters, benzyl ethers, phenyl glycosides, ferulate/coumarate esters, and hemiacetal/acetal linkages (Giummarella et al. 2019; Agrawal and Verma 2020a, b). The biorefineries arena focus on the effective valorization of lignocellulosic materials into valuable products by introducing controlled cleavage of carbon–carbon and carbon–oxygen bonds present in the recalcitrant lignin on the outermost coat. The focus is also maintained on getting rid of various impurities (organic and inorganic).

4.3.2 Starch

Food is stored in plants in the form of starch in seeds, roots, and a little amount in the residual biomass. It is composed of two types of polysaccharides, namely amylose and amylopectin. Amylose is formed by polymerizing D-glucose via α-1,4 linkages linearly, on the other hand in amylopectin, which has branched formation is formed by α-1,4 glycosidic bonds linearly, and α-1,6 glycosidic linkages for branched chains (Whistler and Daniel 1984).

4.4 Enzymes and their Application in Waste

Despite the sustainable availability of biomass for conversion into bioenergy, the process is expensive and time-consuming as it requires lengthy downstream processing for the collection of final products. Before subjecting the different components of waste to hydrolysis, pretreatment of the complex material is required which is the most expensive process in the transformation. It is done to remove the recalcitrant lignin component which remains the major hindrance in exposing the buried cellulose to the saccharification process. The pretreatment involves subjecting the lignocellulosic mass to either high pressure, temperature, or chemical treatment or enzymatic hydrolysis. The chemical pretreatment includes the use of chemicals like organic solvents, concentrated acids/bases, or neoteric solvents which are very harsh and corrosive. Additionally, the process becomes more tedious as many steps are required to separate the final products from the chemicals used in pretreatment. The highly concentrated and corrosive acids and alkali are damaging to the equipment too. There are a lot of unwanted products formed during the process of pretreatment with chemicals that can act as inhibitors to the microbial enzymes used for fermentation. Therefore, the use of hydrolytic enzymes proves to be useful as it poses less cost and also reduces the difficulties faced in the downstream process (Manisha and Yadav 2017). As discussed earlier, the most resourceful feedstock for the production of green fuel is biomass waste, and it has been in regular consideration and experimentation under the biorefinery concept (Azapagic 2014). To switch to industrial symbiosis, i.e., waste from one sector is used as feedstock in another

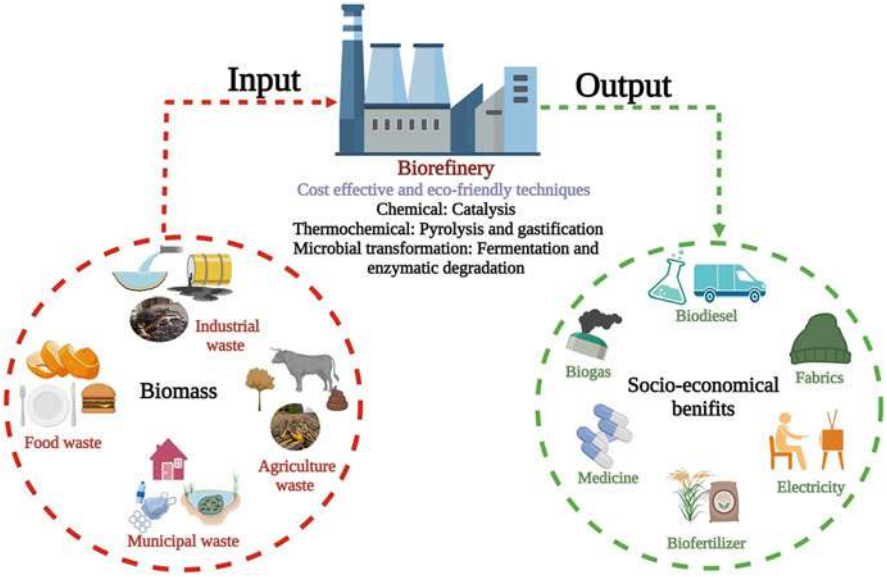

Fig. 4.5 A model of biorefinery concept: Biomass feedstocks from various sources are taken as raw material in biorefinery and converted into various forms of bioenergy and other value-added products

industry; therefore, it is very necessary to identify, characterize, and quantify the residues present in the waste stream (Fig. 4.5).

Microorganisms and their enzymes have long been core tools in the biofuel refineries, being present in all stages, starting from pretreatment, hydrolysis, and fermentation. They can be introduced as biocatalysts with the substrates where they are produced in situ by microorganisms or they are added ex situ in purified form or as enzyme cocktails. In this section, we will be considering the important enzymes, especially the hydrolytic type in saccharification of polymers into monomers and will also discuss the mechanism of their actions.

4.4.1 Hydrolytic Enzymes Involved in the Valorization of Lignocellulosic Waste

The main source of lignocellulosic biomass is the organic residues obtained from human activities such as agricultural waste and food processing industries. Moreover, solid municipal waste which mostly comprises paper and organics can be included as an important lignocellulosic waste stream for valorization into valuable products. The biopolymers included in lignocellulosic biomass constitute cellulose, hemicellulose, and lignin. The major enzymes required to saccharify these polymers

are cellulases, hemicellulases, and lignin-degrading enzymes. These enzymes are classified under the single-family of glycoside hydrolases (glycosidases or carbohydrases), E.C 3.2.1, and are involved in the catalysis of *O*-glycosidic bond hydrolysis (van Wyk et al. 2017; Bhardwaj et al. 2020). The gene corresponding to this class of enzyme is present in all living organisms except in some Archaeans and some unicellular parasites. Glycoside hydrolases cleave the glycosidic bonds via two different mechanisms based on the status of the anomeric configuration during the reaction. The net inversion of an anomeric configuration is achieved as a result of a one-step double displacement reaction between the acidic and basic amino acid groups whereas the retention of the anomeric configuration happens via a two-step double displacement reaction involving acid/base and nucleophilic assistance provided by amino acid residues (Naumoff 2011). All the information concerning genomic, structural, and functional aspects of glycoside hydrolases and their family members is available in a highly curated, knowledge-based database known as the carbohydrate-active enzyme (CAZy) database. This database states that glycoside hydrolases (GHs) are categorized into 135 different families and 14 clans. This classification was based on their overall structural confirmation, amino acid sequence, and function (Lombard et al. 2014).

4.4.1.1 Cellulases

Cellulase is a class of enzymes that hydrolyzes the β-1,4-glycosidic bonds in polysaccharides like cellulose to glucose units and is grouped among glycoside hydrolases (GH). Cellulose from fungus has two domains, namely a catalytic domain, which performs the catalytic activity, and a cellulose-binding domain, which anchors the enzyme to the cellulose substrate. Both the domains are linked together through a linker domain. The hydrolytic action depends upon the synergistic action of three major enzymes, cellobiohydrolase/exoglucanase (E.C 3.2.1.176)/(E.C 3.2.1.91), endocellulase/endoglucanase (E.C 3.2.1.4), and β-glucosidase (E.C 3.2.1.21) (Horn et al. 2012; Kostylev and Wilson 2014). Endocellulases hydrolyze the amorphous area of cellulose to release long-chain oligomers with non-reducing ends which are then acted upon by exocellulase or cellobiohydrolases on the β-1,4-glycosidic bonds liberating β-cellobiose. Cellobiohydrolases act on the oligomers from the reducing ends whereas exocellulase act on the non-reducing ends. β-glucosidase hydrolyzes the smaller glucans or disaccharide cellobiose into the monomeric glucose (Juturu and Wu 2014) (Fig. 4.6a).

Several microorganisms have been found to produce cellulose enzymes. Among bacteria the most important are *Clostridium* species, *Pseudomonas* species, and *Trichoderma reesei* whereas the major cellulose-producing fungi belong to *Aspergillus species*, *Fusarium species*, *Penicillium species*, *Schizophyllum commune*, and *Melanocarpus species.* In anaerobic bacteria, cellulose occurs as cellulosomes, an extracellular aggregated enzyme structure. Endocellulase or endoglucanase belonging to the family glycoside hydrolases (GH) 5 comprises a single catalytic subunit made up of 335 amino acids folding into the active enzyme. The structural

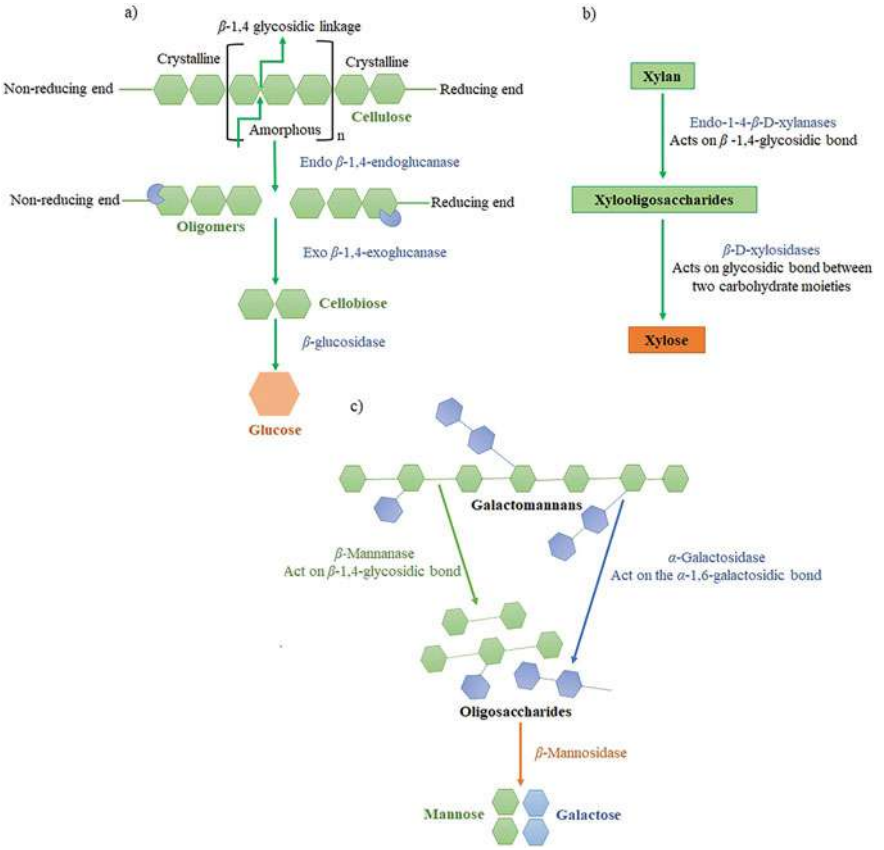

Fig. 4.6 Mechanism of action of cellulases and hemicellulases: (**a**) Endocellulase, exoglucanase, and β-glucosidase act in synergy for the degradation of cellulose. (**b**) The mechanism of action of endoxylanase and β-glucosidase on the degradation of xylan to xylose; and (**c**) synergistic action of β-mannanases, β-mannosidase, and α-galactosidases on the degradation of various units in mannan component of hemicellulose

architecture of endoglucanase has eight $(\beta/\alpha)_8$ barrel-shaped loops along with a short double-stranded anti-parallel β sheet and three single turns helices. The catalytic substrate-binding site has two glutamate amino acid residues at positions 133 (acid-base) and 240 (nucleophile) which are highly conserved and decisive in the first step of the reaction (Lo Leggio and Larsen 2002) (Fig. 4.7a). Cellobiohydrolases the exoglucanases belong to the family GH 7. The three-dimensional structure of cellobiohydrolases is made up of 431 amino acid residues and exhibits a β-jelly roll structure with two anti-parallel β-sheets to each β-jelly roll. Each β-sheet curves to form concave and convex shapes which are connected through four α-helices. Amino acid glutamine at positions 207 and 212 at the active site of the enzyme participates in the acid-base reaction mechanism (Fig. 4.7b) (Muñoz et al. 2001). β-

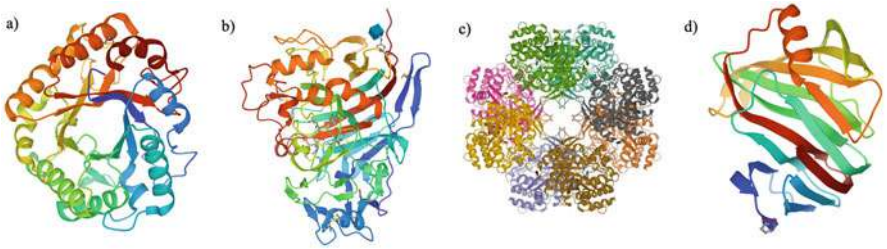

Fig. 4.7 Crystalographic structure of (**a**) endoglucanase enzyme obtained from *Thermoascus aurantiacus* (PDB I.D: 1GZJ) (Lo Leggio and Larsen 2002); (**b**) exoglucanase enzyme obtained from *Phanerochaete Chrysosporium* (PDB ID: 1GPI) (Muñoz et al. 2001); (**c**) *β*-glucosidase enzyme obtained from *Bacillus polymyxa* (PDB ID: 1BGA) (Sanz-Aparicio et al. 1998); (**d**) endo-1-4-β-D-xylanases enzyme obtained from *Trichoderma reesei* (PDB I.D: 1ENX (Törrönen et al. 1994)

glucosidase from the strain *Bacillus polymyxa* (BglA) exists in a tetramer of dimers arranged in an octameric confirmation. The enzymes form aggregates due to their intracellular localization. The substrate-binding site of the enzyme accommodates Glutamine at 166 and 352 which acts as acid/base and nucleophile in hydrolysis reaction, respectively. The substrate binding is also influenced by Histidine residue at 121 as well as tyrosine residue at 296 positions (Sanz-Aparicio et al. 1998) (Fig. 4.7c). Cellulases have been utilized widely in various industries like brewery distilleries, textile processing, paper pulp industries, detergent production, cattle feed processing, and recently been introduced in the production of biofuels.

4.4.1.2 Hemicellulases

This class of enzyme is also known as hemicellulose degrading enzymes. They are involved in the depolymerization of components present in the hemicellulose portion of lignocellulosic biomass such as galactans, xylans, mannans, and arabans. Mannanases, *α*-glucuronidases, and *α*-arabinofuranosidases are widely discussed as well as utilized enzymes.

Xylanases: Xylanase enzymes constitute two major enzymes which include endo-1-4-*β*-D-xylanases (EC 3.2.1.8) and *β*-D-xylosidases (E.C. 3.2.1.37). The endoxylanases hydrolyze xylan into xylooligosaccharides which are further acted upon by xylosidases to yield monomeric xylose (Fig. 4.6b). The structural analysis of endo-*β*-1,4-xylanase II (XYNII) from *Trichoderma reesei* reveals that the enzyme exists as a single domain with 190 amino acid residues folded into two anti-parallel *β*-sheets arranged parallel to each other (Fig. 4.7d). The active site cleft is formed by twisting the β-sheets, and it accommodates two glutamic acid residues at positions 86 and 177 (Törrönen et al. 1994). Some of the accessory enzymes like acetyl xylan esterase (E.C 3.1.1.72), *p*-coumaric esterase (3.1.1.B10), *α*-glucuronidases (E.C 3.2.1.139), *α*-l-arabinofuranosidases (E.C. 3.2.1.55), and ferulic acid esterase (E.C

3.1.1.73) are necessary to hydrolyze the remaining component or side chains of the hemicellulose structure such as glucuronic acid, galacturonic acid, arabinose, galactose, ferulic and coumaric acids (Bhardwaj et al. 2019; Gírio et al. 2010; Beg et al. 2001). The action of α-glucuronidase, α-l-arabinofuranosidases, and acetyl xylan esterase is to remove acetyl and phenolic side branches whereas *p*-coumaric esterase and ferulic acid esterase cleave the ester bonds in xylose. The synergistic enzyme activity of all the xylanases proves significant in opening up the xylan component of the lignocellulosic biomass (Moreira and Filho 2016). The presence of xylanases has been found in many organisms ranging from microorganisms like fungi, bacteria, and yeast to crustaceans, insects, and seeds (Beg et al. 2001). Microbial xylanases are preferred over animal sources. The most notified, experimented, and applied xylanases are from the organisms from the genus *Bacillus, Chaetomium, Nonomuraea, Arthrobacter, Clostridium, Thermomonospora, Dictyoglomus, Fusarium, Streptomyces, Aspergillus,* etc. (Bhardwaj et al. 2019; Sunna and Antranikian 1997).

Mannanases: These enzymes are involved in the depolymerization of mannans which are an integral part of the hemicellulose portion of the cell wall. 1,4-β-D-mannohydrolases or β-mannanases (E.C 3.2.1.78) are endo-acting mannanases that cleave the internal glycosidic bonds on the linear chains liberating short oligosaccharides like β-1,4-manno-oligosaccharides. On the other hand, β-1,4-D mannopyranoside hydrolases or β-mannosidases (E.C 3.2.1.25) are exo hydrolases that choose to act on the non-reducing ends of mannobiose to degrade it into individual mannose units. Lastly, β-1,4-D-glucoside glucohydrolases or β-glucosidases (E.C 3.2.1.21) act on the products liberated from the cleavage of glucomannan and galactoglucomannan specifically cleaving the β-1,4-glucopyranose units from the non-reducing terminal (Dhawan and Kaur 2007; Moreira and Filho 2008). α-galactosidases (E.C 3.2.1.22) and acetyl mannan esterases (E.C 3.1.1.6) are some of the accessory proteins which are required to excise the additional side chains or groups present occasionally on the mannans (Malgas et al. 2015) (Fig. 4.6c).

Mannanase is classified under different GH families (like GH 1–3, GH 5, 26, 27, 113, etc.). Their primary structure is different while they share common spatial arrangements. They all have a canonical $(\beta/\alpha)_8$-barrel protein fold in their active site and based on that they have been included in clan GH-A. The central active site cleft contains two glutamate residues at the C-terminal side (Dawood and Ma 2020). Mannanase is the second most important industrial enzyme after xylanases and has been explored in various industries like textile and paper industries, pharmaceuticals, food, feedstock industries, etc. The bacterial degraders for mannanases among Gram-positive bacteria are from *Bacillus* species, and *Clostridia* species, whereas from Gram-negative bacteria are from *Vibrio*, *Pseudomonas, Klebsiella,* and *Bacteroides.* Among fungal counterparts *Aspergillus, Agaricus, Trichoderma,* and *Sclerotium, Penicillium* species are mostly reported. Actinomycetes from *Streptomyces* species have also been shown to be mannan degraders (Dhawan and Kaur 2007; Chauhan et al. 2012).

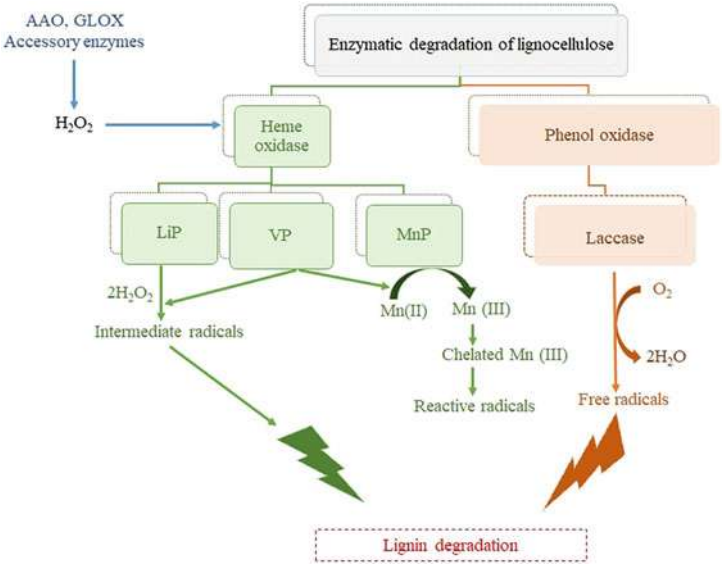

Fig. 4.8 Lignin hydrolysis by lignin-degrading enzymes: Diagram depicting the mechanism of action of different lignin-degrading enzymes for hydrolyzing lignin component

4.4.1.3 Lignin-Degrading Enzymes

They catalyze the conversion of lignin present in lignocellulosic biomass into small aromatic molecules. Lignin is a phenolic polymer containing phenylpropanoid aryl-C3 units links. These can be degraded by the synergistic action of two groups of enzymes, namely lignin-modifying and lignin-degrading auxiliary enzymes. The former includes laccase or phenol oxidases (E.C 1.10.3.2), lignin-modifying peroxidases such as lignin peroxidase (LiP) (E.C 1.11.1.14), versatile peroxidases (E.C 1.11.1.16), dye-degrading peroxidases (E.C 1.11.1.19), and manganese peroxidase (MnP) (E.C 1.11.1.13). All the peroxidases have a unique prosthetic group in the form of protoporphyrin IX (Pollegioni et al. 2015). Auxiliary enzymes in lignin degradation on the other hand include glucose oxidase (E.C 1.1.3.4), cellobiose dehydrogenase (E.C 1.1.99.18), glucose oxidase (E.C 1.1.3.4), aryl alcohol oxidase (E.C 1.1.3.7), pyranose 2-oxidase (E.C 1.1.3.10), and glyoxal oxidase (E.C 1.2.3.5) along with some other enzymes like alkyl aryl etherase, and aryl alcohol dehydrogenase (Bilal et al. 2019; Zhang et al. 2020a, b; Levasseur et al. 2008; Agrawal and Verma 2020a, b) (Fig. 4.8).

Basidiomycetes white-rot fungus is extensively investigated for the production of these auxiliary enzymes (Garcia-Ruiz et al. 2014). Hofrichter and Ullrich stated an action of a new enzyme heme-thiolate haloperoxidases, catalytically identical to other heme-containing oxidoreductases (cytochrome P450 monooxygenases and

catalases), as lignin degrading in cultures of *Ceriporiopsis subvermispora* (Hofrichter and Ullrich 2006).

Although the crystal structure and catalytic cycle of all the ligninolytic enzymes are well-reviewed (Chen et al. 2012; Pollegioni et al. 2015; Wong 2009; Janusz et al. 2017), we have attempted to briefly touch on some of the important aspects in understanding the mechanism of these enzymes.

Laccases: Laccases fall in the group of oxidoreductases that uses oxygen as an oxidizing agent. The four copper ions at the active center of laccases help the enzyme in oxidizing most of the phenolic and aromatic compounds present in the lignin (Mai et al. 2000; Pollegioni et al. 2015). Some metal ions and organometallic compounds are also been reported as the substrate of laccases (Garcia-Ruiz et al. 2014; Zimmerman et al. 2008). All these four copper ions at the T2/T3 site (the T1 Cu and the tri-nuclear Cu cluster (T2 Cu, T3α Cu, and T3β Cu)) have a different electro-paramagnetic resonance which is key to their unique reaction with the random polymeric nature of lignin. The fungal laccases are known to comprise ~520–550 amino acids residues with glycosylation as primary modifications. The three-dimensional structure of fungal laccases demonstrates three tightly arranged cupredoxin-like domains having β-barrel symmetry. The third domain holds the T1 Cu near the surface of the protein, and the T2 Cu, T3 (α and β) Cu are located at the junction of the first and the third domain (Mehra et al. 2018; Sitarz et al. 2016). Laccases are extracellularly, intracellularly as well as periplasmically produced depending on the type of microorganisms producing them. It is found mostly in fungal and bacterial cells.

Lignin Peroxides (LiP): LiPs are generally known to oxidize phenolic and non-phenolic organic compounds instead the enzyme specificity is relatively poor. The structural analysis of LiP isolated from *Phanerochaete chrysosporium* revealed its globular nature. The active site pocket is formed of two domains organized of eight α-helices (major and minor) with restricted β components enclosing a heme-chelating ferric ion (Choinowski et al. 1999). The three-dimensional structure of LiP is further stabilized by four disulfide linkages, two calcium ions, and two glycosylation-specific post-translational sites. Although their enzymatic mechanism is similar to other peroxidases in the same class, they stand effective catalytically due to their very high redox potential when it comes to oxidizing the recalcitrant lignin component (Sigoillot et al. 2012).

Manganese Peroxidases (MnP): MnP was isolated and studied initially from the fungi *Phanerochaete chrysosporium*. Supplementation of Mn ions and other organic compounds like 2-hydroxybutyrate, malonate, glycolate, or glucuronate in the growth medium stimulated the production of MnP in white-rot fungus (Mester and Field 2006). These molecules in particular stabilized the structure of the enzyme. A molecule of heme (iron protoporphyrin IX) is sandwiched between the two domains formed by α-helices very similar to that of the structure of LiP. Very close to the heme porphyrin lies the binding site of Mn^{2+} ion which constitutes one aspartate and two glutamate γ-carboxylic groups. Slightly different from the structure of LiP, MnP consists of five disulfide bridges and two Ca^{2+} ions. The active cycle of MnP varies

from that of LiP, in the oxidation of lignin compounds involving the conversion of $Mn^{2+}$to Mn^{3+}ions (Niladevi 2009).

Versatile Peroxidases (VPs): This enzyme represents a cocktail of LiPs and MnPs. It also constitutes a heme porphyrin group close to the catalytic site. The active center is made of 11–12 α-helixes with four disulfide bridges, two Mn (II) binding sites and two Ca(II) binding sites. This enzyme is capable of oxidizing methoxybenzenes and various non-phenolic lignin compounds with high affinity. The multi-step reaction mechanism of VPs is similar to other peroxidases.

Dye-decolorizing peroxidase (DyP): These enzymes are different from the classical lipid-modifying peroxidases like LiP, MnP, and VPs. They can decolorize a range of molecules including dyes, β-carotene, and aromatic sulfides (Zámocký et al. 2015). They were first investigated and isolated from the cultures of the fungus *B. adusta* (Fernandez-Fueyo et al. 2015). DyPs can oxidize non-specifically all peroxidase substrates and also possess hydrolase and oxidase activity. The structural analysis of DyPs demonstrates the presence of two domains. The catalytic site lies in the cavity of the two domains accommodating the heme cofactor (Colpa et al. 2014).

Glyoxal Oxidases (GLOX): Lignin components like methylglyoxal and glyoxal can be oxidized by the GLOX. These enzymes proceed with the oxidation of their substrate with the formation of extracellular hydrogen peroxide (Yamada et al. 2014). They are moreover required to regulate the peroxidase activity of the lignin-modifying peroxidases. The active center of GLOX is occupied by a copper ion which helps in the aldehyde oxidation of its substrate (Yin et al. 2015).

Aryl Alcohol Oxidase (AAO): This enzyme is a member glucose-methanol-choline oxidase/dehydrogenase family. Structurally, this enzyme is a monomer with two domains non-covalently bound with the FAD cofactor (Fernandez et al. 2009). AAO substrates include various aryl-alcohols (phenolic and non-phenolic), aliphatic (polyunsaturated) primary alcohols, or aromatic secondary alcohols present in the lignocellulose biomass. It also oxidizes the radical intermediates produced by laccase enzymes like guaiacol, sinapic acid, etc. (Mathieu et al. 2016). The oxidative dehydrogenation reaction of AAO is an NADP-dependent reaction and produces H_2O_2 (Ferreira et al. 2010).

Pyranose 2-Oxidase (P2O): These oxidoreductases are involved in the oxidation of aldopyranose compounds. It is produced periplasmically and transported in membrane-associated vesicles (De Koker et al. 2004; Prongjit et al. 2009). They are homotetrameric and have three major conserved regions, namely the binding site for FAD, the substrate-binding region, and the flavin attachment loop. The threonine hydroxyl of Thr169 present at the active site is very important for the oxidation of sugars and flavin molecules. The P2O catalyzes the oxidation of its substrate at the C2 position via a Ping-Pong type reaction mechanism at pH 7 (two half-reactions). The end products are the 2-keto-sugars and hydrogen peroxide. First, a hydride equivalent from the sugar substrate is donated to the protein-bound flavin with the generation of a reduced FAD (FADH) and the 2-keto-sugar, and secondly, a reduced flavin is oxidized by donating two electrons to O_2 thereby forming H_2O_2 (Pitsawong et al. 2010).

4.4.2 *Hydrolytic Enzymes Involved in the Valorization of Food Waste*

Food waste origin is marked starting from the agriculture sector, packaging, and retail, finally till household consumption. These waste streams generated are rich in biodegradable organic matter, which due to their peculiar chemical characteristics like high biological and chemical oxygen demand, content-rich in carbon and nitrogen, are deleterious to the environment when discarded in landfills and aquatic streams. They may cause aquatic pollution, harmful toxic leaching into ground and surface water, altered soil composition, etc. The food processing industries and so their waste is categorized based on the food material they process. Baiano et al. (2014) assessed and estimated the approximate percentage of waste generated by different food processing units or industries (Baiano 2014). According to them, the beverages manufacturing industry generates 26% of food waste, dairy industries make up 21%, fruit/vegetable, cereal edible oils manufacturing and processing as well as meat and fish product processing and preservation industries constitute (12.9%), (3.9%), (8.4%) of food waste, respectively. However, valorization of these compounds to revenue streams like biofuels, other food, and non-food commodities is possible by introducing enzymes catalysis at various steps of their conversions (Andler and Goddard 2018). For instance, esterification of different waste components like oil, sugar, starch, and even flavonoids help in enhancing their values through converting or valorizing them into value-added products like biodiesel, surfactants, biodegradable plastics, and this indirectly prevents their direct disposal to wastewater treatment facilities.

Pectinases: The industries processing vegetables and fruits are rich in crude dietary fibers, carbohydrates, polyphenols, flavonoids, triglycerides, or plant-based fatty acids, etc. The by-product of these industries can be widely valorized through enzymatic and also through other physico-chemical extraction into value-added revenue streams like novel pharmaceuticals, animal feeds, etc. (Mourtzinos and Goula 2019; Fierascu et al. 2020). For instance, soluble and insoluble dietary fibers from citrus fruit pulp have been investigated by a group of researchers to replace fat content in ice cream because of their high phenolic and carotenoid content and more importantly because of their high water and fat retention capacity (de Crizel et al. 2014). Fierascu et al. (2020) have also extensively reviewed the current opinions on utilizing the waste generated from fruits processing industries into useful commodities (Fierascu et al. 2020). Pectinases are important fibrinolytic enzymes, and they are widely utilized in beverages industries for clarification as well as enhancement of color purposes. These enzymes help dissolve pectin structures (Micheli 2001) and are categorized based on their mechanism of bond cleavage: (1) pectin esterases and (2) depolymerase enzymes. Depending upon the substrate on which the pectinases act, pectin esterases are of two types, i.e., pectin acetylesterases (E.C 3.1.1.6) and pectin methylesterase (E.C 3.1.1.11). Pectin depolymerase, on the other hand, are hydrolases, for example, polygalacturonase, PG (E.C 3.2.1.15), and lyases or transeliminase comprising pectin lyase, PNL (E.C 4.2.2.10), and pectate lyase, PL

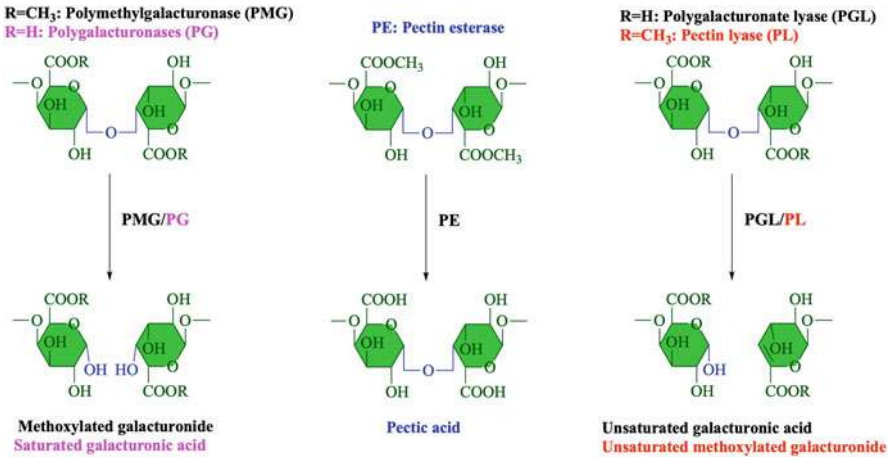

Fig. 4.9 Action of pectinases: Mechanisms of action of polymethylgalacturonase, polygalacturanase, pectin esterases, polygalacturanase, and pectin lyase on their specific pectin substrate

(E.C 4.2.2.2) (Sharma et al. 2013). Pectin methylesterases (E.C 3.1.1.11) convert the methyl groups into pectic acid and the depolymerase enzyme further disintegrates pectic acid into simpler carbohydrates (Fig. 4.9).

Pectinases in common consist of only one domain termed parallel β-helix making a right-handed cylinder. The structure contains three parallel β-sheets. These β-sheets arrange themselves to form a prism-like structure with two β-sheets forming an anti-parallel sandwich while the third one remains perpendicular to the axis. The active site of the enzyme is formed on the exterior side of the parallel β-helix. This structural model is most similar and closest to the structures demonstrated in the referred articles through crystallographic studies in pectinases from various strains (Jenkins et al. 2001; Pickersgill et al. 1994, 1998; Petersen et al. 1997).

Inulinase: Inulinase acts upon the β-2,1-linkage between the fructose units present in the inulin molecule. The polyfructose chain is terminated with a glucose unit attached with α-1, β-2-glycosidic linkage. Inulin is a stored carbohydrate found commonly in roots and tubers of plants like onion, garlic, and also there are reports of bacterial inulin which are comparatively highly branched in nature. Inulinase enzyme is classified into two classes: an endo-inulinase called 2,1-β-D-fructanfructohydrolase (E.C 3.2.1.7) and an exo-inulinase called β-D-fructanfructohydrolase (E.C 3.2.1.80) (Neeraj et al. 2018). The structural analysis of inulinase from *Aspergillus awamori* reveals the presence of two catalytically important residues (Asp41 and Glu241) at the substrate-binding site of the enzymes. These residues play a vital role in the double displacement reaction at the initial hydrolysis step (Nagema et al. 2004). Modern nutrition prospects recommend artificial sweeteners in the place of sucrose in diet to people looking for maintenance of weight/weight loss and lowering blood sugar levels in diabetes. Fructose products

have also made their place as sweeteners in prebiotics popularly known as Greek yogurt. Here's where Inulinase yields its importance in industries generating fructose or fructose oligosaccharides as artificial sweeteners. Inulinase has also been established in the production of a plethora of commercially important products like citric acid, tetrahydrofuran, mannitol, sorbitol and 2,3-butanediol, etc. (Chi et al. 2009).

Lipolytic Enzymes: Wastewater generated from the slaughterhouse, poultry, and fish farms are rich in biodegradable organic matter like oil and grease waste which can result in high BOD and COD. As a consequence, there is an increase in the growth of filamentous microorganisms (bulking), floating and clogging of sludge in the treatment plant, and unpleasant odor. This demands the hydrolysis of the organic matter before its release into the treatment plants for further processing. Even though there are various methods like dissolved air flotation systems, tilted plate separators, grease-trap, and physical-chemical treatment (aerobic and anaerobic pretreatments) employed for the removal of these biodegradable organic matters from the wastewater, their proper implementation counters several setbacks. Therefore, effluents from several origins can be subjected to enzymatic hydrolysis by lipase. Treatment of domestic wastewater rich in oil and grease with lipase obtained from *Candida rugosa* (Jaeger and Reetz 1998) and *Pseudomonas aeruginosa* (Dharmsthiti and Kuhasuntisuk 1998) has been vastly investigated. Lipases are chemically known as triacylglycerol hydrolases (E.C. 3.1.1.3) which catalyze the hydrolysis of triacylglycerol. The active site of lipases is decorated with amino acids like serine, aspartate or glutamate, and histidine (Mateos et al. 2021). They exist as monomeric proteins folded to form β-sheets in the center enclosed by α-helix. The lipase activity is considered maximum at the oil-water interface which is dependent upon its change in conformation from closed to open form upon coming in contact with a hydrophobic surface. Apart from hydrolysis, lipases can catalyze other reactions like esterification, transesterification, acidolysis, and aminolysis.

Lactases: Dairy industries are also major contributors of proteins, fatty acids, and lactose in liquid waste. The major portion of which is considered to be deproteinized cheese whey obtained from cheese producing industries all over the world, which despite no toxic content is a major concern to environmental preservation and safety (Lappa et al. 2019). The lactose component in cheese whey has been elaborately investigated for the production of several commodities like bioethanol, artificial sweeteners, green plastics/polyhydroxyalkanoate polyesters, etc. (Koller et al. 2012), and there are reports of its valorization into disinfectants, electron donors for electricity generation through single chamber microbial fuels cells as well (Banaszewska et al. 2014; Pescuma et al. 2015; Yadav et al. 2015). Lactases are known as β-D-galactohydrolase/β-galactosidases (E.C 3.2.1.23), and they catalyze the cleavage of lactose into glucose and galactose. The most demanding prebiotics, lactulose, and galacto-oligosaccharides (GOS) are produced by lactose through the action of β-galactosidases. Lactose is transgalactosylated by β-galactosidases to produce a mixture of non-digestible forms of mono-, and oligosaccharides that form the GOS. In the reaction, lactose acts both as the donor and acceptor of

transglycosylated galactose. Whereas in lactulose synthesis, lactose only acts as a donor of galactosyl glucose, and the fructose acts as an acceptor.

Protease: Proteases are classified into different groups based on the activity of proteases under various parameters like acidic, alkaline, or neutral conditions as well as the composition of their substrate-binding site (Panda et al. 2013). Alkaline serine protease (E.C.3.4.21–24.99) is most active at pH ranging from neutral to alkaline (Singhal et al. 2012; Varela et al. 1997). Other proteases are the neutral and acidic proteases (Razzaq et al. 2019). Proteases are proven to be a significant tool in bioremediation as well as valorization of waste to value-based products (Page and Cera 2008). The most exploited and studied microorganism for the production of alkaline protease and neutral proteases are *Bacillus species* and acidic proteases are known to be produced by the fungus. These proteases can be efficiently inactivated by PMSF (phenyl methane sulfonyl fluoride). Protein-rich waste is responsible for increasing the biological oxygen demand of aquatic waste. As discussed in earlier sections, the waste aquatic stream is drowned with effluents from food processing industries which constitutes waste from dairy products, waste from poultry, and fish industries as well as textile and leather industries. Leather industries involve alkaline protease with keratinolytic activity to hydrolyze keratin content present in the hair residues for increasing the surface area of the skin. They are also used in bating and clearing undesirable pigments during the preparation of clean skin and hides (Bhaskar et al. 2007; Shankar et al. 2011). Alkaline protease preparations of *Bacillus species* are enormously used in poultry industries to get rid of the feather waste generated in the slaughterhouse. The keratinolytic capability of these proteases has been best utilized in cleaning drainage pipes clogged with hair residues. A cocktail of protease preparation from *Streptomyces species, Bacillus subtilis*, and *Bacillus amyloliquefaciens* along with thioglycolate mixture is available commercially in the market. Alkaline proteases find a crucial place in the degradation of plastics as well as X-ray photographic sheets, particularly for the silver recovery. The active site of the alkaline protease comprises catalytic triad formed by Aspartate and Histidine residues along with Serine residue. Proteases based on their structural and sequence similarity can be obtained and assessed from the database called MEROPS (Rawlings et al. 2006) database. Table 4.1 comprehensively covers the application of different enzymes in the valorization of waste.

4.4.3 Hydrolytic Enzymes in Biodegradation and Valorization of Non-biodegradable Plastics Waste

The monomers utilized for the preparation of most plastics, ethylene, and propylene are derived from petroleum. The most commonly used are polyethylene, polystyrene, polyurethane, polypropylene, polyvinyl chloride, poly (ethylene terephthalate), etc. Out of the total production worldwide, only a small fraction around 20% is getting recycled, thus plastic remains a long-lasting, and major threat to the

Table 4.1 Listed applications of different hydrolytic enzymes in the valorization of waste and their microbial sources

Sl. No.	Name of the enzymes	Microbial source	Industry	Application	Reference
1	Cellulase	Bacteria: *Clostridium species, Pseudomonas* species, *Bacillus species* Fungus: *Aspergillus species*, *Penicillium species, Melanocarpus species*, *Trichoderma species, Schizophyllum commune* Actinomycetes: *Cellulomonas species, Streptomyces species, Thermomonospora species*	Pulp and paper industry, Textile industry Agricultural, and Food Industry Other	Deinking, pulping, bleaching, improve the quality and strength of pulp fibers, reduce the use of energy and chlorine, production of biodegradable sanitary napkins, papers, etc. Bio-stoning of jeans, softening of garments, biopolishing of textile fibers, enhancing quality, stability, and absorbance property of textile fibers, removal of excess dye from fabrics, and restoration of color brightness. Improve seed germination, plant growth and flowering, disease and plant pathogen control, and improve soil quality. Bioethanol production, bio-detergent production	Pere et al. (2001), Kuhad et al. (2011), Singh et al. (2016)
2	Xylanase	Bacteria: *Arthrobacter* species, *Geobacillus species, Pediococcus acidilactici, Bacillus species, Paenibacillus species, Rhodothermus marinus, Staphylococcus aureus, Pseudomonas species* Fungus: *Talaromyces species Thermomyces lanuginosus*, *Thermoascus sp,*	Agricultural, Food Industry, Pulp and paper industry, Textile industry, and Pharmaceutical industry	Improve fermenting process, improve animal feed digestibility, biogas, and bioethanol production, clarification, and maceration of fruit juice, brewing, hydrolysis of agro-residues. Bio-bleaching of pulp, Deinking Textile processing, desizing, and bioscouring. XOS production.	Beg et al. (2001), Bhardwaj et al. (2019)

		Melanocarpus albomyces Chaetomium species, *Fusarium species Humicola* species *Paecilomyces sp., Scytalidium sp.*			
3	Mannanase	Bacteria: *Agaricus bisporus, Aspergillus species, Bacillus species, Bacteroides species, Clostridium species* Fungus: *Aspergillus species, Agaricus species, Trichoderma species*, *Sclerotium species*	Pulp and paper industry, Textile industry, Agricultural and Food Industry	Bleaching of softwood pulps using enzymes, eliminates the use of chlorine and hydrogen peroxide, and enhance the brightness of paper. Desizing and bioscouring, reduce the viscosity of the print paste. Oil extraction from coconut, food additives, oil drilling, improve the nutritional value of animal feed.	Dhawan and Kaur (2007)
4	Aryl alcohol oxidase	Bacteria: *Sphingobacterium* species Fungus: *Thermothelomyces thermophilus, Coprinopsis cinerea, Phanerochaete chrysosporium*	Food and beverages, Polymer industry, and Pharmaceutical industry	Food flavors, e.g., vanillin. Production of bio-based polymer. Production of health beneficial compounds, e.g., to cuminaldehyde (therapeutic effects against cancer and diabetes).	Urlacher and Koschorreck (2021), Serrano et al. (2019), Li and Jiang (2004)
5	Lactase	Bacteria: *Bifidobacterium infantis* *Fungus: Lactobacillus thermophiles, Kluyveromyces fragilis, Aspergillus foetidus*	Food and beverages	Production of lactose-free products reduces the crystallization of ice creams and condensed milk	Saqib et al. (2017), Porta et al. (2010)
6	Alkaline protease	Bacteria: *Bacillus pumilus* Fungus: *Streptomyces fungicidicus*, *Myceliophthora species*	Leather Industry, Cosmetic sector, Pharmaceutical Industry and	Helps in effective bating. Formulation of cosmetic products. Development of ointment compositions, gauze, and new bandage material. Detergents and silver recovery.	Baweja et al. (2016), Sharma et al. (2019), Razzaq et al. (2019)

(continued)

Table 4.1 (continued)

Sl. No.	Name of the enzymes	Microbial source	Industry	Application	Reference
			Other applications		
7	Esterase	Bacteria: *Lactobacillus casei, Pseudomonas species* Fungus: *Aspergillus niger*	Pharmaceutical industry, Paper and pulp industry	Production of chiral drugs, detoxifying toxic residue. Reducing pitch problems during paper manufacture.	Panda and Gowrishankar (2005)
8.	Lignin-degrading enzymes				
	Lignin peroxidase, Manganese peroxidase (MnP), and Glycol oxidase (GLOX)	Fungus: *P. chrysosporium Trametes versicolor, Phlebia radiate, Trichoderma viride, Trametes suaveolens, Phanerochaete sordid* Bacteria: *Bacillus anthracis and Bacillus cereus* Fungus: *Rigidoporus Lignosus* and *Ceriporiopsis subvermispora* Bacteria: *Streptomyces lividans* Fungus: *P. chrysosporium, Pycnoporus cinnabarinus, Bjerkandera adusta, Dichomitus squalene*	Textile industry, Cosmetic industry, Pulp and paper industry, Polymer industry, Food industry, and Other application	LiP and MnP: Decolorize dyes and degradation of a xenobiotic compound. LiP: Decolorize synthetic melanin an alternative to hydroquinone cream. Lip and MnP: Biopulping, biobleaching, delignification in the paper industry. Lip and MnP: Polymerization of acrylamide into a thermoplastic resin (polyacrylamide). LiP and MnP: Delignification of lignocellulose. GLOX: Oxidize aldehydes to a variety of organic acids, carboxylic acids, pyruvic acid, oxalic acid, and formic acid, source of H_2O_2 production during lignin degradation	Marco-Urrea and Reddy (2012), Abadulla et al. (2000), Joon Sung (2020), Agarwal et al. (2018), Iwahara et al. (2000), Daou and Faulds (2017), Falade et al. (2017), Januz et al. (2017)

9	Pectinase	Bacteria: *Bacillus subtilis, Saccharomyces cerevisiae* Fungus: *Aspergillus niger*, *Rhodotorula* species	Food and beverages, Textile industry, and Other applications	Reducing the viscosity and turbidity of freshly collected juice, removing the mucilage from the coffee beans. Bioscouring of cotton fibers, degumming, and retting of fiber crop, oil extraction. Purification of plant viruses.	Nighojkar et al. (2019), Sharma et al. (2019)
10	Insulinase	Bacteria: *Streptomyces species* Fungus: *Kluyveromyces marxianus, Aspergillus niger.*	Food and beverages	Production of metabolites, e.g., lactic acid, sorbitol syrup, and high fructose syrup and oligosaccharides	Singha et al. (2017)

environment, especially to the aquatic streams (Kaushal et al. 2021). Plastics are categorized into two ways: (1) Thermoplastics whose chemical composition remains unchanged at high temperature and have mostly linear carbon chain backbone and (2) Thermosets which are made of other elements along with carbon possess highly cross-linked anatomy and their chemical conversion at high temperatures is irreversible. One of the most common thermoplastics used worldwide is polyethylene (PE). It is composed of ethylene as a monomer unit and is highly crystalline in structure which makes it recalcitrant to biodegradation. Based on the pattern of the linear chain and different densities, PE can be low-density PE (LDPE), high-density PE (HDPE), and also low molecular weight PE (LMWPE) Polypropylene (PP), yet another thermoplastic made by polymerizing propylene gas. PP differs from PE in having a methyl group instead of one hydrogen atom at an alternate carbon atom in the linear carbon backbone. This chemical structure gives it more rigidity in comparison to PE. Both these plastics are included in the category of polyolefin due to their inert and resistant nature to most heating, biological and chemical treatments. It is abundantly utilized as packaging plastics in various industrial sectors (Zheng et al. 2005). Polyvinyl chloride is yet another synthetic plastic that is used in rigid or plasticized forms. They are formed by polymerizing vinyl chloride or chloroethene in linear form. The pollution caused by PVC plastics is noticeable as burning these plastics emits hydrogen chloride fumes which pose serious health hazards. They are more prone to microbial degradation in comparison to other plastics due to the high percentage of plasticizers added to them (Webb et al. 2000). Polystyrene is synthesized by polymerizing styrene as repeating units. They could be thermoplastics or thermosets. They are also widely used in packaging industries due to their foam-like appearance (Tokiwa et al. 2009). Polyurethane and PET (polyethylene terephthalate) both have improved thermostability as they are hetero-atomically branched. PET is the most abundantly produced and used plastic in the modern era. Researchers have claimed that global warming caused by enormous CO_2 emissions and promiscuous usage of PET plastics are two of the most alarming situation in the biosphere (Wang et al. 2020). It is a high molecular weight thermoplastic composed of terephthalate (TPA) and ethylene glycol (EG) via an ester bond. This polymer has great tensile strength, and durability and its production cost are also low. Their structure contains large aromatic rings which make them rigid and resistant to biodegradation (Webb et al. 2013). Polyurethane has heteroatoms with carbamate linkage which could be either ester or ether bonds. They form the major constituents of microplastics making them the most concerning issues in the aquatic system (Shah et al. 2013). Mitigation of these plastics from the environment is carried over by certain physical and chemical methods like incineration, recycling, and dumping them into landfills. All of these methods are not environmentally friendly and they are not even cost-effective. The introduction of plastic hydrolyzing enzymes has opened up hopes for eco-friendly treatments to get rid of this dire environmental pollutant (Verma et al. 2016) (Table 4.2). Varieties of microorganisms like fungi, bacteria, actinomycetes, and algae have been investigated as well as reported to exhibit plastic polymer degrading capacity. Plastic degrading enzymes are mostly obtained from microbial organisms, and therefore they are studied under two categories: intracellular and

Table 4.2 Plastic degrading enzymes and their microbial sources

Sl. No	Enzyme	Microorganism	Plastic substrate	References
1	Laccase	*Rhodococcus ruber* C208 (Mesophilic bacteria) *Bacillus cereus* (Bacteria)	Polyethylene (PE)	Santo et al. (2013), Vimala and Mathew (2016)
2	Manganese peroxidase	*Phanerochaete chrysosporium* ME446 (White-rot fungus) *IZU-154* (Fungi) *Bacillus cereus* (Bacteria) *Penicillium simplicissimum* (Fungi)		Iiyoshi et al. (1998), Sowmya et al. (2015)
3	Lignin peroxidase	*Streptomyces (Bacteria) Phanerochaete chrysospo*rium MTCC-787 (Fungi)		Jeon and Kim (2015), Mukherjee and Kundu (2014)
4	Alkane hydroxylase	Recombinant AH from *Pseudomonas species* E4 expressed in *Escherichia coli* BL21 (Bacteria)		Yoon et al. (2012)
5	PETase	*Chlamydomonas reinhardtii* (Green algae)	Polyethylene terephthalate (PET)	Kim et al. (2020)
6	Cutinase-like enzyme/ IsPETase	*Ideonella sakaiensis* 201-F6 (Bacteria)		Han et al. (2017)
7	Lipase	*Pseudomonas chlororaphis* (Bacteria)	Polyurethanes	Stern and Howard (2000)
8	Polyurethanase	*Serratia marcescens* (Bacteria)		Mankoci et al. (2019)
9	Protease and esterase	*Pseudomonas fluorescens* (Bacteria) *Pseudomonas chlororaphis* (Bacteria)		Hung et al. (2016), Shah et al. (2008), Nakajima-Kambe et al. (1995)
10	Polyurethanase (PUase)	*Curvularia senegalensis* (Fungi)		Crabbe et al. (1994)
11	Polyhydroxyalkanoate depolymerase	*Alcaligenes faecalis* (Gram-negative, rod-shaped bacteria)		Gamerith et al. (2016)
12	Polyester Polyurethane (PUR) esterase	*Comamonas acidovorans TB-35* (Bacteria)	Polyester polyurethane	Akutsu et al. (1998)
13	Phenylacetaldehyde Dehydrogenase	*Pseudomonas fluorescens ST (Bacteria) Pseudomonas putida S12 (Bacteria) Xanthobacter species 124X (Mesophilic bacteria)*	Polystyrene	Oelschlägel et al. (2018)

(continued)

Table 4.2 (continued)

Sl. No	Enzyme	Microorganism	Plastic substrate	References
14	Cytochrome P450 CPY152A1	*Bacillus subtilis* (Gram-positive, catalase-positive bacteria)		Shoji et al. (2007)
15	Cytochrome P450 CPY152B1	*Sphingomonas paucimobilis* (Gram-negative bacteria)		Fujishiro et al. (2012)
16	AlkB (alpha-ketoglutarate-dependent hydroxylase	*Pseudomonas putida* GPo1 (Bacteria)		Hou and Majumder (2021)
17	Alkane monooxygenase	*Geobacillus thermodenitrificans* NG80–2 (Thermophilic bacteria)		Li et al. (2008)
18	Hydroquinone peroxidase	*Azotobacter beijerinckii* HM121 (Lignin decolorizing bacteria)		Nakamiya et al. (1997)

extracellular enzymes. Extracellular enzymes are involved in the depolymerization of long-chain polymers into smaller fractions, viz. oligomers, dimers, etc. Whereas the intracellular enzymes participate in the final conversion of intermediates into the forms which can be assimilated by the microbes as a sole source of carbon. As a result of this process, a valuable emission gas, i.e., methane is released as metabolic products which can be used as fuels and can further be utilized as precursors for the production of organic acids (Amobonye et al. 2021). Furthermore, the wax moth *Galleria mellonella* is known to depolymerize plastics with the help of their gut microbiota containing the fungus *Aspergillus flavus* (Zhang et al. 2020a, b). All these enzymes involved in degrading plastics are hydrolases that catalyze the cleavage reaction in the presence of water (Müller et al. 2005). Esterases, cutinases, laccases, lipases, and PETases are the most extensively studied hydrolytic enzymes concerning the degradation of plastics. Microbial valorization of plastics into value-added chemicals is elaborately reviewed in Ru et al. (2020). The reviewer explained elaborately the microbial metabolic pathway involved in the depolymerization of ester/urethane-containing plastics, aromatic plastics, and linear aliphatic plastics into its monomer constituents and their further assimilation by microbes for the production of value-added chemicals. The chemical structure such as linkages in petro plastics, linearity or branching in carbon chain, type of linkage (ester, ether, or carbamate linkage between the monomers), presence of hydrophobic functional groups, and physical properties like rigidity (crystalline/amorphous) and density plays a significant role in engineering enzymes suitable for biodegradation (Mohanan et al. 2020). Ongoing and present studies for identification of plastic degrading microorganisms and modification of these microbial enzymes through genetic engineering provides a wide opportunity to efficiently recycle or remove

plastic from the environment. Yet advantageous is when these plastics can be converted into more valuable and marketable products.

4.5 Recent Biotechnological Trends in Increasing the Efficacy of Enzymes in the Waste Valorization

Enzymes have been central in various industries like food processing, beverages distilleries, leather, textile, and paper industries for a long time (Sheldon and Woodley 2017). Their involvement in the production of biofuel and value-added products is tremendously surging in recent times (Chapman et al. 2018). Consequently, for obtaining optimal bioprocesses involving enzymes, further enhancement of enzyme stability and functionality is indispensable. Biotechnological breakthrough offers a great deal for enhancing the power of existing enzymes as well as identifying newer enzyme candidates.

4.5.1 Techniques to Decipher Newer Biocatalyst Candidates

Conventionally, enzyme discovery was done by cultivating microorganisms, fractionating cell-free extract, followed by the screening of the enzyme activity, and then recovered enzymes through purification are subjected to mass spectrometric analysis after trypsin digestion. The identified short peptides are then utilized to decipher the corresponding gene from the genomic DNA. Although these methods are dependent on the use of cultivable microorganisms, a significantly important set of enzymes were discovered using this process. State-of-the-art tools are now assessable to scrutiny and tap the vast microbial biodiversity present in nature (Rinke et al. 2013). The introduction of omics such as metagenomics and metatranscriptomics presents a big potential to analyze the diversity of complex microorganisms. These tools help in the development of genomic libraries from environmental DNA for function or sequence-based similarity screening of the enzymes (Gilbert and Dupont 2011; Uchiyama and Miyazaki 2009). For example, a collection of hydrolytic enzymes such as amylase, lipase, oxidoreductase, and epoxide hydrolase have been deciphered using this technique (Knietsch et al. 2003; Rondon et al. 2000). Several bioinformatics strategies such as in silico data mining, the Catalophore™ approach, and de novo enzyme design tools are also helpful in this process (Handelsman 2004). Progressive success in developing methods for genome sequencing like next-generation sequencing has opened up newer approaches to hunt for putative enzymes. Here, genome hunting is based on either searching for the open reading frame or homology alignment of sequences.

4.5.2 Isolation of Enzymes from Extremophiles

Enzymes that can withstand extreme parameters like higher temperature and pH as well as harsh chemicals like a high concentration of salts, metal ions, organic solvent, etc. have great value at industrial levels. There is a large diversity of organisms in the extremophilic regions and as most of these organisms have not been yet cultivated in pure cultures, the characterization of their enzymes from them is comparatively difficult (Pikuta et al. 2007; Cavicchioli et al. 2011). The bacterial isolates obtained from the extremophiles have displayed properties of different hydrolytic enzymes such as amylase, protease, lipase, and xylanase. Extreme thermophiles are widely present in bacterial species like *Thermus, Thermotoga, Clostridium, and Bacillus. Pyrococcus, Thermococcus, or Methanopyrus* belongs to hyperthermophilic Archaea. Hydrolytic enzymes such as Amylase, Xylanase, Lipase, and Protease enzymes are isolated from some halophilic bacterial species like *Halobacterium, Halobacillus, and Halothermothrix* (Moreno et al. 2012).

4.5.3 Genetic Engineering or Recombinant DNA Technology for the Production of Recombinant Protein in a Microbial Host

To obtain desired efficacy in the expression of enzymes, gene-based technology like recombinant DNA technology can be used. In this technology, the desired gene or gene of interest is inserted into the organism *via an* appropriate vector. The gene of interest can be manipulated through the addition of the desired sequence in the endogenous gene or deletion or knockout of undesirable sequence through recombining genes and elements. Rational redesigning and direct evolution are the two different methods that are adapted for modifying enzymes to their desired characteristics. Rational redesigning utilizes site-directed mutagenesis to target amino acid substitution effectively at the active site of the protein for evolving the enzyme into a more efficient one. Whereas the direct evolution method includes repeated oligonucleotide-directed mutagenesis, random mutagenesis through error-prone polymerase chain reaction (PCR), or modification through chemical agents (Manisha and Yadav 2017; Wiltschi et al. 2020) (Table 4.3).

4.5.4 Immobilization of Enzymes

The major confrontation in enzyme technology and its application in industries is bulk production and the question of reusability. These problems can be easily dealt with the immobilization technique. Enzymes can be immobilized by tethering or encapsulating them in an appropriate material that has desired physical, chemical,

Table 4.3 Genetically engineered microbes for increasing the efficacy of expressed hydrolytic enzymes in the valorization of waste

Sl. No.	Enzymes	Improved properties	Organism	References
1	Cellulase	Increased the inherent ability of *Lactiplantibacillus plantarum's* lignocellulose degradation	*Thermobifida fusca*	None and Yadav (2017)
2	β-glucosidase	Improve enzyme activity and thermostability	*Trichoderma reesei*	Lee et al. (2012)
3	Xylanase	T_m improved by 25 °C	*Thermotoga thermarum*	Yang et al. (2017)
4	Laccase	3-fold improved kcat and thermostability	*Bacillus* HR03	Mollania et al. (2011)
5	Lipase	2-fold increase in amidase activity	*Pseudomonas aeruginosa*	Fujii et al. (2005)
6	Lipase B	20-fold increase in half-life at 70 °C	*Candida antarctica*	Siddiqui and Cavicchioli (2005)
7	Pyranose 2-oxidase	Increased thermostability and the catalytic properties	*Trametes multicolor*	Spadiut et al. (2009)
8	Endoglucanase	Increase hydrolytic activity on cellulosic substrate	*Thermoascus aurantiacus*	Srikrishnan et al. (2012)

electrical, or mechanical properties. These materials increase the stability as well as efficacy in terms of better catalytic activity of the immobilized enzymes. Moreover, immobilization can reduce the steps required to separate them from the reaction mixture as well allows substantial reusability without affecting the activity allowing them to be compatible in a continuous process. To examine the enzyme activity of immobilized enzymes two kinetic parameters, namely the Michaelis constant, K_m, and maximal reaction velocity, V_{max} is often examined and compared with the non-immobilized counterpart. The three widely used immobilization techniques are encapsulation or entrapment, carrier-bound attachment, and the formation of cross-linked enzyme aggregates (CLEAs). Encapsulation or entrapment technique, as the name suggests is the immobilization of enzymes using material of varying degrees of porosity and permeability (Bezerra et al. 2015; Jesionowski et al. 2014). Various materials such as a variety of carriers, for example, sol–gels, hydrogels, polymers as well as nanomaterials have been experimented with immobilization of enzymes via encapsulation technique. Carrier-bound immobilization of enzymes is done by physisorption or chemisorption of enzymes on prefabricated organic or inorganic materials. Materials like metal oxides, nanomaterials, ceramic, or silica gels are used for this purpose. Chemisorption technique is preferred over physisorption as covalent attachment reduces the chances of enzyme leaching from the matrix. CLEAs technique is a very recently utilized technique where the soluble enzyme is aggregated using precipitating agents like alcohol, acetone,

ammonium sulfate, etc., and then subsequently cross-linked or co-polymerized with cross-linking agents like glutaraldehyde (Sheldon 2010). Hence, immobilizing enzymes can be well exploited for obtaining desired characteristics to bring increased efficacy in the use of enzyme technology in the industrial arena as well as biorefineries.

4.5.5 Cell Surface Engineering

Genetic technological advances have let us modify microorganisms to express our genes of interest (Table 4.3). Cell surface engineering is another biotechnology tool where the particular microorganism is tailored to express the desired number of enzymes on its surface. This is technique is advantageous in biorefineries where there is a need for multiple enzymes for the conversion of biomass to biofuels. The most utilized and engineered organism includes *Saccharomyces cerevisiae*. Naturally, yeast can ferment sugars to produce alcohols, but it does not possess the enzyme required for saccharification of complex sugars present in the cell wall of the biomass. Cell surface engineering enables the arming of yeast and other organisms with a cocktail of enzymes that help the production of biofuels and other value-added products (Kuroda and Ueda 2013; Ueda 2016; Ueda and Tanaka 2000).

4.6 Conclusions

Developing sustainable approaches toward building a circular economy and safeguarding mother nature has become the need of the hour. Toward achieving this, biomass waste from various sectors like agro-forest, solid municipal waste, food manufacturing, and processing industries can be utilized and profitably and competently converted to bioenergy and value-added products. To reduce the losses incurred by the use of traditional chemical processes in the treatment of waste biomass, enzymes are introduced for the economic and easier hydrolysis of the different waste components. The major portion of waste generated is composed of biodegradable biomass as well as non-biodegradable plastics. Different microbial sources of hydrolytic enzymes are exploited for the valorization of these wastes into value-added products and bioenergy. Cellulases, hemicellulases, lignin peroxidases, pectinases, amylases, proteases, etc. enzymes are widely utilized in this concern. The biorefinery concept is structured to use the by-product from one industry as feedstock for another industry to produce more value-added goods in addition to conversion of waste to bioenergy. Various technological advances like genetic engineering, cell surface engineering, and immobilization of enzymes are exploited to increase the efficacy of the hydrolytic enzymes obtained from microbial sources. Identification of new sources of these enzymes through metagenomic analysis is relevant and very necessary to keep the reservoir filled. In addition to that further

improvement of these enzymes could be done through techniques like metabolic engineering and chemical modification of the enzyme.

Acknowledgments The authors gratefully acknowledge the financial and infrastructural support from the Indian Institute of Technology Palakkad and Department of Science and Technology (DST)-SERB, Govt. of India for conducting research work. AB is supported by the National Post-doctoral fellowship granted by DST-SERB, (PDF/2020/001950) Govt. of India, RS is supported by a scholarship from Council of Scientific and Industrial Research, MHRD, Govt. of India (09/1282 (0003)/2019-EMR-I) and SC is supported by DST-INSPIRE (IF-18022) fellowship, Govt. of India.

Competing Interests There is no conflict of interest.

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Chapter 5
Thermochemical Conversion of Biomass into Value-Added Materials for Effluent Treatment Applications

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Abstract Lignocellulosic biomass has been explored for the synthesis of various value-added materials due to its wide availability and environment-friendly nature. Apart from being studied as a potential feedstock for the synthesis of fine chemicals and generation of biofuels, these biomasses have also shown a wide range of applications in effluent treatment processes. Many agricultural waste biomasses had shown potential in effluent treatment, even in their raw form. Activated carbon prepared by the pyrolysis of biomass has yielded promising results as adsorbents and catalysts support the removal of both conventional and priority pollutants from effluents. Moreover, composite materials including metal oxide composites, magnetic materials, catalyst supports, polymer composites, and graphene composites prepared by the thermochemical conversion of biomass are being explored in tertiary treatment processes for the removal of targeted pollutants from the aqueous phase. Hence, this chapter is aimed to discuss the application of biomass-based value-added materials for effluent treatment applications.

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P. Verma (ed.), *Thermochemical and Catalytic Conversion Technologies for Future Biorefineries*, Clean Energy Production Technologies,
https://doi.org/10.1007/978-981-19-4312-6_5

Keywords Thermochemical conversion · Biomass-based composites · Activated carbon · Effluent treatment · Emerging contaminants

Abbreviations

AC	Activated carbon
BiOBr	Bismuth oxybromide
BOD	Biochemical oxygen demand
COD	Chemical oxygen demand
EIA	Energy Information Administration
TDS	Total suspended solids

5.1 Introduction

Humanity dates back to not a decade or a century but a millennium. Since existence began, the production of wastes in many forms originated particularly from natural sources. The most popular form of waste would be wood or of plant origin. In general, the term "Biomass is defined as matter originating from living plants, including tree stems, branches, leaves as well as residues from agricultural harvesting and processing of seeds or fruits" (Pang 2016). Biomass is considered to carry energy in the form of chemical bonds among hydrogen, carbon, and oxygen moieties (Pang 2019). The sources of biomass are plant products, the residue of crop farming and processing industries, fruit and vegetable waste, agro-industrial waste, household waste, urban waste, and animal waste (Wormeyer et al. 2011). Figure 5.1 shows the classification of biomass resources. They include materials consisting of cellulose, hemicellulose, and lignin (Mohan et al. 2006). As for the elementary composition, 90% of the typical biomass contains 51% carbon, 42% oxygen, and trace amounts of hydrogen, nitrogen, and chlorine (Mandapati and Ghodke 2021b). The biomass resources were calculated to be 146 billion tonnes/annum. Tropical countries like India contribute to the production of about 500 million metric tonnes/year of biomass. This natural carbonaceous resource is mostly used or exploited as a source of fuel.

5.2 Biomass as a Source of Fuel

The rapid consumption of fossil fuel has led to its depletion (Mandapati and Ghodke 2021a; Agrawal and Verma 2022; Kumar and Verma 2021a). Various renewable resources which include biomass-based reserves, wind, solar, and geothermal resources have been explored as alternative fuels. Among these renewables,

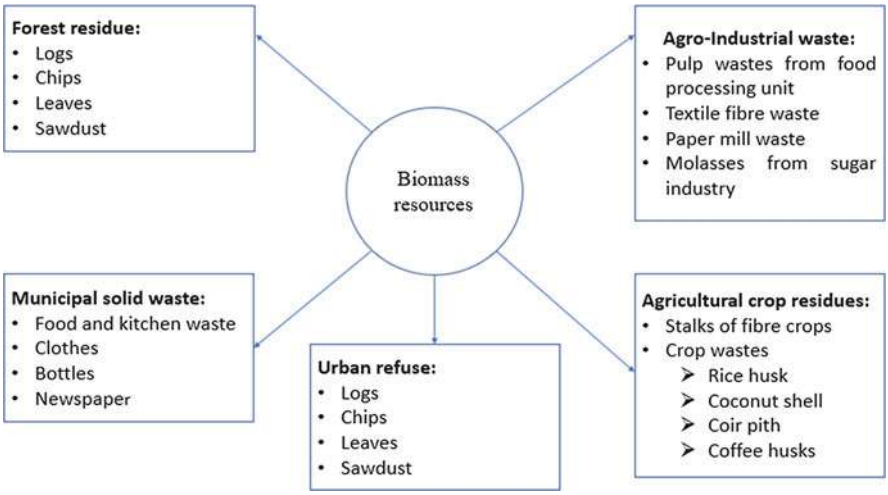

Fig. 5.1 Classification of biomass resources

bioenergy is renewable energy sourced from biomass that is abundant and has a high utilization potential to produce energy (Pang 2019; Kumar et al. 2020; Kumar and Verma 2021b). If 10% of the biomass is exploited for energy production with 50% efficiency, it may have the potential to churn out 3.1 trillion tonnes of oil equivalent energy. This would account for the availability of 200 times the energy consumed worldwide currently (Energy Information Administration, EIA, 2017). On the other hand, the utilization of 10% of biomass for organic chemical synthesis at a 10% conversion rate will lead to the production of 1.6 billion tonnes of chemical feed materials (Pang 2019). Generally, in developing countries, 38% of the energy consumed is primarily from bioenergy produced from biomasses (Sarkar and Praveen 2017).

Biomass is a clean energy source is produced by plants by consuming CO_2 from the atmosphere through photosynthesis (Tekin et al. 2014). Moreover, these biomasses are produced in short duration ranging from months to years when compared to unsustainable fossil fuels which usually require millions of years (Collard et al. 2012). Hence, renewable energy from biomasses proves to be a sustainable source of energy supply that can also address environmental concerns.

5.3 Value-Added Pathway from Biomass for Different Applications

Biomass conversion pathways through various processes are shown in Fig. 5.2. The widely used thermochemical conversion technologies for the conversion of biomasses include but are not limited to gasification, pyrolysis, hydrothermal

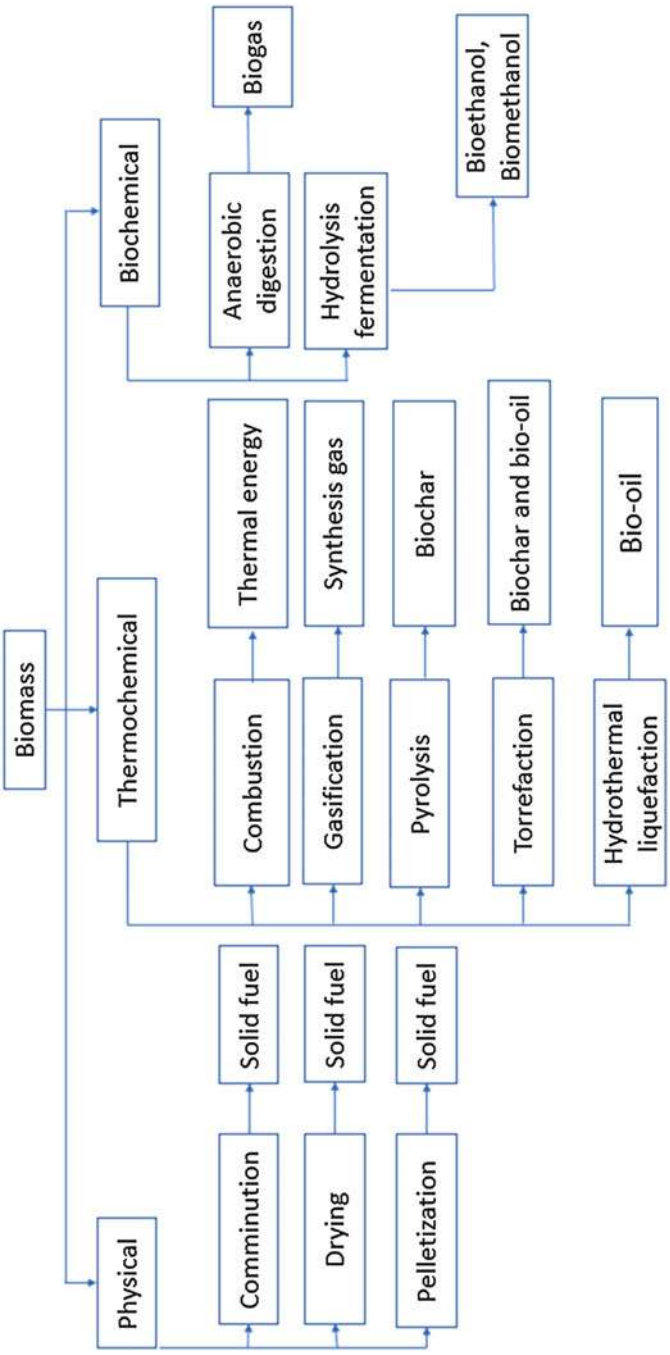

Fig. 5.2 Biomass conversion pathways

liquefaction, and torrefaction (Goswami et al. 2020, 2021a). Torrefaction is a thermal pre-treatment process used in the combustion, pyrolysis, gasification, and liquefaction of biomass (Ghodke and Mandapati 2019). Pyrolysis is heating the biomass at elevated temperatures (573–973 °K) to obtain biochar and bio-oil. The hydrothermal liquefaction process is operated between 523 and 647 °K within the pressure range of 4–22 MPa for the production of bio-oil. Gasification involves the conversion of biomass into syngas at temperatures higher than 973 K.

5.4 An Alternate Strategy for the Utilization of Waste Biomass

Biomass has a great potential to be used as a source of biofuel (Bhardwaj et al. 2021). The total biomass power generation potential of India is estimated to be 17,500 MW. However, at present, only 2665 MW of power is being generated (Kumar et al. 2015). Hence, due to the availability of abundant waste biomass materials, the management or disposal of these substances is itself a major task for environmentalists. In the global arena, "Waste to Wealth" is a term coined to utilize waste material for useful resources. Since the resource is inexpensive, green, and renewable, environmentalists focus on such processes to reduce pollution. There are several studies, where the waste biomass and its derivatives were used as a precursor material for effluent treatment processes. The biomass could be converted into a base material for catalyst preparation in the form of support or as an adsorbent material. They can be utilized for the treatment of effluents by the process of adsorption or by using an advanced oxidation process (AOP). Wherein the preparation of heterogeneous catalyst by supporting the active material on carbon support derived from biomass reduces the cost of the effluent treatment process. Hence, this chapter is aimed to discuss the thermochemical conversion of biomass into value-added materials for effluent treatment applications.

5.5 Biomass for Effluent Treatment Processes

It has been reported that every day two million tons of waste from various sources are discharged into water bodies worldwide. This created an impact that one in eight people worldwide are deprived of safe and clean drinking water as reported in World Water Assessment Programme, World Water Development Report1: "Water for People, Water for Life," Paris (2003). Water pollution is found to be the major reason for diseases and subsequently deaths worldwide (Vairavel and Murty 2020; Goswami et al. 2021b). In India alone, it has been estimated that approximately 580 people die due to water pollution-related illnesses every day (Clark et al. 1996). Major contaminants found in wastewater include but are not limited to dyes and

pigments, heavy metals, phenolic compounds, pharmaceuticals, agrochemicals, and endocrine disruptors (Akhil et al. 2021; Kumar et al. 2019; Kumar et al. 2021). Effluent treatment plants comprise physical, chemical, and biological treatment systems. Generally, all these treatment methods are grouped under primary, secondary, tertiary, and advanced treatment processes. Primary treatment processes aim to remove contaminants by the physicochemical processes like primary clarification (gravity settling) and coagulation/flotation. The secondary treatment processes work on the removal of the residual organics. The secondary treatment processes employ various microorganisms for reducing the COD and BOD of the effluent. Activated sludge treatment methods such as aerobic and anaerobic digestion are some of the secondary wastewater treatment methods. Tertiary treatment methods include membrane separation processes, electrodialysis, advanced oxidation processes, adsorption, biosorption, bioaccumulation, and ion exchange (Sonune and Ghate 2004). Most of these treatment processes like adsorption, biosorption, and advanced oxidation processes utilize functional materials for their operation. These functional materials like adsorbents and catalysts in case of adsorption and oxidation processes respectively are of chemical origin, which leads to secondary pollution. However, the waste biomasses and their derivatives proved to be environmentally friendly precursor materials for the synthesis of adsorbents and catalysts support materials. A simple thermochemical modification of the lignocellulosic waste materials could yield highly functional materials for wastewater treatment processes (Liu et al. 2015).

5.6 Application of Raw Biomass in the Effluent Treatment Process

Adsorption is the most widely used effluent treatment technique This is because, the used adsorbent materials have the potential for regeneration, recovery, and recycling which proved to be an added advantage at the industrial scale of operations. It is not only used for the removal of contaminants but also could be used for the recovery of precious and costly entities from the effluents (Crini et al. 2019). The physicochemical properties of the adsorbent have a major influence on the efficacy of the adsorption process. The chosen or prepared adsorbent should be easily available, economical, non-toxic, and should have good surface characteristics. They must also have high mechanical and thermal stability (Reddy et al. 2017). The lignocellulosic waste biomasses, in their raw or modified form, proved to be potential candidates for the preparation of economical and sustainable adsorbents for effluent treatment (Bhatnagar et al. 2015). Plant and agricultural waste products have earned increased interest for dye and heavy metals removal by adsorption from the aqueous solution because of their natural availability and higher removal efficiency (Garg et al. 2019; Agrawal and Verma 2021). The inexpensive waste products, after basic cleaning or some minor treatment, were explored as adsorbents. Agricultural by-products

especially those containing cellulose exhibit good adsorption potential for removing various toxic pollutants from effluents. There are numerous studies reporting the application of raw lignocellulosic materials like rice husk, oil cakes, banana peel, sugarcane bagasse, powdered leaves, etc. for the removal of different types of dyes, heavy metals, and other priority pollutants. This method of exploiting raw biomass as functional adsorbent materials proved to solve disposal problems associated with the abundant availability of these waste biomasses (Moorthy Rajendran et al. 2020). Few of the studies which deal with the application of these raw biomass materials for effluent treatment is tabulated in Table 5.1.

5.7 Biomass-Derived Activated Carbon for the Effluent Treatment Process

Many studies reported raw agricultural biomass as an adsorbent for the removal of organics and inorganics from simulated effluents. However, most of these raw biomasses were found to lack desired adsorption efficiency and were mechanically unstable. The efficiency of these biomasses could be enhanced by carbonization and activation using thermal and chemical treatment methods. The physical treatment includes carbonizing the material at a temperature of around 500 °C under an inert atmosphere using nitrogen or argon supply. The carbonized material is then activated at higher temperatures using suitable activating agents like steam or CO_2. The chemical treatment method involves the impregnation of the cellulosic biomass with various strong acids and bases like NaOH, KOH, HCl, H_2SO_4, $ZnCl_2$, etc. The impregnated material is then carbonized at higher temperatures at around 500 °C to obtain activated carbon (AC) (Rodríguez-Reinoso and Molina-Sabio 1992). The schematic for the preparation of AC through physical and chemical methods is presented in Fig. 5.3. AC is proved to have superior adsorptive and mechanical properties when compared to raw biomasses owing to increased surface area and porosity (Zhang et al. 2019). AC derived from different biomass has varying properties. The properties of AC depend on the precursor material, type of carbonization and activation, and activation temperature and duration. AC is the widely studied adsorbent material for treating effluents which contain all types of pollutants including the emerging contaminants from effluents (Yahya et al. 2015). Moreover, AC also finds its application as catalyst support in many reported studies in advanced oxidation processes which are discussed in Sect. 5.8.4. The application of biomass-derived AC is not limited to effluent treatment methods, and thus they are used in various fields such as gas purification (Ma et al. 2008), supercapacitors (Yang et al. 2014), and medicinal applications (Lakshmi et al. 2018). Few of the studies on the application of biomass-derived activated carbon for water and wastewater treatment is summarized in Table 5.2.

Table 5.1 Application of raw biomass for effluent treatment

Raw biomasses	Target pollutants	Maximum monolayer adsorption capacity "q_m" (mg/g)	References
Dyes			
Guava leaves	Remazol brilliant blue-R	93.12	Debamita et al. (2020)
Coffee husks	Congo red	38.65	Vairavel et al. (2021)
Neem leaf powder	Coomassie violet	39.64	Divya et al. (2020)
Sugar cane bagasse	Congo red	38.20	Zhang et al. (2011a, b)
Almond shells	Direct red 80	22.42	Ardejani et al. (2008)
Jujuba seeds	Congo red	55.55	Reddy et al. (2012)
Jackfruit peel	Methylene blue	285.71	Hameed (2009)
Lotus leaf	Congo red	45.89	Meghana et al. (2020)
Heavy metals			
Neem leaf	Cd(II)	157.80	Sharma and Bhattacharyya (2005)
Cucumis melo rind	Fe(II)	4.98	Othman and Asharuddin (2013)
Coffee residues	Cd(II)	39.52	Boonamnuayvitaya et al. (2004)
Coconut husk	Pb(II)	2.75	Abdulrasaq and Basiru (2010)
Durian shell	Cr (VI)	117	Edokpayi et al. (2015)
Sugarcane bagasse	Cd(II)	189	Karnitz et al. (2007)
Cassava tuber bark waste	Zn(II)	83.3	Horsfall et al. (2006)
Wheat bran	Cd(II)	101	Ozer and Pirincci (2006)
Phenol			
Tea waste	Phenol	154.39	Pathak et al. (2020)
Garlic peel	Phenol	14.48	Muthamilselvi et al. (2016)
Rice straw	Phenol	5.78	Sarker and Fakhruddin (2017)
Lantana camara	Phenol	112.5	Girish and Murty (2014)
Vegetal cords	Phenol	6.21	Cherifi et al. (2009)
Endocrine disruptors			
Barley husk	Bisphenol A	19.94	Balarak (2016)
Walnut shell	Bisphenol A	38.5	Dovi et al. (2021)
Raw fibric peat	Bisphenol A	6.48	Zhou et al. (2011)

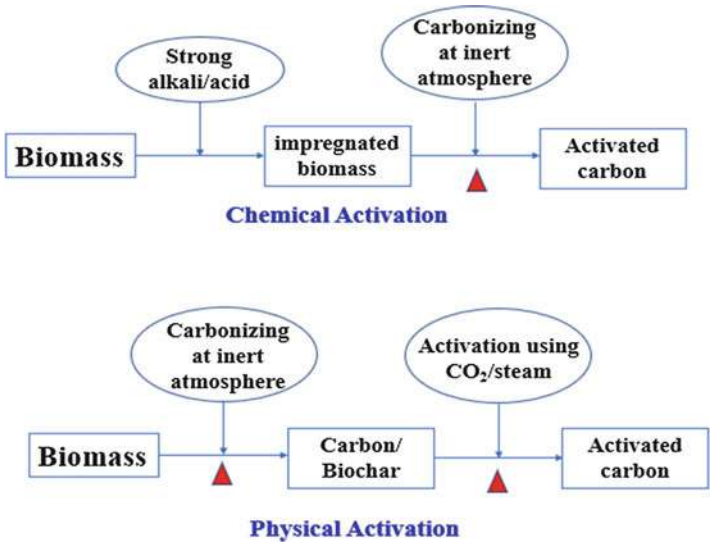

Fig. 5.3 Chemical and physical activation methods for the preparation of biomass-derived activated carbon

5.8 Biomass-Based Composite Materials for Effluent Treatment

Biomass-based materials either in their raw form or with thermal and chemical modification demonstrated to be an efficient low-cost material for the adsorption of various organics and inorganics from simulated effluents. Furthermore, recent studies suggest that these biomasses can also be fused with other functional materials and could be actively applied in various treatment processes. These biomass-based composites include but are not limited to metal oxide composites, magnetic materials, polymeric materials, and graphene-based composites. The schematic representation of the same is presented in Fig. 5.4.

5.8.1 Biomass-Based Magnetic Materials for Effluent Treatment

Generally, nanoparticles have promising potential to be used in various effluent treatment technologies. However, the application of these nanoparticles in water and wastewater purification is limited. This is due to the fact that, the spent nanomaterials after application tends to escape into the aquatic environment causing secondary pollution. The removal and recovery of the spent materials in their nanoform is not economical. Recently, materials coated with magnetite nanoparticles are considered

Table 5.2 Biomass-derived activated carbon for effluent treatment

Biomass-derived activated carbon	Target pollutants	Activation of adsorbents				Maximum monolayer adsorption capacity "q_m" (mg/g)	References
		Physical		Chemical			
		Temperature (K)	Activating agent	Temperature (K)	Chemicals used		
Dyes							
Pomegranate peel	Direct blue 106	–	–	773	$ZnCl_2$, H_3PO_4,	54.05	Amin (Amin 2009)
Orange peel	Direct navy blue 106	–	–	393	H_2SO_4	107.53	Khaled et al. (Khaled et al. 2009)
Pineapple waste	Methylene blue	–	–	773	$ZnCl_2$	288.34	Mahamad et al. (2015)
Rice husk	Acid yellow 36	373	steam	–	–	86.9	Malik (2003)
Sugarcane bagasse pith	Reactive orange	–	–	873	H_3PO_4	3.48	Amin (2008)
Grape processing waste	Methylene blue, Metanil yellow	–	–	873	$ZnCl_2$ $ZnCl_2$	417 386	Saygili et al. (2015)
Apple pulp and peel	Methylene blue	–	–	–	H_3PO_4	278	Hesas et al. (2013)
Palm flower	Amido black 10B	–	–	413	H_2SO_4	4.03	Nethaji and Sivasamy (2011)
Heavy metals							
Dairy waste Sugar beet	Pb(II) Pb(II)	873 873	– –	– –	– –	248 197	Inyang et al. (2012)
Hazelnut shell	Ni(II)	–	–	423	H_2SO_4	11.64	Demirbas et al. (2002)
Canna indica	Cd(II)	–	–	873	HCl	189	Cui et al. (2016)

Corn straw	Cu(II) Zn(II)	– –	– –	873 873	– –	12.5 11.0	Chen et al. (2011)
Pine bark Oak bark	Pb(II) Pb(II)	723 723	– –	– –	– –	3.0 13.1	Mohan et al. (2007)
Peanut shell	Pb(II)	–	–	823	H_3PO_4, HNO_3	35.5	Xu and Liu (2008)
Corn stalk	Cd(II)	–	–	873	$ZnCl_2$	32.4	Youssef et al. (2004)
Phenols							
Bamboo charcoal	Phenol	473	–	–	–	24.96	Ma et al. (2013)
Banyan root	Phenol	–	–	773	KOH	26.95	Nirmala et al. (2019)
Date-pit	Phenol	1173	CO_2	–	–	262.3	El-Naas et al. (2010)
Rattan saw dust	Phenol			973	KOH	149.25	Hameed and Rahman (2008)
Dates' stones	Para-chlorophenol	–	–	773	$ZnCl_2$	102.04	Aldoury and Sabeeh (2014)
Corn husk	Phenol Para-nitrophenol	– –	– –	773 773	$FeCl_3$ $FeCl_3$	8.445 11.668	Mishra et al. (2019)
Pharmaceutical wastes							
Pine sawdust	Sulfamethoxazole	–	–	923	$FeCl_2$, KOH, KNO_3	`19.09	Reguyal and Sarmah (2018)
Tea waste	Sulfamethazine	973	Steam	–	–	10	Rajapaksha et al. (2016)
Giant reed	Amoxicillin	973	Microwave	–	–		Chayid and Ahmecd (2015)

(continued)

Table 5.2 (continued)

Biomass-derived activated carbon	Target pollutants	Activation of adsorbents				Maximum monolayer adsorption capacity "q_m" (mg/g)	References
		Physical		Chemical			
		Temperature (K)	Activating agent	Temperature (K)	Chemicals used		
Cassava waste	Oxytetracycline	–	–	773	KOH	3.33	Luo et al. (2018)
Olive stones	Paracetamol	–	–	773	H_3PO_4	108.3	Garcia-Mateos et al. (2015)
Moringa seed	Diclofenac	–	–	723	H_3PO_4	121.112	Bagheri et al. (2020)
Cauliflower roots	Chlortetracycline	773	–	–	–	81.30	Qin et al. (2017)
Wheat straw	Ketoprofen	–	–	973	HCl	72.46	Wu et al. (2018)
Endocrine disruptors							
Agave Americana leaf fibers in combination with tannin	Tetracycline	1173	–	–	–	87.21	Selmi et al. (2018)
Argun nut shells	Diuron	573	–	–	–	833.33	Zbair et al. (2020)
Argun nut shells	Bisphenol A	573	–	–	–	1162.79	Zbair et al. (2020)
Pistachio nut shells	Caffeine	–	–	493	HNO_3	22.6	Roman et al. (2018)
Montmorillonite/rice husk hydrochar	17α-ethynyl estradiol	–	–	452	Methanol	138	Tian et al. (2018)
Sawdust hydrochar	Tetracycline	–	–	1073	KOH	423.7	Chen et al. (2017)

Potato peel hydrochar	Pramipexole Dorzolamide	– –	– –	473 473	KOH KOH	66 60	Kyzas and Deliyanni (2015)
Rice straw hydrochar	Tetracycline	–	–	573	K_2CO_3	714	Liu et al. (2014)

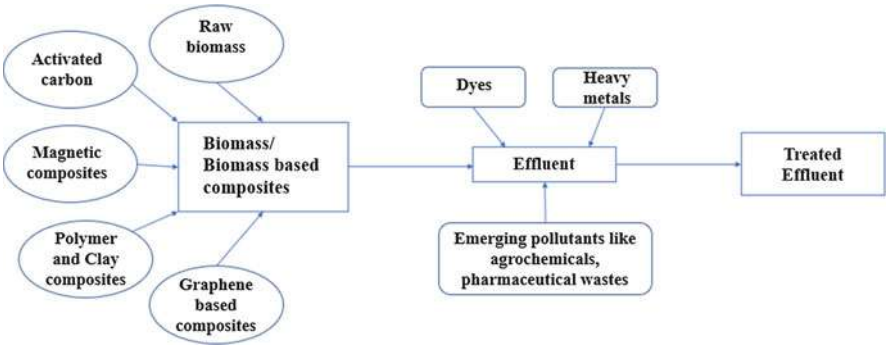

Fig. 5.4 Biomass-based composites for effluent treatment

very promising in the environmental remediation process. Since the magnetic particles are superparamagnetic (they are magnetized only with the external magnetic field), they can be recovered easily by the external magnetic field and reused without any loss of the functional materials (Nethaji et al. 2013). Hence, magnetic materials like iron oxide nanoparticles are extensively studied as functional materials for their application in water treatment owing to their magnetic properties. However, these iron oxide nanoparticles tend to agglomerate in the solution, thereby decreasing their efficiency. Hence, biomass-based materials supported by iron oxide nanocomposites were studied and successfully applied to overcome these drawbacks (Mehta et al. 2015). Biomass-based magnetic composites are mainly used in the adsorption process, and there are few studies which deals on the application of these materials in photocatalytic oxidation (Pang et al. 2016). Though several methods like hydrothermal reactions, sol-gel methods were reported, the co-precipitation method is most commonly employed for the preparation of magnetic composites. In the co-precipitation method, the biomass, or biomass-derived materials like activated carbon (AC) are dispersed along with the iron precursors like ferric chloride or ferrous sulfate. The iron salt in the precursors is then reduced using various reducing agents under continuous stirring, thus depositing the iron oxide nanoparticles onto the matrix of the raw biomass or AC derived from the biomass (Nethaji et al. 2013). The efficiency of these magnetic composites expended for the adsorptive/oxidative treatment of various pollutants from the aqueous phase along with the experimental conditions are shown in Table 5.3. The application of magnetized adsorbents derived from raw biomass and AC is presented in Fig. 5.5. Most of the studies reported an enhanced removal efficiency of the magnetic nanocomposites in comparison with the unmagnetized materials. Few adsorption studies reported a slight decrease in the efficiency, owing to the reduction in the available surface area due to the impregnation of iron oxide particles into the matrix of the biomass. Nevertheless, the ease of separation of the functional material improved considerably, thereby aiding in the regeneration and reusability of the nanocomposite materials.

Table 5.3 Biomass-based magnetic composites for effluent treatment

Biomass-based magnetic adsorbents	Precursor biomass materials	Target pollutants	Surface area (m^2/g)	Maximum monolayer adsorption capacity "q_m" (mg/g)	Inferences	References
Dyes						
Magnetic Corn cob-derived carbon (MCA)	Corn cob	Methylene Blue	153.89 (MCA) 69.45 (unmagnetized carbon)	163.93 (MCA) 103.09 (unmagnetized carbon)	The efficiency of the magnetized and unmagnetized adsorbent derived from corn cob was compared. The magnetized carbon possessed better surface area and adsorption efficiency for the sorption of methylene blue dye.	Ma et al. (Ma et al. 2015)
Magnetic graphene oxide-biomass activated carbon composite	Palm Kernel Shell (PKS)	Acid Blue 113	280.39	32.2	The adsorbent had the saturation magnetization value of 33.74 emu/g as characterized by a Vibrating Sample Magnetometer. A comparison study was also carried out to prove that the magnetized graphene oxide-PKS-derived carbon composites had better removal efficiency than the raw precursor and graphene oxide.	Ying et al. (2020)
Magnetic activated carbon from peanut shell	Peanut shell	Malachite Green	722.34 (CO_2 activation) 448.70 (Without CO_2 activation)	747.03 (CO_2 activation) 270.28 (Without CO_2 activation)	Studies were performed by increasing the iron oxide content in the matrix of the carbon derived from the peanut shell. The surface area, porosity, and adsorption efficiency increased with the increase in the iron oxide impregnation proving that the degree of magnetization is one of the most important parameters.	Guo et al. (2018)

(continued)

Table 5.3 (continued)

Biomass-based magnetic adsorbents	Precursor biomass materials	Target pollutants	Surface area (m^2/g)	Maximum monolayer adsorption capacity "q_m" (mg/g)	Inferences	References
Heavy metals						
Magnetic biochar composites (MB)	Phoenix tree leaves	Chromium (VI)	83.6	55 (Magnetized Biochar) 39.8 (Unmagnetized Biochar) 26.5 (Fe_3O_4 nanoparticles	Magnetized biochar was prepared by hydrothermal method. The O_2 containing groups on the surface of the biochar provided growth sites for Fe_3O_4 nanoparticles.	Liang et al. (Liang et al. 2019)
Magnetic nanoparticle (Fe_3O_4) impregnated onto tea waste	Tea waste	Nickel (II)	22.3 (tea waste) 27.5 (magnetized tea waste)	38.3	This study does not involve the preparation of activated carbon from tea waste. Tea waste is directly incorporated with Fe_3O_4 nanoparticles. The incorporation of iron oxide nanoparticles had a negligible impact on the surface area.	Panneerselvam et al. (2011)
EDTAD-modified magnetic baker's yeast biomass (EMB)	Baker's yeast	Lead (II)/cadmium (II)	–	89.21 (Pb^{2+}) 41 (Cd^{2+})	EMB could be regenerated using both HCl and EDTA with an efficiency of greater than 90% without disturbing the morphological characteristics.	Zhang et al. (2011a, 2011b)
Emerging contaminants						
Fe_3O_4/Douglas fir biochar	Douglas fir	Caffeine (stimulant) Ibuprofen (Inflammatory drug) Acetylsalicylic	468.2 (Douglas fir biochar) 322.0 (Fe_3O_4/Douglas fir biochar)	**Caffeine**: 23.9 (Douglas fir biochar) 73.1 (Fe_3O_4/Douglas fir biochar) **Ibuprofen:** 15.5 (Douglas fir	It was observed that the unmagnetized adsorbent exhibited better surface area when compared to the magnetized Douglas fir biochar. However, the adsorption of all the three pharmaceuticals were better	Liyanage et al. (2020)

		acid (Inflammatory drug)		biochar) 32 (Fe_3O_4/Douglas fir biochar) **Acetylsalicylic acid:** 89 (Douglas fir biochar) 126 (Fe_3O_4/Douglas fir biochar)	using the magnetized adsorbent compared to the unmagnetized char.	

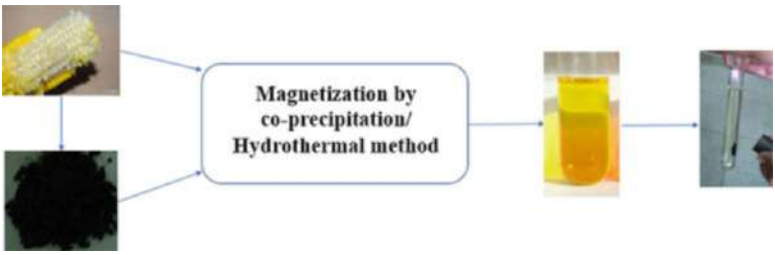

Fig. 5.5 Biomass-derived magnetic composites for effluent treatment

5.8.2 Biomass-Based Polymer and Clay Composites for Effluent Treatment

The efficiency of a functional material in the effluent treatment mainly depends upon its available surface area and surface functionality for adsorptive removal, and oxidative potential in case of advanced oxidation processes. Hence, polymeric materials are potential candidates to be used in the adsorption process, owing to their tunable surface properties (Pan et al. 2009). Naturally occurring biopolymers such as starch, cellulose, chitosan, and alginate were widely explored for their adsorption potential due to their high surface area and variable surface functionality. Among all the above-mentioned biopolymers, chitosan is widely explored for the removal of contaminants from simulated wastewater. It is more preferred since it contains -NH_2 and -OH functional groups on its surface which can act as chelating sites for concentrating organic and inorganic moieties (Wang and Zuang 2018). However, most of these naturally occurring biopolymers exhibit weak thermal and mechanical properties. Moreover, these biopolymers exhibit swelling phenomena when exposed to the aqueous environment. Hence, most of the studies deal with crosslinking these biopolymers with mechanically strong materials like biochar, biomass-derived AC, or clay composites. Natural clay materials like bentonite, montmorillonite, kaolinite, zeolite, etc. are mostly aluminosilicates with the presence of sodium, potassium, magnesium, and calcium. The layered morphology of these clay materials with the charged surface is ideal for the adsorption of ionic contaminants from the effluent. However, these clay minerals have poor potential for the removal of non-ionic contaminants. Nevertheless, they possess strong mechanical and thermal stability. Hence, the biopolymers are generally cross-linked with clay minerals to overcome the shortcomings of both clay and biopolymers (Unuabonah and Taubert 2014). Hence, biopolymers supported with biochar, AC, and clay minerals exhibited superior adsorption efficiency with better thermal and mechanical stability as shown in Table 5.4.

Table 5.4 Biomass-based polymer and clay composites for effluent treatment

Biomass-based adsorbent	Biomass precursors	Target pollutants	Surface area (m^2/g)	Maximum monolayer adsorption capacity "q_m" (mg/g)	Inference	References
Biopolymer composites						
Zeolite derived from chitin	Chitin	Crystal violet, methylene blue, basic fuchsin	–	124 (Crystal violet), 87.45 (methylene blue), 789.10 (basic fuchsin)	The zeolite was synthesized using biopolymer chitin as a mesoporosity agent by the hydrothermal method	Briao et al. (Briao et al. 2018)
Bacteria-derived poly(γ-glutamic acid) (γ-PGA)	*B. subtilis var.* (natto)	Auramine O, rhodamine B, safranin O	–	0.05 dm^3/mg (auramine O), 0.02 dm^3/mg (rhodamine B), 0.19 dm^3/mg (safranin O)	The kinetic data were well in agreement with Boyd's ion-exchange model	Inbaraj et al. (2006)
Chitosan/activated carbon/iron bio-nanocomposite	Chitosan from shrimp shell, activated carbon from grape stalks	Cadmium	834 (activated carbon) 11.6 (chitosan) 419.20 (chitosan/activated carbon/iron bio-nanocomposite)	322.58	The study indicated better interactions between oxygen functional groups of AC, iron ions, and amine groups of chitosan	Sharififard et al. (2018)
Zerovalent iron encapsulated chitosan nanospheres (CIN)	Chitosan	Arsenic (V) and (III)	69 (CIN) 26 (zerovalent iron)	–	The study shows that the adsorbent is porous in nature and iron particles exist in zerovalent state and there exists a complexation reaction among iron, chitosan, and arsenic	Gupta et al. (2012)
Biopolymer clay composites						
Cellulose-montmorillonite composite	Cellulose	Chromium (VI)	87.09	22.2	The interaction between cellulose and montmorillonite has proved the potential application	Kumar et al. (2011)

(continued)

Table 5.4 (continued)

Biomass-based adsorbent	Biomass precursors	Target pollutants	Surface area (m^2/g)	Maximum monolayer adsorption capacity "q_m" (mg/g)	Inference	References
					of the material for the effective adsorption of chromium. The XRD peaks signified the ordered distribution of clay layers in the biopolymer composite	
Montmorillonite-alginate beads	Alginate	Paraquat herbicides	46	0.321 mmol/g	TGA was used to determine the thermal stability of the beads. The results proved that the thermal stability of montmorillonite-alginate beads was much better than alginate beads	Etcheverry et al. (2017)
Guar gum /bentonite bio-nanocomposites	Guar gum	Lead (II), crystal violet (CV) dye	5.533	187.08 (Pb^{2+}) 167.92 (CV)	The TGA results proved increased thermal stability of biocomposites in comparison with guar gum. FT-IR suggested the electrostatic interaction or chelation via hydrogen bond formation between Pb^{2+} or CV and active sites of bio-nanocomposites.	Ahmad and Mirza (2018)

5.8.3 Biomass-Based Graphene Composites for Effluent Treatment

Graphene is composed of single layers of carbon atoms densely packed which attracted tremendous attraction in late 2004. It is a "single layer of carbon atom densely packed in a honeycomb crystal lattice" (Li et al. 2019). Because of its good chemical stability and graphitized basal plane structure, graphene-based materials are widely used in different applications including supercapacitors, fuel cells, batteries, and as adsorbents in effluent treatment systems (Novoselov et al. 2012). Graphene or reduced graphene oxide is mostly synthesized by using Hummers or modified Hummers method. Various functional groups present on the edges of the graphitic planes aid in the interaction of the graphene sheets with the charged contaminants present in the wastewater. However, the graphene sheets generally suffer from stacking and agglomeration problems due to π–π interactions and van der Waals forces in the aqueous phase. Hence, to overcome these limitations and to exploit the desirable properties of the graphene-based materials, various biomass-based graphene composite materials were reported for the removal of organic and inorganic compounds from simulated effluents (Nethaji and Sivasamy 2017). Biomass-derived materials like biochar, AC, and other cellulosic waste biomass were used as composite materials by crosslinking with the honeycomb structure of graphene oxides. There are also studies which had reported on the utilization of iron oxide nanomaterials for acquiring the magnetic properties reducing the stacking problem of graphene layers. The application of these biomass-based graphene composite materials for the removal of organic and inorganic moieties is presented in Table 5.5.

5.8.4 Biomass-Based Metal Oxides Composites such as Catalyst and Catalyst Supports

Heterogeneous catalysis involving metal oxides is a good example of an advanced oxidation process. Literature shows a number of methods for the enhancement of catalyst activity alongside cost reduction and efficiency maximization. Though many paths are sorted for catalyst modification, synthesis of a catalyst supported on materials with a higher surface area without diminishing the activity is a greater concern. Hence, the choice of the support material comes into the picture, wherein it must be cheap, green, and environmentally friendly with enhanced activity. Hence, carbon as catalyst support derived from biomass is a better option. Wherein, the disposal problem of the biomass itself is minimized and the resulting carbon could be used efficiently. However, activated carbon in itself has been used as a catalyst. Juhola et al. (2021) had prepared biomass–metakaolin as granular composite materials for application as a catalyst for the treatment of effluents.

Table 5.5 Biomass-based graphene composites for effluent treatment

Biomass-based graphene composites	Biomass precursors	Target pollutants	Surface area (m^2/g)	Maximum monolayer adsorption capacity "q_m" (mg/g)	Inference	References
Graphene oxide-based carbonaceous nanocomposites	Rice husk	Methylene blue	936	1591	Graphene oxide/ordered mesoporous carbon was prepared from rice husk using mesoporous silica as a template source	Liou and Wang (2020)
Graphene oxide/silica nanocomposites	Rice husk	Rhodamine B	625	147.06	Silica was extracted from rice husk. The hybrid material was prepared by hydrothermal method. The study also claims that the composites not only aid in recycling agricultural waste but also help in the recovery of graphene oxide from the aqueous phase	Liou et al. (2021)
Graphene/activated carbon composite	Glucose	Lead (II)	2012	217.6	Mesoporous graphene/activated carbon composite was prepared from graphene oxide and glucose. The prepared composite had a higher adsorption capacity for Pb^{2+} when compared to the reported literature	Saeidi et al. (2015)
Nitrogen-containing carbon nano-onions-like and graphene-like materials	*Lentinus edodes*	Phenol	35.40	–	Nitrogen-containing carbon materials with different morphologies were prepared using $Fe(NO_3)_3$ through a hydrothermal method. The prepared material was used for both adsorption and photocatalytic oxidation of phenol. The photoelectrons transformed by graphite claim to be reacted with O_2 molecules to form the superoxide radicals ($^{\cdot}O_2$) for the degradation of phenol	Han et al. (2021)

Since the development of nano metal oxides as a photocatalyst under visible light irradiation for environmental remediation (Fujishima and Honda 1972). Various research has been focused on the modification of metal oxides for maximum efficiency. The preparation of metal oxide on catalyst support is highly researched. While a choice for support material activated carbon prepared from biomass is widely preferred. Devagi and Soon (2018) have reported a TiO_2/modified sago bark (biomass) for treating the sago wastewater effluent. The author chose the effluent as it had a higher chemical oxygen demand (COD), biochemical oxygen demand (BOD), total suspended solids (TDS), and was acidic. The sago bark was chosen as a precursor as it is a major waste during the debarking step of the starch extraction. The modified biomass TiO_2 mixture was used as a photocatalyst for 64.92% removal with 0.2 g/L TiO_2/1% MSB (120 min of irradiation). Poudel et al. (2020) has removed As(III) from water by using agro-waste-based biomass impregnated with TiO_2.

ZnO incorporation on biomass-derived activated carbon has been widely researched by various research groups. Cruz et al. (2018) had prepared a ZnO/activated carbon (biomass derives) nanocomposite for the treatment of methylene blue dye. The ZnO nanoparticles were evenly distributed on the surface of the activated carbon. Ramya et al. (2018) has worked on the preparation of activated carbon from tannery sludge biomass. The acquired biomass was used as support material for ZnO-based nanocomposite preparation. The material was used for Cr (VI) removal from the aqueous phase. Supported biomass-based activated carbon for dye degradation has been prepared by hydrothermal technique. Waste biomass was used by Vinayagam et al. (2018) for activated carbon preparation as carbon support. Akpomie et al. (2020) recently prepared a ZnO nanoparticle along with biomass for the treatment of celestine blue dye.

Hybrid bifunctional materials have also been synthesized by various research groups comprising AC and nanomaterials which would serve both as an adsorbent and photocatalytic material. Our group has also worked in this area (Nethaji et al. 2018) and we have reported a bismuth oxybromide (BiOBr)/activated carbon hybrid material as a bifunctional nanomaterial for effluent treatment. It was a good adsorbent material and it even degraded malachite green dye under visible light irradiation. The source of AC was waste polyurethane foam trash from used car seats. The composition of the foam was a polyol with a toluene diisocyanate blend. A simple hydrothermal process was used for the preparation of the bifunctional material.

5.9 Conclusions

Biomass materials are mostly explored for their potential to be used as biofuel. However, the consumption of this biomass for the production of biofuel accounts for less than 10% of the available lignocellulosic materials. Owing to its abundant availability, most of the agricultural waste biomass is considered waste and requires a separate disposal method or a process for the same. But the utilization of these

types of wastes and naturally available renewable material as a source for the production of materials for the effluent treatment itself is a boon to the environment. Most of the studies suggest that these biomasses either in their raw form or modified form proved to be an effective replacement for the synthetic materials conventionally used in effluent treatment operations. Moreover, the hybrid materials which include biomass-based composites could effectively overcome the shortcomings of their synthetic counterparts. The biomass-derived activated carbon was effectively used in various adsorptive and catalytic applications as adsorbent and catalyst support, respectively. The compatible and environmentally friendly nature of the biomasses could be explored for applications in various other unit operations in effluent treatment plants. Hence, these materials derived from the biomasses can be coined as "waste to wealth" and thus prove to be environment-friendly substitutes to treat and overcome effluent treatment problems.

Competing Interests All the authors declare that they have no competing interests.

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Chapter 6
Cellulase: A Catalytic Powerhouse for Lignocellulosic Waste Valorisation

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Abstract Cellulose is the world's most ubiquitous organic compound and has an abundant potential to be transformed into a renewable source of energy and other industrial products. To break down this complex polymer, cellulase has been conventionally used as a biocatalyst. Other than numerous industrial uses, including detergent, paper and pulp, textile, beverages, feed, baking, and biofuel, the application of cellulase for waste valorisation is also a central thrust area. The cellulolytic action of this enzyme has been explored for utilisation of widely available lignocellulosic waste. This reaction has been critical for the cellulose conversion to glucose and then to biofuel. Apart from biofuel, cellulase has also been employed to convert green waste into value-added products such as prebiotic oligosaccharides, organic acids, and biopolymers that can be integrated into the circular economy. Numerous factors affect the catalytic actions of cellulase, like pretreatment of lignocellulosic biomass, structural and compositional variation, working temperature, and pH. This chapter encompasses a detailed insight into the biocatalytic mechanism, applications, and limitations of cellulase as a potent enzyme for waste valorisation.

Keywords Cellulase · Waste valorisation · Lignocellulosic biomass · Circular economy · Biorefinery

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P. Verma (ed.), *Thermochemical and Catalytic Conversion Technologies for Future Biorefineries*, Clean Energy Production Technologies,
https://doi.org/10.1007/978-981-19-4312-6_6

Abbreviations

3Rs	Reduce reuse and recycle
ATCC	American type culture collection
BSG	Brewer's spent grain
CBG	Compressed biogas
CMCase	Carboxymethyl cellulase
CNCs	Cellulose nanocrystals
CNF	Cellulose nanofibrils
CO_2	Carbon dioxide
Co^{60}	Cobalt-60
FPA	Filter paper assay
FPU	Filter paper units
GHG	Greenhouse gas
IFPU	International unit of filter paper activity
IL	Ionic liquid
IU	International enzyme unit
LCA	Life cycle assessment
MNPs	Magnetic nano-particles
MPa	Megapascal
PHA	Polyhydroxyalkanoates
PLA	Poly-lactic acid
RSM	Response surface methodology
SDS	Sodium dodecyl sulphate
SHF	Separate hydrolysis and fermentation
SmF	Submerged fermentation
SMS	Spent mushroom substance
SmSF	Simultaneous Saccharification and Fermentation
SSCF	Saccharification and co-fermentation
SSF	Solid-state fermentation
TCA	Tricarboxylic acid
TEA	Techno-economic analysis
U/g	Enzyme unit per gram
U/mL	Enzyme unit per millilitre
USD	United States dollar

6.1 Introduction

The focus of waste treatment has now shifted to its valorisation, as it promises better management of available resources. The valorisation method, if assisted by biological techniques, becomes a better environment-friendly solution. The utilisation of biological methods for waste valorisation has spawned the concept of a bio-based

circular economy. Cellulase is one such biocatalytic agent which is categorised as endoglucanase (E.C.3.2.1.4), exoglucanase, or cellobiohydrolase (E.C.3.2.1.91), and β-glucosidase (E.C.3.2.1.21).

The source of cellulase can vary from natural to commercial production (Bhardwaj et al. 2021a). The commonly reported bacteria, streptomyces, and fungi for cellulase production are Clostridia sp., *Bacillus licheniformis, Streptomyces argenteolus*, *S. griseorubens, S. lividans, Aspergillus oryzae, Candida cylindracea, Trichoderma reesei, etc.* (Hamdi et al. 2020; López-Mondéjar et al. 2019; Saldarriaga-Hernández et al. 2020; Kumar et al. 2018, 2020a). The genetically developed producers include *E. coli* and *Pichia pastoris*.

The mechanistic insight of cellulase explains the hydrolysis process. Endoglucanase is responsible for the breakdown of β-1,4 glycosidic bond, which exposes new chain ends. The ends so produced are broken by exoglucanase/cellobiohydrolase to produce oligosaccharides, whereas β-glucosidase causes lysis of these oligosaccharides into their monomer units. For a detailed review of the mechanism of action and structure of cellulase, an article by Rabinovich et al. can be referred (Rabinovich et al. 2002). Most of the cellulases have been found to be a multidomain proteins containing a catalytic domain, a cellulose-binding domain and an interdomain linker.

Earlier reports suggest the use of cellulase in waste management such as deinking of paper, degradation of voluminous cotton sludge, and bioconversion of sawdust, vegetable and fruits waste, rice straw, leaves and bamboo, and sawdust. Thus, cellulase-mediated waste valorisation becomes a potent tool for the proficient utilisation of agro-residues, food, paper, and textile industry waste (Khan et al. 2016; Vermelho et al. 2012). The cellulose content of municipal solid waste has also been utilised for biogas production (Demirbas et al. 2016). According to a report, about 10–50 billion tons of dry lignocellulosic waste are produced every year across the globe (Motaung and Linganiso 2018). This waste has been reported to contain about 45% cellulose, 10% hemicellulose, and 10–15% lignin. The agro-waste mainly includes sugarcane bagasse (grown in China, India, Brazil, Thailand, South Africa, and Australia), maize stalks (major crop of countries like South Africa and the United States), rice husk (grown worldwide), and sorghum stalks (tropical and subtropical regions of Asia and Africa). According to Khan et al., lignocellulosic material consists of 30% solid content composed of about 65% holocellulose and 20% lignin (Khan et al. 2016; Kumar and Verma 2021a). The meticulous design of the valorisation method can lead to the recycling of by-products with economic advantage. With the advancement of current technologies, diverse applications of these processes have been explored. These include fossil-fuel alternatives such as bioethanol, biopropanol, aviation fuel, and biogas (Kumar and Verma 2021b). Other platform chemicals, including succinic acid, butyric acid, and enzymes are also being produced by using enzymatic hydrolysis of food and agro-industrial waste. This further extends to the industrial production of innumerable polymers with various commercial applications such as polyhydroxy butyrate, poly-lactic acid, xanthan, pullan, and poly-hydroxy valerate. The application of cellulase for the valorisation of waste from different industries has been summarised in Table 6.1.

Table 6.1 Application of cellulase in the waste valorisation

Industrial waste as substrate	Source of cellulase	Value-added product formed	Method used	Reference
Lignocellulosic biomass	*Bacillus. tequilensis* G9	Bioethanol	Co-culturing of *Bacillus. tequilensis* G9 with *Saccharomyces cerevisiae*	Dar et al. (2019)
Forest waste (spruce and birch)	Obtained from NZYTech Lda.	Prebiotics	Pretreated residues were enzymatically digested by four cellulase	Karnaouri et al. (2019)
Paper sludge	Obtained from Novozymes and Sigma-Aldrich	Bioethanol production	Supplementation of β-glucosidase with a commercial cocktail of enzyme	Gomes et al. (2016)
Lignocellulosic biomass	yeast *Yarrowia lipolytica*	Lipid (ricinoleic acid) production	Consolidated Bioprocessing	Guo et al. (2018)
Sugarcane bagasse	Bacterial consortium from sugarcane bagasse and thermophilic sludge	Methane, hydrogen, and other organic acids	Bioaugmentation of cellulase producing bacteria obtained from lignocellulosic biomass with sludge	Soares et al. (2019)
Spent mushroom and food waste	*A. niger*	Lactic acid	Cellulase produced by *A. niger* led to the breakdown of waste to produce hexoses and pentoses. These were then utilised by *Enterococcus mundtii* CGMCC 22,227	Ma et al. (2021)
Textile waste (cotton-polyester 50:50 blend)	Commercial cellulase and β-glucosidase	Bioplastics, biosurfactants, and other biochemicals	Pretreatment, enzymatic hydrolysis, and recovery step include the use of activated carbon, filtration. Ion exchange chromatography and solvent evaporation	Subramanian et al. (2020)
Tapioca flour commercial waste	NA	Bioethanol gel	Pretreatment, enzymatic hydrolysis, and fermentation	Amalia et al. (2021)
Pomegranate peel	Commercial cellulase and pectinase (Novozyme)	Antimicrobial property for application in therapeutics	High-pressure extraction at 300 and 600 MPa	Alexandre et al. (2019)

(continued)

Table 6.1 (continued)

Industrial waste as substrate	Source of cellulase	Value-added product formed	Method used	Reference
Sugarcane straw	Commercial enzyme (Celluclast and Novozyme 188)	Bioethanol	Steam explosion pretreatment followed by enzymatic hydrolysis	Oliveira et al. (2013)
Sugarbeet	Accellerase (Merck-Sigma Aldrich)	Pectin, phenolic compounds, and succinic acids	Fractionation is followed by enzymatic hydrolysis. The hydrolysate was fermented by *Actinobacillus succinogenes* to produce succinic acid	Alexandri et al. (2019)

This chapter encompasses applications of cellulases in the valorisation of various waste belonging to food, textile, agricultural, and other industrial sectors. The current state of the art pushes toward a bio-based circular economy where the waste stream is maximally recycled to other production input stream.

6.2 Recent Advances in Cellulase Production and Purification

The potential of abundant natural polymer cellulose in nature has been critically known in biofuel production such as bioethanol and biobutanol. However, the major constraint is in the conversion of cellulose to fermentable sugars (saccharification) that can be overcome by treating the biomass with the cellulase enzyme. The cellulolytic enzyme can be obtained from plant, animal, and microbial sources (Zhang and Zhang 2013), among which microbial sources can be used to scale up production.

6.2.1 Microbial Production of Cellulase

Most cellulolytic microorganisms were isolated from the decaying wood and used to produce the cellulase enzyme at a large scale by either solid-state fermentations or submerged fermentations.

6.2.1.1 Solid-State Fermentation (SSF)

The type of fermentation occurs almost without free water; however, the substrate moisture must be present to assist the microorganism's growth. Hence, SSF imitates the natural habitat of maximum number of cellulolytic filamentous fungi. The critical factors that play a crucial role in the extracellular release of cellulase in SSF are moisture content, harvest time, spore count, particle size, type of substrate, incubation time, temperature, and initial pH values that render the adaptation cellulase excretion. The SSF begins with inoculating an optimised number of pure spores of filamentous fungus into the sterile solid medium under the necessary optimised conditions. The cellulase production using *Trichoderma reesei* ZU-02 through SSF by reusing solid substrates in at least three batches yielded the optimum cellulase production (158 IFPU/g koji) was obtained in the second batch of fermentation (Xia and Cen 1999). The comparative investigation of cellulase production in SSF by the two mutant strains of *Trichoderma reesei* showed to be critically varied with the physicochemical parameters like moisture content and the temperature in which *T. reesei* QM9414 strain indicated that only moisture content (70% optimum) had a major influence on the production of cellulase with maximum FPA (filter paper assay) activity (1.1635 U/g). Whereas, temperature (25°C optimum) and moisture (55% optimum) were found to be important in the extracellular cellulase production with maximum FPA activity (2.314 U/g) by *T. reesei* MCG77 strain (Latifian et al. 2007). In addition, Kim et al. had investigated the improvement of cellulase production by *Aspergillus sp.* SU-M15 with the repeated sequential treatment of mutagens Co^{60} γ-rays, N-methyl-N′-nitro-N-nitrosoguanidine, and ultraviolet irradiation to spores of *Aspergillus sp.* The production in wheat bran medium was found to be higher (82.5 U/g) than husk and sawdust by SSF (Vu et al. 2011). Sometimes, a mixture of substrates induces the extracellular cellulase production rather than the individual substrate, as investigated by Barnabe et al., who found that the mixed sludge of paper and pulp that influenced the lignocellulolytic secretions to 7.3 IU/mL from 1.5 IU/mL by *Trichoderma reesei* RUT C-30 (Lai et al. 2017). However, the cost of cellulase production is considerably reduced by utilising the waste residues or low-cost substrates from the municipal corporation, horticulture, kitchen, industrial, or open terrestrial sources as the substrates for SSF by optimising the required physicochemical environment (Bansal et al. 2012; Bharti et al. 2018; Xin and Geng 2010). The purification of extracellular cellulase enzyme can be done by extraction with the circulation of buffer and collection as a crude cellulase preparation that can be used for the saccharification of lignocellulosic materials and other applications.

6.2.1.2 Submerged Fermentation (SmF)

The type of fermentation that occurs in the presence of excess water is known as submerged fermentation (Kumar et al. 2020b). In most industrial-scale enzyme

operations, the production by SmF is of priority because of ease of monitoring and operating. Most of the commercially available cellulases are produced by filamentous fungi; however, some bacteria (Ariffin et al. 2006; Ekperigin 2007; Irfan et al. 2012) and actinomycetes (Das et al. 2014; George et al. 2001; Van Zyl 1985) can also have the ability to produce but in low titre. The aerobic production by SmF depends on several factors like cellulosic substrate, nutrient availability, pH of the medium, inducer concentration, rate of oxygen supply, and fermentation temperature. The media formulation is necessary to result in optimal growth of biomass and cellulase production. The generalised medium used for the growth of filamentous fungi and cellulase enzyme production is Mandel's medium, Vogel's medium. However, to increase cellulase production, these medium needs to be optimised with respect to cellulolytic fungus. The cellulase production can be increased by genetically engineering the cellulolytic strains by either random mutation or site-directed mutagenesis. Separation of extracellular enzymes from the fermented solid biomass is carried out by centrifugation (10,000 × g, 10 min, 4°C) that can be used directly as a crude enzyme for the hydrolysis of cellulosic material (Lai et al. 2017). The cellulase production by SSF and SmF using various substrates has been listed in Table 6.2.

6.3 Immobilisation and Molecular Approaches for Enhanced Cellulolytic Action

The objectives of immobilisation of cellulase enzyme are reusability of the same enzyme for several cycles with excellent efficiency and stability that can substantially reduce the overall cost of the product. The materials used to immobilise the enzymes are both organic and inorganic, including polysaccharides, proteins, polymers, activated carbon, and metals (Datta et al. 2013; Nawaz et al. 2016; Safarik et al. 2016). There are various strategies to immobilise the enzymes, as represented in Fig. 6.1.

Recent reports suggest several methods for cellulase immobilisation for the valorisation of agro-residues, as shown in the above figure (Fig. 6.1). There have been studies for the comparison of conversion efficiency of free and immobilised enzymes for bioethanol production using lignocellulosic residues. The use of nano-structured support for the immobilisation of cellulase has been widely investigated (Ahmad and Khare 2018; Brindhadevi et al. 2020; Han et al. 2018; Husain 2016; Sillu and Agnihotri 2019). These nano-carriers impart reusability and stability of the enzyme and exhibit a larger surface area for enzyme attachment. This facilitates overcoming the mass transfer limitations by increasing the amount of enzyme that can be employed for catalytic conversion. The use of Magnetic Nano-Particles (MNPs) further improvises the process by allowing easy withdrawal of enzymes from the solution utilising an external magnetic field. In another study, the immobilisation was done on MNPs produced using green synthesis. Immobilisation

Table 6.2 Examples of cellulase production using different substrates

Substrate	Microorganism	Fermentation process	Activity			Reference
			FPU	CMCase assay	β-glucosidase	
Wheat bran	*Trichoderma reesei* RUT C30	SSF	1.14 U/mL	14.98 U/mL	0.22 U/mL	Sukumaran et al. (2009)
Enriched Mandel's cellulose medium	*Trichoderma spp.*	SmF	0.396 U/mL	0.488 U/mL	–	Chand et al. (2005)
Solka Floc	*Acremonium cellulolyticus* strain CF2612	SmF	17.8 U/mL	–	40.3 U/mL	Fang et al. (2009)
Wheat bran	*Fomitopsis sp.* RCK2010	SSF	3.49 U/g of substrate	71.6 U/g of substrate	53.6 U/g of substrate	Deswal et al. (2011)
Rice straw	*Melanocarpus sp.* MTCC 3922	SSF	25.3 U/mL	42.3 U/mL	74.1 U/mL	Jatinder et al. (2006)
Pretreated sugarcane bagasse	*Penicillium echinulatum* 9A02S1	SSF	32.89 U gdm^{-1}	282.36 U gdm^{-1}	58.95 U gdm^{-1}	Camassola and Dillon (2007)
Pretreated willow	*Trichoderma reesei* Rut C30	SmF	0.62 FPU/mL	132 U/mL	0.07 U/mL	Kovács et al. (2008)
Sugarcane bagasse	*Trichoderma atroviride* 676	SmF	0.25 U/mL	1.9 U/mL	0.17	Grigorevski-Lima et al. (2013)
Wheat bran	*Penicillium citrinum*	SSF	1.72 U/mL	1.89 U/mL	–	Dutta et al. (2008)

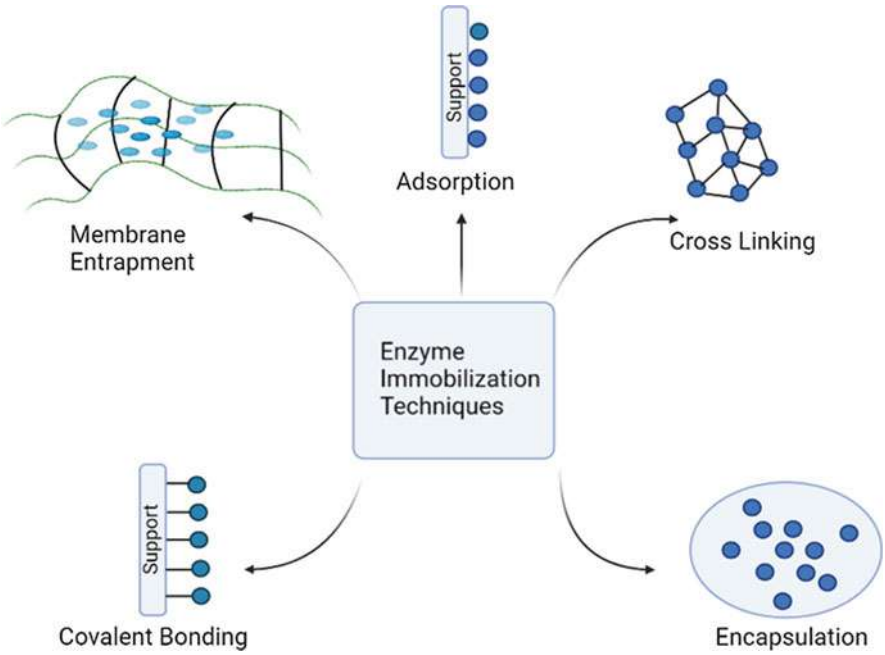

Fig. 6.1 Various methods to adopt immobilisation of enzymes

was secured by the covalent linking of cellulase to MNPs using glutaraldehyde. The results showed higher conversion efficiency of free enzymes (78%) than the covalently bonded enzymes (72%) in the first cycle, owing to the mass transfer limitation. However, subsequent cycles showed stable conversion efficiency, which ultimately is responsible for improved process economics (Ingle et al. 2017). Thus, immobilisation of cellulase contributes to its reusability, stability, and storage.

Cellulase can also be covalently immobilised using glutaraldehyde as a linker. In a significant work, it was immobilised on biochar prepared using sugarcane bagasse and coated with chitosan in different concentrations. This immobilisation process was successful in achieving 90% residual activity of cellulase even after 10 cycles of conversion (Mo et al. 2020). A detailed analysis of the pros and cons of selecting suitable micro- and nano-carriers with different methods of immobilisation is available elsewhere (Rajnish et al. 2021).

6.4 Role of Cellulase in Biorefinery Application

Cellulases are potential candidates for the bioconversion of agro-industrial wastes into several beneficial products. Such wastes are otherwise burnt or used in landfills (Singh et al. 2021). A zero-waste integrated biorefinery would involve cellulases in

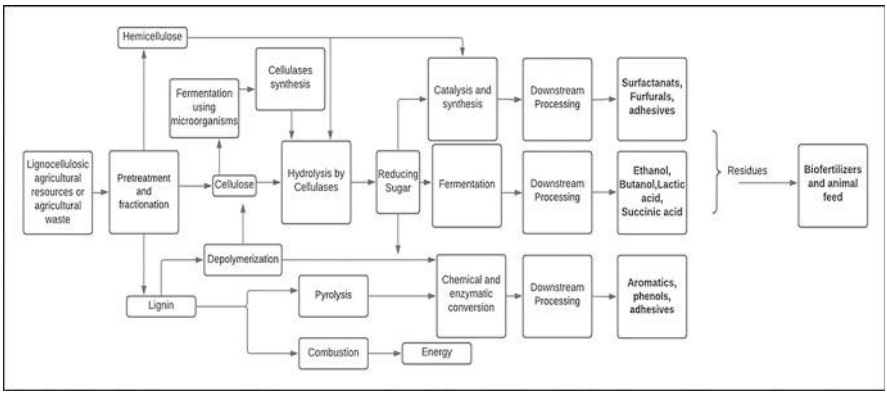

Fig. 6.2 Application of cellulases in a lignocellulosic biorefinery

the sequential production of sugar, organic acids, surfactants, furfurals, and biofuels such as bioethanol, biobutanol, biohydrogen, and compressed biogas (CBG) using agro-industrial residues, and the remaining organic matter can be used as compost or animal feed (Carrillo-Nieves et al. 2020; Kumar and Verma 2021a, b). Commercial enzyme manufacturing companies strive to manufacture enzyme cocktails for industrial biorefineries (Chandel et al. 2012). The biorefinery scheme utilising cellulases is depicted in Fig. 6.2.

6.4.1 Consolidated Bioprocessing for Biorefinery

The integrated approach to biomass conversion is known as consolidated bioprocessing. In this, a single microbe's amalgamation of saccharification and fermentation capabilities reduces environmental losses and expenses in lignocellulosic biorefineries (Bhardwaj et al. 2021a; b). The cellulase production, enzymatic hydrolysis, and bioconversion are combined (Cunha et al. 2020). The method involves hydrolysis of the structural carbohydrates to oligomers using the synthesised enzymes. The oligomers are depolymerised. The residual sugar is then fermented into various valuable products (Daniel et al. 2012). 10–30% of the pretreated liquor contributes as the carbon source for the production of enzymes and propagation of seeds. The enzyme loading is reduced to 10 mg protein/g cellulose (Scarlata et al. 2015). These modifications make the process economical and efficient. In a recent study on the biorefinery approach, consolidated bioprocessing using engineered *Trichoderma reesei* and *Saccharomyces cerevisiae* led to the highest production of 0.5 g/L glucaric acid from steam-treated corn stover in 7 days (Li et al. 2021). In another study, consolidated bioprocessing was used to produce bioethanol using surfactant (Tween 20)-assisted ionic liquid (1-ethyl-3-methylimidazolium methane sulphonate) from pretreated biomass of *Parthenium*

hysterophorus L. The optimisation of pretreated conditions like biomass loading, pretreatment temperature, and time could increase the sugar yield by 40% during saccharification with IL-stable cellulase and xylanase enzymes produced from *Aspergillus aculeatus* PN14 (Nargotra et al. 2019). A novel consolidated bioprocessing method was developed for hyperproduction of butyric acid from delignified rice straw using co-fermentation of *Clostridium thermocellum* ATCC 27405 and *C. thermobutyricum* ATCC 49875. The butyric acid yield was increased up to 33.9 g/L by fed-batch fermentation with a selectivity of 78% (Chi et al. 2018). Therefore, consolidated bioprocessing combines many processes and reduces the overall cost of production by reducing energy requirements and contamination risk as well as maximising cost-effectiveness.

6.4.2 Cellulases in Agro-industrial Waste Biorefinery

Enzymatic pretreatment is an indispensable step of any lignocellulosic biorefinery. Using cellulolytic hydrolysis, the waste from agricultural practices and industries can be converted into several valuable products (Dragone et al. 2020). A cellulolytic enzyme derived from *Bacillus pumilus* is used for sugar production from banana-agro waste in the form of pseudostem and leaves (Kanmani et al. 2011). Cellulases synthesised by *Sporotrichum* sp. LAR5 with 7.88 IU was used to extract reducing sugar from acid-pretreated rice straw (Bajaj et al. 2014). In another instance, cellulases derived from *Penicillium decumbens* were used to hydrolysis corn cobs to produce reducing sugar used for ethanol production (Saliu and Sani 2012). The use of cellulases enzyme for the valorisation of various agricultural and industrial wastes is depicted in Table 6.3.

Cotton waste from textile industries is decomposed using cellulases for safe disposal into the environment. Waste generated from textile industries includes cotton and denim residues from the cotton machining process. These waste streams are treated with cellulase. Such industrial residues are subjected to enzymatic hydrolysis for recycling and production of valuable products such as glucose syrup (Subramanian et al. 2020). Fruit and vegetable wastes are rich in disaccharides and polysaccharides. They are also non-competitive with our food chain. Cellulases can hydrolyse the fruit and vegetable waste for further bioconversion into numerous commercial products (Srivastava et al. 2021). Hemp Hurd is a by-product of the fibre industry. Immobilised cellulase on an activated magnetic support was used to hydrolyse hemp hurds (Abraham et al. 2016). In another study, Liu et al. (2019) investigated the hydrolysis of apple pomace by pectinase and cellulase and further optimised the cellulase hydrolysis parameters by RSM after pectin hydrolysis. They obtained a maximum fermentable sugar production yield of 67.54 ± 1.45%, which provided the highest lipid production of 25.8 g L^{-1} after fermentation by a genetically modified strain *Yarrowia lipolytica* po1f (pex10−mfe− leu+) (Liu et al. 2019).

Table 6.3 Application of cellulases for treating different agro-industrial biorefinery

Agro-industrial waste	Enzyme source	Processing parameters	Observations	References
Paper sludge	Recombinant cellulase cocktail, Opt CelMix similar to Cellic CTec2 and Celluclast	0.015 g/g cellulase cocktail and 10% (w/v) biomass loaded	80% glucan conversion. High glucose yields using low enzyme loading	Malgas et al. (2020)
Newspaper waste	Celluclast 1.5 L and Novozyme 188	Pretreatment using SDS and Tween-80. 15% solid content and enzyme loading was 15 FPU/g waste	29.07 g/L reducing sugar produced after 72 h at 50 °C. 0.42 g/g ethanol produced by co-culture of *Saccharomyces cerevisiae* and *Pichia stipitis*	Xin et al. (2010)
Citrus peel waste	Indigenously from *Aspergillus niger*	30 IU g^{-1} $_{Drm}$ cellulase, processing at 116 °C for 10 min	11.18 mg/ L reducing sugar. 30.7 g/ L ethanol and (339–356 mL/gVS methane)	Patsalou et al. (2019)
Corn stover	Commercial acidic cellulase	Steam-exploded 20 FPU/g dry stover	103 g/L reducing sugar	Lu et al. (2010)
Sugarcane bagasse	*Trichoderma reesei* and *Aspergillus niger*	2.0 IU/g cellulases	0.5 g reducing sugar obtained per dry substrate	Rabelo et al. (2008)
Wastewater sludge	Indigenously from *Trichoderma harzianum*	Bubble column bioreactor and membrane reactor for a system with 30 kDa membrane	73.5% cellulase recovery and 81.37% reduction in chemical oxygen demand	Libardi et al. (2019)
Rice husk	Celluclast	Lime pretreated biomass, 48 FPU/g	0.2 g/g reducing sugar	Saha and Cotta (2008)
Textile residues	Indigenously from *Aspergillus niger* CKB	Alkali pretreated biomass, 0.43 ± 0.01 FPU g enzyme loading	70.2% reducing sugar recovered	Hu et al. (2018)
Sorghum waste	Spezyme-*CP* and Novozyme	10% pretreated (by ammonia) fibres were hydrolysed using 60 FPU Spezyme-CP and 64 CBU Novozyme 188/g glucan	84% digested for a pretreated sample and 38% cellulose digested for untreated biomass 0.24 g ethanol per g dry biomass was obtained for the pretreated sample	Salvi et al. 2010

6.4.3 *Challenges of Cellulase-Based Biorefinery*

Driving down the cost of enzyme production poses the main challenge for agro-industrial biorefineries. Mass bio-prospecting programme for the discovery of efficient cellulase enzyme synthesising microbes can make the production economical in the long run (Jayasekara and Ratnayake 2019). Integrated processing techniques would also be helpful in improving cellulase production and ethanol productivity in a simplified manner (Kuhad et al. 2016). Instead of single enzymes, concoctions and cellulolytic enzyme cocktails should be explored for their applications in hydrolysis (Victoria et al. 2017). In-house enzyme production is an economical option to produce ethanol for biorefineries (Cunha et al. 2017). This would ensure waste to wealth conversion in a sustainable manner.

6.5 Role of Cellulase in Value-Added Product Formation

Lignocellulosic biomass can be transformed into value-added chemicals through various existing chemical, thermochemical, and biological routes. However, the biological method of lignocellulosic biomass conversion using enzymes is a feasible and environment-friendly, and sustainable process. Many bacterial and fungal species have the ability to breakdown the lignocellulosic biomass into simple sugars such as glucose and xylose by secreting enzymes (cellulase and hemicellulase), which can further be utilised to produce several value-added products like acids (lactic acid, citric acids, succinic acid, gluconic acids), butanol, xylitol, microbial polysaccharides, and other fine chemicals (Ning et al. 2021). In combination with other enzymes (utilised pectinase and xylanase), cellulase improves the hydrolysis efficiency and reduces the overall cost associated with the process (Bhardwaj and Verma, 2021). Some of the important value-added products derived from lignocellulosic biomass are mentioned in Table 6.4.

6.5.1 *Lactic Acid*

Lactic acid is used as a green solvent and building block for polylactic acid (PLA), a bio-degradable plastic alternative to petroleum-based plastic. Lactic acid can be produced from simple carbohydrates derived from the hydrolysis of agricultural lignocellulosic biomass. However, these recalcitrant lignocellulosic materials should be pretreated first to ease the cellulosic hydrolysis process governed by the cellulase enzymes. Various pretreatment such as physical (milling, grinding, shredding), chemical (acidic, alkali, ionic liquids), and thermal pretreatments can enhance the cellulosic hydrolysis process based on the nature of the lignocellulosic biomass. Yadav et al. (2021) inspected the role of imidazolium ionic liquid in the pretreatment

Table 6.4 List of some value-added chemicals produced from lignocellulosic biomass

Product	Substrate used	Microorganism	Concentration (g/L)	Yield	Reference
Lactic acid	Rice straw	*L. plantarum* SKL-22	36.75	–	Yadav et al. (2021)
Citric acid	Corn stover biomass	*Aspergillus niger* SIIM M288	136.3	74.9% of cellulose	Hou and Bao (2018)
Succinic acid	Fruit and Vegetable waste	*Yarrowia lipolytica*	43.1	0.46 gg^{-1}	Li et al. (2018)
Butyric acid	Paper mill sludge	*Clostridium tyrobutyricum*	7.0	–	Liu et al. (2018)
Gluconic acid	Microcrystalline cellulose	–	9.60	0.27 gg^{-1}	Ruales-Salcedo et al. (2020)
PHA	Brewer's spent grain	*Burkholderia cepacia*	–	9.0 ± 0.44 mg PHA/g dry BSG	Llimós et al. (2020).

of rice straw and further saccharified with commercial cellulase enzyme for lactic acid production by *Lactobacillus plantarum* SKL-22 strain. They observed that the media optimisation using response surface methodology could enhance the lactic acid yield by 1.11%, with a final yield of 36.75% using rice straw in a single pot bioprocess.

In many bioprocesses, utilised cellulase enzymes are used repeatedly to saccharify lignocellulosic biomass to simple carbohydrates, which could be further used to produce value-added chemicals. This could be a holistic approach for upcoming biorefineries in the future and sustainable product formation. Grewal et al. (2018) explored the one-pot bioprocess of lactic acid production from ionic pretreated lignocellulosic waste saccharified by nano-immobilised cellulase enzyme. In this process, the simultaneous saccharification and co-fermentation (SSCF) process was performed by *Lactobacillus brevis*, which provided a lactic acid yield of 0.22, 0.52, and 0.49 g/g with sugars derived from ionic pretreated cottonseed cake, sugarcane bagasse, and wheat straw, respectively. To reduce a large amount of cellulase requirement in lignocellulosic-based lactic acid production, Chen et al. (2020) established the integration of fed-batch simultaneous saccharification process with membrane separation, in which residual cellulase enzyme present in the waste stream and solid-residue of corn stover were recycled and reused for successive fermentation. Around six rounds of operation were carried out, which increased the lactic acid yield (0.389 g/g) by 1.2 times higher than the conventional process and reduced the cellulase consumption, wastewater discharge and nutrient consumption by 47%, 73.7% and 86.1%, respectively.

In addition to lignocellulosic biomass, other agricultural wastes such as food waste and fungal biomass have also been utilised to produce lactic acid with the assistance of the cellulase enzyme. Ma et al. (2021) studied the co-fermentation of spent mushroom substance (SMS) and food waste to produce lactic acid. They reported that 92.62% of sugar was released from pretreated SMS by *Aspergillus niger* cellulase, which could increase the lactic acid concentration by 22.97% (Ma et al. 2021).

In another study, Abdel Rahman et al. (2019) isolated a thermo-alkaliphilic lactic acid bacterium, *Enterococcus faecium* FW26, which could produce lactic acid from mixed food waste and banana peels with a lactic acid yield of 84% g/g-consumed sugars. *Enterococcus faecium* FW26 secreted the amylase and cellulase enzyme and grew on a mixed substrate of food waste and banana peels without any treatment. In addition, the isolated strain was able to survive and facilitate fermentation under the stress of thermo-alkaline conditions, which might reduce the chances of contamination.

6.5.2 Succinic Acid

Succinic acid is a dicarboxylic acid and an intermediate compound of the tricarboxylic acid (TCA) cycle. It has various uses in the food industry as a flavouring agent,

acidulant, and antimicrobial agent and is also used as a solvent additive, surfactant, foaming agent, detergent extender, coating resins, and ion chelator (Kumar et al. 2019). Glucose, maltose fructose, sucrose, and glycerol have been the most frequently used carbon sources for succinic acid production by fermentation using strains of bacteria such as *Anaerobiospirillum succiniciproducens*, *Actinobacillus succinogenes*, *Mannheimia succiniciproducens,* and recombinant *Escherichia coli* (Karthik and Rathinamoorthy 2017). However, utilisation of cheaper carbon sources such as lignocellulosic biomass with the help of cellulase enzyme could improve the overall process economics. Some microorganisms can utilise partially hydrolysed cellulose and produce valuable products. Jiang et al. (2013) performed the incomplete hydrolysis of cellulose for succinic acid production by *Actinobacillus succinogens*. A succinic acid concentration of 20 g/L with a yield of 64.7% was achieved from 18 g/L of cellobiose and 17 g/L of other sugars obtained from sugarcane bagasse hydrolysate. In another study, Li et al. (2018) hydrolysed the fruit and vegetable waste with a cocktail of enzymes (2% glucoamylase, 2% hemicellulase, 1% cellulase, and 0.25% pectinase) and obtained the final glucose titre of 56. 7 g L^{-1} and a yield of 0.46 g g^{-1}. The obtained hydrolysate was further utilised to produce succinic acid by *Y. lipolytica* PSA02004 with a titre and yield of 43.1 g L^{-1} and 0.46 g g^{-1}, respectively. They improved succinic acid productivity (0.69 g L^{-1} h^{-1}) and concentration (140.6 g L^{-1}) using in situ fibrous bed bioreactor and fed-batch fermentation. Therefore, cellulase facilitates the utilisation of agro-waste for the production of value-added chemicals through biochemical pathways with good efficiency and cost-effectiveness.

6.5.3 Gluconic Acid

Gluconic acid, a non-toxic, non-corrosive, and weak organic acid is industrially produced through the fermentation of glucose by *A. niger* or *Gluconobacter suboxydans*. The gluconic acid production was also reported by various strains of *Pseudomonas*, *Acetobacter*, and *Zymomonas*. Gluconic acid has many applications in the food industry as a flavouring agent, in dietary supplements as different salts (sodium, calcium, and iron gluconate), and in the pharmaceutical and textile industries. Microbial fermentation is the most suitable and chosen method for gluconic acid production due to being less expensive and more efficient than other methods. Large-scale gluconic acid production is mainly affected by three crucial factors: cheaper substrate, oxygen concentration, and pH. Using the lignocellulosic biomass can solve the problem of the cheaper substrate if a cost-effective enzyme production method is developed. Yu et al. (2021) explored the co-immobilisation of multienzymes (cellulase, catalase, and glucose oxidase) on reversibly soluble polymer Eudragit L-100 in a cascade reaction for the direct transformation of corn straw to gluconic acid. The gluconic acid yield was achieved at 0.28 mg/mg, and the rate of conversion of cellulose in corn straw to gluconic acid was obtained at 61.41%. Similarly, Zhou et al. (2019) studied an integrated process of co-production of

gluconic acid with xylooligosaccharides from sugarcane bagasse. After the xylooligosaccharides production from the prehydrolysis, 88.6% of cellulose was converted into simple sugars in fed-batch enzymatic hydrolysis using cellulase (C2730, Celluclast®). The gluconic acid yield of 96.3% was produced by fermentation of sugars using *Gluconobacter oxydans* ATCC 621H. Therefore, approximately 105 g of xylooligosaccharides and 340 g of gluconic acid were co-produced in the integrated process from 1 kg of dried sugarcane bagasse (Zhou and Xu 2019).

6.5.4 Citric Acid

Citric acid is another crucial organic acid used as a preservative and acidulant in the food industries and also used as a starting material in the pharmaceutical and cosmetic industries. It is mainly produced through microbial fermentation from using carbohydrates (mainly starch) or agro-industrial waste. However, due to the restricted supply of food starch, potential alternative sources like lignocellulosic biomass are also being explored to meet future demand. Cellulase plays a vital role in the conversion of these recalcitrant compounds into simple sugars. Citric acid is produced on large scale through fermentation by *A. niger* due to its high citric acid productivity and tolerance to low pH. It is mainly produced through submerged fermentation. However, many researchers have explored the use of agro-residues and lignocellulosic biomass as the starting material for citric acid production. Hou et al. (2018) studied the simultaneous saccharification and fermentation (SmSF) of dilute acid-pretreated corn stover for citric acid production by *A. niger*. In SmSF, pretreated corn stover was hydrolysed by cellulase enzyme, Cellic CTec 2.0, from Novozymes. The highest citric acid concentration (136.3 g/L) was obtained with an overall yield of 74.9% of cellulose in the aerobic SmSF process. This study compared the citric acid production from SmSF with separate hydrolysis and fermentation (SHF) and concluded that the cellulosic citric acid production by SmSF could be highly comparable to starch-based citric acid production.

6.5.5 Microbial Polymers

6.5.5.1 Polyhydroxyalkanoates

Polyhydroxyalkanoates (PHA) are the most potential, degradable, and environment-friendly substitute for petrochemical-based plastics. The main constraint that prevents PHA from commercialisation is the cost of production. Therefore, researchers are focusing on cheap substrates for sustainable PHA production. In this regard, lignocellulosic waste can be used as a possible substrate as it is cheap, readily available, and most importantly, it does not compete with the food requirements of humans. Nowadays, a wide range of lignocellulosic waste generated from industries,

forestry, agriculture, marine biomass, etc. has gained great consideration for PHA production (Obruca et al. 2015).

PHA production using lignocellulosic biomass at the industrial level includes mainly three steps, especially pretreatment, enzymatic/acidic hydrolysis, and fermentation. Many pretreatment techniques are used before the enzymatic hydrolysis of cellulose which includes physical processes for size reduction, chemical treatment (with hydrogen peroxide, acid, alkali, or sodium chlorite) for delignification, physicochemical processes (like a steam explosion, ammonia fibre expansion, etc.) or a biological pretreatment using microbial enzymes. Further, enzymatic hydrolysis is performed for the degradation of complex carbohydrates into simple sugar, which serves as a carbon source for microbes in the successive fermentation step. Later fermentation is carried out using PHA-producing microbes (e.g., *Bacillus sonorensis, Halomonas hydrothermalis, Pseudomonas putida, Bacillus cereus*, etc.) and PHA is recovered. It was suggested that the average sugar and PHA yields were higher in the case of enzymatic hydrolysis as compared to acid-based hydrolysis and cellulase plays an important role in enzymatic hydrolysis (Al-Battashi et al. 2019).

Production of PHA without pretreatment steps is also used to decrease the cost of the process. Israni and Shivakumar (2020) used cellulase, lipase, and amylase positive *Bacillus megaterium* strain Ti3 for the production of PHA without pretreatment step. PHA was produced using 16 different lignocellulosic substrates by submerged fermentation at 30 °C for 48 h at 120 rpm. They demonstrated that ragi husk and sesame oil cake have the most influential factors on the basis of Taguchi orthogonal array. Similarly, Heng et al. (2017) demonstrated a three-step process for PHA production using *Burkholderia cepacia* USM, *Cupriavidus necator* NSDG-GG, and a genetically modified strain of *Cupriavidus necator* H16. They used rice husk as raw material and performed alkaline pretreatment followed by enzymatic hydrolysis and fermentation. For enzymatic hydrolysis, they used a cellulase-based enzymes Celluclast 1.5 L and Novozyme-188 at 50 °C 160 rpm for 72 h. They obtained the highest sugar yield of 87% after enzymatic hydrolysis and obtained 7.8 g/L of dry cell weight and 50% PHA content using a 5 L fermentor.

Llimós et al. (2020) performed two-stage valorisation using Brewer's spent grain (BSG) as a lignocellulosic substrate for PHA production. First, they produced lignocellulolytic enzymes by three fungal strains (*Trichoderma reesei*, *Aspergillus niger* and *Thermoascus aurantiacus*) through SSF of dried BSG. Then these enzyme were used for the hydrolysis of BSG and production of PHA. The PHA was produced by fermentation of sugar rich BSG hydrolysate at 30 °C and 120 rpm using *Burkholderia cepacia* and *Cupriavidus necator*. *C. necator* cells were harvested after 48 h and *B. cepacia* cells were harvested after 72 h. The maximum yield of PHA they obtained was 9.0 ± 0.44 mg PHA/g dry BSG, and the result showed that BSG could be utilised as a suitable raw material for the production of value-added products (Llimós et al. 2020).

PHA is a potential material with a broad range of uses which includes its use as bioplastics, drug carrier, and synthesis precursor, packaging materials, biofuels, implant materials, cosmetic ingredients, disposal cups, and bottles, etc. Presently,

PHA is not well commercialised as compared to fuel-based plastics and products. The main hurdles are the high cost of production and relatively poor characteristics. Still, researchers are searching for the solution to this problem as PHA offers an eco-friendly and sustainable alternative to petrochemical-based products and has a promising future (Wang and Chen 2017).

6.5.5.2 Nanocellulose

In the last few years, remarkable development has been noticed in nanotechnology, especially in nanocellulose production. Nanocellulose produced from lignocellulosic waste is a potential alternative for obtaining various value-added products that can meet the demand of the market and environmental issues (Jaiswal et al. 2021). According to various literature, lignocellulosic biomass like rice husks, wheat straw, cotton, sugarcane bagasse, flax fibres, wood bark, softwood pulp, corncob, hardwood, etc. can be used for the production of nanocellulose (Michelin et al. 2020).

Currently, acid hydrolysis is the most regular method for the production of nanocellulose. However, nowadays production of nanocellulose using enzymatic hydrolysis is gaining significant importance as toxic residues are not produced. Moreover, during enzymatic hydrolysis, mild conditions are used, which makes it a low energy-intensive process (Karim et al. 2017). The process of nanocellulose production by enzymatic hydrolysis contains several steps. The first or pretreatment step consists of delignification where lignin is removed using various mechanical processes (chipping and milling) and chemical processes (alkaline pretreatment and bleaching pretreatment). The second step is enzymatic hydrolysis which is generally carried out at the pH range from 4 to 7 and temperature range from 45 to 50 °C. The last step includes homogenisation, where the enzymatically treated material is homogenised by washing or mechanical homogenisation (Ribeiro et al. 2019).

de Aguiar et al. performed enzymatic hydrolysis of sugarcane bagasse and straw at 50 °C and 200 rpm. The cellulose nanocrystals (CNC) produced by both bagasse and straw showed a crystallinity index of approximately 70% and high thermal stability (de Aguiar et al. 2020). In another study, Yarbrough et al. (2017) reported that the synergistic effect of two or more enzymes shows a better effect than an individual enzyme. They demonstrated that the *Caldicellulosiruptor bescii* with multifunctional enzymes is better in cellulose and nanocellulose production than the classical system of "free enzyme" produced by *Trichoderma reesei*.

The application of only one technique is not as effective as the combination of two or more techniques. For example., high-quality nanocellulose is produced using a coupled ball milling process and enzymatic hydrolysis, which can be further improved when combined with any other technique (Teo and Wahab 2020). Zhang et al. (2020) designed a new approach using poplar wood as lignocellulosic biomass to produce nanocellulose. They used steam explosion and delignification for the pretreatment of poplar wood. Further, the enzymatic hydrolysis was performed using cellulase enzyme followed by ultrasonic treatment. The optimum

condition determined by them for enzymatic hydrolysis was 200 U/g at 50°C for 12h, giving approximately 13.2% yield of nanocellulose. The crystallinity of the composite produced in this study was 61.98% which was higher than the crystallinity of poplar cellulose, and the structure of the composite was not damaged during the preparation steps. Squinca et al. (2020) performed enzymatic hydrolysis using a cellulolytic enzyme complex with the high specific activity of endoglucanase produced by the fungus *Aspergillus niger*. They pretreated the cellulose pulp of eucalyptus by ball milling, followed by enzymatic hydrolysis and sonication, which yielded 24.6% of cellulose nanocrystals (CNCs) by the enzymatic hydrolysis of 96 h. CNC's length, diameter, and crystallinity were 294.0 nm, 24.0 nm, and 78.3%, respectively.

Apart from nanocellulose isolation, cellulases can also be used for the pretreatment of lignocellulosic waste and other pretreatment techniques. Zeng et al. (2020) used endocellulase for enzymatic pretreatment of the bleached pulp of softwood kraft before wet ball milling. Further, they used mechanical grinding for the production of cellulose nanofibrils (CNF).

Nanocelluloses have a wide range of applications in different industries it can be used as material for food packaging in food industries, electronic displays in the electronic industry, paper and coating material in the paper and pulp industry, a supporting material in the medical and tissue industry, absorbent material in the water treatment industry, etc. (Zaki et al. 2021). The enzymatic hydrolysis is a time-consuming process for the production of nanocellulose. However, it has gained the attention of companies and researchers as it has shown better saccharification efficiency and penetration. Despite the challenges, enzymatic hydrolysis for nanocellulose production has great potential because of its unique characteristic and eco-friendly nature (Teo and Wahab 2020).

6.6 Role of Cellulase in a Circular Economy

The application of cellulases for the efficient valorisation of agro-industrial waste has tremendous potential for generating biofuels and biochemicals with low carbon and water footprints that can be recycled back to the earth within the scope of a circular economy (Astolfi et al. 2019). This conceptualises the 3Rs of a circular economic system. In situ resource utilisation, enzyme recycling, and sequential zero-waste biorefinery approaches are commercially viable solutions for sustainable development.

6.6.1 Valorisation via Carbon-Neutral Technologies

Fossil-based resources are now being replaced by alternative renewable feedstocks for the synthesis of cellulases and bioconversion for biofuels, commercially

important chemicals, and biomaterials to envisage a decarbonisation system (Adsul et al. 2020). Millions of tonnes of lignocellulosic waste are generated per annum globally. Their valorisation in an economical and eco-friendly manner forms the core of a bio-based circular economy (Sheldon 2020). In the current scenario, the entire focus lies in the thoughtful conversion of the so-called wastes into valuable products such as reducing sugars, biochemicals, and biofuels, primarily regulated by cellulase enzymes.

6.6.2 *Life Cycle Assessment of Cellulase-Based Biorefinery*

The environmental and economic effects of a process analysed by the life cycle assessment (LCA) technique include all inputs and outputs of material and energy flow at different life cycle stages (Russell et al. 2005; Kumar and Verma 2021c). LCA technology in the assessment of cellulase production and utilisation is limited by rigid system boundaries, data accuracy and availability, variations in statistical techniques, variations in product utility, its selectivity, and local environmental conditions (Borrion et al. 2012). System boundaries of the cellulase manufacturing process focus on the greenhouse gas emissions related to the cradle-to-gate activities for cellulase production (Fig. 6.3). In a case study on cellulase production from corn feedstock, Aspen-Plus and GREET 1.8c.25 were used for estimation of emissions and financial matrices. 258 g CO_2 eq. L^{-1} of bioethanol was estimated for onsite production and 403 g CO_2 eq. L^{-1} for offsite production. The cost estimation was made on the basis of bioethanol plant size and cellulase loading. An additional input like return on investment is used for offsite data. The final production cost was 0.46 USD gal^{-1} of bioethanol for cellulase for on-location production (Hong et al. 2013).

Cellulase production from coffee husk was assessed on the basis of data taken from EcoInvent v3.4 (SimaPro v8.5 software) database. The results showed that the downstream process significantly impacts the environment and economic value (Catalán et al. 2019). In another study, an integrated ethanol production process using spruce loggings was compared to an offsite production scenario. The greenhouse gas emissions were lower in the onsite process as compared with that of the offsite process. The ethanol selling rate was 0.568 euro per litre for the integrated cases and 0.581 euro per litre for the off-location case (Olofsson et al. 2017).

6.6.3 *Increased Circularity via Onsite Application*

The change in the substrate from enzyme production to hydrolysis and offsite enzyme application often alters the enzymatic efficiency. Researchers have found that the microorganisms grown on various carbon sources under different physico-chemical environments produce different isoforms of cellulase, which show behavioural diversity while interacting with the same substrate (Siqueira et al.

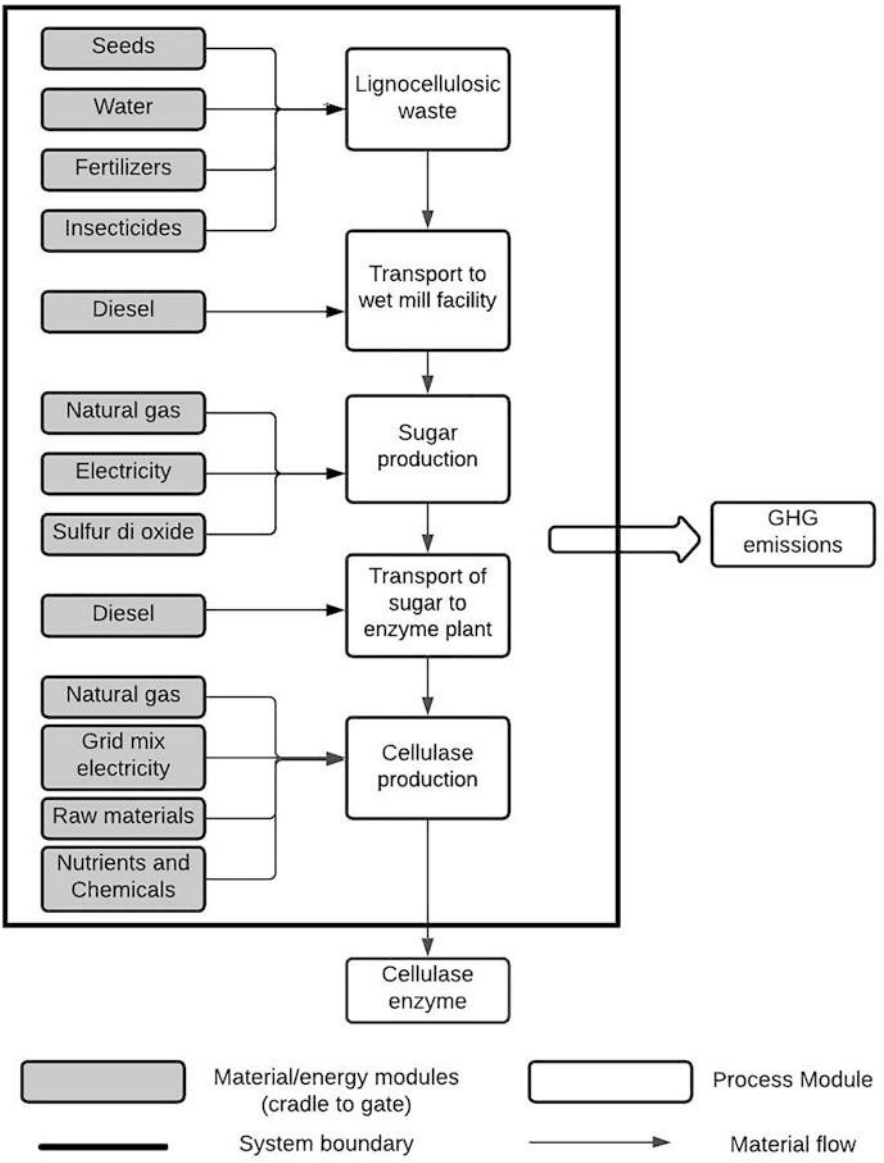

Fig. 6.3 System boundaries for cellulase application in biorefinery

2020; Zhao et al. 2018). The techno-economic feasibility was assessed for the onsite and offsite cellulase synthesis and utilisation for ethanol generation using corn stover as feedstock. The biomass channelised from the ethanol production stream improved the value of onsite cellulase production (Kazi et al. 2010). The comparative routes for onsite, offsite, and integrated biorefinery are shown in Fig. 6.4.

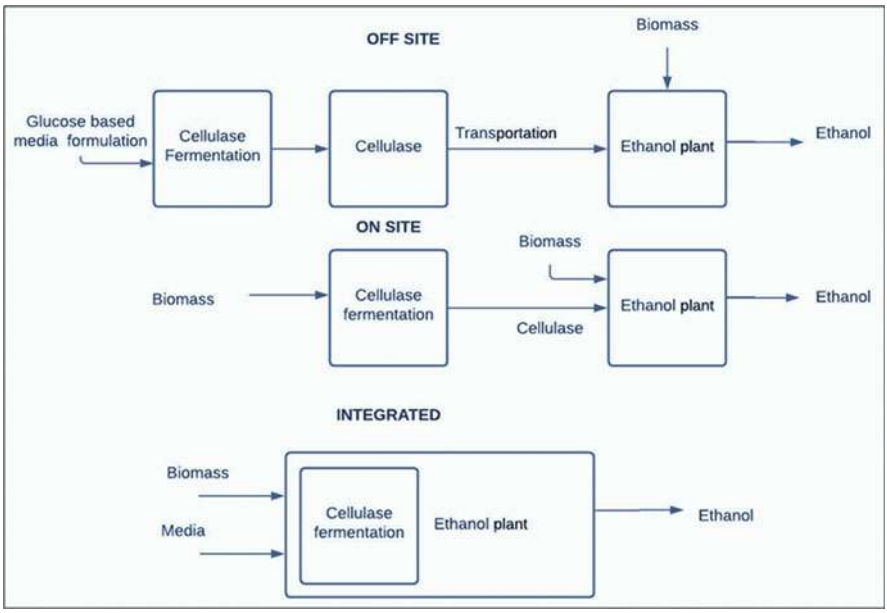

Fig. 6.4 Comparison of offsite, onsite, and integrated biorefinery

Novozyme introduced Cellic CTec3 based on the economic bioconversion of pretreated lignocellulosic substrate (Sun et al. 2015). The techno-economic model for the corn-based ethanol industry revealed the significance of onsite cellulase production for biorefinery. The use of pure glucose, priced $580/t as a carbon source, covers over 50% of cellulases' production cost, which is commercially not feasible (Humbird et al. 2011). Working on a low-cost substrate, non-sophisticated downstream processes, less storage, and transport makes onsite enzyme production eco-friendly and contributes to a circular economy.

6.6.4 Challenges and Perspectives

The circular economic sustainability is challenged by two major threats. These are the lignocellulose biorefinery supply chain and the resistant structure of lignin, which restrict the access of cellulose by cellulases (Garlapati et al. 2020). The last issue significantly affects the cellulose crystallinity and the efficacy of cellulases. Low-cost, energy-efficient pretreatment strategies can resolve the lignin problem. The energy recovery process needs to be accompanied by soil replenishment (Battaglia et al. 2021). In this context, it is essential to re-conceptualise the enzymatic reduction of agricultural lignocellulosic waste for obtaining value-added products through sustainable utilisations. The integrated lignocellulosic biorefinery leads to the circular economic framework through the endorsement of niche markets,

consumer-oriented value generation, and reduction of transportation through supply chain modifications of lignocellulosic feedstocks (Sarsaiya et al. 2019). The ultimate benefits would be a cleaner environment and economic profitability.

6.7 Conclusion

Cellulase plays an important role as it is responsible for the hydrolysis of cellulose into simple sugars, which can be further used as a carbon source for microbial fermentation. Nowadays, valorisation of lignocellulosic waste has gained the attention of researchers, and efforts are made to make this process industrially and commercially feasible as it is eco-friendly and has great potential and a promising future. Cellulases are produced at a large scale using cellulolytic microorganisms either by SSF or SmF. However, industries prefer SmF over SSF as it is easy to monitor and operate. The commonly reported microorganisms for cellulase production are *T. reesei, S. argenteolus, B. licheniformis, Candida cylindracea, Aspergillus oryzae,* etc. Cellulases are a powerful tool for the bioconversion of agro-residues, food, paper, and textile industry waste into beneficial products like surfactants, furfurals, aromatics, phenols, ethanol, butanol, lactic acid, succinic acid, gluconic acid, citric acid, etc. Recently, the use of cellulase is extensively explored for the production of microbial polymers such as nanocellulose, polyhydroxyalkanoates, polyhydroxy butyrate, xanthan, and pullan.

The major challenge behind the use of lignocellulosic biomass in biorefineries is the cost of production. Many strategies have been developed to reduce the cost such as the utilisation of immobilisation techniques for the reusability of cellulase, onsite cellulase production, use of enzyme cocktails instead of a single enzyme, combination of two or more techniques for the pretreatment step, and use of low-cost substrates. Despite many challenges, great progress has been made in the direction of using lignocellulosic biomass as it is a potential renewable alternative for fossil-based resources. Also, advanced tools such as techno-economical analysis (TEA) and life cycle assessment analysis (LCA) provide insight into actual benefits associated with enzyme-based conversion processes over chemical processes. These assessments promise an optimised circular economy approach for maximum fulfilment of societal needs and minimum impact on the environment and ecosystem.

Competing Interests There is no conflict of interest.

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Chapter 7
Technical Criteria for Converting Biomass to High Liquid Bio-Oil Yields

Naval Koralkar and Praveen Kumar Ghodke

Abstract Rising energy demands and depletion of fossil fuels have led the research community to investigate alternative fuel sources. Green and renewable biofuels have evolved to substitute for a non-renewable energy source. Biomass can be utilized as a raw material for producing low-carbon fuels. Although biomass-based fuels can replace fossil fuels, direct use is limited due to the low quality of the fuels and expensive process costs. An unrivaled solution to this problem is an integrated biorefinery concept involving generating hydrocarbon-grade fuels and valuable chemicals from pyrolysis-derived bio-oil. The chapter examines recent breakthroughs in bio-oil up-gradation processes and moisture removal techniques and bio-oil recovery of valuable compounds. One of the widely used and well-developed techniques for producing bio-oil is the fast pyrolysis of biomass. The catalytic cracking process has been identified as a viable technology for converting bio-crude to liquid fuel in bio-oil upgrading. The chapter examines recent trends and advances in the fast pyrolysis technique to improve overall profitability of the process. Critical analysis of the potential and existing techniques and necessary future steps are essential for adopting these methods industrially and in a feasible manner.

Keywords Pyrolysis · Upgraded fuel · Green fuel · Biomass

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P. Verma (ed.), *Thermochemical and Catalytic Conversion Technologies for Future Biorefineries*, Clean Energy Production Technologies,
https://doi.org/10.1007/978-981-19-4312-6_7

Abbreviations

CHNS-O	Analyzer
CO_2	Carbon dioxide
DSC	Differential scanning calorimetry
SEM	Scanning electron microscope; Py-GC/MS- Pyrolysis-gas chromatography/mass spectrometry
FTIR	Fourier transforms infrared spectroscopic analyzer
NMR	Nuclear magnetic resonance spectroscopy
NO_x	Nitrogen oxides
SO_2	Sulfur dioxide (SO_2)
TGA	Thermogravimetric analysis analyzer
XRD	X-ray diffraction analyzer

7.1 Introduction

Biomass is a promising eco-friendly alternative renewable energy source in today's energy landscape. The present global energy supply is primarily dependent on fossil fuels such as oil, natural gas, and coal, with limited reservoirs (Smith et al. 2018; Goswami et al. 2021a). Thus, it has become necessary to evaluate an alternative long-term energy source that can meet the demands of the growing global population and the increasing per capita energy consumption. Also, the evidence of increasing global warming due to greenhouse emissions has raised concern for most developed and developing countries. All these concerns have increased the importance of research for fossil-free alternatives.

Biomass is a renewable energy source that is widely available (Sarkar and Praveen 2017; Agrawal and Verma 2022). Due to environmental concerns and rising energy demands worldwide. Biomass utilization in mainstream energy usage attracts much attention. Biomass is composed of hemicellulose (20–35 wt.%), cellulose (30–50 wt.%), and lignin (15–35 wt.%) (Cheng et al. 2016; Kumar et al. 2020). Apart from carbon-based components, biomass also contains a small amount of nitrogen, sulfur, and inorganic composition ash. As a result, when compared to conventional fossil fuels, biofuel burning produces fewer toxic gas pollutants such as nitrogen oxides (NO_x), sulfur dioxide (SO_2), and soot. Furthermore, biomass fuel combustion can produce zero or negative carbon dioxide (CO_2). emissions since CO_2 generated from bio-oil combustion can be recycled back into the plant through photosynthesis (Debalina et al. 2017; Goswami et al. 2020a, 2021b).

Different routes are available by which biomass can be converted into biofuel. These include thermal, biological, and physical processes. Among the different available processes, pyrolysis has emerged as a promising technology for producing liquid fuel products due to its storage, transport, and versatile applications such as combustion engines, boilers, and turbines. Managing solid biomass and waste is a

complex and cost-intensive task that encourages pyrolysis research. However, it is still in its early stages of development and faces various technological and economic challenges to compete with existing fossil fuel-based technologies (Tang et al. 2020; Goswami et al. 2020b). An extensive investigation of the production of bio-liquids and other products using different biomass species has been carried out in the past.

The quality bio-oil production from different biomass is challenging and requires the application of advanced technologies such as catalytic pyrolysis. Thermochemical conversion techniques can efficiently and economically transform biomass into energy-rich compounds used in various applications (Mandapati and Ghodke 2021). Different routes are available for converting biomass into usable fuel. These technologies include thermochemical conversion of biomass via combustion, pyrolysis, and gasification. The disadvantage of the gasification process is that the produced synthesis gas needs to be utilized at the place of production as it becomes economically inviable or costly for storage and transportation through pipelines (Mandapati and Ghodke 2020).

The chapter discusses the different routes of pyrolysis and its technical specifications and the advances in the pyrolysis technology used for biomass conversion. The chapter covers a detailed description of the pyrolysis technique, its technical specifications, and the technology involved. Pyrolysis has emerged as an essential technology in turning biomass into solid, liquid, and gaseous fuels. A yield of around 60–65% bio-oil production has been reported in literature through the pyrolysis process utilizing a fluidized bed reactor.

7.2 Pyrolysis

Pyrolysis is the process in which both thermal and chemicals effects the conversion of organic materials in an oxygen-depleted atmosphere such as nitrogen. It is an endothermic reaction (Basile et al. 2015). The term pyrolysis was derived from two Greek words: pyro, which means fire, and lysis, which denotes degradation into essential components. During pyrolysis, components of biomass begin to decompose at 350 °C–550 °C due to rapid heating and progress to 700 °C–800 °C, resulting in the generation of a variety of products such as liquids, solids, and gases (Das et al. 2015). Biochar and bio-oil are the solid carbon-rich product and the volatile fraction of pyrolysis that is partly condensed to a liquid fraction product, respectively. The pyrolysis process produces hydrogen, carbon dioxide, methane, and carbon monoxide, among other gases (Moorthy Rajendran et al. 2020). These products are intriguing because they could be used as alternative energy sources. Pyrolysis has emerged as a critical method in converting biomass into solid, liquid, and gaseous fuels. The literature reported a yield of roughly 60–65 wt.% bio-oil generation through the fast pyrolysis process using a catalytic fluidized bed reactor. However, pyrolysis-produced bio-oil has numerous unfavorable qualities that directly use bio-oil as an engine fuel challenge. Thus, bio-oil is enhanced by the hydrotreating

or catalytic cracking process to make it compatible as a drop-in fuel molecule (Shihadeh and Hochgreb 2000).

7.2.1 Different Routes of Pyrolysis

Pyrolysis processes may work efficiently under a variety of conditions. Thus, there are various pyrolysis processes, such as fast, slow, flash, intermediate, ultra-flash, vacuum, and catalytic pyrolysis (Wei et al. 2021). Especially, vacuum pyrolysis occurs at negative or low pressures, whereas another pyrolysis of biomass occurs at atmospheric or controlled positive pressure (Chintala et al. 2018). The ideal primary fuel for the pyrolysis process is waste material, such as forest debris, woody biomass, and agricultural waste. Longer residence times and high temperatures increase biomass conversion to gas. Pyrolysis conditions can be varied depending on the desired product type (Bridgwater 2012; Dhyani and Bhaskar 2017). Several different modes of biomass pyrolysis depending on conditions, such as heating rate and residence time of biomass, are being actively developed (Hu and Gholizadeh 2019; Kiliç et al. 2014; Adelawon et al. 2021; Dhyani and Bhaskar 2017). However, the most generally used classification of pyrolysis processes is slow, fast, and flash pyrolysis.

7.2.1.1 Slow Pyrolysis

Slow pyrolysis or conventional pyrolysis has been used to convert diverse feedstock biomasses into charcoal or biochar at slow heating rates for a lengthy residence time, around 5–30 min, and at temperatures below 300 °C since the beginning of the pyrolysis process (Ahmad et al. 2014). Feedstocks, wheat straw, pinewood, dried algae, and green garbage were employed. This procedure can also make bio-oil or liquid fuels. Slow pyrolysis is typically carried out at atmospheric pressure. The heat required for the process is provided from an external source, usually produced from partial combustion or combustion of the produced gases or biomass feedstock (Ghodke and Mandapati 2019). The process results in the development of vapor phase components that continue to react, resulting in the formation of charcoal and other liquid products. Slow pyrolysis produces roughly 35 wt.% biochar, 30 wt.% bio-oil, and 35 wt.% gaseous products. However, due to technological restrictions such as cracking of the primary product due to high residence time (Demirbaş 2005; Jahirul et al. 2012), bio-oil is produced through slow pyrolysis is not suitable for direct use as a liquid fuel.

7.2.1.2 Intermediate Pyrolysis

Intermediate pyrolysis is a type of pyrolysis that occurs halfway between fast and slow pyrolysis. It has an excellent product distribution and may be employed in the coproduction of biochar, bio-oil, and gas (Meng et al. 2019). Intermediate pyrolysis is flexible to diverse materials and has good product distribution. The product is a two-phase separable bio-oil, high quality, and biochar compared to other pyrolysis categories (Bridgwater 2003; Kumar et al. 2021). Researchers have also discovered that it has easily distinguishable liquid phases, with the organic phase exhibiting biodiesel-like qualities. The organic phase can be combined with up to 50 wt.% (Xiong et al. 2009). Its aqueous phase, which comprises C2–C6 sugars, hydroxy acids, oligomers, and water-soluble phenols, is also effective in manufacturing biogas and ethanol. The process has the advantage of requiring less bio-oil upgrading than quick pyrolysis oil and allowing for complete usage of all products. Because of the extensive interaction with steam, intermediate pyrolysis can treat high moisture content feedstock, and when this happens, the biochar takes on the properties of activated carbon (Gao et al. 2020).

7.2.1.3 Fast Pyrolysis

Fast pyrolysis is the rapid thermal degradation of biomass at more excellent heating rates, such as 1000 °C min^{-1}. Fast pyrolysis has a short vapor residence time of less than 2 s (Bhattacharya et al. 2009). Bio-oil is the main product of rapid pyrolysis. However, the amount of product created depends on the feedstock composition, ranging from 60% to 75% oily products, and 10–20 wt.% gaseous (CH_4, CO, CO_2, H_2, and light hydrocarbons), and 15–30 wt.% solids products. Rapid heating and quenching produce bio-oil, and the high reaction rates reduce char formation and favor the generation of either gas or liquid products. Higher temperatures, heating rate, short vapor residence time, rapid cooling of vapors for high bio-oil production, and precise control of reaction temperature are the essential characteristics of the fast pyrolysis process (Collins and Ghodke 2018). Furthermore, a fast pyrolysis process requires reducing the water content of feedstock and reducing the particle size to less than 2 mm. In fast pyrolysis, rapid and systematic separation of solids (char), rapid gas removal, and cooling pyrolysis product favor the formation of bio-oil or liquid products (Ponnam et al. 2021). This liquid product can be readily and inexpensively transported and stored, decoupling solid biomass handling from consumption (Malode et al. 2021).

7.2.1.4 Flash Pyrolysis

Ultra-fast pyrolysis is another name for the process. Flash pyrolysis is a thermal breakdown of large molecules into smaller molecules that occurs in an inert

Table 7.1 Summary of the classification of the pyrolysis method

Method	Residence time	Temperature (°C)	Heating rate (°C/s)	Major bioproducts
Slow pyrolysis	5–30 min	400–500	10	Char, bio-oil (tar), gases
Ultra-fast/flash pyrolysis	<0.5 s	750–1000	>500	Bio-oil and gases
Fast pyrolysis	0.5–2 s	500–650	100	Char, good bio-oil, gases

atmosphere such as nitrogen at a rapid heating rate of 1000–10,000 °C per minute and residence time is less than 1 s. An excellent feed particle size (2 mm) is required to perform this procedure at an optimal rate. The degradation of biomass produces a significant amount of coke and aerosol vapors during flash pyrolysis. A dark brown liquid (bio-oil) is produced after cooling and condensation, with a heating value of half diesel. In contrast to prior procedures, this is a novel technology with well-regulated parameters that generate high liquid yields (Gandidi et al. 2018). Flash pyrolysis in fluidized bed reactors can produce a high liquid output of up to 75 wt.% bio-oil (Gómez et al. 2018). However, there are certain drawbacks to flash pyrolysis, such as low thermal stability, particles in the oil, and the oil's corrosiveness, which results in the generation of oils with high viscosity, density, low calorific value, and carbon residues (Huang et al. 2013). Table 7.1 depicts the summary of different pyrolysis technologies available and can be used to produce different bioproducts.

7.2.2 Technical Specifications: Pretreatment, Characterization, and Mechanism

7.2.2.1 Pretreatment

Pretreatment is an important stage in the biochemical and thermochemical conversion of biomass. It requires structural changes to overcome biomass's recalcitrance. Improved biomass features are necessary to increase the biomass's energy use efficiency (Wu et al. 2021; Bhardwaj and Verma 2021). The primary treatment process requires the heating of lignocellulosic biomass (cellulose, hemicellulose, and lignin) to convert into polymeric and aromatic constituents. In contrast, the heterogeneity in atoms and inorganic oxides element components of biomass act as catalysts, resulting in the generation of a biofuel product with different carbon structures and significant reforms that increase yield during the bioconversion process (Kim 2018; Kumar and Verma 2020a, b). Current pretreatment systems have two significant challenges: high costs and obtaining a processed product with essential component degradation. Past and ongoing research and development efforts have failed to meet these problems. Pretreatment treatments must be tailored to the type of biomass and how it will be utilized in bioconversion and biorefinery

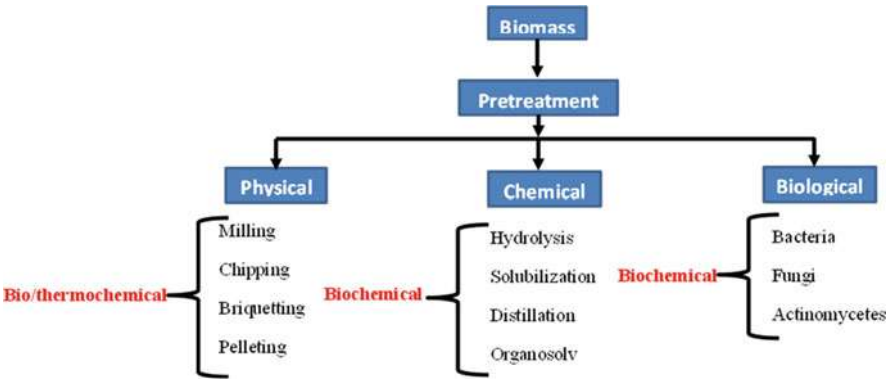

Fig. 7.1 Different pretreatments methods for biomass

processes. Figure 7.1 depicts the types and pretreatment processes required for biomass conversion technologies (physical, chemical, and biological). The subsections that follow go into the various types of preprocessing in greater depth.

Physical Treatment

Physical pretreatment of biomass aims to improve the surface area and pore size by reducing particle size through mechanical comminution. Physical processing reduces cellulose crystallinity and polymerization degree in lignocellulosic materials. Prior to both enzymatic and thermochemical biofuel production, this step is required. However, little is known about how physical preprocessing procedures function, particularly how biomass chemical content or bond structure are affected. The lignocellulosic biomass application determines the physical pretreatment technology to be undergone or utilized. Biochemical conversion of lignocellulosic biomass, for example, should undergo a reduction in particle size through milling, shredders, and cutters in order to improve biochemical digestibility. Milling of biomass is necessary before use in the thermochemical conversion process to reduce the size of biomass. Palletization, densification, and heat treatment torrefaction are the prior pretreatment process of thermochemical conversion technologies. Prior size reduction is essential in both biochemical and thermochemical conversion pathways to minimize mass and heat transfer constraints. Another physical pretreatment approach is chipping when employing waste wood biomass or agricultural residue as a feedstock in thermochemical conversion technologies. The thermochemical conversion technologies require feedstock with 50 mm or less diameter, feedstock with 50 mm or less is required.

Torrefaction, densification, and pelletization are examples of physical pretreatment procedures for biomass (Reckamp et al. 2014; Chi et al. 2021). These preprocessing procedures use heat to cause changes in the biomass, resulting in improved biomass characteristics. The physical pretreatment procedure has the

drawback of removing the lignin content from lignocellulosic biomass materials, rendering the cellulose composition inaccessible. Another disadvantage is the high heat-energy requirements for pretreatment and not economically feasible commercial use. According to research findings, the process of removing lignin from lignocellulosic biomass materials can be led to an increase in the energy demand. Thus, thermal pretreatment treatment method could influence the total energy effectiveness/cost of a biorefinery process (Lewandowski et al. 2020).

Chemical Pretreatment

In chemical pretreatment methods, organic or inorganic chemical compounds are used to disrupt the chemical bonds of lignocellulosic biomass by interacting with the intrapolymer and interpolymer bond connections of the organic components are known as chemical pretreatment of biomass. Biomass, particularly lignocellulosic components, is considered recalcitrant because it is resistant to chemical breakdown. The structural diversity and complexity of biomass such as its crystalline nature and the degree of lignification, all contribute to its recalcitrance (Mai et al. 2014; Bhardwaj et al. 2020). In preliminary chemical pretreatment method, the chemical structural recalcitrance of the lignocellulosic nature of biomass is disrupted, resulting in cellulose crystalline phase reduction along with lignin breakdown. Before biochemical conversion, chemical preprocessing of biomass, particularly lignocellulosic biomass, is frequently employed to extract the biopolymeric constituents of the feedstock. Acids, alkalis, organic solvents, and ionic liquids are some of the chemicals that have been used to pretreat biomass chemically and have had a significant impact on its structure (Basak et al. 2020).

Biological Pretreatment

Biological preprocessing/pretreatment of the organic composition of biomass is always related to the activity of synthetic enzymes or enzymes produced from an organism that potentially can break down or depolymerize the hemicellulose, cellulose, and lignin components of biomass. The biological pretreatment method has many benefits over other physical/chemical pretreatment processes are particularly uses energy consumption and produces little or no toxic chemical. Biological pretreatment produces a high yield of required/valuable products. But major disadvantages are substrate and process reactions are very sensitive (Cao et al. 2013; Agrawal and Verma 2020). However, its significant drawbacks in biological pretreatment are that the method chosen was too slow and requires meticulous management of fungus growth conditions and the enormous amount of area required to complete the process (Kan et al. 2016). It was observed from the literature that the residence period or time required for biological activities in pretreatment was between 11 and 15 days. Moreover, because microorganisms consume the organic components of biomass, biological pretreatment operations have technological

challenges and are seen as less economically appealing than other pretreatments (Robak and Balcerek 2018; Chintala et al. 2019). In the biological pretreatment of biomass, different fungi such as white-rot, brown-rot, and soft-rot fungi are used widely. Apart from fungi, actinomycetes and bacteria are used to pretreat the biomass. The fungi are notably applied to eliminate hemicellulose and lignin composition of biomass while causing minor damage to cellulose biomass. White-rot and brown-rot fungi have a wide degradation mechanism for gaining access and destroying the lignocellulosic biomass such as waste wood and agricultural residue. Their extraordinarily powerful metabolism or mechanism has been successfully employed in industrial commercial operations. White-rot and brown-rot fungi have been demonstrated to brighten hardwood kraft pulp, potentially lowering bleaching chemical costs and reducing the environmental effect of paper manufacturing operations (Phillips et al. 2017; Ghodke and Mandapati 2017; Bhardwaj et al. 2021).

Even though various preprocessing methods have been studied and more are in progress, evaluating pretreatment technologies is challenging. The primary reasons for challenging pretreatment techniques are upstream and downstream processing of biomass. Additionally, capital expenditure, waste treatment systems, and complicated chemical recycling (Shen 2015; Ghodke 2021).

The thermochemical conversion uses a variety of feedstocks, including wood waste, energy crops such as sweet sorghum, short rotation forestry, and agricultural residues. The critical technical parameters for thermochemical processing suitability are ash, moisture, and other physical characteristics. The two most essential economic elements are cost, including collecting, production, transportation, and availability. Competing uses such as pulp and combustion, board manufacture, recycling, and material recovery, rather than energy recovery, are also a concern.

7.2.3 Feedstock Drying

In most circumstances, pyrolysis necessitates a feedstock with a moisture level of less than 15 wt.% although there is a trade-off between moisture levels and conversion production efficiency. The moisture content essential for conversion differentiates between conversion plants. When biomass is delivered, the moisture level will be 50–60 wt.% range (Praveen et al. 2015).

Passive drying during summer storage can cut this by 30%. The moisture content of a silo can be reduced to as low as 12 wt.% with active silo drying. Drying can be done in various ways, including near-ambient solar drying, waste heat flows, and specially constructed dryers that operate at the location. Commercial dryers come in various shapes and sizes, but rotary kilns and shallow fluidized bed dryers are the most prevalent.

7.2.3.1 Characteristics of Feedstocks

Table 7.2 summarizes the essential physical features of biomass. Notably, the relatively low bulk density, high moisture content, and wide particle size range are notable traits.

7.2.3.2 Biomass Characterization

Biomass heterogeneity is still a natural feature. The biomass quality determines the possibility and viability of extracting products from it. The two conversion techniques such as biochemical and thermochemical conversions are primarily applied to recover commercially valuable products from biomass. The attributes of biomass also affect the conversion route chosen. Thus, characterization is required to effectively understand the underlying physicochemical properties of biomass. Depending on its characteristics, one can evaluate its suitability for bioconversion. The physical properties are critical for the efficient use of biomass in biofuel production methods (Dutta et al. 2015).

However, the fundamental organic components of biomass influence its features, which vary based on the source of biomass composition, biomass source, species, soil condition, climatic condition, and other factors. Depending on the end-use of biomass, proximate and final analyses are routinely determined and reported using a variety of analytical processes. The data is crucial for assessing biomass's diverse application potential, notably its energy production potential when third-generation biomass is used as a biofuel in thermochemical conversion operations (Sharma et al. 2020). Among the characteristics analysis techniques used in physicochemical characterization investigations involving organic composition are the following:

- XRD- X-ray diffraction analyzer
- FTIR- Fourier transforms infrared spectroscopic analyzer
- TGA- Thermogravimetric analysis analyzer
- NMR- Nuclear magnetic resonance spectroscopy
- CHNS-O analyzer
- DSC-Differential scanning calorimetry
- SEM-Scanning electron microscope
- Py-GC/MS- Pyrolysis-gas chromatography/mass spectrometry

Table 7.2 Essential physical features of biomass

Feedstocks	Forestry residues	Poplar tree	Waste wood	MSW	Straw
Moisture content (%)	20–50	10–40	5–30	20–30	5–25
Density (kg/m^3)	350	450	350	450	250

7.3 Future Prospects

The replacement of fossil fuels with biomass resources on a wide scale is a hot topic not just for energy generation but also for manufacturing chemicals, bioproducts, and materials. Moreover, and since biomass is extensively accessible, commercial products/biofuels from lignocellulosic materials can be produced in most geographic locations. Biomass characteristics determine the economic viability and effectiveness of the value-added product production, the primary treatment method used. However, biomass heterogeneity, high capital and operating costs associated with bioconversion, and the mechanisms underlying the biomass conversion process are all issues with using biomass for heat, chemical products, and fuels generation. As a result, efforts should be made at all levels to build more user-friendly and cost-effective technologies to stimulate the broad use of biomass and attract investment in this field. Furthermore, because ideal biomass pretreatment conditions are rarely published, nothing is known about them.

7.4 Summary

Biomass preprocessing and characterization is critical in determining that biomass materials are utilized effectively in biofuel production. An improved comprehension is required of the origins of biomass and its recalcitrance. Further the impact of primary treatment to maximize the different biofuel production pathways. It requires an assessment of biomass using cutting-edge analytical techniques capable of providing knowledge. Based on the features of the pyrolysis products, criteria for assessing the feasibility of biomass for a pyrolysis conversion process to produce solid, liquid, and gaseous fuels are developed. The applicability of various biomass for pyrolytic transformation is investigated using this technique. It is discovered that various biomass is suitable for diverse applications such as combustion of fuel, liquefaction, gasification, and the production of char adsorbents depending on the chemical composition of biomass.

Conflict of Interest All the authors declare that they have no competing interests.

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Chapter 8
Biomass Pyrolysis and its Multiple Applications

Shivangi Pathak, Anil Kumar Sakhiya, and Priyanka Kaushal

Abstract In the twenty-first century, the growing demands of a modernized and growing population have led to the rapid expansion of agricultural and industrial sectors around the world. This expansion leads to produce a massive amount of biomass materials and various organic and inorganic pollutants. Biochar has promising potential in tackling such global concerns and can serve as a low-cost adsorbent for accomplishing sustainable development goals (SDGs). Biochar, a carbon-rich solid product, can be obtained by slow pyrolysis of biomass under an oxygen-limited atmosphere. Produced biochar can be used as an adsorbent to remove organic and in-organic pollutants from groundwater and industrial wastewater. However, biochar has a lower surface area and limited surface functional groups, which results in lower adsorption capacity. Hence, activation is required. Apart from adsorbent biochar is also used as a soil additive to sequestrate carbon to mitigate climate change and enhance its fertility and water retention capability hence lowering the frequency of irrigation in the field. In this chapter, the fundamentals of slow pyrolysis, its process parameters, product yield distribution, and various application of biochar will be discussed in detail.

Keywords Pyrolysis · Biochar · Activation · Adsorbent · Soil amendment

Abbreviations

ACB	*Araucaria columnaris* bark
BSA	Biochar soil amendment
CEC	Cation exchange capacity
DCFC	Direct carbon fuel cell
DFT	Density functional theory

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P. Verma (ed.), *Thermochemical and Catalytic Conversion Technologies for Future Biorefineries*, Clean Energy Production Technologies,
https://doi.org/10.1007/978-981-19-4312-6_8

dw	dry weight basis
fw	Fresh weight basis
GRSB	Granular Rice Straw biochar
JPB	Jujube pit biochar
MB	Methylene blue
MBC	Magnetically modified rice husk biochar
OM	Organic matter
PAHs	Polycyclic aromatic hydrocarbons
PBB	Powdered bamboo biochar
PRSB	Powdered Rice Straw biochar
RE	Removal efficiency
RO	Reverse osmosis
SA	Sulfonamides
SPS	Spent *P. ostreatus* substrate
SSS	Spent shiitake substrate
TC	Tetracycline
TPH	Total petroleum hydrocarbons
WHC	Water holding capacity

8.1 Introduction

The world's food, water, and energy demand are increasing rapidly as the global population increases. Large amounts of biomass waste (crop residues, forest residues, organic) are generated daily due to high living standards and economic development. Globally, the approximately 140 Gigaton biomass waste generated and managing such a huge amount of waste is a great challenge. Most of the biomass waste is discarded and open burning in the field, which negatively impacts the environment (Tripathi et al. 2019; Goswami et al. 2020a; Agrawal and Verma 2022). The conversion of biomass waste to biochar through pyrolysis is a feasible solution and creates value addition in society.

Biochar is a "carbon-rich solid product" formed by pyrolyzing biomass at high temperatures (300–600 °C) in an inert environment (Sakhiya et al. 2020; Goswami et al. 2020b, 2021). Biochar can be used for a variety of energy and environmental purposes, including clean solid fuel, fuel cells, catalysts, soil amendments, composting additives, groundwater and wastewater purification, air purification, carbon sequestration, hydrogen storage, etc. (Dillon and Heben 2001; Sakhiya et al. 2021a, b; Baghel et al. 2022). The pyrolysis product yield distribution is influenced by a variety of factors, including pyrolysis temperature, heating rate, residence time, and particle size. Presently, biochar is used as an economical adsorbent for removing organic (PAHs, pesticides, dye, surfactants, pharmaceutical) and inorganic (heavy metals such as As, Ni, Pb, Zn, and Cu) contaminants from groundwater and wastewater (Cha et al. 2016). Moreover, biochar has a good water

holding capacity and nutrient retention capacity, improving agriculture sustainability and product yield. Biochar produced from agricultural waste and used in the soil can return the nutrients and develop a circular economy (Zhou et al. 2021).

However, there are certain limitations to using biochar as an adsorbent and other environmental application due to cation exchange capacity, limited surface functional groups, low porosity, and surface area. The activation is a process that improves the physicochemical properties of biochar using different activation agents and heating in the temperature range of 500–900 °C. According to the report "Global Activated Carbon Market Forecast & Opportunities 2017," demand for activated carbon is predicted to grow at a rate of higher than 10% per year for the next half-decade, reaching a market value of 3 billion dollars by 2025 (Park et al. 2013). The sustainable utilization of biomass to produce bioenergy and activated charcoal would not only assist in alleviating the environmental issues due to coal mining but also cuts down the price of producing efficient sorbents.

This chapter describes the biochar production through the pyrolysis, types of pyrolysis process, parameters influencing the pyrolysis. Activation of biochar via physical and chemical activation is also discussed in detail. Additionally, biochar has a wide array of uses such as solid fuel, catalyst, the adsorbent in water purification, additives in composting, and hydrogen storage were described in detail.

8.2 Pyrolysis

Pyrolysis is a thermochemical conversion technology in which biomass is thermally heated at elevated temperature and produced three different products: biochar, bio-oil, and gas. In addition to its efficacy, pyrolysis produces multi-products compared to other thermochemical conversion processes (Tripathi et al. 2016).

8.2.1 Physics of Pyrolysis

In the pyrolysis process, organic material is thermally decomposed under an oxygen-limited environment. It's a particularly complicated process with a lot of various reactions in the reacting zone. Biomass contains three major constitutes, including hemicellulose, cellulose, and lignin (Kumar and Verma 2021a, b). The heating of biomass, biomolecules of hemicellulose, and cellulose break down in the form of volatile matter and produce bio-oil by condensation. The heating of biomass under an oxygen-limited environment allows a rise in temperature greater than its limit thermal stability and produces a more stable solid product called biochar.

The pyrolysis process mainly consists of two steps, i.e., primary and secondary pyrolysis. During primary pyrolysis, heat causes biomass molecules to cleave and devolatilize, forming numerous functional groups such as hydroxyl, carboxyl, and carbonyl. The process of devolatilization takes place by dehydration of biomass,

followed by decarboxylation and dehydrogenation. Secondary pyrolysis begins after the completion of the primary pyrolysis process in which heavy hydrocarbon breakdown into condensable (bio-oil) and non-condensable gases (CO, CO_2, H_2, and CH_4). The cracking of heavy hydrocarbon compounds can be presented by the following reaction (Tripathi et al. 2016):

$$(C_6H_6O_6)_n \rightarrow (H_2 + CO + CH_4 + \ldots + C_5H_{12}) + (H_2O + CH_3OH + CH_3COOH + \ldots) + C \quad (8.1)$$

Where the initial part of the products in the reaction represents the non-condensable gases, the later part represents the mixture of condensable gases, and the last part is the char yield.

8.2.2 *Types of the Pyrolysis Process*

The process parameters help in classifying the pyrolysis process into four categories: slow, fast, flash, and intermediate. Each type has its own set of benefits and drawbacks. The basic characteristics of each type of pyrolysis and the operating parameters are discussed in the following section. Table 8.1 represents the types of pyrolysis processes based on operating conditions.

8.2.2.1 Slow Pyrolysis

It is a conventional method and widely used to produce charcoal in history and characterized by a modest heating rate and a prolonged residence time. It is generally carried out from 400–600 °C to get higher biochar yield with a small amount of

Table 8.1 Operating conditions of types of pyrolysis processes

Operation condition	Slow	Fast	Flash	Intermediate
Temperature (°C)	550–950	850–1200	900–1300	400–650
Heating rate (°C/min)	0.1–1.0	10–200	>1000	1.0–10
Residence time (min)	300–500	0.5–10	<1	0.5–20
Particle size (mm)	5–50	<1	<0.5	1–5
Reference	Bahng et al. (2009), Demirbaş and Arin (2002)	Bahng et al. (2009), Demirbaş and Arin (2002)	Bahng et al. (2009), Demirbaş and Arin (2002)	Zhang et al. (2010), Kebelmann et al. (2013)

condensed bio-oil and non-condensable gases such as CO_2, CO, CH_4, and hydrocarbons (C_1–C_2) (Al Arni 2018). The slow pyrolysis is characterized by longer residence time (>60 min), lower heating rate (0.1–1.0 °C/min), a particle size of 5–50 mm, and performed at ambient pressure. The longer residence time provides an appropriate atmosphere and time to complete the secondary pyrolysis reaction. Additionally, a longer vapor residence time permits vapors formed during the secondary reaction to be evacuated, which results in higher biochar yield. Biomass with larger particle size, high lignin content, and lower ash content is best suited for the production of biochar (Demirbas 2004).

8.2.2.2 Fast Pyrolysis

Fast pyrolysis is mainly used to produce the bio-oil (yield >50%). Biochar and gas yields are overshadowed by bio-oil production. It has a high heating rate as compared to slow pyrolysis. Fast pyrolysis is mainly performed within the temperature of 850–1200 °C, with a heating rate ranging from 10–200 °C/min, the particle size of <1 mm, and residence time less than 10 s (Greenhalf et al. 2013). Different reactor designs are utilized for fast pyrolysis, including rotating cones, bubbling fluidized beds, circulating beds, ablative reactors, etc. The main objective of fast pyrolysis is to raise the temperature of the feedstock to a point where thermal cracking takes place while decreasing the exposure time, which promotes biochar formation. The bio-oil produced through fast pyrolysis is corrosive because of its low pH value. Moreover, bio-oil contains a high amount of water fraction, which lowers its heating value. As a result, before using bio-oil, it must be upgraded (Xu and Etcheverry 2008).

8.2.2.3 Flash Pyrolysis

Flash or rapid pyrolysis is a modified variant of fast pyrolysis and is characterized by a high heating rate, shorter residence time, and extreme reaction temperature. Rapid is carried out at a heating rate > 1000 °C/min and a short residence time of 1–10 s (Demirbaş and Arin 2002). Such an extreme condition is arranged in flash pyrolysis to obtain a high bio-oil yield with lower water fraction and biomass conversion efficiency of up to 70%. In flash pyrolysis, heat and mass transfer processes, chemical-reactions kinetics, and biomass transition phase behavior play a significant role in product yield distribution.

The most difficult aspect of implementing this pyrolysis on a large scale is designing a reactor in which the input biomass can be heated at an extreme heating rate for a short residence time. A major issue is the stability and quality of the bio-oil as it is heavily influenced by the presence of biochar in the product. The presence of biochar in bio-oil leads to catalysis of the polymerization reaction, resulting in a higher viscosity of bio-oil (Tripathi et al. 2016).

8.2.2.4 Intermediate Pyrolysis

Intermediate pyrolysis is usually carried out to achieve the balance of product yield between fast pyrolysis and slow pyrolysis. It has a decent product yield distribution, and therefore, it can be used in the co-production of biochar, bio-oil, and gas. The process conditions of this pyrolysis lie between fast and slow pyrolysis. The pyrolysis is carried out in the temperature range of 400–650 °C with a heating rate of 1–10 °C/min and residence time of 15–20 min. The advantages of the conditions in intermediate pyrolysis are that they prevent the development of high molecular tar compounds with excellent quality bio-oil and generate dry biochar which is appropriate for soil amendment and bio-energy production (Kazawadi et al. 2021). The bio-oil produced through this pyrolysis can be used in engines and boilers directly because it does not have a high amount of reactive tar. This is a significant benefit of intermediate pyrolysis over fast pyrolysis (Mahmood et al. 2013).

8.3 Effects of Process Parameters

8.3.1 Process Temperature

Temperature is the most crucial element to control the reaction process, and it directly influences the biochar physicochemical properties and yield. The increment in pyrolysis temperature negatively impacts biochar yield because it facilitates the thermal cracking of heavy hydrocarbon compounds leading to a rise in liquid and gas yield (Ahmad et al. 2014). Biochar developed in the primary pyrolysis reaction takes part in the secondary reactions and enhances the bio-oil and gas yield at the expense of biochar. Hence, a lower pyrolysis temperature is suitable for higher biochar yield. Moreover, biochar has a graphene-like structure when it is produced above the 300 °C temperature. Graphene holds a flat-polyaromatic and monolayer carbon structure, high electrical conductivity, and stability index. Additionally, biochar produced at higher temperatures enhances physicochemical properties such as pH, surface functional groups, and BET surface area (Wu et al. 2012). Biochar produced at higher temperatures can also have high aromaticity and recalcitrant carbon fractions in biochar which improves the stability.

8.3.2 Heating Rate

In biomass pyrolysis, the heating rate affects the product yield and physicochemical properties to some extent. The pyrolysis process is mainly classified based on the heating rates. The process with a lower heating rate can minimize the secondary pyrolysis reaction and hence, it confirms that no thermal cracking arises, resulting in

higher biochar yield. In contrast, higher heating rates promote the fragmentation of feedstock and increased the bio-oil and gas yield by restraining the possibility of char formation. The higher heating rate also enhances the depolymerization of lignocellulosic constitutes into primary volatile compounds, which reduces the biochar yield (Tripathi et al. 2016).

Dilek Angin performed the pyrolysis of safflower seed press cake for biochar production by altering the heating rates (10–50 °C/min) (Angin 2013). It was noticed that as the heating rate increased the biochar yield decreased. Similar results were observed in the literature (Ateş et al. 2004; Huang et al. 2017; Zhao et al. 2018).

8.3.3 Residence Time

Residence time has a crucial part in the minimalism of the product yield distribution, product properties, reaction mechanism, and product quality. The biochar production can be carried out at various scales of residence time ranging from a few minutes to several days. Low temperature combined with longer solid residence time is suitable for char production (Cha et al. 2016). The higher residence time supports the depolymerization of lignocellulose composition by offering them adequate reaction time. In contrast, lower residence time in the pyrolysis process minimizes the depolymerization reaction, which results in lower biochar yield (Park et al. 2008).

Residence time influences the char yield and the characteristic and physicochemical properties of biochar such as surface area, micro- and macropore development, and surface functional groups. It was reported that longer residence time in biomass pyrolysis enlarged the pore size (Tay et al. 2009). Pyrolysis temperature, particle size, heating rate, and other variables frequently govern the influence of holding time. This makes it difficult to give a clear picture of the role of residence time in the development of biochar.

8.3.4 Particle Size

Particle size is also one of the essential factors in the biomass pyrolysis process as it controls the reaction rate and heat transfer rate. The heat transfer rate of input feedstock decreased from the outer surface to the core of the material by increasing the particle size, which results in higher biochar yield (Encinar et al. 2000). Additionally, when particle size increases, the vapor released during the thermal breakdown of feedstock travels a greater distance inside the biochar layer, which triggers secondary pyrolysis and leads to an increase in biochar yield.

Hong et al. (2020) studied the effect of temperature and particle size on biochar yield using various agriculture waste. The biochar yield increased with increasing the particle size (Hong et al. 2020). Demirbas also studied the influence of particle

size on char yield through pyrolysis of agriculture waste. It was observed that char yield improved from 19.3 to 35.7% for olive husk and 5.6–16.7% for corncob by rising particle size from 0.5 mm to 2.2 mm (Ayhan Demirbas 2004).

8.4 Biochar Activation

8.4.1 Physical Activation

In physical activation, biochar produced thermochemically from biomass is processed with activities such as steam, CO_2, and air/O_2 at a temperature range of 700–900 °C. Biochar porosity increases in an oxidizing environment at an elevated temperature during the activation process. Activation increases the surface area and pore size of biochar, which improves its adsorption capacities. The oxidizing agents penetrate the biochar layers and gasify the carbon atoms, causing inaccessible pores to expand and open (Tripathi et al. 2016). Unlike air activation, steam and carbon interaction is an endothermic reaction, making it simpler to curb (Demirbas 2009). Activation temperature, degree of activation, biomass feedstock, and activation agent strongly controls the physical activation. The generic trendline observed in the literature shows that with the increase in process temperature and time, porosity growth enhances. Moreover, this leads to an increase in pore size distribution. Using the air as an activation agent shifts the reaction in the direction of combustion because of the synergistic effect of air and biochar. An unregulated reaction can cause excessive ash formation and reduced activated carbon yield (Dawson et al. 2003). Tables 8.2 and 8.3 show the mechanism of the activation agent and the effect of the activation agent on biochar BET surface area, respectively.

Table 8.2 The mechanism of activation agent on biochar BET surface area

Oxidizing agent	Chemical reaction	Process	Reference
Steam/ H_2O	$C + H_2O \rightarrow C(O) + H_2$ $2C + H_2 \rightarrow 2C(H)$	Chemisorption	Ahmed et al. (2016), Belaroui et al. (2014), Mendoza-Carrasco et al. (2016)
CO_2	$C + CO_2 \rightarrow C(O) + CO$ $C(O) \rightarrow CO$ $C + CO_2 \rightarrow 2CO$	Carbon gasification by carbon dioxide	Aworn et al. (2008), Betancur et al. (2009)
Air/O_2	$C + O_2 \rightarrow CO_2$	Carbon gasification by oxygen	Jung et al. (2015), Singh et al. (2008), Xiao and Pignatello (2016)

Table 8.3 Effect of activation agent on the BET surface area of biochar

Feedstock	Activation agent	Activation temperature (°C)	Activation time (h)	BET surface area (m^2/g)	Reference
White wood (Spruce)	Steam	750	2.46	643	Azargohar and Dalai (2008)
Populus nigra wood	Steam	800	1	322	Shim et al. (2015)
Mixed hardwood	CO_2	800	–	730	Contescu et al. (2018)
Rice husk	Steam	700	0.75	236.7	Mayakaduwa et al. (2017)
Aspen wood	CO_2	800	3.6	910	Veksha et al. (2015)
Poplar	Air	250	0.5	570	Suliman et al. (2016)
Waste rubber	Air	500	0.08	240	Heras et al. (2009)

8.4.2 Chemical Activation

The biochar is doped with a different chemical agent to improve its physicochemical characteristics including surface area, pore volume, and fictional groups. The functional group, pore volume, and the surface area are changed according to the impregnation ratio of the chemical agent. The chemical activation of biochar generally takes place within the temperature range of 450–900 °C (Sakhiya et al. 2020). This activation of biochar is broadly classified into two categories: single-step and multi-step chemical activation. The chemical activation mechanism is ambiguous in comparison to physical activation. Mainly two types of reactions occurred on the surface of biochar during the chemical activation, i.e., dehydration and oxidation. The main advantages of chemical activation in comparison to physical activation are greater carbon yield, low temperature, high surface area, high porosity structure, and high efficiency. Chemical agents also suppress tar formation. Over a long period, the corrosion and depletion of equipment take place due to the corrosive nature of chemical agents, which is the major limitation of chemical activation. Even at high temperature, the corrosion increases and more rapidly harm the equipment. After chemical completion of activation, washing of biochar is mandatory which makes the process costlier as compared to physical activation.

There are different types of chemical activation methods available in the literature according to the desired application. If oxidation of surface functional group required acidic chemical agents were used (nitric, hydrochloric, phosphoric acids, hydrogen peroxide). Similarly, if basic modification is required NaOH and KOH agents are used for activation. Different modifications of biochar such as sulfonation, amination, and impregnation of various metals ($FeCl_3$, $ZnCl_2$, MgO, CaO, ZnO, etc.) were also used (Sajjadi et al. 2019). Among the above listed chemical agents, the

KOH is more suitable for activation due to lower process temperature, higher surface area (up to 3000 m^2/g), high product yield, and superior microporous structure (Li et al. 2020). The CH_3COOK is a non-toxic chemical agent and can be used for biochar activation. Sakhiya et al. (2021b) studied the comparative study of steam and CH_3COOK-activated biochar for heavy metal adsorption. Results indicated that CH_3COOK-activated biochar had a higher surface area and adsorption capacity in comparison to the steam-activated biochar (Sakhiya et al. 2021b).

8.5 Biochar Applications

Biochar, a low-cost carbonaceous material has a stable carbon matrix capable of retaining materials such as water, air, organic compounds, and metals. Biochar has specific thermal and electrical properties that are still being investigated. With so many different characteristics, biochar is an efficient, eco-friendly, cost-effective alternative with a wide range of applications (Schmidt and Wilson 2014). Figure 8.1 shows the various applications where biochar can be used as a substantial alternative.

8.5.1 Biochar as an Absorbent

Biochar has emerged as a cost-effective alternative to other carbonaceous materials for removing various inorganic and organic pollutants from gaseous, aqueous, and solid phases, including heavy metals, aromatic dyes, polycyclic aromatic hydrocarbons (PAHs), phenols, and antibiotics (Oliveira et al. 2017).

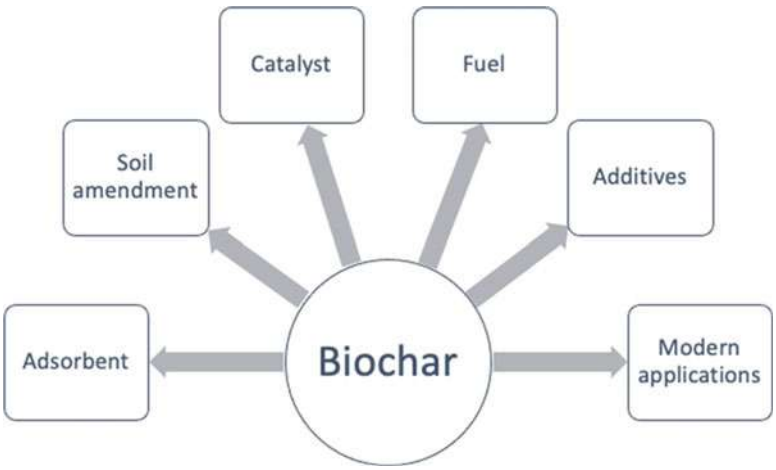

Fig. 8.1 Various applications of biochar

8.5.1.1 Biochar for Wastewater Treatment

Large volumes of wastewater effluent containing hazardous chemicals are generated by industries and are routinely deposited into adjoining environmental water sources, either directly or indirectly. Therefore, effluents must be treated before discharge to remove contaminants in order to safeguard the aquatic ecosystem and human health (Singh et al. 2020a, b). The global population is rising by 80 million citizens each year, causing a need for safe drinking water of around 64 billion cubic meters; the world must focus on creating methods for a safe water supply (Alam et al. 2014).

Some traditional wastewater treatment techniques necessitate the use of dangerous chemicals, which are both expensive and harmful to the environment (Khin et al. 2012). These processes often affect the environment by producing toxic and non-eco-friendly end products with considerable initial and ongoing capital costs (Singh et al. 2020a, b). The most used wastewater treatment processes are adsorption, reverse osmosis (RO), and membrane filtering. These processes have drawbacks such as membrane deformation, high operational costs, complex instrument handling, the development of undesirable sludge, and other disposal issues. As a result, an alternative, improved, substantial, and cost-efficient wastewater treatment technique is required.

Economically and environmentally sustainable wastewater remediation setups are based on biomass's highly efficient and ecologically sustainable materials. Biochar has specific characteristics such as high porosity, large surface area, and holding water for a longer time making it a suitable substitute for wastewater treatment (Yargicoglu et al. 2015). This section of the chapter emphasizes biochar's potential to remove undesired and hazardous species such as organic contaminants and heavy metals from wastewater. Figure 8.2 demonstrates the adsorption mechanism of heavy metals and organic compounds onto biochar's surface.

Biochar for Heavy Metal Removal

Heavy metals in wastewater have the ability to cause damage to the environment. Even at minor concentrations, long-term exposure to heavy metals can cause major health concerns. (Ahmed et al. 2016; Sakhiya et al. 2022). According to recent research, biochar generated from plant wastes and animal manure can effectively absorb heavy metals from waste and drinking water (Dai et al. 2017; Higashikawa et al. 2016; Tan et al. 2016; Zhou et al. 2017).

The functional groups such as OH and −COOH on the surface of biochar show a strong affinity towards heavy metals. The π conjugate aromatic structure of biochar allows it to change negative charge in π-orbital, resulting in losing electrons of a functional group more efficiently, and adsorption becomes more significant (Wang et al. 2018). Samsuri et al. (2014) showed that the polarity index, functional groups

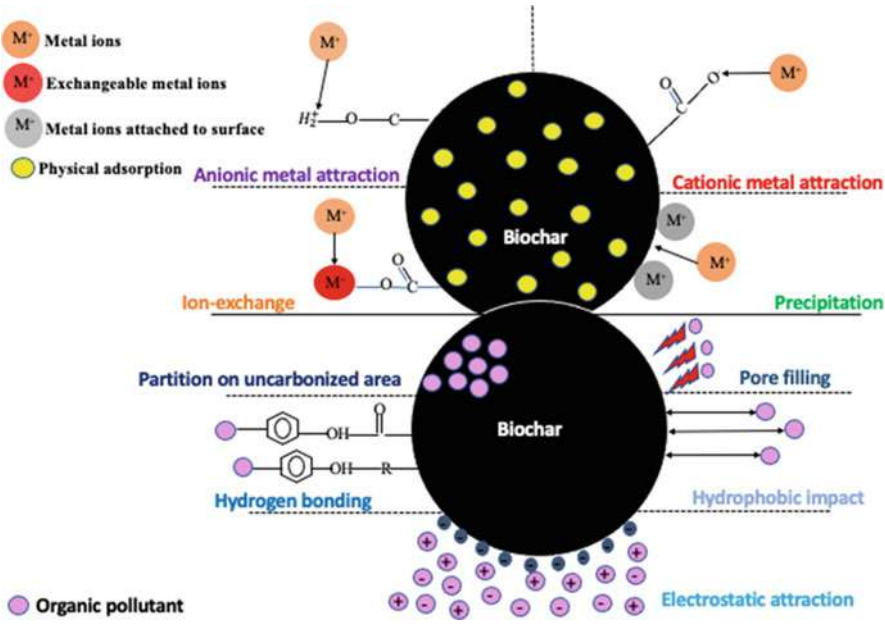

Fig. 8.2 Adsorption mechanism of organic pollutants and heavy metals onto biochar

containing oxygen or O/C molar ratio has an important role in the heavy metal adsorption process (Samsuri et al. 2014).

Arsenic is found in both wastewater and drinking water and is highly toxic. Van Vinh et al. (2015) impregnated $Zn(NO_3)_2$ over biochar, and the results revealed an increase in adsorption capacity of As^{3+} from 5.7 to 7.0 mg/g (Van Vinh et al. 2015). Furthermore, fresh and dehydrated banana peels biochar was used to remove Pb^{2+} from wastewater, removal efficiencies obtained were of 359 mg/g and 193 mg/g, respectively (Zhou et al. 2017). Higashikawa et al. (2016) studied the effect of pyrolysis temperature on Cd^{2+} adsorption using a mixture of biochar derived from rice husk, sugarcane straw, chicken manure, and sawdust. Rising the pyrolysis temperature from 350 to 650 °C increases the percentage of Cd^{2+} removed (Higashikawa et al. 2016).

Removal of Organic Pollutants

Organic contaminants are widespread in wastewater. Dyes, phenols, PAHs, and antibiotics have recently gained high attention due to their complex aromatic structure, high toxicity, and biodegradable resistances in the environment.

Globally, the textile sector is estimated to be worth $1 trillion, accounting for approximately 7% of total global exports and engaging roughly 35 million people (Desore and Narula 2018). The water pollution caused by the textile industry has a

significant impact on the environment. According to the literature, biochar application can be an economical and environment-friendly solution to remove dyes from the aqueous solutions with more than 80% efficiency (Srivatsav et al. 2020). Various operational factors (such as temperature, solution's pH, biochar dosage, and concentration of dye) have a critical role in altering the adsorption of dye using biochar (Chu et al. 2020; Mahmoud et al. 2020; Park et al. 2019). Experiments were conducted to determine the threshold pH values, and the findings revealed that biochar could withstand pH levels as low as 2 and as high as 11. Despite this, removal efficiencies were higher than 80% (Srivatsav et al. 2020). Fan et al. (2017) used BC prepared from municipal sludge to extract methylene blue (MB), accurately represented by a pseudo-second-order model, and had a removal efficiency of up to 100% (Fan et al. 2017).

Furthermore, after three cycles, the clearance rate of MB has remained at 60%. The adsorption capacity increased with increasing pH throughout the adsorption phase is attributed to electrostatic interaction. Furthermore, Si–O–Si on BC can offer adsorption sites and interact with MB's functional group containing nitrogen. Moreover, MB can create a hydrogen bond with BC's hydrogen. Kelm et al. (2019) studied the performance of biochar derived from wood residues for adsorption of Indosol Black NF1200 dye and results indicated that at low pH values, biochar could be an efficient adsorbent for azo dyes removal from textile industries (Kelm et al. 2019).

Phenols are being widely used at an industrial scale, which leads to the release of phenolic pollutants in industrial wastewater (Mohammadi et al. 2015). PAHs are also released from various industries and are toxic, carcinogenic, mutagenic, and persistent. Phenols and PAHs have a complex aromatic structure, making them biodegradation resistant (Busca et al. 2008), thus emerging the need to remove these pollutants before moving into the aquatic system. Biochar is used to remove PAHs and phenols from aqueous solutions due to its high adsorptive ability. Various factors such as surface area, adsorbent and adsorbate concentrations, pore-volume, and size affect the adsorption of phenols and PAHs on biochar.

Recently, Chandola et al. (2021) conducted a study for removing phenols from aqueous solutions using biochar produced at different temperatures from *Araucaria columnaris* bark (ACB), 100% phenol removal was achieved with the biochar produced at a temperature of 500 °C (Chandola et al. 2021). A study showed that 57% of PAHs dissolved in sewage sludge can be removed using biochar (Oleszczuk et al. 2012). A review by Lamichhane et al. (2016) stated that more than 98% adsorption capacity could be achieved using biochar as adsorbent for PAHs removal (Lamichhane et al. 2016).

Antibiotic contamination and the emergence of antimicrobial-resistant microorganisms are significant environmental concerns across the world. Given the rising use of antibiotics, reducing their presence in the environment is critical (Krasucka et al. 2021).

Peng et al. (2016) studied the use of biochar for the adsorption of seven antibiotics in an environmental concentration of aqueous solutions. A significant amount of antibiotics were removed, and the adsorption energy increased significantly using

the density functional theory (DFT) as the number of rings increased, showing the relevance of π–π interactions in the adsorption process (Peng et al. 2016). Peiris et al. (2017) studied the removal of Sulfonamides (SA) and Tetracycline (TC) using biochar and investigated the adsorption mechanism in detail. Electron donor–acceptor interactions of electron-withdrawing compounds with surface aromatic rings are the most common adsorption mechanism (Peiris et al. 2017). Fan et al. (2017) pyrolyzed rice straw at different temperatures to examine if BC could remove common antibiotics like TC. Due to its wide specific surface area and porosity, BC produced at a higher temperature showed a maximum adsorption capacity of 50.72 mg/g (Fan et al. 2017). Table 8.4 shows literature on biochar applications for removing heavy metals, PAHs, phenols, dyes, antibiotic pollutants.

8.5.1.2 Biochar for Air Purification

When gaseous chemical pollutants are released into the atmosphere, they cause serious human health and environmental threats; hence, we need to prevent their emissions. Fabric filters, electrostatic precipitators, and activated carbon injections are the few techniques used to reduce the emission of toxic gaseous contaminants in the environment (Yang et al. 2018). The high maintenance and installation costs of these techniques limit their application on a large scale. According to recent research, biochar can also remediate gaseous pollutants (Bamdad et al. 2018). Biochar derived from palm kernel, eucalyptus wood, cotton stalk, and pine efficiently removed CO_2 with adsorption capacity ranging from 3.22 mmol/g to 7.32 mmol/g (Chatterjee et al. 2018; Heidari et al. 2014; Nasri et al. 2014; Zhang et al. 2014). Similarly, the H_2S removal efficiency of more than 95% was achieved using biochar derived from various feedstocks (Bhandari et al. 2014; Das et al. 2019; Sun et al. 2017). Alkali medium is favorable for achieving high H_2S adsorption. The interaction with biochar's surface functional groups COOH and OH is responsible for H_2S adsorption (Shang et al. 2013). Table 8.5 shows the various recent studies which used biochar for removing toxic gaseous contaminants removal.

Biochar is also used as an adsorbent for the remediation of pollutants from soil. Since it is a very vast application, it has been reviewed in detail in the next section.

8.5.2 Biochar as a Soil Amendment

Various studies have recommended biochar as an efficient soil additive in agricultural soils. Biochar application improves soil aggregate stability and enhances its capacity to hold water for a more extended period by improving its pore characteristics, surface area, particle, and bulk density. The kind of soil and its texture also plays an important role. Biochar amendment effects are more noticeable in soil having high sand-sized particles than in soil rich in clay (Blanco-Canqui 2017; Kavitha et al. 2018).

Table 8.4 Biochar produced from various feedstocks is used to remove heavy metals and organic pollutants

Biochar feedstock	Pyrolysis temperature (°C)	Pollutants	Residence time (h)	Adsorption temperature (K)	R.E[a]/Q_{max}[b]	Reference
Long-root Eichhornia crassipes	500	Pb(II) Zn(II) Cu(II) Cd(II)	4	298	39.09 mg/g 45.40 mg/g 48.20 mg/g 44.04 mg/g	Li et al. (2018)
Spent *P. ostreatus* substrate (SPS), spent **shiitake** substrate (SSS)	700	Pb(II)	2	298 ± 0.5	326 mg/g	Wu et al. (2019)
Sesame straw	700	Cd, Cr, Cu, Pb, Zn	4	298	8 6 mg/g 65 mg/g 55 mg/g 102 mg/g 34 mg/g	Park et al. (2016)
Mixed municipal discarded materials	300	Cu(II)	12	303	4–5 mg/g	Hoslett et al. (2019)
Chicken manure	700	NAP	2	298	84.3%	Liu et al. (2021a, b)
Jujube pit biochar (JPB)	800	Pb(II)	2	298	137.1 mg/g	Gao et al. (2020)
Wheat straw	600	NAP	1	298	69.6 mg/g	Jiang et al. (2014)
Soya bean straw	700	NAP	3	298	25.66 mg/g	Hu et al. (2019)
Magnetically modified rice husk biochar (MBC)	500	PHE	2	298	97.6 mg/g	Wei Guo et al. (2018)
Excess sludge	700	PHE PYR	1.5	298	87.32 mg/g 80.88 mg/g	Guo et al. (2017)
Rice straw	550	Naphthenic acid	2	298	8.6617 mg/g	Singh et al. (2020a, b)

(continued)

Table 8.4 (continued)

Biochar feedstock	Pyrolysis temperature (°C)	Pollutants	Residence time (h)	Adsorption temperature (K)	R.E[a]/Q_{max}[b]	Reference
Pine fruit shell	550	Phenols	1	298	99%	Mohammed et al. (2018)
Pine chips	300	Acetaminophen and naproxen	0.25	298	94.1% and 97.7%	Jung et al. (2015)
Alternanthera philoxeroides	600	Ibuprofen	1	298	97%	Du et al. (2021)
Soybean dreg	600	Methylene blue	1	318	1273.51 mg/g	Ying et al. (2021)
Palm Kernel shell	350	Crystal violet	0.33	298	24.45 mg/g	Kyi et al. (2020)
Poplar wood	750	Trichloroethylene	0.25	298	98%	Puppa et al. (2020)

[a]*R.E* Removal efficiency
[b]Q_{max} Maximum adsorption capacity

Table 8.5 Literature of gaseous pollutants removed by biochar

Feedstock	Preparation mechanism	Pollutant	Removal rate	Reference
Hickory chips	By simply ball milling of pristine biochar with ammonium hydroxide, N-doped biochar was prepared.	CO_2	N-doped biochar resulted in 31.6–55.2% higher adsorption than the corresponding pristine biochar	Xiaoyun Xu et al. (2019)
Waste wood/ brominated flame retarded	Brominated biochar was prepared using one-step pyrolysis at 600 °C and biomass to plastic ratio 1:1 (mass basis)	Elementary mercury	40% removal efficiency was achieved	Xu et al. (2018)
Black spruce and white birch residues	Activated biochar was prepared using KOH, CO_2, and superheated steam	SO_2	The highest adsorption capacity of 76.9 mg/g was obtained using steam-activated white birch biochar.	Braghiroli et al. (2019)
Biomass	Biochar was produced by pyrolysis at 550 °C under an inert (N_2) environment at 12–15 min residence time.	Gaseous ozone	The removal efficiency obtained was 55 ppbv	Zhou et al. (2018)
Neem	Biochar was prepared through a moderate pyrolysis N_2 environment	Toluene	Adsorption capacity obtained was 65.5 mg/g	Kumar et al. (2020a)
Pinecone	Biochar was prepared at 500 °C with 5 °C/min N_2 flow for 90 min Activation was done using hydrogen peroxide impregnation	Formaldehyde	The removal efficiency obtained was 89%	Yi et al. (2018)

Biochar addition to soil affects its physicochemical parameters such as surface area, tensile strength, pH, cation exchange capacity (CEC), and water-holding capacity, which have a direct impact on plant growth (Chan et al. 2008; Lehmann and Rondon 2006; Zong et al. 2014). Various studies showed that biochar addition improves plant growth by facilitating the nitrogen (N), phosphorus (P), and potassium (K) biochemical cycle (Chan et al. 2008; Gul and Whalen 2016) and influencing soil microbial activities. The elements such as carbon (C), hydrogen (H), sodium (Na), calcium (Ca), magnesium (Mg), N, P, and K present in biochar (Zhang et al. 2015) supply nutrients to plants for sustainable growth. Biochar decomposes slowly in soil due to its long residence life of about 3000 years. Yuan and Xu showed that factors such as biochar's alkalinity, functional groups present on its surface, and strong pH buffering capacity help to regulate soil acidity (Yuan and Xu 2011).

Table 8.6 Biochar application as a soil amendment

Biochar feedstock	Contaminant	R.E. (%)	Reference
Rice straw	Cd	87.1	Tang et al. (2020)
Clover	TPH	18.6	Abbaspour et al. (2020)
Rice straw	PAHs	40–58.84	Zhang et al. (2020)
Poplar wood	Cu, Cd, Pb, and Zn	72.8	Chen et al. (2020)
Wheat straw	TPH PAHs	45.83 30.34	Han et al. (2016)
Rice straw	TPH	84.8	Qin et al. (2013)

R.E. Removal efficiency, *TPH* total petroleum hydrocarbons

Biochar amendment improves the nutrient cycle of a plant. A review by Tesfaye et al. (2021) showed a significant impact of biochar soil amendment (BSA) on plant P uptake and soil available P by 55% and 65%, respectively (Tesfaye et al. 2021). Scheifele et al. (2017) reported an increase in nodule dry matter and biological nitrogen fixation (BNF) by 1.8 and 1.2 folds, respectively, in soybean plants with the amendment of maize and wood biochar (Scheifele et al. 2017).

Biochar having a large surface area and pore volume has been an excellent means to remove heavy metals present in polluted soil (Ahmad et al. 2018). The soil amendment using biochar improves soil pore fraction, which provides more space for microorganisms to grow, and N, P, Ca, and K present in biochar provides nutrients for plant growth (Sakhiya et al. 2020). On the other hand, Warnock et al. (2007) examined the effect of BSA on microorganisms present in the soil. BSA results in a reduction in overall microbial biomass (Warnock et al. 2007). Few recent studies of remediations of various contaminants from soil using biochar have been mentioned in Table 8.6.

8.5.3 Biochar as a Catalyst

Biochar having high surface area and specific surface functional groups can be prepared by functionalization or activation. Biochar has plenty of potentials to be utilized as a flexible catalyst or catalytic assist in various chemical processes because of its unique chemical structure. Biochar can be utilized as a catalyst in biogas upgrading, biodiesel production, improved syngas production, biomass conversion to chemicals and biofuels, de-NO_x processes, and microbial fuel cell electrodes (Cao et al. 2017; Lee et al. 2017). The biochar properties are enhanced by activation or functionalization procedure for being used as a catalyst. The biochar's physico-chemical characteristics, mainly its specific surface area, pore-volume, and pore size distribution, can be enhanced to various degrees depending on the activation techniques used (Cao et al. 2017). According to a study by Do Minh et al. (2020), the electrical and chemical configuration of biochar, when correctly controlled, makes it a great photo-, electro-, and chemo-catalyst that might even be used in modern

applications. Biochar can be used in combined catalysis with other phases due to its unique characteristics of semiconductivity (Do Minh et al. 2020).

Zhu et al. (2015) prepared chemically and physically activated biochar from rice husk. The evaluation of the biochar characteristics indicated that it could be employed as catalytic support. In methane catalysis, the activated biochar-assisted Ru (Ru/ABC) catalyst excelled or was equivalent to the standard AC-assisted Ru catalyst. Under the optimum reaction conditions, 98% of CH_4 selectivity was achieved and 100% CO conversion (Zhu et al. 2015).

8.5.4 Biochar as an Alternative to Fuel

Biomass has enormous potential as a fuel source and a source of both thermal and electrical energy. Because of the limited reserves of conventional energy resources, lignocellulosic biomass is becoming the central focus of the modern period, which does not need any energy storage systems (Kumar et al. 2020b, c). Biochar is typically high in carbon and can be a pollution-free solid biofuel (Kane et al. 2016).

The high moisture content, bulkiness, low energy density, hygroscopic nature, and high transportation cost in various cases are certain drawbacks of raw biomass when utilized as a fuel (Abdullah and Wu 2009; Tsai et al. 2007), making it unsuitable for a variety of industrial applications. On the other hand, because of their biodegradability, environmental friendliness, and long-term viability, these organic materials have emerged as a leading contender for biofuels and bioenergy production. Pyrolyzing biomass at high temperatures to various value-added and energy-rich products is a preferable solution. Biochar, which has entirely different characteristics than the respective feedstock, has the potential to make significant and long-term improvements in guaranteeing a future supply of green energy and turning bioenergy into a carbon-negative sector (Kwapinski et al. 2010). Many studies have been carried out on producing biochar from various agro-residues for being used in fuel applications. Biochar's heating value (16–35 MJ/kg) is equivalent to, or nearly twice the raw biomass and many low-grade coals heating value for any given feedstock (Mullen et al. 2010; Sukiran et al. 2011). Around three billion people worldwide rely on conventional stoves, for example, three stone and open fires, to meet their cooking needs. These cookstoves emit hazardous fumes and are responsible for four million fatalities per year (Sakhiya et al. 2020). Biochar-fired cookstoves can minimize carbon and few other hazardous gas emissions in cookstoves when used for heat and cooking (Birzer et al. 2014). Compared to an open cooking fire, biochar stoves lowered particulate matter emissions by 92% and carbon monoxide emissions by 87% in laboratory tests (Schultz 2013).

8.5.5 Biochar as an Additive

8.5.5.1 Biochar Used as an Additive in the Construction Sector

In recent years, the spread of industry and urbanization has heightened the need for concrete for building purposes. The building industry, especially the cement industry, has been identified as one of the significant contributors to CO_2 emissions, accounting for around 7% of all GHG emitted into the atmosphere (Andrew 2018; Benhelal et al. 2013; Gupta et al. 2018). As a result, rising importance for developing greener solutions to decrease the company's carbon footprint and raw material usage (Miller et al. 2018). Several researchers have utilized biochar as an eco-friendly filler in cement manufacture and cement-based construction products to reduce carbon emissions. Biochar has poor heat conductivity, excellent chemical stability, low flammability, and conductivity, making it a suitable candidate for construction material and a filler in cement mortar products (Gupta and Kua 2017). Suarez-Riera et al. (2020) utilized biochar microparticles as a filler and a replacement for cement powder in cement paste and mortar composites. The results indicated that 2 wt% biochar fragments are adequate to improve the strength and resilience of cement and mortar mixtures. When used instead of cement, mechanical properties equivalent to the reference samples were achieved (Suarez-Riera et al. 2020). Another research uses biochar made from wood, food waste, and rice as a carbon-sequestering additive in mortar, attaining comparable mechanical strength results by adding 1–2 wt% biochar to the control mix.

Gupta and Kua (2019) also discovered that adding finer biochar particles at the start guarantees an increment in early strength and water tightness when utilized in cement mortar fusions. It was reported that timber waste biochar can be utilized as a filler material in concrete structures to improve strength and moisture resistance (Gupta and Kua 2019).

Poor thermal conductivity, directly impacted by the availability of broad arrays of pores on biochar, depends mainly on the process temperature and biochar's feedstock (Brewer et al. 2014). Extraction of oxygen functional group from the biochar's surface reduces the energy sites, forming the biochar to cause less hazardous reactions when blended with concrete mixes (Cross and Sohi 2013).

8.5.5.2 Biochar as an Additive in Composting

The swift development of humans and lifestyle changes have caused high waste creation. Furthermore, the animal farm business is expanding, posing its own set of problems. Biochar production involves transforming biodegradable carbon (biomass) into aromatic carbon (biochar), which is less degradable. Therefore, along with waste management, biochar production has an additional quirk of being an atmospheric carbon sequestration technique. Composting also promotes a more orderly breakdown of organic waste materials biologically and physicochemically.

An integrated technique employing biochar in composting reduces ecological concern throughout the waste treatment process by decreasing harmful chemical leaching and emission (e.g., heavy metals, H_2S, NH_3) as well as pathogen levels (Antonangelo et al. 2021).

Biochar has several advantages as a compost addition, including boosting composting and humification performance, promoting microbiological activities, lowering GHG and NH_4 emissions, and immobilizing heavy metals and organic contaminants (Guo et al. 2020). Various studies showed that biochar helps reduce nitrogen losses (Awasthi et al. 2018), as compounds like NH_3 and NH_4 are adsorbed by biochar (Janczak et al. 2017). The temperature goes up quicker in the course of the composting process in the presence of biochar, and the thermophilic phase lasts longer. When biochar is added at the beginning of the composting process, it enhances the water holding capacity (WHC), assuring that most composts have the required moisture content of 50–60% w/w. The carbon to nitrogen (C/N) ratios of various feedstock-derived biochar and composts vary, which has a direct impact on the rate of organic matter (OM) breakdown (Godlewska et al. 2017). Biochar has a high specific surface area (SSA) and a highly porous structure, providing nutrients for soil microorganisms to grow. Various functional groups on the biochar surface result in high cation exchange capacity (CEC), which acts as an electron carrier, making it easier to transfer and transport electrons (Antonangelo et al. 2019). Biochar application rates to compost have ranged from 5 to 10% (mass basis) to 50% or more (Jindo et al. 2012). A dose of more than 20–30% biochar (mass basis) is not encouraged since an excessive quantity of biochar compared to the composting material might obstruct biodegradation. Biochar has been proven to speed up the composting process when used in sufficient amounts, primarily by enhancing the consistency and structure of the mix and boosting microbial activity in the composting mixture. This enhanced activity results in higher temperatures and shorter compost development time (Camps and Tomlinson 2015). Table 8.7 shows the impact of biochar addition to compost in various other studies.

8.5.6 Other Modern Applications

Besides the above-discussed applications, biochar can be used in energy storage gadgets such as supercapacitors, lithium, and sodium-ion batteries etc. Biochar is used as electrode material in supercapacitors. Biochar activation was done to increase its specific surface area, resulting in an increased capacitive performance of biochar (Tan et al. 2017). For supercapacitor fabrication, Jin et al. (2014) prepared biochar from corn stover using microwave-assisted slow pyrolysis coupled with KOH activation. At a current density of 0.1 Ag^{-1}, the biochar had a specific capacitance of 246 F g^{-1} (Jin et al. 2014). Biochar is a carbon-rich material, highly porous and conductive, which makes it a suitable material to be used for sulfur-carbon (S/C) cathode composite for lithium-sulfur (Li-S) batteries (Vivekanandhan 2018). The activation process enhances biochar characteristics such as surface area,

Table 8.7 Impact of biochar on composting

Biochar feedstock	Scale	Biochar dosage	Compost material	Impact on the composting process	Reference
Corn biochar		1% w/w	Corn waste compost, corn biochar (1:1)	Shoot concentration, N, P, and K availability increased by 16, 38, and 15%, respectively, after biochar + compost addition over control soil	Liu et al. (2021a, b)
Powder and granular rice straw and bamboo biochar	Lab	10% (fw[a])	Pig manure, wheat straw (10:1)	• When compared to GRSB,[b] PRSB,[c] and PBB,[d] GBB had the lowest CH_4 and N_2O emissions • GBB had the highest cumulative NH_3 emissions (957 mg/kg)	He et al. (2019)
PRSB (500 °C)	Field	10% (dw[e])	Pig, dry chicken manure, rice straw (10:10:1 fw)	• The GWP achieved from biochar treatment was 19.8% lower than that obtained from the control treatment • Biochar reduces energy consumption in turning piles and has the potential to enhance the oxygen supply	He et al. (2017)
Rice hull biochar	Lab	20% fw	Chicken manure, hardwood sawdust, and rice hull biochar (7:1:2)	• BM drastically reduces N_2O and CO_2 emissions by 27% and 35%, respectively • Biochar amendment in chicken manure compost reduces soil N_2O emissions significantly by controlling soil organic stabilizing and functional group activities	Yuan et al. (2017)
Bamboo biochar	Pilot	3% w/w	Pig manure, wood chips, and sawdust (3:2)	• The addition of biochar lowers the NO_2-N concentration and also reduces the NO_2 emissions from pig manure composting	Wang et al. (2013)

(continued)

Table 8.7 (continued)

Biochar feedstock	Scale	Biochar dosage	Compost material	Impact on the composting process	Reference
				• Manure has lower moisture content and higher pH • The addition of biochar significantly changed the number of denitrifying bacteria	
Broad-leaved tree konara oak biochar	2% v/v	Field	Poultry manure, apple pomace, rice husk, oak bark (2:5:2:1)	• Biochar increased the amount of carbon collected by humic substance extraction by 10% and decreased the amount of water-soluble carbon by 30% • Phosphate, urease, and polyphenol oxidase activities improved by 30–40% due to biochar blending despite lower biomass	Jindo et al. (2012)
Eucalyptus grandis biochar	50%	Pilot	Biochar, coffee husk, and sawdust (1:1 fw)	Biochar amendment in poultry manure reduces the loss of nitrogen. This enrichment allows process optimization through odor emission and nitrogen loss reduction via nutrient balancing	Dias et al. (2010)

[a] *fw* fresh weight basis
[b] *GRSB* Granular Rice Straw biochar
[c] *PRSB* Powdered Rice Straw biochar
[d] *PBB* Powdered bamboo biochar
[e] *dw* dry weight basis

carbon content, surface functional groups, porosity, and pore volume, which improves metal encapsulation. Sajib et al. (2017) used KOH-activated biochar derived from canola meal as cathode composite. At the 0.05 °C rate (83.75 mA/g), the biochar cathode composite showed a high initial discharge capacity of 1507 mAh/g (Sajib 2017). Further, biochar is gaining a huge interest for fuel cell applications. Biochar can be used as a fuel in the electrolyte, as the chemical energy accumulated in biochar is a source for electricity generation, in direct carbon fuel cell

Table 8.8 Biochar applications in energy storage devices

Feedstock	Energy storage device	Biochar role	Reference
Fish scale	Supercapacitor	Electrolytes	Senthil and Lee (2021)
Walnut shell	Supercapacitor	Electrodes	Xiaoyang Xu et al. (2017)
Coconut shell	Supercapacitor	Electrodes	Jain and Tripathi (2014)
Cornstalks	Li-ion battery	Anode	Shengbin Wang et al. (2015)
Ginkgo leaves	Li-ion battery	Anode	Ou et al. (2016)
Pinecone	Sodium-ion battery	Anode	Zhang et al. (2017)
Sepals of palmyra palm fruit	Sodium-ion battery	Anode	Damodar et al. (2019)
Cherry petals	Sodium-ion battery	Anode	Zhu et al. (2018)
Banana peel	Li-S battery	cathode	Yang et al. (2016)
Almond shell	Li-S battery	cathode	Benítez et al. (2018)

(DCFC) (Huggins et al. 2016). Table 8.8 shows the application of biochar in various energy storage devices.

Altogether, enhancing biochar's physical and chemical properties through activation has many applications, such as energy storage devices. Utilizing biochar is a sustainable approach; therefore, we can expect exponential growth in its usage in the coming years.

8.6 Conclusion

The world's population is increasing at an alarming rate (approx. 80 million people annually), leading to the increased demand for food, energy, safe water supply, etc. Agricultural production has been increased around 50% in the last two decades, resulting in the generation of a massive amount of biomass waste daily. The majority of biomass waste is thrown or burned openly in the field, which has a severe influence on the ecology and public health. Biochar is a cost-effective and sustainable option to address the above-mentioned problems. This chapter reviewed different types of pyrolysis processes for biochar production and the factors affecting the biomass pyrolysis process.

The chapter also discussed the different biochar applications such as adsorbent in water purification, air purification, soil amendment, additives in composting and construction sector, an alternative to fuel, catalyst in various processes such as syngas production, biogas upgradation, and biodiesel production. Moreover, there are certain modern applications including, electrodes and electrolytes in supercapacitors, anode, cathode composites in lithium and sodium-ion batteries, and hydrogen storage. The application of biochar is highly dependent on its physicochemical characteristics such as porosity, specific surface area, and surface functional groups. These properties of biochar are enhanced using various types of

activation processes: physical and chemical. Worldwide biochar gets attention due to its desired physicochemical properties which can be useful in different types of applications to generate the circular economy.

Conflict of Interest Statement The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this chapter.

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Chapter 9
Recent Advances in Direct Catalytic Thermochemical Gasification of Biomass to Biofuels

Rupesh S. and Deepanraj

Abstract Biomass gasification is the sub-stoichiometric thermochemical transmutation of biomass into gaseous biofuel. The gasification products mainly consist of syngas, tar, and char. In general, conversion of tar to useful fuel constituents is energy intensive as it requires a higher process temperature (>800 °C) due to the higher activation energy requirement for cracking. Catalyst takes a crucial position in biomass gasification by increasing the quality and yield of gaseous biofuel at the expense of undesirable tar and char, at a lower process temperature at a faster rate. Among the different catalysts, commercial Ni-dolomite combinations exhibit remarkable tar reforming capability of 89–99% by mutually mitigating the deactivation of nickel and CH_4 reformation insensitivity of dolomite. In this view, Ni-dolomite combinations can economically nourish producer gas through simultaneous tar reformation and CO_2 sorption. Recent practices and future scopes for catalytic performance enhancement through new catalyst configurations and novel technologies are also discussed.

Keywords Biomass gasification · Gasifier · Tar reforming · Catalysts · Deactivation

Abbreviations

ASH	Ash
ATR	Autothermal reforming
d_{af}	Dry ash free
ER	Equivalence ratio
FC	Fixed carbon

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P. Verma (ed.), *Thermochemical and Catalytic Conversion Technologies for Future Biorefineries*, Clean Energy Production Technologies,
https://doi.org/10.1007/978-981-19-4312-6_9

FT	Fischer–Tropsch
GR	Gasifying ratio
GT	Gas turbine
M	Moisture
ms	Milliseconds
MTG	Methanol-to-gasoline
Nm^3	Normal cubic meter
PAH	Polycyclic aromatic
SBR	Steam to biomass ratio
VM	Volatile matter
WGS	Water gas shift

9.1 Introduction

Environmental sustainability and fossil fuel utilization are two inevitably conflicting requirements for the better being of mankind. Complete reliance on conventional energy sources, to meet the global energy demand, leads to both an energy crunch and adverse environmental issues due to the surplus emission of greenhouse gases like carbon dioxide (Goswami et al. 2020, Goswami et al. 2022). The estimated rise in world energy demand for 2021 is 4.6%, with an associated increase of 33 billion tonnes of carbon dioxide emission. Thus, depleting nature of conventional energy sources and their unfavorable environmental impacts encouraged energy extraction from replenishable energy sources. Consequently, in 2020, the global utilization and electricity generation from renewable resources is raised by 2% and 3%, respectively. Moreover, an 8% improvement in renewable energy to electricity conversion is targeted in 2021 to attain an output of 8300 TWh (Rupesh et al. 2021). Among the renewable energy sources, biomass is the highest energy distributer, approximating 15% of primary global energy consumption. However, the practical applicability of biomass can be ensured by tackling its low energy density through sustainable and eco-friendly energy conversion routes. Among the feasible bio-chemical, physiochemical, and thermochemical conversion methods, thermochemical means (pyrolysis, combustion, and gasification) are attractive because of their faster and continuous conversion. In thermochemical conversion, biomass gasification is a potential and practically feasible pathway to extract and concentrate energy embedded in biomass. In gasification, biomass is transmuted to gaseous fuels and upgraded chemical products in sub-stoichiometric conditions, without leaving any net adverse impact on the environment. The gaseous fuel obtained from gasification is mainly a mixture of hydrogen and carbon monoxide, turned into synthesis gas or syngas, which can also serve as a chemical feedstock for synthesizing other liquid fuels and value-added chemicals. In biomass gasification, the extent of biomass conversion and, the quality and quantity of the fuel generated are significantly influenced by the reactor, operating parameters, and presence of catalysts. This chapter deals with

various aspects of biomass gasification and the role of catalysts in augmenting the quality and yield of syngas.

9.2 Biomass Gasification

Biomass is non-fossilized, renewable, and biodegradable organic matter derived from animals, plants, or microorganisms. Major biomass sources consist of terrestrial and aquatic biomasses, agricultural, municipal, and solid waste, and forest residue (Sikarwar and Zhao 2017; Kumar and Verma 2021a, b). Especially, non-edible fibrous plant derivatives composed of lignin, cellulose, and hemicellulose, known as lingo-cellulosic biomass, threaten proper waste management or lead to adverse environmental impacts on direct combustion. This can be properly addressed by converting them to energy through appropriate energy conversion routes (Bridgwater 2003; Bhardwaj et al. 2021a, b; Bhardwaj and Verma 2021). Biomass gasification is the sub-stoichiometric thermochemical transmutation of biomass to gaseous fuel and upgraded chemical products. During gasification, biomass is subjected to thermal decomposition followed by chemical reactions with the participation of a medium, known as a gasifying agent, in a reactor termed as gasifier. Basically, gasification is a four-stage process comprising drying, pyrolysis, partial oxidation, and reduction. The steps in gasification are mentioned in Table 9.1.

The gasification product's yield firmly relies on the biomass, gasifying medium, reactor configuration, and process parameters. In general, the gasification products comprise a non-condensable gaseous mixture mainly consisting of H_2, CO, CO_2, CH_4, known as producer gas, condensable higher hydrocarbon species collectively termed as tar, and solid constituents such as char and ash. The overall biomass gasification process can be represented as:

Table 9.1 Different steps in biomass gasification (Sansaniwal et al. 2017)

Name	Temperature range (°C)	Process
Drying	100–150	The moisture content in biomass (5–35 wt.%) is transformed into steam
Pyrolysis	150–700	Thermal dissociation of biomass structural constituents such as lignin, cellulose, and hemicellulose into condensable and non-condensable volatiles and char
Partial oxidation	700–1500	Sub-stoichiometric combustion or oxidation of pyrolysis products that release heat to drive the other three endothermic steps
Reduction	800–1100	The various condensable and non-condensable gases, char, and gasifying agents chemically interact to yield gaseous constituents of producer gas

$$\text{Biomass} + \text{Gasifying agent} + \text{Heat} \rightarrow H_2 + CO + CO_2 + CH_4 + H_2O + \text{Tar} + \text{Char} \quad (9.1)$$

9.2.1 Gasification Chemistry

The biomass gasification chemistry is too intricate with numerous reactions and is not yet completely explored (Pfeifer and Hofbauer 2008). The main homogenous and heterogeneous chemical reactions in gasification are listed in Table 9.2. The

Table 9.2 Main gasification reactions (Basu 2010; Valderrama Rios et al. 2018;Silva et al. 2019)

S. No.	Reaction name	Stoichiometry	Enthalpy of reaction (kJ/mol)
1	Drying	Wet Biomass + Heat → Dry Biomass + H_2O	–
2	Pyrolysis	Dry Biomass + Heat → Gas + Tar + Char	–
Oxidation reactions			
3	Char oxidation	$C + O_2 \rightarrow CO_2$	−394
4	Partial char oxidation	$C + 0.5O_2 \rightarrow CO$	−111
5	Hydrogen oxidation	$H_2 + 0.5O_2 \rightarrow H_2$	−242
6	Carbon monoxide oxidation	$CO + 0.5O_2 \rightarrow CO_2$	−283
7	Methane oxidation	$CH_4 + 2O_2 \rightarrow CO_2 + 2H_2O$	−520
8	Tar partial combustion	$C_mH_n + (0.5m + 0.25n) O_2 \rightarrow mCO + 0.5nH_2O$	–
Reduction reactions			
9	Boudouard	$C + CO_2 \leftrightarrow 2CO$	+172
10	Hydrogasification	$C + 2H_2 \leftrightarrow CH_4$	−74.8
11	Char steam reforming (Water gas reaction)	$C + H_2O \leftrightarrow CO + H_2$	+131
12	Water gas shift (WGS) reaction	$CO + H_2O \leftrightarrow CO_2 + H_2$	−41.2
14	Methanation reactions	$2CO + 2H_2 \rightarrow CH_4 + CO_2$	−247
15		$CO + 3H_2 \leftrightarrow CH_4 + H_2O$	−206
16		$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$	−165
Tar decomposition reactions			
17	Thermal cracking	$C_mH_n \rightarrow aC_{m-x}H_{n-y} + bH_2$	–
18		$C_mH_n \rightarrow 0.25C_{m-x}H_{n-y} + 0.25(4n - m) H_2O$	–
19	Steam reforming	$C_nH_m + nH_2O \rightarrow \left[n + \frac{m}{2}\right]H_2 + nCO$	–
20	Dry reforming	$C_nH_m + nCO_2 \rightarrow \frac{m}{2}H_2 + 2nCO$	–
21	Hydrogenation	$C_nH_m + 2(n - 0.25m)H_2 \rightarrow nCH_4$	–
22	Carbon formation	$C_nH_m \rightarrow nC + 0.25mH_2$	–

reactions can be classified as drying, devolatilization or pyrolysis, oxidation, reduction, and tar reforming reactions. The extent of involvement of each reaction in the process relies on biomass composition, type of gasifying medium, reactor design, and process parameters. Overall, gasification is an endothermic process; is termed autothermal if the heat required is supplied by the combustion reactions, within the reactor, or allothermal if supplied externally.

9.3 Gasifying Agents

The role of gasifying medium or agent is pivotal in the yield and quality of gasification products. Air, steam, air-steam (both air and steam), oxygen, and steam-oxygen (both steam and oxygen) are the mainly used gasifying agents in biomass gasification. A comparison of the merits and limitations of air, steam, and oxygen as gasifying agents is listed in Table 9.3. In general, the quantity of gasifying agent used is referred to using non-dimensional terms defined with respect to the quantity of biomass used. Equivalence ratio (ER), steam to biomass ratio (SBR), and gasifying ratio (GR) are the non-dimensional terms, respectively, used for air, steam, and steam-oxygen mixture.

$$\text{Equivalence ratio (ER)} = \frac{\text{Actual air} - \text{fuel ratio}}{\text{Stoichiometric air} - \text{fuel ratio}} \tag{9.2}$$

$$\text{Steam to biomass ratio (SBR)} = \frac{\text{Mass of steam}}{\text{Mass of biomass}} \tag{9.3}$$

$$\text{Gasifying ratio (GR)} = \frac{\text{Mass of (steam} + \text{oxygen)}}{\text{Mass of biomass}} \tag{9.4}$$

Table 9.3 Comparison of different gasifying agents (Sansaniwal et al. 2017)

Agent	Merits	Demerits
Air	• No external heat is required as heat for gasification is obtained from partial combustion. • Economical	• Low calorific value due to N_2 dilution • Can be used only for direct burning applications
Oxygen	• The gasifying medium can enable partial oxidation, so no external heat is required. • H_2, CO, and CH_4 enriched producer gas with minimum tar and no N_2 dilution • Better carbon conversion	• Comparatively, expensive handling is required • Safety issues
Steam	• Producer gas enriched with H_2 • A higher H/C ratio of producer gas is suitable for the synthesis of liquid fuels	• Higher tar content • Energy-intensive as external heat is required to enable gasification

Table 9.4 Influence of gasifying medium on gas composition

Medium	Gas composition (vol.%)					Gas yield (Nm^3/kg_{daf})
	H_2	CO	CO_2	CH_4	N_2	
Air	5–16.3	9.9–22.4	9.0–19.4	2.2–6.2	42–62	3.7–61.9
Steam	38–56	17–32	13–17	7–12	0	60–95
Steam and oxygen	13.8–31.7	42.5–52	14.4–36.3	6–7.5	0	2.2–46

Air gasification is autothermal as the heat input for gasification is derived from partial oxidation of biomass. But the quality of the producer gas obtained is low as it is diluted with N_2 contained in the air. Consequently, the heating value of the producer gas from air gasification is limited to 4–7 MJ/Nm^3. Self-sustained gasification with H_2, CO, and CH_4 (without N_2) enriched producer gas enables the achievement of a superior calorific value of 12–28 MJ/Nm^3 in oxygen gasification. On the other hand, the use of steam results in producer gas with a higher H/C fraction having a calorific value (lower heating value) of 10–18 MJ/N m^3 (Basu 2010). Combinations of different gasifying agents are used to merge their advantages to improve the producer gas characteristics. Gas yield and composition using different gasifying agents are depicted in Table 9.4.

9.4 Types of Gasifiers

The gasifier, the reactor in which gasification takes place, has a vital impact on the quantity and quality of producer gas generated. The design, construction, and operation of gasifiers differ depending on the biomass attributes (moisture content, ash content, size, shape, etc.), quality, and yield of syngas to suit various downstream applications. The reactor is generally classified into fixed bed, fluidized bed, entrained flow, and plasma gasifiers.

9.4.1 Fixed Bed Gasifier

9.4.1.1 Updraft Gasifier

In an updraft gasifier, the gasifying agent supplied from the bottom reacts with the descending biomass loaded from the top to generate producer gas rising through the bed. The reactor is termed updraft, due to the upward flow of gas, whereas the opposing gas flow relative to biomass in the reactor signifies its alternate name countercurrent gasifier (Loha et al. 2018). In updraft gasifiers, biomass is sequentially subjected to drying, pyrolysis, reduction, and partial oxidation in the respective

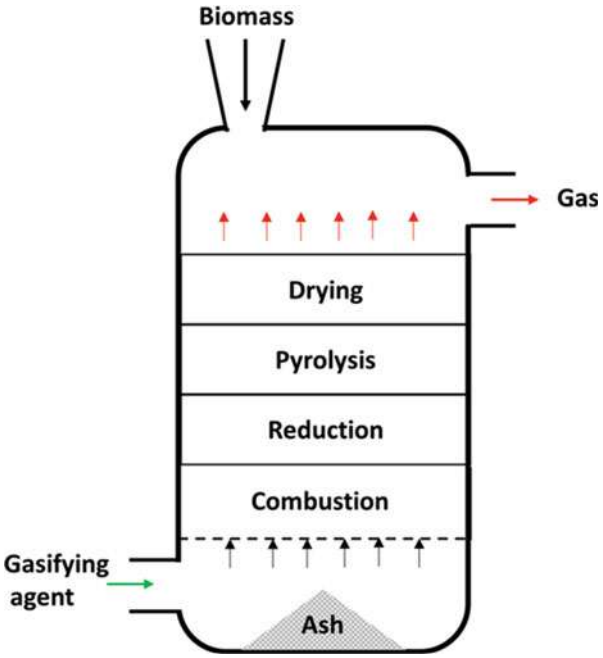

Fig. 9.1 Updraft gasifier

distinct zones to generate producer gas as shown in Fig. 9.1 (Ren et al. 2019). Generally, in the oxidation zone, the ash is melted at 1200 °C.

The flow of raw gas generated through a relatively low-temperature pyrolysis zone laden the producer gas with uncracked tar precursors, resulting in lower syngas yield with higher tar content. During this process, after passing through the fuel bed, the hot gases leave at a lower temperature range of 200–400 °C (Basu 2010; Surisetty et al. 2012). As the gas flows from a high-temperature oxidation zone to low-temperature pyrolysis and drying zones, the heat content in it can be utilized for drying and pyrolysis, thereby achieving improved thermal efficiency. Thus, updraft reactors can accommodate feedstock with higher moisture content (up to 60% by weight) and are well suited for downstream applications that can accommodate producer gas with high tar content, low dust at high flame temperature (Sansaniwal et al. 2017).

9.4.1.2 Downdraft Gasifier

In this gasifier, both biomass loaded from the top and gas generated move parallelly downward and hence the name downdraft gasifier. Due to this unidirectional movement of biomass and producer gas, this reactor is also known as a co-current gasifier (Sansaniwal et al. 2017). The tar concentration in the producer gas is relatively low due to the gas flowing from the low-temperature drying zone to the high-temperature

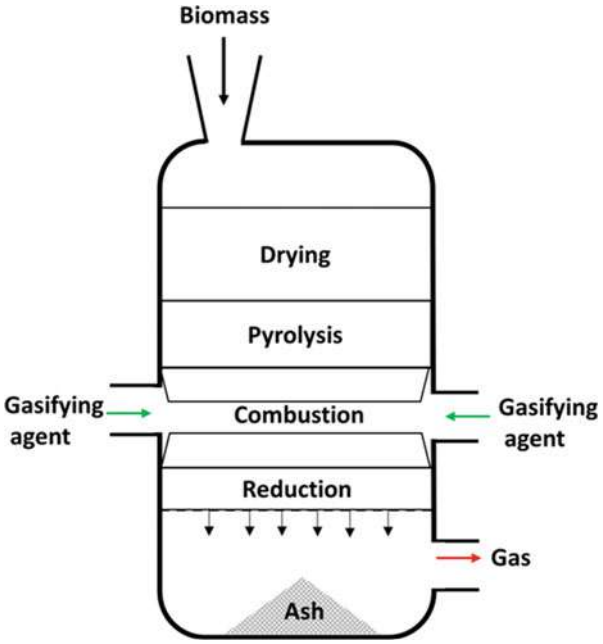

Fig. 9.2 Downdraft gasifier

combustion zone and thereby cracking the tar formed from the pyrolysis more effectively (Bermudez and Fidalgo 2016).

Thus, producer gas passing through the high-temperature (900–1000 °C) zones aids in the partial combustion of tar and leaves the gasifier at 700 °C (Basu 2010; Devi et al. 2020). However, pyrolytic gas combustion to support endothermic reactions lowers the heating values of produced gas (Ruiz et al. 2013). Moreover, incompetence in accommodating biomass with high moisture (<25%) and ash (<6%) content limits its usage (Surisetty et al. 2012). But producer gas with low particulate and tar content makes it appropriate for small-scale power generation. The schematic of the downdraft gasifier is shown in Fig. 9.2.

9.4.1.3 Crossdraft Gasifier

These gasifiers are named so due to the mutually perpendicular flow directions of biomass and producer gas with respect to the reactor, as shown in Fig. 9.3. Here feedstock is introduced from the top, while the entry of gasifying agent and exit of producer gas takes place from diametrically opposite sides of the gasifier respectively side (Devi et al. 2020).

The fuel is subjected to drying, devolatilization, and gasification as it descends the reactor. The high-velocity gasifying agent directly fed to the oxidation zone reacts with char to generate a very high temperature (<1500 °C). As gasifying agent enters through the oxidation zone and the generated producer gas leaves through the

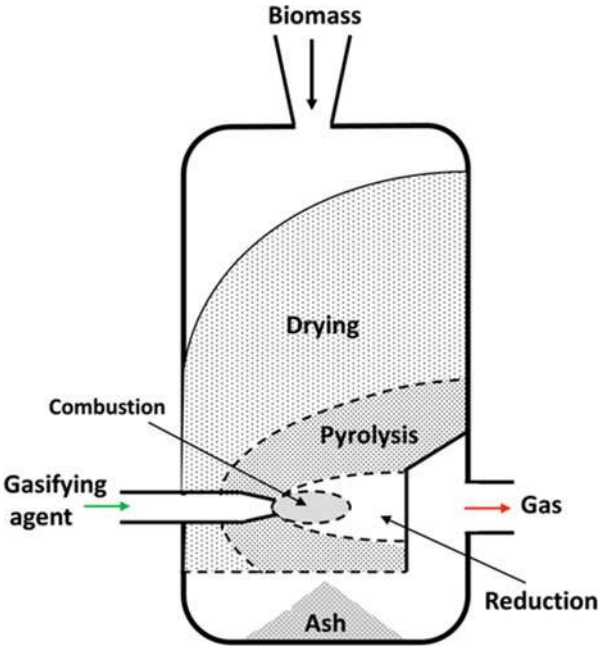

Fig. 9.3 Crossdraft gasifier

reduction zone, pyrolysis, drying, and ash disposal zones act like separate zones thereby restricting the fuel type (Loha et al. 2018). Moreover, the high temperature of exit gas results in higher CO_2 and lower H_2 and CH_4 concentrations, respectively, limiting its practical usage. Even though, this type of gasifier offers a quick response against load, better producer gas flexibility, easy and quick start-up, its incapability to handle biomass with high moisture (<20%), ash content (<1%), volatiles, and lower particle size further limits its application (Sansaniwal et al. 2017). Also, product gases leaving the gasifier at 900–1250 °C lower the energy efficiency by up to 75%, along with slagging issues (Basu 2010; Bermudez and Fidalgo 2016).

9.4.2 Fluidized Bed Gasifier

A fluid-like behavior of both the bed material and biomass particle is experienced in this gasifier. The bed made up of fine particles like silica sand, dolomite, etc. acts as fluid suspension when acted up by the fluidizing media like air, steam, oxygen, or their combination. The entire bed is supported over a plate provided with a large number of holes, known as distributed plate, through which a uniform distribution of gasifying medium at sufficient velocity is provided. In these gasifiers, fluidizing medium is maintained at a minimum velocity, minimum fluidization velocity, to ensure proper fluidization of bed material. The random movement of bed particles in a fluidized bed promotes proper mixing, thereby achieving better heat and mass

transfer (Sansaniwal et al. 2017). The higher heat and mass transfer associated with fluidized beds ensure better temperature uniformity, syngas quality, cold gas efficiency, and fuel flexibility, making it the most promising reactor (Ren et al. 2019).

Moreover, the vigorous mixing in the bed leads to non-distinguishable reaction zones with overlapping gasification stages leading to homogeneous product gas. Furthermore, around 95% carbon conversion efficiency and excellent scaling up suitability enhance the adequacy of fluidized bed gasifiers. Fluidized bed gasifiers of capacity ranging from 5 to 50 MW are widely used in small- to medium-scale applications (Devi et al. 2020). The main two variants of fluidized bed gasifiers are as follows.

9.4.2.1 Bubbling Fluidized Bed Gasifier

The schematic of the bubbling fluidized bed gasifier is depicted in Fig. 9.4. This is the most extensively used variant owing to its flexibility to adapt parameters like capacity, temperature, feedstock, etc. The fluidized medium like oxygen, air, or steam is forced through the plenum over the inert bed made up of fine sand or alumina particles with minimum fluidization velocity to counterbalance the weight of the solid particles (Ren et al. 2019; Devi et al. 2020). On further increasing the gas velocity, the bed material got suspended in upward flowing gas forming the fluidized

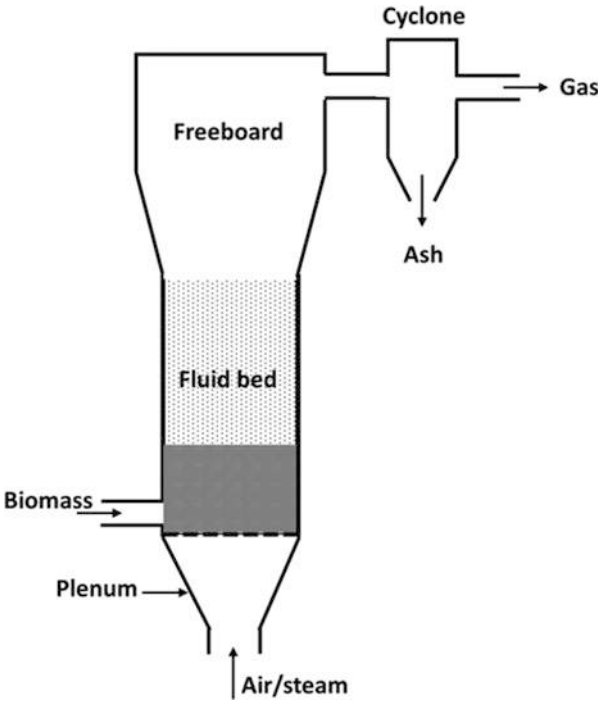

Fig. 9.4 Bubbling fluidized gasifier

bed. At this point, the weight of the bed particles is counterbalanced by the drag force between solid particles and fluid, resulting in a pressure drop. On further increasing the velocity, the bubble formation starts in small sizes, grows bigger, and moves through the bed. These bubbles set the particles in motion by withdrawing particles from their surrounding as they move up. While reaching the free surface of the bed, these bubbles are subjected to bursting, letting the gas free board. The rapid, random, and repeated movement of solid particles maintain a uniform temperature distribution with minimum particles escaping the gasifier along with the gas. A cyclone separates solid particles from the producer gas such as escaped bed materials, char, and ash, thereby providing particle-free producer gas. (Sansaniwal et al. 2017; Loha et al. 2018).

Usually, for bubbling fluidized bed gasifiers, fluidization velocity is maintained in the range of 1–3 m/s to guarantee the suspension of solid particles. In a bubbling fluidized bed, maximum syngas content is achieved above 1200 °C, and maximum H_2 or methane is obtained below 1000 °C (Devi et al. 2020) and can process medium-sized feedstock (Silva et al. 2019). High heat exchange and reaction rates due to compact construction, high ash content with a low melting point, proper adaptation to moisture and ash content, and other fuel characteristics are the significant merits of using the same. The main setbacks of the gasifier include melting of ash, bed agglomeration, poor solid conversion due to back mixing, high tar, and dust content, and high temperature of producer gas. Nevertheless, these reactors are apt for medium capacity applications greater than 5 MW (Surisetty et al. 2012; Bermudez and Fidalgo 2016).

9.4.2.2 Circulating Fluidized Bed Gasifier

This reactor mainly comprises a riser, cyclone, and a return leg as in Fig. 9.5. The biomass introduced through the side of the reactor mixes with the hotbed. Here, the fluidization velocity is well above that of the bubbling bed beyond the bubbling bed in the range of 5–10 m/s (Ren et al. 2019). In this velocity range, the solid particles are spread over the entire riser region, the majority being entrapped by the gas (Basu 2010). The raw producer gas exiting the reactor is supplied to a cyclone where the gas and solid particles are segregated. These segregated char and bed material is fed back to the reactor bed through the return leg, thus forming a loop of circulating solids (Surisetty et al. 2012). This, a solid circulation loop with enhanced gas velocity is established by maintaining suitable pressure differences across the reactor. Thus, enhanced heat and mass transfer characteristics, compared to that of the bubbling type, can be achieved by increasing the residence time through particle recycling (Loha et al. 2018; Ren et al. 2019). Besides high cost and erosion, these reactors are well suited for scale-up applications up to 100 MW.

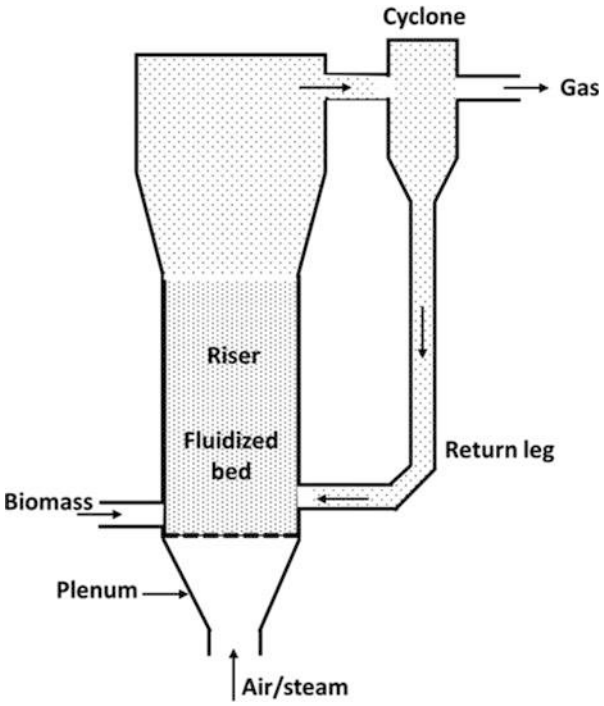

Fig. 9.5 Circulating fluidized gasifier

9.4.3 Entrained Flow Gasifier

This gasifier establishes a co-current flow of very fine biomass particles and gasifying agents introduced at the top of the reactor as in Fig. 9.6. Here, gasifying agents like oxygen, air, or their combinations are used at velocities higher than 10 m/s. The gasifying agents entrap fine biomass particles along with the flow to form a thick cloud within the gasifier. The very low retention of biomass inside the gasifier demands high operating temperatures in the range of 1200–1500 °C. The extreme turbulent flow thus existing in the reactor causes the rapid conversion of fuel and high throughput (Loha et al. 2018). The higher operating temperature provides high-quality producer gas with low tar content (Silva et al. 2019). Even though entrained flow gasifier has fuel flexibility, the oxygen consumption is reduced by biomass with less ash and moisture content. Apart from the cost incurred in sizing the biomass, these reactors are preferred for large-scale operations with applicability greater than 100 MW (Ruiz et al. 2013).

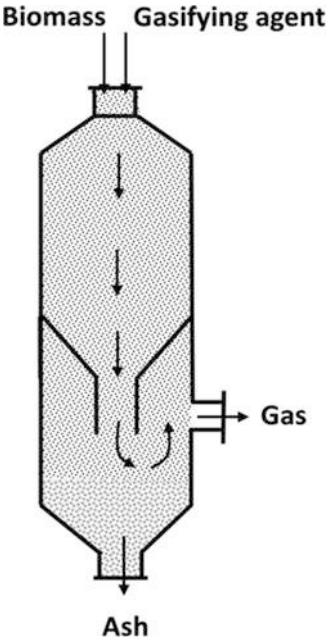

Fig. 9.6 Entrained flow gasifier

9.4.4 Plasma Gasifier

This gasifier is mainly designed for municipal solid waste management. In a plasma gasifier, the crucial part plasma torch is placed towards the bottom of the gasifier as in Fig. 9.7. The biomass is introduced to the plasma zone from the top of the gasifier and reacts with the ionized gasifying agent introduced next to the plasma torch. An electric discharge is experienced when the temperature within the gasifier reaches 10,000 °C, causing degradation of matter at an atomic level. Thus, the fuel breaks down into its constituent elements converting all organic ingredients into gaseous fuel (Ren et al. 2019). The resulting gas is taken out from the top of the gasifier and the residue is melted and is removed as slag (Loha et al. 2018).

Any toxic or poisonous solid waste can be gasified through a plasma gasifier without any prior segregation or treatment. Using these gasifiers, waste reduction can be attained on large scale with leach-resistant inert slag as the by-product, used as a building material. But the overall energy production from plasma gasification is low even at high-energy consumption and capital cost. Moreover, the rapid reaction with fewer syngas contaminants makes it suitable for large-scale applications like municipal or hospital waste gasification (Munir et al. 2019). A comparison between various characteristics of gasifiers is as in Table 9.5.

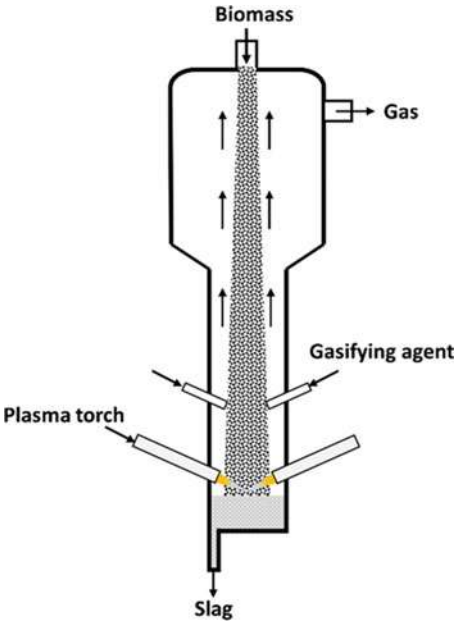

Fig. 9.7 Plasma gasifier

Table 9.5 Comparison of gasifiers (Moustakas et al. 2005; Fabry et al. 2013; Gabbar et al. 2020; Kuo et al. 2020)

Gasifier	Feed size (mm)	Temperature (°C)	Cold gas efficiency (%)	Tar (g/N m^3)	Output (MW)	Capacity
Fixed bed or moving bed gasifiers						
Updraft	5–100	200–700	80	30–150	1–10	Small
Downdraft	20–100	700	80	0.015–3	0.01–1	Small
Crossdraft	5–20	1250	80	0.01–0.1	< 10	Medium
Fluidized bed gasifiers						
Bubbling	10	800–900	89	1–3	< 25	Medium
Circulating	0.18–0.23	800–900	89	< 5	60	Medium
Entrained and Plasma gasifiers						
Entrained	< 0.075	1200–1500	80	~ 0	> 100	Large
Plasma	25–200	1200–5000	38.3–47	<0.01	12–100	Large

9.5 Tar

Tar is a dark, viscous, and sticky liquid with a boiling point of 150 °C, formed by condensation of volatiles at the low-temperature zones of the gasification system. It is the most undesirable product of gasification as it leads to coking, clogging, and corrosion in end-use devices like turbines, engines, heat exchangers, pipelines, and other contact surfaces when condensed. It also reduces the syngas heating value and gasification efficiency, thereby increasing maintenance and process cost (Rakesh and

Dasappa 2018). In actual practice, the constituents of tar are enormously diverse in the chemical constitution and are generally differentiated as a group of organic formulations with a molecular weight greater than that of C_6H_6 (Maniatis and Beenackers 2000).

9.5.1 Composition

The typical composition of tar from biomass is depicted in Fig. 9.8.

Tar can be classified into four tar groups based on the presence of aromatic rings in the constituents. The details of different forms of tar for a residence time of 300 ms are as follows: (Evans and Milne 1997)

- Primary group: consists of cellulose, hemicellulose, and lignin-derived compounds comprise syringols, acids, phenols, aldehydes, sugars, etc. They are initiated at 500 °C and gradually decomposed mainly to secondary tar between 500 and 800 °C.
- Secondary group: Generated between 500 and 800 °C by the decomposition of primary tar to phenols and olefins. Beyond 800 °C they decompose to tertiary tar.
- Tertiary group: More than 90% of tertiary tar is observed in the temperature range of 500–800 °C. Aromatics derived from methyl groups such as methyl acenaphthylene along with indene, methylnaphthalene, and toluene form this group.
- Condensed tertiary: They are poly-cyclic aromatic hydrocarbons (PAHs) lacking substituents for hydrogen-like C_6H_6, $C_{10}H_8$, $C_{12}H_8$, $C_{14}H_{10}$, $C_{16}H_{10}$, etc.

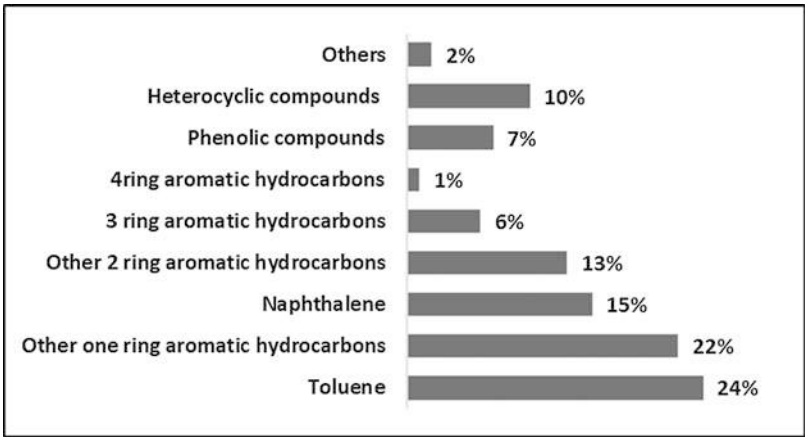

Fig. 9.8 Composition of tar (Devi et al. 2005a; Anis and Zainal 2011; Guan et al. 2016)

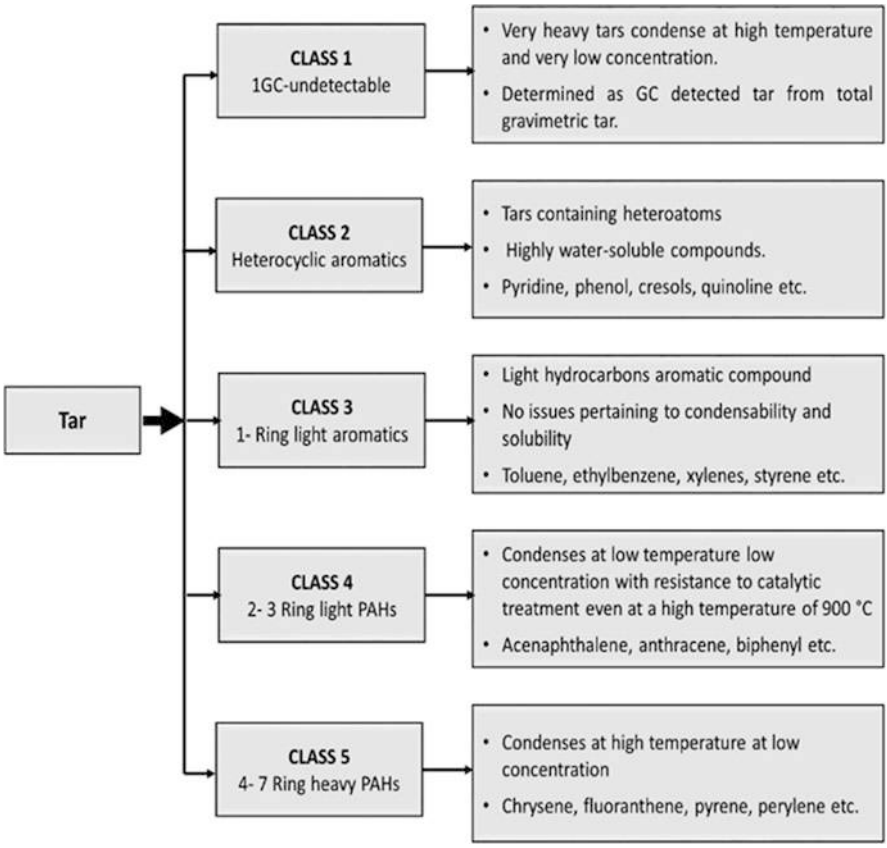

Fig. 9.9 Classification of tar is based on condensability and solubility (Devi et al. 2005c; Anis and Zainal 2011)

Information regarding condensability and solubility of tar constituents is very critical as it predicts the tar fouling and tar removal strategies. Based on this, tar can be classified into five distinct classes, as given in Fig. 9.9.

9.5.2 Effects of Operating Conditions on Tar Yield

Apart from the reactor design, operating conditions such as temperature, pressure, gasifying agent, and residence time have a significant role in the yield and conversion of tar during biomass gasification. Temperature is the most vital parameter that determines the yield and composition of tar by driving tar decomposition reactions. In general, an overall quantity of tar and char decreases with an increase in temperature. The yield of oxygen comprising tar species, such as C_6H_6O (phenol), C_7H_8O

(cresol), and C_8H_6O (benzofuran) decreases with temperature, mainly below 800 °C. The formation of aromatic formulations without substituents such as benzene and naphthalene are promoted with temperature. Moreover, the production of substituted 1 and 2 and 3 and 4 ring aromatics decreases and increases, respectively, with temperature (Devi et al. 2003). The total reduction in tar yield with complete eradication of phenols accompanied by a decrease in poly-cyclic aromatic hydrocarbons is reported with an increase in pressure between 8 and 21.4 bar (Knight 2000). The choice of gasifying agent significantly influences the tar formation. It is reported that tar yield is lowest for air and highest for steam with respective values of 2–20 and 30–80 g/Nm^3 (Basu 2010). Injection of steam reduces the process temperature whereas the addition of air or oxygen can increase process temperature and reduce tar formation at the expense of heating value, by promoting the oxidation of volatiles. A 50% tar reduction was reported by Narváez et al. (1996) at 800 °C when ER is increased from 0.2 to 0.45. Apart from all the operating conditions discussed, residence time has a consequential role in tar yield. A tar reduction of 75% is achieved by varying residence time from 1 to 5 s due to the increase in the duration of tar exposure with a gasifying agent.

9.5.3 Application Limits

As tar can cause operational issues, the quantity of tar content in producer gas determines the potential end-users of the gas. The acceptable tar content in producer gas for different applications is depicted in Table 9.6.

From Sect. 9.3, it is clear that the tar yield with different gasification agents is appreciably high compared to the permissible limit of tar content for different applications. Furthermore, tar constituents are stable carcinogens with adverse environmental impacts (Guan et al. 2016). In view of the same, it is inevitable to reduce the tar content to suit the gas for different applications and to safeguard the health and environment.

Table 9.6 The permissible limit of tar content in producer gas for different applications (Fabry et al. 2013; Valderrama Rios et al. 2018)

Application	Tar content (mg/Nm^3)
Direct combustion	No limit
Compressors	50–500
I C engines	Less than 100
Gas turbine	Less than 5
Fuel cells	Less than 1
Methanol synthesis	Less than 0.1
Fischer–Tropsch synthesis	Less than 0.1

9.6 Effect of Key Operating Parameters on Gasification

The product distribution and conversion efficiency of biomass gasification mainly depend on the operating parameters and gasifier design. This section is dedicated to the various key operating parameters and their influence on biomass gasification.

9.6.1 Biomass/Feedstock

The primary factor that influences the gasification performance and product gas composition is the type of biomass used for gasification. A preliminary comparison of the gasification performance of different feedstock can be done from their structural, chemical, and gross constitution. Structurally, lignocellulosic biomass is composed of lignin (20–30%), cellulose (33–51%), and hemicellulose (19–34%) (Van Maris et al. 2006). Biomass with higher hemicellulose and cellulose contributes to more gas yield whereas, lignin, being a cementing agent, is considered the possible source of tar (Valderrama Rios et al. 2018). So, biomass with a higher value for the ratio of the sum of cellulose and hemicellulose to lignin is preferred for biomass gasification. The composition of biomass in weight percentage of elements such as C, H, O, N, and S plus moisture and ash content can be determined by performing ultimate analysis. The elemental composition thus obtained can be used to estimate the stoichiometric air requirement and higher heating value of the feedstock (Rupesh et al. 2020). Feedstock with higher oxygen content is reported to yield syngas with lower calorific values (Rupesh et al. 2015). On the other hand, the gross composition of biomass in terms of fixed carbon (FC), volatile matter (VM), moisture (M), and ash (ASH) obtained from the proximate analysis can also serve as an indication of gasification performance. Feedstock with higher moisture content demands additional heat to vaporize the moisture, leading to lower operating temperatures. Even though biomass with the moisture content of less than 35% is acceptable, that in 10–15% is the most suitable (Kaushal and Tyagi 2012). On the other hand, biomass with higher ash content reduces gas yield and demands more ash handling.

Feedstock particle size and density also significantly influence the gasification process. Smaller particle size possesses a larger surface area, which in turn leads to enhanced heat and mass transfer. Thus, biomass with smaller particle size leads to better carbon conversion, H_2 yield, and lower tar content in the producer gas at the expense of energy spend for sizing the biomass (for particle size <1 mm) (De Lasa et al. 2011; Chan et al. 2019). Moreover, biomass with lower density exhibit invariable temperature throughout the biomass particle leading to homogenous gas generation. Whereas, the existence of temperature gradient in denser feedstock leads to non-homogeneous gas yield (Kirubakaran et al. 2009). Thus, it is clear that the physical and chemical properties of biomass have a predominant role in gasification

and hence feedstock characterization is the most important prerequisite for any gasification study.

9.6.2 Temperature

Being an endothermic process, temperature has a vital role in determining the char conversion, tar content, gas yield, and producer gas constituents. Increasing temperature up to 600 °C favors tar yield, due to enhanced devolatilization of biomass, and decreases thereafter (Fagbemi et al. 2001). When the temperature is increased from 700 to 850 °C, tar yield is found to be decreased (Narváez et al. 1996). This is due to the combined influence of enhanced tar cracking and tar reformation reactions at higher temperatures. Consequently, H_2 and CO concentrations were increased and that of CO_2 was decreased. Similarly, an increase in temperature from 700 to 900 °C increases carbon conversion and gas yield, respectively, by 18.5% and 76.9%, with a corresponding reduction of 7.3% in gas calorific value (Lv et al. 2004). The higher char conversion at higher temperatures is mainly promoted by endothermic Boudouard and char steam reforming reactions. The hydrogen concentration in producer gas increased with temperature to a maximum of 45% at 950 °C and decreased thereafter. The decrease in H_2 concentration beyond 950 °C (at higher temperatures) is attributed to the promotion of WGS reaction in the reverse direction justified by Le Chatelier's principle (Gai et al. 2016; Niu et al. 2017). The influence of temperature on calorific value depends on whether the process is autothermal or allothermal. In the former, the calorific value degrades with temperature as higher temperature demands enhanced combustion, leading to a reduction in H_2 and CO, and consequent increase in CO_2 and N_2 (Wu et al. 2009). On the other side, being energy-intensive, indirect heating or allothermal operation can alleviate the problem economically if heat is supplied from an external renewable source. Apart from the merits of high-temperature gasification, elevated temperature promotes the melting of biomass ash with alkali metals and leads to agglomeration, sintering, corrosion, and clinkering (Wang et al. 2008). Also, higher operating temperature demands higher equipment costs and advanced operational strategies.

9.6.3 Pressure

Based on the operating pressure, gasifiers can be distinguished as atmospheric and pressurized gasifiers. Gasification at elevated pressure can decrease the yield of char by promoting char conversion. Mixed observations were made by researchers regarding the effect of pressure on tar yield. A decrease in syngas tar content in pressurized biomass gasification was reported by Wang et al. (2008). On the contrary, an experimental investigation of fluidized bed gasifiers by Mayerhofer et al. (2012) reported an increase in tar content when pressure is increased in the

range of 0.1–0.25 MPa. Higher second law efficiencies of pressurized gasification with feeble variation in syngas calorific value and yield were identified by Motta et al. (2018). However, pressurized gasification is preferred for downstream applications that demand high-pressure syngas such as engines and turbines. An increase in overall turbine efficiency by 52.17% is observed when gasification pressure is increased from 1.013 to 4 bar (Fryda et al. 2008). However, pressurized reactors are preferred only to industrial-scale gasification units owing to their operational complexities and cost.

9.6.4 Residence Time

The quantity and configuration of tar generated during gasification are significantly affected by residence time, the mean time for which feedstock particles are retained inside the gasifier. An increase in residence time reduces oxygen-containing compounds as well as compounds with one and two aromatic rings, with a corresponding increase in compounds with three and four aromatic rings. An increase in residence time increases the extent of completion of all reactions, especially for heterogeneous reactions with a limited rate like char gasification (Martin-Sanchez et al. 2017). Thus, an increase in residence time promotes char conversion and improves syngas yield with lower tar content. However, the initial increase in the yield of gas constituents, such as H_2, CO, and CH_4, got stabilized beyond a specific residence time, with insignificant temporal change thereafter, as the reactions approach completion (Guan et al. 2016). Based on the design, fixed bed gasifiers can have more residence time whereas, in a fluidized bed reactor it is decided by the minimum fluidization velocity (Agu et al. 2019). A smaller biomass particle size is preferred for reactors such as entrained gasifiers with a lesser residence time of the order of 1–2 s to ensure the entire gasification within the stipulated residence (Zou et al. 2018). So, an appropriate residence time should be selected to balance the gas yield with gasification efficiency and downstream application requirements (Cao et al. 2020). This can be achieved with the proper design of reactors and forced draft units (blower or fan) associated with the same.

9.6.5 Equivalence Ratio

Equivalence ratio (ER) is the dimensionless representation of actual air supplied to gasification, compared to the corresponding stoichiometric requirement, given by Eq. (9.2). ER is the parameter that significantly influences the producer gas quality, especially in autothermal air and air-steam gasification. In an autothermal reactor, a supply of sufficient air is required to maintain the required gasifier temperature through partial oxidation of feedstock. Hence, smaller ER values lead to lower gasification temperature (Lv et al. 2004), leading to lower conversion efficiency

and gasification performance degradation, as discussed in Sect. 9.6.2. On the other hand, higher ER leads to the oxidation of char and tar to CO_2 instead of converting them to combustible constituents such as H_2, CO, CH_4, and other lighter hydrocarbons. Thus, the shifting of the process from gasification to complete combustion adversely affects the producer's gas calorific value (Mansaray et al. 1999). Moreover, the N_2 contained in the supplied air dilutes the producer gas, thereby, significantly reducing its energy content. So, for better gasification performance, an optimum ER ranging between 0.2 and 0.4 should be maintained based on the other performance parameters (Narváez et al. 1996). Lv et al. (2004) varied ER from 0.19 to 0.27 in air-steam biomass gasification to analyze the influence of ER on producer gas calorific value and yield. During the initial variation of ER from 0.19 to 0.23, the lower calorific value and gas yield is increased in the ranges of 8.82–8.84 MJ/Nm^3, and 2.13 to 2.37 Nm^3/kg, respectively. On the further increase of ER, from 0.23 to 0.27, calorific value and gas yield is decreased to 7.3 MJ/Nm^3 and 1.9 Nm^3/kg, respectively. This is attributed to enhanced oxidation of combustible constituents such as H_2, CO, and CH_4, and consequent increase in CO_2 composition.

Thus, an ER of 0.23 is proposed as the optimum value for biomass gasification. Similar studies were carried out by other researchers to explore the effect of ER on the gasification process. Parallel observations were made for syngas calorific value with tar reduction by Narváez et al. (1996) when ER is varied from 0.25 to 0.45. An ER of 0.26 is identified as the optimum value for maximizing hydrogen concentration in the syngas. In another comparable work, Skoulou et al. (2008) observed the shifting of CO enhancement to CO degradation when ER is increased from 0.2 to 0.4. An ER corresponding to 0.2 is revealed as the optimal value for maximum H_2 yield. So in biomass gasification, an ER has to be selected to convert feedstock to fuel gas rather than feed stock to flue gas.

9.6.6 Steam to Biomass Ratio

Steam to biomass ratio (SBR) (defined in Eq. 9.3) is the predominant parameter that governs H_2 yield in biomass gasification. It is the dimensionless representation of quantitative steam addition with respect to that of the biomass. H_2 and CO_2 concentration in producer gas increase with steam addition and that of CO and hydrocarbons decreases. The enhancement of H_2 and CO_2 concentration is mainly because of the WGS reaction and steam reforming of tar. With the availability of excess steam, the CO formed is in turn consumed in the water gas shift reaction for the further improvement of H_2 and CO_2 concentration (Lv et al. 2004). Apart from the advantage of higher H_2 concentration, an increase in incombustible CO_2 with steam addition leads to inferior gas quality and greenhouse gas emissions. Moreover, steam generation is energy-intensive (requires external heat addition) and steam injection reduces the gasification temperature, adversely affecting the gasification performance. Thus, an optimum SBR value should be maintained for higher

gasification efficiency with low carbon emission. Several studies reported the influence of SBR on producer gas attributes and tar yield. Tar content in the gas is reduced with an increase in steam addition (Mayerhofer et al. 2012; Song et al. 2012). A decrease in tar yield by 55.8% with lower aromaticity is reported when SBR is increased from 0.49 to 2.66 (Qin et al. 2015). Excessive SBR in the range of 0.35–4.04 reported a reduction in gas yield, calorific value, and carbon conversion due to a drop in gasification temperature (Lv et al. 2004). In steam biomass gasification, H_2 concentration is increased by 20% with an increase in SBR from 0 to 0.5, the variation in concentration being insignificant beyond an SBR of 0.5 (Cao et al. 2020). The addition of an oxidant in the form of air or oxygen with steam makes the process autothermal, with required gasification temperature, with a compromise in H_2 and CO concentration (Gil et al. 1997). The higher CO_2 concentration associated with steam addition can be reduced by using CO_2 sorbents like CaO. CaO sequestrates CO_2 to form solid $CaCO_3$ through a carbonation reaction, given by Eq. (9.5), thereby improving H_2 concentration in the producer gas (Rupesh et al. 2016).

$$CaO + CO_2 \rightarrow CaCO_3 \tag{9.5}$$

These operating parameters take a pivotal role in the design, development, and performance prediction of gasification systems. Further improvement in gas quality, yield, and H_2 concentration can be achieved by using different catalysts to enhance steam reforming of char and tar.

9.7 Need for Catalytic Biomass Gasification

As mentioned in Sect. 9.5, tar is an undesirable and unavoidable by-product of thermochemical biomass conversion, which significantly reduces the heating value of synthesis gas. It can be commercially converted to non-condensable and simpler combustible constituents by two methods (1) thermal cracking and (2) catalytic cracking. Thermal cracking is the splitting of complex hydrocarbon tar molecules into simpler ones by heating the product gas above 1100 °C. This additional heat required is either supplied from an external source or internally generated by providing more oxidizer, making the process energy-intensive and reducing the syngas quality, respectively. On the other hand, catalytic cracking (hot syngas cleaning) is the process of simultaneous cleaning and quality upgradation of raw gas in the presence of a catalyst (Basu 2010). The cleaning and quality improvement are mainly due to compositional upgradation of the gas achieved by reforming higher hydrocarbons (tar) to simpler gaseous constituents like hydrogen and carbon monoxide (Sutton et al. 2001). The presence of catalysts mainly reduces the activation energy of the tar cracking reactions, thereby achieving the reactions at lower temperatures. Generally, in catalytic cracking, the tar in the vapor phase is reformed to H_2 and CO using steam (Eq. 9.6), carbon dioxide (Eq. 9.7), or both. Catalysts can

also enable the reforming of methane in the gas, using steam (Eq. 9.8) and carbon dioxide (Eq. 9.9), especially when the desired product is syngas (a mixture of H_2 and CO).

Steam reforming (tar)

$$C_nH_m + nH_2O \rightarrow \left[n + \frac{m}{2}\right]H_2 + nCO \tag{9.6}$$

Dry reforming (tar)

$$C_nH_m + nCO_2 \rightarrow \frac{m}{2}H_2 + 2nCO \tag{9.7}$$

Steam reforming (methane)

$$CH_4 + H_2O \rightarrow CO + 3H_2 \tag{9.8}$$

Dry reforming (methane)

$$CH_4 + CO_2 \rightarrow 2CO + 2H_2 \tag{9.9}$$

Thus, catalytic gasification can upgrade the product gas quality and yield by transmuting undesirable tar to syngas constituents with lower energy expenditure. The different types of catalysts in biomass gasification and their characteristics will be discussed in Sect. 9.8.

9.8 Types of Catalysts

Catalysts of biomass gasification can be broadly classified based on their use and composition. Based on use, the catalyst can be identified as primary catalysts and downstream or secondary catalysts. Primary catalysts are either present in the reactor bed where biomass is added, or it is mixed with the biomass before feeding to the reactor, as in a fluidized bed gasifier. Thus, primary catalysts are mainly meant for promoting the gasification process and reducing tar. Whereas, secondary catalysts are mainly provided in a separate secondary reactor downstream of the reactor to promote reforming of tar and CH_4, thereby increasing syngas yield (Chan and Tanksale 2014a).

9.8.1 Natural Mineral Catalysts

Mineral catalysts are compounds that contain oxides of metals like aluminum, calcium, iron, magnesium, etc.

9.8.1.1 Dolomite

Dolomite ($MgCO_3 \cdot CaCO_3$) is a cheap and easily available compound that serves as both a primary and secondary catalyst. Constitutionally distinct dolomites can exist based on their geographical origin. The comparative analysis of the catalytic activity of different dolomite variants was conducted by Narváez et al. (1996) and found that Norte and Seville variants, respectively, have the highest and lowest tar conversion of 95% and 77%. Higher syngas yield was obtained from variants with larger Fe_2O_3 content and pore size. This is due to the promotion of WGS reaction and tar cracking by the Fe content, and larger surface area contributed by the porous morphology of the catalyst. The porosity and reactivity of dolomite can be further improved by calcination above 900 °C, as the oxides formed can reduce tar by enhancing steam and dry CO_2 reforming reactions. The content of CaO and MgO in dolomite is an indirect measure of its tar cracking efficiency, the presence of MgO also retards the catalyst deactivation due to coking. The H_2 in the syngas is improved to 20–25% when dolomite is used in bed (Miyazawa et al. 2006). Calcined dolomite fails to reform methane even though it can achieve tar conversion completely. Apart from the tar reforming capability, fragmentation due to thermal shock, attrition, and elutriation limit the use of dolomite.

9.8.1.2 Limonite

It is a mineral in powdered form rich in FeOOH. The iron content in limonite (Fe_2O_3) improves its acceptance as a catalyst in gasification. On increasing the temperature to 673 K, raw limonite is reduced to Fe_2O_3, which exhibits high activity towards tar reforming (Li et al. 2007). This reduced limonite generates a maximum gas yield of 46.9 mmol/g. On further temperature rise, at 700 °C, H_2 rich gas yield of 74.1 mmol/g can be attained when steam is supplied. On the other hand, calcinated limonites under a steam environment, hinder carbon deposition and improve syngas production (Ren et al. 2019). But impurities in the form of talc mineral, dispersed in the limonite in powder form, limit its usage.

9.8.1.3 Olivine

Olivine ($(Mg, Fe)_2SiO_4$) is a non-porous silicate mineral containing magnesium and iron (Rapagnâ 2000; Corella et al. 2004; Devi et al. 2005a). Its outstanding

mechanical strength, along with its non-toxic and attrition resistance, make it an excellent choice for bed material in fluidized bed gasifiers (Rapagnâ 2000; Pfeifer and Hofbauer 2008; Fredriksson et al. 2013). Apart from the limited tar reforming due to the non-porous structure, the presence of Fe and magnesite content imparts moderate catalytic activity to olivine (Devi et al. 2005a; Soomro et al. 2018). For an increase of temperature from 700 °C to 820 °C, the tar content is reduced approximately by a factor of 10 accompanied by a 1.5 times increase in the dry gas yield. Even though pure olivine is insensitive to methane reforming, olivine combined with nickel activates the same (Devi et al. 2005b). When used as in-bed catalysts, Ni-olivine combination can exhibit a tar reduction of 75% at 850 °C (Pfeifer and Hofbauer 2008). Deactivation of Ni-olivine catalyst at a high weight hourly space velocity of benzene can be mitigated by combining nickel with olivine powder and calcium aluminate cement to form nickel-modified olivine (Ni/MO) (Yang et al. 2019).

9.8.1.4 Calcite and Magnesite

Calcite ($CaCO_3$) and magnesite ($MgCO_3$) are naturally existing carbonates of calcium and magnesium. After calcination, calcined calcite (CaO) and magnesite (MgO) can be used as the catalyst for tar reduction. Delgado et al. (1997) made a comparative study of calcined dolomite, calcined magnesite, and calcite as the downstream catalyst for reforming raw producer gas from a fluidized bed gasifier. The catalytic reactivity, tar reduction capability, and gas yield were found to increase, respectively, for calcined dolomite, calcined magnesite, and calcined calcite. In steam naphthalene gasification, 75% carbon conversion is achieved with a mixture of CaO and MgO (in the ratio of 1:9), which is 25% and 17% higher than that with pure MgO and CaO, respectively (Alarcon et al. 2021).

9.8.2 Alkali Metal Catalysts

Alkali metals (Li, Na, K, Rb, etc.), alkali earth metals (Be, Mg, Ca, Ba, etc.), and alkali metal carbonates (K_2CO_3 and Na_2CO_3) together constitute the alkali metal catalysts (Dayton 2002). They exhibit dual catalytic function thereby capable of enhancing syngas yield and tar reforming. Alkali metal catalysts are considered to be the active catalytic components that promote char gasification, steam-tar reforming, hydrocarbon reforming, and water gas shift reactions; however, alkali earth metal has a predominant role in enhancing WGS reaction (Long et al. 2012). Bio-char that contains alkali earth metals formed during the steam-tar reforming can be used as a catalyst for ash treatment and value-added syngas production (Ren et al. 2019). The rate of biomass char gasification can be further increased by combining alkali carbonates with active metal-based catalysts (Baker and Mudge 1984). Complete elimination of tar and a higher yield of H_2-rich gas ($H_2/CO > 10$) can be obtained

from biomasses like wood, sawdust, castor stalk, etc. with K_2CO_3 supported Al_2O_3 or SiO_2-Al_2O_3 catalysts (Mandal et al. 2019). The usage of alkali metals is constrained by particle agglomeration due to quick evaporation at elevated temperatures and catalytic activity loss. Moreover, the recovery difficulties and higher ash content make alkali metal catalysts less desirable for commercial use (Dayton 2002).

9.8.3 Metal-Based Catalysts

9.8.3.1 Ni-Based Catalysts

The higher effectiveness of nickel in tar reforming and quality improvement of syngas through methane reforming, water gas shift, and reversing ammonia makes it the most significant catalyst for biomass gasification (Guan et al. 2016). The capability of a nickel catalyst to reform methane makes it superior to naturally occurring mineral catalysts. Nickel catalysts can find its application as the primary catalyst in the gasifiers (in situ) and as a secondary catalyst in the downstream reactor (in post gasification). As a primary catalyst, nickel can perform both tar reforming and syngas cleaning, eliminating downstream reactor and extra heating (Chan and Tanksale 2014a). But in this case, the catalytic activity cannot be prolonged as a result of deactivation due to carbon formation, sulfur poisoning, and sintering (Baker and Mudge 1984). This can be addressed by using the same as a secondary catalyst in the downstream rector, thereby extending the spell of catalytic activity by minimizing coke formation. Further coke formation can be minimized by using catalytic support. Better dispersion of nickel with other supporting compounds enhances its catalytic activity. Generally, Ni is supported by Al_2O_3, olivine, ZrO_2, TiO_2, CeO_2, MgO, SiO, MgO, CaO, K_2O, and Fe_2O_3 to reduce carbon formation and these supported Ni catalysts are added with second metal additives for further improvement in stability and activity (Świerczyński et al. 2007).

The coke formation reactivity of steam reforming naphtha catalysts, UCI G90-C and ICI 46-1, with 15% Ni on alumina support, towards tar, is in the order benzene > toluene > anthracene > pyrene > naphthalene (Coll et al. 2001). For G-90B, a ceramic-supported 11% nickel catalyst, the methane reformation is tremendous methane above 700 °C. Moreover, at 800 °C, 86% of the benzene and 97% of the naphthalene reformation is achieved (Kinoshita et al. 1995).

9.8.3.2 Fe-Based Catalysts

Iron species show excellent activity towards tar conversion (Torres et al. 2007). Metallic iron (Fe^o) is the most active iron species metallic iron, FeO, Fe_2O_3, and Fe_3O_4 in toluene and benzene hydrogenation due to its C-C bond dissociation capability (Nordgreen et al. 2006). Whereas, oxides of Fe^3 and Fe^2 species exhibit the most catalytic reactivity in the steam phenol reforming (Richardson et al. 2012).

In addition to tar conversion, the reduced form of Fe_3O_4 further enhances the significance of Fe-based catalyst in biomass gasification by promoting WGS reactivity between 700 and 900 °C, (Newsome 1980). The performance similarity exhibited by 20 wt.% Fe with olivine to that with 3.9 wt.% Ni with olivine facilitates the possibility of replacing the usage of Ni with less toxic Fe in fluidized beds. Even though the Fe-olivine catalyst can prevent coking, it leads to a loss of about 5 wt.% of iron due to a drop in its reducible form, limiting its activity (Virginie et al. 2012). NH_3 decomposition is an added feature of Fe-based catalysts. Fe catalyst can accomplish a maximum NH_3 decomposition of 87% fixed bed secondary reactor at 900 °C (Hongrapipat et al. 2012).

9.8.3.3 Metal and Metal Oxide Catalysts

The catalytic activity of Ni-based catalyst can be improved by assisting it with noble metals such as Pt, Pd, Mn, Rh, and Ru, metal oxides (CaO, Al_2O_3, MgO, La_2O_3, CeO_2, TiO_2, etc.) or bimetal oxides (Ru/CeO_2, n$(CeZr)_xO_2$, etc.) (Ren et al. 2019). Compared to Rh, Ru, and Pd, Pt exhibits superior methane reforming in steam gasification due to its better alloying with Ni (Nishikawa et al. 2008). Noble metal-supported Ni inhibits sintering and maintains steady catalytic activity while alloying of Fe with Ni suppresses coke deposition. In general, Ni supported with metal oxides promotes steam reforming of tar, lifetime and overall catalytic activity conversion up to 99.95% and Al_2O_3-supported Ni/MgO catalyst can achieve a tar conversation of 99.95% in the range 720–760 °C (Ren et al. 2019, 2020). The activity order of metal oxide supports like MgO, CeO_2, ZrO_2, etc. is $Ni/CeO_2 - ZrO_2 > Ni/\gamma\text{-}Al_2O_3 > Ni/CeO_2 > Ni/ZrO_2$ (Ren et al. 2020). Further improvement in tar reforming can be attained by combining support material with another metal. A higher benzene conversion is attained at 850 °C, using bimetallic catalyst Ni/ZrO_2 with La, Sr, and Fe which in turn improves tar conversion. (Keller et al. 2016). Furthermore, a bimetallic $MgAl_2O_4$-supported IrNi catalyst exhibits superior stability during steam reforming of methane and tar due to a substantial reduction in coking and sintering (Dagle et al. 2016).

9.8.4 Zeolites

Zeolite, a crystalline aluminosilicate material occupying ions and water molecules within the cavities in its structural framework, comes under the category of fluid catalytic cracking catalysts, which have the potential to eliminate tar. Zeolites are acidic in nature with better hydro-thermal stability, good nitrogen, and sulfur resistance, low cost, and easy regeneration ability (Buchireddy et al. 2010). Zeolites usually find their application as supports to Ni-based or other metal-based catalysts in tar reforming. Among the different variants of zeolites like Zeolite Y, Zβ, ZSM5, ZY-5.2, the variants ZY-5.2 and Zeolite Y show better naphthalene conversion due

to their acidic nature and larger pore size, respectively. But, nickel-supported zeolite (Ni/ZY-80) can attain a naphthalene conversion rate of 99% with prolonged activity (Kathiraser et al. 2016). Moreover, the addition of Fe or Ni in mesoporous SBA-15 catalyst improves the formation rates of H_2, CO, CO_2, and CH_4 whereas the addition of Ni in mesoporous MCM-41 can achieve the lowest coke deposit of 7 wt% of the catalyst, thereby, mitigating the major limitation of zeolite (Wu and Williams 2011a).

9.8.5 Activated Char Catalysts

Bio-char, a gasification residue concentrated with metals like K and Ca contributed from the base biomass feedstock, can act as a catalyst. These alkali or alkaline earth metals can promote WGS reaction and enhance tar reforming through H_2O absorption (Hu et al. 2015). Moreover, tar reforming contributed by some functional groups containing oxygen in the bio-char makes it a suitable catalyst for biomass gasification (Shen et al. 2015). Furthermore, the tar removal efficacy of bio-char can be improved up to 97% by incorporating it with Ni (Wang 2013). A better gas yield of nearly 90 mg per g of biomass and carbon balance of 98% is attained using Ni dispersed brown coal char at a lower temperature range of 400–550 °C (Li et al. 2010). Additionally, a more potent way of acquiring high-quality syngas is by combining char with Ni-Co catalyst, Ni-Co altering the quality and rate of syngas. (Yang et al. 2019).

9.9 Comparison of Different Catalysts

This segment deals with the comparison of different catalysts discussed in Sect. 9.8.

9.9.1 General Comparison

Initially, a general comparison of natural mineral, alkali metal-based, metal-based, zeolite, and char catalysts are incorporated, as in Table 9.7.

9.9.2 Comparison of Gas Yield and Tar Conversion

The tar reforming capability of a catalyst improves the yield and quality of syngas. The following section depicts the comparison of gas yield and tar conversion from different catalysts.

Table 9.7 General comparison of various catalysts

Classification	Catalyst	Merits	Demerits	References
Natural minerals	Dolomite	• Cheap, • Higher tar conversion rate	• No methane reformation • Fragmentation, attrition, and elutriation	(Gil et al. 1997; Zhou et al. 2009; Ren et al. 2019)
	Olivine	• Mechanical strength • Non-toxic • Attrition resistance	• Limited tar reformation • Insensitive to methane reforming	(Rapagnâ 2000; Devi et al. 2005a; Pfeifer and Hofbauer 2008)
	Limonite	• High tar reforming • Inhibit coke formation	• Presence of impurities	(Li et al. 2007, 2010)
Alkali metal-based	Alkali metal	• Enhances water gas shift reaction • Higher yield of H_2 rich gas	• Particle agglomeration • Higher ash content	(Dayton 2002; Long et al. 2012)
Metal-based	Ni-based	• Higher tar reforming • Ability to reform methane • Enhances water gas shift reaction • Can be used as both primary and secondary catalyst	• Coking, sulfur poisoning, sintering	(Baker and Mudge 1984; Guan et al. 2016)
	Metal and Metal oxide	• Ability to reform methane • Higher benzene conversion • Suppress coking and sintering	• Expensive • Deactivation due to sulfur poisoning	(Nishikawa et al. 2008; Ren et al. 2019)
Zeolite	Zeolite	• Better hydrothermal stability • Easy regeneration	• Intricacy in synthesis	(Buchireddy et al. 2010; Ren et al. 2019)
Char	Bio-char	• Ample availability • Obtained as a by-product of gasification • Promotes water gas shift reaction	• Susceptible to gasification	(Li et al. 2010; Yao et al. 2016)

Table 9.8 Comparison of natural mineral-based catalysts on gas yield

Catalyst	Reaction temperature (°C)	Gas yield (%)	References
Pt -Ni/dolomite	800	79.19	Chaiprasert and Vitidsant (2009)
Fe-Ni/dolomite	800	78.12	
Co-Ni/dolomite	800	49.53	
Ni/dolomite	770	69.1	Sato and Fujimoto (2007)
Natural dolomite	800	44.67	Islam (2020)
Calcined dolomite	800	61.33	
Natural olivine	800	36.60	
Calcined olivine	800	46.67	

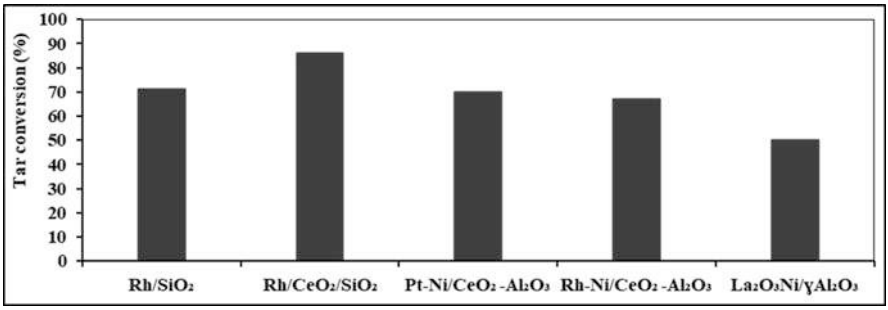

Fig. 9.10 Comparison of noble metal-based catalysts on tar conversion (Tomishige et al. 2004)

9.9.2.1 Natural Mineral-Based Catalysts

The comparison of natural mineral catalysts on gas yield is given in Table 9.8. It is clear that the tar reforming capability of both natural dolomite and natural olivine is improved when calcinated. Consequently, the gas yield is increased by 37.2% and 27.5%, respectively, for calcined dolomite and calcined olivine when compared with their natural form. Thus, the acceptance of calcined dolomite interns of gas yield is 31.4% more than that of calcined olivine. Moreover, the tar reforming as well as gas yield of dolomite can be augmented by combining it with metals like Ni, Fe, Pt, Co, etc. The addition of Fe and Pt with Ni/dolomite contributes comparable improvement in gas yield, with the former prompting coking and the latter being expensive.

9.9.2.2 Metal-Based Catalysts

The comparison of noble metal-based catalysts on tar conversion is represented in Fig. 9.10 CeO_2 has a vital role in enhancing tar conversion in a noble metal-based catalyst. Addition of CeO_2 in Rh/SiO_2 increased tar conversion by 21.12%. The tar

Table 9.9 Comparison of nickel-based catalysts on gas yield

Catalyst	Reaction temperature (°C)	Gas yield (%)	References
Ni/MgO	800	81	Xu et al. (2010)
NiO-MgO	800	47	Wang (2013)
NiO/MgO	850	51	Dong et al. (2017)
Ni-Mg-Al	800	52.9	Nahil et al. (2013)
Ni-Mg-Al-CaO	800	54	Nahil et al. (2013)
Ni-Mg-Al-Quartz	800	48.6	Nahil et al. (2013)
Ni/Al catalysts	650	77	Bimbela et al. (2013)
Ni/Al_2O_3	850	53	Wu and Williams (2011a)
Ni ZnAlOx	800	48	Ren et al. (2019)
$Co\text{-}Ni/Al_2O_3$	650	68	Nishikawa et al. (2008)
$Ni–Ce/Al_2O_3$	900	71	Fu et al. (2014)
$Ni/CeO_2\text{-}Al_2O_3$	650	82	Efika et al. (2012)
$Ni/CeO_2–ZrO_2$	800	70	Feng et al. (2017)
$Pt\text{-}Ni/CeO_2\text{-}Al_2O_3$	650	82	Efika et al. (2012)
$Rh\text{-}Ni/CeO_2\text{-}Al_2O_3$	650	83	Efika et al. (2012)
$Ni/La_2O_3\text{-}\alpha Al_2O_3$	700	96	Remiro et al. (2013)
Ni- Fe K/dolomite	800	78.12	Chaiprasert and Vitidsant (2009)
Ni/MCM-41	800	51	Wu et al. (2013)

conversion capability of CeO_2/SiO_2 supported catalyst increases in the order Pd < Pt < Rh.

A significant variation in the reforming potential of catalysts can be achieved by combining different catalysts in various proportions and permutations. As the most effective tar reforming catalyst prone to easy deactivation, nickel is combined with other metal and mineral compounds to enhance its properties. The impact of various combinations with Ni catalyst is emphasized in Table 9.9.

The addition of magnesium to nickel improves the mechanical strength, attrition resistance, and porous structure of the catalyst considerably, with a modest reduction of 14% in gas yield (Sutton et al. 2001). The oxides of Mg improve the catalyst stability by increasing the metal dispersion of Ni and thereby evading the nickel aggregation. A substantial reduction in coke formation is detected with improved water absorptivity and OH surface mobility by adding magnesium (Wu and Williams 2011a). The compounds of Mg find its application as a CO_2 sorbent despite being used as primary and secondary catalysts (Arnold and Hill 2019). Dolomite is a potential mineral source that can practically incorporate Mg with Ni to form catalyst combinations with the aforementioned merits.

Aluminum and aluminum oxides are the most used catalytic support for Ni due to their excellent steam reforming reactions and initial catalytic activities amidst a wide range of impurities. Rapid deactivation faced by Al_2O_3 can be retarded by keeping

the catalytic weight to biomass flow ratio higher than 0.65. While $NiAl_2O_3$ catalyst with 33% Ni shows high gas yield and tar reforming stability, Ni-Al catalyst with 28% Ni exhibits better H_2 production and reaction time (Wu and Williams 2011a). Further improvement in catalytic activities of Al can be achieved by supporting it with alkaline earth oxides, natural mineral catalysts, rare metal oxides, etc. An excellent tar conversion of more than 99% with higher gas quality can be attained by supporting Ni/Al with LaO_3 (Zhang et al. 2018a, b). The presence of Mg in Ni/Al improves the catalytic resistance to attrition and sintering. However, K has only a feeble effect on catalytic activity compared to Mg. Also, oxides of La and Co can significantly enhance the stability of the catalyst along with increased carbon conversion, reduced coke formation, and a higher syngas yield when used as Ni/Al support (Wu and Williams 2011b). A tremendous improvement in H_2 yield with higher tar conversion and coking resistance can be attained by adding Ce to Ni/Al catalysts (Nishikawa et al. 2008). Even if the addition of noble metals like Pt, Ru, Ir, etc., significantly contributes towards the stability of the nickel sites and reduction in coke formation, the effect of Pt is commendable over the others (Wu and Williams 2011a).

In general, the oxides of Zr and Si improve H_2 yield from gasification without appreciable tar reduction. However, there it with Ni catalyst inhibits coke formation. Similarly, Ca with Ni imparts increased H_2 yield, CO selectivity, and decreased CO_2 selectivity (Inaba et al. 2006). On the other hand, the presence of Fe in Ni catalyst improves the WSG reaction and hence the gas yield.

The addition of dolomite with Ni shows excellent catalytic activity and stability for tar reforming. Among the various Ni/dolomite variants, the catalyst with 15% Ni exhibits the optimum tar reforming (Srinakruang et al. 2006). The coking resistance of Ni/dolomite was found to be superior to Ni/Al_2O_3 and Ni/SiO_2 (Chaiprasert and Vitidsant 2009). In addition to choking resistance, the inhibition of sulfur poisoning makes Ni/dolomite unique among the other catalysts. Furthermore, MgO and CaO in calcined dolomite can act as potential CO_2 sorbent along with its excellent tar reformation.

The well-defined porous structure, significant surface area, and high thermal stability of zeolite improve the reforming and thermal cracking of hydrocarbons when used with Ni. Zeolite support in Ni catalyst reduces the tar content with the penalty of coke deposit. The coke formation owing to the acid nature of the catalyst can be inhibited by introducing metals like Ce, Mg, etc. (Wu and Williams 2011a).

A comparative assessment of commercial nickel catalysts on tar reforming is demonstrated in Table 9.10. In most commercial catalysts, the Ni O content is maintained below 25% to achieve better tar reforming. In addition to the oxides of Mg, Ca, Si, and K, Al_2O_3 has a prominent fraction in all commercial nickel catalysts. Along with the percentage fraction of the various constituents, reaction temperature also influences the tar conversion rate. The commercial catalysts experience good stability at a higher temperature which can be prolonged to around 60 h (Caballero et al. 1998). Moreover, the coke removal rate in this catalyst is higher than the coke formation rate at high temperatures. Furthermore, using steam or CO_2 gasification, deactivated catalysts can be regenerated with much ease (Wang 2013).

Table 9.10 Tar conversion of commercial nickel-based catalysts

Catalyst	Composition	Reaction temperature (°C)	Tar conversion (%)	Reference
BASF—G1-25/1	(25% NiO- 8% CaO-66%Al_2O_3-1% K_2O)	785–850	98–99	Chan and Tanksale (2014b)
BASF G1-50	(20% NiO- 11% MgO-16% CaO-32%Al_2O_3-7% K_2O – 32% $MgAl_2O_3$)	660–800	89–99	Corella et al. (2004)
Haldor Topsoe—R-67	(15% NiO-0.1% SiO_2-85% K_2O)	780–840	95–100	Corella et al. (2004)
ICI Katalco—46-1	(22% NiO-11% MgO-13% CaO-26% Al_2O_3- 16%SiO_2-7% K_2O)	700–875	73–100	Corella et al. (2004)
BASF—G1-25S	(12–15% NiO->70% Al_2O_3)	785	88–97	Caballero et al. (1998)
Haldor Topsoe—RKS-1	(15% NiO-85% K_2O)	785–800	92	Caballero et al. (1998)
Haldor Topsoe—R-67-7H	(15%NiO-85% $MgAl_2O_4$)	690–780	99	Mullen and Boateng (2008)
Sud Chemie—G-90LDP	(14% NiO-13% CaO-73%Al_2O_3)	850–900	99	Pfeifer and Hofbauer (2008)
Sud Chemie—G-90EW	(14% NiO- 10% CaO-65% Al_2O_3)	850–900	99	Pfeifer and Hofbauer (2008)
Sud Chemie—G-90B	14% NiO-6%CaO-76%Al_2O_3	650–900	99	Pfeifer and Hofbauer (2008

Pertaining to the aforementioned comparison, it can be presumed that Ni, the best tar reforming catalyst under dry and steam atmosphere owing to its excellent catalytic activity towards tar reduction at low cost, is the most accepted catalyst in biomass gasification. But, rapid catalytic activity loss due to carbon deposition confines its application in biomass gasification. Even though doping Ni catalysts with alkaline metals, noble metals or dolomite can influence its overall catalytic performance, agglomeration of alkali metals and sulfur poisoning of noble metals enables the coke resistant dolomite to the more suitable support for Ni catalysts. Thus in a nickel dolomite combination catalyst, the low methane reforming of dolomite and rapid coking of Ni are mutually resolved due to the superior methane conversion and coke resistance of Ni and dolomite, respectively. Additionally, the CaO content in dolomite acts as a sorbent for noncombustible CO_2 there by improving the syngas quality. So a combination of Ni and dolomite can perform as an excellent tar reformer and CO_2 sorbent. Owing to imparting ample

value-addition to syngas, Ni/dolomite is supposed to be the better choice among the biomass gasifying catalysts, which can be in the mixed or impregnated form (Di Felice et al. 2009; Xu et al. 2010).

9.10 Catalytic and Deactivation Mechanisms

Catalysts enhance the quality of syngas from gasification by reforming higher hydrocarbons and CH_4 into high-valued gas constituents without contributing any pollutants (Gao et al. 2020). This is mainly achieved by reducing the activation energy of reforming reactions, thereby lowering the consumption of gasifying medium. The effectiveness of a catalyst rests on its structure, elemental constitution, reaction mechanism, deactivation resistance, and stability. The catalytic cracking mechanism for tar is a series of (Ren et al. 2019).

- Dissociation of hydrocarbons and CH_4, and absorption on the metal site.
- Dehydrogenation of hydrocarbons and CH_4.
- Oxidation of hydrocarbon through OH radicals derived from hydroxylation of water.
- Production of syngas, CH_4, and light hydrocarbons.

9.10.1 Catalytic Mechanism

Due to the inherent intricacy of tar, the tar cracking mechanism may be proposed by considering it from two perspectives (i) as real tar and (2) as a tar model represented by toluene.

9.10.1.1 Reforming Mechanism of Real Tar Using Char Catalyst

Due to the concurrent involvement of acid, alkali, homogeneous and heterogeneous reactions, and catalytic and deactivation effects, the tar cracking mechanism is relatively perplexed. Deposition, dehydrogenation with soot generation, and soot gasification are the main reaction routes involved in the tar cracking mechanism with char, where the alkali-alkaline earth metal variants (AAEM) in the char influence the soot generation rate. In the tar cracking reactions, no further aromatics are formed from benzene and naphthalene, whereas lighter aromatics are formed from alkyl-atomic compounds. The tar components adsorbed at the char active sites, undergo polymerization, and release gaseous product leaving soot.

The H/O/OH-free radicals formed from the dissociated steam and carbon dioxide are adsorbed on the char active sites to initiate tar reforming. The catalytic tar decomposition is further enhanced with additional free radicals from the electron

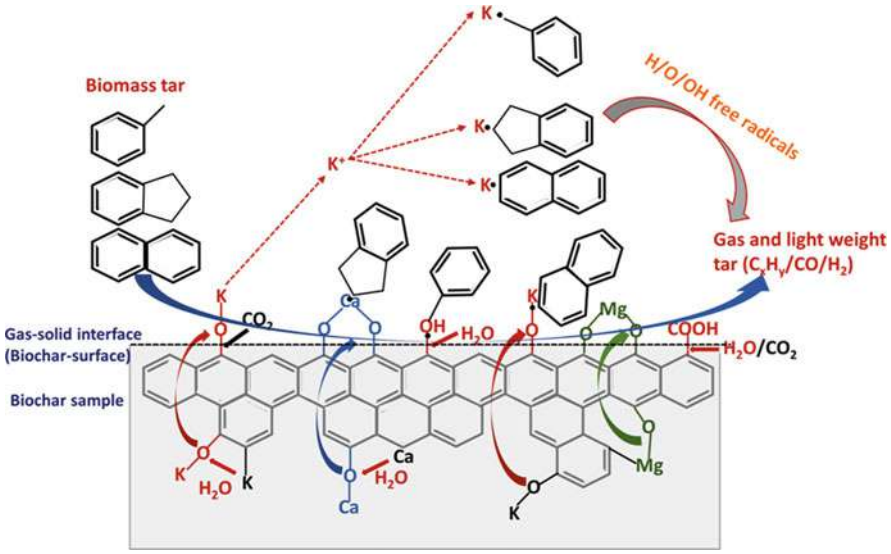

Fig. 9.11 Bio-char enabled tar cracking mechanism at 800 °C (Zeng et al. 2020)

pair shift towards small tar molecules, where H_2O and CO_2 dissociate the carbon bonds in smaller aromatic rings. The vacant sites resulting from this bond dissociation are again occupied by H/O/OH radicals, thus allowing many radicals into the char carbon structure. The AAEM species is bonded with the carbon element in the char through oxygen element forming AAEM-Oxygen bond (e.g., K-O bond shown). When tar is adsorbed, the AAEM-Oxygen bond is dissociated, due to the gasification reactions between H_2O, CO_2, and carbon, and the resulting AAEM species move to a solid-gas boundary, occupying the active sites on the fragmented tar. This AAEM species is further replaced by H/O/OH radicals thereby converting tar to lighter hydrocarbons and gas as shown in Fig. 9.11.

9.10.1.2 Steam Reforming Mechanism of Tar Model (Toluene)

In this section, the mechanism is discussed by representing biomass tar as toluene with LSNFO perovskite catalyst (Oemar et al. 2012). The mechanism is explained in three steps, namely (i) adsorption, (2) reaction, and (3) desorption as shown in Fig. 9.12. During adsorption, water present on the support site is dissociated to form adsorbed H and OH. This OH is then split to form adsorbed H and O, replacing the existing O in the support site. On the metal site, toluene (C_7H_8) is decomposed into adsorbed CH_2, and benzene (C_6H_6), followed by decomposition of benzene to C_2H_2. Subsequently, these CH_2 and C_2H_2 react with adsorbed oxygen on the support site to form CH_2O and C_2H_2O groups. The CH_2O gets further decomposed into CHO and H, and CHO is combined with available adsorbed oxygen to form H and

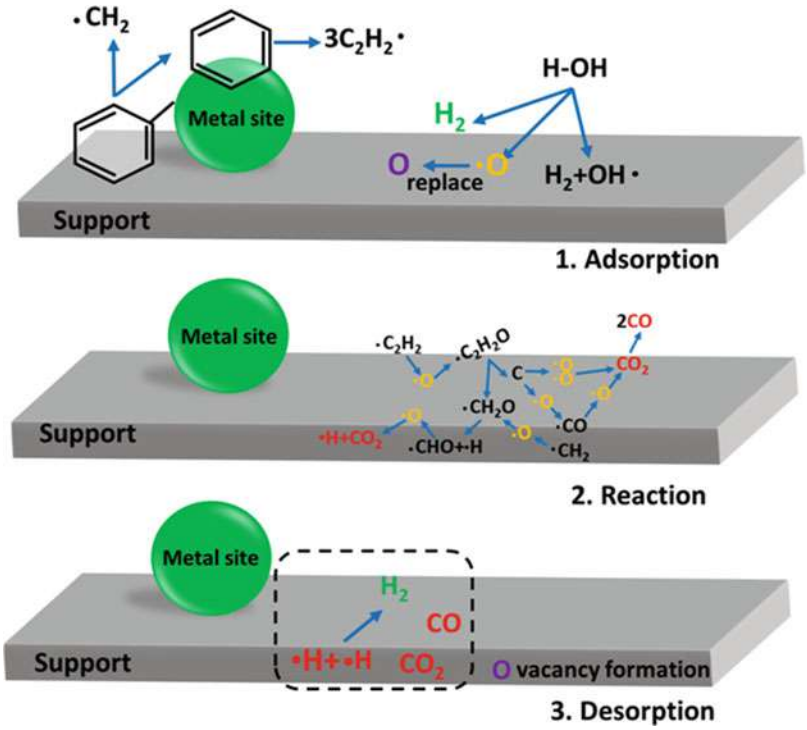

Fig. 9.12 Catalytic reforming mechanism of toluene

CO_2. The resulting free radicals of H combine to form hydrogen. On the other hand, C_2H_2O combines with the adsorbed oxygen to form CH_2O and C. Carbon further reacts with oxygen to form CO and CO_2. Finally, these resulting H_2, CO, and CO_2 desorb to form a support site for the gaseous phase.

9.10.2 Deactivation Mechanisms

Deactivation is the loss of catalytic activity over time due to usage. The loss associated with frequent replacement of catalysts can be minimized by using deactivation-resistant catalysts. Thus, a proper understanding of catalyst deactivation is inevitable in designing and developing deactivation inhabitant catalysts. The physical and chemical mechanism leading to the deactivation of tar cracking catalysts can be mainly classified into three, coking, sulfur poisoning, and sintering.

9.10.2.1 Coking or Coke Formation

It is the most common deactivation of catalyst encountered due to the deposition of coke or carbon on the active metal centers and/or pores on the catalyst, thereby reducing the catalytic activity by limiting the interaction between catalyst and reactants (Forzatti and Lietti 1999). Coking is due to the encapsulation of catalysts by carbonaceous deposits originating from the cracking or condensation of hydrocarbon or carbon monoxide and methane. The major reactions that promote coking are (Quitete and Souza 2017; Liu et al. 2019),

$$2CO \leftrightarrow C_{(s)} + CO_2 \tag{9.10}$$

$$CO + H_2 \leftrightarrow C_{(s)} + H_2O \tag{9.11}$$

$$CH_4 \leftrightarrow \ C_{(s)} + 2H_2 \tag{9.12}$$

$$C_nH_m \leftrightarrow nC_{(s)} + \left[\frac{m}{2}\right]H_2 \tag{9.13}$$

Amorphous and filamentous are the two variants of carbon deposition on catalysts. The formation of amorphous carbon leads to quick deactivation by encapsulating metal centers whereas, there is no rapid deactivation due to filamentous carbon unless a bulk deposit is formed, leading to reactor clogging (Tan et al. 2020). In general, calcined dolomite is deactivated by an amorphous variant but when calcined dolomite is loaded with Ni, filamentous carbon assumes deactivation.

The main factors that influence coking are the size of the active site, temperature, steam to carbon ratio (S/C) and tar composition. Size of metal particles that form the active site influence the deposition of carbon on the catalyst. Active metal sites with larger particle sizes are more vulnerable to carbon deposits. More carbon deposition was reported on Ni sites with bigger particle sizes when incorporated into MCN-41 mesoporous support (Wu et al. 2013). A similar observation is made when toluene is steam reformed with Fe-Ni/Palygorskite (Zou et al. 2018). An increase in temperature decreases coking, for instance, coking was completely absent beyond 750 °C (Islam 2020). S/C has a significant role in coking as the addition of steam enhances water gas shift reaction (2) and Boudouard reaction, thereby completely regenerating coked catalyst accompanied with improved gas quality (Quitete and Souza 2017). Improvement in hydrogen yield and reduction in carbon deposit was found in Ni/Al_2O_3 when steam to carbon ratio is increased (Cao et al. 2020). Tar composition determines the type of carbon formed on catalysts due to coking. Atomic carbon α-C, which readily reacts with steam, is formed by the reforming of phenol whereas, other forms of carbon β-C and γ-C, lesser reactive with steam, is resulted from naphthalene when Ni-Fe alloy combined with olivine is used as a catalyst for reforming (Meng et al. 2018). Also, filamentous and aromatic carbon is generated when the toluene benzene mixture and toluene naphthalene mixture is subjected to reforming in the presence of Ni catalyst.

Coking of a commercial catalyst can be depressed by selecting suitable catalytic composition and optimal operating conditions. Higher steam to hydrocarbon ratio and improved hydrogen partial pressure are used to restrain carbon-induced catalyst deactivation. The catalytic composition can also restrict coking as ions of alkali metals like potassium when integrated with catalyst catalyzes carbon deposit, thereby facilitating bed catalyst regeneration. Similarly, Pt/Al_2O_3 can be modified to $Pt\text{-}Re/Al_2O_3$ to attain prolonged aversion against coking.

9.10.2.2 Sintering

Sintering is a thermally initiated process leading to a reduction in active sites of supported and non-supported catalysts through surface modifications. Particle migration or coalescence and atomic migration are the sintering mechanisms for catalysts based on thermal diffusion of the catalyst constituents. In the former, two particles are transported along with the support, and their collision with each other leads to the formation of a bigger particle. Whereas, in atomic migration, a metal ion discharged by one particle is captured by another. For Ni, particle migration is predominant at temperatures less than 700 °C while at higher temperatures particle migration becomes more prevalent. Sintering reduces surface area and porosity and makes the active sites inaccessible for the reactants. The reverse of sintering, termed dispersion, is the phenomenon of reduction of particle growth due to O_2 and Cl_2 or both, due to the generation of oxides or chlorides of the metal followed by their splitting.

Sintering mainly depends on the temperature, atmosphere, carriers, promoters, and porosity (Bartholomew 2001; Morris and Bartholomew 2015). The sintering rate escalates exponentially with temperature notably in the presence of steam resulting in coarser and diverse particle sizes (Ochoa et al. 2018). Higher calcination and reaction temperatures favor thermal-driven metal growth and agglomeration. H_2 atmosphere promotes sintering in base metals whereas both O_2 and H_2 support the same in noble metal catalysts. Lower thermal stability and weak metal-carrier interaction promote the sintering mechanism. The thermal stability of metal with carrier improves in the order carbon $<$ silicon dioxide $<$ aluminum oxide. Unsupported CuO is subjected to sintering compared to supported CuO used for syngas reforming at 800 °C (Huang et al. 2013). Addition of promoters that increase (e.g., fluorine, lead, sulfur, oxides of barium, calcium) and decreases (oxygen, oxides of Ce, Ba, Ca) the metal mobility reduces sintering. The possibility of sintering impends when the pore size is comparable with that of metal crystallites. Thus, the tendency of catalyst sintering can be minimized by using thermally stable catalyst composition, avoiding metal-carrier interactions, proper choice of atmosphere and promoters.

9.10.2.3 Sulfur Poisoning

In general, poisoning is the degradation is catalytic activity resulting from firm chemical adsorption of impurities to the active metal sites from the feed stream. During thermochemical processing of biomass, the sulfur contained in the feedstock is converted to hydrogen sulfide which in turn converted to metal sulfide at the active metal sites. Consequently, the catalyst is deactivated as the active metal is encapsulated by inert metal sulfide, leading to deactivation due to sulfidation. Moreover, the lower thermal stability and better mobility of metal sulfides promote sintering. Especially, Ni-based catalysts are prone to this deactivation as hydrogen sulfide formed reacts with the Ni to form nickel sulfide.

$$\mathrm{Ni} + \mathrm{H_2S} \leftrightarrow \mathrm{NiS} + \mathrm{H_2} \tag{9.14}$$

The reaction is strongly influenced by temperature, above 900 °C nickel irreversibly combines with hydrogen sulfide, whereas at lower temperatures, sulfur forms only polysulfide that can be released easily in a sulfur-free environment (Hepola and Simell 1997). Adding oxygen or steam, or increasing temperature can successfully crack NiS formed, but the use of oxygen or steam leads to the formation of inert NiO or $NiSO_4$, an alternate source of deactivation (Li et al. 2007).

Apart from nickel, sulfur poisoning can also inhibit the reactivity of noble and transition metal-based catalysts. Sulfur poisoning reduced the activity of Rh–$LaCoO_3$ catalyst, used for tar reforming of syngas, due to the formation of Rh and Co sulfides (Ammendola et al. 2012). Deactivation of Co-based catalyst was also reported by Gao et al. (2020) due to inert cobalt sulfide formation

$$\mathrm{Co} + \mathrm{H_2S} \leftrightarrow \mathrm{CoS} + \mathrm{H_2} \tag{9.15}$$

Ammonia, potassium sulfate, potassium chloride, zinc chloride, and formulations of biomass-derived fly ash are the other compounds that can cause deactivation (Albertazzi et al. 2008). Normally, steps are taken to prevent or minimize sulfur poisoning as the poisoned catalyst is difficult to regenerate. One fundamental step to achieve the same is to subject the feed to suitable pretreatments like hydrodesulfurization or zinc oxide beds to remove sulfur compounds from the stream. Effective reduction of poisoning can be achieved by deploying guard beds ahead of the principal bed.

9.11 Products and Application of Biomass Gasification

Much focus is concentrated on the commercial application of biomass gasification to generate energy due to the renewability, carbon neutrality, and ample availability of biomass. Also, being the only renewable carbon source, biomass will be the future

feedstock for chemical and transportation fuel production (Boerrigter and Rauch 2006). This section deals with the significant applications and products derived from biomass gasification.

9.11.1 Products

Biomass gasification can provide syngas (termed bio-syngas) as the primary product, which can be further processed to form secondary products such as electricity, chemicals (methanol, ammonia, etc.), liquid, and gaseous biofuels. Liquid biofuels like ethanol, gasoline, and diesel, and gaseous biofuels like methane and hydrogen are produced through direct conversion of syngas. These secondary products find application as industrial chemical feedstocks and energy conversion fuels (Demirbaş 2001; Arcoumanis et al. 2008).

9.11.1.1 Bio-syngas

Being the primary product, the syngas, a mixture of H and CO, mainly finds their application as a chemical and energy feedstock. Syngas is an essential precursor of methane, hydrogen, and valuable chemicals like ammonia, methanol, etc. The production of bio-syngas through steam reforming reaction results in bulk production of hydrogen followed by carbon monoxide, which further undergoes shift reaction to generate additional H_2 and CO_2. The H_2/CO ratio determines the formation of desired products like gasoline, methanol, etc. from syngas. Thus, syngas act as a carrier for power generation and an intermediate product for biofuel production. (Basu 2010; Molino et al. 2016).

9.11.1.2 Heat and Electricity Generation

The producer gas generated from gasification can be consumed in boilers and furnaces to generate heat, in ICE to produce small–medium scale electricity, and in GT to generate small–large scale electricity. In GT, the exhaust gas can be further recovered using a heat recovery steam generator and can be used to generate additional electricity (Bauen 2004).

9.11.1.3 Methanol

Methanol (CH_3OH), formed during the catalytic synthesis of syngas, acts as a potential transport and chemical industry feedstock. The primary use of methanol includes making formaldehyde, acetic acid, etc. and converting fats and oils to biodiesel through transesterification (Kumar et al. 2009). The catalytic conversion

of syngas to methanol is carried out with Cu or Ni-Al-based catalysts. Steam reforming of methane leads to the production of methanol, which can in turn be converted to gasoline (Kumar et al. 2009).

$$CO + 2\,H_2 \rightarrow CH_3OH \tag{9.16}$$

9.11.1.4 Ammonia Synthesis

Ammonia (NH_3), synthesized from the combined effect of H_2 and N_2 on a catalytic bed, is widely used in fertilizers, disinfectants, refrigerants, etc. Iron catalysts with added promoters have a vital role in the synthesis of ammonia. The gas formed from biomass gasification is made to endure the shift reaction followed by a scrubbing and regeneration process before converting to NH_3 (Basu 2010).

$$N_2 + 3H_2 \rightarrow 2NH_3 \tag{9.17}$$

9.11.1.5 Ethanol

Ethanol (C_2H_6O) is a prominent biomass-derived transport fuel used in the transportation industry. It can be used as a single fuel or is used in spark-ignition engines along with gasoline. Ethanol is produced by the thermochemical conversion of syngas with Rh-based or Cu-based catalysts. But the energy loss occurring during the conversion of syngas to these liquid fuels inversely affects their overall energy conversion efficiency (Kumar et al. 2009; Basu 2010).

9.11.1.6 Gasoline

A hydrocarbon mixture that has a carbon number within the range of 5–11 constitutes gasoline. Using fixed and fluidized beds of zeolite catalysts, gasoline conversion from methanol is exercised through the methanol-to-gasoline (MTG) process. Gasoline is obtained by a double dehydration process with the production of dimethyl ether intermediate (Molino et al. 2016).

9.11.1.7 Diesel

A high-quality (Cetane number $>$ 70) diesel oil, with a similar composition to petrodiesel (produced from petroleum), can be developed by employing the FT process. The diesel thus produced can be used in compression ignition engines along with petrodiesel (Molino et al. 2016).

9.11.1.8 Methane

Membrane technology can produce methane in which the compressed syngas is fed to some polymer permeation modules (Molino et al. 2016). The average operating temperature of FT synthesis is between 693 and 893 °C. The high temperature of FT also favors methane production, which can be further converted into H_2 through catalytic dehydrogenation (Huffman 2011).

9.11.1.9 Hydrogen

Hydrogen can be derived from syngas through catalyzed WGS reactions. Cr supported Fe-based catalysts, and Cu-based catalysts are usually used to enhance the shift reaction (Molino et al. 2016). In automobiles, H_2 is employed with zero-emission using fuel cells that can attain high efficiency of about 60%. Moreover, H_2 can be used to prepare chemicals and intermediate products like ammonia, methanol, etc. (Kumar et al. 2009).

9.11.2 Applications

The gasification process exercises the conversion of biomass feedstock to a suitable gaseous fuel product. The flexibility of this alternative energy conversion method to produce energy and a variety of fuels with fewer greenhouse emissions tremendously widens its possibilities (Winther et al. 2012). The significant areas of application of biomass gasification include boilers, gas turbines, internal combustion engines, and the synthesis of liquid transport fuels and useful chemicals (Kumar et al. 2009).

9.11.2.1 Boilers

In boilers, energy is harvested from syngas through direct combustion without causing any adverse environmental effects. Even though syngas finds ample applications in power plants, the low overall electric energy efficiency necessitates a cogeneration unit for heat reuse. Similarly, in power plants, biomass gasification products can be burned along with conventional fuels (co-combustion), limiting the weight percentage of the former in the range of 5–15 (Molino et al. 2016).

9.11.2.2 Gas Turbines

Syngas finds application in gas turbines (GT) with a relatively higher electrical conversion efficiency of 40% compared to conventional steam cycles. This improved conversion efficiency is attributed to the better combustion characteristics of gaseous fuels. An additional improvement in energy efficiency by up to 60% can be achieved using an additional energy recovery system, and GT is considered the most effective electrical-generation system (Jiang et al. 2016; Ren et al. 2020). However, a rapid turbine blade deterioration due to high-temperature flue gas demands a higher calorific value and appropriate cleaning methodologies.

9.11.2.3 Internal Combustion Engines

The biomass-derived syngas with high calorific value and fewer pollutants are required for the appropriate performance of internal combustion engines. Along with combustion, syngas is also employed for co-combustion with biogas achieving an overall energy conversion efficiency of 45%. But the fruitful application of syngas in engines demands modified fuel supply systems (Shah et al. 2010; Zhang et al. 2018a, b).

9.11.2.4 Fuel Cells

Yet another important application of syngas is its usage in high-energy fuel cells. In fuel cells, the electricity is generated through chemical energy conversion with an energy conversion efficiency greater than 40%. Moreover, direct feeding of fuel cells with syngas or with a mixture of H_2 and CH_4 adds flexibility (Molino et al. 2016). Furthermore, the noncombustible usage of syngas in high-temperature fuel cells makes the approach sustainable and eco-friendly (Pierobon et al. 2014).

9.11.2.5 Fischer–Tropsch Synthesis

Fischer–Tropsch (FT) synthesis is the catalytic transmutation of syngas to long-chain hydrocarbon. Catalytic support for the FT process is provided by iron, cobalt, and nickel-based metals (Basu 2010). The name of the process is coined as a portmanteau of the names of Franz Fischer and Hans Drops who developed the process in 1920. Hydrocarbons of varying molecular weight can be obtained by the reaction between H_2 and CO on feeding syngas to the FT reactor. A wide variety of oxygenated compounds with carbon number spanning from 1 to 35 (inclusive of gases and solid waxes) are produced from FT reactions in which hydrocarbons in the span C_5–C_{10} serves as synthetic fuels, and higher range hydrocarbons favor gasoline

products. The standard operating temperature and pressure of FT reactions are between 200 to 350 °C and 20–300 atm.

$$(2x + 1)H_2 + xCO\ (\text{catalyst}) \rightarrow C_xH_{(2x+2)} + xH_2O \tag{9.18}$$

Where $C_xH_{(2x\ +\ 2)}$ indicates a hydrocarbon mixture spanning from methane to wax depending on the pressure, catalyst, and temperature maintained in the reaction.

9.12 Recent Advances and Future Perspectives of Catalytic Biomass Gasification

The previous section discussed various catalysts, their classification, mechanisms, and their effect on tar reforming and gas yield. This section deals with the recent trends and future scope in catalytic biomass gasification. Further improvement of catalytic performance through new catalyst configurations and novel technologies are discussed.

9.12.1 Recent Advances

One of the most recent advancements in catalytic biomass gasification is the reactive flash volatilization approach to improve the uneconomical options of fluidized bed gasifiers with a downstream catalytic reactor for tar cleaning or a downstream catalytic reformer with a pyrolysis reactor. A fixed bed gasifier with a significantly higher mass flow rate and carbon space velocity is used in reactive flash volatilization, making it cost-effective on a small scale (Colby et al. 2008). Reactive flash volatilization with Rh-based catalyst is appropriate for cellulose gasification and is a promising approach towards superior syngas production. But the high cost of Rh catalyst confines its application to bench-scale (Chan and Tanksale 2014b). Similarly, a carbonless decomposition of H_2 and CO can be attained from a non-volatile feed in less than 50 ms through reactive flash volatilization with Rh-Ce catalyst. The rapid oxidation of decomposition products prevents the condensation reactions and hence the carbon formation. As a result, higher feed conversion with H_2 and CO components is achieved with a lower C/O ratio (Salge et al. 2006). Another prospective technology for potential tar reforming is hybrid plasma-catalysis due to its high tar conversion rate and syngas yield. Furthermore, dry reforming of tar to H_2 and autothermal reforming (introduction of O_2 to promote tar cracking, thereby limiting C deposition) are alternative emerging technologies to replace the conventional steam reforming at the expense of catalytic deactivation and oxygen purification cost, respectively (Sinaei Nobandegani et al. 2016).

9.12.2 Future Perspectives

With reference to the prior studies, Ni-based catalysts are identified for their outstanding tar reforming capability. The value-addition of Ni catalysts by incorporating noble metals or dolomites is worth researching for further development; Ni-dolomite configurations seek more research attention due to their instant availability and affordability (Wu and Williams 2011a). As a commercially viable process, the gasification of lignocellulose is another area of future scope, where more robust and effective Ni catalysts may take a crucial position (Chan and Tanksale 2014a).

Currently, studies are focusing on the improvement of sintering, sulfur poisoning, and coking resistance of catalysts. But the complexity of developing a catalyst by integrating all of these is still challenging. So, synthesizing a multi-functional catalyst with anti-coking, anti-sintering, and anti-poisoning properties is a highly relevant field that requires more attention. Furthermore, poisoning resistance towards halogen and other organic nitrogen species along with catalytic regeneration and recycling is yet another field for further research (Gao et al. 2020).

Even though attempts continue to optimize the laboratory-scale reactors and catalysts, scaling-up of the same is more complex. The optimization of space velocity, unstable flow rate, etc. on scaled-up reactors is another area of futuristic interest (Nabgan et al. 2017). Moreover, scaling-up of sorption enhanced steam reforming (SESR) with the integrated sorbent, from laboratory to commercial-scale, also entails much research interest (Gao et al. 2020).

Another vital area requiring much attention in the industrial application of biomass gasification is the stability in tar reforming. Sustained tar reforming with simultaneous catalyst regeneration through chemical looping involves relevant consideration (Artetxe et al. 2017). Apart from this, further research should be done in reactor design optimization by incorporating sophisticated techniques employed in bubbling, decoupled triple bed, and countercurrent fluidized bed reactors (Gao et al. 2020). Additionally, elevated temperature steam reforming performance demands further studies on improved reactor material and design. Also, alternative novel improving technologies like dry reforming and autothermal reforming (ATR) are to be explored to their full extent (Sinaei Nobandegani et al. 2016).

Another emerging area with promising research opportunities is the mechanism of tar cracking. The actual tar cracking mechanism should be considered with various types of model compounds in varying amounts accounting for their complex structure containing five-ring aromatic hydrocarbons, oxygenates, and polycyclic aromatic hydrocarbons, rather than a single first-order tar model compound (Ren et al. 2019). Along with the catalytic reforming mechanism, the mechanism involving preparation and pre-treatment of catalyst and combined interaction mechanism of metals supports, and promoters are other relevant areas that demand further research (Gao et al. 2020). Furthermore, research focusing on syngas production as a potential secondary feedstock for synthetic fuel production necessitates a one-step chemical fuel synthesis from biomass tar steam reforming with optimized

parameters and a suitable catalyst, opening the possibilities of a new precinct in biomass gasification (Liu et al. 2019).

9.13 Summary

This chapter deals with various aspects of biomass gasification and the requirement for catalytic producer gas enhancement. Biomass gasification is a complex transmutation of biomass to gaseous fuels and chemical feedstock. The performance of the process is mainly governed by reactor design and key operating parameters such as gasifying agent, type of feedstock, temperature, pressure, and residence time. The fruitful application of producer gas can be ensured only if the undesirable tar content in it is reduced beyond specified limits. Reforming tar using catalysts is identified as the most economical and commercially viable route for achieving the same. The chapter also covers catalytic activation and deactivation mechanisms, and a performance comparison of different catalysts in tar reforming and enhancement of gas yield. It is inferred that the selection of a suitable catalyst not only enhances gas yield through tar reforming but also acts as a CO_2 sorbent to sequestrate incombustible CO_2, further enhancing producer gas quality. In this view, Ni-dolomite combinations are identified as an economical and potential catalyst that nourishes producer gas through simultaneous attainment of tar reformation and CO_2 sorption. Further improvement of catalytic performance through new catalyst configurations and novel technologies are also discussed.

Conflict of Interest All the authors declare that they have no competing interests.

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Chapter 10
Recent Advances in Fast Pyrolysis and Oil Upgradation

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Abstract In this chapter, a brief introduction to various pyrolysis processes and a detailed analysis of fast pyrolysis has been explored. A wide variety of feedstocks suitable for the fast pyrolysis process and physicochemical properties of bio-oil has been incorporated. Various types of pyrolyzers that are available in the papers are also mentioned here. Furthermore, enrichment of bio-oil using various upgradation techniques and its physicochemical properties are also discussed. This includes the processes such as steam reforming, catalytic cracking, and supercritical extraction. The application of bio-oil as a fuel requires enhancement in its properties which is achieved using blending with other fuels. Thus, this chapter also strives to explain the recent advances made in bio-oil properties enhancement. Also, a brief analysis of the techno-economic feasibility of bio-oil production and its environmental sustainability is specified. This chapter attempts to give an overview of the whole concept of bio-oil production to its application.

Keywords Pyrolysis · Bio-oil · Fast pyrolysis reactor (pyrolyzer) · Upgradation and enhancement of bio-oil · Techno-economics · Environmental sustainability

Abbreviations

BCO	Bio-crude oil
CE	Capillary electrophoresis
CFBR	Circulating fluidized bed reactor
CR	Cassava rhizome
CS	Cassava stalk
CSBR	Conical spouted bed reactor

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P. Verma (ed.), *Thermochemical and Catalytic Conversion Technologies for Future Biorefineries*, Clean Energy Production Technologies,
https://doi.org/10.1007/978-981-19-4312-6_10

dAPCI-TOF MS	Dopant-assisted atmospheric pressure chemical ionization time-of-flight mass spectroscopy
FTIR	Fourier transform infrared
FT-NMR	Fourier transform–nuclear magnetic resonance
GC	Gas chromatograph
GPC	Gel permeation chromatography
HAD	Hydrodearomatization
HDM	Hydrodemetallation/hydrodemetallization
HDN	Hydrodenitrogenation
HDO	Hydrodeoxygenation
HDS	Hydrodesulfurization
HPLC	High-performance liquid chromatography
HTC	Hydrothermal carbonization
HTG	Hydrothermal gasification
HTL	Hydrothermal liquefaction
ICFBR	Internally circulating fluidized bed reactor
iPEPICO	Imaging photoelectron photo ion coincidence spectroscopy
LC	Liquid chromatography
LCA	Life cycle assessment
LQIT-dAPCI-MS	Linear quadrupole ion trap dopant-assisted atmospheric pressure chemical ionization mass spectroscopy
MBMS	Microreactor for pyrolysis connected with molecular beam mass spectrometer
MDGC	Multidimensional gas chromatography
NEV	Net energy value
RTP	Rapid thermal pyrolysis
SFCs	Supercritical fluids
SOC	Soil organic carbon
SPI-TOFMS	Single-photon ionization time-of-flight mass spectroscopy
SVUV-PIMS	Synchrotron-based vacuum ultraviolet photoionization mass spectroscopy
TAP	Temporal analysis of products

10.1 Introduction

As human society progresses towards a new height of technological advancement, the need for per capita energy requirements is also growing tremendously. To meet this necessity of energy, exploration of renewable sources is being highlighted and proposed to fulfill the demands. The dependency of society on non-renewable sources of energy such as fossil fuels will not be accepted in the near future due to scarcity and an increase in population. Also, fossil fuel utilization leads to greenhouse gas emissions that lead to climate change (No 2014). Renewable sources of

energy are explored to overwhelm petroleum and its related product (Kumar and Badgujar, 2019, Kumar et al., 2022). Lignocellulosic biomass is a promising renewable source that can be considered feedstock for biofuel (Patel and Kumar 2016; Bhardwaj et al. 2021; Kumar and Verma 2021a, b). Biomass is converted to solid, liquid, and gaseous biofuel through chemical, thermal, thermochemical, and biochemical processes. The thermochemical process has some leverage over the biochemical process in terms of reaction time and conversion. In the case of thermochemical, both liquefaction and pyrolysis can produce bio-oil. Still, pyrolysis has an advantage in terms of capital cost, making this process more suitable for converting biomass to bio-oil (Nanduri et al. 2021; Agrawal and Verma 2022; Goswami et al. 2020a, 2021). Biomass can be converted to bio-oil through a fast pyrolysis process. Bio-oil cannot be used directly with petrol or diesel due to its non-miscibility with hydrocarbon, polar, and unstable nature (Patel and Kumar 2016; Goswami et al. 2020b). Other bio-oils, oil from vegetables, and animal wastes cannot be used in raw form in conventional engines. Raw bio-oil obtained through pyrolysis can be used in boilers and furnaces as a replacement fuel instead of fuel oil (Nanduri et al. 2021). But on further upgradation of bio-oil, it can be used as a transportation fuel in diesel engines, fuel for combined heat and power generation, and fuel for gas turbines (No 2014). Depending upon the upgradation technique of pyrolysis oil, it will also result in valuable chemicals (Jacobson et al. 2013).

10.2 Bio-oil

Bio-oil is found in liquid form with dark brown color, which comprises oxygen-rich compound (Mohan et al. 2006; Goswami et al. 2022). Bio-oil produced from pyrolysis of biomass is also referred with various terms in the literature, such as wood pyrolysis oil, wood oil, wood distillates, liquid wood, wood liquid, liquid smoke, bio-crude, bio-crude oil (BCO), bio-fuel oil, pyrolysis liquid, pyrolysis oil, pyrolytic bio-oil, pyrolytic oil, fast pyrolysis bio-oil, fast pyrolysis liquid, biomass fast pyrolysis fuel, biomass pyrolysis liquid, biomass flash pyrolysis liquids (BFPL), pyroligneous tar, and pyroligneous acid (No 2014; Krutof and Hawboldt 2016; Isahak et al. 2012). In this chapter, a liquid produced from pyrolysis of biomass will be termed bio-oil. If bio-oil is from other sources such as plastic, it will be referred to as pyrolysis oil.

10.2.1 Feedstock for Bio-Oil

Bio-oil or pyrolysis oil can be obtained not only from biomass but also from animal waste, vehicular residue, plastic, and paper waste from paper mills each has been discussed in the latter portion of the chapter. Various feedstock categories are cellulose and lignin, bark, wood, nuts and seeds, agricultural wastes/residues,

algae, forestry residues, grasses, and miscellaneous. After harvest, the biomass is pretreated, such as removing moisture content and reducing particle size, before feeding the reactor to increase the bio-oil yield. There are various studies where co-pyrolysis, instead of using a single feedstock, two or more feedstock were used for the fast pyrolysis process to enhance the bio-oil properties (Lu et al. 1994; Mohan et al. 2006; Abnisa and Wan Daud 2014).

10.2.1.1 Lignin, Cellulose, and Forest Residue

Mohan et al. (2006) reviewed the work of Fullana et al. who performed the pyrolysis of cellulose, lignin, and sewage sludge and analyzed the product obtained on multidimensional gas chromatography (MDGC). In the same review, fast pyrolysis of forest residue and grasses was also discussed. Dalla et al. (2016) produce the bio-oil from forest residue, softwood (*Picea abies*), and (*Eucalyptus* sp.). Intermediate, fast, and catalytic fast pyrolysis was performed on these feedstocks. Fast pyrolysis of hardwood and softwood residues shows bio-oil yields of 69% and 70%, respectively.

10.2.1.2 Bark and Wood Trunk

Pinto et al. (2018) performed fast pyrolysis on the bark of Pinus radiata, catechin, and water-insoluble (W-I) tannins from the bark of *Pinus radiata* and compared the fraction of phenol and organosolv-lignin in pyrolysis oil from the given feed materials. Specific compound catechols show the highest percentage in the pyrolysis oil of W-I tannins.

Santos et al. (2018) shows the fast pyrolysis of stump wood and trunk wood from *Eucalyptus urograndis* with 450–480 °C reactor temperature. Combining light and a heavy fraction of pyrolysis oil yield from stump wood and trunk wood is 43.4% (31.6% light fraction and 11.8 heavy fractions) and 47.1 (34.1% light fraction and 13.0 heavy fraction), respectively.

10.2.1.3 Nutshell Waste

Das and Ganesh (2003) showed the yield of pyrolysis oil from cashew nutshell that was in the range of 37% (at 400 °C) to the highest of 42% (500–550 °C) and then dropped to 36% (600 °C) (Das and Ganesh 2003). Another paper related to this study characterized the bio-oil obtained from pyrolysis of cashew nutshell. Shell was first heated up to 175 °C, and oil was collected, represented as CO1. After the removal of CO1, it was further pyrolyzed under a vacuum at 500 °C to get the maximum quantity of bio-oil, denoted by CO2. Both bio-oil CO1 and CO2 show complete miscibility with diesel (Das et al. 2004).

A pressed *Jatropha carcass* was used by Jourabchi et al. (2014) for pyrolysis to study the bio-oil yield in a fixed bed system. The system's temperature was in the range from 573.15 °K to 1073.15 °K with a heating rate of 50 °K/min in the presence of nitrogen. The maximum yield of bio-oil from this is around 50%.

10.2.1.4 Seeds

Seeds are also used for the production of pyrolysis oil. Onay and Kockar (2003) used rapeseed for the production of bio-oil. A maximum bio-oil yield of 46.7% was found in slow pyrolysis at the temperature of 550 °C with a heating rate of 30 °C/min without any sweeping gas in the Heinze retort. This bio-oil yield was increased to 51.7% by introducing sweeping gas, i.e., nitrogen, at a flow rate of 100cm^3/min. In fast pyrolysis, the maximum bio-oil yield was 63.1% at 550 °C at 300 °C/min with a sweeping gas flow rate of 100 cm^3/min. This yield was increased to 68% at similar conditions with 0.6–1.25 mm particle size and the sweeping gas flowrate was between 100 and 200 cm^3/min. On the other hand, flash pyrolysis shows the maximum bio-oil yield of 73% at 600 °C with a sweeping gas flow rate of 100 cm^3/min.

10.2.1.5 Agricultural Residue

Pattiya and Suttibak (2012) experimented on agricultural residue, i.e., cassava rhizome (CR) and cassava stalk (CS). Fast pyrolysis was done on this waste in a fluidized bed reactor in the presence of nitrogen gas. For CR, the optimum temperature is 475 °C, at which bio-oil yield was maximum which was 69.1%, whereas, for CS, the temperature was 469 °C, and yield was 61.4%. In another related study, fast pyrolysis in a free-fall reactor in the presence of nitrogen gas on agricultural residue, i.e., sugarcane and cassava residue, was done by Pattiya et al. (2012) for CR and CS maximum bio-yield was 50.3% and 52.3% at 400 °C and 450 °C, respectively. For sugarcane leaves and sugarcane tops, bio-yield was 53.3% and 44.1% at 400 °C and 350 °C. Yanik et al. (2007) demonstrated fast pyrolysis on oreganum stalks, corncob, and straw at 500 °C in a fluidized bed reactor. Bio-oil yield for oreganum stalks, corncob, and straw was 39%, 41%, and 35%, respectively.

10.2.1.6 Algae

Miao et al. (2004) utilized the microalgae for the fast pyrolysis process. The experiment parameter was as follows: the reaction temperature was 500 °C, the heating rate was 600 °C/s, sweeping gas (nitrogen) flow rate 0.4 m^3/h, and residence time for vapor was 2–3 s. The bio-oil yields from microalgae *M. aeruginosa* and *C. protothecoides* were 23.7% and 17.5%, respectively.

10.2.1.7 Animal Waste

Even some study shows the production of bio-oil from animal waste. Das et al. (2009) showed the production of bio-oil from the fast pyrolysis of chicken manure. Woo et al. (2015) generate bio-oil from swine manure using the fast pyrolysis method. Emrah et al. 2019 produced bio-oil, using goat manure as feedstock for the pyrolysis process.

10.2.1.8 Other Feedstocks

Bio-oil is not only produced using biomass or organic waste, but it can also be generated using industrial waste, plastic, and other sources of feedstock. Papermill residue is also used to generate pyrolysis oil using a fast pyrolysis process, as mentioned by Charusiri (2015). A comparative study on automobile shredder residues (ASR) was carried out after processing it through conventional and fast pyrolysis (Zolezzi et al. 2004). This study shows that fast pyrolysis of ASR produces a high yield of pyrolysis oil, i.e., 58% at 800 °C. Wong (2017) performed the distillation of pyrolysis oil produced from plastic wastes. Fast pyrolysis was conducted at 500–800 °C in a laboratory to obtain the pyrolysis oil. Prurapark et al. (2020)

explored the effect of temperature on pyrolysis oil generated from HDPE (high-density polyethylene) and PET (polyethylene terephthalate). In the case of HDPE, the highest pyrolysis oil yield was seen at 450 °C, i.e., 40.5 L of pyrolysis oil from 100 kg of HDPE. All the above feedstocks are suitable to produce bio-oil or pyrolysis oil. This also shows that feedstock for fast pyrolysis is not limited to just biomass.

10.2.2 Bio-oil from Pyrolysis

The pyrolysis process is done in an oxygen-free environment at a temperature range of 200 °C–900 °C. Pyrolysis process depends upon the thermal pretreatments, peak temperature, heating rate, carbonization particle size, and residence time. Pyrolysis can be of a slow, mild, and fast nature. In all cases, biomass goes under thermal decomposition to give biochar, bio-oil, and syngas [C_1–C_2 hydrocarbon, hydrogen, carbon mono oxide, carbon dioxide] (Baghel et al., 2022; Sakhiya et al., 2020). Pyrolysis products are the transformation of biomass components such as hemicellulose, cellulose, and lignin at various temperature ranges as shown in Fig. 10.1. There are various types of pyrolysis and related processes that mainly produce pyrolysis oil are mentioned in Table 10.1. (Cha et al. 2016).

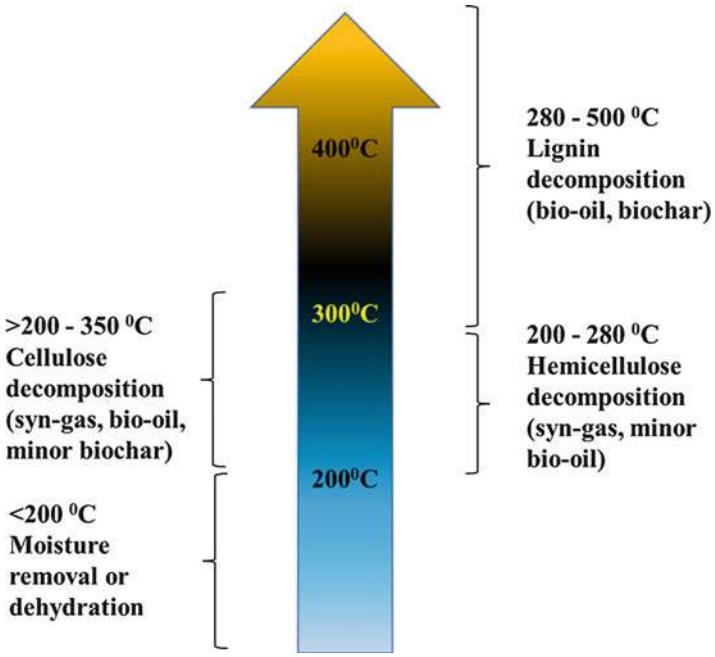

Fig. 10.1 Temperature range for the breakdown of biomass components (Zaman et al. 2017)

10.2.2.1 Slow Pyrolysis

Slow pyrolysis, this process aims to produce high carbon content solid product. The residence time can vary from minute to hour; peak temperature can be in the range of 200 °C–600 °C, and the heating rate is generally less than 50 °C/min, and whole process occurs in an inert environment. Generally, the inert environment is created using nitrogen gas (Antal 2003; Cha et al. 2016).

10.2.2.2 Steam Pyrolysis and Vacuum Pyrolysis

Commonly, nitrogen gas is passed through a pyrolysis reactor to establish an inert environment, also known as sweeping gas. If this nitrogen is replaced with steam, the process is modified from slow pyrolysis to steam pyrolysis. In comparison, steam pyrolysis gives more bio-oil yield than slow pyrolysis (Özbay et al. 2008).

When slow pyrolysis is performed under the vacuum, then this process is modified to vacuum pyrolysis, which can also be used to produce bio-oil. In steam and vacuum pyrolysis, fundamental parameters such as heating rate, residence time, and peak temperature are similar to slow pyrolysis (Carrier et al. 2011; Özbay et al. 2008).

Table 10.1 Generalized values of process parameters for bio-oil production (Khan et al. 2021; Zhang et al. 2020; Farai et al. 2018; Joardder et al. 2014; Yu et al. 2014; Mohan et al. 2006; Tang and Huang 2005; Franke 1994; Anthony 1990)

Process	Process variation/Environment	Pressure	Temperature	Heating rate	Residence time	Product Priority
Slow pyrolysis (conventional)	With nitrogen	Atmospheric	200–600 °C	<50 °C/min	Minutes to hours	Biochar
Steam pyrolysis	With steam	Atmospheric	200–600 °C	<50 °C/min	Minutes to hours	Bio-oil
Hydro-pyrolysis	With hydrogen	Above atmospheric	200–800 °C	<100 °C/min	Minutes to hours	Bio-oil
Methano-pyrolysis	With methane	Above atmospheric	200–800 °C	<100 °C/min	Minutes to hours	Bio-oil
Vacuum pyrolysis	With vacuum	Below atmospheric	200–600 °C	<50 °C/min	Minutes to hours	Bio-oil
Hydrothermal carbonization	With water	Above atmospheric	<250 °C	Slow	Minutes to hours	Hydro Char
Hydrothermal liquefaction	With water or methanol	Above atmospheric	250–400 °C	Slow	Minutes to hours	Bio-oil
Hydrothermal gasification	With water	Above atmospheric	>400 °C	Slow	Minutes to hours	Syngas
Torrefaction (Mild pyrolysis)	Oxygen-free environment	Atmospheric	200–300 °C	Slow	Minutes to hours	Bio-coal
Fast pyrolysis	Oxygen-free or in a nitrogen environment	Atmospheric	650 °C	Very high	0.5–5 s	Bio-oil
Flash pyrolysis	Oxygen-free or in nitrogen environment	Atmospheric	1000 °C	Very high	<1 s	Bio-oil
Flash-hydro-pyrolysis	With hydrogen	Above atmospheric	<500 °C	High	<10 s	Volatile Matter
Vacuum flash pyrolysis	With vacuum	Below Atmospheric	400 °C	Medium	2–30 s	Bio-oil

Microwave-assisted pyrolysis	With nitrogen	Atmospheric	Around 300 °C	Medium	Seconds—minutes	Bio-oil
Fast microwave-assisted pyrolysis	With nitrogen and premixed catalyst	Atmospheric	300–1000 °C	High	Seconds—minutes	Bio-oil
Flash microwave-assisted pyrolysis	With nitrogen and non-premixed catalyst	Atmospheric	300–1000 °C	Very high	Seconds—minutes	Bio-oil
Solar-assisted pyrolysis	Oxygen-free environment	Atmospheric	Around 500 °C	Medium	Minutes	Bio-oil
Plasma pyrolysis	With nitrogen	Atmospheric and above atmospheric	Around 1000 °C	Very high	Seconds—minutes	Syn-gas
Gasification	Oxygen-free environment	Atmospheric and above atmospheric	Around 1000 °C	Very high	Seconds—minutes	Syn-gas

10.2.2.3 Hydro-Pyrolysis and Methano-Pyrolysis

In hydro-pyrolysis and methano-pyrolysis, pressure is also high compared to conventional or slow pyrolysis, which ranges from 5 bar to 50 bar, and other parameters such as heating rate, residence time, and peak temperature are similar to slow pyrolysis (Franke 1994). When sweeping gas is replaced with hydrogen and methane, it is known as hydro-pyrolysis and methano-pyrolysis, respectively. Franke (1994) experimented on coal with these processes, i.e., methano-pyrolysis, hydro-pyrolysis, and conventional pyrolysis with and without impregnation of lithium oxide which acts as a catalyst. In methano-pyrolysis, methane was mixed with nitrous oxide in a 4:1 (methane to nitrous oxide) ratio and then fed to the reactor. Maximum liquid conversion was achieved in methano-pyrolysis which is higher than the yield produced in hydro-pyrolysis.

10.2.2.4 Hydrothermal Carbonization, Liquefaction, and Gasification

The hydrothermal carbonization process is not a single process, but it is a part of the hydrothermal process. This type of thermochemical process is achieved in a close reactor where biomass and water are mixed, and its pressure can go from atmospheric to 350 bar, and temperature generally is in the range of 200–400 °C. Here, the feedstock is mixed with water in a specific ratio and then fed to the hydrothermal reactor. The hydrothermal process is a combination of processes at various temperature ranges, below 250 °C called hydrothermal carbonization (HTC), in between 250 °C and 400 °C is hydrothermal liquefaction (HTL), and above 400 °C is hydrothermal gasification (HTG). These stages HTC, HTL, and HTG have a max yield of biochar, bio-oil, and gases, respectively. The bio-oil yield from HTL ranges from 7.9% to 67% under different conditions with various feedstocks. Some researchers have shown that liquefaction is also performed using methanol instead of water (Tong et al. 2021; Xu and Li 2021; Cha et al. 2016; Xiao et al. 2012; Kruse 2009).

10.2.2.5 Torrefaction

Torrefaction is mild pyrolysis that involves oxygen-free or very minute concentrations at a temperature range of 250–375 °C. This process yields low carbon valued products its percentage varies from 51% to 55%. The product of torrefaction may be used for fuel purposes, and its product can be used as biocoal (Uemura et al. 2011; Meyer et al. 2011; Ghiasi et al. 2014; Cha et al. 2016).

10.2.2.6 Fast Pyrolysis

In fast pyrolysis also termed ultra-pyrolysis or rapid pyrolysis, the particle size is between 1 and 2 mm, moisture content should be less than 10% by weight, and vapor residence time is in seconds. The heating rate can go as high as 1000 °C/s. Fast pyrolysis aims to produce a high percentage of bio-oil; the yield of bio-oil is in the range of 60–75%, which is very high in comparison to other pyrolysis processes (Mohan et al. 2014, Meyer et al. 2011, Gollakota et al. 2016, Mohan et al. 2006, Shen et al. 2020; Mok et al. 1985).

10.2.2.7 Flash Pyrolysis

Flash pyrolysis, sometimes referred to as ultra-fast (ultra-rapid) pyrolysis, consider a process with very high heating rates which can go up to 10,000 °C/s with an intermediate temperature range between 500 and 600 °C and very short residence times less than a second. Around 75% of bio-oil yield can be achieved from this process. An enormous heating rate diminishes the char and tar formation. Due to rapid quenching, liquid products condense before the breakdown of higher molecular weight compounds that may transform into gaseous products (Results 1993; Goyal et al. 2006; Jahirul et al. 2012; Energy Institute 2020).

10.2.2.8 Vacuum Flash Pyrolysis and Flash-Hydro-Pyrolysis

Furthermore, if flash pyrolysis is performed under a vacuum, it is known as vacuum flash pyrolysis. The reason behind this is to get a high yield of bio-oil from this process and, it diminishes the secondary decomposition and inhibits further gas formation. When flash pyrolysis is performed along with hydrogen gas at high pressure (maximum limit of 20 MPa), it is known as flash-hydro-pyrolysis (Pecha et al. 2017; Goyal et al. 2006; Tang and Huang 2005).

10.2.2.9 Microwave-Assisted Pyrolysis

Microwave-assisted pyrolysis is generally performed to maximize the yield of bio-oil. This is different from conventional pyrolysis, as heating is performed using a high-frequency wave, penetrating the feedstock and increasing temperature from inside to outside. This process has advantages over conventional pyrolysis is in terms of response rate, heating rate, heating efficiency, and thermal inertia (Yu et al. 2014; Zhang et al. 2020). It is also modified to microwave-assisted fast pyrolysis or premixed method and ultra-fast (flash) microwave-assisted pyrolysis or non-premixed method. The selection of the method depends on the high heating rate and the way microwave absorbing material or catalyst is used in the process

(Tsubaki 2020). Fast microwave-assisted (premixed) pyrolysis is where feedstock is premixed with a catalyst which rises the temperature quickly due to the fast-absorbing capacity of the microwave by catalyst or microwave absorbent (CaO, NiO, Fe_2O_3, MnO_2, $CuCl_2$, NaCl, $MgCl_2$ γ-Al_2O_3, and TiO_2, etc.). Another method is flash microwave-assisted pyrolysis (non-premixed), here catalyst (such as activated carbon (AC), graphite, and SiC) is poured into the reactor to achieve the desired temperature. Then the material or feedstock is fed to the reactor for instant heating (Zhang et al. 2020).

10.2.2.10 Solar-Assisted Fast or Flash Pyrolysis

In solar-assisted fast or flash pyrolysis, the feedstock is fed to the reactor present at the focal point of the parabolic solar panel acts as a solar concentrator. Heat is applied externally to reach the expected temperature for pyrolysis. This process is more eco-friendly and energy efficient (Joardder et al. 2014). A 50% bio-oil yield is obtained with date seed at 500 °C with a residence time of 2 h. Goyal et al. (2006) mentioned in their paper that Lede and Bouten (1999) reported solar flash pyrolysis of samples of cellulose.

10.2.2.11 Plasma Pyrolysis

Plasma pyrolysis of biomass is performed at high temperatures (around 1000 °C) to produce syngas and biochar. Generally, carbon monoxide and hydrogen percentages are high, and methane percentage is low in syngas from plasma pyrolysis. The power required for this process depends on the nature and amount of feedstock. As plasma torch is the only heat source in this process, controlling it is helpful during the varying nature of feedstock (Tang and Huang 2005; Farai et al. 2018).

10.2.2.12 Gasification

Another way to get a high yield of gas is from gasification. The partial oxidation process of converting biomass into char and syngas and temperature can go as high as 1000 °C. This process focuses mainly on gaseous products (hydrogen, carbon mono oxide, carbon dioxide, nitrogen, etc.). Although carbon content in final residue can reach up to 95%, this process emphasizes gaseous products more (Taba et al. 2012; Tay et al. 2013).

10.2.3 Physicochemical Properties

Bio-oil properties mainly depend on the nature of the feedstock. Physicochemical properties change as feedstock is altered. The nature of bio-oil is determined through various physical and chemical properties. Physical parameter determination is necessary because that will help during the usage, storage, and handling of bio-oil. Different physical parameters are mentioned in Table 10.2 (Fagemas 1995).

Chemical properties include the quantification of various chemical compounds present in bio-oil. Different components of bio-oil can be identified using analytical methods of gas chromatography (GC), liquid chromatography (LC), high-performance liquid chromatography (HPLC), Fourier transform infrared (FTIR), proton(H^1), and carbon (^{13}C) Fourier transform-nuclear magnetic resonance (FT-NMR) spectroscopies, Gel permeation chromatography (GPC), and capillary electrophoresis (CE). Advanced technology includes a combination of two or more technologies, also known as hyphenated methods, for example, GC/MS (gas chromatography with mass spectroscopy), LC/MS (liquid chromatography with mass spectroscopy), GC/FT-IR, and GC/FT-NMR (Fagemas 1995).

Recent advances in an experimental setup that helps in understanding the fast pyrolysis mechanism on the molecular level that can be either catalyzed or non-catalyzed pyrolysis, which includes small level pyrolyzer combined with advanced analytical instruments that are mentioned below (Nanduri et al. 2021):

Table 10.2 Physical properties of pyrolysis oil (Mohan et al. 2006;Elliott et al. 2012;Oasmaa et al. 2012)

Parameters	Value	Test Method
Water (% by weight)	20–30	ASTM E203
Solids (% by weight)	0.01–1.00	Ethanol insoluble, methanol-dichloromethane insoluble
Ash (% by weight)	0.01–0.2	ASTM D482
Nitrogen (% by weight)	0–0.4	ASTM D5291
Sulfur (% by weight)	0–0.05	ASTM D4294
Viscosity at 40 °C (cSt)	15–35	ASTM D445
Density at 20 °C (g/mL)	1.1–1.3	ASTM D4052
Pour point (°C)	from −9 to −36	ASTM D93
Flash point (°C)	45 minimum	ASTM D93 procedure B
Gross heat of combustion (MJ/kg)	15 minimum	ASTM D240
pH	>2.5	ASTM E70–07
Distillability	Not distillable	
Stability	Viscosity can increase and can phase separate	80 °C for 24 h 40 °C for 1 week
Elemental analysis (CHN) (%by wt.)		ASTM D5291
Conradson carbon residue (% by wt.)		ASTM D189

- Micro pyrolyzer combined with GC-MS.
- Micro pyrolyzer interfaced with dopant-assisted atmospheric pressure chemical ionization time-of-flight mass spectroscopy (dAPCI-TOF MS) system.
- Pyro probe coupled with linear quadrupole ion trap dopant-assisted atmospheric pressure chemical ionization mass spectroscopy (LQIT-dAPCI-MS).
- Pyrolysis furnace combined with bio-crude oil (BCO) multidimensional gas chromatography (MDGC), cassava rhizome (CR), cassava stalk (CS), hydrothermal liquefaction (HTL), and above 400 °C is hydrothermal gasification (HTG). Hydrothermal carbonization (HTC), gas chromatography (GC), liquid chromatography (LC), high-performance liquid chromatography (HPLC), Fourier transform infrared (FTIR), proton (H^1), and carbon (^{13}C) Fourier transform-nuclear magnetic resonance (FT-NMR) spectroscopies, gel permeation chromatography (GPC), capillary electrophoresis (CE), dopant-assisted atmospheric pressure chemical ionization time-of-flight mass spectroscopy (dAPCI-TOF MS) linear quadrupole ion trap dopant-assisted atmospheric pressure chemical ionization mass spectroscopy (LQIT-dAPCI-MS).
- Synchrotron-based vacuum ultraviolet photoionization mass spectroscopy (SVUV-PIMS).
- Pyrolysis combined with single-photon ionization time-of-flight mass spectroscopy (SPI-TOFMS) system.
- Microreactor for pyrolysis connected with molecular beam mass spectrometer (MBMS).
- TAP (Temporal analysis of products) reactor connected with imaging photoelectron photo ion coincidence spectroscopy (iPEPICO).

Hundreds of organic compounds such as alkanes, aromatic hydrocarbons, phenol derivatives, ketones, esters, amines, alcohols, ethers, and sugars with a molar ratio higher than 1.5 for H/C. Most of the organic compounds present in bio-oil come from the different biomass fractions, as shown in Fig. 10.2. A complete fraction of bio-oil present in the literature is mentioned in Table 10.3. Inorganic species are present in trace amounts. Inorganic component of bio-oil includes calcium, silicon, potassium, iron, aluminum, sodium, sulfur, phosphorous magnesium, nickel, chromium, zinc, lithium, titanium, manganese, lanthanum, barium, vanadium, chlorine, etc. (Goyal et al. 2006; Isahak et al. 2012).

10.3 Fast Pyrolysis Reactor/Pyrolyzer

In previously found research, various reactors have been employed to enhance bio-oil production. These types of reactors are not just limited to biomass waste, but they are also compatible with plastic waste, tires, and other related waste (Isahak et al. 2012; Basu 2010; Cai et al. 2021; Jahirul et al. 2012; Lewandowski et al. 2019).

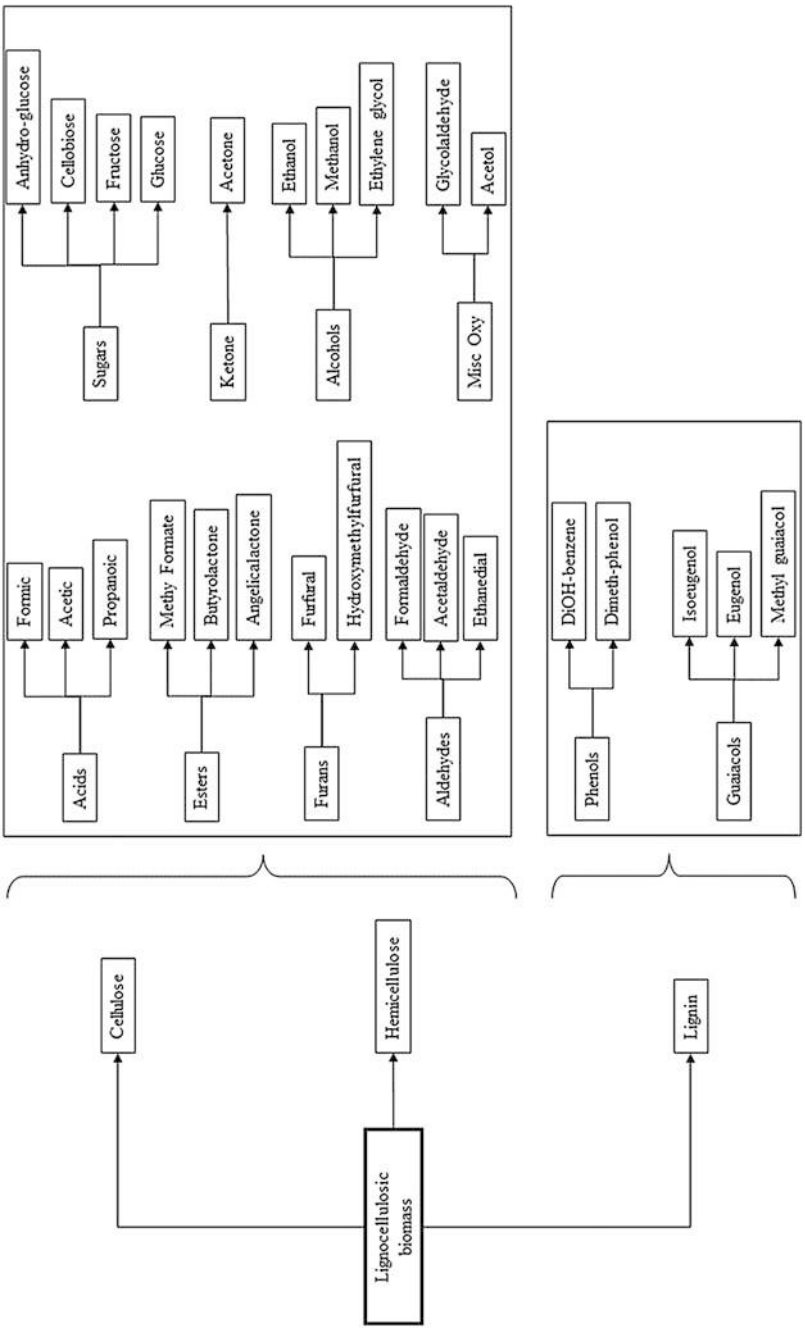

Fig. 10.2 Various components of bio-oil from the different fractions of biomass (Liu et al. 2020)

Table 10.3 The organic groups that are present in bio-oil (Goyal et al. 2006)

Functional group	Related compounds
Acids	Formic, acetic, hexanoic, benzoic, propanoic, etc.
Alcohols	Methanol, ethanol, iso-butanol, 2-propene-1-ol, etc.
Aldehydes	Formaldehyde, acetaldehyde, pentanal, 2-butenal, ethanedial, etc.
Alkenes	2-methyl propene, dimethyl cyclopentene, alpha-pinene, etc.
Aromatics	Benzene, toluene, naphthalene, xylenes, phenanthrene, fluoranthene, chrysene, etc.
Esters	Methyl formate, butyrolactone, methyl propionate, methyl n-butyrate, velerolactone, etc.
Furans	Furan, 2-methyl furan, furfural alcohol, 2-furanone, furfural, etc.
Guaiacols	2-methoxy phenol, 4-methyl guaiacol, eugenol, ethyl guaiacol, etc.
Ketones	Acetone, 2-butanone, 2-cyclopentanone, 2-pentanone, 2-hexanone, 2,3 pentanedione, cyclo-hexanone, etc.
Nitrogen compounds	Ammonia, methylpyridine, methylamine, pyridine, etc.
Other oxygenates	Hydroxy acetaldehyde, dimethyl acetal, hydroxy acetone, acetal, methyl cyclopentenone, etc.
Phenols	Phenol, methyl-substituted phenols
Sugars	Levoglucosan, glucose, D-arabinose, fructose, D-xylose, etc.
Syringols	Hydroxy acetaldehyde, dimethyl acetal, hydroxy acetone, acetal, methyl cyclopentenolone, etc.

10.3.1 Fixed Bed Reactor

A fixed bed reactor for pyrolysis works similarly as in the case of the gasifier. It is a feasible system for small-scale combined heat and power applications. This type of reactor is equipped with a gas cooling system and cleansing system. Cleansing systems consist of the cyclone, wet scrubber, and dry filters as shown in Fig. 10.3. The raw material is fed from the top of the reactor and comes in contact with the gas stream moving upward, which is counter to the movement of raw material. Generally, steel, concrete, or firebricks can be used for the construction of this type of reactor. Fuel introducing unit, ash removal unit, and the gas outlet are also incorporated into this reactor. It operates at the lower side of gas velocity, and ash carries over. A recent study also shows the usage of catalysts for further conversion of tar (Chopra and Jain 2007; Jahirul et al. 2012; Zaman et al. 2017).

Laboratory scale fixed bed reactors deals with a minimal amount of sample, which is in grams. This type of reactor operates in batch mode. They can be either horizontal or vertical reactor types. In some cases, these reactors are combined with a catalyst for the enhancement of bio-oil production. An approximate representation of a fixed bed reactor for fast pyrolysis is shown in the given Fig. 10.4. The system consists of the following parts: the feeder, the reactor, the catalysis portion, and the condensation part. The catalyst is in the lower portion of the reactor in a basket. Construction material for pyrolysis reactor is generally stainless steel. Product, i.e.,

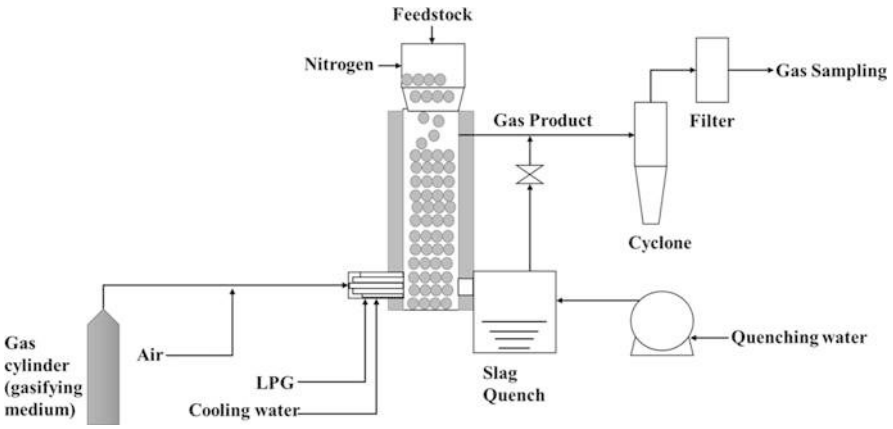

Fig. 10.3 A pilot-scale fixed-bed system (Chopra and Jain 2007)

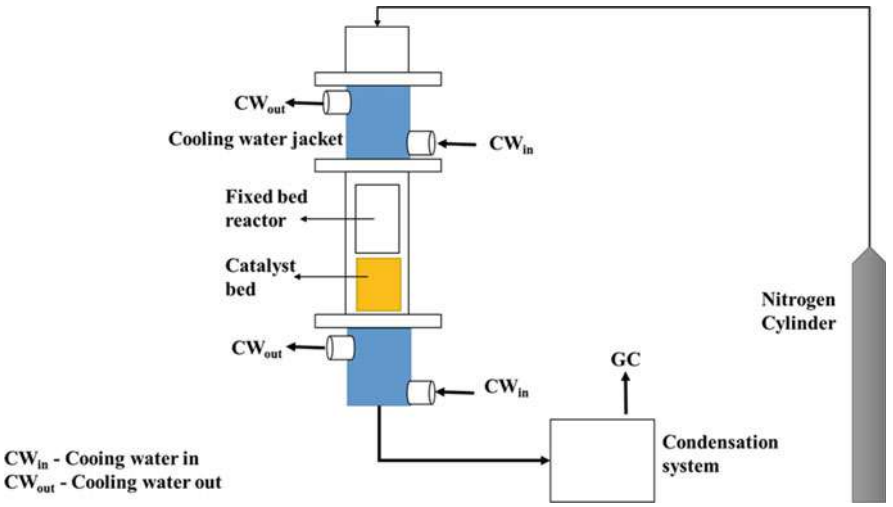

Fig. 10.4 Laboratory scale fixed bed reactor system with catalyst bed for fast pyrolysis (Cai et al. 2021)

gasses (contain bio-oil and flue gas), flow out of the reactor, whereas char remains within the reactor (Cai et al. 2021). Fixed bed combined with other technologies such as induction, microwave, catalyst, and solar, which can be modified to induction-heating fixed bed reactor, microwave fixed bed reactor, two-stage catalytic fixed bed reactor, and solar fixed bed reactor, respectively (Lewandowski et al. 2019; Tsai et al. 2006).

10.3.2 Fluidized Bed Reactor

In fluidized bed reactors, pressurized gas passes through the solid bed of feedstock, creating a solid-gas mixture that increases the contact area between solid and gas. This system provides a reasonable heat transfer rate, better reaction control, vapor residence time, high relative velocity between gas and solid phase. The various categories of fluidized bed types are bubbling fluidized bed reactor, circulating fluidized bed reactor, conical spouted reactor, internally circulating fluidized bed reactor, and rapid fluidized bed reactors (Jahirul et al. 2012; Lewandowski et al. 2019).

10.3.2.1 Bubbling Fluidized Bed Reactor (BFBR)

An illustration of BFBR is shown in Fig. 10.5. Fine particle of biomass is fed on hot fluidized sand. Fluidization is achieved either by inert gas or recycled flue gas. Fluidization velocity ranges from 0.5 to 3.0 m/s to keep the particle bed under suspension. Overhead space in the reactor, also known as the freeboard, can be greater than the diameter of the particle bed in size. The range of bio-oil yield is between 70% and 75%. Biochar is separated rapidly and does not accumulate in the

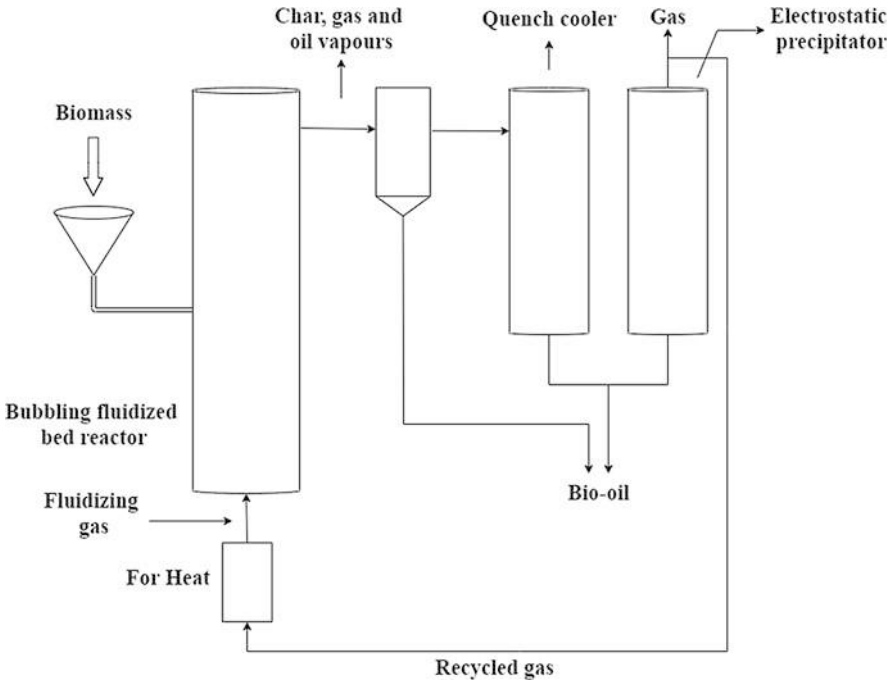

Fig. 10.5 Bubbling fluidized bed reactor (Mohan et al. 2006)

bed. This type of reactor shows product quality and consistent performance (Jahirul et al. 2012; Mohan et al. 2006; Basu 2010). Wang et al. (2018a, b) studied the combined effect of alkali metal oxides and HZSM-5 in fast pyrolysis of bamboo sawdust in a BFBR, combined with a fixed bed reactor of catalyst for enhancing the quality of bio-oil.

10.3.2.2 Circulating Fluidized Bed Reactor (CFBR)

A schematic representation of CFBR has been shown in Fig. 10.6. The working principle is similar to the bubbling fluidized bed reactor. It provides short vapor residence time the uniform temperature distribution with reasonable control. The regime of hydrodynamic for this reactor is referred to as a fast bed. Gas velocity in this reactor is higher than BFBR. The particle bed of this reactor is enlarged, and it has a loop seal which provides an external loop for solids, hence recyclization of the particle occurs, which in turn increases the particle residence time in comparison to BFBR (Basu 2010; Zaman et al. 2017; Lewandowski et al. 2019).

10.3.2.3 Conical Spouted Bed Reactor (CSBR)

Alvarez et al. (2014, 2016), performed fast pyrolysis of sewage sludge and rice husk in a conical spouted bed reactor. A pilot-scale plant for this type of reactor has been picturized in the given Fig. 10.7. CSBR can be used for the pyrolysis of lignocellulosic biomass, tires, plastics, and other waste. The feedstock of irregular textures, sticky solids, and fine particles can be handled easily. It provides low vapor residence time in the spout zone and a little higher in the annulus zone. Bio-oil yield is above 60% for rice husk (Alvarez et al. 2014, 2016). It can be modified to CSBR with an integrated superheater, CSBR with a separate superheater, CSBR

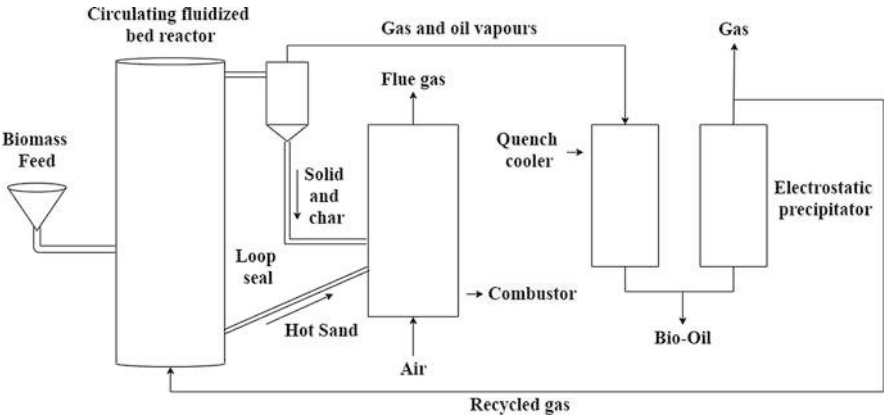

Fig. 10.6 Circulating fluidized bed reactor (Mohan et al. 2006)

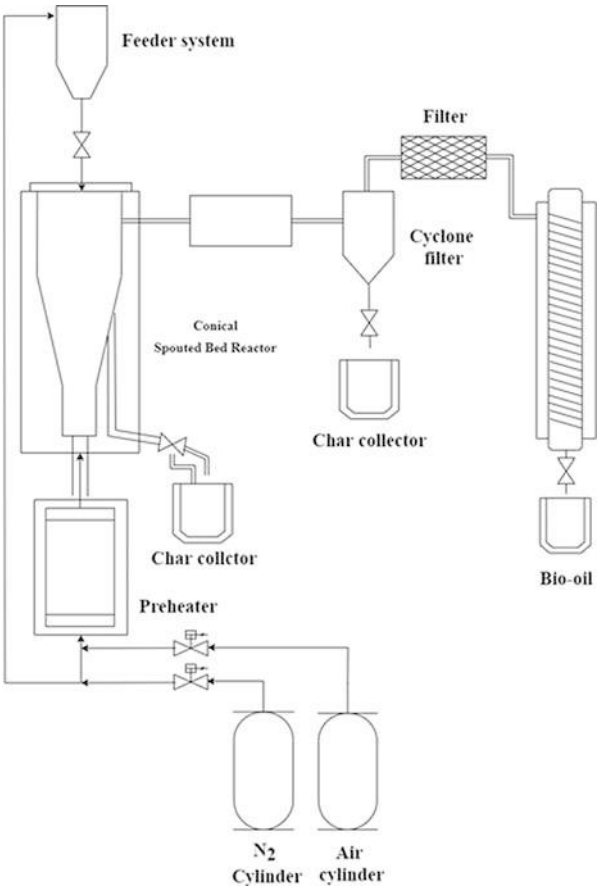

Fig. 10.7 Conical spouted bed reactor (Lewandowski et al. 2019)

with draught tube, CSBR with catalyst, CSBR under vacuum, dual conical spouted reactor (Lewandowski et al. 2019; Fernandez-akarregi et al. 2014).

10.3.2.4 Internally Circulating Fluidized Bed Reactor (ICFBR)

It is classified into the category of ultra-fast or flash pyrolysis due to meager vapor residence time. Here riser and sand heater are combined in a single unit. The raw material is introduced with the riser gas and meets the hot sand particle carried from the annulus to the mixing zone at the bottom of the riser, as shown in Fig. 10.8. The temperature here ranges between 780 and 860 °C with a residence time of 400–600 ms, and the yield for volatile is greater than 90%. It can also produce oil from plastics and tires (Briens et al. 2008; Lewandowski et al. 2019).

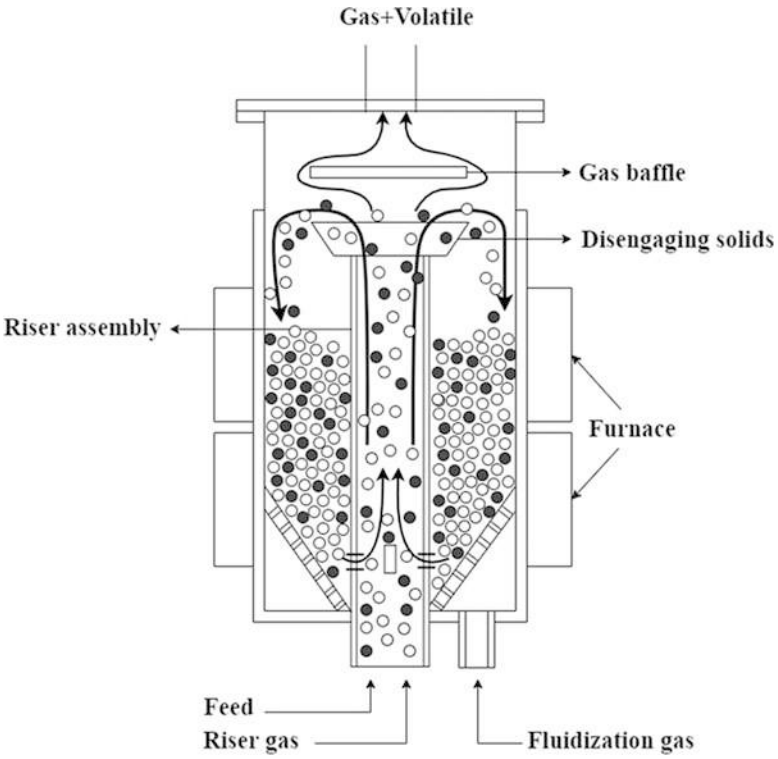

Fig. 10.8 Internally circulating fluidized bed reactor (Lewandowski et al. 2019)

10.3.2.5 Rapid Fluidized Bed Reactors

This type of reactor is used for rapid thermal pyrolysis (RTP). Here feedstock is introduced to high-velocity uprising hot sand (sometimes referred to as Fast Fluidizations or Jet Spouted Beds) at 500 °C, and then it is cooled down within seconds. The heating rate is 1000 K/s and vapor residence time ranges from a few hundredths of milliseconds to 5 s. Here bio-oil yield for wood is in the range of 83% (Lewandowski et al. 2019).

10.3.3 Ultra-Rapid Pyrolyzer

This type of reactor provides a high heating rate, very short residence time and generates a high bio-oil yield. Given Fig. 10.9. shows a pictorial representation of an ultra-rapid pyrolyzer. It provides a concise mixing time (10–20 ms), short residence time (70–200 ms), and quench time (20 ms). Despite the 650 °C temperature of the reactor, liquid yield can reach up to 90%. Preheated inert gas at 100 °C is made to

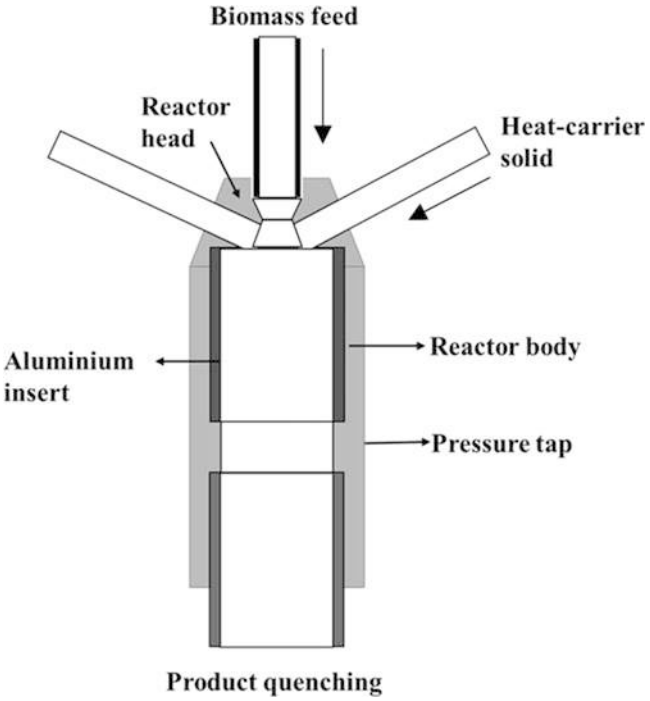

Fig. 10.9 Ultra-rapid pyrolyzer (Basu 2010)

colloid with biomass stream in the reactor. Externally heated thermal carrier, i.e., sand, is bombarded on biomass which enters through multiple jets. This type of collision creates a very high heating rate, and biomass is pyrolyzed within a millisecond. The vapor leaving the reactor is quenched quickly to restrict the cracking or secondary reaction to prevent gas conversion (Basu 2010).

10.3.4 Free-Fall Reactor

The free-fall reactor is also referred to with many other terms: drop tube reactor, entrained flow reactor, a flow reactor, tubular reactor, and a downflow reactor. Biomass or other feedstock is fed from the top of the reactor, which is co-current with the gas stream and falls under gravity. During the fall, sample gets pyrolyzed along the way. It is a relatively simple design for a reactor and easy to scale up; that is why many researchers adopt it. High temperature in the reactor is achieved either using electrical heating or combustible gas such as air and propane mixture. A schematic diagram for a fast pyrolysis system with a free-fall reactor is shown in Fig. 10.10 (Lu et al. 1994; Jones et al. 2009; Papari and Hawboldt 2013; Billaud et al. 2016). Pattiya et al. (2012) worked on a free-fall reactor for fast pyrolysis on

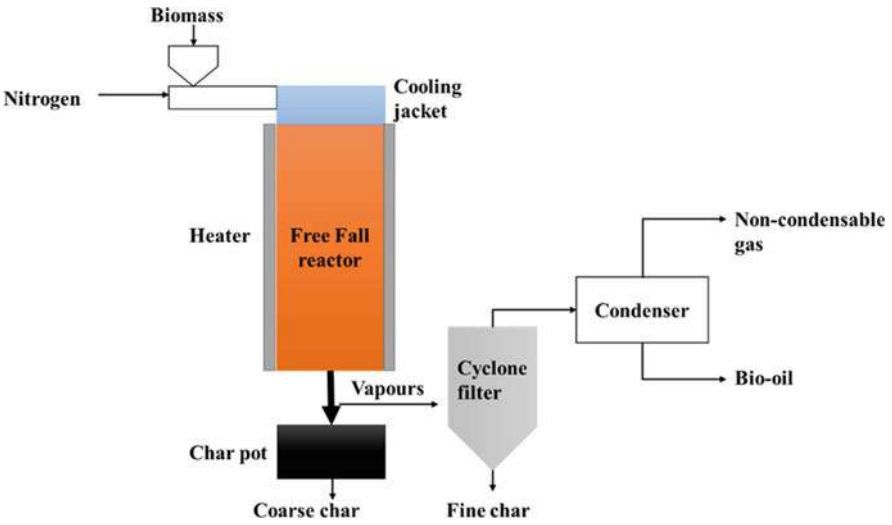

Fig. 10.10 Free-fall reactor (Papari and Hawboldt 2013)

sugarcane and cassava residues. The bio-oil yield of 70% can be produced through fast pyrolysis of cassava stalk in a free-fall reactor at 450 °C.

In a study by Mok et al. (1985) demonstrated increased heating efficiency of biomass by using Thermovortactor on the top of the reactor. Two conical vessels termed vortical contactors, or Vortactors, are used with the entrained flow reactor. One Vortactor at the top of the reactor transfers the heat from preheated nitrogen gas or suspended particles known as Thermofor stream to biomass feed. Other Vortactor supplied with liquid nitrogen, known as cryovortactor, is present at the end of the reactor for fast quenching of vapor products. (Mok et al. 1985).

10.3.5 Rotating Cone Pyrolyzer

This reactor has been in development since the early 1990s in The Netherlands at the University of Twente. Heat carrier, i.e., sand and the biomass feed, is introduced from the top and falls on the base of a rotating cone that revolves at 360–390 revolutions per minute and provides excellent mixing. Biomass pyrolyzed in a small annular volume at a very high heating rate of 5000 K/s. The particle moves spirally along the hot wall up to the lip of the cone due to centrifugal force. A solid particle containing char and sand is spilled over the lip of the cone and generated vapors containing bio-oil move towards the condenser through the other tube. Spilled hot sand and char move towards the bottom of the reactor, where char is combusted in a fluidized bed, as shown in Fig. 10.11. This helps in heating the cone and sand. It provides a short residence time for vapor (0.3 s), and an excellent bio-oil yield ranges from 60% to 70% on dry feed as this reactor does not require any inert

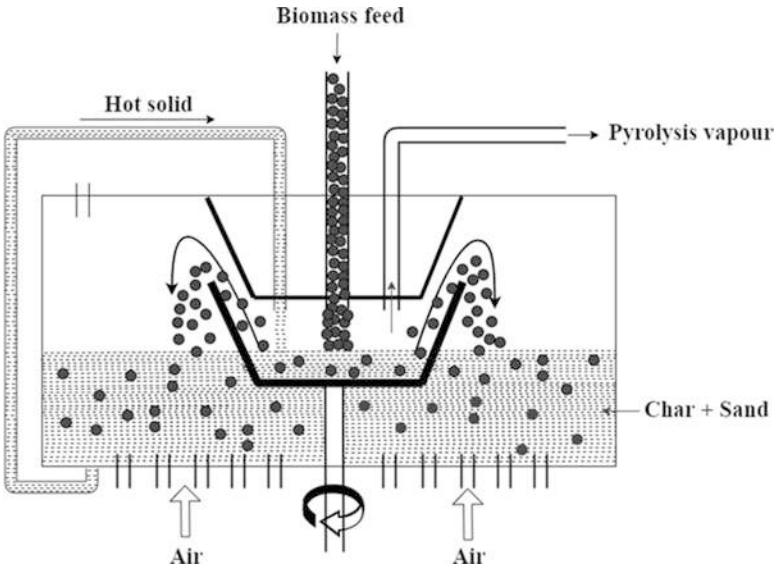

Fig. 10.11 Rotating cone pyrolyzer (Basu 2010)

gas, resulting in small downstream equipment. In Malaysia, a plant with a rotating cone pyrolyzer is commissioned to operate at a capacity of 2×10^6 tons per day using empty fruit bunch of palm oil. (Isahak et al. 2012; Jones et al. 2009; Basu 2010).

10.3.6 Ablative Pyrolyzer

This type of pyrolyzer comes in the category of advanced type of fast or flash pyrolysis. Here heat transfer takes place through direct contact with a hot rotating disk or a hot surface of the reactor. Biomass is pressed or impacted against this hot surface through mechanical pressure. This type of pyrolyzer can handle large particles compared to another pyrolyzer. Depending upon the requirements, size can vary up to 20 mm. Mainly two types of reactor come under this: Vortex type reactor and rotating disk reactor (Lewandowski et al. 2019; Jahirul et al. 2012).

10.3.6.1 Vortex Reactor

In a vortex reactor biomass particle is fed along with a stream of inert gas or steam as shown in Fig. 10.12. It is injected tangentially into the cyclone reactor (cylindrical reactor) at high pressure with a velocity of 400 m/s. The biomass particle faces the high temperature of the reactor wall which is in the range of 625 °C. High centrifugal

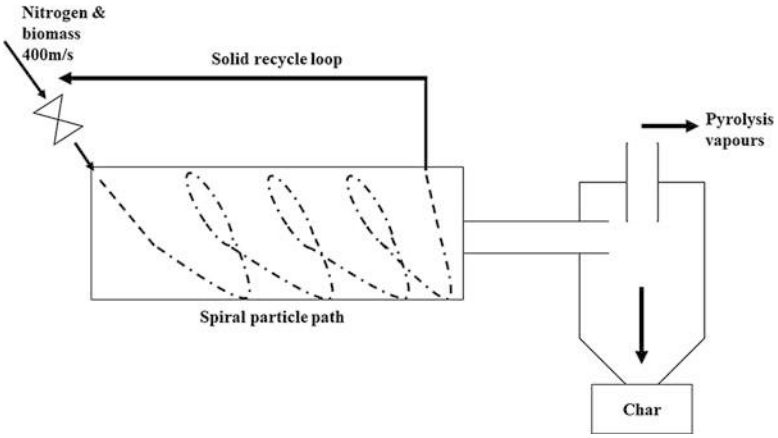

Fig. 10.12 Vortex reactor (Lu et al. 1994)

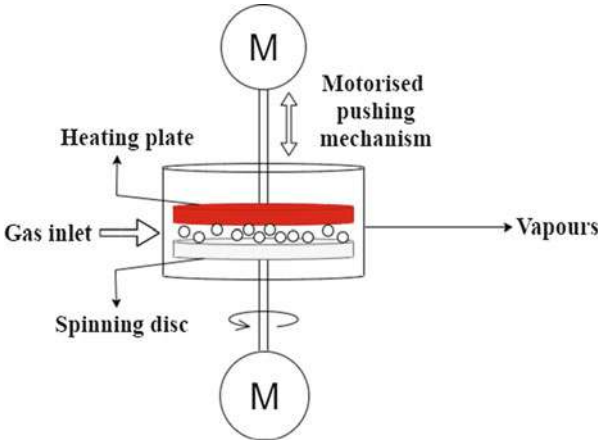

Fig. 10.13 Rotating disk reactor (Cai et al. 2021)

force is experienced by the particle and induces a high ablation rate. The liquid film is formed at the wall of the reactor and evaporates rapidly. Generated vapor is carried away by sweeping gas very quickly within 50–100 milliseconds. Particles that are not converted but are recycled through the recycle loop. Complete conversion can be seen in 15 cycles and the bio-oil yield reaches up to 80% (Lewandowski et al. 2019; Lu et al. 1994; Jahirul et al. 2012).

10.3.6.2 Rotating Disk Reactor

In this type of reactor, biomass is fed forcefully on top of a hot rotating disk. Biomass is pyrolyzed when it comes in contact with the rotating disk, which melts the biomass and produces vapor, as shown in Fig. 10.13. An inert gas may or may not

be used as sweeping gas. Biomass does not require extensive grinding and can save energy. The yield of bio-oil from this reactor of large size biomass is similar to that of a fluidized bed with a particle size of less than 1 mm (Cai et al. 2021; Jahirul et al. 2012; Jones et al. 2009).

10.3.7 Auger Reactor or Moving Bed Reactor

Auger reactor is also called a moving bed reactor, which uses a screw-based reactor that helps move and pyrolyze the biomass particles in a cylindrical tube heated in an oxygen-free environment, as shown in Fig. 10.14. This reactor reaches a temperature range of 400–800 °C, responsible for the devolatilization of biomass particles. This reactor can handle a large amount of biomass, also provides reasonable control of residence times, and generates a high quality of bio-oil. The screw in the reactor can be modified into Twin Screw (a twin-screw mixer reactor) and a concentric screw (dual concentric rotary cylinder reactor) (Jahirul et al. 2012; Henrich et al. 2016; Fu et al. 2018; Cai et al. 2021).

10.3.8 PyRos Reactor

PyRos reactor is equipped with a rotational filter within a cyclonic reactor to produce particle-free bio-oil, as shown in Fig. 10.15. The working temperature range is 450–550 °C, and residence time varies between 0.5 and 1 s to stop further cracking. Biomass particles mixed with preheated sand particles (an inert material) in the ratio of 1:15. A mixture of sand that acts as a heat carrier, biomass, and inert gas is fed into the reactor. Pyrolysis is achieved using direct contact with the heat carrier. Solid particles and gas are separated with the help of centrifugal force. Solid particles are

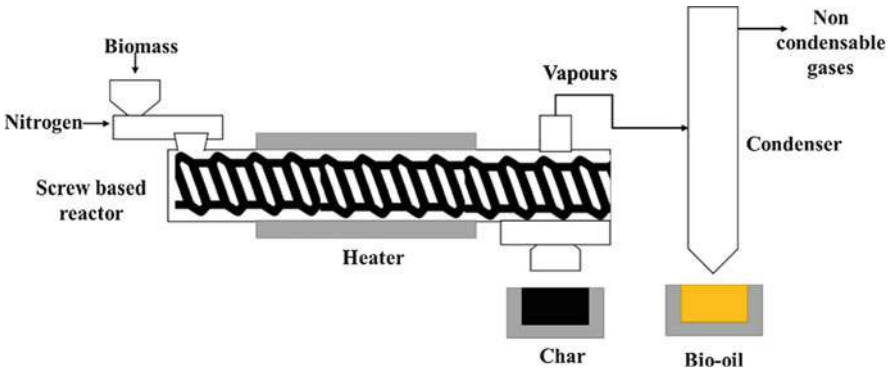

Fig. 10.14 Auger reactor or moving bed reactor (Cai et al. 2021)

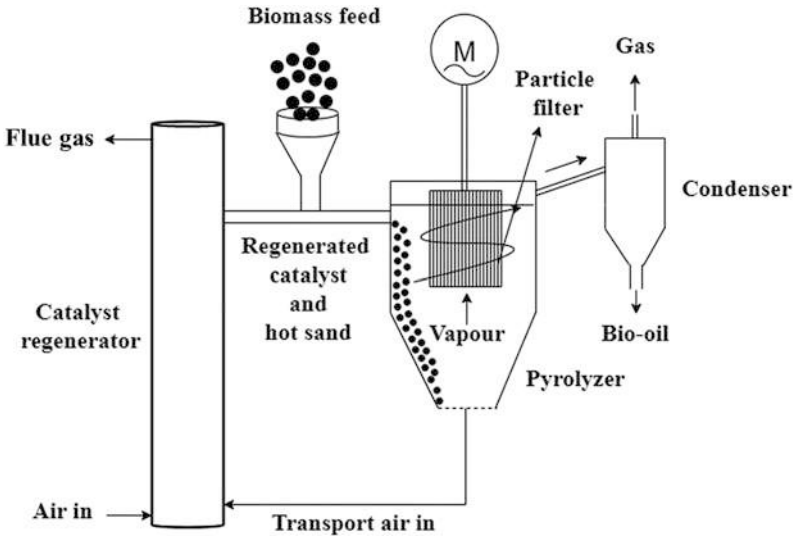

Fig. 10.15 PyRos reactor (Brem and Bramer, 2007)

thrown towards the wall, which slides down. During this time, efficient heat transfer takes place which results in devolatilization. The solid residue is sent back to the regenerator to complete the cycle. The removed gas is passed through a rotational filter, particle-free vapor from the reactor is transformed into bio-oil using a condenser. This reactor produces bio-oil yield of 70–75%. Also, it is low-cost and compact in comparison to other reactors. Along with inert material or heat carrier, a catalyst such as sodium-based alumina can also be used in this reactor (Brem and Bramer, 2007; Jahirul et al. 2012; Imran et al. 2018).

10.3.9 Multiple Hearth or Vacuum Pyrolysis Reactors

Vacuum pyrolysis is combined with a multiple hearth reactor commonly known as a vacuum pyrolysis reactor. Rector is divided into various sections, as shown in Fig. 10.16. The top section of the hearth has a temperature of 200 °C whereas the bottom section of the hearth is around 400 °C. Here, biomass is fed from the top of the multiple hearth reactor. Biomass moves downward due to gravity and a mechanically driven scraper. During downward movement, it passes through various sections of different temperatures. At the bottom of the reactor, only char remains, which can be removed easily. The vapor is removed instantaneously from the reactor using a vacuum pump that works at a 1 kPa pressure. In this process, the heat transfer rate is lower, but vapor residence time is shorter. Due to the low heating rate, bio-oil yield ranges from 30 to 45%, which is very low compared to other reactors. Various

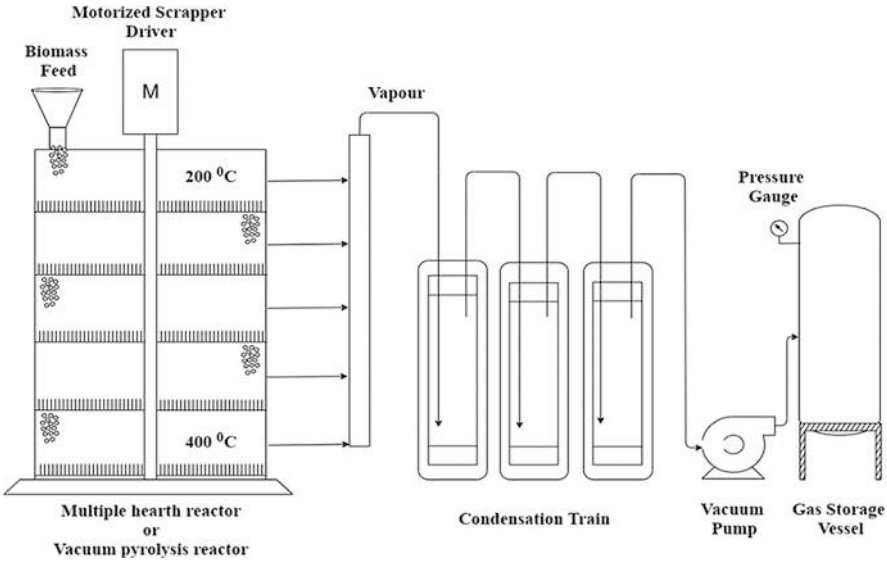

Fig. 10.16 Multiple hearth or vacuum pyrolysis reactor (Lu et al. 1994; Basu 2010)

study shows that scaling up of this reactor will involve considerable expense due to high capital cost and maintenance cost as it will require an increased capacity vacuum pump and large mechanical transporter (Lu et al. 1994; Basu 2010; Isahak et al. 2012).

10.3.10 Microwave Reactor

Microwave-assisted fast pyrolysis reactor consists of a quartz reactor placed inside a microwave oven, as shown in Fig. 10.17. As discussed earlier, it can be either premixed or non-premixed microwave-assisted fast pyrolysis. In both cases, absorbed microwave energy is used to pyrolyze the sample. This type of reactor has efficient heat transfer, heating process with exponential control, and enhanced chemical reactivity. Sometimes hotspot appears in the microwave, which is responsible for the production of syngas. A wide range of biomass and industrial waste can be used as feedstock in this reactor, generating a high yield of bio-oil and syngas. Various modification is seen in microwave-assisted pyrolysis, such as the recent study by Wang et al. (2018a, b) demonstrated continuous fast microwave pyrolysis, and Zhang et al. (2018) worked on two-step microwave-assisted catalytic fast pyrolysis (Jahirul et al. 2012; Wang et al. 2018a, b; Zhang et al. 2018; Lewandowski et al. 2019).

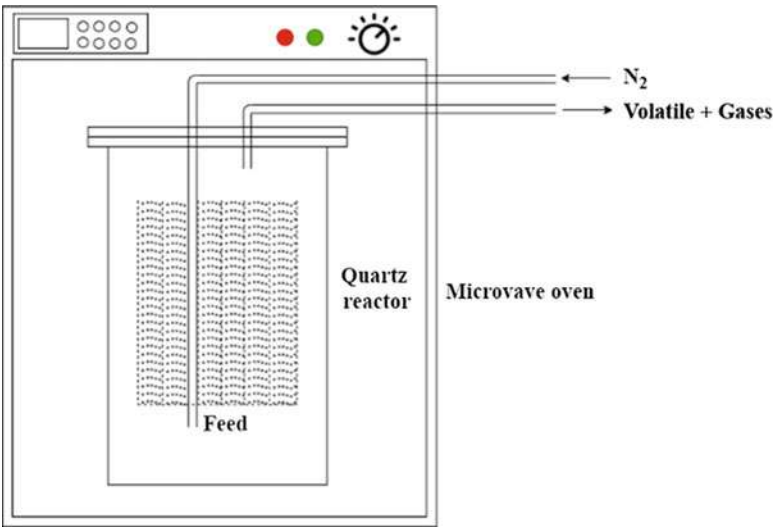

Fig. 10.17 Microwave reactor (Lewandowski et al. 2019)

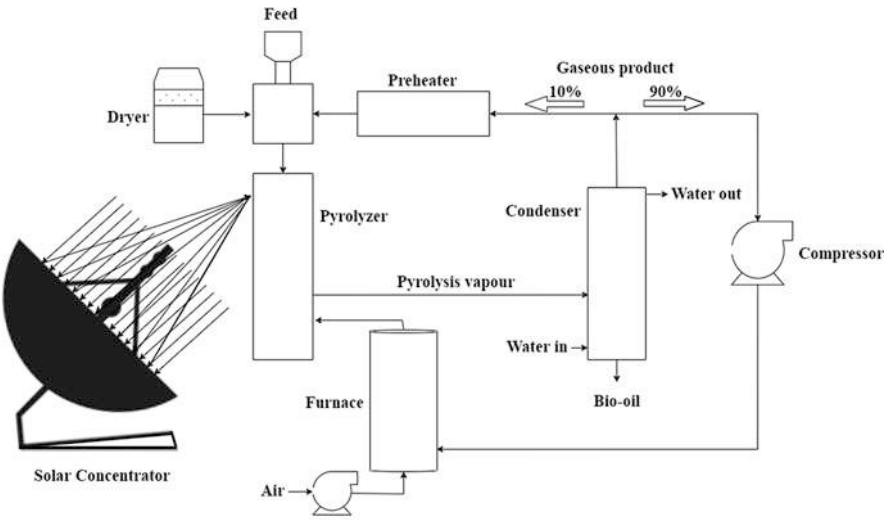

Fig. 10.18 Solar pyrolyzer (Joardder et al. 2014)

10.3.11 Solar Reactor

The construction material that is used for the reactor is quartz. The reactor is opaque, located at the focal point of the concentrator, as shown in Fig. 10.18. The reactor temperature can reach higher than 700 °C. Solar reactors offer a quick start-up and

shut-down period in comparison to slow pyrolysis reactors. Joardder et al. (2014) showed the various arrangements of the reactor and solar concentrator. The reactor can be equipped with rotating capability and sliding capability along with a solar concentrator for continuous heating (Jahirul et al. 2012; Joardder et al. 2014).

10.3.12 Plasma Reactor

A plasma reactor is made from quartz in the form of a cylindrical tube surrounded by copper electrodes. Biomass is fed in the middle of the tube using a screw feeder present at the top of the tube as presented in Fig. 10.19. The reactor is filled with inert gas (nitrogen, argon) to create an oxygen-free environment. Electrical energy is provided through electrodes to generate plasma from the inert gas. This plasma is used to pyrolyze or gasify the biomass present in the tube. From the reactor, pyrolysis vapors are removed using a variable speed vacuum pump. On the one hand, the high operating cost of the reactor and high electricity consumption are limitations of this process and on the other hand, it generates high energy density within the reactor. This form of energy creates a high temperature that eliminates the tar formation by cracking (Tang and Huang 2005; Van Oost et al. 2006; Jahirul et al. 2012).

10.3.13 Pulse Shock Tube

Fast pyrolysis can also be performed on a shock tube using pulses of high energy. This process focuses on producing tar-free gas, mainly CO_2, CO, CH_4, C_2H_2, and

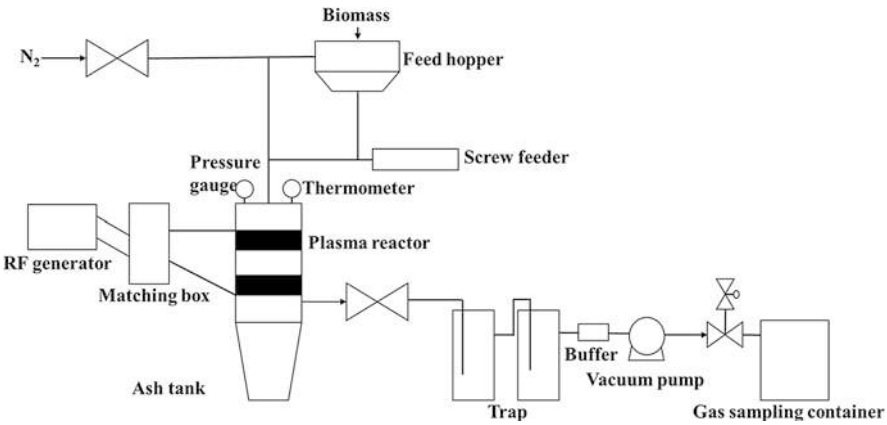

Fig. 10.19 Line diagram of a radio frequency (RF) plasma pyrolysis rector system (Tang and Huang 2005)

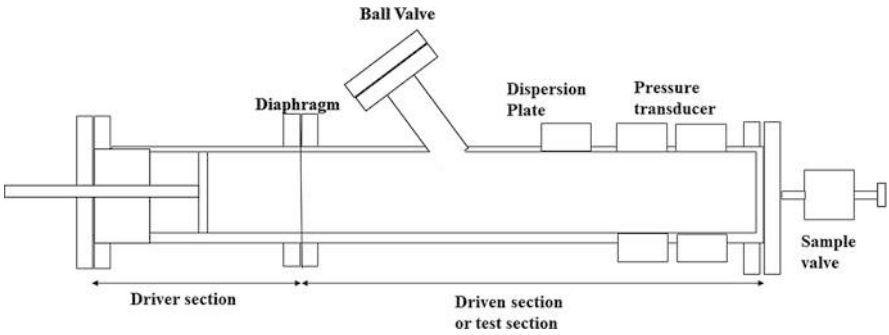

Fig. 10.20 A schematic of a shock tube (Mebklin 1994)

C_2H_4. The temperature in the shock tube is greater than 700 °C. The heating rate can reach as high as 10^4 °C/s, and the reaction time is less than 0.5 s. The shock tube consists of two main sections: the driver and test (driven unit) sections separated from the flanged diaphragm, as shown in Fig. 10.20. Helium was used in the driver section as high-pressure gas, and argon was used in the driven section as a low-pressure gas. Diaphragm gets ruptured due to high pressure from the driver section, which creates a shock wave that heats the sample in the test section and is heated again by reflected shockwave to reach the desired temperature for fast pyrolysis (Mebklin 1994).

10.4 Enhancing Bio-Oil Properties

Raw bio-oil or unprocessed pyrolysis oil is not fit for direct application. During bio-oil storage for an extended period, it may lead to oxidation that deteriorates bio-oil quality. Due to the high viscosity of bio-oil balancing pressure drops during its transports, it can be overcome at the expense of high maintenance of the instruments or equipment. Low pH indicates the acidic nature of bio-oil that can lead to corrosion of storing equipment. The increased presence of various component that differs in chemical nature is the reason for phase separation or layering, which is responsible for creating hindrance during filtration. The presence of alkali metal in the bio-oil leads to depositions in the boilers and in combustion chambers that affect the equipment's efficiency. High moisture content in bio-oil reduces calorific value and stability, proceeds to phase separation, and complicates in nature of viscosity. To eliminate these problems bio-oil needs to be upgraded. It requires some chemical change for stabilization, viscosity reduction, deoxygenation, and reduction of molecular weight that can be achieved using various upgradation techniques (Gollakota et al. 2016; Jacobson et al. 2013).

10.4.1 Bio-Oil Pretreatment Methods

In some cases, pretreatment of bio-oil is required for further upgradation. Pretreatment includes the removal of moisture, and fine particles (char) that improve viscosity, calorific value, density, and reduce catalyst poisoning. Fine particles can be removed using a centrifuge. Moisture can be removed from bio-oil through various means such as distillation, dehydration, reactive rectification, and physico-chemical separation. (Wang et al. 2009; Jacobson et al. 2013).

Dehydration of bio-oil can be performed using the chemical sodium sulfate (Na_2SO_4). Na_2SO_4 was mixed with bio-oil in a ratio of 1:3. This technique removes water partly and also increases the concentration of sulfur in bio-oil. In reactive distillation, lower molecular weight carboxylic acid (volatile organic acid) is converted to ester and is separated using reactive distillation. Also, a layer of water and the non-volatile compound is formed. This process shows the water reduction from 33% to 11%. Fractionation of bio-oil can be done with the help of excess water when a large quantity of water is added in bio-oil two-layer form. The top layer consists of high polarity compounds and some carbohydrate derivatives (Jacobson et al. 2013).

Fine particles can be removed directly from vapor termed as hot vapor filtration (HVF) and if the fine particle is removed from bio-oil then it is termed liquid phase filtration. HVF can be in situ or ex situ; in situ filtration is performed within the reactor, and ex situ filtration is performed outside the reactor. Liquid filtration is achieved using a membrane (ceramic) after pyrolysis condensation (Kumar and Strezov 2021).

10.4.2 Upgradation Technique

There are various methods as mentioned below for upgrading bio-oil or transforming it into a resourceful fuel. (Patel and Kumar 2016; Zhang et al. 2013; Kumar and Strezov 2021; No 2014):-

- Catalytic pyrolysis
- Hydroprocessing
  - Hydrotreating
  - Hydrocracking
- Steam reforming
- Supercritical fluids
- Esterification
- Emulsification and blending
- Molecular distillation
- Electrochemical or electrocatalytic upgrading of bio-oil

10.4.2.1 Catalytic Pyrolysis

The fast pyrolysis process with catalyst, also called the catalytic process, provides upgraded bio-oil. Mainly, oxygenated molecules are transformed into less oxygenated molecules or hydrocarbons. There are three variants of this process that can be found in the literature which are mentioned below (Kumar et al. 2019):

- The in situ catalytic upgrading process involves mixing feedstock and catalyst before feeding it to the reactor, as shown in Fig. 10.21(a). This type of upgradation is generally incorporated with fixed bed reactor, fluidized bed

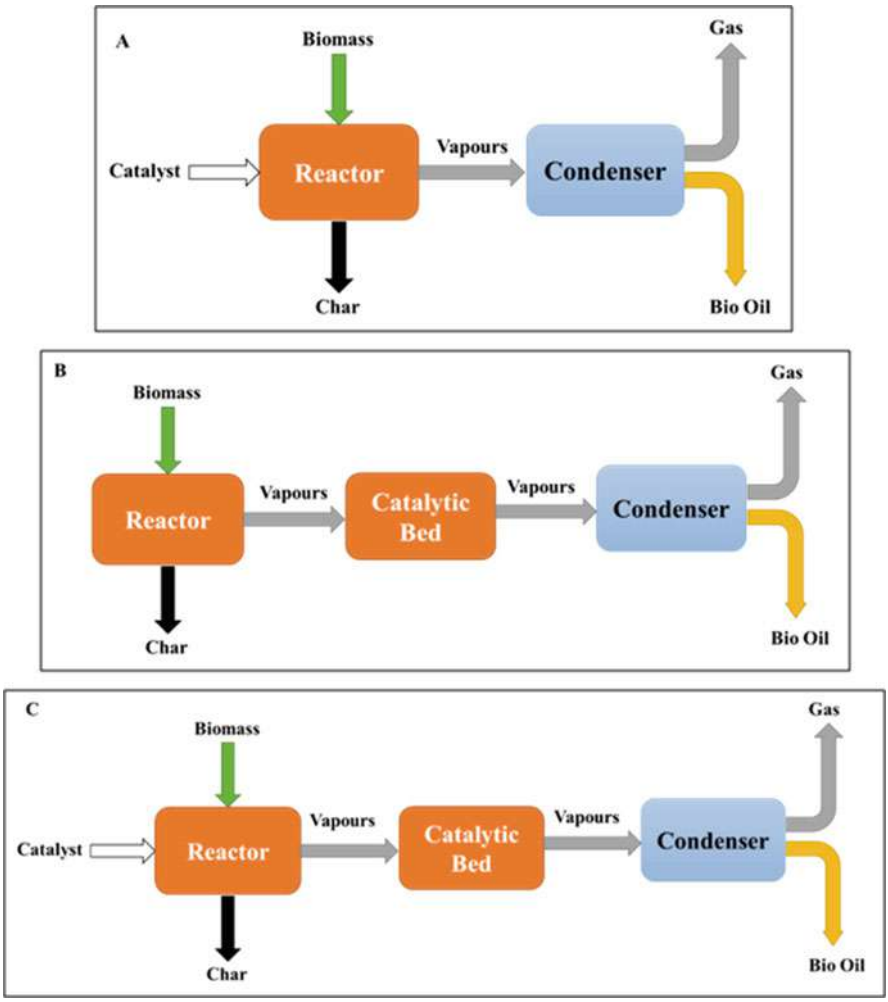

Fig. 10.21 Above picture represents various configurations of catalytic processes: in situ (**a**) ex situ (**b**) and combined in situ and ex situ (**c**) process. (Imran et al. 2018; Kumar et al. 2019)

reactor, microwave reactor, etc. The required catalyst is high to maximize the contact between generated vapors and the catalyst surface. This can be either in continuous or batch reactors. (Fan et al. 2018; Imran et al. 2018; Wang et al. 2014).

- Ex situ catalytic upgrading process: The pyrolysis and catalytic (reactor with catalytic bed) reactors are separate. Here vapor generated from the pyrolysis reactor is passed through the downstream catalytic reactor for upgradation, as shown in Fig. 10.21(b). Individual reactors provide reasonable control over temperature. Due to separate reactors, vapor does not mix with char during the upgradation process. Catalyst consumption is lower in comparison to the in situ method (Fan et al. 2018; Imran et al. 2018).
- Combination of in situ and ex situ process: As the name suggests, it is the combination of in situ and ex situ processes as shown in Fig. 10.21(c). The combined upgradation technique can achieve remarkable transformation of the oxygenated compound. Kumar et al. (2019) performed a study on a combined process and completed 98% conversion of oxygenated compounds higher than the individual in situ and ex situ processes (Kumar et al. 2019).

Conventional catalytic cracking in the petroleum industry referred to the transformation of heavy distillate to light and medium distillate. The same process can be used to reform the large and highly oxygenated molecule of bio-oil. This process is set up under high pressure, temperature above 350 °C in the presence of a catalyst (e.g., HZSM-5) along with hydrogen gas. Catalytic cracking has been performed in two patterns. One is only catalytic cracking, and the second is the combination of catalytic pyrolysis and catalytic cracking (Jacobson et al. 2013; Zhang et al. 2013; Baloch et al. 2018).

10.4.2.2 Hydroprocessing

Conventional catalytic cracking in the petroleum industry referred to the transformation of heavy distillate to light and medium distillate. The same process can be used to reform the large and highly oxygenated molecule of bio-oil. This process is set up under high pressure, temperature above 350 °C in the presence of a catalyst (e.g., HZSM-5) along with hydrogen gas. Catalytic cracking has been performed in two patterns. One is only catalytic cracking, and the second is the combination of catalytic pyrolysis and catalytic cracking (Jacobson et al. 2013; Zhang et al. 2013).

Hydroprocessing, also referred to as catalytic hydrogenation, is the combined process of hydrotreating and hydrocracking, as shown in Fig. 10.22 (Patel and Kumar 2016).

- Hydrotreating unit: The preliminary stage for removing heteroatoms from the feed before introducing them to the hydrocracking unit. Here, the reaction occurs in the presence of hydrogen and catalyst, whereas temperature varies between 300 and 400 °C and pressure is in the range of 50–200 bars. It involves the following reactions (Patel and Kumar 2016; Jacobson et al. 2013).

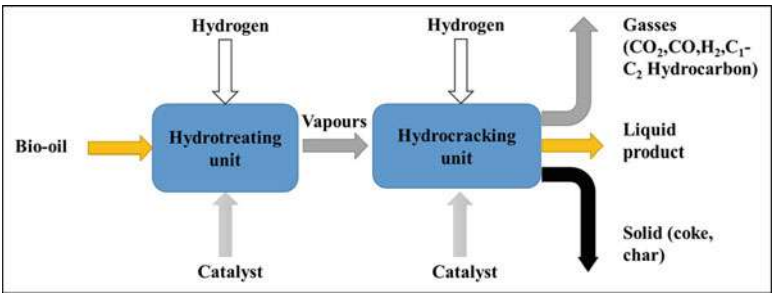

Fig. 10.22 Hydroprocessing flow diagram (Patel and Kumar 2016)

– Hydrodesulfurization (HDS): It involves the removal of sulfur from the compound and transforming it into hydrogen sulfide as mentioned in the given reaction:

$$R - SH + H_2 \rightarrow R - H + H_2S \tag{10.1}$$

– Hydrodenitrogenation (HDN): It involves the removal of nitrogen from the compound and transforming it into ammonia as mentioned in the reaction:

$$\text{Pyridine} + H_2 \rightarrow \text{Pentane} + NH_3 \tag{10.2}$$

– Hydrodeoxygenation (HDO): The hydrotreating process can be of high severity, meaning complete hydrodeoxygenation, and low severity, which means partial hydrodeoxygenation depending upon temperature. This process removes oxygen from the compound and converts it to water, as mentioned in the reaction:

$$R - OH + H_2 \rightarrow R - H + H_2O \tag{10.3}$$

– Hydrodearomatization (HDA): It involves transforming polynuclear aromatics into three rings, then two rings, then one ring, and then the end products (The Crankshaft Publishing, n.d.).
– Hydrodemetallation/Hydrodemetallization (HDM): Removal of metals and transforming them to relative metal sulfide (The Crankshaft Publishing, n.d.; Jacobson et al. 2013).

- Hydrocracking unit: This unit further improves the quality of bio-oil. Reaction in this unit occurs at elevated temperature and pressure in the presence of hydrogen and catalyst. The following reactions take place in this unit:

– Hydrodealkylation: It involves the removal of an alkyl group from the compounds as shown in the reaction:

$$R - C_6H_5 + H_2 \rightarrow C_6H_6 + R - H \tag{10.4}$$

– Hydrocracking: Cleavage of high molecular weight compound results in the formation of two lower hydrocarbons as mentioned in the reaction:

$$R1 - CH_2 - CH_2 - R2 + H_2 \rightarrow R1 - CH_3 + R2 - CH_3 \tag{10.5}$$

– Isomerization of alkanes: It involves rearrangement of molecules such as:

$$\text{Pentane} \rightarrow \text{Isopentane} \tag{10.6}$$

– Hydrodecyclization: Reduction of the number of cyclic compounds.

• Another reaction takes place in both units.

  – Decarboxylation: Removal of a carboxylic acid group from the compound.

$$R - COOH \rightarrow R - H + CO_2 \tag{10.7}$$

– Decarbonylation: Removal of aldehyde group from the compound.

$$R - CHO \rightarrow R - H + CO \tag{10.8}$$

– Water−gas shift reaction:

$$CO_2 + H_2 \rightarrow CO + H_2O \tag{10.9}$$

– Methanation: Carbon monoxide and carbon dioxide convert to methane in the presence of hydrogen.

  – Coke formation:

$$\text{Polyaromatics} \rightarrow \text{Coke} \tag{10.10}$$

10.4.2.3 Steam Reforming

Steam reforming or catalytic steam reforming process helps to produce hydrogen from bio-oil simultaneously upgrading the bio-oil in the presence of steam in a temperature range of 350–1000 °C. This process is affected by steam to carbon ratio, temperature, and material space velocity. A high amount of steam leads to a water-gas shift reaction. At low temperatures, generated hydrogen reacts with carbon

monoxide to form methane. The problem associated with this is the formation of coke that deactivates the catalyst. A fluidized bed reactor or fixed bed reactor is generally used in this process. Other reactors are spouted bed reactor, tubular quartz microreactor, and combined two-stage pyrolysis-reforming reactor. Sometimes the process can be modified to use two reactors of the exact nature or different nature, for example, two-stage fixed bed reactor, fixed bed reactor combined with fluidized bed reactor, spouted bed reactor combined with fluidized bed reactor (Kumar and Strezov 2021; Zhang et al. 2013; Wu et al. 2008).

10.4.2.4 Supercritical Fluids Extraction

Supercritical fluids (SFCs) provide a medium to upgrade the bio-oil with low viscosity and high calorific value. Various solvents such as ethanol, methanol, water, and CO_2 are used to improve the quality of bio-oil. Further enhancement in bio-oil can be achieved by adding catalysts such as aluminum silicate and HZSM-5. Upgrading of bio-oil can be performed with an autoclave reactor. SCFs, catalysts, and bio-oil are mixed and fed into the reactor. The reactor is purged with a gas (e.g., hydrogen) to remove oxygen. Then the reaction is performed at high temperature (563 K) and high pressure (2 MPa) with a certain value of residence time (5 h). The disadvantage of this process is that it is only applied on a laboratory scale, due to economic feasibility it cannot be applied on a large scale (Li et al. 2011; Zhang et al. 2013).

10.4.2.5 Esterification

The presence of carboxylic acid creates an opportunity to transform it into ester with the help of catalytic esterification. The process improves bio-oil quality by lowering its viscosity, acid number, and water content. Its stability and corrosion properties are also promoted. This process is similar to the supercritical fluids process. The only difference is the priority of targeting species, i.e., carboxylic acids, and the temperature, which is lower in comparison to SFCs (60 °C) (Hong-you et al. 2010; Zhang et al. 2013).

10.4.2.6 Emulsification and Blending

Emulsification and blending are the physical upgrading techniques for bio-oil. In emulsification, two immiscible liquids are mixed, where one liquid forms tiny droplets and suspends on the second liquid. If droplet size is between 1 and 10 μm, it is simply emulsion, and when droplet size is in the range of 1–100 nm, it is known as a microemulsion. Bio-oil is emulsified with diesel and biodiesel in a particular ratio along with surfactant (Span 80), and co-surfactants (methanol) that decrease the viscosity increases the calorific value, and cetane number. Mixing can

also be performed using ultrasound. Emulsified diesel and biodiesel can be introduced as fuel for the combustion engine. Bio-oil blended with diglyme and alcohols (such as methanol ethanol and isopropyl alcohol), decreases the value of viscosity, flashpoint, pour point, and density but increases its heating value (No 2014; Zhang et al. 2013; Kumar and Strezov 2021).

10.4.2.7 Molecular Distillation

It is also termed short path distillation because the distance between evaporation and condensation surface that is less than the mean free path of the molecule. This process is performed under a high vacuum to reduce the boiling point of the liquid. Due to a very short path molecule that is evaporated, condensed before even their collision with another molecule. Wang et al. (2009) use the molecular distillation apparatus KDL-5 developed by UIC Corporation, Germany. Bio-oil was divided into three categories, i.e., light middle and heavy fraction. The bio-oil recovery rate was higher than 97% (Wang et al. 2009).

10.4.2.8 Electrochemical or Electrocatalytic Upgrading of Bio-oil

This process is also termed electrolytic hydrogenation converts carbonyl and oxygenated compounds into valuable hydrocarbon. It uses an electrolytic cell that comprises cathode and anode compartments divided by a membrane. It can be either a cationic or anionic exchange membrane. Water is reduced to proton and oxygen at the anode, and bio-oil is hydrogenated through the addition of proton and evolution of hydrogen at the cathode as shown in Fig. 10.23. This evolved hydrogen can be

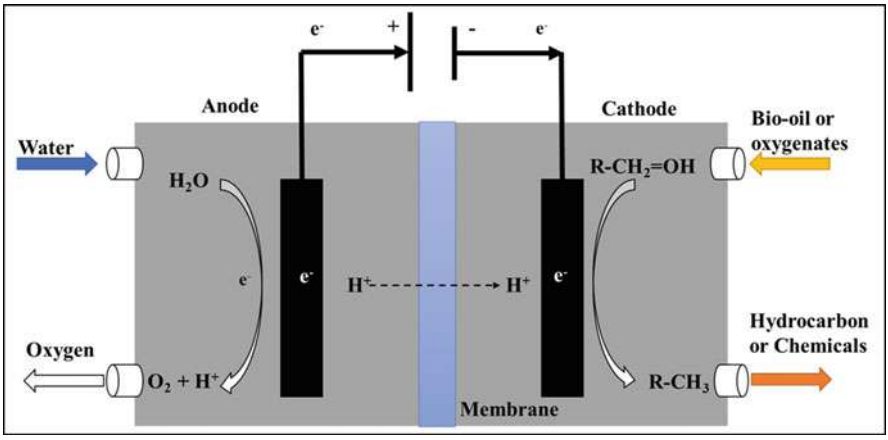

Fig. 10.23 Bio-oil upgradation through the electrochemical cell (Kumar and Strezov 2021)

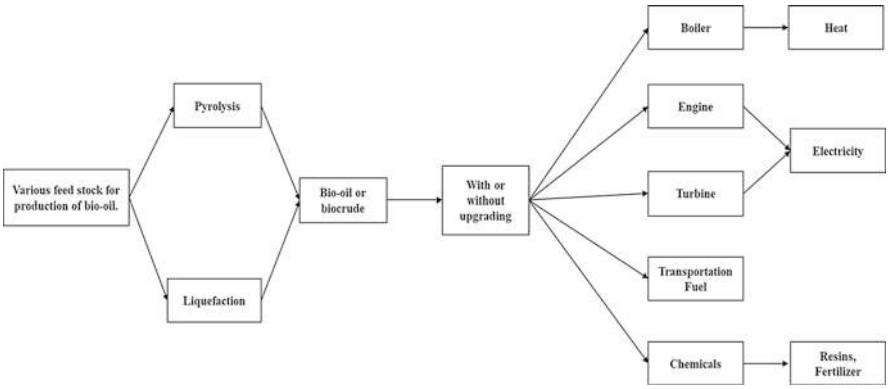

Fig. 10.24 Production to application of bio-oil (No 2014; Jahirul et al. 2012)

reduced by increasing the potential that increases conversion efficiency (Kumar and Strezov 2021).

10.4.3 Applications of Bio-oil

Upgraded bio-oil has the following application (Fig. 10.24) (Gollakota et al. 2016):

- It can be used as fuel for combustion.
- It can be used as fuel for power generation.
- It can be used as a transportation fuel (directly or blended with diesel).
- It can be used for the production of chemicals, resins, and anhydrous sugars (levoglucosan).
- It can be used as a binder for briquetting and palletization.
- It can be used as a wood preservative.

10.5 Techno-Economics and Environmental Sustainability of Bio-oil

Techno-economics analysis of production and upgradation of pyrolysis oil for various applications has already been stated in multiple research projects. Generally, the techno-economic analysis includes capital cost, operating cost, and cost of production per unit. Wright et al. (2010) studied the techno-economics for producing transportation fuel from biomass through fast pyrolysis. The plant was operated at 2000 tpd (ton per day) corn stover capacity. The upgradation technique involves in this process is hydroprocessing. Two scenarios involved one where hydrogen for upgradation is manufactured within the system using bio-oil, and the other is where

Table 10.4 Comparison of two processes (Wright et al. 2010)

Type of process	Includes hydrogen production unit	Includes purchased hydrogen
Capital cost	$287 million	$200 million
Operating cost	$109 million	$123 million
Fuel yield	134×10^6 L/year	220×10^6 L/year
Cost of fuel (equivalent to gasoline)	$3.09/gallon or $0.82/L	$2.11/gallon or $0.56/L
Production of bio-oil	104×10^6 gallon/year or 394×10^6 L/year	104×10^6 gallon/year or 394×10^6 L/year

hydrogen was purchased for upgradation. Various techno-economic parameters are discussed in Table 10.4 (Wright et al. 2010).

Another techno-economic simulation was performed on in situ and ex situ upgradation technique of pyrolysis oil with a plant capacity of 2000 MTPD (metric ton per day) by Li et al. (2015). Bio-oil yield generated was 438, 439 kg per BDMT (bone dry metric ton), and biofuel yields were 152.6 and 143.5 liters per dry metric ton (Li et al. 2015).

Zhu et al. (2014) simulated the techno-economic analysis of biofuel produced by upgrading the bio-oil derived from hydrothermal liquefaction. Here bio-oil was upgraded through hydroprocessing which involves hydrocracking and hydrotreating. Here plant capacity is 2000 MTPD of feedstock. The total capital cost of the plant turned out to be $512 million, whereas the operating expense was $100.64 million. The minimum fuel selling price comes out to be $4.44 per gallon gasoline equivalent. It was also mentioned that the cost of biofuel is not competitive enough to beat the pricing of conventional gasoline (Zhu et al. 2014).

Lam et al. (2019) studied a microwave non-premixed pyrolysis system to produce bio-oil using activated carbon and under a vacuum. As the process is non-premixed, activated carbon is used as the radiation absorber before feedstock (waste plastic and waste cooking oil) is fed to the reactor. Two studies were performed on a lab scale; one is in the presence of nitrogen, and the other is without it. Results have shown that without using nitrogen in the process, the liquid yield was high. Process without nitrogen was scaled up from 0.1 kg/h to 100 kg/h. Total capital cost and the annual operating cost were $3146 and $30,233. The cost of a liquid fuel unit per liter was found to be $0.25 (Lam et al. 2019).

Hsu (2012) has structured a study on LCA (life cycle assessment) on diesel and gasoline produced through fast pyrolysis of forest residue and hydroprocessing as an upgradation technique. GHG emission in the case of gasoline derived from bio-oil was 117 g/km, which is much lesser than conventional gasoline 300 g/km (in 2005), and net energy value (NEV) was 1.09 MJ/km. In the case of diesel derived from bio-oil, GHG was 98 g/km, and NEV was 0.92 MJ/km. If the hydrogen and electricity generation plant were built within the system, the GHG emission could be further lowered to 62 g/km for diesel and 74 g/km for gasoline (Hsu 2012).

Fan et al. (2011) estimated the GHG emission on conventional Rankine power plants using fast pyrolysis bio-oil and fossil fuel. In this scenario, CO_2 that was absorbed by feedstock was also considered. Study shows that GHG emission for pyrolysis oil was 77–99% lower than that of fossil fuel. A more efficient system can be developed by generating electricity within the system and using it in pyrolysis (Fan et al. 2011).

Shemfe et al. (2016) investigated the greenhouse gas (GHG) emission generated by one-ton biofuel production from the upgradation (hydroprocessing and zeolite cracking technique) of bio-oil obtained from the fast pyrolysis of *Miscanthus*. This was further categorized based on soil organic carbon (SOC) sequestration rates (feedstock CO_2 absorption): excluding (SOC), low (SOC), and high (SOC) for the best and worst scenarios. Hydroprocessing technique for worst-case generated 738, 627, and − 268 kg CO_2 equivalent per ton of biofuel for exclusion, low and high SOC, respectively. For the best case 390, 212, and −1221 kg CO_2 equivalent per ton of biofuel was generated for exclusion, low and high SOC, respectively. Similarly, it was calculated for zeolite cracking for the worst scenario it was 1328, 1104, and −697 kg CO_2 equivalent per ton of biofuel was generated for exclusion, low and high SOC, respectively, and for the best-case scenario, it was 627, 268, and −2614 kg CO_2 equivalent per ton of biofuel was generated for exclusion, low and high SOC, respectively (Shemfe et al. 2016).

10.6 Conclusion

This study mentions that there are no boundaries from where we can take the feedstock to produce bio-oil or pyrolysis oil. Still, most research focus on biomass, animal manure, or non-organic wastes such as plastics. Recent advances provide enough data to justify that fast pyrolysis and hydrothermal liquefaction are the best source for bio-oil production. In terms of economic feasibility, hydrothermal liquefaction may not be a good idea to scale up without some improvements. To understand the complex fast pyrolysis process, various advance experimental setups are present that help in the understanding of the process on a molecular level. On large-scale setup and lab-scale setup, various fast pyrolysis reactors or pyrolyzers are mentioned in various studies that can be used to produce bio-oil. However, some of the reactors do not come to the category of fast pyrolysis, but their bio-oil yield is high, such as (multiple hearth reactor) and some of the reactors are under the category of fast pyrolysis reactor (plasma reactor and pulse shock tube), but their product priority is other than bio-oil, i.e., gases. A thorough study on the reactor should be attained before applying any reactor to the field, either for research or industrial application. Upgrading technology also showed promising results. Upgrading the bio-oil for various applications, not just for fuel, but various chemicals that can also be obtained from the bio-oil. It was also mentioned in some of the studies that upgraded biofuel has been used as a fuel for transportation and electricity generation. Most of the studies over techno-economics of the process

discussed are simulated, but they provide an idea for total capital and annual operating costs. In terms of environmental sustainability, it was shown in many studies that GHG emissions are lower in comparison to fossil fuels. This is mainly because a large amount of CO_2 is absorbed during plant's growth period. The above study provides enough information showing the possibility of fossil fuel independency from various stationary and mobile application sectors, which can be moved towards the bio-oil sector.

Competing Interests All the authors declare that they have no competing interests.

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Chapter 11
An Overview on Organosolv Production of Bio-refinery Process Streams for the Production of Biobased Chemicals

Veluru Sridevi, Dadi V. Suriapparao, Hemanth Kumar Tanneru, and K. S. N. V. Prasad

Abstract The potential of renewable energy and chemical sources is more important than ever before due to the combination of diminishing crude oil supplies and population increase. The bio-refinery concept is evolving from a fascinating notion to a viable replacement for a variety of fossil-fuel-based goods. Pre-treatment processes designed for a comprehensive bio-refinery shall show selective dissociation of each constituent of a biomass feedstock, ease of access to and detachment of the constituents after separation, high yield revival of every component, process components readily available for conversion into chemicals with negligible purification, as well as economic feasibility. These criteria are typically met by organosolv pre-treatments. To be broadly accepted by markets and the public, the generation of renewable chemicals, as well as biofuels, should be price and performance competitive employing crude oil-derived counterparts. The focus of this study is on developing a biomass conversion technique that maximizes the transformation of lignocellulosic biomass into commercially viable high-value products, allowing for effective translation to an economically feasible commercial process.

Keywords Bio-refinery · Biomass feedstock · Economy · Organosolv technology · High-value products

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P. Verma (ed.), *Thermochemical and Catalytic Conversion Technologies for Future Biorefineries*, Clean Energy Production Technologies,
https://doi.org/10.1007/978-981-19-4312-6_11

Abbreviations

AFEX Ammonia fiber explosion
IEA International Energy Agency
LCB Lignocellulosic biomass
LHW Liquid hot water

11.1 Bio-refineries

The scientific community's major goals for the development of green-type businesses are bio-refineries as well as biobased "green" chemistry. Another goal of the bio-refinery is to maximize or optimize the economic, environmental, and social advantages by utilizing all synergies for an efficient and viable operation. The projected future expansion of biofuel industry, as well as the development of novel biofuel production technologies, need the construction of the new integrated bio-refineries.

The present work on biodiesel, ethanol, and sophisticated biofuel production to displace a fraction of the vast quantity of shipping gasoline and diesel consumed yearly in the USA addresses the energy objective. Fuel, however, is a low-value product despite its enormous volume. As a result, achieving the bio-refinery's economic aim will be difficult due to the low return on investment on the biofuel-only operation (Goswami et al. 2021; Kumar and Verma 2021a**,** b). Integrating chemical products into bio-refinery's portfolio, however, poses two major problems. The manufacturing of biobased chemicals is hampered by a lack of conversion technologies. (i) Renewable carbon to chemical conversion is the least developed and most difficult of all bio-refinery activities when compared to nonrenewable hydrocarbon conversion methods. The existence of the requisite conversion technology is not required for promising hypothetical situations involving fuels and chemicals. (ii) An oversupply of targets poses a difficulty to biobased chemical synthesis. Engineering process analysis is great for setting pricing objectives and selecting technologies that provide the best possibilities for the research investment as the molecular configuration of the intended product is understood. Yet, because of the basic difference between chemical research and fuel, these analytical approaches are less helpful when applied to multi-product chemical scenarios (Fig. 11.1).

Bio-refinery is a novel word that refers to two primary topics: bioproducts and bioenergy, both of which are critical to a more biobased society. The use of lignocellulosic materials in bio-refineries as a substitute for fossil-fuel refineries necessitates effective fractionation and product recovery technologies (Bhardwaj et al. 2021a, b; Kumar et al. 2020). The focus of the bio-refinery idea for biomass use has changed from the construction of more or less energy-driven bio-refineries to considerably more flexible facilities that can generate chemicals and energy carriers.

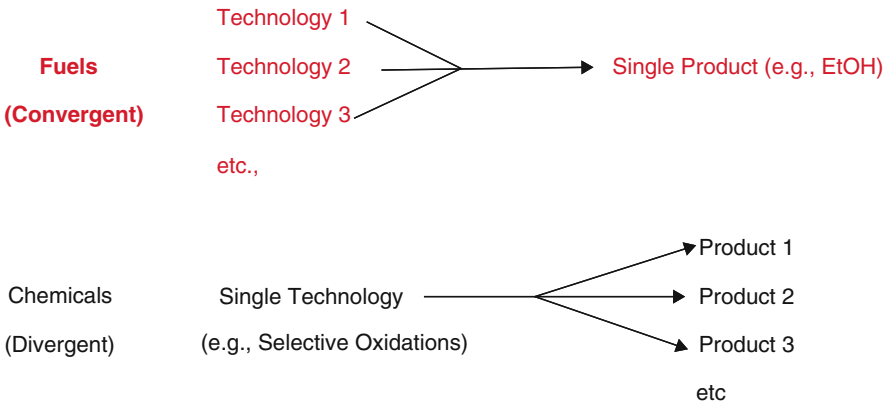

Fig. 11.1 Biobased fuels and chemicals research methods

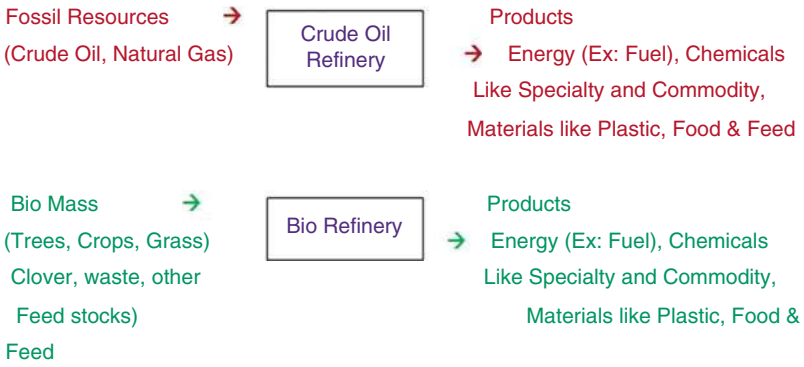

Fig. 11.2 Biomass as a renewable feedstock for bio-refineries

According to the huge number of articles published, biomass pre-treatment is critical for the full-scale implementation of the bio-refinery idea.

Bio-refinery is defined as "the viable conversion of biomass into a variety of biobased goods and bioenergy" by the International Energy Agency's (IEA) Bioenergy Task (Fig. 11.2) (International Energy Agency 2012). It implies that a bio-refinery can be an idea or a facility or a process or a plant, or a cluster of facilities that necessitates the integration of several disciplines of expertise, including chemical engineering, biomolecular engineering, chemistry, biology, and biochemistry (Clark et al. 2006). If a fully integrated strategy is established, biorefining can give a viable route to value products while also improving biomass processing costs and environmental footprint.

The growing biobased economy is a very attractive industry with a lot of potential for the future and a lot of business prospects (Luoma et al. 2011; Mehariya et al.

Table 11.1 A list of problems that must be overcome for bio-refineries to be implemented successfully

Issues	Key points
Increase the size of the project to an industrial level	It necessitates a considerable financial commitment. Strong financial motivation is required. Investors believe the return on investment is too low
Uncertainty about the future	Laws and rules are ambiguous
Construction and design	Plant construction delays equipment evaluations.
Availability of biomass	Ensure that appropriate resources are available at all times of the year Possibility of running on several materials
Supply and logistics	Reliable storage and transportation are required
Data for the development of processes	Data transfer from a small to a large scale
The degree to which a process has reached maturity	Handling and feeding under high pressures

2021). There are several definitions for bio-refinery, but in general, the term refers to the use of renewable raw resources (such as biomass) to generate energy and a variety of common commodities in an economically and environmentally sound manner (Dermibas 2009; Aresta et al. 2013; Himmel 2008; Agrawal and Verma 2022; Goswami et al. 2020). Fossil fuels are the world's primary energy source; nevertheless, the most pressing issue of one of the contemporary societies is reducing our reliance on fossil-fuel sources while also promoting rural development. The bio-refinery idea tries to use the same technologies which have been utilized to refine crude oil into biomass conversion (Dermibas 2009). Bio-refineries are viewed as a highly promising path to achieving our goals of long-term development and environmental preservation. Bio-refineries should comprise a variety of facilities, unlike oil refineries, which are huge industrial complexes (Rodrigues 2011). In the near future, renewable energy sources will be necessary for the long-term growth of our civilization (Dewulf and Van Langenhoven 2006). Plant biomass is the world's most abundant source of renewable resources and a major source of renewable energy.

Table 11.1 summarizes the obstacles that have been identified for the effective deployment of future bio-refineries. According to Sanford et al. (2016), the first step toward a major financial commitment is to scale up a solid small-scale operation into a large-scale bio-refinery. As a result, there has to be a very strong monetary advantage to justify the expenditure. Furthermore, investors understand the poor return on invested money as well as an uncertain future scenario, since regulations governing biofuels and bio-chemicals are still short-term stable, implying that the financial groundwork for major investments is not yet complete. They also find that many biotechnology firms that had a successful early-stage procedure have faced serious issues as a result of scale-up hurdles and the consequences of delays in building, testing, and operation. Capital expenditures have been reduced in certain situations by adapting existing facilities. A few of the significant factors are (i) biomass availability, which comprises a year-round flow of lignocellulosic

biomass at a reasonable price, as well as access to a biomass of a comparable sort to operate a bio-refinery within a limited working range. (ii) All logistics and a fully functional supply chain, such as transports of low-density materials and storage facilities, must be provided in order for continuous operation to be viable. The most significant expenditures connected with the bio-refinery idea are feedstock management and shipment. Feedstock accounts for 40–60% of full-scale biofuel production expenses (Humbird et al. 2011; Joelsson et al. 2016). Tao et al. (2011) analyzed 6 biomass pre-treatment methods from a process and techno-economic standpoint.

11.1.1 Processing for the Bio-Based Economy: Bio-refineries

The biggest hurdle to the expansion of the biobased chemicals industry, according to scientists, is the cost of manufacturing, which is still greater than their traditional equivalents. Furthermore, some biobased chemicals are still regarded as high risk in terms of upfront infrastructure investments. Policy interventions designed to stimulate these markets could include investment incentives, making the production of their fossil-fuel counterparts more expensive—for example, through carbon taxes—or attempting to make the use of biobased chemicals mandatory in certain industries, thereby increasing demand.

The goal of the bio-refinery idea is to make the most of plant components. Energy generation is not the main application of biomass in this idea, but rather an optional one. To maximize the value of available functions and biomass usage, feedstock selection, transportation, and biorefining processes are utilized. Intricate input-output chains aid in realizing the best social and economic outcomes. This is accomplished by first producing (small volume) high added value items, which are then followed by less-valued products (Fig. 11.3).

Green bio-refineries, which feed grass through a series of processing steps, provides a new option for grass feedstock processing. The mechanical separation of grass into a liquid phase containing soluble chemicals and a solid phase mostly

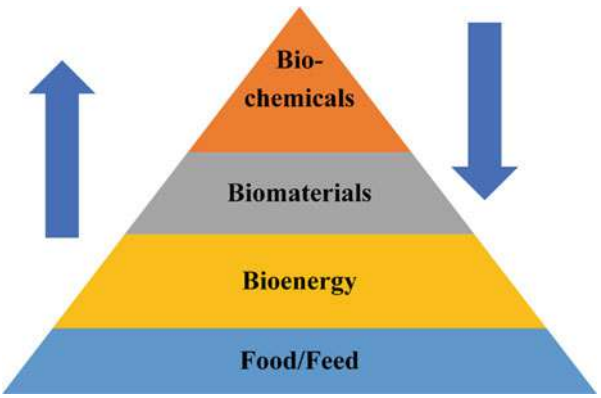

Fig. 11.3 Biobased "products" market pricing vs. market volume (De Jong et al. 2009). *The up arrow indicates Market Price and the down arrow indicates Market Volume*

Table 11.2 Characteristics of the most common bio-refinery types

Bio-refinery	Feedstock and conversion	impact	Remarks
Whole crop	Cereal crops, dry or wet milling	Although there is a link between monomer and polymer manufacturing, large-scale production competes with food production. Straw that may be used in lignocellulosic bio-refineries. Straw that may be used in lignocellulosic bio-refineries	Mainly from maize, wheat, moderately capital intensive
Oleochemical feedstock bio-refinery	Oil crops (rape seed, soybean, oil palm)	Links to production of chemicals, functional monomers, lubricants, and surfactants. Direct completion with food	Close to full commercialization. Capital intensity is moderate
Lignocellulosic bio-refinery	Lignocellulosic crops, residues of food and feed crops	Reduced completion with food, feed production, high water use efficiency, high potential for GHG emission reduction	Not yet on commercial scale. Capital intensive
Green bio-refinery	Grass	Links to the production of proteins, sugars, and fibers. No direct completion with food	R & D phase

composed of fibers is critical. The economic return of the fibers determines the bio-refinery's overall economic efficiency (De Jong et al. 2009). Table 11.2 summarizes the key features of the most common bio-refinery processes.

To make bio-refineries more cost-effective, chemicals made from biomass, a viable feedstock, are highly sought as a replacement for petrochemicals. Improved interest in becoming less energy reliant than in the past has been fueled by a dependency on fossil-based energy sources, diminishing crude oil accessibility, a desire to preserve the environment from devastating carbon emissions, and an ever-increasing global population. These concerns have prompted researchers to explore methods to replace crude oil derivatives with renewable resources. Biomass processing in integrated bio-refineries is the best way to compete with fossil-fuel refineries. To generate biofuels and biobased chemicals, the integrated bio-refineries used a variety of biomass feedstocks and conversion methods. A bio-refinery ought to require the most effective conversion methods for the production of high-value chemicals and biofuels from an industrial standpoint (Ragauskas et al. 2006a, b; Huber et al. 2006; Stephanopoulos 2007). Agricultural residues and wood chips, for example, are affordable renewable feedstocks for commercial large-scale bio-refineries because they are widely accessible and can store carbon.

11.2 Biomass Feedstocks

Hard and softwoods have long been the world's primary raw materials for pulp manufacturing, and they consistently produce high-quality results. However, due to a variety of causes, including the newly generated demand for bio-refinery applications, the cost of these raw materials has skyrocketed in the previous decade. As a result, alternative raw materials which can compensate for the absence of low-cost wood have been actively explored. As a result, each person should be able to select the best biomass source for bio-refinery applications. Plants with high productivity, like grasses, have the ability to provide low-cost biomass to fulfill present demand (Vilela et al. 1997; Vilela et al. 2001; Paulino et al. 2007; Mazzarella 2007). Presently, biomass sources are classified as either woody or non-woody. In North America, Europe, and other temperate regions, forest and agricultural wastes have been the primary targets. In tropical regions, such as Brazil, there is a lot of potential for using fast-growing, high-productivity woody and non-woody biomass to implement the bio-refinery idea.

Biomass as a variety of bio-refinery feedstocks, biomass from trees, agro-forest leftovers, grasses, plants, aquatic plants, and crops, as indicated in Fig. 11.4, is a flexible and essential renewable feedstock for the chemical industry. Plants convert CO_2 and water into primary and secondary metabolite bio-chemicals through the photosynthesis process. Both of these compounds are significant in the industry. Carbohydrates and lignin, known as lignocellulose, are the primary metabolites

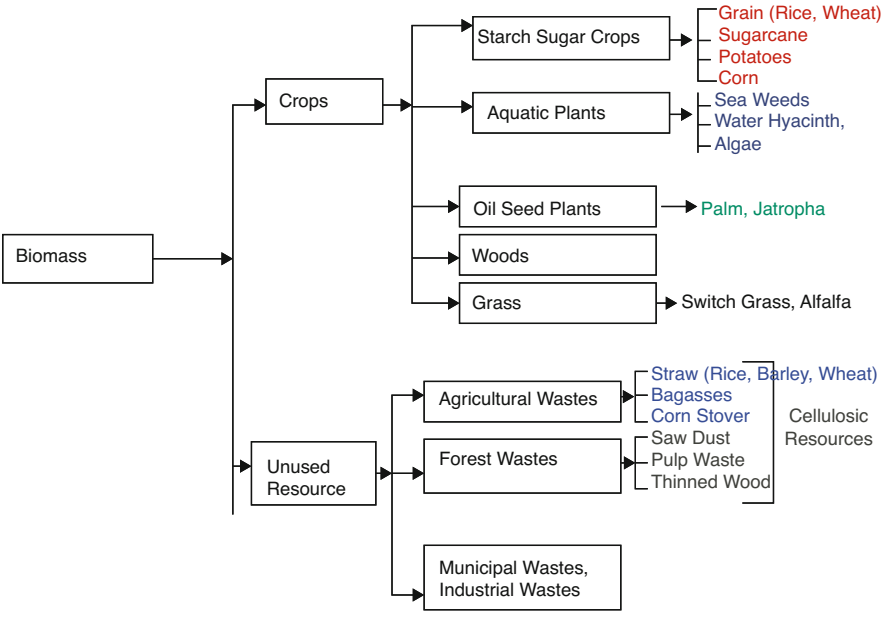

Fig. 11.4 Biomass as a viable bio-refinery feedstock

found in large amounts of biomass. Biofuels may be produced from lignocellulosic biomass. High-value bio-chemicals such as gums, alkaloids, resins, plant acids, rubber, tannin, waxes, triglyceride, terpenes, steroids, terpenoids, and other secondary metabolites are found in little amounts in plants (Ragauskas et al. 2006a, b). Using an integrated processing approach, secondary metabolites may be used to make high-value compounds such as food flavors, feeds, medicines, cosmeceuticals, and nutraceuticals, among other things.

11.3 The Full-Scale Operation of the Bio-refinery Concept

A bio-refinery is a renewable version of a crude oil refinery, with the primary distinction being the raw material used. Biomass may be turned into a variety of chemicals and energy carriers in a bio-refinery, and it can also help to build a circular economy; this notion is based on the idea that lignocellulosic materials used to make biobased goods can be recovered (to a degree) and recycled (Capolupo and Faraco 2016). Biorefining is defined as "the viable conversion of biomass into a range of marketable biobased goods and bioenergy" by International Energy Agency (2012). When it comes to a large-scale production plant, however, the many types of raw materials provide a significant difficulty. Figure 11.5 depicts a schematic diagram of a possible bio-refinery for the synthesis of energy carriers and chemicals.

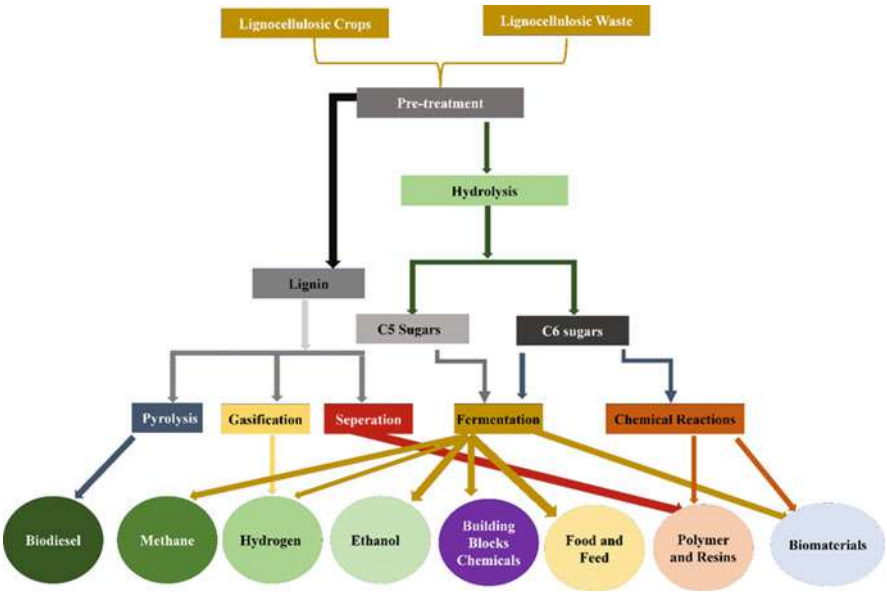

Fig. 11.5 A schematic overview of potential energy carriers and chemicals is produced in a bio-refinery

Some significant considerations have been made in order for the bio-refinery idea to become a viable path toward a society that is less reliant on fossil fuels. Bio-refineries are an important part of the future integration of fuels, food, chemicals, and energy production.

11.4 Biobased Chemicals

Biobased products are often believed to be greener substitutes to crude oil-based materials, which are non-biodegradable, have the potential to harm animal and marine life, and, for the most part, have an intrinsically hazardous life cycle from manufacture to disposal (Werpy and Petersen 2004; Chemical and Engineering News 2006). Biobased products are commonly marketed as being made from "renewable" resources, even though biomass production requires nonrenewable inputs, such as fossil fuels, and locks up other limited resources like land and water. The notion that biobased products are more environmentally friendly than their crude oil-based equivalents is being extensively investigated (Frankfurt 2011; US Department of Agriculture 2008).

The quantity and variety of compounds that may be generated using a biomass source and/or bioprocessing method are astounding. The US Department of Energy selected 12 compounds or chemical classes in 2004 as possible building blocks from which numerous value-added chemicals might be made. Because of this variety, it is hard to develop a policy framework that is the same for extremely low production volume compounds like enzymes and a biobased version of ethylene, the world's greatest production volume organic molecule.

11.4.1 Cost and Performance of Biobased Chemicals

It is hard to sell biobased compounds only on the basis of their environmental credentials. In a world where the triple-bottom-line is king, economics and societal concerns must also favor bio-over petrochemicals. This, however, implies that this relatively new business will have to compete directly with the petrochemicals industry, which has had decades to refine its methods. As a result, if biobased chemicals provide a long-term triple-bottom-line benefit, society should anticipate having to reward the sector until it can compete on price and performance.

By disrupting these chemical connections, biomass can be used as a prospective energy resource for humans. Biomass is considered a viable energy source, thus making use of this perspective energy resource is a significant focus of study. The simplest and most popular approach is to convert this energy into heat through a simple combustion process. The present focus is on turning biomass into a form with a greater energy value as a crude oil fuel alternative. Biomass has been studied in a variety of ways, and first-generation biofuels have even experienced success in

industrial settings. Biodiesel, ethanol, and tiny quantities of biogas are among the first-generation biofuels produced. The present biofuel industry, on the other hand, is dominated by fuels generated from food sources, which has caused heated discussion about their influence on biodiversity, land usage, and competition with food crops. Furthermore, others argue that first-generation biofuels do not achieve the greatest reduction in greenhouse gas emissions. This chapter will concentrate solely on the context of fuels generated from the processing of lignocellulosic biomass (LCB), often known as second-generation biofuels, for the reasons stated above. Forest remnants that are developed, especially to create bioenergy, agricultural leftovers like maize stover, rice, wheat straw, etc. are also sources of LCB. Second-generation biofuels are presently not cost-competitive and are not being produced commercially. Many of the issues presented by first-generation biofuels appear to be addressed by the creation of second-generation biofuels. Biomass is made up of several kinds of lignin in addition to varied quantities of hemicellulose, lignin, and cellulose. As a result, any effort to use biomass in a processing atmosphere must be strong enough to accept the diversity of biomass forms.

The availability of various forms of lignocellulosic materials varies greatly between countries and continents. Forests are numerous in certain areas, whereas agricultural plants are more frequent in others. The composition of lignocellulosic components varies depending on the species. The fundamental components, however, are essentially the same, but the amounts of specific carbs, aromatics, and other compounds vary: around 50–60% are carbohydrates, 20–30% lignin, and the remainder is extractives, fatty acids, ash, etc. (Ståhl et al. 2018). The fundamental components, however, are essentially the same, but the amounts of specific carbs, aromatics, and other compounds vary: around 50–60% are carbohydrates, 20–30% lignin, and the remainder is extractives, fatty acids, ash, etc. (Sjöström 1993). Hemicelluloses have higher hydrophilicity than cellulose, making them simpler to hydrolyze. Lignin is the most important non-carbohydrate component of lignocellulosic materials, with a complex structure of aromatic compounds. It is linked to both cellulose and hemicelluloses, and it is a big reason why lignocellulosic materials are so strong.

11.5 Biomass Pre-treatment's Objectives

The primary focus of the biomass pre-treatment phase has altered in recent years; before, the primary focus was on using lignocellulosic materials for bioethanol synthesis. The other major components, lignin, and hemicelluloses drew less attention. Today, it's critical to figure out how to increase the total yield of the important chemicals found in lignocellulosic materials. Pre-treatment techniques that allow for effective carbohydrate and lignin recovery are desirable; however, this is dependent on the circumstances and the ultimate product. The energy requirements of the manufacturing process must be satisfied in any case, either by externally or internally integration of high-energy streams, such as in a pulp mill where surplus lignin is the

primary source of process energy. It is anticipated that between 20% and 30% of the lignin in an efficient mill is accessible for purposes apart from internal energy requirements (Mistra, 2003).

11.5.1 Lignocellulosic Biomass Physical and Chemical Characteristics

Agricultural wastes are sometimes known as biomass or lignocellulosic materials. While referring to high-grade plants, such as soft or hardwood, the phrase "lignocellulosic biomass" is used. Hemicellulose, lignin, and cellulose are the major components of lignocelluloses, with minor quantities of pectin, protein, extractives, ash, and water. Within the lignocellulose complex, cellulose is essential for the crystalline fiber structure, hemicellulose is located between the cellulose chains, and lignin is essential for the matrix's structural role (Srivastava and Lisle 2004).

Cellulose is the most important structural component of plant cell walls, providing chemical and mechanical stability. The process of photosynthesis absorbs solar energy and stores it as cellulose. Whereas hemicellulose is a co-polymer of various C_5 and C_6 sugars; lignin, on the other hand, is an aromatic compound polymer that provides a protective coating for plant walls (Fig. 11.6).

11.6 LCB Pre-treatment Technologies

The major objective of LCB pre-treatment is to break the refractory structure to allow enzyme access in subsequent downstream processing, as shown in Fig. 11.7. There are a variety of ways to achieve this, and each technique has various outcomes. Enzymatic hydrolysis, for example, may necessitate the removal of hemicellulose due to the lack of hemicellulases, but consolidated bioprocessing makes use of organisms that naturally release hemicellulases and can efficiently manage hemicellulose (Bhardwaj and Verma, 2021). Each of these processes might make use of biomass that has been pretreated differently. As a result, identifying a single pre-treatment strategy that will serve as the industry's "gold standard" is nearly

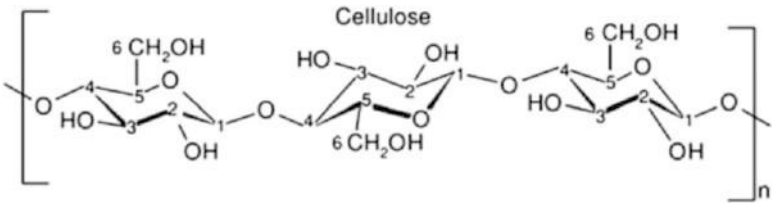

Fig. 11.6 Structure of cellulose molecule

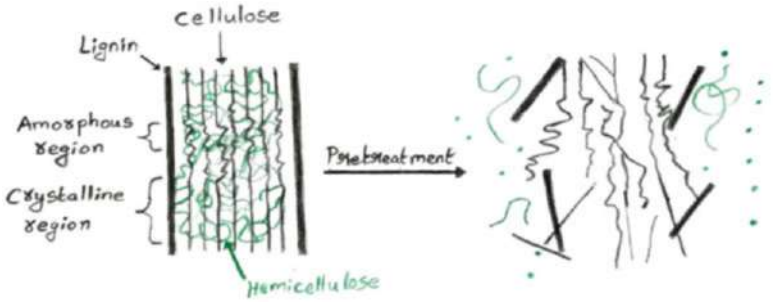

Fig. 11.7 Pre-treatment of LCB

impossible. However, the advantages and disadvantages of each will be recognized, and this knowledge may be utilized to develop a system-wide biomass use strategy.

Pre-treatment is required to break the microstructure of lignocellulosic biomass, which is made up of hemicellulose, lignin, and cellulose, to make carbohydrates more available. The goal of pre-treatment is to eliminate lignin and hemicellulose, lessen cellulose crystallinity, and enhance biomass porosity. A good pre-treatment must prevent the degradation of carbohydrates, as well as the emergence of inhibitors to the resulting hydrolysis and fermentation processes, while also improving the creation of sugars or the capacity of pretreated material to form sugars by water treatment, all while remaining cost-effective. The optimal pre-treatment relies on the expected usage of the primary biomass components, among other things. Physical, chemical, and biological pre-treatments, as well as combinations of these, are classified as physical, chemical, and biological pre-treatments, with physicochemical pre-treatments receiving special attention.

11.6.1 Mechanical Comminution

The goal of mechanical pre-treatment of biomass is to improve its digestibility. Cutting, grinding, etc. are some of the mechanical methods which can be used to minimize particle size, enhance the accessible specific surface area, through various fractions. In terms of energy consumption, this approach has certain drawbacks. The energy needs for mechanical comminution are determined by the desired end particle size and biomass properties.

11.6.2 Steam or Water Vapor Explosion

The most frequent physicochemical technique of biomass preparation is steam or water vapor explosion. Biomass is to be treated by high-pressure steam and then

rapidly decompressed in this technique. Following that, an explosive reaction occurs, culminating in the breakdown of hemicellulose and the rupture of the lignin matrix. The crystallinity of cellulose may decrease, and the surface area of the substrate may rise, increasing cellulose digestibility (Li et al. 2007).

11.6.3 Liquid Hot Water Pre-treatment (LHW)

Liquid hot water (LHW) procedures are biomass pre-treatments that employ pressure to maintain extreme temperatures (160–240 °C) in water. LHW used water as a reaction medium at relatively high reaction temperatures. The liquid state is maintained by increasing the pressure. Changing the process temperature and pressure alters the dielectric strength and ionic product of LHW and effectively removes hemicelluloses from the lignocellulosic matrix at 220 °C. Increasing Ro (severity factor measures the combined effect of temperature and time in each pre-treatment) increases the yield of undesired by-products. Hence, compromise must be found between biomass solubilization and concentration of undesired degradation product.

11.6.4 Ammonia Fiber Explosion (AFEX)

AFEX is a physicochemical pre-treatment in which lignocellulosic biomass is treated for a length of time with high pressure and temperature for liquid ammonia, and then the pressure is rapidly decreased. The AFEX technique is quite similar to the processing of steam explosions. The chemical action of ammonia under pressure causes the biomass to expand, resulting in an increase in accessible surface area and a decrease in cellulose crystallinity at the same time (Mosier et al. 2005a, b).

11.6.5 CO_2 Explosion Pre-treatment

The pre-treatment procedure for carbon dioxide explosions is comparable to steam and AFEX. It was created in an attempt to enhance lignocellulosic biomass pre-treatment by using a green, low-temperature, and low-cost technique.

11.6.6 Wet Oxidation Pre-treatment (WOP)

By oxidizing soluble suspended materials with oxygen at elevated temp (150–350 °C), wet oxidation pre-treatment was utilized in the industry for

wastewater treatment and soil remediation (Zerva et al. 2003). It was later proposed as an alternative to steam explosion for the processing of lignocellulosic biomass.

11.6.7 Acid Hydrolysis

Strong acids, such as sulfuric acid, have also been used to treat lignocellulosic materials since they are potent cellulose hydrolysis agents that do not require the use of enzymes to achieve acid hydrolysis and generate fermentable sugars. They are poisonous, dangerous, and corrosive, which makes the pre-treatment procedure highly costly.

11.6.8 Peroxyformic Acid

Peroxyformic acid pre-treatment is a chemical pre-treatment technique for oxidative delignification. By combining formic acid with hydrogen peroxide in situ, peroxyformic acid is produced. This combination is then supplemented with lignocellulosic biomass and left to sit for many hours. Formic acid dissolves hemicellulose chains and functions as a solvent for lignin. Peroxyformic acid causes oxidative delignification by increasing lignin solubility. The temperature of the reaction is raised and the majority of the delignification happens. Any residual lignin is degraded in the last step.

11.6.9 Alkaline Hydrolysis

Pre-treatment of lignocellulosic materials is possible with certain bases. Alkaline pre-treatment treatments are appropriate. Sodium hydroxide or lime (calcium hydroxide) is commonly utilized. The biomass is soaked in an alkaline solution and combined for a period of time at a low temperature in the alkaline pre-treatment. Other pre-treatment processes use higher temperatures and pressures, but alkali pre-treatment uses lower temperatures and pressures. It can be done under normal settings, but it takes a long time to process.

11.6.10 Ozonolysis

The lignin composition of lignocellulosic biomass, as well as trash, is reduced by ozone pre-treatment. During carbohydrate breakdown, lignin absorbs the majority of

ozone. The degradation is primarily restricted to lignin, however, hemicellulose is impacted in a little way, while cellulose is unaffected.

11.6.11 Organosolv Pre-treatment

The use of organic and aqueous organic solvent mixtures having inorganic acid catalysts is used to pretreat lignocellulosic biomass using organic solvents. Catalysts can also be organic acids like oxalic acids. Methanol, acetone, and ethylene glycol are common solvents. Pre-treatment is generally done at a high temperature (up to 200 °C) and under high pressure (Chen et al. 2015; Kabir et al. 2015). With alcohol, the catalytic process is identical to that of the autohydrolysis pre-treatment. Lignin and lignin–carbohydrate linkages are hydrolyzed by the solvent. Lignin is eliminated in large amounts, and hemicellulose is almost entirely dissolved, while cellulose remains solid. Organic acid pre-treatment accelerates delignification and hydrolysis of cellulose, as well as lignin breakdown, by dissociating hydrogen ions. At high temperatures, the inclusion of a catalyst is not required in this situation. A large portion of soluble carbohydrates is broken down further into by-products like furfural, which hinder fermentation bacteria.

11.6.12 Biological Pre-treatment

Biological pre-treatment is a secure and ecologically acceptable pre-treatment that involves the use of enzymes produced by fungi and bacteria that can transform lignocellulosic biomass into more easily hydrolyzed chemicals. To break down lignin, hemicelluloses, polyphenols, etc., microorganisms are employed. Brown rots target cellulose primarily, whereas white and soft rots target both cellulose and lignin. Lignin degradation enzymes are involved in the process of lignin breakdown (Eggert et al. 1997). Table 11.3 lists the benefits and drawbacks of several lignocellulose biomass processes.

11.7 Organosolv Technology Outperforms Other Pre-treatment Methods

Early elimination of the raw material into smaller components is critical to both processes, allowing intermediates to be more adaptable and easier to utilize than the raw material itself. natural gas and crude oil are transformed into fuels through physical separations through cracking as well as chemical transformation. Pre-treatment is a term used to designate similar activities that allow biomass

Table 11.3 Different pre-treatment techniques for lignocellulose biomass: advantages and drawbacks

Pre-treatment technique	Advantage	Drawback
Milling	The level of polymerization and crystallinity of cellulose are decreasing Particle size reduction to improve specific surface area and pore size	More power and energy consumption
Steam explosion	Hemicellulose solubilization, lignin transformation are the results The two-step technique produces more glucose and hemicellulose	Generation of toxic compounds Partial hemicellulose degradation
Liquid hot water	Biomass size reduction is not needed No chemicals or corrosion-resistant materials are generally required	High energy and water requirements Formation of toxic compounds
Ammonia filter expansion (AFEX)	Increase accessible surface area Low inhibitors formation. Does not require a small particle size of biomass	Not that much is effective for the biomass having high lignin content High cost of large amounts of ammonia Very high-pressure requirement
CO_2 Blast	Improve accessible surface area Low cost No inhibitory compounds Not flammable Easily recoverable after extraction	Pressure requirements are high
Wet oxidation	More degree of solubilization of hemicellulose and lignin	High cost of oxygen and alkaline catalyst
Concentrated acid	High glucose yield Ambient temperatures	High cost of acid Corrosion-resistant equipment is required
Diluted acid	Recovery of sugars at the end of the process is high	The concentration of reducing sugars is relatively low degradation products
Alkali	The reduced degree of polymerization and crystallinity of cellulose Disruption of lignin structure	High cost Not used for large-scale plants
Ozonolysis	Successfully removes lignin content	Cost of ozone is more
Organosolv	Cause lignin and hemicellulose hydrolysis	High cost
Biological	Reduction in the degree of polymerization of cellulose Partial hydrolysis of hemicelluloses	Reduced process rate Treatment rate is less

downstream processing in a bio-refinery. Pre-treatment methods have been created and evaluated in a wide range of ways (Mosier et al. 2005a, b). Future bio-refineries will adopt the petrochemical industry's paradigm, combining the production of low-value fuel with the manufacture of high-value chemicals generated from each of lignocellulose's major components.

As a result, pre-treatment procedures will need to place a much higher focus on the yield and purity of the various bio-refinery process streams utilized in chemical synthesis. While more selective techniques may incur greater costs, the capability to combine valuable chemical products into an integrated operation will generate a revenue stream capable of covering the expense of improved selectivity upfront (Bozell 2008). Pre-treatment processes optimized for an integrated bio-refinery will demonstrate selective dissociation of each constituent of a biomass feedstock, easiest access to and segregation of the constituents after separation, high yield recuperation of each component, process components ready for transformation to chemicals with minimal purification. Pre-treatments with organosolv often satisfy these criteria. Pre-treatment of the cellulosic with solvents has been the topic of many reviews covering technique and mechanism and was first mentioned in a patent in 1932 outlining the use of ethyl alcohol for wood segregation (Zhao et al. 2009a, b; McDonough 1993; Kleinert and Tayenthal 1932; Johansson et al. 1987; Jimenez et al. 1999). Several feedstocks have been utilized with formic acid, acetic acid, and peroxyformic acid (Poppius-Levlin et al. 1991). Organosolv methods are typically omnivorous in terms of the raw material they work with and have been used to pretreat hardwoods, softwoods, and grasses (Pan et al. 2006; Munoz et al. 2007; Jimenez et al. 2008). Organosolv technology has a variety of benefits over more traditional pulping methods, according to reports. Organic solvents lower the viscosity of the pre-treatment medium, allowing for better penetration into the biomass, more effective lignin removal, and less lignin recondensation and molecular weight rise during fractionation (Oliet et al. 2002; Pye and Lora 1991; Sarkanen 1990). Although cellulose separated from eucalyptus following an ethyl alcohol pre-treatment has been observed to have redeposited lignin, the presence of solvent can delay the redeposition of lignin onto the other biomass components once the separation is complete. pH control or alkali washing of the cellulose has been used to combat redeposition (Oliet et al. 2001; Xu et al. 2007; Paszner and Cho 1989; Zhang et al. 2007). Organosolv cellulose is more easily purifiable. This is essential in the paper industry as a solution to environmental concerns related to pulp bleaching, as well as in the chemical sector, which typically demands high purity starting materials. Furthermore, as compared to cellulose produced utilizing traditional methods such as kraft or soda, the cellulose had better bleachability and viscosity retention. Organosolv cellulose's enhanced characteristics have been used in the manufacture of viscose and carboxymethylcellulose (Cronlund and Powers 1992; Shatalov and Pereira 2007; Sixta et al. 2004; Ruzene et al. 2007).

11.7.1 Different Solvents for Organosolv Pulping

The need for more viable manufacturing techniques for commodities like paper and cardboard has increased as industrialized countries' environmental consciousness has grown. Traditional pulping methods generate enormous volumes of polluting waste known as "black liquor." As a response to this, the experts are increasingly

focusing on developing new pulping processes. These processes, which are referred to as "organosolv processes" in general, allow for the manufacture of high-quality pulp and paper with minimal capital investment, high yield. Because alcohol is the most commonly used solvent for organosolv pulping, this chapter examines the limited research that has been done thus far on the use of other solvents.

The first scientific reference to organic solvent delignification originates from 1893. Organosolv methods accepted the dominance of classic chemical pulping techniques in those days. During the 1970s, however, scientists began to find alternatives to the traditional methods' numerous disadvantages, including foul aromas. Initially, efforts were concentrated on improving the pulping process; later, alternative methods that did not utilize sulfur as a reagent was created. However, these efforts ran into additional issues, such as the difficulty in recovering chemicals and the waste's contaminating character. New organic solvent-based methods began to develop in the 1980s. The fact that they could make the best use of the raw materials was their biggest advantage. Despite the fact that the Kraft process was still in use in the 1990s, the global environmental concerns it produced and the large expenditures required concluded that other pulp manufacturing methods must be developed. The Kraft or sulfate process is the most widely used pulping method in the industry. Wood, particularly softwood is by far the basic raw material for this procedure, whose main drawback is that a few of them have high ash content, which also causes serious troubles in black liquor recovery circuits. Although the true agent that works during the delignification reaction is the sulfur that is produced, sodium sulfate is used as a replacement reagent in this procedure. The process may be separated into two parts: (i) pulp manufacturing and (ii) chemical recovery. Some of the black liquid from Kraft pulping can be recirculated and utilized as a pulping solution. In certain situations, black liquor can make up 40–60% of the pulping solution without impacting the pulping yield or the properties of the pulps produced. By doing so, a portion of the reagents may be reused without an expensive evaporation step, the chemicals' penetration into the chips is aided, and the black liquor's heat energy is used.

The best pre-treatment technique should be chosen based on feedstock properties such as relative hemicellulose, lignin, and cellulose proportions, as well as manufacture capability and intended product kinds. Organosolv pre-treatment has recently received more attention due to its effectiveness in eliminating refractory particles from lignocellulosic biomass. The major advantage of organosolv pre-treatment is its capacity to take out pure lignin, known as organosolv lignin, which is then used as a useful co-product rather than an unwanted by-product. As a result, a viable and renewable energy market is formed, with economic and decarbonization advantages.

11.8 Overview of Organosolv Pre-treatment

The technique of extracting lignin from lignocelluloses using organic solvents is known as organosolv pulping. Organosolv pulping has piqued attention since the 1970s, owing to the fact that traditional pulping processes, such as Kraft and sulfite, have severe drawbacks. Pre-treatment with organosolv is comparable to pulping with organosolv; however, the level of delignification for pre-treatment is not needed to be like that of pulping. Likewise, organosolv pre-treatment provides the following benefits: (i) organic solvents are easy to distill and reuse for pre-treatment; (ii) chemical recovery in organosolv pulping procedures may separate lignin as a solid substance and carbohydrates as syrup, both of which have potential as chemical feedstocks (Aziz and Sarkanen 1989). Organosolv pre-treatment appears to be more viable for lignocellulosic biomass bio-refinery, which addresses the use of all biomass components. The organosolv pre-treatment does, however, have certain disadvantages. To shun the accumulation of dissolved lignin, which leads to complex washing arrangements, pretreated particles must always be washed with an organic solvent prior to water washing. Organic solvents are usually costly, therefore as much as feasible should be recovered, but this increases energy consumption. Due to the volatility of organic solvents, organosolv pre-treatment must be done under effective supervision. Because of the inherent fire and explosion risk, no digester leaks may be permitted. As a result, organosolv pre-treatment is now too costly to be utilized for biomass pre-treatment. It may be carried out in a wide range of organic solvent systems using additional catalysts at temperatures ranging from 100 to 250 °C, whereas organic peracid pre-treatment may be carried out at much lower temperatures. Solvents with low boiling points, as well as a range of alcohols with greater boiling points and other groups of organic molecules, have been investigated. It is thought that organic acids produced from the biomass function as catalysts for the breakdown of the lignin–carbohydrate bond in most organosolv procedures if the pre-treatment is carried out at greater temperatures, while acid catalysts are introduced, however, the level of delignification is enhanced, resulting in greater xylose yields. Mineral acids are effective delignification catalysts, whereas organic acids can also be employed. The majority of hemicellulose and lignin is dissolved, whereas cellulose remains solid. To cut costs, the organic solvents which were used must be recycled. Solvents, on the other hand, must be removed from the system since they may impede the development of organisms, enzymatic hydrolysis, and fermentation. Dry lignin, an aqueous hemicellulose stream, and a reasonably pure cellulose fraction are all separated by the organosolv process.

11.9 The Chemistry of Organosolv Delignification

Organosolv pulping is gaining popularity due to its potential for cost-effectively increasing additional pulp production capacity as well as potential environmental benefits. Delignification of wood in non-aqueous media, also known as organosolv pulping. Since the idea was established early in the century, it has been the focus of a lot of research. Much of this activity has occurred in recent years, however, and most has been empirically directed toward the identification of efficient solvent systems and optimum process conditions. Until recently, very little work is done regarding fundamental aspects of these systems, so little detailed information is available on their mechanisms. On the other hand, the mechanisms of the Kraft and sulfite pulping processes and their variants have been studied in detail, and there has been considerable basic work on non-aqueous lignin solvolysis, although most of it has not been primarily directed at understanding the related industrial processes. For example, numerous studies have been done to elucidate lignin structure by analyzing its solvolysis products. As a result, there exists a substantial amount of information that can serve as a basis for inferences concerning organosolv pulping mechanisms. In addition, increased levels of basic research on the subject during the past few years have added to a growing store of theoretical knowledge that should facilitate further development of organosolv pulping technology.

11.9.1 The Nature of Organosolv Pulping

Traditional chemical pulping techniques rely on the capacity to gradually break down and alter the lignin macromolecule until the resultant molecular fragments are small enough to dissolve in the aqueous pulping fluid for them to be effective. It's possible that organosolv pulping delignifies by physically dissolving lignin without first chemically fragmenting it by substituting most or all of the water with an organic solvent. In reality, no solvent has yet been discovered that can achieve this desired result, and all organosolv methods rely on chemical lignin breakdown before dissolving it.

11.10 Advantages and Disadvantages of Organosolv Pre-treatment

Traditional pulping methods, yield good-quality pulps with a huge cellulose concentration, whereas liquid lignin-hemicellulose fractions comprising 50–55 % dry weight of lignocellulosic biomass are utilized by low-value applications such as direct burning (Vila et al. 2003a, b; Xu 2006). Organosolv pre-treatment is an improved pulping technique used in the manufacture of second-generation

bioethanol (Mesa 2011). It is a biomass pre-treatment process that primarily eliminates lignin and hemicellulose while also making cellulose more digestible (Cybulska 2015). It is very efficient when used with lignocellulosic biomass for refractory material removal and cellulose saccharification (Zhang et al. 2016; Pande and Bhaskarwar 2012; Jimenez 2004; Garcıa et al. 2014). Furthermore, it leads to an enlarged surface area and huge pore volume (Zhao et al. 2009a, b). Because the lignocellulosic biomass's stiff structure is broken down, the hydrolysis time is decreased and enzyme usage is lowered (Geng et al. 2012; Kim and Pan 2010). Table 11.4 summarizes the basic benefits and drawbacks of organosolv pre-treatment. The basic mechanism of organosolv pre-treatment is to handle lignocellulosic biomass with an organic solvent with or without a catalyst, in order to separate lignin fractions in a liquid state from cellulose fractions in solid form (Sun and Cheng 2002; Taherzadeh and Karimi 2008). Figure 11.8 depicts the overall flow diagram of the organosolv pre-treatment. Solvent and water are combined to provide a solvent's concentration of 35–70% then added to the lignocellulosic biomass with such dry biomass to solvent/water mix proportion between 1:4 and 1:10. To speed up the process, a catalyst can be introduced. In general, the working temperature range is 120–200 °C, the pulping duration is 30–90 min, and the median pH is 2–3.8 (Koo 2011; Behera 2014; Kumar 2009). During the organosolv process, lignin bonds and lignin-carbohydrate linkages hydrolyze, resulting in a solid phase mostly composed of cellulose and hemicellulose. To prevent lignin precipitation, this prepared material must be rinsed with an organic solvent.

Following this, the pretreated material is washed using water to eliminate the organic solvent. Following this, filtering separates the solid and liquid phases, and the pretreated solid is transported to a saccharification and fermentation reactor to generate bioethanol. Waste liquor is mostly composed of ethanol, water, other by-products. The wasted liquor is pumped into a distillation column to recover the solvent. The solvent-free liquid is diluted using water after distillation to precipitate the lignin, which is subsequently separated by filtering from water-soluble fractions.

Table 11.4 Advantages and disadvantages of organosolv pre-treatment

Advantage	Disadvantage
• As a feedstock, both woody and non-woody biomass might be utilized • The yield of enzymatic hydrolysis is increased when lignin is separated • The amount of water, energy, and reagents required is smaller than that of other pre-treatment techniques • Solvents may be recovered simply • Small-scale applications are less expensive than other pre-treatment techniques • The environmental effect is considerably reduced because it is Sulfur-free • By-products are transformed into valuable goods	• High solvent usage is required. As a result, solvents must be recovered • Energy usage rises as a result of solvent recovery • Due to the volatile nature of the components, process control must be exercised with utmost caution

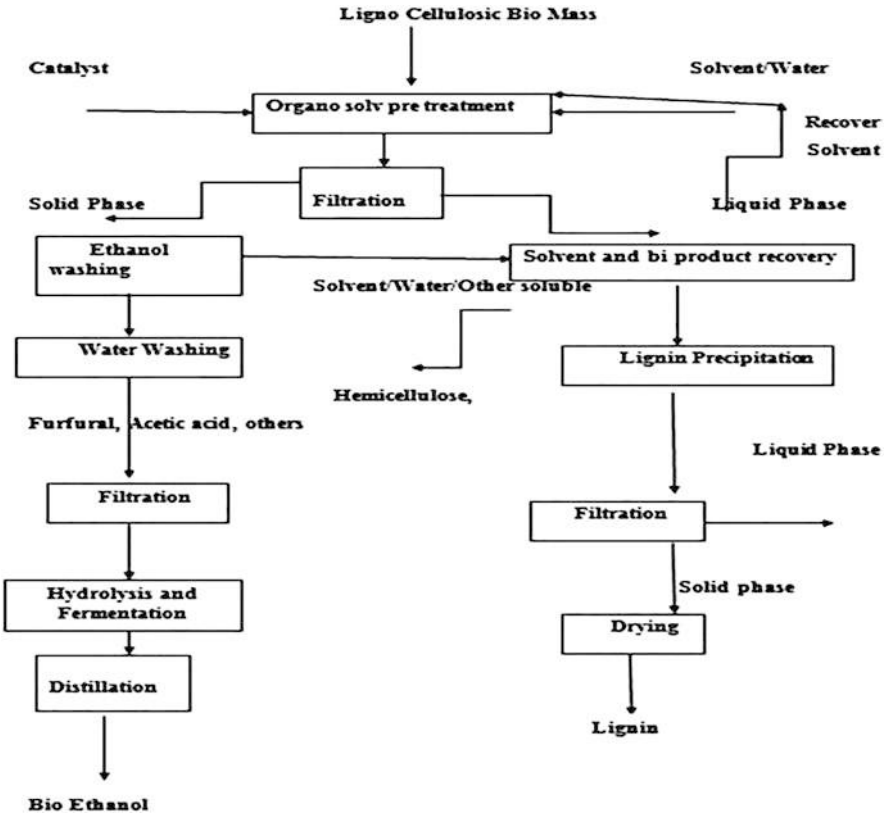

Fig. 11.8 The diagram of the pre-treatment of organosolv

It is rinsed in water to eliminate impurities before being dried to produce high-purity lignin. Because all of the biomass molecules involved are quickly translatable to high-grade fuels and chemicals. It is one of the most ideal pre-treatment procedures for use in LCF bio-refineries (Li 2012; Perttunen et al. 2001). Furthermore, the moderate pre-treatment conditions of temperature, pressure, and pH reduce the quantity of carbohydrate breakdown. In the solid phase of the organosolv pre-treatment, nearly pure cellulose is produced with just a little degradation, while hemicellulose and lignin are formed in the liquid phase. In the solid phase, the main constituents are glucan (60–65%), Klason lignin (25–30%), xylan (5–10%), arabinan (0.1–3%), and acetylated forms (1–3%), and in the liquid phase, monosaccharides, oligosaccharides, and degradation products. The concentration. of these components varies depending on the kind of feedstock used and the operating circumstances (Ruiz 2011). Furfural, HMF, etc. are among the main hemicellulose degradation products in increased bioethanol production efficiency (Wang 2012; Sannigrahi et al. 2010; Hallac et al. 2010; Lloyd and Wyman 2005; Pan et al. 2005). In contrast to typical pre-treatment procedures, organosolv

pre-treatment produces a high yield of hemicelluloses. A variety of useful chemical compounds such as bioethanol, furfural, and xylitol may be produced from this.

The advantages of employing organic solvents for the delignification of certain cellulose materials are highlighted. The main features of published organosolv pulping techniques are described, and softwood pulp yields and strengths are compared to equivalent values of Kraft and sulfite pulps. Despite these benefits, the Kraft method has some significant disadvantages. Some of them, such as the issue of malodorous material emissions, are so serious that they have effectively prevented the Kraft process from being implemented in Germany.

One more significant issue is connected to the product; while better in terms of strength quality, Kraft pulp needs a very efficient bleaching process in order to achieve the necessary levels of brightness. As a result of colorful and poisonous effluents, particularly chlorinated lignin particles from bleaching, severe water pollution issues have arisen. At the opposite end of the spectrum, technological constraints permit the max size of the boiler, resulting in most contemporary mills today aiming for a modular size of close to 300,000 tonnes of pulp per year. Although the Kraft process employs dissolved organics as fuel, making it energy self-sufficient, the bulk of the energy generated is required for the concentration of these wasted liquors before combustion. Furthermore, lignin alone accounts for the higher calorific value of the dissolved material. Chemical pulp mills employing traditional technology have grown prohibitively costly. There is an obvious need to create innovative pulping technologies to minimize the amount of capital required to establish new chemical pulp mills while also using less wood, energy, and chemicals. One solution to current difficulties is to remove lignin from lignocellulosic raw material using organic solvents rather than reacting with inorganic chemicals. The significant conceptual benefit of employing an organic solvent for lignin

11.11 Organosolv Pre-treatment for Biofuel Production

The second generation biofuels derived from lignocellulosic biomass are commonly regarded as a means of meeting future transport needs without raising food costs (Field et al. 2008). Because lignin cannot be fermented, the utilization of lignocellulosic materials results in lignified leftovers that can be burned or used as co-products. To make cellulose to be saccharified for fermentation, lignin must be separated from cellulose during a pre-treatment phase (Bommarius et al. 2008; Zhu et al. 2008). Typically, lignocellulosic biomass is pretreated in one or two stages to fractionate the various polymers and enhance glucose yield (Pan et al. 2005; Panagiotopoulos et al. 2013). Softwoods produced responsibly and away from food markets are good raw materials for biofuel generation (Shuai et al. 2010). To allow saccharification, their lignin content, which is generally 30% of the dry matter, must be decreased. A delignification phase utilizing organosolv pre-treatment increased softwood saccharification yields (Arato et al. 2005; Mabee et al. 2006).

Some organisms can ferment the saccharification products of non-cellulosic polysaccharides. However, xylose and, more specifically, its breakdown product furfural are unusable or poisonous to many of the most often utilized bacteria (Pienkos and Zhang 2009). As a result, pentosans and xylose may have a negative value rather than being helpful co-products. The bio-refinery idea is based on the efficient synthesis of a variety of commercially viable co-products, and realizing this concept is a significant twenty-first-century problem (Ragauskas et al. 2006a, b). New pathways to lucrative co-products would thus be pleasing, other than the possibilities are dependent on the raw material (Pan et al. 2005).

11.11.1 Organosolv: A Potential Pre-treatment Technology for Bioethanol Production

When compared to the standard Kraft process, organosolv pulping methods provide relatively few advantages. However, organosolv methods, which seek to delignify and open the cell wall matrix, may prove to be promising (Kautto et al. 2013). According to Murinen et al. (2000), a successful organosolv method for kraft process substitution includes the following properties: (i) completely sulfur-free, (ii) most of the lignin is dissolved with little loss, (iii) operating parameters should be kept to a minimum, (iv) chemical recovery method that is both efficient and easy, (v) there are no environmental issues, (vi) the process's optimum size-tiny in comparison to the kraft process, (vii) adaptable to a wide range of raw materials, (viii) recuperation of useful by-products, (ix) superior pulp quality, (x) excellent bleachability without the use of chlorine, (xi) elevated yield of pulp, (xii) the procedure consumes little energy, and (xiii) the process has a closed chemical cycle.

The quest for pulping procedures that might meet the aforementioned parameters resulted in the invention of numerous organosolv techniques capable of generating pulp with characteristics similar to kraft pulp. The discussed technologies were all pilot or full-scale tries, but none of them resulted in continuous production (Lora and Aziz 1985; Young and Baierl 1985; Dahlmann and Schroeter 1990; Funaoka and Abe 1989; Gottlieb et al. 1992; Hamelinck et al. 2005). Organosolv methods are thus particularly appealing for non-woody raw materials, and at least one of them, the formic acid procedure, has been commercialized (Rousu et al. 2002). The Lignofibre (LGF) process is a unique flexible organosolv technique that meets the majority of the parameters stated above, and it applies to both annual plants and wood raw materials (Liitiä et al. 2011).

11.12 Conclusions

Bio-refineries will be an important part of a resilient and viable economy, ideally with feasible small-scale alternatives to help marginal and rural areas thrive economically. The development of bio-refineries and bioprocesses for the manufacture of biobased chemicals and polymers should be based on a sustainability assessment that takes into account feedstock availability along with techno-economic, and social implications. One of the most significant roadblocks to the development of effective biomass-based bio-refineries that can race with existing crude oil refineries is the proficient breakdown and transformation of lignocellulosic material into chemicals and fuels. The discussed methods are all under research at the laboratory scale. In comparison to other pre-treatment techniques, the organosolv pre-treatment enables high-efficiency ethanol and lignin synthesis from biomass. The rate of delignification of the solid phase, glucose retrieval yield from the solid phase, and lignin retrieval yield from the liquid phase are three parameters that can be used to describe the efficiency of the organosolv pre-treatment. The most significant challenge they confront is the cost of treatment procedures. To address this, first and foremost, cost-cutting is required. Efficient solvent recovery, holding out the organosolv reaction at the air pressure, efficient by-product recovery, identification of marketplace for bioproducts, and rises in the sales price of value-added products should all lead to more widespread industrial use of organosolv-based bio-refineries. This will contribute significantly to the establishment of a viable green economy, decarbonization, and climate change mitigation. The pulp business looks to be a very attractive potential since it already has an effective method for separating the lignin and extractives fractions from the polysaccharides; this cracking is highly appropriate for bio-refinery applications and is required. For the manufacture of glucose and its by-products, organosolv pre-treatments were effectively used to Sitka spruce wood. This strategy is in line with the bio-refinery idea, which calls for the efficient production of a variety of commercially viable co-products. The holistic use of biomasses and the reduction of pre-treatment costs should be the emphasis of prospective organosolv pre-treatment development. This may be accomplished by lowering the quantity of organic liquid needed in pre-treatment, improving the value of by-products, and optimizing the entire process, as well as certain other factors connected to energy and chemical usage reduction. As a result, this study can give some data and suggestions for future organosolv pre-treatment development.

Competing Interests All the authors declare that they have no competing interests.

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Constraint Handling in Metaheuristics and Applications pp 231–249

Role of Constrained Optimization Technique in the Hybrid Cooling of High Heat Generating IC Chips Using PCM-Based Mini-channels

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Chapter | First Online: 13 April 2021

275 Accesses | **3** Citations

Abstract

Optimization has gained enormous importance in the area of electronics to control the temperature of the high heat-generating electronic components. The present study emphasizes the transient numerical simulations on seven asymmetric IC chips kept next to the Left–Right-Bottom-Top (LRBT) and Left–Right-Bottom (LRB) mini-channels fabricated on the SMPS board using the three different phase change materials (PCMs); Suntech P116 (Tm: 49.5 °C), Paraffin wax (Tm: 44 °C), and n-eicosane (Tm: 40.5 °C). The objective is to minimize the maximum temperature of the configuration (arrangement of the seven IC chips) and to

maintain it below the critical limit (≤125 °C). The results suggest that the configuration maximum temperature has dropped by 15.33–22.2% using the n-eicosane (lower melting point PCM). A correlation is established for the dimensionless configuration maximum temperature excess (θ) in terms of the IC chips heat input (q_o), PCM volume content (v_o), and IC chips size (δ). The optimal temperature of the configuration is identified using the GA-based constrained optimization strategy. A sensitivity analysis is carried out to study the effect of the constraints on the optimal temperature of the IC chips. It confirms the dependency of the IC chips temperature on their size, heat input, and PCM volume content.

Keywords

Constrained optimization **Genetic algorithm**

IC chips **Mini-channel** **Phase change material**

Thermal management

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About this chapter

Cite this chapter

Mathew, V.K., Patil, N.G., Hotta, T.K. (2021). Role of Constrained Optimization Technique in the Hybrid Cooling of High Heat Generating IC Chips Using PCM-Based Mini-channels. In: Kulkarni, A.J., Mezura-Montes, E., Wang, Y., Gandomi, A.H., Krishnasamy, G. (eds) Constraint Handling in Metaheuristics and Applications. Springer, Singapore. https://doi.org/10.1007/978-981-33-6710-4_10

.RIS .ENW .BIB

DOI

https://doi.org/10.1007/978-981-33-6710-4_10

Published	Publisher Name	Print ISBN
13 April 2021	Springer, Singapore	978-981-33-6709-8

Online ISBN	eBook Packages
978-981-33-6710-4	Computer Science Computer Science (R0)

wherein cohorts were limited to people with a subsequent health visit within the 14 to 90 day period after diagnosis (as shown in the appendix of the Article).[1]

With the benefits of the longer follow-up that is now available compared with when we did the study, we ran an additional sensitivity analysis in which the same cohorts of patients (ie, with COVID-19 and influenza) as in our primary analysis were followed up until Feb 12, 2021. This analysis allowed us to distinguish people who were potentially lost to follow up in the 3 months after their diagnosis from people who had not yet made contact with a health-care organisation from the network. In this additional analysis, there was substantially less difference in the follow-up rates between cohorts, particularly at 15 days, where the number of people who were at risk of psychiatric illness differed only by 0·2% (ie, 62·2% of the COVID-19 cohort *vs* 62·0% of the influenza cohort). In this analysis, at 90 days, the relative risk of having had a psychiatric illness in the COVID-19 versus influenza cohorts was 1·86 (95% CI 1·65–2·06), which is similar to that in the primary analysis. This similarity provides further evidence that differences in loss of patients to follow-up do not account for the observed differences in rates of psychiatric diagnoses.

Regarding the exclusion of patients from the analysis who had died after the index event, we included these patients as part of a sensitivity analysis in a follow-up study.[2] Similar findings were obtained when patients who had died were included.

SL is an employee of TriNetX. All other authors declare no competing interests. MT and PJH accessed and verified the data in the study and the corresponding author had final responsibility for the decision to submit for publication.

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India needs rigorously tested, culturally adapted cognitive behavioural therapy

As of 2017, the Global Burden of Disease study estimated that approximately one in seven people living across 29 states in India were affected by mental illness.[1] Some challenges that were highlighted were the mental health treatment gap, the need for community-based psychological interventions, and the need for cultural adaptation of psychological interventions like cognitive therapies, keeping in mind the existing plurality in approaches to treatment. Beck and colleagues[2] emphasised the need for increased acceptance of, and access to, culturally adapted models of cognitive behavioural therapy as a potential solution to the vast unmet mental health treatment gap in low-income and middle-income countries. In support of adaptation of psychotherapy for indigenous populations, Bhargava and colleagues[3] reported that the integration of Indian theories such as karma and rebirth, drawn from the Bhagwad Gita, with cognitive behavioural therapy have been beneficial. Other systems, such as the Patanjali Yoga philosophy, the Siddha philosophy, and the Ayurveda, describe aetiology, stages of progression, and treatment for mental disorders, and aim for holistic mental health. In India, these systems of wellbeing are widely accepted and popular. Although attempts to infuse Eastern concepts and practices into Western psychotherapy have been promising, cultural adaptations of psychological therapies are limited to language of delivery and context and cultural orientation of the therapist, rather than content.[4] Models of adaptation of Western psychotherapies need to be improved through studies of systematic adaptation in conformance with specific requirements of a population.[5]

One of us, DS, is a practicing psychotherapist, who, to improve acceptance for psychological therapies, has introduced the concept of therapy in the non-clinical setting of coffee shops in Pune, India. DS has felt the need for indigenisation of cognitive behavioural therapy, not only for rural populations but also for middle and lower socioeconomic groups in urban centres. In India, concepts of Karma-phal (cycle of actions and consequences), Prarabdha (sins connected to past lives), Moh (attachment), and Dukkha (suffering) are preferred over sophisticated but unfamiliar western philosophy, to explain and cope with psychological suffering. Psychotherapists improvise to include the client's religious and cultural concepts in therapy, compromising its empirical basis.

We urge organisations and researchers to endeavour to create and test a comprehensive model of psychotherapy that combines principles of cognitive behavioural therapy with the philosophy and practices from the Bhagwad Gita, the Patanjali Yoga Darshan, and the Ayurveda. Psychotherapy in India needs to shift from an individual and ad hoc approach to an empirical and standardised one to improve mental health outcomes.

DS has a private psychotherapy practice. BPC declares no competing interests.

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Published **Online** March 16, 2021 https://doi.org/10.1016/S2215-0366(21)00070-5

The COVID-19 pandemic has reinforced the need for community mental health-care models in Uganda

As seen elsewhere, the COVID-19 pandemic in Africa has created an urgent need for an isolated space in which to manage patients with COVID-19. In high-income countries such as China, the UK, and the USA, standalone units were built to cater for the extra isolation space required to manage a surge in COVID-19 confirmed cases. In much of Africa, building such units was not possible because of financial, infrastructural, and human resource constraints.[1] Mental health units that conventionally stand alone, far from other health services, were deemed appropriate for the isolation of patients with COVID-19. In Uganda, the only national referral inpatient mental health unit (Butabika Hospital, Kampala, Uganda) has only 638 inpatient psychiatric beds for a population of almost 50 million people. Between Jan 1 and Dec 31, 2018, the bed occupancy rate of Butabika Hospital was 149%.[2] Even with such a high demand for beds in Butabika Hospital, lower level regional psychiatric units were converted into isolation units for COVID-19.[3]

The transformation of psychiatric units into isolation centres in low-income countries such as Uganda requires mental health providers to adopt new care models that do not involve these standalone units. Models for community mental health care, such as integrating psychiatric care into everyday clinical practices, are crucial during this pandemic. Integrated mental health care is an attempt to combine behavioural health services wholly or partly with general and specialty medical services.[4] Care models that ensure staff and patient safety while managing psychiatric and non-psychiatric patients together should also be promoted. For example, the Safewards model aims to reduce conflict between patients and health-care workers so that coercive or restrictive measures are not put in place.[5] Musisi and colleagues have even argued for the introduction of outreach mobile mental health clinics.[6]

Community mental health care needs to become the primary form of care for patients with psychiatric illness in African countries both during and after the COVID-19 pandemic. With a surge of the pandemic in African countries, the need for isolated spaces and the alternative use of inpatient psychiatric beds for patients with COVID-19 are only expected to rise. An expected increase in the incidence of mental health-related complications will also pose a challenge because of the inadequate space; yet existing patients need ongoing care. Now rather than later, a discussion of community models of care for patients with mental illness need to be expedited. Because of the insufficient financing available before the onset of the COVID-19 pandemic, alternative care models were never fully developed in low-resource settings such as Uganda.[1] More substantial funding is required to scale up community mental health services, integrated care, and the Safewards model. This scale-up will ensure that after the COVID-19 pandemic, health systems can help reduce the possibility of a second pandemic, this time of mental illness.

We declare no competing interests.

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Sociocultural influences on interventions for anorexia nervosa

In *The Lancet Psychiatry*, M Solmi and colleagues reported a network meta-analysis, which found that psychological interventions are associated with modest, but clinically significant improvements for adults with anorexia nervosa.[1] However, none of the well known specialty interventions were better than treatment as usual. Solmi and colleagues suggest investigating patient level and therapist level factors that might influence outcomes. In an associated Comment, Evelyn Attia recommends mechanism-based

community members. They are the most marginalised members of south Asian society and are suffering from the crisis of COVID-19 lockdown.[2]

Social stigmatism forces hijras to earn their livelihood by doing informal activities such as collecting money from shops or blessing newlywed couples and newborn babies,[1] and many earn money as sex workers. A survey found that because of lockdown, most hijras were anxious about money (94%) and food (68%).[4] Another survey related to the effect of lockdown on the hijra community found that about 82% of respondents had not earned "a single penny in the last two weeks", and that 59% did not get any support from aid programmes.[5] About 16% of hijra respondents experienced mental abuse, but relatively few faced physical torture and violence.[2]

The multiple and intersecting forms of discrimination and the consequent vulnerabilities experienced by hijras place them at a higher risk of mental health problems, which might trigger various illnesses and long-term psychological complications.[4]

During the lockdown, there was discrimination in who received primary health-care treatment, especially for hijra people.[2] The medical infrastructure is under pressure; obtaining treatment and tests for severe acute respiratory syndrome coronavirus 2 is challenging for hirjas because hospital wards are classified for men or women, with no centre for hijra community members. Hirjas often do not access medical health care because of discrimination, not having legal identification documents, and patient rights violations in health-care settings. Health-care providers' insensitive attitudes towards hirjas make them uncomfortable, such that Hijra people tend not to seek timely COVID-19 treatment, compounding their health risks.[5]

There is a need for basic safety net programmes, free counselling services specifically for the hijra community, and nationwide education campaigns to increase mental health literacy and reduce stigmatisation and discrimination.

I declare no competing interests.

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Mental health insurance in India after COVID-19

In India in 2017, 197·3 million people had been diagnosed with mental disorders, which equates to one in seven of the population, with the most frequently affected being those aged 30–49 years.[1] However, the Indian Government and private insurers have been extremely slow to introduce mental health insurance. In April, 2017, India passed the National Mental Health Act, which mentions provision of health insurance for people with mental illnesses.[2]

India has adopted universal health coverage and has introduced schemes like Ayushman Bharat, a National Health Protection Mission to provide health insurance to 500 million people,[3] which includes financial support for screening and treating mental illnesses. Those who need care for mental illness should be referred to a high-level facility, which will provide inpatient and outpatient services, counselling services to patients and their family, dispense medicines to patients with psychiatric disorders, and send patients to addiction treatment centres.[3]

Up to the end of 2019, a search of the internet, newspapers, and journals (by MG) found no signs of private insurers introducing mental health policies. Surprisingly, we observed the effect of the COVID-19 pandemic with lockdown in India from March 25, 2020, changed the scenario. On June 2, 2020, the Insurance Regulatory and Development Authority of India instructed all insurance companies to provide policies for people with mental illnesses by Oct 1, 2020.[4]

As of October, 2020, insurance plans for mental health treatment are being provided by HDFC ERGO in the my:health Suraksha Plan.[5] The situation for other private insurers is not clear. The Ayushman Bharat scheme of the Government of India included the costs of outpatients and inpatients because of a mental illness. Private insurers like HDFC ERGO have excluded outpatient treatments, which comprise the majority of costs for treatment because of the chronic nature of many mental disorders.

We believe India has an unmet need for insurance of mental health. Stigma associated with mental illnesses and little awareness of patients' rights stop many patients from reporting their condition or asking for insurance. The management of chronic mental illnesses requires regular, and sometimes lifelong, investments in medicine and treatment. Out-of-pocket expenditure can lead to untimely cessation of treatment and subsequent relapse. Unless the stigma of mental disorders is removed and insurance is provided for mental illness, universal health coverage in India will not meet its inherent promises.

We declare no competing interests.

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19 Nanotechnology Industry

Scenario of Intellectual Property Rights

Rakesh A. Afre

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19.1 INTRODUCTION

Nanotechnology is the engineering of matter at the atomic and molecular scale where size is measured in billionths of a meter (one nanometer = one-billionth of a meter). Nanotechnology is a very diversified field with a range of technologies at the nanoscale, such as materials, pharmaceuticals, biotechnology, genomics, neuroscience, robotics, and information technology, etc. In the last three decades, developed countries like the United States, Japan, Germany, France, and Korea have invested several billion dollars in research and development in this atomic-scale technology. All this enthusiasm played an important role resulting in several thousand patents of revolutionary new products and applications. Furthermore, there will be new developments which will make efficient energy sources at nanoscale with potential storage capacity like batteries and solar cells, and drug delivery systems, chemotherapy, stents, etc. While nanotechnology as a revolutionary domain has a number of unique attributes that are particularly susceptible to the development of patent bottlenecks, the problem with it is not new. A patent does not guarantee the right to make something or do anything with it. Instead, a patent gives the owner the right to

Current Pathobiology Reports (2021) 9:133–144
https://doi.org/10.1007/s40139-021-00227-z

NANOPARTICLE-BASED DRUG DELIVERY (M GOGOI, SECTION EDITOR)

Toxicology of Nanoparticles in Drug Delivery

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Accepted: 19 October 2021 / Published online: 24 November 2021
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Abstract

Nanoparticles have revolutionized biomedicine especially in the field of drug delivery due to their intriguing properties such as systemic stability, level of solubility, and target site specificity. It can, however, be both beneficial and damaging depending on the properties in different environments, thus highlighting the importance of nanotoxicology studies before use in humans. Different types of nanoparticles have been used in drug delivery, and this review summarizes the recent toxicity studies of these nanoparticles. The toxicological evaluation of three widely used nanoparticles in drug delivery that are metal, lipid, and protein nanoparticles has been discussed in detail. Studies have recorded several toxic effects of various nanoparticles such as metal-based nanoparticles have been linked to increased oxidative stress and have the potential to infiltrate the cell nucleus and protein-based nanoparticles have been observed to have hepatotoxicity and nephrotoxicity as their adverse effects. Considering the increasing application of nanoparticles in drug delivery and the growing concerns of regulatory authorities regarding the toxicity of nanocarriers in living organisms, it requires urgent attention to identify the gap in toxicity studies. The review highlights the gap in toxicity studies and potential focus areas to overcome the existing challenges.

Keywords Nanoparticles · Nanotoxicology · Toxicity assessment · Protein nanoparticles · Metal nanoparticles · Lipid nanoparticles

Abbreviations

DXR	Doxorubicin
HSA	Human serum albumin
RA	Rheumatoid arthritis
CLT	Celastrol
CPP	Cell-penetrating peptide
GNPs	Gelatin nanoparticles
RFRTs	Ferritin derivative apoferritin
TIMP-GLIA	Tolerogenic immune-modifying nanoparticles containing gliadin
FNPs	Fibroin nanoparticles
SFN-DXR	Silk fibroin nanoparticle bearing doxorubicin
TSFN	Tween 80–coated silk fibroin nanoparticles
SF	Silk fibroin
HPLC	High-performance liquid chromatography
IC50	Half maximal inhibitory concentration
LD50	Median lethal dose

This article is part of the Topical Collection on *Nanoparticle-based Drug Delivery*.

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Introduction

The recent years have marked an unprecedented growth in nanotechnology due to its wide range of applications [1]. The rising popularity can be attributed to their astonishing properties which sets them apart from any other material. This has allowed their application to be expanded in the field of biomedicine, for the purpose of drug delivery, nutraceuticals, diagnostics/imaging, production of biocompatible materials, and much more [2, 3]. The technological advancements using nanoparticles have gained importance in such a short period due to impressive physical and chemical properties. A particle having size less than 100 nm (any one dimension) is characterized as a nanoparticle as per the new definition. These nanostructures possess great surface

to area ratio, show quantum effects, and are highly mobile when present in free state [4]. The conventional drugs face problems like low specificity, less bioavailability, and high toxicity. These problems can be substantially overcome by use of nanoparticle-based drug delivery systems [5]. Metal, non-metal, polymers, and biopolymers have been used for preparation of nanoparticles/nanoformulations [6, 7]. For therapeutics, a nanoparticle can be used in two ways by acting itself (1) as a drug or (2) as a carrier for another substance [7, 8]. Since these nanoparticles are entering the biological system, they interact with various biological pathways and biomolecules found within living beings. Where on one side the unique properties of nanoparticles makes them ideal for drug delivery, the same properties also raise challenges, if not optimized efficiently [9]. As mentioned before, nanoparticles have a great surface to area ratio, which in one case provides benefit of efficient drug loading, but on the contrary, it can also increase chances of interaction with other biomolecules when present in a living organism. Some of the other properties that attract the concerns of scientists when using nanoparticles as drug delivery systems are their particle size (which allows easy access to various organs), charged surface (increasing chances of non-specific interaction), solubility, surface coating, and more. Various researches over the years support the claim that nanoparticles can be a bane or boon. We mostly know about the tremendous benefits that nanoparticles offer, but there is very limited information about toxicity, non-specific protein interaction, translocation to secondary target organs, etc. [10]. Some of the in vivo and in vitro studies have already highlighted the toxic effects of silica, gold, silver, and titanium nanoparticles. Since the size of the nanoparticles used in these studies is similar to the size used in biomedicine, it becomes imperative to assess the toxicological parameters. This review tries to highlight toxicity studies for some of the commonly used nanoparticles in drug delivery (Figs. 1 and 2).

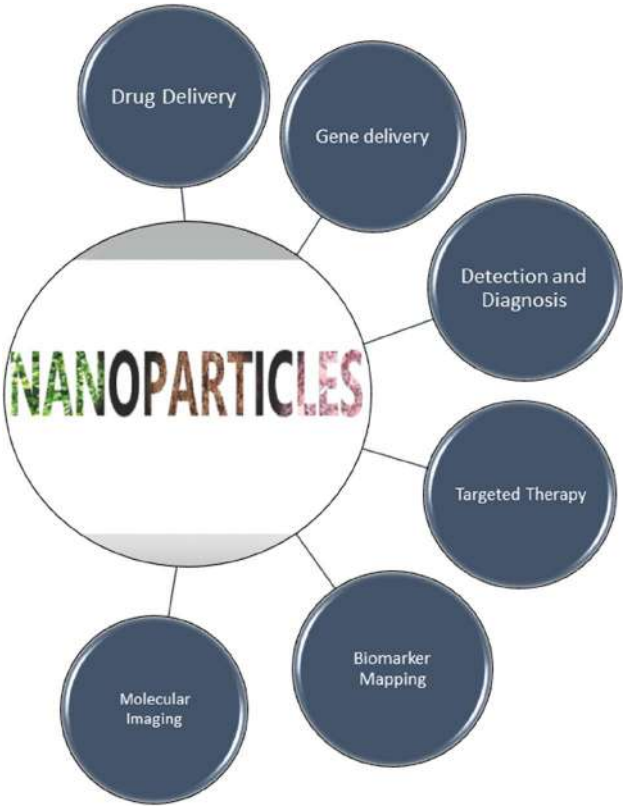

Fig. 1 Biomedical applications of nanotherapeutics

Carbon-Based Nanoparticles

Nanoparticle-based drug formulations have become prominent in recent years due to their properties of targeted and sustainable drug release, enhanced solubility of the drug, and minimum or no adverse drug reactions. Among numerous nanocarriers, carbon nanotubes have been one of the major focuses because of their wide application in cancer therapies [11]. However, the application of carbon nanotubes has been also expanded to non-anticancer drugs, either as a carrier or as an adjuvant [12]. Carbon nanotubes are made from graphene sheets. These sheets are rolled into different shapes (such as ellipsoid, hollow, sphere, and tubes) and sizes. The carbon nanotubes are majorly classified into two types that are SNWCT (single-walled carbon nanotubes) and MWCNT (multi-walled carbon nanotubes) [11, 13, 14]. The advanced physical, mechanical, and chemical properties of CNT raise some concern among researchers when used in drug delivery systems. This is because CNTs can move across the cell membranes while commuting the carrier biomolecule. Therefore, understanding the cytotoxicity of CNTs becomes very crucial [15].

Studies have shown that CNTs can cause neurotoxicity, pulmonary toxicity, immune toxicity, embryotoxicity, genotoxicity, hepatotoxicity, and cardiovascular toxicity [16–22]. Gholamine et al. studied the toxicological impact of SWNTs and MWCNT in mice. The mice were randomized into five groups and injected with MWCNT and SWNTs suspended in PBS (phosphate-buffered saline). After 2 weeks of observation, mice of each group were evaluated for a brain factor known as the brain-derived neurotrophic factor (BNDF). The factors support overall neuron health by promoting sprouting, improving survival, as well as controlling other aspects such as memory, learning, locomotion, and more. SWNTs and MWCNT showed anxiogenic and depression effects, while also impacting the gene expression of BNDF [16]. Fujita et al. conducted studies to understand the in vitro and in vivo pulmonary toxicity caused by SWNTS. It was found that SWNTS induced inflammation and slower recovery and

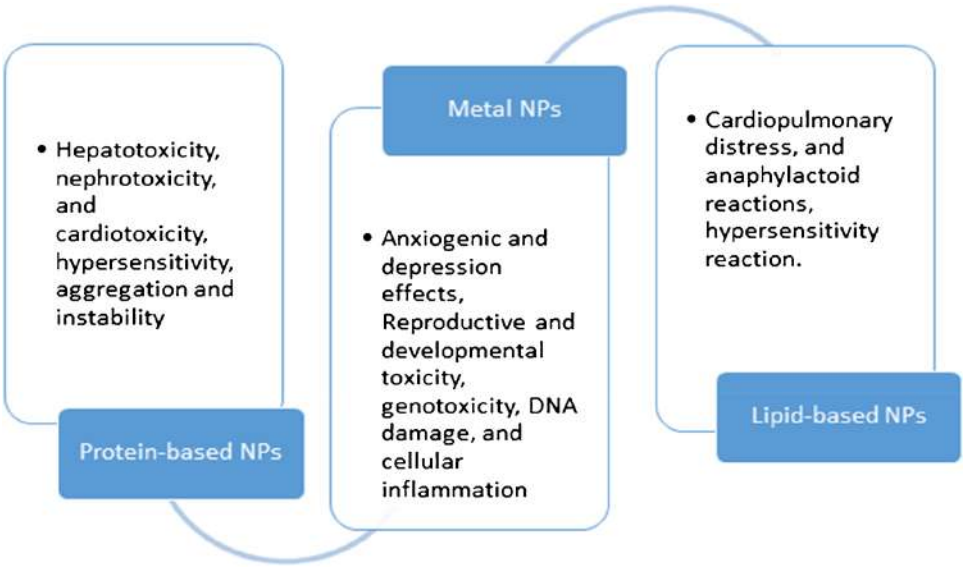

Fig. 2 Toxicity recorded by protein-based nanoparticles, lipid-based nanoparticles, and metal nanoparticles

enhanced the expression of genes governing cell proliferation, inflammation, and reactive oxygen species generation However, the level of toxic impact is dependent on the size of SWNTs used [23]. Reproductive and developmental toxicity of CNTs was observed in mice after intravenous administration. Fetal malformations, miscarriages, and teratogenic and high levels of ROS were some of the observations [24]. A study by Shang et al. highlights damage caused to the kidney and brain by SWCNTs by promoting ROS in mice [25]. Zeinabad et al. studied the CNTs interaction with tau protein and PC12 cells. Results showed that both MWCNT and SWCNT induced different modes of cell death pathway. Moreover, CNTs led to neurotoxicity and protein conformational changes [26••]. Cao et al. mention about the toxicological impact of CNT to the vascular system, wherein they induce formation of atherosclerotic plaque, poor heart rate, and vasomotor dysfunction among animal models [27]. CNTs have been used effectively to deliver anti-tumor drug paclitexal in animal models. The study showed significant tumor suppression and effective release of the drug with minimal side effects. However, the study lacks any toxicity assessment for short-term or long-term period [28].

Metal-Based Nanoparticles

Superparamagnetic iron oxide (SPIONS) is one of the FDA-approved nanoparticles, used widely in diagnostics as well as therapeutic application (hyperthermia, magnetic drug delivery, treatment of anemia) [29]. However, the recent years have observed expansion of SPIONs as potential drug delivery systems to treat cancer and other diseases [30]. But, the concerns related to toxicity of SPIONS have somehow limited their use until their property is enhanced. This is the reason that some of the previously FDA-approved SPIONS for use in medical imaging have been pulled off concerning their toxicity issues and anaphylactic reactions. Existing studies have demonstrated challenges like ROS, LDH leakage, inflammation, DNA damage, and alteration in mitochondrial functioning [31–33]. Currently, Feraheme™ or ferumoxytol of AMAG pharmaceuticals is the only approved drug used in anemic patients suffering from chronic kidney disease (CKD) [34–36]. Umirem, Gastromark, and Feridex are some of the other FDA-approved SPIONs used as imaging agents [34, 37].

SPIONs are also known to impact the complement system leading to innate immunity-related side effects. One such SPION-based drug known to alter the complement system is Feridex™, which is now discontinued by FDA [38–40]. Another concern with use of SPIONs is they show well-known Haber–Weiss and Fenton reactions as the free ions ($Fe2+$) released after lysosomal ingestion react with oxygen or hydrogen peroxide in the powerhouse of cell (mitochondria), forming free radicals which are responsible for genotoxicity, DNA damage, and cellular inflammation [41, 42].

Some of the recent studies have highlighted that the toxicity of SPIONs can be reduced by use of coating agents. Polymers like polyethylene glycol (PEG), poly vinyl (PV), dextran, and chitosan can enhance the half-life and stability of SPIONs and prevent aggregation within cells [42, 43]. Recently, Ferreti et al. evaluated the biocompatibility of magnetic particles. The study finds that when magnetic iron nanoparticles are coated with zwitter-ionic ligands, they showed good stability and biodistribution and required levels of renal clearance [44]. Toxicity of SPIONs is a critical issue which needs to be considered before its extensive use as a drug delivery system. Though lots of research have been carried out to understand the toxicity relation, it is crucial to

dig deeper to enhance their property further. This will lead to more safe use of SPION nanoparticles and FDA approval for drug-based purposes. Similar to SPIONs, other metal nanoparticles like titanium, gold, silver, and zinc have been used for drug delivery but showed significant toxicity.

Lipid-Based Nanoparticles

Alec D Bangham discovered liposome back in the 1960s at the University of Cambridge (Babraham Institute). Liposomes are small and spherical structures forming an enclosed compartment, composed of phospholipid layers. The cavity formed at the center due the interaction between hydrophilic head and hydrophobic tail allows them to act as amphipathic nanocarriers for a wide range of drugs in therapies [45, 46]. About more than 40 liposome-based drugs are either approved for use in the market or are undergoing clinical trials [47, 48]. This increases interest in understanding the interaction of liposomes within the living system and so does their toxic effects. Upon entry into the living system, liposomes interact with a wide range of biomolecules such as LDL, HDL, and opsonins. The encounter with opsonins leads to activation of RES recognition, which further tries to eliminate it from the system. Other lipoproteins in the blood are known to reduce the stability of liposomes within the biological system by leading to rearrangement of surface lipids. This is one of the major concerns when using liposomes as a drug delivery system [49]. To overcome this challenge, liposomes are PEGylated as they prevent opsonization and support evasion from phagocytic cells [47, 50]. Doxil® is an FDA-approved PEGylated liposome-based drug used for treatment of tumor [51]. However, Gabizon et al. highlighted that encapsulated doxorubicin alters particle clearance and phagocytic functioning of cells in the liver. Recently, FDA has approved emergency use of two lipid nanoparticle-based mRNA COVID vaccines of Pfizer-BioNTech, proven to be 95% effective [52, 53]. However, a major challenge of vaccine instability has been observed. Adjustments of lipid nanoparticle structure would be necessary to enhance and improvise the vaccine stability.

In 2018, FDA EC approved another lipid RNAi-based drug named ONPATTRO by Alnylam Pharmaceuticals for diseases caused due to altered TTR (Transthyretin) protein. Risk and safety studies associated with the drug use indicate infusion-related reactions, which can be reduced with vitamin A supplementation and premedication with certain drugs like antihistamines and corticosteroids. Some of the common adverse reactions observed with use of drugs include respiratory symptoms, headache, and change in blood pressure. However, there are limited studies to understand the use of this drug among pregnant women, patients with renal issues, etc. [48, 54, 55].

Some of the PEG-liposomal formulations are negatively charged; this often leads to complement activation, hypersensitivity reactions (HSRs), cardiopulmonary distress, and anaphylactoid reactions. Hoven et al. studied the impact of negatively charged PEGylated liposome nanocarriers. Liposomes with variations in PEG in terms of liposomal size, chain length, surface concentration, and anchor molecule were done. While other variations caused no to mild effects, PEG anchored with cholesterol showed maximum complement activation [56]. Another similar study is conducted by Szebeni et al., where they evaluated the doxil and hynic PEG liposomes for complement activation and hypersensitivity [57].

Protein-Based Nanoparticles

Protein-based nanoparticles have received much attention in recent times due to their biocompatibility, amphiphilicity, easy biodegradability, and reduced toxicity. Albumin, gelatin, ferritin, fibroin, and casein are some of the widely used protein-based nanoparticles for drug delivery [58].

Human serum albumin is a flexible protein that is used to make albumin-based nanocarriers (ANCs) for the administration of cancer treatments [59, 60]. Because of the various drug binding sites found in the albumin molecule, a large amount of drug can be integrated into the particle matrix with serum albumin nanoparticles [61]. HSA may be a valuable analytical indicator for people with autoimmune diseases. Thanks to breakthroughs in biotechnology and protein science, many albumin-associated pharmaceutical preparations, such as nab-paclitaxel (Abraxane®), albumin nanoparticles manufactured utilizing nab technology, are already on the market [62]. The FDA has also approved a paclitaxel formulation (Abraxane). Abraxane differs from previous paclitaxel formulations in that it is attached to albumin particles, which confer water solubility; previous formulations had to be administered in the solvent Cremophor®, which raised allergic reactions and limited dosing [63].

While looking at the preferred outcomes, we must not ignore the drawbacks. Albumin NPs have been claimed to be unstable and extremely damaging to healthy cells. To overcome this and to lengthen their half-life in an aqueous environment and/or limit the development of protein macroaggregates, the freshly synthesized albumin NPs must be stabilized or cross-linked. Thermal treatment, high hydrodynamic pressure, and enzymatic cross-linking using genipin or transglutaminase are some of the ways that can be used to stabilize protein nanoparticles. Glutaraldehyde cross-linking is one of the most commonly utilized ways for stabilizing albumin nanoparticles in general [64]. Steinhauser et al. used heat denaturation of HSA to produce particles loaded with ASOs and found out that HSA nanoparticles cross-linked

with glutaraldehyde exhibited no toxicity in cell culture at concentrations of up to 5 mg/ml [65].

Langer [66], used PH and buffer titration studies were used to assess the stability and electrical behavior of the HSA nanoparticles, with a typical titration profile of prepared nanoparticles throughout a pH range of 3 to 10. The HSA nanoparticles' isoelectric point (pI) was calculated to be 5.05. The nanoparticles became unstable at pH values around pI, as evidenced by PCS: particle diameter increased from 250 nm to roughly 2.7 m (Langer, 2003). Even at higher pH values when the nanoparticles had a strong surface charge, the particle aggregation remained largely irreversible. They concluded that pH values that lead to neutral particle surface charges should be avoided when handling protein-based nanoparticles. In an HSA nanoparticle suspension, the pH value, as well as the buffer content, was found as critical criteria for particle stability [66].

Albumin nanocarriers are used in the treatment of a variety of disorders in addition to cancer. HSA-coupled TRAIL delivery, according to Byeon et al., could be a promising therapy for rheumatoid arthritis (RA), a chronic autoimmune disease marked by extreme synovial hyperplasia and joint destruction. They also found that rats given CLT-encapsulated HSA nanoparticles had milder hepatotoxicity, nephrotoxicity, and cardiotoxicity than rats given CLT alone, according to serum analysis and histopathological examination [67].

Mesken et al. recently released a study on plasmid transmission in HEK 293 T cells loaded onto HSA nanoparticles coated with cell-penetrating peptide (CPP). They were prepared using the desolvation method. There was little cytotoxicity and no significant change in performance when nanoparticle-mediated transfection was examined at low plasmid concentration levels [68]. Even though numerous studies and tests have been conducted to assess the efficiency of HSA nanocarriers and their potentially hazardous effects at various doses, sufficient evidence suggesting the detrimental toxicity of this nano-carrier is still lacking.

Gelatin nanoparticles have recently been offered as a feasible option for parenteral formulations due to their low cost, biocompatibility, biodegradability, minimal antigenicity, and application in a variety of formulations [69]. The US Food and Drug Administration considers gelatin to be a natural, biodegradable, and biocompatible substance that can be utilized to deliver resveratrol, cycloheximide, doxorubicin, and other medications for tumor therapy [70].

In terms of nanoparticle formation, gelatin has low mechanical strength and a fast breakdown rate. To boost mechanical strength and minimize the rate of breakdown and solubility in aqueous solutions, gelatin nanoparticles must be physically, physiologically, or chemically cross-linked with various cross-linking agents, such as GA [71]. In comparison to uncross-linked particles, cross-linking of GNPs is necessary to provide stability, spherical shape, and increased in vivo circulation time. GNPs that have not been cross-linked have been discovered to be unstable and to agglomerate during storage. Furthermore, substantial applications of GNPs as a drug/vaccine delivery vehicle have been explored in a variety of sectors. However, there is still a serious issue with the usage of animal-derived gelatin, which poses a danger of infection with transmissible spongiform encephalopathy [72].

Experiments on the toxicity of GNPs after administration have been carried out. In a study, paclitaxel-loaded gelatin nanoparticles were used to treat dogs with intravesical bladder cancer, and they achieved a 2.6 higher drug concentration in tumors than control dogs given Cremophor EL with commercial paclitaxel injection. Intraperitoneal injections of doxorubicin-loaded glutaraldehyde cross-linked gelatin nanoparticles into rats were used in the experiment. The electrocardiograms and body weights of the animals were monitored for side effects, and the results revealed that control nanoparticles (no medication) had no toxicity. Although the anticancer drug's potency was increased, repeated administration of the formulation (doxorubicin-loaded gelatin nanoparticles) revealed substantial cardiotoxicity [73].

In another study, healthy rats were exposed to doxorubicin-loaded gelatin nanoparticles that had been cross-linked by glutaraldehyde. The researchers discovered that linking DXR to gelatin nanoparticles boosted the drug's cardiotoxicity in the lab. The significant toxicity of DXR-loaded nanoparticles might be due to the drug's covalent binding to the carrier since DXR was attached to the protein matrix of nanoparticles through glutaraldehyde [74].

Gelatin nanofibers have also been proposed for wound healing processes in tissue engineering. Although electrospun pure gelatin nanofibers have been successfully developed, their poor mechanical characteristics and quick disintegration profile have limited their use in wound healing. Many people have tried to combine gelatin with other natural and synthetic polymers to take advantage of gelatin's excellent biocompatibility while increasing the mechanical and physical qualities of nanofibers [75].

Ferritin has unique qualities in various disciplines, such as restricted synthesis, nanodevices, disease detection and therapy, drug delivery, vaccine development, and bioassay. These qualities make ferritin a suitable and powerful nanoplatform [76]. Given its presence not just within every cell of the human body but also in the extracellular space and circulating plasma, the iron storage protein ferritin is likely the greatest prospect for therapeutic use among NPs. The production of ferritin nanoparticles in microorganisms necessitates time-consuming purifying procedures. As a result, producing ferritin nanoparticles in large quantities is challenging. Furthermore, ferritin nanoparticles' high drug loading capacity is limited by their tiny size [77].

The most widely studied technique for anticancer drug delivery is doxorubicin (DXR) encapsulation in ferritin nanocages. DXR is a commonly used cytotoxic medication that has good anticancer activity and is used to treat a variety of solid cancers. However, because it is linked with numerous significant toxicities when supplied at large dosages, its usage is dose-limited. This tailored nanoformulation enhances the drug tumor uptake and accumulation, tumor growth suppression, and circulation half-life and decreased DXR cardiotoxicity [78].

In the experiment conducted by Todd et al., an exceptional tumor inhibition rate of 83.64 percent was re-counted when a genetically improved ferritin derivative apoferritin (RFRTs) was photo-irradiated by a 671-nm laser. Toxicity of the skin is one of the most prevalent side effects of photodynamic treatment; with this RFRT formulation, they found low toxicity to the skin and other tissues [79].

Although ferritin-based drug delivery has been employed for cancer therapy, ferritin was nearly always supplemented with recognition ligands to accomplish tumor-specific targeting in practically all published studies. It causes problems because these additional surface changes undermine the inherent tumor-specific binding of natural ferritin and disrupt its in vivo performance and biocompatibility due to the changed surface physicochemical characteristics of ferritin nanoparticles. Furthermore, many existing approaches for drug loading into ferritin include dismantling ferritin nanocages under a harsh acidic pH, which irreversibly destroys ferritin protein cages and causes harm to the spherical protein surface. Ferritin's irreparable destruction will significantly impact their in vivo stability and medication delivery efficiency [80].

Despite the fact that numerous researches, in their clinical trials, have revealed that this NP can decrease the harmful effects of cytotoxic medications, there is little information on its harmful effects. Before the probable application of this substance in humans, the safety profile, including pharmacology and toxicology, should be thoroughly examined. The development of gliadin NPs has benefitted medication delivery and controlled release applications [81]. Although the desolvation approach can be used to make gliadin particles, it has significant drawbacks, such as limited drug loading efficiency and the difficulty to separate particles from the aqueous phase [82]. Antisolvent precipitated gliadin NPs are vulnerable to pH, heating, and salt effects, resulting in aggregation and instability. They were particularly susceptible to aggregation at pH 6.0–7.0, with a rapid rise in particle size and even the formation of a precipitate [83].

Furthermore, when it comes to achieving the desired outcome with the usage of gliadin nanoparticles while avoiding serious side effects, it is critical to pay attention to the dosage. TIMP-GLIA tolerance was investigated in mice after immunization by Freitag et al. TIMP-GLIA was given several hours after priming (day 0) and again after immunization on day 7. While 0.025 mg/mouse (1.25 mg/kg) therapy failed to show effect, dosages of 0.25–2.5 mg/mouse (12.5–125 mg/kg) were linked to significant reductions in ear edema when compared to controls. Experiments using gliadin recall also revealed a dose-dependent impact. T cell proliferation was unaffected in spleen cells from animals given 0.025 mg/mouse TIMP-GLIA, but two infusions of 0.25–2.5 mg/mouse/dose resulted in a considerable reduction of T cell growth. TIMP-GLIA had no observed adverse impact level of 75 mg/kg, according to the study [84].

Multiple studies have been conducted to determine the stability of GNPs [85]. In line with the research, the gliadin nanoparticle suspensions showed fair stability to aggregation and sedimentation over a very slim pH range that is from pH 4.5 to 6.0, when exposed to a selection of food processing conditions. Short-term thermal handlings at temperatures above 40 °C disrupted particle suspensions, increasing the size of the particle and sedimentation. The increased hydrophobic attraction between the protein particles and/or the increased particle collision frequency may have caused the instability seen at higher temperatures. The protein nanoparticles' poor aggregation stability in pH, salt, and temperature conditions often encountered in food products would be a serious issue for their use in many real-world food systems. As a result, more tactics are needed to improve aggregation stability. Coating gliadin nanoparticles with pectin is a viable method of creating stable functional ingredients, particularly for application in the food sector [86].

The creation of fibroin nanoparticles (FNPs) for a variety of biomedical applications has recently received a lot of attention. FNPs can encapsulate a variety of medicinal chemicals, including small and large molecules, proteins, enzymes, vaccines, and genetic elements, due to their versatility and chemical modifiability [87]. Pandey et al. used the MTT assay to assess the cytotoxicity of the medication containing silk fibroin nanoparticles on the rat and human glioblastoma cell lines C-6 and LN-229, respectively. They also calculated the capacity of free and drug-bearing nanoparticles to impede cell growth. SFN-DXR had an IC50 value of 3.68 M and 1.44 M for 24 and 48 h against C-6 rat glioma cell lines, respectively, which was lower than DXR. Similarly, when TSFN (Tween 80–coated silk fibroin nanoparticles) with DXR is compared to free DXR, the TSFN with DXR has a considerably larger cytotoxicity impact [88].

Similarly, Mishra et al. used solvent precipitation procedures to make curcumin-loaded SF nanoparticles and tested their efficiency in triggering apoptosis in a metastatic breast cancer cell line in vitro. Curcumin release from nanoparticles indicated an initial burst release of more than 50% in the first 24 h, with curcumin release continuing for up to 7 days. Although lower concentrations of curcumin-loaded SF nanoparticles seemed to be less hazardous after 96 h of

treatment compared to 24 h of therapy, prolonged exposure to greater concentrations of curcumin-loaded SF nanoparticles resulted in noteworthy cell viability reductions [89].

Several previous studies have shown that fibroin nanoparticles can be used as a nanocarrier to minimize toxicity and improve the bioavailability of many medications. Although the silk fibroin protein nanoparticles have many advantages, they also have certain drawbacks. Dong et al. employed HPLC to measure the amount of free and encapsulated ibuprofen in liposomes after separation and learned that silk fibroin-coated liposomes had a lower drug encapsulation efficiency than liposomes. It was found out that the presence of SF reduced drug encapsulation, which could be the result of SF hydrophobic chains being inserted into lipid bilayers, causing breaking in the liposomes' outer layers, resulting in medication leakage following methanol treatment. The drug release rate was positively associated with SF dissolving time, but negatively with SF concentration, according to the results of an in vitro release investigation [90].

The delayed degradation of the fibroin crystalline antiparallel sheet domain has also been reported to be a drawback in some applications that need the removal of nanoparticle carriers quickly and completely. Furthermore, because fibrin is a protein, it can be attacked by immune systems such as macrophages and giant cells, resulting in encapsulation and granuloma development inside these cells, which can lead to drug release outside the target. Finally, while fibroin can be extracted from a variety of sources, comprising those that are similar to other natural products, the nature of each batch may differ slightly due to post-conversion process alterations in both species and entities [91].

Casein is a fascinating protein because it has excellent structural and physicochemical features that make it ideal for drug delivery. Gandhi et al. made casein nanoparticles with doxorubicin, an anticancer medicine. The team used fluorescence studies with varied quantities of DXR combined with a 2% casein solution to maximize DXR binding to casein. Casein NP was found to be an efficient drug delivery carrier at both physiological and acidic pH levels [92].

Microspheres made of hydrophobic proteins, casein, may easily encapsulate hydrophobic pharmaceuticals like progesterone, and the release rate and amount of drug molecules may be regulated by adjusting the glutaraldehyde-to-protein ratio and particle size [89, 93].

Pealva et al. explored the use of casein NPs as resveratrol transporters. Once the nanoparticles were created by coacervation, they were then refined and spray-dried. The mean size of NPs was calculated to be roughly 200 nm with a resveratrol payload of roughly 30 g/mg. The experiments that took place in vitro indicated that the release of resveratrol from casein nanoparticles did not show any effect by the pH and followed a kinetic of zero order. When resveratrol was loaded with casein nanoparticles, its oral bioavailability resulted in 10 times greater than the polyphenol delivery as an oral solution, thus proving the hypothesis of improved efficacy of casein nanoparticles as oral carriers [94].

Toxicity and therapeutic efficacy such as the IC50, LD50, and EC50 of the casein can be evaluated by standard pharmaceutical techniques. A 3-month dose-repeated toxicity test was conducted to examine the safety of casein nanoparticles when administered orally to animals. It was reported that the males and females treated with the maximum dose of 500 mg/Kg bw developed comprehensive hyperchloremia after almost a month. A few cases of hypernatremia in the females were also reported. It was concluded that the formation of drug aggregates with casein nanoparticles results in highly localized concentrations at the sites of deposition that are associated with local toxicity [95]. This study indicates the side effects of protein-based nanoparticles, casein, although further studies are required for the detailed analysis of casein nanoparticle's toxicity and adverse effects.

Polymeric Nanoparticles

Polymeric nanoparticles are made from natural or synthetic polymers and are especially evident in smart drug delivery. They are being studied extensively in the pharmaceutical industry as carriers for controlled/sustained release in medication delivery systems. Safety difficulties, toxicity threats, inadequate biocompatibility, and physiological challenges all contribute to the activity of these nanopharmaceuticals being limited [96]. The downside of these nanoparticles is toxic monomer aggregation, residual material linked with them, and toxic degradation process [97].

Polymeric NPs' toxicity is affected by quantum size effects, which are linked to oxidative stress, cytotoxicity, and genotoxicity [98]. Upon investigation of the toxicity of a variety of poly(lactide-co-glycolic acid) (PLGA) nanoparticles on human-like THP-1 macrophages, Nadège et al. found that the slightly cytotoxic chitosan polymer conferred significant cytotoxicity to PLGA nanoparticles when used as a nanoparticle stabilizer. Poly vinyl alcohol and poloxamer 188 polymers also conferred significant cytotoxicity to PLGA nano. These findings revealed that stabilizers employed in the formulation of PLGA nanoparticles have a significant toxicological role when used at high concentrations, which could have consequences for local toxicity of PLGA-based nanomedicine [99].

The differential lung toxicity of positively and negatively charged PEG-polylactic acid (PLA) nanoparticles was explored following daily endotracheal instillation of BALB/c mice while examining the impacts of nanoparticle surface charge for polymeric particles. PEG-PLA nanoparticles based on cationic stearylamine induced higher local and systemic harmful effects [100]. Synthetic nanoparticles

and organic solvents that have been demonstrated to be harmful are employed to manufacture these nanoparticles. Natural polymers and synthesis methods using less harmful solvents have been investigated as potential alternatives to synthetic polymers [101]. Because of their poor circulation stability and targeting inefficiency, using polymeric NPs as chemotherapeutic drug delivery vehicles is generally difficult [102]. Multiple experiments have been going on in order to address these issues such as Palanikumar et al. developed biocompatible and biodegradable pH-responsive hybrid NPs. A cross-linked bovine serum albumin shell was added to these nanosystems based on a drug-loaded PLGA core to decrease interactions with serum proteins and macrophages. As a result, the drug-loaded NPs demonstrated strong anticancer action both in vitro and in vivo while causing no harm to healthy tissue [103].

Silica Nanoparticles

Properties like ease of surface modification, synthesis, tunable pore size, efficient thermal stability, and biocompatibility make silica nanoparticles a great choice for drug delivery [104, 105]. Recently, silica nanoparticles have been used to deliver cargos like anti-cancer drugs (camptothecin and doxorubicin in breast cancer treatment), nucleic acid, and proteins (e.g., RGD peptide and aptamers) via controlled released method [106–108]. The growing application often demands assessment of potential risks of toxicity associated with the nanoparticles. Some studies have highlighted the toxicity of silica nanoparticles which leads to lung diseases and neurotoxicity and impacts the immune system. The physicochemical properties of silica nanoparticles are known to induce toxicity at cellular level. In a study by Bancos et al., the impact of silica nanoparticles on macrophages was evaluated using the RAW 264.7 cell line. Exposure to 10-nm silica nanoparticles led to reduced phagocytosis and impacted cytokine production [109]. Gonzalez et al. identified that silica nanoparticles induce cytotoxic effects on lung carcinoma cells [110]. Similar studies by Yang et al. highlight cytotoxicity in human vascular endothelial cells due to silica nanoparticles [111]. Micronucleus assay and in vitro comet-based analysis have been carried out in some studies to understand the damage caused by silica nanoparticles at DNA level. It was observed that silica nanoparticles show dose-dependent genotoxic effects in these studies on lung cells [112]. In another study, Lucarelli et al. used U-937 cells and ELISA-based assay to evaluate the change in response to silica nanoparticles; they observed M1 polarization and altered IL-1 beta and TNF-alpha production [113]. Xifei et al.'s study highlights that silica nanoparticles lead to increased levels of reactive oxygen species and depletion of glutathione at intracellular levels [114]. Thus, it is very important to evaluate and regulate the toxicity assessment of nanoparticles before use in humans.

Challenges and Future Prospects

The field of nanotechnology has seen remarkable improvement post-1980 as many nano-based drug delivery products have been approved in the market. However, a range of challenges are faced before these nano-based agents enter into the market which include efficient development of nano-carriers, toxicity assessment, and ethical and regulatory requirements. The safety and risk ratio both to the patient and to the manufacturer is not yet clearly defined. The risk/benefit ratio itself faces challenges in laying the details as a strong framework for evaluation has not been demarcated. For nano-based products to be used, it is very crucial that the pre-clinical and clinical testing guidelines be set in detail. Nanoscience has recently stepped in drug delivery and understanding of toxicity, pharmacology, and immune responses of these nano-products still need a lot of deep research.

One common challenge in defining the safety and risk of nanoparticles is that they have different properties depending upon their size, surface area, and surface charge, making them difficult to categorize the risks. Moreover, nanoparticles can move within the biological system easily due to their effective biocompatibility. They can cross cell membranes, placenta, blood–brain barrier, and more, thus increasing the chances of non-specific interaction, which can eventually lead to unwanted accumulation and toxicity at cellular level. Clinical trials to assess these risks can be of great advantage, but the current scenario faces challenges of efficient risk assessment, risk management, and risk communication. Sometimes, extrapolation of animal-based studies into humans can be detrimental. For example, a study involving monoclonal antibodies showed severe illness among the human subjects, even though the results were promising in animal subjects. Learning from these kinds of cases needs to be considered when working with nanomedicines. Post-clinical trials, the side effects need to be informed to the respective safety agency to avoid potential risks.

Despite the above challenges, it can be anticipated that the efficiency of nanomedicines will make their demand grow further in coming years. The application of nano-carriers in cancer treatment has attracted the eye of many researchers, which becomes more evident from the fact that most of the FDA-approved nano-based drugs were cancer related. All in all, the coming years will see a rise in the application of nano-based drug delivery agents for a variety of diseases, and there will be significant growth in the market size of nanomedicines.

Conclusion

Over decades, nanoparticles have been considered to be a wonder of modern science due to their wide applications. The use of nanoparticles in biomedicine has very well marked that standard, as superior therapeutic systems have emerged, helping us conquer various diseases and enhance the quality of human life. However, there still exist many challenges to effectively utilize the full potential of nanoparticles. Toxicity of nanoparticles is one the major barriers that need urgent attention. The safety assessment is vital for use in living beings to recognize potential risks and create preventive measures. As we have seen, there is very limited data available for certain types of nanoparticles used in drug delivery. Therefore, before pushing nanoparticles into the therapeutic market, it is crucial to assess the risk to benefit ratio. Another reason is that the property of nanoformulations within a biological system is yet to be studied in great detail. This has made the regulatory authorities take necessary steps which emphasizes understanding the safety and toxicity concerns associated with nanoparticles used in drug delivery systems. Thus, toxicological studies are needed to provide a more detailed analysis of nanoparticles used in drug delivery. It will help researchers to enhance the properties of nanoparticles further to reduce the toxicity and create nanoformulations which are effective but also non-toxic for therapeutic use.

Declarations

Conflict of Interest Swati Sharma, Roza Parveen, and Dr. Biswa Prasun Chatterji declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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Content list available at: www.benthamopen.com/TOSCJ/

DOI: 10.2174/1876893801805010031, 2018, *5*, 31-46

REVIEW ARTICLE

Ocular Stem Cells to Treat Retinal and Corneal Disorders

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Received: July 17, 2018 Revised: October 12, 2018 Accepted: October 18, 2018

Abstract:

Background:

According to WHO, 285 million people are visually impaired out of which, 39 million are classified as blind and the remaining 246 million people have low vision which comprises of moderate vision impairment and severe vision impairment. Therapies to treat major disorders leading to visual impairment like Age-related Macular Degeneration (AMD), Stargardt's Disease (STGD), Retinitis Pigmentosa (RP) and corneal scarring are required.

In the last decade, many advances have been made to treat these disorders using stem cell therapy. For corneal damage by accidental burns, scarring or limbal stem cell deficiencies which can lead to partial or total blindness, are treated with a risky intervention like keratoplasty. To overcome issues like graft rejection caused by keratoplasty as well as have a better outcome, limbal stem cell therapy has been introduced. Similarly, Retinal Pigment Epithelium (RPE) is a supporting tissue essential in nutrient transport, production of growth factors, phagocytosis of the photoreceptors and retinol cycling.

Discussion and Conlusion:

Degeneration of this monolayer causes many diseases that have no prevailing treatment; however, research is being carried out to replace this simple epithelial monolayer primarily with an autologous source of cells and currently using stem cells. This review discusses the advances made in the field of ocular stem cell therapy with regards to development, cultivation and novel methods used to deliver these cells to replace the corneal and retinal epithelium as a new standard for treatment.

Keywords: Cornea, Retina, Retinal pigment epithelium, Corneal stem cells, Retinal stem cells, Limbal stem cells, Keratoplasty, Stem cell therapy.

1. INTRODUCTION

According to WHO, 285 million people are visually impaired out of which 39 million are classified as blind and the remaining 246 million people have a low vision which comprises of moderate vision impairment to severe vision impairment [1].

Blindness is primarily caused by the loss of supportive glia which is due to the reduction in neurons. The retina is a part of the central nervous system which shares its regenerative abilities with the brain and spinal tissues thus there are no techniques to transplant lost Retinal Ganglion Cells (RGC) and photoreceptor cells, similarly, axogenic or neuroprotective techniques are unavailable [2]. Retinal Pigment Epithelium (RPE) is a supporting tissue essential in nutrient transport, production of growth factors, phagocytosis of the photoreceptors and retinol cycling. Degeneration of this monolayer leads to diseases like Age-related Macular Degeneration (AMD), Stargardt's disease, Retinitis Pigmentosa (RP) which ultimately cause loss of vision. There are no present remedial treatments for these conditions; however, a great deal of research is being carried out to replace this simple epithelial monolayer primarily with an

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autologous source of cells predominantly using stem cells [3].

Cornea, a translucent layer concealing the eye serves a vital function of channelling light towards the retina. Corneal scarring that leads to corneal opacity is the major cause of visual impairment; that can be treated by corneal transplantation (keratoplasty) [4]. The corneal surface is comprised of five layers within which the corneal epithelium forms 10% of the layer that is replenished by stem cells, located at the limbus region of cornea [4, 5]. The remaining 90% of layer of the corneal surface is composed of stroma which largely consists of water, collagens and keratocytes or quiescent fibroblasts [5].

The cornea has an essential role during the occurrence of wounds and the generation of tear. The presence of corneal nerve is responsible for the sensation of pain and touch. Therefore, any dysfunction of cornea can lead to blindness, where corneal opacity is considered to be the second most causative agent for blindness [6].

Chemical burns that are alkali or acid burn can lead to damage in cornea. The damaged cornea is unable to regenerate causing partial or total blindness. Stevens-Johnson Syndrome (SJS) of the eye may have rare incidence but its severity is high. In this syndrome, continuous as well as increased inflammation and fibrosis of the cornea, causes opacity and a deficiency of stem cell in the affected region eventually evoking to blindness. A study in US from 2010 to 2012 reported incidence of 3834 people suffering from SJS. Considering this incidence rate in the US, it can be expected that approximately 86,500 new cases of SJS are registered every year in the world. Similarly, chemical burn incidents were calculated using the US figure which suggest down to 357,710 burn accidents all over the world [7].

Corneal blindness caused by chemical burns, SJS *etc*. can be the initiating event for total or partial corneal and/or limbal stem cell deficiency which is frequently treated with keratoplasty. Apart from the difficulty in finding an appropriate donor, a post-surgery complication of allograft rejection is often observed due to corneal vascularization, retinal disease, diabetes, adherent leukemia and bilateral blindness [8]. Thus to attain successful transplantation of cornea and regain its functions, new techniques of cultivation and application of stem cells have been increasing worldwide.

This article briefly summarizes the arising new techniques to combat corneal and retinal blindness. A detailed discussion of all the diseases and conditions leading to visual impairment is outside the scope of the current review and thus we focus on age-related macular degeneration, Retinitis Pigmentosa, Stargardt's Disease and corneal degeneration. These are the preliminary targets for stem cell therapy, therefore we focus on treatments wherein stem cells are cultured and utilized to treat these disorders in the below review. The paper contains tables which help us summarise the patents more efficiently. The patents used herein have been obtained from the World intellectual property organization, Espacenet and Google patents.

1.1. Age-related Macular Degeneration

Age-related Macular Degeneration (AMD) is categorised as the primary cause of blindness affecting people above the age of 55 years and it is estimated to infringe 196 million people worldwide by the year 2020 [9]. AMD can be labelled as wet AMD (neovascular choroidal) accounting for 10-15% of the cases and dry AMD (geographic atrophy) computing for 80-85% of the total cases [3, 10]. RPE cells are situated below the photoreceptors which perform essential functions as mentioned earlier; these cells are majorly affected by AMD, making it a complex degenerative disease caused as a result of a low-grade inflammation in the retina leading to deterioration of RPE and Bruch's membrane. RPE is involved in phagocytosis of photoreceptor outer segments (OS) disc; overtime the RPE degenerates due to senescence and incomplete digestion of phagosome activating the accumulation of lipofuscin which interferes with RPE functioning and ultimately causing apoptosis. Between the ages of 10-90 years, the Bruch's membrane undergoes thickening bringing about the defect in nutrient transport weakening the RPE to adhere to the basement membrane [3, 11]. Conventional treatment for wet AMD involves the intravitreal injection of Anti-Vascular Endothelial Growth Factor (VEGF) but on discontinuation of the treatment, relapse is observed. The other treatments that are practised includes photodynamic therapy and laser photocoagulation. Although the success rate is low as the RPE cells are previously damaged and surgery might lead to the further removal of healthy cells [12]. Stem cell therapy is a potential treatment for AMD as it can persuade improvement of visual perception [10].

1.2. Retinitis Pigmentosa

Retinitis Pigmentosa (RP) is an inherited retinal disorder that can have X-linked, dominant or recessive pattern of inheritance. It is a rare genetic disorder characterised by 100 defined mutations that cause the defect in the

photoreceptor cells. These mutations can affect the cells ability to produce the required protein for its regular functions or provoke production of a toxic protein which would be fatal for the cells, thus, damaging the photoreceptor cells. RP can cause severe symptoms like night blindness, fundus appearance, tunnel vision, posterior subcapsular cataract that can eventually lead to blindness. RP has various subtypes, the most common subtype is a mutation in the gene coding for rhodopsin which affects 1 in 4000 patients. Some of these subtypes are caused due to mutations in genes coding for RPE, this can be potential targets for stem cell therapy to replace RPE [3, 13, 14].

1.3. Stargardt's Disease

Stargardt's disease is the most commonly inherited retinal disorder. Children are majorly affected by this retinal disorder with a prevalence of 1:8,000-10,000, arising during the first and second decades of life. The highly prevalent form, STGD1 is autosomal recessive in nature and occurs due to mutations in ABCA4 gene, that is responsible for encoding a transporter protein expressed on photoreceptors. The other forms like STGD3 and STGD4 are also caused due to mutations in ELOVL4 gene and PROM1 gene respectively where both of these genes code for proteins present in the PRs [3]. One of the symptoms presented by patients suffering from Stargardt's includes bilateral central vision loss with characteristic yellow-white flecks at the posterior edge of the RPE in the macular region which indicate buildup of lipofuscin in the PRs and later in the RPE. Currently, there is no treatment available for this disorder; research is being carried on to treat this disease with stem cell therapy, gene replacement therapy and various pharmacological approaches [15].

1.4. Stem Cells to Treat Retinal and Corneal Disorders

A mucosal epithelial overlying and underlying the eyelid is known as ocular surface [16]. In normal condition, the surface has three layers namely corneal, limbal and conjunctival epithelia, where every layer has a different cell type [17]. Corneal epithelial is made up of non-keratinised squamous epithelial cells; the property of this layer is to undergo a continuous custom of renewal and regeneration [16]. Stem cells present in these layers split and proliferate whenever there is a need for tissue repair. During splitting into two daughter cells, one cell retains the characteristic of rejuvenation while the other differentiate which is destined to be part of corneal epithelium also known as 'transient amplifying cells'. These cells are generally available at the basal layer of limbal and corneal epithelium [18].

Stem-cell deficiency can occur in case of chemical burns, SJS, aniridia, various endocrine deficiencies as it causes scarcity of stromal microenvironment to support stem cell function. Corneal cell deficiency can be identified by immunohistochemistry to detect any lack of corneal differentiated cells and an increase in goblet cells containing mucin [16, 19]. Thus, Stem cell-based therapy should be an ideal treatment to treat blindness as it would have a low failure rate; it will not require immunosuppression and will be reproducible using a wide range of phenotypes. The sources for these cells include pluripotent as well as multipotent cells [3].

2. UNDERSTANDING ROLE OF STEM CELLS IN OCULAR SURFACE DISORDERS

2.1. Role of Induced Pluripotent Stem Cells

Reprogramming of mouse somatic cells by identification of essential transcription factors was first demonstrated by Takahashi and Yamanaka that urged the development of Induced Pluripotent Stem Cells (iPSCs) which have been used for various purposes, one of them being for the development of RPE cells [20]. Followed by this discovery, Yamanaka and colleagues succeeded in integrating transcription factors using retroviruses within human fibroblast cells to convert them into pluripotent stem cell-like state. With the successful conversion of hESCs into RPE, researchers used similar protocols for iPSCs obtained from prenatal and adult fibroblast to obtain RPE and retinal cells. These cells are evaluated based on common criteria that include; hexagonal cell morphology, observed pigmentation on cells, formation of cell polarity, tight junction formation and growth factor exudation [21]. The genetic profiles of these iPSCs are similar to ESCs but they do not have limitations similar to these cells *i.e.* ethical issues of embryo-derived cells and limited immune rejection as they are derived from the patient; making iPSCs a worthwhile opportunity for regenerative medicine. Various sources apart from human fibroblast can be used for cell derivation like NSCs, primordial blood cells and peripheral blood monocytes. Currently, Sendai viruses and episomal vectors are utilised to integrate the transcription factors to produce most of the iPSC cell lines [11].

The first clinical trial for AMD utilizing iPSCs was initially performed in Japan. The study was suspended within a year due to reported mutations in the derived iPSCs which were absent in the original patient fibroblast cells [11].

iPSCs, however, are considered to be comparable to hESCs as they have been shown to harbour differences in gene expression and DNA methylation. Chromosomal telomeres are observed to be significantly shorter in iPSCs compared to the native RPE indicating DNA aging. Thus, the cells might not have the desired lifespan, requiring one or more transplantations to attain effective results. Diseases like Stargardt's which occurs due to a single gene defect may prove to be a challenge for iPSC based therapies as the cells generated from the patient will contain the genetic defect. These defects need to be corrected prior to cell differentiation and transplantation. Whereas in AMD such genetic manipulations are not needed as it is a polygenic disease and no individual gene correction would be able to provide a cure [3].

Preparation of viable retinal precursor cells is vital for treating any disorders; patent CN103627669 defines a method for differentiation of human iPSCs into retinal precursor cells using a specific medium containing nitrogen followed by transfer to a neurogenic medium comprising knockout serum substitute as well as nicotinamide. The patent describes the morphological changes occurring within the cells in great detail making it easier for understanding. The cells can also be used for drug screening, treatment for AMD and gene interference experimentation (Table **1**) [22]. Patent US 2015/0368713 A1 also describes a method for the production of high-efficiency RPE cells from induced somatic cells which also include fetal RPE cells. The patent initially references culturing of embryoid bodies on medium containing inhibitor for Nodal pathway and two inhibitors for Wnt pathway. The second medium has four variations wherein they lack fibroblast growth factors, Wnt and Nodal inhibitors, in addition to Noggin and knockout serum replacement. The cells are further cultured in selective medium to produce high-efficiency cells. Another embodiment mentions characterization of the cells and determining agents which affect the induced cells by increasing gene expression as well as help in RPE survival under stress (Table **1**) [23]. Similarly, invention CN105274049 discloses a protocol for the culturing and differentiating of human iPSCs-3D retina cells to retinal ganglion cells. The patent is a first of its kind, providing methods and media formulations for directional differentiation of cells helping us understand as well as explore the regulatory mechanisms of iPSCs. These cells will not only be useful to treat major diseases specifically to treat optic nerve injury but also help to study tissue engineering of cells in the nervous tissue (Table **1**) [24]. As these discoveries are in the infantile stages, the studies that are being carried out using humans are limited and have great scope for positive clinical trials.

Table 1. Summary of patents filed for iPSCs used for treating ocular disorders.

No.	Cell Type	Patent No.	Disease	Summary	Route of Administration	Refs.
1	iPSCs	CN103627669	Retinal neuronal disorders *e.g.* AMD, RP	Method of inducing human pluripotent cells to obtain retinal precursor cells like pigment epithelial precursor cells and neurofilament progenitor cells. The invention induces the cells with Neurobasal medium, knockout serum replacement and nicotinamide so as to overcome low differentiation efficiency and reduce the cell differentiation time period.	-	[22]
2	iPSCs	US2015/0368713A1	Retinal degenerative disorders like AMD, RP, Stargardt's disease, Glaucoma and diabetic retinopathy	Method to utilize iPSCs (somatic cells) to obtain retinal pigment epithelium cells for their use in screening assays, therapeutics with their pharmaceutical compositions described in the invention and to study RPE. The iPSCs are produced from somatic cells such as fetal retinal pigment epithelial stem cells.	Polymeric scaffold/extracellular matrix and cell suspensions	[23]
3	iPSCs	CN105274049	Treatment and recuperation of optic nerve injury	The invention provides a procedure to induce directional differentiation of iPSCs-derived 3D retinas into retinal ganglion cells *in vitro* wherein a nerve cell layer is digested into a single cell which is used as seed junction to produce functional RGCs using various media compositions.	-	[24]
4	iPSC's	US20140127803A1	Bilateral Corneal diseases	This patent describes the procedure to transform induced pluripotent stem cells to corneal epithelium cells in presence of amniotic membrane.	-	[27]
5	iPSC's	US20180010093A1	Damaged or diseased ocular surface	This invention relates to a method for inducing the differentiation of corneal epithelial cells in serum-free media from human pluripotent stem cells.	-	[28]
6	iPSC's	WO2018037161A1	Corneal diseases	The patent describes methods to differentiate pluripotent cells into corneal cells.	-	[29]

iPSCs are competent to individuate themselves into three layers namely, mesoderm, ectoderm and endoderm. This

capability of iPSCs was first discovered in 2006 by the Nobel laureate Yamanaka since then its features has been used in various cell therapies including corneal surface disorders [25]. In in-vitro conditions, the pluripotent cells are immortal thus acting in favour of regenerative medicines [26].

Patent US 2014/012780A1 describes the method to form eye precursor cells from iPSCs. The inventors were able to grow iPSCs without the help of feeder cell layer. The cells were subjected to a medium which contained Bone Morphogenetic Protein – 4 (BMP-4), Tumour Growth Factor-Beta (TGF-β) inhibitor and Fibroblast Growth Factor (FGF). The culturing of the above cells in mentioned medium leads to the development of eye precursor cells. After this development, the cells were then grown in culture in absence of TGF-β, FGF and BMP-4 but in presence of Epidermal Growth Factor (EGF), hydrocortisone, insulin, isoproterenol, and tri-iodo-thyronine which aids in formation of corneal epithelial precursor cells and on maturation it forms corneal stratified epithelium cells (Table **1**) [27]. In Osaka University, inventors were able to formulate a procedure by which these iPSCs were able to differentiate themselves into corneal epithelium stem cells. Pluripotent stem cells were exposed to the reprogramming factor in serum-free medium to acquire iPSCs which were able to differentiate themselves into corneal epithelial cells. The factors used in this medium were a stromal cell or amnion-derived cells. The cells which showed the presence of corneal epithelium cell surface marker having an inexhaustible life were selected. These selected cells can further be cultivated to form corneal epithelium sheet for the treatment of corneal surface disorders (Table **1**) [28]. This invention guided the inventors to formulate a similar method to produce corneal epithelium cells. Here, the iPSCs were grown in a flask or a dish where substrate was made up of one or more elements. These elements were collagen, fibronectin, laminin or laminin fragments, vitronectin, basement membrane matrices, gelatin, hyaluronic acid, polylysine, vitronectin, and hyaluronic acid. Here, the media was like the earlier invention, a serum-free medium; however, this media did contain one or more of very minute amounts of BMP-4, TGF or activin. By this procedure, iPSCs formed a concentric zone of ectodermal lineages cells that contained eye precursor cells. To obtain only corneal epithelium cells from eye precursor cells, first, the cells were grown in media which consist of rock inhibitor, keratinocyte growth factor (KGF) and serum which gave corneal progenitor epithelium cells. These progenitor cells were screened for the presence of K12, pax6 and MUC16 markers on the cells that were selected for induction of corneal epithelium cells [29]. These corneal cells can be cultivated into sheet format which can then be incorporated into the defected eye surface as an alternative cell regenerative treatment in patients [28, 29].

2.2. Role of Human Embryonic Stem Cells

Human Embryonic Stem Cells (hESC) individuate into functional RPE by replicating with close morphology to endogenous tissue as well as acquiring cell surface markers specific to RPE. These cells display physiognomies, similar to the innate cells like polarity, phagocytosis, retinol cycling and similar tight junctions. Embryonic stem cells were used for the first time to restore visual function in Royal College of Surgeon rats; a widely used animal model to study disorders of the RPE as well as AMD, where embryonic stem cells derived from primates were used for replacement of RPE to treat AMD [3, 30]. In humans, Schwartz *et al.* were the first to successfully transplant ESCs derived RPE into two patients suffering from varying forms of AMD and Stargardt's disease where no signs of graft rejection were observed within the first four months of transplantation. Further trials showed the long-term survival of a graft in 18 patients, the study demonstrated improved visual acuity and quality of life measure [9]. The efficiency of the protocols in use is limited for production of hESC. To improve these, 3D hydrogels have been used to culture the cells. The research indicated improved derivation of RPE from 0.5% RGD-alginate hydrogel. This type of hydrogel scaffold can also help in transport as well as transplantation of RPE and also help in the formation of neural tissue [31].

Human embryonic stem cells are produced using various methods; Patent WO2008/129554A1 describes a method for production of RPE cells from human stem cells majorly embryonic stem cells. The invention utilises Transforming Growth Factor-β (TGF-β) superfamily for preparation of RPE. The cell culture system comprises a medium enhanced with TGF-β superfamily, where the stem cells are promoted for directed or augmented differentiation. The patent provides a culture media comprising matured RPE, a method for harvesting these cells and for transplanting them into a subject to help treat retinal disorders due to loss of RPE or injury (Table **2**) [32]. Similarly, Patent WO2009/051671A1 provides methods for production of RPE on a large scale from hESC and iPSCs using various media compositions like addition of growth factors along with methods for purification of the cultured RPE as well as to sub-culture these cells at various concentrations. The patent also gives a characterization of the cells and comparison of RPE with ones derived from other sources *e.g.* adult and fetal RPE. The invention also helps identify cell maturing agents which assist in various screening assays and the cells can also be used to treat various retinal disorders (Table **2**) [33].

Table 2. Summary of patents filed for hESCs used for treating ocular disorders.

No.	Cell Type	Patent No.	Disease	Summary	Route of Administration	Refs.
1	hESCs	WO2008/129554A1	Retinal dysfunction, injury or the loss of retinal pigment epithelium	A process of differentiation of hESCs to RPE in a basic medium consisting of various TGFβ superfamily. The patent claims method for RPE cell production in bioreactors and further used to prepare therapeutic compositions to treat retinal disorders.	Intraocular transplantation	[32]
2	hESCs	WO2009/051671A1	AMD, Stargardt's disease, RP and diabetic retinopathy	The invention describes method to obtain a substantially purified form of human RPE cells induced from hESCs or other human stem cells. The patent comprises of pharmaceutical compositions of these RPE cells as well as cryopreserved preparation thereof. The cells are also used for screening assays based on the different markers like RPE-65, PEDF, Otx2 *etc*.. The pharmaceutical compositions are further used to treat retinal disorders.	Intraocular transplantation using suspension, matrix, substrate or injection into subretinal space of the eye	[33]
3	hESCs	EP2484308 (A1)	Keratitis, corneal ulcer, corneal abrasion, snow blindness, corneal infections, corneal dystrophy *etc*.	The invention refers to a mode to obtain a 3D synthetic cornea from parthenogenically activated human oocyte followed by transplantation of the cornea to treat various disorders as well as studying the effects of therapeutic agents on the cornea.	Implantation of synthetic cornea	[35]
4	Human placental or human umbilical cells	US7412734B2	Ocular degenerative conditions like RP, glaucoma, optic nerve trauma, ocular lesions *etc*.	Method to utilise postpartum-derived cells for differentiation into a neural cell fate which can further be characterized based on the presence of cell surface receptors. The neural cells are used to treat ocular disorders and the patent also describes formulations as well as routes of administrations thereof.	Surface of the eye or interior of the eye using a cannula or device, implantation using scaffolds/extracellular matrix containing cells	[36]
5	hESCs	US2007/0031386A1	AMD, RP and related retinal degenerative disorders	The invention provides a technique for derivation of eye cells like neural, retinal, corneal cells *etc*. from embryonic stem cells. The patent also embodies characterization of the derived cells based in various parameters followed by their used to treat various retinal disorders as well as Parkinson's disease.	Transplantation into subretinal space	[37]
6	hESC	CN106754717A	Damaged or diseased ocular surface	The invention provides an inducing culture medium to produce corneal endothelial cells. The method does not involve a feeder layer so that other animal origin pollution is avoided, and the biological safety is high; the corneal endothelial cells are used as corneal endothelium seed cells in tissue engineering.	-	[38]
7	hESC	CN106167790A	Damaged or diseased ocular surface	This patent illustrates a method to induce the human embryonic stem cells to differentiate into human neural crest stem cells, inducing the human neural crest stem cells to differentiate into the human corneal endothelial cells.	-	[39]

The Patent US2007/0196919A1 describes a protocol for differentiation of hESC into retinal progenitor cells. These cells are multipotent *i.e.* they can give rise to neurons, photoreceptors *etc*. The cell population obtained after differentiation can be used for screening assays. These cells are allowed to transform in the presence of growth factors, bone morphogenetic protein, an antagonist to wnt signalling pathway and an IGF1R agonist to produce embryoid bodies. Genetic modifications are carried out to introduce traits useful for selection process like a selectable marker or can be used to improve the efficiency for tissue regeneration on transplantation. The patent also describes a kit with the cells and formulations for their optimal growth which can be cultured and used as grafts for treating glaucoma and AMD (Table **2**) [34]. A procedure to develop synthetic cornea from hESCs has been described in patent EP2484308 (A1). The cornea obtained is 3D sensory scaffold transplantable in humans. The patent also mentions a protocol wherein the stem cells are obtained from human oocyte and differentiated with the help of a mitogen, bFGF or a combination of both. The invention further describes methods for treating as well as replacement for the cornea (Table **2**) [35].

The Patent US7412734B2 refers to the derivation of retinal stem cells from human placental or umbilical cells which are cultured in L-valine rich medium. The patent also mentions methods for treatment for ocular disorders by providing a kit consisting of a pharmaceutical carrier, the cells as well as reagents for culturing the cells. The compositions given can be administered to the interior of an eye or to the surface (Table 2) [36]. Another invention

US2007/0031386 A1 discussed methods for culturing RPE cells and their use for treatment thereof. The cells are characterized based on phenotyping, functionality, pluripotency of the cells based on transplantation in various animal models and the expression markers present on RPE. The invention describes a method of production of genetically modified trabecular meshwork to treat glaucoma along with methods to treat Parkinson's diseases with embryonic stem cells derived RPE (Table **2**) [37].

Embryonic stem cells can be induced to differentiate into corneal epithelial cells by various techniques. Human embryonic stem cells can be differentiated into corneal endothelial cells in DMEM media without any feeder cells, avoiding any contamination of cells. In this DMEM media, mercaptoethanol, glutamine, basic FGF, Knockout Serum Replacement (KSR) and Retinoic Acid (RA) were added. Addition of RA in this culturing media was important as it directed human embryonic cells to form neural crest leading to corneal endothelium cells. The cells which showed similarities in human corneal cells were selected and expanded (Table **2**) [38]. Embryonic stem cells can differentiate into corneal endothelial cells by first developing into neural crest stem cells. A procedure developed by Chinese inventor, cultivated human embryonic stem cells in a medium containing mouse embryonic cells as a feeder layer. Proliferative human embryonic stem cells were separated and then cultivated in human corneal stromal cells as another feeder layer, leading to the induction of human embryonic cells to neural crest cells. Induced neural crest cells were able to differentiate themselves into human corneal epithelial cells by utilising the described human corneal endothelium culture medium (Table **2**) [39].

2.3. Role of Mesenchymal Stem Cells to Treat Retinal Disorders

Mesenchymal Stem Cells (MSCs) belong to the subtype of stromal cells which have the capacity for self-renewal as well as differentiation into multiple lineages [40]. They are derived from various tissues like umbilical tissue, embryonic mesoderm, bone marrow, adipose tissue, endometrial polyps [40, 41]. MSCs have the ability to clone into adherent fibroblast cells possessing unique cell surface receptors [41]. MSCs can differentiate into endoderm, mesoderm and ectoderm; under regulatory control of various transcription factors. Regulatory genes also help in differentiation of progenitor cells to specific lineage. MSCs have widely been cultured using various biomaterial scaffolds making them ideal candidates for stem cell-based therapies as patient autologous cells can be cultured [40]. Bone marrow-derived MSCs have been injected in the sub retinal cavity in mouse models of Retinitis Pigmentosa; these stem cells proved to be helpful as improvement of morphology was observed in the mice models [3]. Bone marrow-derived MSCs were cultured from mice and targeted to differentiation into cells expressing markers for retinal cells (Holan et. al.). These cells were checked for anti-inflammatory effects as well as growth factor production. From the results, it was concluded that MSCs could support survival of the healthy cells within the diseased retinal cells by producing growth factors. Retinal damage is further associated with local inflammatory reactions which hinder wound healing, MSCs act by inhibiting production of cells causing inflammatory reactions mainly cytokines utilizing their immunosuppressive properties. MSCs are ideal for stem cell therapy due to their inherent ability to differentiate into various cell types; this diverging capacity was tested by incubating bone marrow-derived MSCs with retinal tissue extracts and cultures of stimulated spleen cells. The cultured MSCs expressed markers for retinal cells for *e.g.* Rhodopsin, Rlbp, Calb2 and S-antigen; making them quintessential for stem cell therapy for retinal disorders [42]. In another study bone marrow-derived, MSCs were used to treat patients suffering Retinitis Pigmentosa through intravitreal injection of the stem cells. The injection of the autologous cells did not meet safety standards and resulted into GFAP over expression, retinal folding, and detachment of the RPE and macrophage filtration. Thus further investigation needs to be carried out for use of the stem cells in humans [43].

The Patent US 20060280729A1 describes a method for treating ocular disorders using mesenchymal stem cells which are differentiated on the basis of surface markers specific to the retinal phenotype. The screening is done for cells expressing CD29, CD44, CD105 and CD166. Simultaneously, cells should also lack at least one of these markers; CD14, CD34 and CD45. These cells when transplanted at the target location they provide support for the cells and may differentiate into retinal cells with surface markers except rhodopsin and the cells do not form PR layers (Table **3**) [44].

Table 3. Summary of patents filed for MSCs used for treating ocular disorders.

No.	Cell Type	Patent No.	Disease	Summary	Trial Status	Route of Administration	Ref.
1	MSCs	US20060280729A1	Brain trauma, AMD, RP, optic nerve trauma, glaucoma *etc*.	Approach for treating patients by administration of pharmaceutical compositions of mesenchymal stem cells that are characterized by the presence or absence of surface markers wherein the cells help survival in situ but do not have similar structure as photoreceptor cells and do not express markers specific to such cells.	Pre-clinical	Surface of the eye or interior of the eye using a cannula or device, implantation using scaffolds/extracellular matrix containing cells	[44]

2.4. Role of Adult Stem Cells

Adult stem cells are characterised by their ability for self-renewal as well as the potential to differentiate into mature cells with specialized functions. Adult stem cells are rare and maintain cell homeostasis, replace wounded and dead cells. They are found in various tissues but their means of origin have not yet been identified, with the proposed theory that these cells are set aside during the differentiation phase during embryo development. Some tissues with reported adult stem cells are retina, cornea, bone marrow, peripheral blood. In this section, we discussed about hematopoietic stem cells and somatic stem cells both of which are classified as adult stem cells [45].

In patent WO2007/130060 A2, a method for isolation of adult retinal stem cells from extra-retinal ocular tissue has been described. These cells are isolated from vestigial tissue from a mammalian donor suffering from fatal vasculature. The patent further describes a method for treating retinal disorders using these retinal stem cells isolated from adult stem cells and a method for kit production for harvesting as well as culturing the extra-retinal ocular tissue (Table **4**) [46].

Table 4. Summary of patents filed for Adult Stem Cells used for treating ocular disorders.

No.	Cell Type	Patent No.	Disease	Summary	Trial Status	Route of Administration	Refs.
1	Adult stem cells	WO2007/130060 A2	Photoreceptor degeneration like AMD, RP *etc*., night blindness, colour blindness, retinal detachment, ischemic retinopathy, infectious conditions	Methods for isolation of adult stem cells from vestigial tissue of a donor suffering from persistent fetal vasculature. The invention further provides procedures to culture these cells to obtain retinal stem cells. The patent also offers a kit for harvesting these extra-retinal ocular tissues to improve survival rate.	Pre-clinical	Injection or transplantation of retinal stem cells in retinal site, subretinal space, vitreal cavity, optic nerve using a biodegradable carrier	[46]
2	Adult stem cells	CN102604886 (A)	AMD, RP, diabetic retinopathy	Human somatic cells are used in the presence of plant extracts and protein inducible autologous retinal stem cells to induce reverse differentiation to yield retinal stem cells having similar markers to retinal cells.	-	-	[47]

Similarly, in patent CN102604886 (A) human body cells (blood mononuclear cells) are cultured in presence of plant extracts and proteins which induce reverse differentiation of these cells to autologous retinal stem cells and autologous retinal cells. The autologous retinal cells have similar markers as the retinal cells and differentiating abilities. The autologous stem cells can be used to treat various ocular disorders and the method described for the production of these cells can be possibly scaled up for large-scale industrial production. The invention also describes a method for kit production of these reverse differentiated cells (Table **4**) [47].

2.5. Hematopoietic Stem Cells

Hematopoietic stem cells originating from the bone marrow are present in the ratio of 1:10,000-15,000. HSCs are capable of migrating through blood and are even found in fetal liver and spleen [2]. These cells are capable of forming blood vessels and are identified by the surface markers like $CD34^{+}$, Thy^{+}, Lin^{-}; as cells containing these markers are most likely stem cells and can be used for transplantation [2].

The Patent WO2004/01059 A2 describes a method to isolate lineage negative (Lin^{-}) hematopoietic stem cells

containing endothelial progenitor cells (EPC) from adult bone marrow, upon transplantation these cells get incorporated into the vasculature and help in blood vessel formation, they additionally help in stabilizing retinal vasculature in diseased conditions like AMD and diabetic retinopathy. Further, in the study, they transfected the isolated Lin^- cells with a therapeutic gene of angiogenesis inhibiting peptides which prevented abnormal blood vessel formation in conditions like AMD and diabetic retinopathy (Table **5**) [48].

Table 5. Summary of patents filed for hematopoietic stem cells used for treating ocular disorders.

No.	Cell Type	Patent No.	Disease	Summary	Trial Status	Route of Administration	Refs.
1	Hematopoietic stem cells	WO2004/01059A2	Ocular vascular disease *e.g.* AMD, diabetic retinopathy	Isolated bone-marrow derived Lin^- hematopoietic stem cells comprising endothelial progenitor cells with markers CD31 and c-kit that can be used for development of retinal vessels as well as prevent degeneration of these vessels. The cells are transfected with a therapeutically beneficial gene which specifically targets neovasculature and inhibits new retinal vessel formation acting as a vasculotrophic and neurotrophic agent.	Pre-clinical	Intravitreal injection	[48]
2	Hematopoietic stem cells	US2010/0068141A1	Pre-proliferative retinopathy, diabetic retinopathy, choroidal neovascularization, glaucoma, AMD, RP, Stargardt's disease, Best's macular dystrophy *etc*.	The patent claims a method for treating ocular diseases recruiting stem cells which are induced by heat shock, within which the heat shock is induced by a small compound or an expression vector. Further the invention portrays modes of administration and pharmaceutical compositions for ameliorating ocular diseases.	Pre-clinical	Oral, rectal, topical, intraocular, buccal, intravaginal, intracisternal, intracerebroventricular, intratracheal, nasal, transdermal, device/implants, or parenteral routes	[49]

The Patent US 2010/0068141 A1 describes composition and methods useful for the treatment of various ocular disorders using hematopoietic stem cells, in a part of the patent they describe a method of recruiting HSC in the Retinal Pigment Epithelium (RPE) by subjecting them to sub-visible threshold lasers. These cells trans differentiate into RPE cells and express surface markers found on RPE. The study utilised GFP labelled HSC for transplantation and demonstrated the ability of HSC to migrate to the RPE. The laser-induced heat shock proteins, VEGF and stromal derived factor (SDF-1) helped the cells to acquire morphological features of RPE (Table **5**) [49].

2.6. Role of Neural Stem Cells

Neural stem cells have a characteristic regenerative ability that gives rise to three cell types; neurons, astrocytes and oligodendrocytes. These cells give renewed hope for repair after injury or disease in the CNS [50]. The differentiation property of the particular cells is enhanced when they are cultured on a matrix or scaffold containing growth factors; the cells differentiate into neural stem cell-derived neurons which integrate at the site of implantation [2]. In a study in humans, neural retinal cells were transplanted to check for three major parameters; improvement in visual acuity, safety of the transplantation procedure and to check for rejection of the transplanted cells. Eight patients with RP and one with AMD received subretinal transplants out of which none experienced any postoperative side effects like retinal detachment, infection, retinal vasculitis, extensive bleeding or intraocular inflammation. This led the researchers to conclude, that the transplantation procedure is safe and indicated high graph tolerance but visual improvement was not observed in any of the nine patients [51]. In another study, neural stem cells derived from umbilical cord-derived mesenchymal stem cells were used for transplantation into the vitreous body of the rat models. It was observed that the transplanted cells reduced leakage in the retinal blood vessel demonstrating their ability to control endothelial cells and pericytes which may lead to improvement of microcirculation in diabetic retinopathy. The number of surviving retinal ganglion cells increased after transplantation and helped in curtailment of disease progression, promising for future treatment of neurodegenerative disorders using neural retinal cells [41].

The Patent US 2003/0207450 A1 reports a novel method for the isolation of neuroretina-derived retinal stem cells (NRSC) from human and murine models. These cells are isolated from the neuroretina region of the RPE possessing the potential for self-renewal and multipotent differentiation. On transplantation in diseased retina, these cells differentiated into photoreceptor cells making them true retinal cells and viable for treating various retinal disorders (Table **6**) [52]. Similarly, in patent US 2003/0148513 A1, a novel method for isolating mammalian multipotent neural cells (MNSCs) is depicted. The invention describes method for isolating said cells from neural tissue and procedure for culturing these cells to form cluster of mammalian stem cells. Further, the invention provides pharmaceutical compositions and acceptable carriers useful for administering these cells for disease conditions and tissue regeneration (Table **6**) [53].

Table 6. Summary of patents filed for Neural Stem Cells used for treating ocular disorders.

No.	Cell Type	Patent No.	Disease	Summary	Trial Status	Route of Administration	Refs.
1	Neuroretina-derived Retinal Stem Cells (NRSC)	US2003/0207450A1	Retinal dystrophy	The patent explains a system for the first successful isolation of stem cells from neural retinal tissues which have capability of self-renewal, multipotency and retina-specific differentiation. In one of the embodiments, the neuroretinal tissue from aged donors was also used for stem cell production.	Pre-clinical	-	[52]
2	Mammalian Multipotent Neural Stem Cells (MNSCs)	US2003/0148513A1	Disorders affecting vision like, neurodegenerative disease, autoimmune disorder, corporal disease, neurotoxic disease, a demyelinating disorder *etc*.	The invention defines reagents and methods used to produce neural stem cells, to treat neurological and corporal disorders. The art also provides pharmaceutical compositions which can be administered to animal models to check for effect and efficiency. For production of retinal stem cells the stem cells are cultured in a media containing growth factors like TGF-b3, CNTF and IGF-1 individually or in combination which is used to treat retinal defects.	Pre-clinical	Injection to the target site or transplantation	[53]
3	Neuroretinal Cells	US2011/0004304A1	-	The patent illustrates a bioreactor comprising of three layers made of microchannels, polymer scaffold and thin film membrane embedded with RPE cells used for culturing retinal cells or tissues. The bioreactor layers mimic the retinal structure and offer spatial organization.	-	-	[54]

The Patent US2011/0004304 A1 describes a bioreactor device consisting of three layers; first comprising of a network of micro channels, a polymer layer forming a scaffold and the final layer of a thin-film membrane impregnated with RPE which is sandwiched between the first two layers with the RPE facing the scaffold. The patent claims that neuroretinal cells can be cultured on these bioreactors composed of artificial plexus and micro structured scaffold. These bioreactors mimic layers of the retina and help in spatial organization. The scaffold also provides nutrients as well as oxygen which further helps in assembly of the cells seeded on the bioreactor (Table **6**) [54].

2.7. Role of Amniotic Membrane in Treating Corneal Disorders:

Degeneration of eye, abnormalities or injuries like scarring can be jeopardy to a person's eyesight [55]. Ocular surface disorders are the second most leading cause of blindness [56], thus it is the need of hour to come up with innovative interventions. In conventional treatment, keratoplasty is conducted where healthy corneal epithelial cells are procured from either a healthy donor's eye or from the patient's eye itself and used as an auto graft or allograft respectively. The procurement of a large amount of healthy cells poses a risk to the eye from where they are secured [55].

In case of SJS, its primary stage shows a defect in conjunctiva and reduction in limbal stem cell deficiency. The described invention can help to treat this primary stage rather going to secondary stage. In this art, from an eye biopsy

of the conjunctiva, cells were cultured wherein conjunctiva stem cells were isolated and expanded. The cells obtained from the biopsy were nurtured in a medium with human amniotic membrane as a substrate. During the period of cultivating conjunctiva cells, three types of media were used at various stages of isolation and expansion. However, hydrocortisone, Epidermal Growth Factor (EGF) and insulin were present in all three mediums. Various culturing steps were used to achieve well-characterised conjunctiva cells [57]. These could be transplanted to patient's eye by using Pterygium, Trabeculectomy and Seton surgery [58]. This invention was improvised by Devi et al in the year 2007, their methodology made it possible to cultivate conjunctival stem cells in one media rather three. The media used in this invention was Dulbecco's Modified Eagle's Media (DMEM) with 15% nutrient serum addition and didn't require any feeder layer (Table **7**) [57].

Table 7. Summary of patents filed for Corneal Epithelial stem cells used for treating ocular disorders.

No.	Cell Type	Patent No.	Disease	Summary	Route of Administration	Refs.
1	Corneal Epithelial stem cells	US20020039788A1	Damaged or diseased ocular surface	This patent describes the methods to form a bioengineered corneal epithelial composite graft on an extracellular matrix. This graft can aid in the treatment of damaged ocular surface especially corneal epithelium.	Implantation to the target site.	[55]
2	Conjunctival stem cells	US20070218039A1	Damaged or diseased ocular surface	The patent characterise a method where with the help of a simple single medium culture scheme, and a support material a tissue system is formed which is transplanted to the diseased or damaged ocular surface.	Implantation to the target site.	[57]
3	Corneal Endothelial cells with amniotic membrane	US2005O214259A1	Corneal edema, keratoleukoma and keratoconus.	Corneal endothelial cells were harvested, multiplied and grown on a collagen layer (amniotic membrane) to form corneal endothelium-like sheet.	Implantation to the target site.	[59]

Patent US 2007/0218039A1 demonstrated isolation of corneal epithelium cells from a healthy eye. A biopsy was conducted on the healthy eye at the limbus region. The cells found in this region were epithelium cells; possessing several stem cell-like properties. To cultivate these cells, the tissue from biopsy was cultured in DMEM containing glutamine, foetal calf serum, hydrocortisone, EGF, ABAM and cholera toxin. The corneal cells were placed on 3T3 feeder cells; upon colony formation in this medium, the cells were transferred to new serum-free medium in absence of feeder cells, which contained Keratinocyte Growth Factor (KGF) and low calcium for expansion of the cells. These cells were transferred to an Extracellular Matrix (ECM) made up of fresh human amniotic membrane. Once the cells grow on this ECM, they are processed with either growth factor or one attachment factor like hydrocortisone, KGF, fibrin, collagen, laminin, EGF and hepatocyte growth factor. This selection made sure that a multi-layered polarity of corneal cells was nurtured. H and E, immunocytochemical staining was organized to ensure that the cells adhered were corneal epithelium cells. This composite graft was then applicable to use in keratoplasty surgery (Table **7**) [55].

Similar to patent US 2007/0218039A1, another invention by US 2005/O214259A1 was able to cultivate a corneal epithelium-like sheet on an ECM made up of amniotic membrane and collagen. This invention made it possible to treat ocular disorders which required corneal epithelium transplantation (Table **7**) [59].

A retrospective study by Koizumi *et al.*, in 11 patients suffering from severe stem cell deficiency due to SJS; underwent transplantation of corneal epithelial cells with an amniotic membrane as a carrier. Within 48 hours, no epithelial defects were observed suggesting that the complete transplantation took place. After 6 months of transplantation, the patients showed progressive clear vision indicating that amniotic membrane can be used as ECM for successful graft transplantation [60].

2.8. Use of Cultured stem Cells in Various Devices

In ocular surface disorders, corneal scarring causes inability of corneal epithelial cell to regenerate causing opaqueness of the eye steering to hindrance in proper vision. To improve such disrupted vision, several devices and hydrogels are being invented.

One such device promises to improve vision by corneal overlay. In this invention, corneal epithelial cells or limbal stem cells are extracted from the patient itself, which will require the corneal appliance. This reduces graft rejection and toxic reactions along with the stem cells, small portion of corneal epithelium is taken leaving out Bowman's membrane or stroma of the cornea. The appliance consists of lens and epithelial cells fixed on the lens forming a layer or a flap.

This contact lens can be made of synthetic stroma having desired power, toric lens or by using hydrogels. This ocular device is then placed on the affected area of cornea which then aids in improving the visual acuity (Table **8**) [61].

The main cause in disruption of cornea is epithelial dysfunction, wherein the tissue layer of cornea is intact but there are some voids in the cell layer. To treat this condition, a system is invented where the healthy cells are delivered to these voids. The system consists of a biodegradable film made up of biopolymer which is suitable for the eye as well as supports and initiates expansion of healthy epithelial cells placed on it. The corneal epithelial cells used for transplantation are extracted from a healthy part of patient's eye in a small amount. These cells are then cultured, following placement on the film. The complete film is placed on the patient's cornea where the film acts as a transport system to deliver healthy epithelial cells. Once the film is fixed, it dissolves over time leaving behind only healthy functional cell layer over the cornea, completing the cell replacement therapy (Table **8**) [62].

For corneal injury treatment, few Chinese inventors have developed a biological cornea patch. This cornea patch was composed of silk protein layered with human bone marrow mesenchymal stem cells. The patch with a good amount of seeded stem cells was grafted on damaged cornea for treatment of injury (refer Table **8**) [63]. Another method to transport healthy corneal stem cells to the wounded and injured ocular surface is by using hydrogels. In an invention, the hydrogel consists of 1.1-1.3% strontium alginate which is in thin layer or disc-shaped. These hydrogels contain encapsulated healthy human corneal stem cells in water-soluble pore which is directly applied on the site of ocular surface injury for transporting healthy cells and forming its layer for treatment (Table **8**) [64]. Using this same hydrogel concept, baiji sulfated *Bletilla striata* polysaccharide can also be used to treat ocular damage. Umbilical cord mesenchymal stem cells of passage 3 to passage 5 were infused into polysaccharide of density 1 x 10^5 to 5 x 10^5 number of cells/ ml. This combination of cells and polysaccharide promoted the mesenchymal cells to differentiate and proliferate into corneal epithelial cells. Thus, this preparation has competence to repair ocular surface damage (Table **8**) [65].

Table 8. Summary of patents filed for Limbal Stem Cells used for treating ocular disorders.

No.	Cell Type	Patent No.	Disease	Summary	Route of Administration	Refs.
1	Limbal Stem Cells	US20050080484A1	De-epithelialized Cornea	Invention of corneal appliance is explained here. Wherein the lens body consists of endothelial cells procured from limbal stem cells.	Implantation to the target site.	[61]
2	Corneal endothelial cells	US20090222086A1	Corneal endothelial defects and disorders	The invention provides a resolvable corneal button comprised of a biodegradable polymer which is capable of supporting the growth and expansion of endothelial cells on its surface for use in transplantation of healthy corneal endothelial cells to cornea tissue in need of a transplant and a method of using same.	Implantation to the target site.	[62]
3	Mesenchymal Stem Cells	CN104941001A	Ocular surface disorder like ophthalmology blinding corneal ulcers	In this patent, bone mesenchymal stem cells which are derived from allograft are planted on a silk protein biological film to develop the corneal patch used for curing corneal injuries; such as corneal ulcer.	Implantation to the target site.	[63]
4	Corneal stem cells, MSC or ESC	US20140072601A1	Damaged or diseased ocular surface	The patent describes a hydrogel prepared encapsulating live cells like corneal stem cells, mesenchymal stem cells or embryonic stem cells.	Topical application at the target site.	[64]
5	Umbilical cord MSC	CN105147722A	Damaged or diseased ocular surface	This patent talks about a preparation containing sulphated *Bletilla Striata* polysaccharide and umbilical cord mesenchymal stem cells used for remarkably promoting the reproduction of corneal epithelial cells	Topical application at the target site.	[65]
6	Mesenchymal stem cell	CN107412266A	Conjunctival and Corneal ulcers	Invention of small pet eye drops made up of mesenchymal stem cells that can promote production of corneal epithelial cells on its application.	Eye drops applied at the target site.	[66]
7	Living endothelial cells	US20100274257A1	Damaged or diseased ocular surface	This corneal endothelial tissue inserter associates to a medical device that works with eye tissue which retracts an extremely thin layer of donor endothelial eye tissue and places this tissue within the eye of a recipient.	Device acts like an Injection	[67]

In-vitro cultivation of umbilical cord mesenchymal stem cells can be incorporated into pet eyes drop. These stem cells releases cytokines, which helps the healthy corneal endothelial cells present in damage cornea to proliferate, and heal the wound, making it an easy drug delivery system (Table **8**) [66]. Cultured healthy corneal endothelial cells can be transferred to wounded cornea without any trauma or minimal trauma by using a device created by American inventors. This device has a longitudinal irrigation tube which is placed inside a handle. At one end it has a button where the handle is wider and on the end of the handle, it has a tapered nose. From this tapered nose, a forward tube is projected outward. The irrigation tube and forward tube consist of epithelial cells which are delivered to the damaged cornea by the surgeon by pressing the button on top of the handle (Table **8**) [67].

CONCLUSION AND FUTURE PROSPECTS

Ocular surface disorders are tormenting and lead to visual impairment. Stem cell transplantation for ocular diseases is currently progressing from over a decade of preclinical to the early stages of clinical trials. The review describes many patents wherein the method of culturing and obtaining these cells has been given and formulations for their use in tissue regeneration as well as treating retinal disorders with different approaches have been described. More studies need to be carried out to observe the immune response on transplantation as well as clinical trials need to be conducted to test these formulations and their efficacy [3]. Regularizing the different parameters between patients and stem cell transplantation is difficult to accomplish. However, if in the next ten years, studies conducted by numerous researchers, patients treated and results published of this work will help the field to be generally accepted as a conventional method to treat ocular surface disorder.

Another important aspect to be addressed is the cost to benefit ratio of these studies as culturing of these grafts is an expensive affair wherein in the future when these grafts will be commercially produced they might not be profitable to these organizations as this form of treatment is not affordable for all patients. In countries with publicly funded healthcare systems, the process of graft generation has a high cost-benefit ratio. Thus, major work needs to be carried out to not only successfully use of stem cells to treat ocular disorders but also to make this form of therapy a conventional one which would be affordable to all patients [68].

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

Declared none.

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CHAPTER 2

Taxol To Nanotaxol: A Journey Towards Enhanced Drug Delivery

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Abstract: Drug delivery in the field of cancer has undergone a continuous revolution over the past few decades. Development of novel chemotherapeutic agents without the method of delivering them to the tumor site would find no practical application in uprooting the fatal disease of uncontrolled cell proliferation, cancer. This makes the development of drug carriers exceedingly essential for diagnostics and therapy alike. Nanotechnological science has gained impetus in the recent past and has found applications in a plethora of fields. It has managed to create an impact in the field of diagnostics, drug delivery and therapy, equally. Taxol®, a chemotherapeutic agent that was initially obtained from the bark of *Taxus brevifolia*, moved on to the semi-synthetic approach for its synthesis to address the shortage of its natural source. This drug is partially soluble in water and its initial formulation with Cremophor EL manifested as anaphylactic reactions. To do away with these problems and others such as lower circulation time in blood and non-specificity, nanotechnology is now being looked at as a promising solution. Nanotechnological carriers aim at enhancing target-specificity by functionalization, drug stabilization and preventing its degradation due to physiological conditions, pH, enzymes, etc., demonstrating an Enhanced Permeability and Retention (EPR) effect, prolonged blood circulation and thus better anti-tumor activity, while the side effects being almost negligible. The patents in this chapter aim to highlight how nanotechnology can find practical applications and how one or more than one drugs could be administered *in vivo* in a sustained fashion. The step-wise development in using this potent anticancer drug (Taxol) involved the use of human serum albumin associated compositions (Abraxane®), cremophor-free formulations (Capxol™, Genexol-PM™), numerous oil-in-water emulsions, liposomes and micelles, use of graphene quantum dots (GQDs) for bioimaging and drug delivery and the use of single-walled and multi-walled carbon nanotubes. It also allows the readers to explore nanodevices that can be turned on and off as and when the need be for localized drug delivery. Enabling the nanocarriers to modulate the pharmacokinetic and pharmacodynamic properties of the drug is another notable feature that some of these nanocarriers possess.

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ISBN: 978-91-89764-00-2 | (Issued by an international ISBN agency REDSHINE)
DIP: 18.10.9189764005 (Digital Indentify Password® developed by dotRED International foundation)
DOI: 10.25215/9189764005

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Published in Category of Edited Book
March, 2023(First Edition)

ISBN: 978-91-89764-00-2 | ISBN-10: 91-89764-00-5 | DIP: 10.25215/9189764005 | DOI: 10.25215/9189764005

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Published by:-

Sinhgad Technical Education Society's
Sinhgad College of Architecture, Pune
Address: - S.NO. 44/1, Vadgoan (Bk.,), Off Sinhgad Road, Pune 411041
Website: - www.sinhgad.edu
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Contact No: - 020-24350465/0468/0541

Printing by:-

Swarswat Arts &Printer's
Address: - 495/96, Ellina Apartment,
Shaniwar Peth, Near
Ashirwad Mangal Karyalaya,
Pune 411030
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ISBN NO: 978-81-929293-1-6

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ISBN: 978-93-92774-00-3

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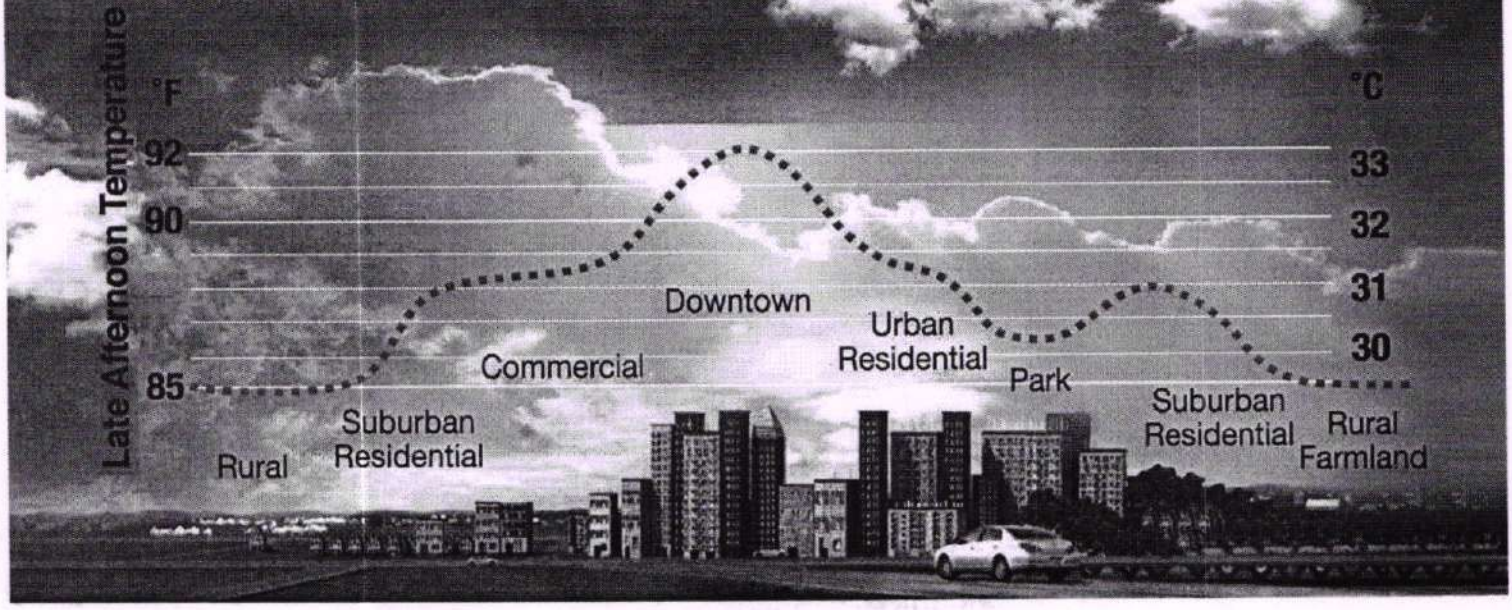

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Conference Proceedings of
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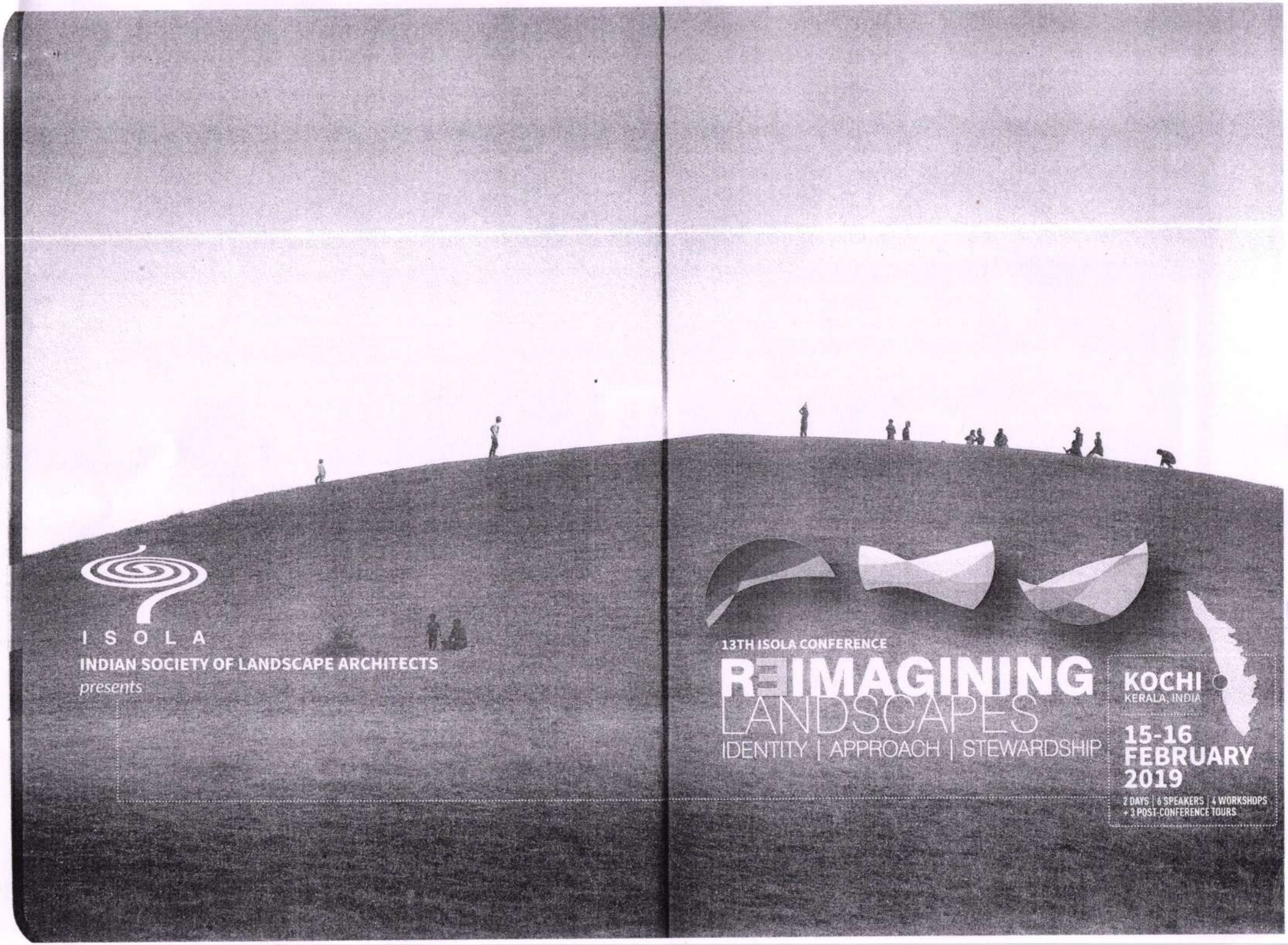
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REIMAGINING LANDSCAPES: IDENTITY | APPROACH | STEWARDSHIP
13TH ISOLA CONFERENCE 2019 | KOCHI, KERALA

DAY 0: THURSDAY | 14 FEBRUARY 2019

14 FEB

VENUE: **BOLGATTY PALACE & ISLAND RESORT**

10:30 AM – 11:00 AM	PRESS MEETING AT PRESS CLUB
02:00 PM – 04:00 PM	ISOLA NATIONAL EC MEETING & EB MEETING
04:00 PM – 07:00 PM	EC AND ALL EIGHT CHAPTER ELECTED MEMBERS MEETING & WORKSHOP BRIEFING
07:30 PM – 09:30 PM	PRE-CONFERENCE DINNER

DAY 1: FRIDAY | 15 FEBRUARY 2019

15 FEB

VENUE: **GRAND HYATT KOCHI BOLGATTY**

08:30 AM – 09:30 AM	REGISTRATION
09:30 AM – 10:30 AM	INAUGURAL SESSION
	WELCOME SONG
	WELCOME & PRESIDENTIAL ADDRESS
	LIGHTING THE LAMP
	INAUGURAL ADDRESS BY CHIEF GUEST
	NATIONAL CONFERENCE BRIEF
10:30 AM – 10:45 AM	TEA
10.45 AM – 12.45 PM	SPEAKERS SESSION 1: IDENTITY
	DONOR PRESENTATION
12:45 PM – 01:45 PM	LUNCH
01.45 PM – 03.45 PM	SPEAKERS SESSION 2: APPROACH
	DONOR PRESENTATION
03:45 PM – 04:00 PM	TEA
04:00 PM – 06:00 PM	WORKSHOPS 1,2,3 & 4
	WORKSHOPS PAPER PRESENTATION
07:00 PM – 08:00 PM	CULTURAL PROGRAM:
	TRADITIONAL ART FORMS OF KERALA
08:00 PM – 10:00 PM	GALA DINNER WITH FUSION INSTRUMENTAL MUSIC

DAY 2: SATURDAY | 16 FEBRUARY 2019

16 FEB

VENUE: **GRAND HYATT KOCHI BOLGATTY**

09:30 AM – 11:00 AM	WORKSHOPS 1,2,3 & 4
	WORKSHOPS PAPER PRESENTATION
11:00 AM – 11:15 AM	TEA
11:15 AM – 01:15 PM	SPEAKERS SESSION 3: STEWARDSHIP
01:15 PM – 02:15 PM	LUNCH
02:15 PM – 03:00 PM	AWARDS FUNCTION
03:00 PM – 03:30 PM	WORKSHOP CONCLUSION
03:30 PM – 03:45 PM	TEA
03:45 PM – 05:00 PM	PANEL DISCUSSION
	CONFERENCE ROUND-UP BY CONVENOR
	FELICITATIONS
	VOTE OF THANKS BY KERALA CHAPTER CHAIRPERSON

DAY 3: SUNDAY | 17 FEBRUARY 2019

17 FEB

SINGLE DAY TOURS

07:30 AM – 02:30 PM	TOUR | T1 KUMARAKOM RESORTS
09:30 AM – 04:30 PM	TOUR | T2 KOCHI-MUZIRIS BIENNALE
07:30 AM – 02:30 PM	TOUR | T3 IRINGOLE KAVU & PANIYELI PORU

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International Journal of Technology Engineering Arts Mathematics Science
Published by:
AISSMS IOIT-Research
e-ISSN: 2583-1224

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Analytical Study of Materials with Potential Possibility for Light and Ventilation without an Opening

Laukik Nalawade[1] and Prof. Prathama Jhaveri[2]
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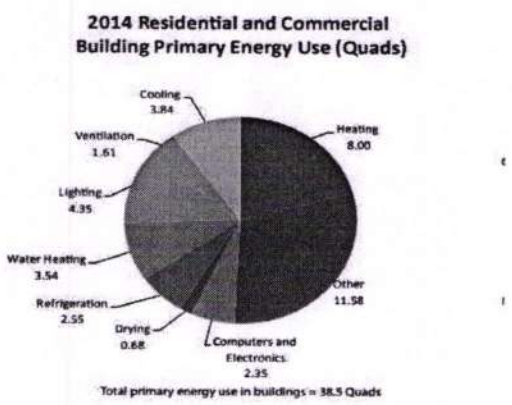
ABSTRACT: Natural light and ventilation are the major points that affect the energy consumption of a building. It plays a very crucial role in the health and behavior of the user. Many of the firms trying to have a sustainable design with innovative ideas. Advance building materials can be used to achieve light and ventilation where the openings are not possible. This research will focus on the Advance building materials for light and ventilation-Translucent Concrete and Meta-material, manufacturing, construction techniques using these materials, the uses and efficiency of working and, also critical analysis of those materials in terms of use, cost and, workmanship. The aim is to introduce more materials that can help this industry to achieve sustainable design to reduce environmental impact.

Keywords: *Advanced Building Materials-Translucent Concrete-Metamaterial-Sustainable Design.*

1. INTRODUCTION AND BACKGROUND OF A STUDY

Windows and openings are the techniques that are being used since humans started to build their shelters. It is among the dominant strategy that is being used to have Natural light and Ventilation. In the modern construction era. These strategies are lagging to fulfil the requirement resulting in more power consumption. Energy conservation is a key and emerging global.

An issue for sustainable infrastructure development. Translucent concrete and metamaterial are the materials that can be used to have both light and air, naturally and it is an innovative solution towards significantly reducing the need for artificial lighting and ventilation. (specifyconcrete, 2019)



Key: **Quad** = quadrillion Btu; **Btu** = British thermal unit

Figure 1

(Abdul Rahman, 2020)

2. LITERATURE REVIEW

Natural light and ventilation can reduce building construction and operation costs and reduce energy consumption. Moreover, it would also ensure safe, healthy, and comfortable living conditions. Providing a comfortable and healthy interior

April 2022 | ISBN: 978-93-90951-55-0

Topic-Translucent Concrete Innovative Building Material to Achieve Sustainability

Pooja Sandeep Galande[1] and Prof. Nikita Pawar[2]
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Fourth Year B. Arch, 2022
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ABSTRACT: As the concept of green architecture has become a common interest in many disciplines, innovative materials are continuously developing to meet the requirements of green architecture. The solution lies in sustainable development, which leads to far less waste than any other type of materials, such as translucent cement, meaningless landfill waste, less energy consumption, and less impact on the environment. where cement absorbs the sunlight in the day and emits it in the nighttime which makes it a viable material for reducing power consumption. This paper totals and evaluates the previous studies in terms of translucent concrete applications and deals with the advantages it brings in the field of smart construction. We identified possible areas for future research and suggested recommendations in the conclusion to fill the gaps between studies examining the results on the environment.

Keywords: *Green Architecture, Innovative Materials, Sustainable, Smart Construction.*

1. INTRODUCTION AND BACKGROUND STUDY

World-wide, concrete is the most widely used building material However, researchers and manufacturers have been working together to produce different types of concrete to improve the overall quality and the economic value of construction.

As a result, translucent concrete has gained popularity in many industries across the world. As a resource-saving, eco-friendly building material, Light Transmitting or translucent concrete is increasingly used in high-end architecture and interior cladding.

1.1 What is Translucent Concrete?

Translucent concrete is designed using the Nano-Optics principle, in which optical fibers act as slits to transmit light from one side of the surface to the other. These optical fibers are spread evenly through the concrete and are visible on both sides of the block. As patterns form on one side of the surface, they appear as shadowy outlines through the concrete.

1.2 A Brief History of Translucent Concrete

Translucent concrete dates back to the early 1900s, when advances in polymer-based optical fibers led to its invention. Light-transmitting concrete has been an idea for years, but the actual concept of translucent concrete was introduced by Hungarian architect Aron Losonczi in 2001. A pioneer of translucent concrete, Losonczi was able to produce the first transparent concrete block within two years after pitching the idea.

This new material was called LiTraCon (short for Light Transmitting Concrete), and soon became popular in countries including Italy, Germany, and even China.

1.3 How is Translucent Concrete Manufactured?

Translucent concrete is made by combining two major materials; fine concrete (with cement and aggregates like sand) and optical fibers.

Optical fibers replace other aggregates in concrete and conduct light from both artificial and natural sources even at angles of incidence greater than 60 degrees. The optical fiber consists of three layers—the buffer coating, the cladding, and the core, which transmits light.

April 2022 | ISBN: 978-93-90951-55-0

Cost Effective Single Family Housing Unit for Middle Income Group in Context with Life Cycle Process of Building Materials

Ar. Anagha Shah, Prof. Aparna Mhetras and Dr. Amit Kaur

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ABSTRACT: In India, housing units add a large amount of energy consumption. Thus, there is a need of energy efficient design to reduce the demand in energy consumption. While designing energy efficient houses, there are chances of cost getting on higher side due to the sustainable construction materials and renewable energy. The main aim of the paper is to find out the parameters for cost-effective single family housing unit for middle income group. This study will focus only on the materials required for wall construction. This is an analytical and applied research which will prove the applicability of these materials as cost effective through life cycle process. The methodology adopted for the research is background study, market survey, and comparative analysis of conventional & sustainable construction materials and their life cycle process. It will lead us to apply this study in the practical field for the awareness and benefit of clients.

Keywords: *Cost Effective, Single family, Residential, MIG, Wall Materials, Life Cycle Process.*

1. INTRODUCTION

1.1 Background Study

Majority of people in India are more inclined towards affordable housing. The built size ranges in India for Affordable housing is generally correlated with the income status and these guidelines are set by the Ministry of Housing and Urban Poverty Alleviation. (Bhavya Kattamudi, 2018). There were various government schemes which have been launched in India for affordable housing in past years. The 'Pradhan Mantri Awas Yojana' is currently focusing on 'Housing for All' till 2022. Previous government schemes were adopted majorly for rural population, EWS and LIG sections of the society. Indian government has launched a scheme for middle income housing group for affordable housing named 'Pradhan Mantri Awas Yojana (PMAY)' which is a credit linked subsidy scheme, in 2016 and issued the guidelines in January 2017. In this scheme, they have defined the MIG in two parts, MIG-I and MIG-II on the basis of their annual income and specified the dwelling unit carpet areas. Even if the government schemes support affordable housing in India, there has no mention of sustainability principles in the construction process.

GRIHA (Green Rating for Integrated Habitat Assessment) India, is working towards making affordable housing sustainable in lines with government's PMAY-Housing for all by 2022. It has launched a new rating variant 'GRIHA for affordable Housing'. Though, there are guidelines regarding the sustainable housing issued by government or aligned agencies, there is no social awareness about it. To understand the importance of sustainability, one needs to understand the meaning of it.

Sustainability can be defined as meeting the needs of today without compromising the needs of future generations. Sustainable housing has the potential to produce good quality housing at a price that is affordable both in the short and long term. Thus, sustainable housing must aim at economic, social and environmental sustainability from planning to implementation phase and at the same time result in housing that is affordable, accessible and environmentally less damaging. (Vijai Shanker Singh, Deep Narayan Pandey, 2012). The criteria for the further study has been considered from the derived variables are as follows: a. Housing for middle income group, b. Hot and dry climate of Maharashtra, c. Materials and d. Life cycle process of materials.

1.2 Research Gap

The extensive background study of the various researches has been carried out and it came to notice that the previous studies/researches on the topic do not encompass the sustainability factor, parameters for cost effectiveness with a focus on

April 2022 | ISBN: 978-93-90951-55-0

To Identify the Cause of Water Scarcity in the Bhukum Village

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ABSTRACT: Villages are an important part of India. And most of the villages are developed near the water bodies like river, lakes etc. but now a day villages are mostly affected because of lack of water. And this condition directly affected the economy and human lives of villagers. Bhukum has an origin of Ram River near Shree Rameshwar temple. In 19th century, water was getting collected near this temple and it was satisfying all needs of water for villages. In 2009, the condition of village was so they needed water tankers for fulfilling their water needs. In this study, we first have studied the Bhukum village, which is located near Pune, to understand groundwater change, factors influencing groundwater levels in the past few years.

The gap is to identify the cause of reduction in water level, so it is necessary to know the reason of reduced water level in the Bhukum village. So the study will focus on the cultural aspects and history of village, according to climate and water availability. Also identify the problems faced by the villagers. For this, the survey has been conducted through Stratified random sampling method. In which the villagers identified previous scenario of the village and current conditions faced by them. And to carry out a hydrology study and case study to identify actual reasons of decrease in water level in Bhukum village. The research will conclude with the identification of causes of lack of water in Bhukum Village.

Keyword: *Ground Water, Bhukum, Water Scarcity, Water Recharge, Causes of Scarcity.*

1. INTRODUCTION

Groundwater is directly or indirectly related to living organisms and the sustainable development of a society since it plays a vital role as a water resource. About more than two billion human beings in the world depend on groundwater for their daily water supply with a huge quantity of the world's agricultural and commercial sectors being subjected to water necessities which are catered through groundwater. Sustainable development and efficient management of this scarce resource has been challenging task in India. Rapid growth in urban, industrial, and agricultural water usage concerning groundwater requirements, in several countries, has especially resulted in those arid and semi-arid zones, which are experiencing water shortages thereby causing the imbalance between its demand and supply. Groundwater is not only influenced by climate change but also by human interventions. As per earlier studies investigating the response of groundwater systems to climate change has been a smart and productive move. Lower groundwater levels are a warning to the environment since its causing hindrance in economic development worldwide. In this study, we select one village which is located near Pune that is Bhukum. The growth rate of Pune city from the last 40 years is observed to be 40% and it has been estimated that it will be much more in the future. With a growing population, there is an increasing demand for water from agriculture, industry, and domestic use. In this study, we first have studied the Bhukum village, which is located near Pune, to understand groundwater change in the past few years. Also, we understand the problems that villagers are faced because of changes in groundwater levels. Then, we analyse variations in factors influencing groundwater levels and identify the major factors.

Background of the Topic: The location of Bhukum is in Mulshi Taluka in Pune District of Maharashtra State, India. It belongs to Pune Division. Bhukum gets water from Sarovar Lake and Khatpevadi Lake. All the rainwater flow from the west southwest to the east side of Bhukum. Mulshi has an average rainfall of 2000 mm. So the water table in that area is good. Because of heavy rainfall, Mulshi had many small lakes with very good water tables for the whole year. In Mulshi taluka, there are more than 10 lakes. The Ram River originates in the Western Ghats at an elevation of 957 m above mean sea level. It then flows in the northwest direction eventually merging with the Mula River at an elevation of 550 m. The Bhukum village is the main place where the ram river origin then the flow of the Ram River is through, Bhugaon and Pirangut along with the Bavdhan, Aundh, Pashan and Karvenagar suburbs of Pune lie in the Ramadi watershed (Kopal Kumar and Anargha Dhorde, 2013).

April 2022 | ISBN: 978-93-90951-55-0

Potential of E-Waste Byproducts in Architecture

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ABSTRACT: Advances in the field of science and technology brought about the industrial revolution in the 18th century which marked a new era in human civilization. With the advancement in technologies and expansion of software, Industries have led to the production of e-waste in a large amount which is increasing day by day. This hazardous waste is a threat to human health and the environment and hence needs to be addressed for achieving sustainable development. This study aims at the different processes to recycle E-waste whose by-products can be used in architecture. This study not only aims at the processes but also gives an overview in understanding the different aspects of sustainability and how E-waste By-products obtained after the recycling process can be used in architecture.

Keywords: *Electronic-waste, Recycle, Sustainability, Construction Material.*

1. INTRODUCTION

The electronic industry is the largest and fastest-growing industry in the whole world. Rapid expansion in any form has its own set of unavoidable repercussions. E-waste is described as discarded electrical and electronic equipment that is near or at end of its useful life. Information technology has tremendously transformed our day-to-day lives. It has provided innumerable benefits to all the users by reducing the communication gaps providing many facilities on their fingers and making our lives easier and more comfortable. In the last two decades with the increase in demand and rapid changes in equipment, features, and capabilities product obsolescence with a decrease in prices and increase in internet use has generated a huge quantity of out-of-date electric and digital devices. However, it has also resulted in unfettered resources consumption and alarming waste generation. E-Waste encompasses a whole range of electrical and electronic equipment such as computers, printers, scanners, monitors, mobile phones, refrigerators, Tv's, cameras, batteries, CD-DVD, automobile equipment, etc. Many of them contain hazardous metals and chemicals which are a threat to human health and the environment and hence need to be addressed while maintaining economic viability growth and improvement of one's quality of life. Maharashtra being the top contributor of E-waste in India raises a concern for the management to be done properly. The objective of the paper is to study the various parameters by which management of e-waste is done and what are the by-products obtained in the process can be used in architecture by contributing to overall sustainable development.

2. HISTORY AND BACKGROUND

The electronic revolution began in the mid-century when advances and inventions were being made, and the electronic industry took off in many nations, with digitalization in many sectors seen as a step toward the countries' progress. The electronics manufacturing business has established itself as one of the most innovative in the world. It is constantly developing and implementing new technology. This has also facilitated the development of what is known as inherent product obsolescence. As it is generally cheaper to buy new items than to fix or update a damaged or outdated one, a growing number of electronics and electrical appliances are being discarded. This resulted in the generation of e-waste.

3. LITERATURE REVIEW

Electronic gadgets are nowadays an integral part of human lives and with advancement in technology and competition in the market, the rate of production of gadgets has increased which is resulting in the generation of e-waste as there are no alternatives or designs produced which can replace the old gadgets in such a way that after the damage it should not be dumped. The software industries along with educational institutes to government organizations switched over to digitalization. Studies suggest that around 95% of e-waste is handled in the informal sector and most of the processes are crude waste processing techniques, which are adopted leading to various health and environmental hazards. E-waste generation globally has increased over the past 10 years, where India ranks 3rd in the e-waste generation in Asia generating 1.8 Million tons of

April 2022 | ISBN: 978-93-90951-55-0

Analyzing the Potential of MR (Mixed Reality) and AI (Artificial intelligence) in Space Interpretation

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ABSTRACT: With the increase in technological advancement, its involvement in designing our living spaces has greatly increased. Researchers have explored the benefits and applications of mixed reality (MR) and AI (Artificial intelligence) in various scenarios. It possesses great potential and its application in architecture has lots of fields to uncover. The spatial perception of any space can be skillfully altered as per the needs and proficiency of the designer. Eventually, it will help envision the future aspects and their outcomes, in shaping the future for the human personal and social life. And finding options on how newer technologies can help to spread the outreach of the work done using the research ideas.

Keywords: *AI, NFT, Machine Learning, AR, VR, MR, Photogrammetry.*

1. INTRODUCTION AND BACKGROUND OF THE STUDY

Human society has come a long way on the path of development. Making an overall observation we can consider interactions or connections playing a major role in this development which happens in a staged manner:

Stage 1: Humans were generally individually living species that eventually started living in bigger communities.

But what brought them together was the very first interactions via some random sign language, which further developed into a written and oral way of communication. Just through stage one, human society moved from the stone age to forming larger n larger empires. Individuals started sharing personal experiences, which helped in developing and evolving ideas and technologies easing the day-to-day lives of humans. Stage 1 reached its peak once humans began creating and sharing written manuscripts. But still there remained a scene of disconnect between people over long distances

Stage 2: As technology developed, the sharing of P-S interactions became easier. Initially, this medium was through locally fixed instruments such as radio, fax, and telephones. The pager technology made sharing of information motile. Eventually, leading to the evolution of current-day keypad phones. It enabled us to share both vocal and textual information over long distances.

Stage 3 (ongoing): The range of these connections further expanded with the creation of the internet. Initially, the internet just demonstrated static information, vaguely also called "Web 1". As more and more people had access to the internet evolution of dynamic and interactive websites began. The information highway got a major boost with the creation of mobile utility devices like laptops and eventually our current day smartphones. This all is considered to be a part of "Web 2.0"

Stage 4 (ongoing and future): But still, there is a huge gap between what we call digital/virtual and real interactions.

Here, the development in the concepts of virtual reality augmented reality, mixed reality, and blockchain technology, portray the start of the so-called "Web 3.0".

Objectives:

The study will look into various AI & MR options which can help.

Interpret space with the help of various immersive reality hardware and software technologies.

Study how machine learning algorithms work.

Understanding human special recognition and view ways to achieve higher spatial consciousness skills in an already existing space.

April 2022 | ISBN: 978-93-90951-55-0

The Effects of Slums on Cityscape in Metropolitan City

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ABSTRACT: India is a fast-developing country with many of its metropolitan cities growing at a higher pace in terms of their population. During last two decades, migration from villages and small towns to urban areas has increased tremendously in India. This had led to the degradation of urban environment by growth of slums, especially in the metropolitan cities. Pune is one of the many metropolitan cities in India where urbanisation has a great boost. Slums cause many adverse effects not only to the surrounding but also the architecture of that place, its identity, and its skyline as well. This paper articulates the consequences of rapid increase in slums, on the surrounding and the overall cityscape in Pune. The research will be carried out by identifying slums in Pune, analysing with respect to living conditions, socio-economic factors, selecting a study area and interviewing people within it. The paper concludes with recommending ideas to glorify the cityscape.

Keywords: *Slums, Cityscape, Population, Internal Environment, Urbanisation, Introduction & Background of Study.*

1. INTRODUCTION

In India, the rapid growth of slums in urbanized areas, especially in metropolitan areas, has become a major problem due to urbanization. The surge in slums is primarily due to the outflow of people from rural areas and the tensions over unused, unprotected, inappropriate public and private land. This includes poverty, unemployment, lack of access to clean water, lack of permanent housing/increase in temporary housing, traffic congestion, pollution, poor and unhealthy living conditions, poor sanitation, and lack of land. It leads to many serious problems, such as improper use of land, destruction of cityscape, etc.

Pune is the second-largest city in the state of Maharashtra and one of the eight-mega cities in India regarding population, with a population growth of 7.8% during 2001–11. Pune attracts thousands of immigrants because of a couple of variants of monetary sports and most of them falling in the class of lower strata forming low-income groups, who are compelled to stay in slum due to poor affordability. These immigrants begin encroaching areas may it be a public land, private land, land besides heritage sites, riverbanks, and many other, which ultimately destroys the cityscape and cultural price of the metropolis. To study the effects of rising slums in Pune and finding an appropriate solution to retain the identity of the place and adding value to the skyline is main lineup of this research. The study bars to the cityscape & living conditions of slums in Pune.

The concept of slums has both physical as well as social aspects, and thus its definition varies according to different countries, places, housing situations, and governing authorities. A compact area of at least 300 populations or about 60–70 households of poorly built congested tenements, in unhygienic environment usually with inadequate infrastructure and lacking in proper sanitary and drinking water facilities (Census of India, 2001 and National Slum Policy). In simpler terms, slums are densely populated regions within an urban area, which are informally inhabited by individuals of the low-income group or by the people below poverty line (BPL). They are a set of various colonies having colloquial names as vasati, etc. The acute problem of slum formation is found not only in the big cities but, also in medium and small cities/towns in India due to rapid urbanization, poor housing planning and increasing migration of people from rural to urban areas (Jitendra Kumar, 2014).

Reasons for generation of slums:

- Unemployment.
- Literacy.
- Socio-economic status.
- Growth in population.
- Unaffordability of modern housing.
- Pandemic.
- Natural disasters.

April 2022 | ISBN: 978-93-90951-55-0

The Impact of Green Architecture on the Environment of the Neighborhood

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ABSTRACT: Today in the 21st century, the world is developing faster, resulting in serious problems of environmental and human health. Buildings becoming a huge consumer of energy and land which leads to serious topics such as global warming, heat island effect, etc. Green building design is becoming widely adopted and is being promoted for sustainable health. It improves the environment as well as heals human health through various methods. They must not be only eco-friendly but should also be functional to provide a comfortable, workable, living, and calm environment for human activities. It is realized that green buildings as a universal response to spread awareness and knowledge of the role of human activity in global climate changes, environmental degradation with maximum expression in LEED Certification and other assessing methods. This paper aims at highlighting the impact on the building environment which encompasses the scope of the positive environment by studying the materials, benefits, and techniques of green building globally.

Keywords: *Maximum, Global-Warming, Green Building, Sustainable, Environment, Certification.*

1. INTRODUCTION AND BACKGROUND STUDY

India is a developing country which leads to an increase in population and rapid development. The robust increase in technological advancement and the growth of GDP has brought in huge demand for residential buildings. Development has led to focal changes in world development, which mainly includes natural, social, economic, and Environments on the green land become a problem to the world's environmental condition. Buildings interact with the environment in numerous ways as the traditional construction methods and materials are damaging the ecosystem because they release greenhouse gases, dust and consume resources in form of water, energy, materials, etc. The construction industry is one of the largest sectors for environmental degradation. A large quantity of carbon dioxide gas is emitted throughout construction. It is calculable that 40 percent of energy consumption and one-third of all greenhouse gases emissions worldwide (United Nations Environment Programme, 2009) is caused by the sector. Nearby 40-50 percent of electricity is consumed domestically for air conditioning (the National Climate Change Secretariat, 2012).

Hence, buildings are major pollutants that affect the ground coverage, air quality and are accountable for climate change. To resolve these issues, it is necessary to change the construction techniques & to consider green building design. It also ensures that the human standard of living can be constant and not scarce. The three effective factors are environmental, economic, and social. Environmentally, A green design decreases the use of energy, water, waste, prevents environmental degradation, and promotes the use of natural resources. Economically, the total operation and maintenance cost is reduced which is usually spent on productivity. And socially, it has a positive impact on the ground coverage and neighboring infrastructure. Green buildings are meant to be beautiful and we live, play, and work in that ecosystem, it has a mental and physical connection to our health and environment in numerous ways.

1.1 Topic

The impact of green architecture on the environment of the neighborhood.

1.2 Research Question

Can green buildings principles and materials help achieve an impact on the building environment?

1.3 Aim

Design green buildings by blending spaces between nature and humans using modern technology.

International Journal of Engineering Research
Volume No.8, Special Issue No.6

ISSN: 2319-6890 (online), 2347-5013(print)
1 & 2 March 2019

Study the Technological Applicability of Vertical Forest in Indian Urban Context

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Abstract : The Vertical Forest is a model of vertical densification of green and built within the city" (Stefano.B, 2014). It is a model that operates correlated to the policies for reforestation and naturalization of the large urban and metropolitan borders. The presence of trees, bushes and green walls distributed throughout the facades of the building, contributes to the regeneration of the environment and urban biodiversity while limiting the expansion of the city. India with its increasing population rate has led to vertical housing construction due to the decrease in amount of horizontal land parcels. With increasing demand for shelter and the need for survival in this country has led to negligence of the environmental conditions. Ruthless cutting down of trees, increasing pollution due to increasing vehicles, usage of non-biodegradable items, has led to pollution of air, health disorders and bad living conditions. The need for landscape has increased but gets restricted on the horizontal plane, thus need for vertical landscape takes the front row .This research paper discusses the technological applicability of vertical forest in Indian urban context by understanding the concept of Vertical Forests. It intends to study the construction technology, cost and time needed for integrating Vertical Forest in Residential buildings, analyzing "tree typologies "on the basis of vertical forest concept, climate and location, and to finally determine the feasibility of vertical forest concept in India. The Bosco Verticale, in Milan, Italy ,Europe is the only residential building in the world of its kind .Due to the pollution issues faced by the people of Milan; Architect Stefano Boeri initiated the concept of vertical forest . India having a similar need may benefit from this concept conclusively .This paper provides much of a foundation of knowledge for the students and professionals that would be interested in landscape technology and environmental aspects.

Keywords: Vertical Forest, India, Residential building

1.1 Introduction

The synergy of the urban sprawl for the environmental survival is useful to reconstruct the relation between nature and city. Vertical Forest acts by subverting the principle of enclosed green spaces (gardens, parks) typical of the tissue of cities and proposes a diffused yet adaptable system of vertical green.Each vertical forest equals, in amount of trees, a large area of level and forest.

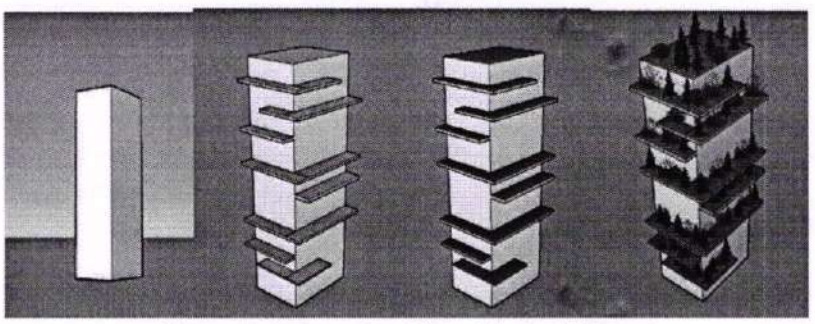

Figure 1: Understanding a Vertical Forest

The concept of vertical forest can be adopted to different typologies of intervention, private or public residential buildings, tertiary, representative or public buildings. It can also add value to abandon buildings or buildings whose construction has been interrupted.

Vertical forest aids in the creation of a micro - climate and to filter small particles of dust from the urban environment. The diversity of plants and their individual characteristics produce humidity, absorb carbon dioxide

International Journal of Engineering Research
Volume No.8, Special Issue No.6

ISSN: 2319-6890 (online), 2347-5013(print)
1 & 2 March 2019

Climate Vulnerability - Understanding and Addressing Impacts of Vulnerability on Ecosystems and Human Societies, Kothrud Ward Pune

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Abstract : The impacts of global climate change are increasingly being felt around the world. The extent of the impacts depends upon the magnitude of climatic changes affecting a particular system (exposure), the characteristics of the system (sensitivity) and ability of people and ecosystems to deal with the resulting effects (adaptive capacity of the system or area).These three factors determine the vulnerability of that system or area. This paper introduces what is vulnerability and how it affects the environment contributing to climate change on ward level. Kothrud ward was selected as it experiences the greatest pressure because of migration of population, which also impact land use pattern of Kothrud ward. Vulnerability index is measure of the exposure of a population to some hazard. To calculate vulnerability index of Kothrud ward, Leopold matrix is used. Simple interaction matrix is presented for factors which are under exposure, sensitivity and adaptive capacity. As a result of simple interaction matrix we can clearly say that adaptive capacity of Kothrud ward is very low hence the Kothrud ward area is highly vulnerable.

Keywords: Exposure, Sensitivity, Adaptive Capacity, Vulnerability Assessment

1. Introduction

The impacts of global climate change are increasingly being felt around the world. The extent of the impacts depends upon the magnitude of climatic changes affecting a particular system (exposure), the characteristics of the system (sensitivity) and ability of people and ecosystems to deal with the resulting effects (adaptive capacity of the system or area).These three factors determine the vulnerability of that system or area. Over the past decades, methods of vulnerability assessment have been developed in the wide range of development related fields ranging from natural hazards research, food security research and poverty analysis, sustainable livelihoods research and related fields. Experience with this framework suggest that vulnerability is a complex subject that has many dimensions which may often have overlapping effects that make it difficult to ease out precise cause-effect relationship.

The term vulnerability is often used to describe the potential (adverse) effects of climate change on ecosystems, infrastructure, economic sectors, social groups, communities and regions (IPCC 2007). In the course of increasing global temperature and extreme weather phenomena the intergovernmental panel on climate change (IPCC) has been founded by the united nations environment program (UNEP) and the world meteorological organization (WMO) for a better understanding of climate change and meeting concerns of these observations.

2. What is Vulnerability?

Vulnerability (IPCC 2007): Vulnerability is 'the degree to which a system is susceptible to and unable to cope with adverse effects of climate change, including climate vulnerability and extremes. Vulnerability is a function of the character, magnitude and rate of climate change and variation to which a system is exposed, its sensitivity, and its adaptive capacity.'

Exposure (IPCC 2007): the presence of people, livelihoods, species or ecosystems, environmental functions, services, and resources, infrastructure, or economic, social or cultural assets in places and settings that could be adversely affected.

Sensitivity (IPCC 2007): the degree to which a system or species is affected, either adversely or beneficially, by climate variability or change. The effect may be direct (e.g. a change in crop yield in response to a change in the mean, range or variability of temperature) or indirect (e.g. damages caused by an increase in the frequency of coastal flooding due to sea level rise).

International Journal of Engineering Research
Volume No.8, Special Issue No.6

ISSN: 2319-6890 (online), 2347-5013(print)
1 & 2 March 2019

Development of Barrier-Free School Facilities

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Abstract: ***Education provides a base of development to a country. As per Universal Declaration of Human Rights, education is a fundamental right for all including learners with special educational need. Each child has a desire to go to the school where he/she can learn lessons that are relevant to his life but approximately 59 million children in India have no access to schools. Besides psychological and social barriers the lack of accessible facilities in terms of road infrastructure and transport infrastructure debarred many disabled children from enjoying equal opportunities in education. The school premises as well as the road infrastructure inside the school has to be barrier free so as to ensure accessibility for all including children with special needs It has been observed that in most of the cases the road environment does not take care of this aspect. So there is a need to develop guidelines for barrier free pedestrians infrastructure for successful inclusion for all including children with diverse disabilities. School facilities should allow all students, with or without handicaps, to fulfil their education without hinderance. A prevailing concern in inclusion of special educational needs is lack of access to education for both children and adults with special educational needs. The aim of present study is to find out the problems and strategies to facilitate inclusive education among learners with special educational needs. This study also focuses on current and past scenario of inclusive education for better learning and also it provides a clear insight about the various problems and suggests the strategies to develop better inclusive educational practices for learners with special educational needs. schools must be acquainted with the diverse needs of the students, ensuring quality education through a flexible curriculum, organizational arrangements and teaching strategies. Through proper policy planning and implementation of strategies as well allocation of resources inclusion of learner with special educational needs can overcome all the barriers. Access and equity are the basic requirement for the process of inclusion at different educational levels.***

Keywords : People with disabilities; barrier free campus; universal design; campus environment; Student with Disabilities

1.1 Introduction :

The school facility is a place for study and life for students to spend most of the time during the day time. Therefore, it is necessary to maintain schools as a comfortable and well off space for students as well as to secure the health and safety of students. This research paper suggests ways of designing and managing school buildings to improve accessibility for students with disabilities.

The facilities should also act as a base for the local community's lifelong learning experience, and should always be ready to be utilized as an emergency shelter in case of fires or earthquakes. Therefore, moving forward with making school facilities barrier-free is an important challenge.

Major barriers in including the learners with special educational needs are lack services related to early identification and intervention services, negative attitudes of parents and teachers, inadequate teacher training, inflexible curriculum, inadequate assessment procedures.

To prepare the school system for inclusive education, with an intension that all children have the right to attend school in a least restrictive environment and the responsibility of the school is to accommodate differences among learners with special educational needs. To meet the challenges, it is required to ensure access which is relevant and good in quality.

This effort aims to bring about awareness of the issues faced by the physically challenged people when using public buildings and spaces. It also incorporates concerns of the elderly, children and of people facing temporary mobility problems.

International Journal of Engineering Research
Volume No.8, Special Issue No.6

ISSN: 2319-6890 (online), 2347-5013(print)
1 & 2 March 2019

Analysis on Spatial Planning of Wadas in Pune

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Abstract: ***The traditional form of residence of peshwas in their ruling period was wadas. It has unique design features like courtyard planning, spatial materials, decorative pillars and arches, well planned openings for good light and ventilations etc. Wadas were the administrative and dominant structures under peshwa period. Almost three generations of Maratha's rule their empire from this structure. During this period, after the establishment of the power of peshwas in Maharashtra several small, medium and some large scale traditional dwelling of a particular style came into existence. Today the wada style of architecture is became huge part of the heritage, which is deteriorating in the old areas of cities, losing its identity and importance due to introduction of concrete structures under the name of development and modernism. This research paper mainly focused on wadas of pune and their spatial planning in terms of features, circulation and surroundings which are being demolished due to present high rising real- estate value, due to which it requires a gentrification in today's urban context. The research also includes study of wadas on the basis of geographical, economic, social and surrounding aspects as they influence the development and evolution of traditional architecture. The paper emphasizes on the planning module of central courtyard and rooms, there spatial planning which is modified in today's contemporary world as need of the users. The paper also considers technical, legal and owners/renters constraints in preserving and protecting the structures. A various live case studies of wadas in pune conducted to identify various aspects of design. Which have been lead to gentrification of this structures. The case studies explore the features and characteristics of traditional architecture which transformed into contemporary architecture in today's conditions. The spatial planning of wadas has become obsolete because of its lack of maintenance and deficiency to fulfil contemporary aspirations of the users. This research gives reasons and solutions to the problems which are associated with gentrification in terms of renovations and improvement of structure or distinct so that it comforts to middle class people who owning or renting the residence of wadas.***

Keywords: Spatial planning, Features, Urban Context, Gentrification.

I. Introduction

The literal meaning of 'spatial planning' is space optimization of area considering surrounding urban aspects with integration of features, style and tradition of that period. Wadas was the popular type of residence which are designed with combination of several spatial aspects. This structure evolved under reins of peshwas, its style was a fusion. Where features from various architecture styles were combined with local construction techniques. Settlement developed around the residence was divided in wards known as 'Peths'. Streets and roads in the settlements were narrow as designed for non-motorised vehicle. The roads were never straight as the growth of this settlement is radial. The plots for construction of wadas were rectangular located next adjacent to the streets.

To understand the transmission of traditional design styles in today's urban context a case method was undertaken. Case of vishrambaug wada and bhau saheb rangari wada which located in heart of the pune city was selected for study as its design inspired from typical wada typology. Vishrambaug wada constructed in '1807' by peshwa bajirao II for his residence which is one of the royal residential structure of that period. It has three centrally located courtyard surrounded with kholi's (Room). Bhau saheb rangari wada constructed in '1870' by madhav(bhau) rangari which is the head quarters of peshwas where meetings and strategies where made against british government. This wada designed in small rectangular plot which has no courtyard but several kholi's are constructed with narrow access. This both wadas are provided with several traditional features and decorative fixtures.

International Journal of Engineering Research
Volume No.8, Special Issue No.6

ISSN: 2319-6890 (online), 2347-5013(print)
1 & 2 March 2019

Analysis on Strategies Used For Planning and Space Optimization in Bus Terminus in Urban Areas

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Abstract : ***Development of city depends on various factors such as lifestyle, standard of living, transportation, infrastructure etc. Transportation is one of the measuring aspect or scale to measure the development of city in urban areas. There are different types of transportation modes such as metro, railway, airplanes, roads etc. Mainly cities or regions or areas are basically connected by roads. So government provides various modes of transport by road which are buses on city level and outside of city level. So bus terminus is one of the major factor of city. To design bus terminus various factors have to be consider such as surrounding, location, roads, amenities, services, vehicular movement, pedestrian movement, accessibility etc. Bus terminus consist of different spaces such as space for terminal staff, space for bus staff and spaces for passengers, service area etc. It is also important that maintain privacy in each space. In bus terminus along with buses, its circulation, its parking other factors/spaces also have to consider such as spaces for hawker's/vendors, parking for outsiders, space for rickshaw, taxi, cycles etc. So while designing passenger area it includes toilets, drinking water, waiting area etc. For terminus staff/bus staff member's rest room, revenue office, toilets, canteen, locker rooms etc. are required. For maintenance and repair of buses emergency service area also required. While design bus terminus barrier free design is also take into consideration for that different signage, audio systems and materials are required to be used. The research paper aims to standardize the design procedure for bus terminus, making it efficient for designers as well for users. The scope of the study is restricted to the bus terminus where buses are only for city level. These study through literature and surveys would help framing a definition for a bus terminus along with its different spaces.***

Key words: Transportation, Bus terminus, Different spaces, design procedure.

1. Introduction

City provides various modes of transport which are may be private or government like railway, airplanes, buses, taxi, autorikshaw etc. So it is important to develop such mode of transport according to the city population, needs, lifestyle etc. To reduce traffic, air pollution, sound pollution government provide transportation in form of buses. Buses helps to connect various areas of cities in city level as well as outside of city level. So to promote usage of buses it is important to provide bus terminus with all necessary services and facilities. A bus terminus is a designated place where a bus or coach starts or ends its scheduled route. The terminus is the designated place that a timetable is timed from.So it is important to design attractive bus terminus in every urban area to satisfy users' requirements which also provide economy to government with reducing traffic and pollutions. So the aim of the research paper is to understand design philosophies used for bus terminus and providing solutions related to its location and surrounding. For that following objectives should need to be fulfil like **to study surrounding that affects bus terminus, to understand design problems or issues of bus terminus, to study solutions of the problems and circulation design, to study various design philosophies which are important for bus terminus and find appropriate solutions.**

International Journal of Engineering Research
Volume No.8, Special Issue No.6

ISSN: 2319-6890 (online), 2347-5013(print)
1 & 2 March 2019

Barrier-Free Environment for Handicapped People in Restaurants

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Abstract : This research is about to make people and designers aware about the need of handicapped people while designing a space, especially restaurants. Design is something which is user friendly, meaning universal design which can be easily used by all type of users. It also facilitates independent functioning of individuals, so that they can live life with dignity, self-respect and participate in all activities of life independently. Barriers make an environment unsafe and cause a high level of difficulty to the user. But more importantly barriers cause spaces to be out of reach, denying people the opportunities of participation in various spheres of life. This loss of opportunity is not only a loss for the person but also society's loss which misses out on their contribution. Simply put, a barrier causes exclusion and its removal is necessary for ensuring inclusion and participation of all the society. While designing a restaurant (public building) the design standards used should not create a barrier for the handicapped people. Considering this, these people also have equal rights to see, eat and feel a space. The public places (restaurants) designed are not helping them to overcome their pain and disability. It is neither creating a sense of safety and security nor leisure. This is in turn increasing their hesitation in using a particular building. This research includes case studies, charts, tables, design standards, guidelines and also design parameters for how restaurants can be made barrier-free. Restaurants will be able to use this research proposal to find the appropriate ways to improve consumer's satisfaction and also to increase profits. The study includes only physically challenged especially wheelchair bound people of all age groups. The research includes guidelines for planning, interior design, materials for barrier-free environment. Designing barrier-free spaces will not harm the activities of a normal human being but it will definitely benefit the ones who are disabled. By this we are making them independent, building up their self-confidence and helping them in Overcoming their Disability by Considering Everyone's Ability in Our Designs.

Key words: Disability, Barrier-Free, Restaurants

Introduction

The research topic is about handicapped people, someone who is disabled has an illness, injury or condition that restricts the way they can live, especially by making it difficult for them to move about. While designing any public place especially restaurant where all the users are entertained like children, teenagers, adults, elderly, handicapped, etc. the design principles used should avoid multiple barriers and avoid creating an uncomfortable environment for the user. For any group of user- the size and space for approach and use should be anthropometrically correct, the space should have an equitable use and should be flexible, the design should be simple but effective which is easy to understand regardless of the user's knowledge, experience and language, the space should communicate necessary information effectively to the user, the design should minimize hazards and should not incorporate more physical effort for the user

.**What is Disability?** Disability is a condition or function which limits the person to experience usual day to day activities. Persons with disabilities include those who have long-term physical, mental, intellectual or sensory impairments which in interaction with various barriers may hamper their full and effective participation in society on an equal basis with others. As per Census 2011, in India, out of the 121 Cr.

International Journal of Engineering Research
Volume No.8, Special Issue No.6

ISSN: 2319-6890 (online), 2347-5013(print)
1 & 2 March 2019

Role of Native Plants for Greenway Mapping in Charholi Budruk, Pune

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Abstract : ***It is a proven fact that there is steady rise in the population of cities. The semi urban regions are now transforming into urban cities in faster pace. This trend of shift from rural to urban will continue and more people will come to stay in the cities. In this given factual situation, it is imperative to make cities more livable by providing more pollution free green areas, from singular plants to continuous corridors and green urban forests. The coming years will be of great challenge for environmental experts to contribute in the well-being of humans living the urban and semi urban cities. In this context, the present study focuses on identification and analysis of existing trees, native and non-native, for promoting green spaces. It is well understood that the enhancement of the urban fabric through native trees will play vital role in the city development plan. Therefore it becomes essential to map the native trees which in turn will create a basis for establishing a continuous greenway corridor. For the said subject under review, this study conducted in Charholi Budruk, in the city of Pimpri Chinchwad, Pune. The study conducted in Charholi Budruk explores the viability of creating continuous greenway corridor through existing and proposed native trees, using the overlay technique. Thus it is concluded that it is possible to create such greenway corridor and insert it in the development plan of the city, which will help to enhance the livability of the citizens through conscious environmental up gradation.***

Keywords: Native plants, Greenway, Overlay method, Charholi Budruk

Introduction

The concept of Native plants has been broadly accepted, but the detailed meaning of "native" is doubtful to be found in agreement. (Web, 1985). Web Defines nativeas:*a native plant is one that has arrived before Neolithic times, or has arrived since without human agency* (Web, 1985). Though the context is referred for UK, it may be accepted as a generic definition. Thus, nonnative species are those which have been introduced intentionally or unintentionally after this time. (Mark, 2011).

The importance of nativity in plants and the dangers of non-native and exotic species have been argued under the following five heads (Hitchmough and Dunnett, 1996; Gilbert and Anderson, 1998; Worrell, 1998).

- Natives are efficient in growth compared to exotic species
- Exotic specieseventually become invasive and outgrow native species
- Natives plants support ancillary species and promote their growth
- Native plants exhibit biodiversity and contribute to world native seeds
- Native plants define the landscape character of the region

(Kendel and Rose, 2000)

Odum (1971) defines the ecosystem as, a set of living organisms in a given area which interact among themselves and between them and the physical environment in which they are inserted, resulting in energy flows and material transfers through the various levels of the food chain.

This paper will understand the contribution of native plants in the urban ecosystem. Charholi Budruk is the selected study area to conduct the research. Data collection is done through Google earth imagery and on site verifications with the help of guide books and expert comments. Analysis of the data is done through overlay method in CAD and GIS software for evaluating the role of native trees in greenway mapping. A proposal is presented to prove the possible insertion of a continuous greenway through policies and plantations in the missing links. Livability index of the city is thus enhanced through a continuous greenway of the existing water stream and the native plants.

Urban Ecosystem and Native Plants

International Journal of Engineering Research
Volume No.8, Special Issue No.6

ISSN: 2319-6890 (online), 2347-5013(print)
1 & 2 March 2019

GIS based Visual Value Analysis for Mapping Greenways: A Case of Charoli Village, Lohegaon, Pune, Maharashtra, India

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Abstract : ***Visibility and scenic landscape analysis of a particular region or a state is an important field of study with direct implications in urban and regional spatial planning, landscape planning and management. More specifically, GIS-based landscape visibility analysis can be a tool to protect, manage, and plan cultural urban landscapes, focusing on scenic and visual values. As an important component of urban landscape planning, visual impact assessment is mainly concerned with: A. the direct impacts of the development upon views of the landscape through intrusion or obstruction; B. the reactions of viewers who may be affected; C. the overall impact on visual amenity, which is often cumulated by plenty of minor process and can be range from degradation through to enhancement or vice versa. Visual aspects are fundamental in urban planning and environmental conservation, since each plan choice can generate manipulation or obstruction of urban elements, producing negative effects on the image of the city. Using visibility maps it is possible to predict the visual impact of transformations, locate interventions on the basis of visual sensitivity, and protect landscape of bio-physical importance, cultural heritage and significant landmarks visible from selected viewpoints. The visual value is defined by biological and physical (or biophysical) values, while the perception-based approach emphasizes the human view (subjective) of the landscape. This paper outlines a methodology to identify and study of visual values of Charoli for development of conservative zoning and conservation subdivision design development, an approach we call growing greener using GIS as a tool to identify the potential Viewshed from a potential viewpoint.***

Keywords: Conservative zoning, GIS, Visual values, Viewshed

Introduction

Charoli is an ancient village. Charholi issituated in the proximity of Alandi a town in Pune, Maharashtra which is popular place of pilgrimage and also holds a samadhi of St. Dnyaneshwar. It is settled under the foothills of Wagheshwar hill in the west and Indrayani River to the north. The picturesque Charoli village is been documented in a book "Aashi amchi Charoli" which states that Charoli was a small village surrounded by a mass of fertile land, green pastures, hills, mountains and the holy river Indrayani. These visual natural landscapesmake Charoli village a place of significance with natural heritage which include wide expanse of ecosystems, providing habitat to wildlife, rare plants, animal species, wetlands, streams, rivers, etc. In terms of smart urban planning finding land resources with cultural and visual value; conservation of these aspects is of prime importance before going ahead with any kind of urban development. The urban wave of development from city has now approached this village's boundary. Therefore, adoption of greenways as a key concept in the current scenario is of utmost significance as an ecologically responsive and adaptive planning.

International Journal of Engineering Research
Volume No.8, Special Issue No.6

ISSN: 2319-6890 (online), 2347-5013(print)
1 & 2 March 2019

Components of Built Environment Impacting Human Health With Respect to Green Buildings in India

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Abstract : "We shape our buildings and thereafter they shape us" . Buildings' indoor environment is where people spend most of their time. The design of homes, schools, hospitals and other buildings can impact directly and indirectly on health. The relationship between indoor environmental parameters and human health is same all over the world, however, the extent of impact depends on many region specific factors/variables- climate, ambient air quality, ambient noise levels, dietary habits and lifestyle .There is not much data available on built environment in India with respect to their impact on occupants' health especially considering Indian scenario. The objective of this study is to establish connection between green buildings in India and human health and wellbeing through literature review and identify strengths and gaps to address all relevant aspects of occupants' health through mapping of LEED rating system in India. A limitation of much of the research is the reliance on indirect, lagging and subjective measures of health. Study highlighted strengths that rating systems like LEED always had human health and wellbeing as one of the integral facts in their overall approach towards sustainability. It also revealed the gaps such as existing framework of this rating system can be expanded to integrate health aspects and it can be achieved by developing new credits in existing criteria. Though there are various rating systems In India, study is limited to application of framework to one rating system, LEED (USGBC) which can be applied to other rating systems In future research to identify the gaps and overcome identified issues.

Keywords: Rating systems, Occupant Health and wellbeing, indoor environment, LEED

Introduction

Most people are unaware of the impact that the built environment can have on their health.built environment provides several opportunities to positively impact occupants' health and wellbeing.As mentioned, indoor environment quality influences occupants' health directly. Therefore to provide better indoor environment quality, we need buildings with less impact on environment and human health. Green building concept is a comprehensive strategy to approach to this aim. Howard (2003) has defined green building as practice of Increasing the efficiency with which buildings and their sites use energy, water, and materials and reducing building impacts on human health and the Environment through better sitting, design, construction, operation, maintenance and removal the complete building life cycle. Materials which are used in green building construction process not only are environmentally friendly but also they are healthier and cleaner for the residents. Green buildings with having exclusive features such as better ventilation, using more sunlight and higher indoor air quality provide healthier environment and increase occupants' productivity and wellbeing compared with non-green buildings. Enhancing the quality of life and social equity over all would certainly lead to a healthier population. Reversing climate change would absolutely improve health, as major weather events are mitigated and air quality improves. Protecting and restoring water resources would undoubtedly improve the both quality of life and health outcomes for the human population, across urban and rural, developed and underdeveloped areas. This paper is not intended to include a review of these factors in detail, but to cite a few important aspects here to highlight the breadth of the issue and to strengthen human health aspect in existing products.

To address this, study proposesa framework of health and wellbeing Indicators for green buildings in India and recommendations on how to identify and integrate these to LEED rating system which can be used with other rating systems for future studies of buildings and health.

ISSN: 2319-6890 (online), 2347-5013(print)
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study. Some owners have rented first floor of the bungalows to tenants. The owners are mostly middle-aged and senior citizen couples having children while tenants are either singles or married couples. There are two municipal gardens in close proximity to the society and Rose Garden is within the society boundaries. There are other facilities like supermarket, restaurants, swimming pool, library and hospitals located within a 1km radius.

In this case, residents know their immediate neighbors and 20-30 people except immediate neighbors. Social interaction observed in this case is mostly between 2-3 people and only in case of planned events does it exceed. Residents mostly interact with residents immediately next to their homes, either over low-height compound walls or by visiting neighbor's homes. Interaction with others is planned.

Public spaces where social interaction takes place in this case are the pavements, benches along pavements, gardens, nearby restaurants and community halls. Feeling of safety, sense of belonging to the society and provision of various amenities near the society are the contributing factors in bringing residents together. Privacy of residents is well maintained in this case, but the frequency of social interactions as well as formation of social relations can be thought to be hindered by the same.

Conclusion

Frequency and amount of social interaction in residential neighborhoods is an essential indicator of sense of community among the residents. Determinants of social interaction can be categorized as universal and case-specific. As typology of the neighborhood changes, general determinants remain applicable to all cases while the case-specific ones vary.

From the analysis, location of the neighborhood with respect to municipal amenities, location of public space with respect to arrangement of the units (central, linear) and in relation to staircase/lifts, scale of the space with respect to number of users and type of interaction, physical distance from the residential unit, presence of seating facility, design of spaces with activities for all age groups (socially inclusive) were the universal physical factors determining the nature and frequency of social interaction taking place in a residential neighborhood. Age, privacy concerns, cultural norms and associations, feeling of safety, presence of a dedicated committee to initiate events at neighborhood level were the non-physical factors identified which were also case specific.

In case of traditional wada, age, small number of occupants, need for privacy due to cultural norms followed by residents (user group profile) largely determined the type and frequency of social interaction taking place. It also resulted in potentially sociable spaces like central courtyard becoming used for non-sociable purposes only, like parking and garbage collection centres.

In case of chawl, physical factors were the major determinant of social interaction. Lack of designated spaces resulted in the shared corridor being used as the public space, with delineations of territory created by the residents with use of furniture elements. These furniture elements further improved the sociability of the space. The semi-openness of the corridor also is an important factor, since it brings sense of safety and comfort due to daylight in an otherwise linear space. But in this case, the violation of privacy is acceptable to the residents.

In case of apartment housing, provision of socially inclusive spaces varying in scale, activities and varying in nature of openness provide increased opportunities of social interaction among residents. The physical design of lobby spaces to incorporate daylight, with seating elements and with doors of neighbors opening in front of another create possibilities for frequent encounters. In this case, presence of a committee which initiates community interaction through events is also a major determinant of social interaction among residents.

In case of detached housing, proximity to various facilities and public amenities and need for privacy largely determine the quality and quantity of social interaction among residents.

Thus, design of residential neighborhoods can incorporate certain features in order to create meaningful public spaces which improve social interactions and thus social relations:

1. Provision of public spaces catering to all members of the user group with seating facility and to accommodate different number of users.
2. Locating public spaces of appropriate scale and dimensions along the regular movement pattern of residents and making it easily accessible to all users, at preferably 2km radius maximum distance from their homes.
3. Ensuring good daylight in public spaces for comfort and safety of users.

International Journal of Engineering Research
Volume No.8, Special Issue No.2

ISSN:2319-6890 (online), 2347-5013(print)
28th& 29th Feb. 2020

Study of Existing High Rise Buildings Shadows on the Basis of their Geometry

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Abstract: High rise buildings are a symbol of modern life, wealth to the city and also introduces modernity. However, they are known for their shape and form. In high rise buildings, casting of shadows plays an important role as they tend to cover their neighboring buildings firstly because of their humongous height but also with their concrete mass.To attain these purposes, comparison of existing high-rise buildings will be analyzed to consider shadow pattern from different forms i.e. triangle, square, oval, rectangle and circle and at different heights. In conclusion, the results will be summed up for the best appropriate geometry for high rise buildings on the basis of shadow length and area underneath shadow.

Keywords: High rise buildings, shadow pattern,neighboring, geometry.

I. Introduction

The high-rise building, more than any other building type, has the capacity to capture the public imagination, its sheer scale, visual prominence, and symbolic potential have rendered it a speculative enterprise, economically, technologically and architecturally. The skyscraper is representation of cities around the world. From New York to Kuala Lumpur, London to Hong Kong and Singapore to Sao Paolo, high-rise buildings define the image of metropolis. The high-rise buildingshave been called a "social condenser" and a "vertical machine" and yet it has traditionally been seen as a building type outside the mainstream of architectural culture.

Despite its close relationship to development forces the multistory buildinghas created as provocative and unique range of architectural approaches to its design. Perhaps because of those very ties, it serves as a barometer of contemporary culture.Multistory buildings can also stimulate issues like wind flow, view, sunshine and air pollution. High-rise building can get a sun solar easily especially the upper floors because of its height. What about the impacts of the building on the surroundings area, making shadow and avoiding getting sun solar directly from the sun are the appear points that many researchers concede about it. The effects of this shadow and shading are change in different climate and block the sun with change the access, sun light and solar sun.

As clear that this shading goes back to a number of factors as:

- Differences in height of buildings (sky line)
- Tall buildings direction and location according to surrounding neighborhoods (with sun path and time)
- Mass and form (geometry)
- Urban space between the buildings (distance)

High rise buildings, although they are mostly well-known symbolic figures of metropolises, have been criticized since their first development. Primarily, opponents to tall building construction cite their huge mass as contrary to human nature; with their excessive energy consumption; the extra pressure they cause on city infrastructure; and the exacerbation of already difficult issues such as traffic and parking. Further, opponents object to their unnatural indoor conditions that adversely affect occupant health, and their role in the lowering of the economic viability of surrounding land by blocking the light, air, and view (Bronin, 2009).

The effects of shading by one building upon another can be either positive or negative depending upon the site-specific circumstances of the properties involved. A potential benefit of shading for adjacent structures may be a cooling effect gained during warm weather. Negative consequences of shading include the loss of natural light for passive or active solar energy applications or the loss of warming influences during cool weather. Factors influencing the relative impact of shadow effects are site-specific and include differences in terrain elevation

International Journal of Engineering Research
Volume No.8, Special Issue No.2

ISSN:2319-6890 (online), 2347-5013(print)
28th & 29th Feb. 2020

Micro-Climate Analysis of Bhopal through Bioclimatic Chart, Radiation Square, Wind Rose and Sun Path Diagram to Evaluate Passive Design Strategies

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Abstract: ***The concept is to develop climate-appropriate design to make building climate responsive and increase the human comfort level of indoor spaces. This research paper will provide suitable data to analyze the micro-climate of Bhopal to evaluate different design strategies. The average pattern of variation in elements such as temperature, humidity, solar radiation and wind over long periods of time will be measured. The use of Bioclimatic Chart is to identify human comfort and design strategies. Radiation Square provide hourly radiation data to design solar system and facade material. Wind rose provides direction, speed and frequency of wind for different seasons. Sun path diagram will show the path of sun in the sky-dome as projected on to a horizontal surface and to design shading devices. These manual techniques will be interrelated to each other for appropriate passive design strategies and climate responsive buildings.***

Key Words: Bioclimatic chart, radiation square, wind rose, sun path diagram, passive design strategies, micro-climate analysis.

I. Introduction

Today's building are more focused on adapting glass construction style without considering the geo-location, micro-climate and surrounding context of proposed site which leads to high energy consumption in various seasons. Buildings are responsible for the 40% of world energy consumption (Serghides, 2014). Therefore, energy conservation is a fundamental concern nowadays. To reduce the energy consumption architects, engineers, public and private sector must have the understanding to make buildings climate responsive which will escalate indoor and outdoor environment quality for humans, animals and living organisms. The goal is to make building climate responsive, initial step will be to study the micro-climate of proposed site so to understand the impact of nature on building throughout the year. And also to achieve good human comfort condition in indoor spaces and evaluate proper design strategies that can help in improving the energy performance of new and existing buildings.

The main aim of this research paper is to use bioclimatic chart, radiation square, wind rose and sun path diagram in order to determine the appropriate technique and strategies to achieve climate responsive building at Bhopal. The climatic data of Bhopal is referred from India Meteorological Department (1971-2000). The data is plotted on quantitative and qualitative bioclimatic chart to identify most critical month in terms of human comfort level. The hourly solar radiation (W/m2) is considered on horizontal roof and differently orientated walls to suggest photovoltaic and hot water solar system to increase energy performance of building. The wind rose helps to design the position and size of inlet and outlet of fresh air. Sun path diagram helps to design shading devices for critical month and to understand percentage of sunlight penetrating from non-opaque surfaces.

According to climatic conditions in which people are bioclimatically comfortable condition consists of: relative humidity between 20% and 75%, temperature between 20°C and 27°C and wind speed up to 5m/s.

Climatic Parameters

Bhopal lies on 523m height above mean sea level. The latitude is 23°17' and longitude is 77°21'. The climate type of Bhopal city comes under composite according to Bureau of Energy Efficiency - Energy Conservation Building Code 2017.Bhopal experience 5 and half months of winter mid-September to February, 3 and half months of summer March to mid-June, 3 months of monsoon mid-July to mid-September. The Dry Bulb Temperature ranges from 14.5°C-38.5°C throughout the year. The lowest temperature 10.5°C in January and highest temperature 40.7°C in May month. The relative humidity ranges from 20% to 88% throughout the year. Lowest relative humidity experienced by May month ranges from 20%-37% and highest in August month

International Journal of Engineering Research
Volume No.8, Special Issue No.2

ISSN:2319-6890 (online), 2347-5013(print)
28th & 29th Feb. 2020

RESEARCHING CARBON SINK FOR PUNE CITY- TIME TO VALUE THE OLD

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Abstract: As Pune is trying to become carbon neutral city by 2030, it is trying to reduce its carbon emission by adopting all its measures like use of sustainable resources, preservation of natural resources, alternatives to fossil fuels, tree planation etc., this type of rapid development of the city is harming the ecological cycle of the urban system in the city. This study mainly focuses on the carbon sink of the city and how well they can act as a carbon sequester ,if carbon sinks are well studied and planned , it can be managed to make carbon as a resource for the better ecological system of the city. The research will mainly talk on the solutions for the better carbon sink, which is well thought for acceptance and to value the principles of sustainability i.e. Social, economical and environmental dimensions of the city.

Keywords : Carbon Sink, Carbon Sequestration, Native Trees, Ecological cycle, Sustainability, Biodiversity, Indian Culture

INTRODUCTION:

The concept of carbon sink is to capture carbon by growing trees. The Pune city, in hurry to accomplish its carbon neutrality by 2030, started growing native trees which a good step is taken with active support of the citizens. But there is a broad thinking for carbon sink in other countries, which talks about the grasslands and its importance in carbon sink and how ecology plays important role in sequestration with respect to grassland.

India has a long history and connection with its grassland and its ecological system. Due to the invaders and influence of the western culture, the country has lost its heritage of worshiping nature and forgot its rural - urban relationship. In this paper the research is aiming to recall and establish a lost heritage of country , which has emerged as a solution for the carbon sequestration , which will also try to open up connections , linkages with government and support given to public for better establishing relation with biodiversity.

The research will talk about how better the carbon sequestration can be done in different ways by proper management and unique ideas for the storage of carbon, to help not to participate in climate change. Pune Municipal Corporation has an area of 243.84 sq.km. 20 - 22 % of its land area has forest cover, which shows the ideal percent coverageof green area for a city. The World Health Organization recommended the availability of a minimum of 9 m^2 of green space per individual with an ideal UGS value of 50 m^2 per capita. These statistical values correlate with a number of UGS standards, including: linkages between sustainable cities and better health [1]. The Pune city has its population of 3132143 in number according to PMC census data 2011 [2].The present study indicated that Pune city emits 7.8 lakh tons of carbon per year.

The civic body of Pune Municipal Corporation has counted around 4009623 trees in 2019 news reported in Hindustan time's newspaper. The plantation is still in process to increase carbon sink under the notice of PMC. The municipal corporation has also set rules for Townships, NGO's etc. for tree plantation to promote indigenous species of trees to support the native biodiversity in ignorance of exotic species. As Pune is covered by hills and ranges it has good forest cover and green patches of grassland which is home to several animals and support too many herbivores for grazing their food. Grassland to be protected for balancing ecological cycle of animal to human relation, which is degrading day by day impacting humans health mentally and physically as human in its busy urban life has no time even to look at nature around it peacefully. World's healthy milk (27%) and animal breed (23%) comes from grassland [3]. Grassland provides livelihood to many poor people and nomadic

International Journal of Engineering Research
Volume No.8, Special Issue No.2

ISSN:2319-6890 (online), 2347-5013(print)
28th & 29th Feb. 2020

Feasibility Of Groundwater Recharge For Maintaining Groundwater Table In Charholi Budruk, Pune

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Abstract: ***Groundwater is the main source of domestic water requirement in India and its overexploitation has led to water scarcity. Because of rapid urbanization, land uses have changed which is affecting low infiltration rates of rainfall into the soil and has diminished the natural recharging of aquifers. To respond to the growing crisis of groundwater, the artificial recharge of aquifers has become widespread in India. In this paper, the feasibility of Groundwater recharge for the Charholi area was assessed. From literature review, it was found that in rainy season groundwater table in this area is 2-5m but the level goes down up to 10m in the summer season. Case studies from Maharashtra were studied to identify how effective groundwater recharge methods are and which type of methods are suitable in this area and also the relation between recharge methods, geographical conditions and topography are studied. The research paper gives various methods for groundwater recharge in Charholi area.***

Key words – Groundwater, artificial recharge, water table, Charholi

I. INTRODUCTION

The main resource which is needed to survive on earth is Water. Only 3% of the world's water is freshwater and the problem of water scarcity is worldwide. Groundwater is the major source which is used for domestic purpose. In the last three decades, exponential growth in the number of groundwater structures has been observed. This has lead to an enormous withdrawal of groundwater for various uses of agricultural, industrial and other domestic needs (CGWB,2011). Increased use and limited recharge have contributed to the lowering of the water table (Ramaswamy, 2007). Groundwater recharge is a hydrologic process, where water moves downward from the surface to the underground. There are two types of Groundwater recharge-

1. Natural Groundwater recharge- through rainfall, water bodies like lakes & rivers. The natural replenishment of groundwater reservoir is slow.
2. Artificial Groundwater recharge- The term artificial recharge refers to the transfer of surface water to the aquifer by human interference. Therefore, any man-made structure which adds water to an aquifer is called as artificial recharge. Artificial groundwater recharge is becoming increasingly important in India, where over-pumping of groundwater has led to underground resources becoming depleted.

Recharge is an important process for sustainable groundwater management since the volume-rate abstracted from an aquifer in the long term should be less than or equal to the volume-rate that is recharged (Ajoy Mishra). An aquifer is a geologic formation that contains sufficient saturated, permeable material to yield significant quantities of water to wells and springs. There are generally two kinds of aquifers. 1. Unconfined Aquifers- It also called a water-table aquifer, which is an aquifer that has the water table as its upper boundary. Unconfined aquifers occur near the ground surface.

2. Confined Aquifers- A confined aquifer is an aquifer that is bounded above and below by confining beds. Confined aquifers generally occur at significant depth below the ground surface (Molly Maupin & Nancy Barber, 2000).

As cities grow urban area expands geographically, characterized by impervious surfaces like roads, pavements and buildings. Rapid development and construction in these areas result in the loss of greenspaces. This decreases the cities capacity to absorb water, decreases groundwater, causes urban flooding, worsens the status of wetlands, disturbs the entire hydrological cycle and ecological area. Proper pre-planned groundwater management in urbanizing areas can save these areas from water scarcity in the future hence preplanning of artificial recharge methods is important in Urbanizing areas.

II. THE STUDY AREA

According to 2011, the Census of India, Pune district is the fifth urbanized district in Maharashtra state. Rapidly increasing population in the Pune city is creating a burden on natural resources like water. Pune city and its neighboring villages which are urbanizing are already facing water scarcity problems. The study area is Charholi Budruk, an ancient village near Alandi, in Pune district adjacent to Pune city. Charholi lies in the southern bank of the Indrayani river with 1752 Ha. Area. The main occupation of people living in Charholi Bk. is farming and water used for farming is from wells hence, maintaining

International Journal of Engineering Research
Volume No.8, Special Issue No.2

ISSN:2319-6890 (online), 2347-5013(print)
28th & 29th Feb. 2020

Role of Transit Hubs in Social A Developing Smart City

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Abstract:The literature review shows, that among the three facets of sustainability; environmental, economic and social, the third has always been the neglected one by scholars and policy makers. This paper draws attention on the relation between transit and social sustainability. The paper argues, while considering transit's sustainability, only transit systems are studied whereastransit hubs are equally significant. A survey was carried out to find, how people of different age groups, gender and economic class perceive these transit hubs in terms of equity, quality, interconnectedness and access. Paper concludes by elaborating the relation between transit hubs and social sustainability.

Keywords: *sustainability, transit, social sustainability, transit hubs, smart cities, transit oriented developments*

Introduction

Sustainability has been a wide subject of interest for scholars for past few decades, which is further subcategorized into three dimensions which are, Environmental, economic and social (Partridge, 2012; Lucas , Marsden, Brooks & Kimble, 2007; Gomaa & Sakr, 2015). Though the term sustainability has included the social aspect, for two decades this aspect of the sustainability has been neglected(Vallance, Perkins, & Dixon, 2009). Few scholars define social sustainability as development which leads towards harmonious evolution of the society(Gomaa & Sakr, 2015).

This paper argues that the social dimension of sustainable development is also important in order to achieve sustainability as a whole. Social sustainability still lacks the recognition whereas it's an independent dimension which is Equally significant to the economic and environmental sustainability (Omann & Spangenberg, 2002).

The literature review shows that there's a relation between transit and social sustainability. Transportation system is a very important factor which shapes the city and which impacts all three aspects of the city's sustainability. (Bamwesigye & Hlavackova, 2019).

Literature review suggests, that more research needs to be done on transit system's sustainability in general and social sustainability in particular. The social elements are in-fact significantly affected by transit systems.

(Pucher , Korattyswaropam, Mittal & Ittyerah, 2005; Lucas et al., 2007; Mattioli, 2015).

Transit oriented development (TOD) has been an emerging topic in urban India. The study shows that the cities in India which have tried to incorporate TOD, have failed to consider social sustainability.(Joshi, Joseph, Patel, & Darji , 2017). Joshi (2017) suggests that TODs in India shall be inclusive of social sustainability to achieve their goal.

This research argues that, while considering sustainability of transit systems, only macro level planning is taken into account, where the micro level aspects are also equally significant. Here, transit systems and transit corridors are termed as macro and transit hubs such as train stations, bus stands are termed as micro(Elshater & Ibraheem, 2014). Few scholars argue that TODs worldwide and in India have not really focused on the transit hubs. Transit hubs are nodes where different modes of transport are exchanged(Elshater & Ibraheem, 2014).

The government of India recently announced "Smart Cities Mission" where transportation has been given a great importance(Bamwesigye & Hlavackova, 2019). Few scholars argue that very less is done in order to promote public transit in these cities. They claim that the transit routes that are developed are unknowingly encouraging the usage of private vehicles among people (Ramos , Vicente , Passos, Costa & Reis, 2019).

This research proposes, that Maximum focus shall be given on increasing the amount of public transit usage and reducing the number of private motor vehicle users in India. This reduction of private vehicles will result in low carbon emission, safe environment, social equity and economic sustainability of these cities. This paper argues, that equal importance shall be given to the transit hubs and how they affect social sustainability of that city. (Srinivasan, 2019).

International Journal of Engineering Research
Volume No.8, Special Issue No.2

ISSN:2319-6890 (online), 2347-5013(print)
28th & 29th Feb. 2020

Review about traditional courtyard as a micro climate in the improvement of human thermal comfort in hot and dry climate

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Abstract: ***Courtyards as a traditional strategy were used to create a suitable microclimate for dwellers, but in contemporary architecture, inadequate knowledge of form and features of courtyards makes them deficient to serve the purpose as an element to provide thermal comfort. This paper focuses on traditional courtyard considering them as a micro climate and discusses about the various physical parameters affecting the human thermal comfort such as courtyard geometry, orientation, wind effects and features of courtyards like its height, etc. for hot and dry climate. Each parameter is discussed in detail along with various factors affecting those parameters. The key findings of this research paper are identifying the optimized contemporarily-built environment with the climate based on traditional factors to reach sustainable contextual courtyards the for achieving and improving the human thermal comfort in hot and dry climate.***

Key words – Traditional courtyard, microclimate, thermal comfort, physical parameter.

Introduction:

Several studies reported that, in past ten years, the earth surface temperature has increased drastically, and human are facing a heat phenomenon as global warming. This heat creates negative impact on city residents and causes a severe thermal discomfort [i]. Currently, the energy demand for construction industry is 34% of total energy in the world comparing it to global industrial and mobility energy needs, which is also emitting significant greenhouse gas emissions [ii]. Therefore, residential buildings have the major impact in global climate change. Courtyards in residential buildings have a major role in providing thermal comfort and space quality for inhabitants. In various climatic conditions, courtyard control temperature and energy consumption rates in buildings. Hence, courtyard is considered as a heart of the house in hot and dry climate. Courtyard must be compatible with environment and act as a temperature regulator and microclimate in spatial structure [iii]. But in contemporary architecture, inadequate knowledge of form and features of courtyards makes them deficient to serve the purpose as an element to provide thermal comfort.

This paper focuses on traditional courtyard considering them as a micro climate and has compiled the literature review of research papers which had discussed about the various physical parameters affecting the human thermal comfort such as courtyard geometry, orientation, wind effects and features of courtyards like its height, etc. for hot and dry climate. Each parameter is discussed in detail along with various factors affecting those parameters. The key findings of this research paper are identifying the optimized contemporarily-built environment with the climate based on traditional factors to reach sustainable contextual courtyards for achieving and improving the human thermal comfort in hot and dry climate.

Research Method:

In this paper quantitative type of research method is used to address the theme by using combination of simulation and experimental results derived from various case studies available in the literature. This paper is an attempt to review about the various physical factors affecting the human thermal comfort for traditional courtyard like direction and orientation of the courtyard, wind speed and courtyard height, solar radiation, vegetation and albedo. These factors are addressed one by one along with related articles along their significance from which the results and conclusions are drawn as discussed below.

Literature Review:

The history of courtyard uses, and its application goes back thousands of years and extends across different parts of the world. Courtyards are the microclimate transformer in the house due to their capability to moderately maintain high temperature, wind breeze and adjust the relative humidity. Various design elements of courtyard such as size, shape, details, proportions, and surrounding wall vary based on hot and dry climate. These variations arise from various external factors like social, economic, cultural and environmental factors [iv]. The courtyards not only serve the purpose of social economical gatherings, but also, they induce the source for airflow and maintain the thermal comfort to inhabitant. Also, the thermal lag and the properties of self-shading are used to reduce the heat gain. The courtyard surrounding walls and floor is a very good

International Journal of Engineering Research
Volume No.8, Special Issue No.2

ISSN:2319-6890 (online), 2347-5013(print)
28th & 29th Feb. 2020

Use of Perforated Screen as Double Skin Façade for Office Building in Jaipur

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Abstract: ***Jaali as perforated screen for light and ventilation in buildings have been extensively used in India. Similarly, Double skin facade is an envelope construction comprising of two transparent skins separated by an air corridor. In today's time contemporary building designs use Jaali as double screen façade as a passive method for natural ventilation. This article aims to study the factors that affect Jaali screen façade and understand the effect of Jaali as a passive strategy for building envelope for a composite climate. The research shows that Jaali screens with 30% to 50% perforation on South Facades in a composite climate provide an improved daylight quality in office buildings.***

Keywords – Double Skin façade, Perforated Screen, Jaali, Passive strategy, Daylight, Natural Ventilation.

INTRODUCTION

This research study explores the performance of Jaali screen façade as double skin façade in terms of daylighting, natural ventilation and cooling (by shading). The motivation for using double skin façade concept in buildings is a solar passive design concept.

Energy represents a major component in any country's economy and hence it affects the world's economy. Saving energy consumption, especially in buildings, directly affects national economy. The shortage in the traditional energy sources motivates the design of energy saving buildings. Many design treatments are developed to achieve energy saving through buildings.

Building envelope plays an important role in the energy consumption of the building as major parts of the building are exposed to the sun radiation. If treated properly this can considerably help in controlling the thermal comfort inside the building. An energy-efficient building envelope refers to an effective boundary between the conditioned interior space of the building and environment beyond it. Passive or climate responsive architecture uses the natural processes of a site to reduce or replace the need for mechanical systems in buildings.

City Character: Jaipur represents rich and vibrant lifestyle and tradition of Rajasthan. Jaipur being financial center and capital of Rajasthan, is dominated with office buildings. It is famous for its archetypal architecture and beautiful Jaali screens. As per ECBC climate zones Jaipur comes under Composite climate. It means it experiences extreme climate in both summers and winters. The city's climatic evaluation as per the bioclimatic zone necessitates requirement of passive strategies for reducing heat gain and this research paper is a step in this direction.

The Double skin façade is a passive design option for buildings used to improve the energy efficiency and indoor thermal comfort. It consists of a internal façade, an air cavity and an additional external skin. It presents the range of natural ventilation and daylight performance. It is a European architectural trend that is currently gaining momentum throughout the world. The form and construction of façade system have a strong influence on both the energy consumption of a building and the comfort level of its occupants. According to the source book of Belgian Buildings Research Institute (BBRI) "An active façade is a façade covering one or several stories constructed with multiple skin. In this kind of façade, the air cavity situated between the skins is naturally or mechanically ventilated. The air cavity ventilation strategy may vary with time."

'Jaali' means a fine and ornamented web, generally found in Indian, Indo- Islamic and Islamic Architecture. Jaali as a perforated screen is used for permitting light and ventilation in buildings. These screens provide protection from direct solar radiation and regulate social interaction between spaces. Presently, these screens are used in commercial buildings to reduce glare and control solar heat gain through fenestration. When properly designed, these systems can not only improve visual and thermal comfort but also greatly reduce electric lighting, cooling and heating energy consumption (Lee, DiBartolomeo, Selkowitz 1998). Previous studies on the Jaali as Screen façade show that the amount of energy reductions attained varies depending on shading geometry and climatic variations.

METHODOLOGY

This study adopts three tools to reach the outcome. The first method is a case study of existing building, second method is analyzing the Jaali types and perforation found in the traditional architecture of the city and third method employs simulation for daylight of

International Journal of Engineering Research
Volume No.8, Special Issue No.2

ISSN:2319-6890 (online), 2347-5013(print)
28th & 29th Feb. 2020

Mental mending and Mindfulness through Architecture

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Abstract: ***Growing technological developments have harbored the mental growth of human beings. Societal pressures to acquire a particular status in terms of materialistic ambition is seen as success, leading towards a downfall of physical and mental health. In this fast-going and digital age where there is a pressure of reaching somewhere, the meaningful and crucial information is being ignored.***

Increasingly an individual's inability to step away from technology can have serious consequences, one major effect being anxiety. Recent surveys have shown increasing cases of anxiety and stress with the growing and developing world. Along with the fast age, there is an increasing ignorance of self-awareness, human connections and personal well-being.

Here in the paper we will understand what the problem is, in the context of psychological conditions. What are the causes and consequences of the current situation of the society? What actually mental well-being and mindfulness is? Role of architecture in mental wellness and how it can act as a solution and therapy along with psychology and act of senses. Studying and analyzing case studies of such spaces will help understand the solutions to social and self well-being. Concluding factors and sense of designing for human well-being on a deeper level using Architecture and Psychology as tools for a better society.

Keywords- Technology, Social media, Anxiety, Mental well-being, Mindfulness, Mindful Architecture

1. INTRODUCTION

The evolution of social media has come to shape the technology sector as well as real-world social interactions. Social media platforms are some dominating fields today. Along with the birth of "Smartphone", social media seems almost unavoidable as it gets easy to connect to the internet anywhere. It is becoming difficult to separate men from their machine. As society's attention is focused on online praise or impending tragedy through a screen, the connection to the present moment and casual personal encounters are lost. Thus, the technology being so beneficial on one side, and on the other side, it is also deteriorating the mental health of today's society. People want online validation and to match the world they try to be updated as much as possible. And the inability to step away from the technology causes anxiety. In the fast pace and over-stimulation of our digital age, it has become increasingly difficult to focus on meaningful and relevant information. As we live life through a screen and more isolated from our peers and ourselves, adverse effects on our physical and mental health emerge. Human connections and self-awareness is important for maintaining interpersonal well-being and psychological balance where the community is grounded in meaningful experiences within the public and private domains.

Considering the current status of societal stress, the return on investment in mental health, and different practices that address these issues, there appears a marked lack of architecture designed to address society's changing needs. Architecture has the power to promote a sense of presence, forcing users to pay attention to the immediate environment or simply fading into the background to allow users to think on their own. Place influences emotions, which control our lives and could also impact behavior. By crafting mindful architecture, the need to constantly be connected to outside factors can be reduced. Through design, mental state and behavior can be influenced in a way that promotes wellness and therefore potentially reduces the anxiety the user feels towards things beyond his or her control. Mindful architecture is a method and practice of architectural discovery. It seeks to reverse this trend and redirect our focus back to the duality of the mind and body by focusing on the tangible and intuitive qualities of the physical world. Architecture can contribute to well-being as a

International Journal of Engineering Research
Volume No.8, Special Issue No.2

ISSN:2319-6890 (online), 2347-5013(print)
28th & 29th Feb. 2020

Study of Cooling Strategies of Animals and its Application in Habitable Spaces in Arid Climate

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ABSTRACT: Bionics', the science of emulating nature's strategies, has remarkably contributed to solving many of human problems in different fields. This paper discusses the use of various strategies and adaptations that animal species in arid condition use for better living and survival. Emphasis is on animal behaviour and then study expands to the living patternsinto five viewpoints which consist of form, material, way of construction, function and process. Ultimately by expanding the results into architecture finds new solution to get sustainable architecture.An analytical study is done to understand which strategy can be used for natural cooling and further studied in detail. This strategy further can be implemented to reduce energy consumption by humans used forcooling in desert regions. Analysis of selected thermal strategy or adaptation, existing in animal species is done.A hypothesis is built on the conclusion of the comparative and analytical study of strategies. Bionics and architecture are strongly related and hence bionics can be used in architecture to make it more sustainable and energy efficient.

KEYWORDS:bionics, arid, thermal strategies, natural cooling, skin, energy efficiency, sustainability.

INTRODUCTION:

Bionics is an interdisciplinary science crossing biology and technical science, developedwithin the recent 30 years.The term 'bionics' can be traced back to the 1950s. An American major, Jack E. Steele, coined this term in 1958 and further expanded its usage in 1960 with many other bionics' scholars at the first bionics seminar, held in America. This seminar marked bionics as a formal subject and laid the foundations of bionics. At that time, 'bionics referred to transferring technologies into life-forms, also known as biomimetics, diagnosis, biomimicry or biotical creativity engineering. Later, the term was also applied to bionic design, which is the harmonious relationship between the human and nature in industrial design, and also a major path to systematically study and improve the way of life.Human thermal comfort is defined as a condition of mind, which expresses satisfaction with the surrounding environment. High temperatures and humidity provide discomfort sensations and sometimes heat stress (i.e., reducing the body's ability to cool itself). Moreover, discomfort and heat stress reduce productivity and may lead to more serious health problems, especially for aged persons. In hot summers of arid regions, high outdoor air temperatures due to intensive solar radiation and low relative humidity are present. Consequently, discomfort sensations and heat stress are expected. Therefore, persons should take care when they go outside in hot summer to protect their health from heat and/or sunstroke.The indoor environment also plays an important role on the person's comfort and in arid conditions it is more important to work on the indoor habitat and energy consumption is high to keep the space lively and comfortable. To reduce the energy consumption and start a walk towards sustainability using the natural techniques that animals use in arid zones. Bionics is somewhere it not only implements the strategies but also puts in the research and development of that built environment.

METHODOLOGY:

International Journal of Engineering Research
Volume No.8, Special Issue No.2

ISSN:2319-6890 (online), 2347-5013(print)
28th & 29th Feb. 2020

Landscape As a Part of Sustainable Streetscape

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Abstract - Sustainable streetscape ensures that spaces are long lasting and function as a part of the greater ecosystem implementing technologies that reduce carbon footprint. Sustainable streetscape plays an important role in development of urban areas. The streetscape inurban areas which are built for the public interaction, physical activities, traffic control etc. In this context, the research aims to create sustainable streets with the use of landscape elements to provide an attractive and healthy environment. In Pune, many streets in core of the city are very crowded and have heavy traffic flow, so with the help of landscape, making street more user friendly and sustainable is necessary. The research consists of the study of different streets with different character and the study of landscape elements useful for making streets more sustainable. The research concludes the importance of developing urban environment in Pune by creating sustainable streetscape through landscaping.

Keywords - sustainable – streetscape –landscape - ecosystem- urban development – user friendly.

Introduction - Nowadays, urban development is increasing rapidly. It majorly affect to climaticchanges. Streetscape is an important part of urban development. And by making streets more sustainable with the help of trees, shading devices, planters, water features .etc.one can help reduce pollution and carbon footprint. The research paper focuses on how does landscaping help in making streets more sustainable. As an answer to this question, the case studies have been selected from Pune. Case study includes J.M road and D.P road as sustainable streets.

Sustainability means use of natural or recycled materials, application of energy efficient methods in our design that is beneficial for public and also for future generations to live with healthy environment. Sustainable streetscape means the spaces we are designing are sustained or long lasting, user friendly, functional for all age groups andhelps to reduce carbon footprint.Sustainable streetscapes are beneficial to create better places and environment for present and future development.

Research Objectives –

- To study the advantages and principles of sustainable streetscape.
- To study the different landscape elements and their effects on climate tomake street more sustainable.
- Toidentify design principles for landscaping to make street sustainable.
- Formulate design recommendations for developing street landscaping of one street in Pune.

Research Methodology –

The research based on the study of sustainable streetscape and providing conceptual design for one street in Pune. The details of methodology of this paper given below:-

- Primary data - Collection of all the theoretical data required for the study of landscape elements used in sustainable streetscape.
- Secondary data – Collection of data through live case study and interviews and then list down the observations of case study. Case study of four streets includingtwo streets with landscaping and two streets without landscaping.
- Analysis of collected data –Analysing the data to understand the climatic conditions and design elements used in street design. Which will help to develop the landscaping of street in Pune.
- Design recommendationfor making street sustainable through landscaping.
- Conclusion.

Advantages of sustainable streetscape:

- Sustainable streetscape creates healthy environment for user.
- It helps to create interactive public and gathering spaces.
- It creates a visual image of particular area.
- It helps to reduce heat effect, glare and improve air quality.
- It helps to reduce air and sound pollution.
- Improves stromwaterrunoff by increasing permeable/ porous surfaceand landscaping.

Principles of sustainable streetscape :

International Journal of Engineering Research
Volume No.8, Special Issue No.2

ISSN:2319-6890 (online), 2347-5013(print)
28th & 29th Feb. 2020

Feasibility Study for Integrating Natural Ventilation Through Skylights in Pune

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Abstract :The earliest kind of skylights were the open holes in domed ceilings known as the Oculi. As domes developed, the Oculi disappeared replacing the same with glass skylights which would stop the rain from entering the interiors. These skylights continued through the middle ages and are still used even in the modern era. In this modern era, sustainable structures are an important aspect of architecture, making natural ventilation and natural light the most important factors in a building. The increase of migrant population to urban areas has led to growing urbanization all over the world. It is observed that many areas in urban conglomeration ,the buildings or houses are placed very near to each other due to which natural light and ventilation have no enough space to travel through the windows. Hence, the other source through which light can be explored is through the roof. Skylights can be used for daylight and also as a natural ventilator. These are one of the architectural elements which are less studied and practiced. This study checks the feasibility of integrating skylights and natural ventilation which would help to reduce the use of electricity and contribute to sustainability. By the experimental method, this paper gives the feasibility of the integration of natural ventilation and skylights in a room in Pune.

Keywords -natural ventilation , skylights , urban congestion, ECBC,Pune.

I. Introduction

The idea of creating openings in the roof and using sun as the source of natural light to light up a room was developed in ancient Rome and continued through the middle ages. Oculi was the earliest type of skylight that was used in Pantheon. Oculus were the openings in roofs that would let in light as well as rain. Thus the use of Oculus gradually stopped as it was not practical to keep this kind of openings in a building where weather conditions are not favorable. This led to use glass as covering for these openings. Romans were the first to use glass in architecture. Thus adding a lantern shaped structure on top of the building, provided some insulation and also gave the benefits of natural light. The light channeling through these openings would create drama in the interiors. As architecture progressed, use of roof openings in different shapes increased which were later known as skylights (ii).

Coming through the ancient ages to the modern era, people are more concerned of sustainability which could be achieved by using natural elements that are favorable for environment. Using less artificial light and ventilation, would take a step forward towards sustainability. Using daylight and natural air in a building by strategically placing the openings, have become an important factor while designing a building. Windows or openings could be placed anywhere in the structure including the roof to let the air and light inside. The areas where window to wall ratio is very less , openings in the roof can be acceptable mode of natural ventilation and daylight. This includes the use of skylights in the ceilings or roofs which will give diffused light into the interiors and also act as an aesthetic feature. But these require more study.

Light effects of skylights depend on the materials that are used as covering of skylights. Skylight materials are divided into basic materials like glass and plastic and types of each respectively. Each material have different effects on light. The use of these materials will depend on the requirements of each project or as per the design. The material should also contribute to sustainability of the building. Growing urbanization has led to the increase in density of the areas leading to close placing of houses.Thus the light cannot penetrate through such small space and then through the windows to the interiors. Openings in roofs are an appropriate option for such conditions. This

International Journal of Engineering Research
Volume No.8, Special Issue No.2

ISSN:2319-6890 (online), 2347-5013(print)
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Architecture of Traditional Ali in Pune, A Case Study of Tambat Ali

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Abstract: ***Pune, the cultural capital of Maharashtra, has a long legacy of Alis. Alis are the craft skill by lanes. During the Peshwa rule in 18th century, Kasba Peth was the centre of Pune, where the supporting trades like Tambat, Burud, Kumbhar, Kasar, Badhai, etc. got located. Tambat Ali (an Ali named after Tambat community) is an area where copper and brass items are manufactured. It has been observed that, in Pune these trade practices, which carry heritage value are undergoing rapid changes. For example Pagadband Ali where Pagadbands used to make Pagdis (caps), which had great importance particularly in Peshwa period, is now on the verge of decline. In order to avoid the loss of such significant heritage skill, it has become necessary to record the existing picture of Alis. The aim of this paper is to give analysis of changes in the evolution of the Tambat Ali. This paper attempts to document, the existing working craftsmen's in Tambat Ali, along with a set of recommendations that would help to preserve the character of this area.***

Keywords: Tambat *Ali*, Pune, Architecture, Craft skill, coppersmiths, building typology,

Introduction:

Pune is a historical city and Kasba Peth is the oldest part of the city. Down the centuries, Pune has been ruled by several dynasties. In 2003 some artefacts were found in Kasba peth area from the Satvahana period which evidences the settled life in that area around 200BC. One can find the references to the Pune region in copper plates dated 758 and 768 A.D. issued by Rashtrakuta ruler, in these copper plates Pune is referred as 'Punya vishay' and 'Punaka Vishay' respectively (i). Punya meaning 'holy' and Vishaya meaning 'region'. It was so described because the city has a confluence of two rivers Mula and Mutha, which is considered holy in the Hindu religion (ii). From 9th century Pune region became the part of Yadava Empire, which was defeated by the Mughals in the 11th century. During the 12th and 13th century, Pune was under the rule of Allaudin Khilji and later Muhammad Tuglaq, for a long time Pune was under Muslim ruler (ii).During 1600-1817 v AD Marathas ruled Pune. Pune city was the seat of Peshwa, the prime minister of Maratha king from 1713-1818 AD, Nine Peshwas ruled Pune city during this period. In 1735 Peshwa I shifted capital city from Satara to Pune which increased the political and economic importance of the Pune city. Over 18 Peths were established during the 18th century. The Maratha power ended in 1818 and the British set up an empire in the Pune city (ii).

According to the author of the Gazetteer, the starting point of the settlement in Pune was near the river bank of Mula-Mutha, he reasons that the presence of river attracted the settlement. According to the local tradition, there were three original villages-'Kasarli', 'Kumbharli' and 'Punewadi' (iii). It is more likely that they were small hamlets rather than villages. We can still find these three villages as Kasar *Ali* (Tambat *Ali*), Kumbhar wadi and Kasba peth respectively. By the time of Peshwa rule these small hamlets had grown and became a Kasbe Pune.

The Bara Balutedar system was a hereditary village servant system of twelve trades which existed at the time of Peshwa rule. The Balutedari system was important part of socio-economic life of city of Pune. Kumbhars (potters) and Tambats (copper smiths) were one of the important members of Balutedari system. Peshwas invited people with specialised skills from different areas to come and settle in Pune. People of one community carrying one trade lived together and their area of residence were named on the basis of the trade they carried out, like Tambat *Ali*. This neighbourhood settlement gave rise to very typical cell-like formation of Indian urban life. The cell like localities were further emphasise by their shrines and temples for the worship of community deities, like the Goddess Kali temple belonging to the Twasta Kasar community(3). Kumbhar *Ali*, Tambat *Ali* and other trade basedAlis such as Bhoi *Ali*, Shimpi *Ali* and some other are all a few minute walk from each other. This shows the compact placement of Kasba Peth. The streets named after profession or trade still exists. The documentation of existing workshops is done by site visit along with survey method.

Tambat *Ali*:

Alis are the craft skill by lanes - places where groups of people with special skills lived together and carried out their trade. These *Alis* were named after the trade or profession they carried out. All the *Tambats* (coppersmiths) used to live together in *Tambat Ali*. The name '*Tambat*' came from the Marathi word '*Tamba*' which means copper and hence people working in copper are called as *Tambats* and place where they live together is called as *Tambat Ali*. Peshwasinvited *Tambats* some 400 years backfrom Konkan region of Maharashtra to come and settle down in Pune (v).

International Journal of Engineering Research
Volume No.10, Special Issue No.1

ISSN:2319-6890 (online), 2347-5013(print)
7th&8thMay. 2021

A Study of Biophilic Design to Build Educational Spaces tor Autism

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Abstract: ***Autism Spectrum Disorder (ASD) is a complex developmental disorder that involves persistent challenges in social interaction, verbal and non-verbal communication and restricted or repetitive behaviors. Autism is a lifelong condition and there is no cure for it. In the case of facilities for children with autism, the line between healthcare environment and educational environment becomes obscure.***

Recent research has shown that the educational environments have a profound effect on learning and performance among autistic children (Msotafa, 2008). Universal design was formalized in the 1960's with the thought of making places accessible for those with physical disabilities. It is now time to think along the lines of inclusive design to address a wider range of needs and experiences. We have not been very successful as a nation to provide for better living and public spaces for autistic patients.

The basic objective of this paper is to study the specifications of an educational center for autism and to survey if biophilic design approach is beneficial for the development of autistic children. This research paper is divided into two parts. Part one focuses on interviews with parents and caregivers of autistic children and case studies on autism centers and schools for the better understanding of the requirements for designing the same and to study methods of incorporating biophilic design in educational spaces specific to autism. The second part shall focus on evaluation and presentation of these case studies and to suggest a new set of standards for designing an educational space for autistic people with a biophilic approach. The conclusions shall be used to re-think educational and awareness centers for children with autism and shall encourage people to broaden their views about universal design.

Keywords: Autism, architecture, biophilic design, spatial needs, sensory environment, educational environment.

INTRODUCTION

According to the Rehabilitation Council of India, 1 in 100 children are suffering from autism and these are the statistics of the cases that have been recorded. In India we have accepted physical disabilities and almost all building designs have special considerations for people with physical disabilities but what about them with mental disabilities or disorders? Any kind of mental disorder or even developmental disorder is still considered as a taboo. Autism Spectrum Disorder is a developmental disorder. A person experiencing the same faces challenges in his or her day-to-day activities like social interaction, verbal and non-verbal communication and restricted or repetitive behavior. It is one such disorder which is by and far the most challenging developmental disorder which has been overlooked by the architects as a condition that influences building design. (Msotafa, 2008)

Recent research has shown that educational environments have a profound effect on learning and performance among autistic children. (Msotafa, 2008) Every patient with autism behaves differently and might not have the same issues but a school that helps and guides them through their initial years will make a huge difference in their lives.

WHAT IS AUTISM?

Autism Spectrum Disorder (ASD) is a complex developmental condition that involves persistent challenges in social interaction, speech and non-verbal communication and restricted or repetitive behaviors.

The effect of ASD and the severity of the symptoms are different in each person. ASD is usually first diagnosed in childhood with many of the most-obvious signs presenting around the age of 2 or 3 years. (J. Nathan Copeland, 2018) Autism is a lifelong condition. However many children diagnosed with ASD go on to live an independent, productive and fulfilling life if they are provided with the required guidance and help in the early stages of their lives. How about instead of considering autism as an obstacle we architects are supposed to tackle, we focus on finding the means to build spaces that can be occupied by all groups of people. As architects in what way can we help build a better world for them? There are various interior elements that are added to their educational spaces to make them feel comfortable. How about we incorporate these additions into our designs and help them feel calm and concentrate more on daily basis. One such technique is Biophilic Design.

International Journal of Engineering Research
Volume No.10, Special Issue No.1

ISSN:2319-6890 (online), 2347-5013(print)
7th&8thMay. 2021

Understanding The Problems Faced And Recommends Solutions to Implement Traditional Facades In Modern Architecture

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Abstract: Facades play an important role in showcasing the architecture and history of an area. This research paper provides an insight into the challenges faced while incorporating traditional façades into urban designs. Two cities which have strong architectural character which have rules and regulations implied upon Jaipur & Ahmedabad and two cities which do not have any rules and regulations regarding maintaining the historic structures surroundings, Shaniwar Wada, Pune and also Sindhpur, Gujarat where houses aged hundred years old are in abundance which are constructed in Wada architecture style & Bhori architecture. These houses when redeveloped or reconstructed can be built while adopting traditional facades contemporary to that period to preserve the history and character of that area or can also be restored. However, to cater to the rising population and urbanization, the buildings need to have more FSI leading to the construction of many multi-storeys buildings which destroys the neighborhood character.

Keywords: **façade, policy, character, preserve, wada architecture, modern architecture.**

INTRODUCTION

Elevation is the most common view used to describe the external appearance of any building. In this case we are considering a specific area surrounding Shaniwar wada & the area in Sindhpur where the type of architecture found is very old hence it is very similar to each other. So, when reconstructed, redeveloped or restored the external appearance can be kept similar to conserve and preserve the character of that area. By doing a case study on a similar historic place i.e., Jaipur & Ahmedabad where both come under world heritage UNESCO sites.

CASE STUDY 1

JAIPUR, RAJISTHAN: UNESCO, JHCPR (Jaipur Heritage Conservation & Protection Regulations), 2020

The policy in Jaipur that is by sub-clause (iii) of clause (g) of section 46 and section 336 & 337 of Rajasthan municipalities act, 2009 for city which states as follows:

i] Roles and responsibilities of technical heritage committee:
- To facilitate proposed adaptive reuse plan of heritage assets.
- To enter into agreement with private with conservation and adaptive reuse of heritage sites.
- to oversee the working of heritage cell.

ii] The heritage assets are categorized in three grades as follows:
- Grade-I: National importance, ASI protected and other similar heritage sites.
- Grade-II: State importance, state owned and other similar heritage assets.
- Grade-III: Local importance, private properties and other similar heritage assets.

iii] Building Parameters and Control Guidelines:

- Setback line: as per prevailing building line. No projection which is balcony or any covered area to be constructed on public street except where it is permitted by competent authority.

- Ground coverage area: Within plot or property area. The character of open spaces to be maintained as per originally constructed. No modification permitted.

- Height: Roads main bazars (15m) G+3 height. Internal (12m) G+2 height. No coverage of veranda allowed and also no construction above shops (ekdhalya).

-Built Up area ratio: As achieved within permissible ground coverage and permissible maximum height or no. of floors.

- Parking: as per parking policy of walled city. Parking area to be identified and parking to be allowed in such designated areas with levy or fee prescribed as per JMC.

- No basements allowed in special cases for parking, MRT'S etc. special permission to be taken from the state government

International Journal of Engineering Research
Volume No.10, Special Issue No.1

ISSN:2319-6890 (online), 2347-5013(print)
7th&8thMay. 2021

Sustainability In High Rise Architecture.

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ABSTRACT:

In developing countries, places where builders are countingly facing with challenges related to poor infrastructure, obstacles effecting constructability, and a lack of political will and state resources, the sustainable tall building provides a unique opportunity for economic analysis and social reflection. In India, especially, the social structure is defined by the economic structure. As cities cope with rapid population growth—adding 2.5 billion dwellers by 2050—and grapple with expansive sprawl, politicians, planners, and architects have become increasingly interested in the vertical city pattern.

It is concluded that since tall buildings consume massive energy, designers of the next generation of tall buildings will incrementally aim for "zero energy" design. In this approach climate is used to advantage and the building becomes a source of power. It is possible that tall buildings will someday even produce excess energy and transfer the excess to the city's power grid for use in other ways.

Sustainable architecture is environmentally conscious,energy-saving, and utilizes responsive and renewable materials and systems (Newman, 2001). Ecological and environmental concerns have expanded beyond the issue of the consumption of non-renewable energy sources. Sustainability essentially aims for ecological balance.

This paper will review and examine shortfalls of tall buildings found in the literature to inform future developments. The paper will gather a vast amount of fragmented criticism and concerns, and organizes them around the three pillars of sustainability: social, economic, and environmental. Mapping out the "unsustainable" aspects forms the foundation for addressing them in future research and tall building developments. the authors will argue that a truly sustainable approach to design acknowledges interdependence and embraces diversity in economics, necessitating that a successful "green" tall building be an integrated part of a society's financial, technological and cultural advancements.

Keywords: Sustainability; High-Rise Developments; Economic Shortfalls; Environmental Problems, steel structure.

INTRODUCTION:

The rapid growth of the economy of the leading countries is contributing to the development of big cities. In connection with intensive internal and external migration of people to big cities, the population density is increasing. As a result, the number of modern multi-storey buildings and high-rise buildings is increasing. [9]

One of the important components of the living environment of a city is the residential environment. The unified system "man - apartment - building - neighbourhood – residential area of the city" defined in the scientific literature as "residential environment" has complex features and mechanisms. A human being, interacting with the residential environment, performs non-productive activities on the territory of populated areas. In big cities, the development of the living environment, mainly consisting of multi-storey buildings, high-rise buildings and modern public spaces, causes variation in temperature and wind conditions of the terrain and aggravates the environmental situation. [8], [9].

The comprehensiveness of the sustainability concept is apparent in one of the earliest and most frequently used definitions created by the United Nations' Brunt Land Commission in 1987.The commission defined sustainability as "meeting the needs of the present withoutcompromising the ability of future generations to meet their own needs". Concisely, this definition resonates the root meaning of the word "sustain", which is to "provide with nourishment" or to "keep going", as defined by Merriam-Webster Dictionary. Therefore, in line with the urban planning profession, sustainability emphasizes the long-term implications of all human activities. It also presumes that resources are finite and that we should use them conservatively and wisely

International Journal of Engineering Research
Volume No.10, Special Issue No.1

ISSN:2319-6890 (online), 2347-5013(print)
7th&8thMay. 2021

The Study of Acoustical Traditional Japanese Landscape Elements by Recommending Design Interventions For Pune-Okayama-Friendship Garden, Pune, India.

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Abstract: ***Urbanisation is taking over, resulting in a drastic shift in the population from rural to urban areas. Per medical records, stress has increased extensively among adults, children and the elderly. Urban Development and the lifestyle accompanying it has negative health effects which pose as a major challenge for the global society. The aural effect has an impact on the quality of the natural and green spaces and affects our well-being. One such example that further validates the importance of green spaces is Pune-Okayama-friendship garden. Pune-Okayama-friendship garden possesses a natural flow of water from a canal that spreads across the garden. The observations made, are based on the analysis of how sound is used to enrich tranquillity in four gardens based out of Japan. Readings made with the use of SPL meter in specific spaces of the Pune-Okayama-friendship garden support the comparative study.***

Key words: Sound scape Design, urban lifestyle, tranquillity, Japanese gardens

INTRODUCTION

As per medical records, stress has increased extensively among adults, children and the elderly. Urban Development and the lifestyle accompanying it have negative health effects, which pose a major challenge for the global society. Although some amount of stress is inevitable in an individual's life, an ample amount of it could lead to adverse health effects like depression, cardiovascular disease, chronic fatigue, insomnia etc. For gardeners, landscape architects, and designers worldwide, Japanese gardens have always been sources of inspiration. Gardens originally influence the Japanese garden tradition in China. It has a long history with several unique designs, including the karesansui dry landscaped garden, the kaiy ¯ u-shiki-teien stroll garden and the cha-niwa-teien tea garden. The Japanese sense of aesthetics is characterized by asymmetry, symbolism, geometry, meticulous detailing and the use of natural materials and is a diverse practice. For several hundred years, Japanese gardens' green and natural habitats have been a significant part of the urban fabric. These gardens provide a contrast to modern life's pressures and the increasingly densified communities that frequently surround them. Soundscape research adopts a comprehensive understanding of the sound environment, including problems as well as positive experiences focusing on the qualitative and subjective perception of sound environments; it is a wide and interdisciplinary field. In the current research, one of the available methods called Soundscape behaviour was applied to study sonic experiences in the
Pune- Okayama- friendship garden. The paper suggests that the notion of the soundscape in a garden plays an important role in visitors' overall experience. The goal was to substantiate Soundscape behaviour in the Pune- Okayama friendship garden as a design tool and to increase the understanding of peaceful soundscape design. Analysis of four traditional Japanese gardens in terms of soundscape forms the foundation of the research. The objective of the paper is to describe and recommend a few design interventions in terms of elements to be provided in the Pune-Okayama-Friendship garden for enhancements of the soundscape by studying the case studies briefly and the existing aural environment of the garden.

MATERIAL AND METHOD

The present research is focused on empirical material collected in the Pune- Okayama friendship garden using an on-site analysis. The material was collected during the visits made to the garden. A profound and detailed study was done involving five literature reviews in order to understand Japanese gardens in-depth in terms of soundscape landscape. Most of the gardens studied have existed for several hundred years, during which time the gardens and their soundscapes (not least the soundscapes surrounding the gardens) may have changed to various degrees. It should be noted that the aim of the study is not to contribute to historical perspectives as to how gardens in Japan were developed at various times or areas. Instead, tradition is used as a sense in which general understandings of soundscape landscape can be taken from, which can then be extended to other (contemporary) gardens and green areas. It could well be that some of the results encountered in the gardens were not the consequence of their designer's deliberate actions. This does not, however, make it any less important for the purposes of the review. Field notes were collected from the Pune- Okayama friendship garden in the form of digital recordings and SPL readings, which were

International Journal of Engineering Research
Volume No.10, Special Issue No.1

ISSN:2319-6890 (online), 2347-5013(print)
7th&8thMay. 2021

Role Of Womens' Lifestyle In Spatial Planning In Pol Houses, In Ahmedabad

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Abstract : ***Lifestyle of people residing in a community is directly reflected in the architecture and spatial planning of the spaces. With the progression of time, the spatial requirements alter in turn to manifest these changes. In this due course of time, the societal role of women has significantly changed. The lifestyle of women is becoming distinct and evolving considerably. In Indian society, women have an important role to play in the household. With the current time, women are not limited to household, but are also actively pursuing their professional careers. In the older days, the role of women was mostly limited to the household and revolved around their families. Women are a major part of the family, residing in the houses during the majority of the time of the day. This required an important aspect of design to be linked to the individual lifestyles of these women. The societal role of women was an important factor to impact the spatial planning and organization the spaces which formed the house as a whole. The traditional pol houses are an instance of intricate planning design based on lifestyle and the societal role of women. The foundational idea of these houses is based on a vast factor of social norms, cultural aspects and climatic conditions. The traditional pol houses are a very ideal example of women centric design. Eventually, there was an increase in requirements in the present day, and these horizontal houses transformed into vertical residences, majorly constituting high rise apartments. This paper aims to study the evolution of household spaces with the societal evolution of the women's lifestyle and how the women are an important component about how the houses are designed, taking an example of the traditional pol house, which developed horizontally to the modern-day high-rise apartments, which have evolved vertically.***

Keywords : Woman, pol houses, spatial organization, lifestyle

1. Introduction

1.1 History

The walled city of Ahmedabad was established in 1411, by Sultan Ahmed Shah. In his rule, he established the city for reasons of trade, since the location of the city was strategic to the former trade routes. This is one of the reasons, why the population of the city to date remains trade oriented, and the economy was balanced on the basis on the trades established. The trades carried out in the pols ranged from a huge array of jewelers to merchants to food businesses and so on. The day to day trade and business was carried out in the houses itself, which was reflected in the how the nature of spaces was implemented.

Every Pol house had a different aspect about itself, but the pols were never segregated based on the financial background of the residents.

1.2 Spatial planning

Pol houses are based on a similar ideology, but they incorporate so many factors. The economic background gets reflected. The design of a pol house is completely apt for the kind of temperature Ahmedabad experiences. Certain other aspects that require study are the socio-cultural aspects which affect the designing of a pol house.

The spaces in the pol houses were each of a distinctive characteristic and could still be modified according to the requirements of people. The pol house is not just a residence, but it also has an economic aspect, based on the occupation of the residents. Every house in the pol is separate based on the spatial planning as well the connection of spaces. The spaces have been designed to completely incorporate the needs of the family and also provide spaces which can be modified as and when required. Certain spaces included in the planning are multipurpose and can be used by various members for a diverse range of activities.

The pol houses are set in such a historic time period, that in some way or the other the socio-cultural are yet reflected in the design and planning. The social background of the time was completely different as compared to today's modernization. Hence, like it is for every type of traditional architecture, the designing of these houses might have been significantly designed to incorporate the needs of the family at the time.

One striking aspect to be considered in these spaces is the incorporation of the need of women of the household. The

International Journal of Engineering Research
Volume No.10, Special Issue No.1

ISSN:2319-6890 (online), 2347-5013(print)
7th&8thMay. 2021

Bamboo: A Futuristic Construction Material

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Abstract: ***Global warming is a concern, not only for the generations that live but also for the generations to come. We, the human beings are the cause of this rising global issue and needless to say, the sufferers too. Global warming has far-reaching effects on us all and it becomes our sole responsibility to safeguard ourselves and the generations to come by reducing carbon emission and so, we strictly need to adopt and implement methods and materials which has low carbon footprint, sustainable and pollution free, in short, methods that are less or not hazardous to the earth as a whole.***

With reference to the same, this paper talks about one of the most unrecognized and underestimated sustainable material - Bamboo, about how bamboo can withstand natural disasters like earthquakes. The paper also throws light on a study of bamboo and its features as its strength to weight ratio, energy required for construction, pollution aspect, etc. and so, how bamboo can be considered as a futuristic material for humankind.

Keywords : Global warming, carbon footprint, pollution, need for sustainable material, bamboo, futuristic material.

INTRODUCTION :

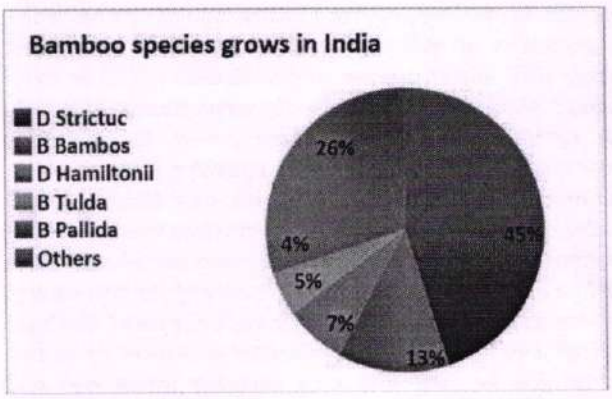
Construction world is ever-evolving, from design, construction methods to the construction materials. Taking a building into consideration, from Architect, to Builder, to building owner, everyone is demanding for a strong, durable, cost efficient, sustainable and easily available construction material. And bamboo is a material which can right tick all of this with few additional benefits.

Bamboo is a gaint grass from gramineae family, which grows in tropical, sub-tropical and mild temperate zones. Large tracts of bamboo forest occur in tropical Asian countries including India, Myanmar, Thailand and China. In India, it grows naturally in almost all parts of country except Kashmir. 125 indigenous and 11 exotic species of bamboo from 23 different genera are reportedly found in India.

Bambusa bambos and Dendrocalamus strictus are two genera constitute the highest proportion of bamboo forest in India. D. strictus is one of the predominant species of bamboo in Uttar Pradesh, Madhya Pradesh, Orissa, and Western Ghats.

Following pie chart shows the percentage of different bamboo species grows in India.

Figure 1 :



Bamboo is a fast growing natural building material, which is easily available. It is sturdy, easy to use, inexpensive, reusable, and can be treated to give different shapes while growing, hence flexible.

LITERATURE REVIEW :

Here are some of the statements shared by PWD authorities, construction team of BRTC and bamboo experts to TOI.

"Till now, bamboo was seen as a cosmetic material and was used for furniture and small canopy or sheds only, but now we have included new items which will promote use of bamboo even in construction of buildings and complexes. Not only PWD, but other departments will be able to promote it in construction activities," **said Ulhas Debadwar, principal secretary, PWD.**

"These items were included in the SSR(state schedule rates) taking a cue from Bamboo Research Training Centre (BRTC) in Chichpalli in Chandrapur. Entire BRTC is made out of bamboo. For the first time, RCC columns and beams of bamboo were put to use as non-scheduled items. We documented them and now have put in the SSR," **Debadwar told TOI.**

"Bamboo has a long and well-established tradition of being used as a construction material throughout the tropical and sub-tropical regions of the world. Bamboo is a critical resource as it is very efficient in sequestering carbon and also

International Journal of Engineering Research
Volume No.10, Special Issue No.1

ISSN:2319-6890 (online), 2347-5013(print)
7th&8thMay. 2021

Reduction of Carbon Footprint By Planning A New Settlement With Sustainable Materials

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Abstract:

The extraction of huge extents of materials is been very common, in building projects. Construction of new buildings by extraction of materials leads to consuming embodied energy (EE) and it releases carbon dioxide (CO2) in large amount which is hazardous for the environment. Materials like cement and steel have been proven to emit high percentage of CO2, so what if by replacing such materials and bringing sustainable materials like mud blocks , fly ash or slag, etc which can reduce CO2 emissions to some extent. Looking at this situation, we should ask to ourselves is there really a need to always locate in the core of the city, what if we plan a settlement away from the main city area? Can't we develop a new town where residential structures up to two storeys can be built with such materials which emit low carbon emissions? This will help in reducing the carbon emissions at a mega level. Thus, we can create a module which is sustainable and can be repeated in different areas according to the need. Decentralisation is appropriate vision for planning and is also the tool to control carbon emissions, so by using these sustainable materials we can plan a new settlement which will benefit us in reducing the carbon emissions at higher percentage.

Keywords: Embodied energy, carbon dioxide, local materials, Decentralisation.

Introduction

Sustainability is "meeting the needs of the present without compromising the ability of future generations to meet their own needs."

This guide provides information that will not only increase the community's sustainability, but also help to mitigate the adverse impacts of climate change. The world's main construction materials are cement and bricks which, are produced in all countries because of their geographic profusion. The manufacturing of cement emits CO2 from the combustion of fossil fuel(coal) and also due to the use of electricity which involves the burning of coal.

Buildings are responsible for a large amount of energy consumptions and environmental emissions throughout its life cycle. However, majority of the research focus has been on material selection and constructing a small settlement with low carbon emitting materials to minimise emissions from buildings. Promoting the development and use of the low embodied carbon building material and services, the energy efficiency of construction machines, as well as the renewable energy use are identified as three main pivotal opportunities to reduce the carbon emissions of the construction sector. **(Huang, L.; Krigsvoll, G.; Johansen, F.; Liu, Y.; Zhang January 2018).**

Cities are the major contributors to carbon emissions and therefore are at the forefront of achieving tangible reductions. Tools that quantify emissions in cities are required to provide the baseline knowledge to allow coherent future planning targeted towards low carbon footprint.

Methodology

It is noted that most of the energy rating tools have given importance towards selection of greener/sustainable materials and utilisation of energy efficiency methods to enhance the building occupancy conditions with less emphasis on construction stage emissions and impacts. Understanding the complete building process is very important in justifying CO2 emissions. These processes include extraction, manufacturing, transportation, construction, maintenance, and disposal. Wide ranges of material are utilized in buildings that use energy and release CO2 through its life cycle, which is regarded as embodied energy and embodied carbon. As part of mitigation measures, assessment of embodied carbon of building materials is one of the fundamental approaches that can have a positive impact on carbon footprint. The selection of appropriate sustainable building materials can reduce about 30% of embodied CO2 emissions over a lifespan of the building.

(Khozema A; Mardiana A; Yusri Y,2020)

International Journal of Engineering Research
Volume No.10, Special Issue No.1

ISSN:2319-6890 (online), 2347-5013(print)
7th&8thMay. 2021

Paper In Architecture

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Abstract: ***With the increasing population a shortage of space is observed. Increasing population escalates the need of construction, which then causes adverse effects on the ecosystem. To make the building sustainable, fast to construct and cost-effective paper can be used in various forms and as various structural members. Paper buildings can be easily constructed, demolished and even recycled making it an ideal material. This research paper suggests that paper is a versatile, flexible, sustainable material which can be used for the construction of various elements. Replacing paper from conventional building materials can help reduce excessive construction waste, is dismantlable and can be recycled which provide sustainability and diversity.***

Key words: sustainable, paper forms, building material, recycle, ease of construction

INTRODUCTION

Paper is a material which is used extensively in our day to day lights. Right from newspaper to packaging boxes, paper is used in many forms and varieties. Paper was first in China to the emperor of the Han dynasty by Cai Lung. It has been primarily used as an information carrier and packaging material. However, the architectural applications of people have been known since the eighth century A.D.
Growing awareness of the scarcity of fossil fuels and natural resources, the need to curb CO2 emission and the necessity of reducing the ecological burden caused by the use of materials such as polythene and its derivatives, concrete, steel is encouraging people to find more environmentally friendly solutions, including economical factors.
Paper and its derivates can overcome the ecological burden. But due the growing digital age paper is replace by electronic devices. Since the use of paper has no limits it can be used to only for communication or packaging purposes but its use in architecture can be experimented.

LITERATURE REVIEW:

To understand the paper and its forms for architectural use, it was necessary to study researches already conducted and the architects working with paper. Research paper b y Lawrence C. Bank, Terry D. Gerhardt talks in detail about the paper board tubes. The Research paper by Jerzy F. Latka explains elaborately the various paper products, its derivatives, properties and its application in architecture.

METHODOLOGY

The objective of the paper was to understand how paper can be used in architecture and to what extent it can be used. To conduct this study, literature study and case studies were the primary material of the study. The literature study helped in deriving the use of paper products and its varieties in buildings whereas the case study helped explain the applications of paper in architecture.

MAKING OF PAPER

The most common raw material for paper production is pulp of the wood. It can also be produced from other cellulose containing materials like straw, bamboo and cotton. Paper production is divided into two steps. Step one is preparation of paper pulp. Step two is processing the pulp and paper mills to form paper sheets. Following is the flowchart of making of paper in brief:

PROPERTIES OF PAPER

The basic properties of paper are defined by weight and density, moisture content, physical characteristics, strength properties, optical properties and different criteria.

Paper is classified as paper and cardboard. Paperboards are distinguished per the fibers employed in the assembly, the assembly and pulping ways and also the weight of the paper. There are many grades of paper, whose categorization depends on properties like weight, usage, conversion, staple or pulping ways.

The following weight based grades of paper are recognised:

Tissue: low weight < 40 g/m²; Paper: medium weight 40–120 g/m²; Paper board: medium high weight, one 120–200 g/m²; Board: high weight >200 g/m²

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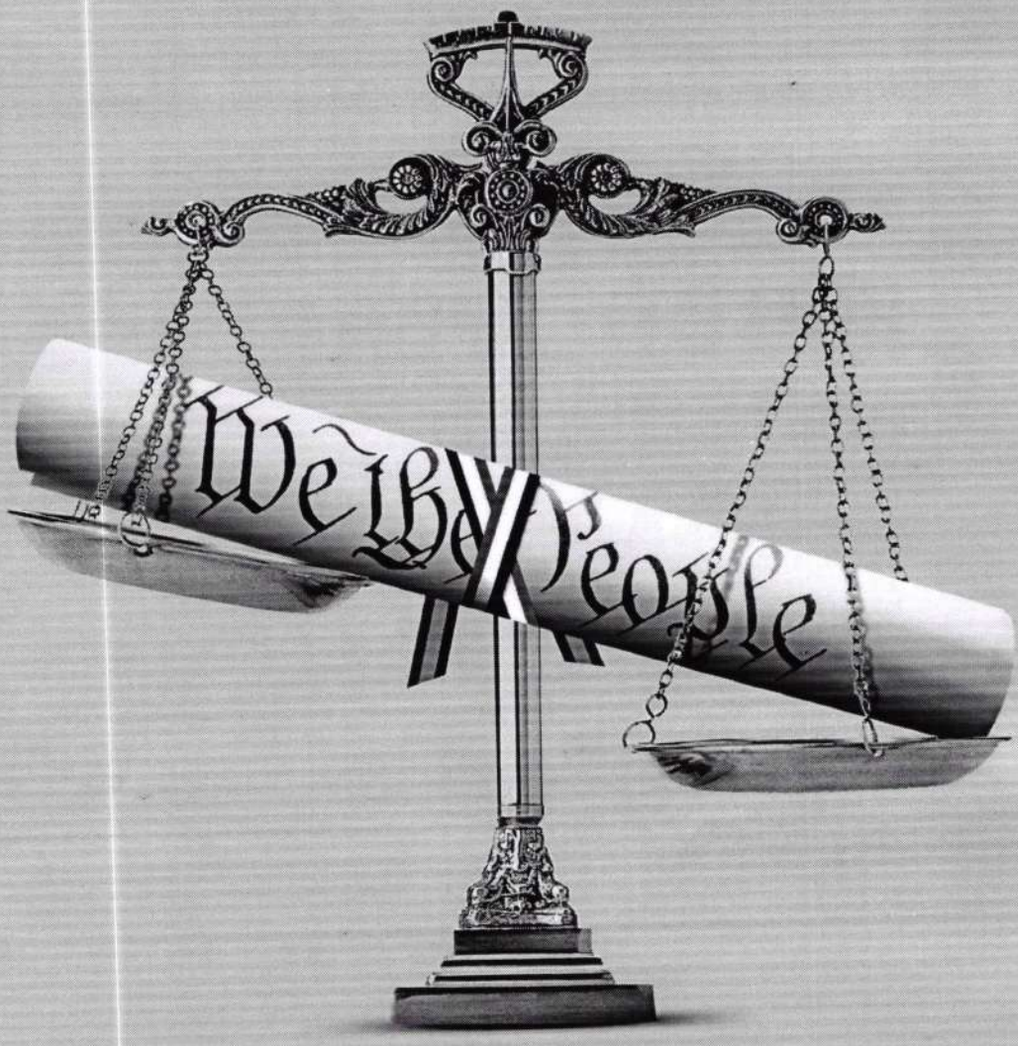

Published by

Government Law College, Mumbai

SCHOOL OF LAW
RUFEEKYAD. Y. PATIL UNIVERSITY

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Problems and Challenges in Implementation of World Constitution: An Overview

Dr. Nagesh H. Sawant & Prof. Damodar M. Hake

ABSTRACT

In Sanskrit, there is the phrase Vasudhaiva Kutumbakam, which means the world is one family treating the world as a single entity. Realization of the phrase is possible if there is a system to unite all states under one umbrella, for this purpose, a world constitution may provide a system to govern the states. However, drafting a constitution with the consensus of the States and its implementation is a herculean task. There are a lot of concerns in drafting the world constitution and even more about the implementation. Hence, envisioning the world constitution is like a daydream. This article emphasizes and highlights some major snags and challenges in the execution of the world constitution.

1. Introduction

The European Union (EU) is considered the best example of the unification of states in one continent under one flag. It gave hope for the unification of the states with the help of the World Constitution. In the words of Fulvio Attina, "The political unification of the world is the product of the exportation of the rules and institutions of the European states system to the world planet".[1] Apart from the classical and successful model of the EU, there are other regional organizations such as the Organization of African Unity (OAU), the Organization of American States (OAS), and the Association of Southeast Asian Nations (ASEAN). These organizations/unions signed and ratified convention/agreements, instituted commissions, and courts. But these regional organizations are not as efficient as the EU due to numerous reasons. However, the BREXIT raised many uncertainties and is contemplated as a major setback for the unification of the states under one banner. In light of this, thinking about a world constitution raises many questions, doubts, problems given the practical realities across the globe. This article ponders upon problems and challenges in the implementation of the world constitution

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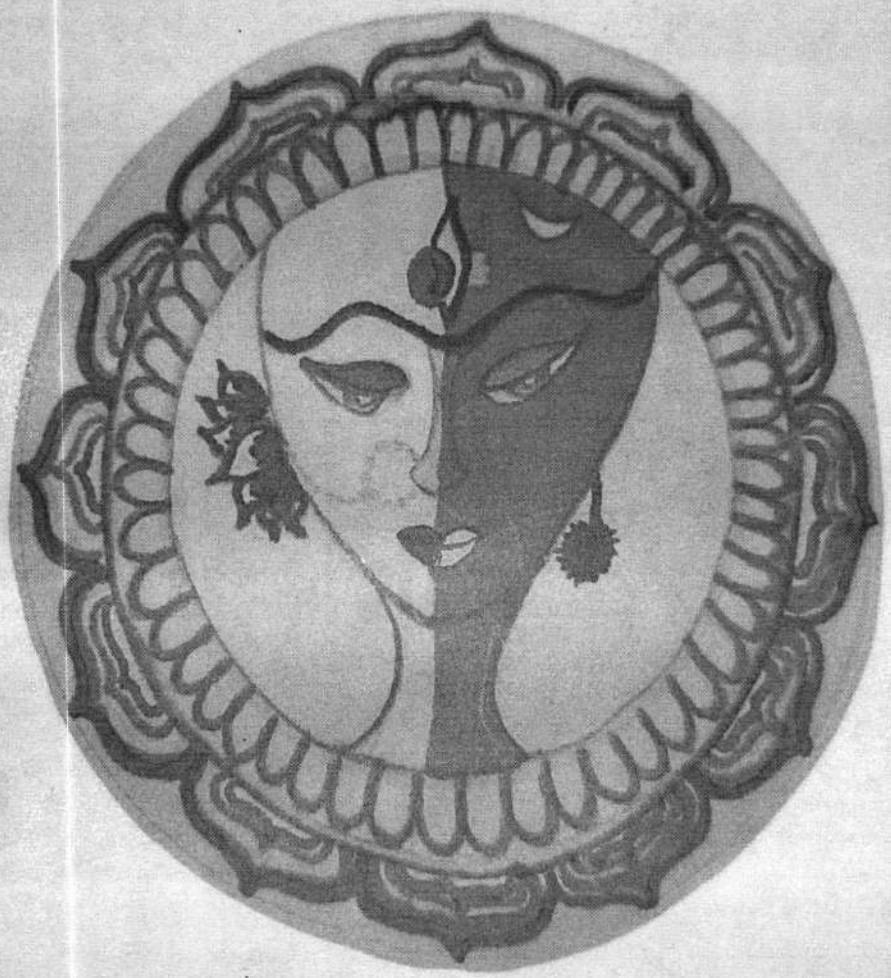

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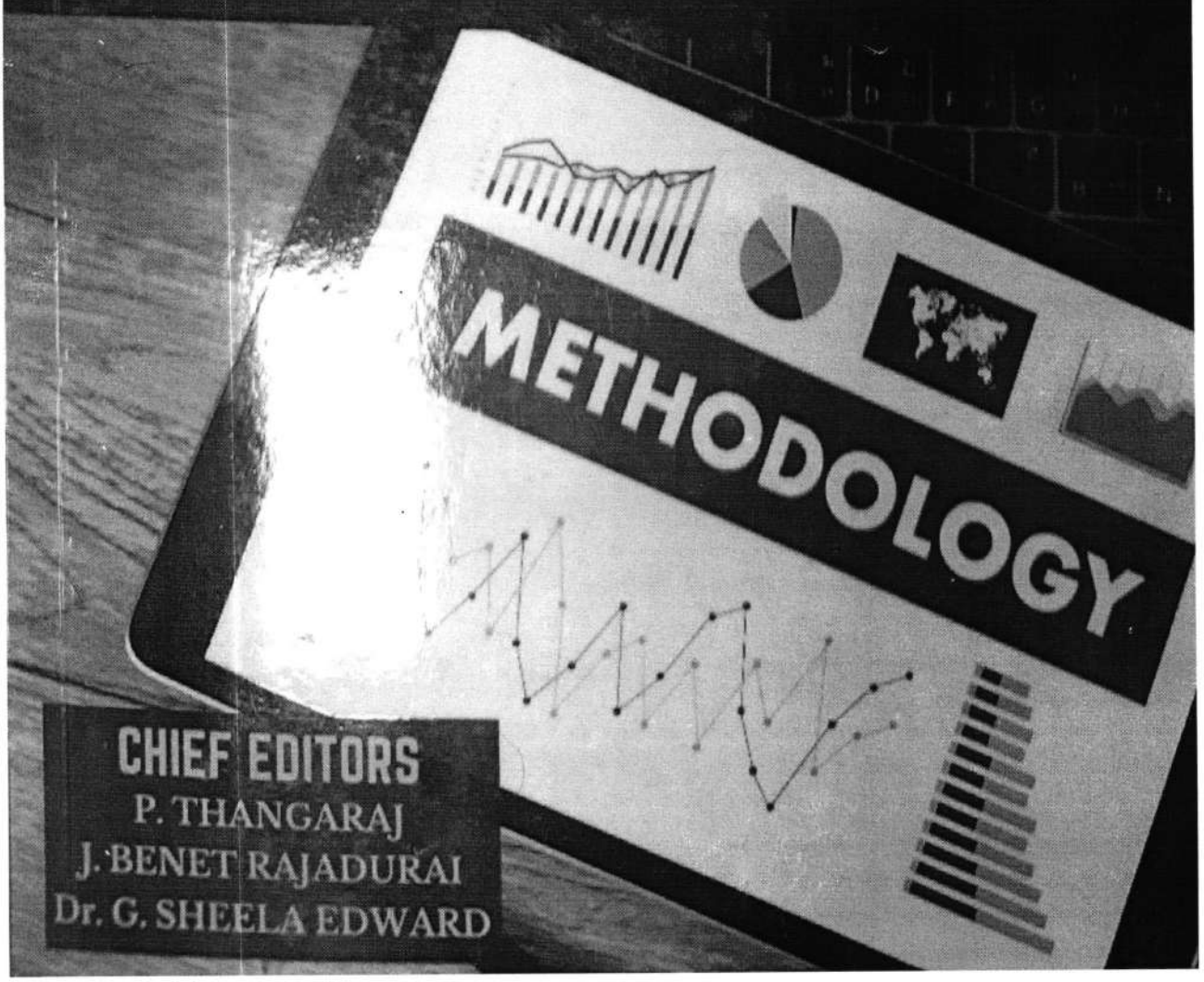

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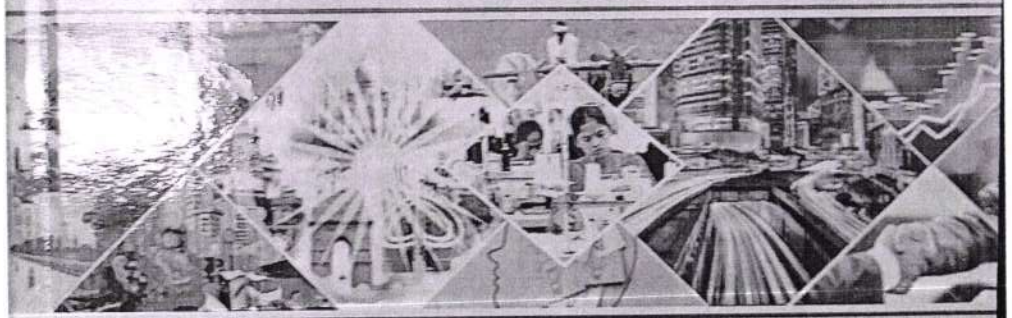

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A Study of Customer Preference of Online Shopping over Traditional Offline Shopping in Pune

*Siddhant Salve & Chinmoy Goswami***

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Abstract

Due to adaptation of the technological innovations by industries in recent years the way people shop has definitely changed. Most people tend shop online as it is more convenient. Offline shopping seems more time consuming to the majority of the population than online shopping because of which many retail giants have incorporated technological advancements into their businesses. The use of the internet has allowed companies to cut marketing costs, reduce the price of the products and improve communication with their consumers. Now a days, consumer uses the mobile and other internet device through range of products and services with huge selection and choices all through their devices from the comfort of their home. This research paper aims to compare recent online shopping with exiting traditional offline shopping in Pune city region and the behavior of the consumers to towards these type of shopping.

Keywords: - *Consumers satisfaction, Online shopping, Technological, Physical shopping, Products etc.*

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Online advertisements through the internet are helping with promoting the

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Introduction

Online advertisements through the internet are helping with promoting the

products to the masses. Businesses are guaranteed a successful endeavor if their enterprise system is tech based.

A rivalry has been established in last couple of years between online and offline stores as customers have the choice of choosing from of the two different modes of shopping to satisfy their shopping needs. Geographical limitations, fraud, product delivery, tangibility, information about the goods, subjectivity to tax, etc. are some factors that distinguish between these two shopping methods.

Factors Affecting Online Shopping

Risk: When a consumer buys a product online, they are basically rolling a dice on whether the product is worth buying or not. There is a chance that the goods were falsely promoted, that the color or quality of the product may have changed from what was depicted online, and that it may have other flaws. The consumer has little recourse if the items are delivered on schedule as a result of unavoidable circumstances.

Convenience: Preferring to shop online brings more convenience to the consumers than offline shopping. Taking your car out for spin and visiting the store then walking through different aisles browsing for the right product can very consuming of your time. It's quite handy to shop online from the comfort of your own home and on the device of your choice. The ease of the payment process and the delivery of the merchandise are two tempting features of online shopping.

Offers: Online shoppers get products offered at lower price and also get offered discounts associated with the bank or the brand. Offline stores offer discounts seasonally or during stock clearance. Shopping online gives offers and discount regardless of the season or festival.

Tangibility of the product: Offline shopping provides the customer with the privilege of tangibility of the product this is something online shoppers cannot have. Sometimes the touch and feel of a product determines whether the consumer is going to buy it or not. Online shopping lacks in this area, customers at times have second thoughts about buying the product through websites as they have not felt the touch of the product and are not able the gauge the quality or the worthiness of the product.

Factors Affecting Online Shopping

Time consuming: Customers who shop offline must visit multiple different businesses in order to find the ideal product. Offline shopping

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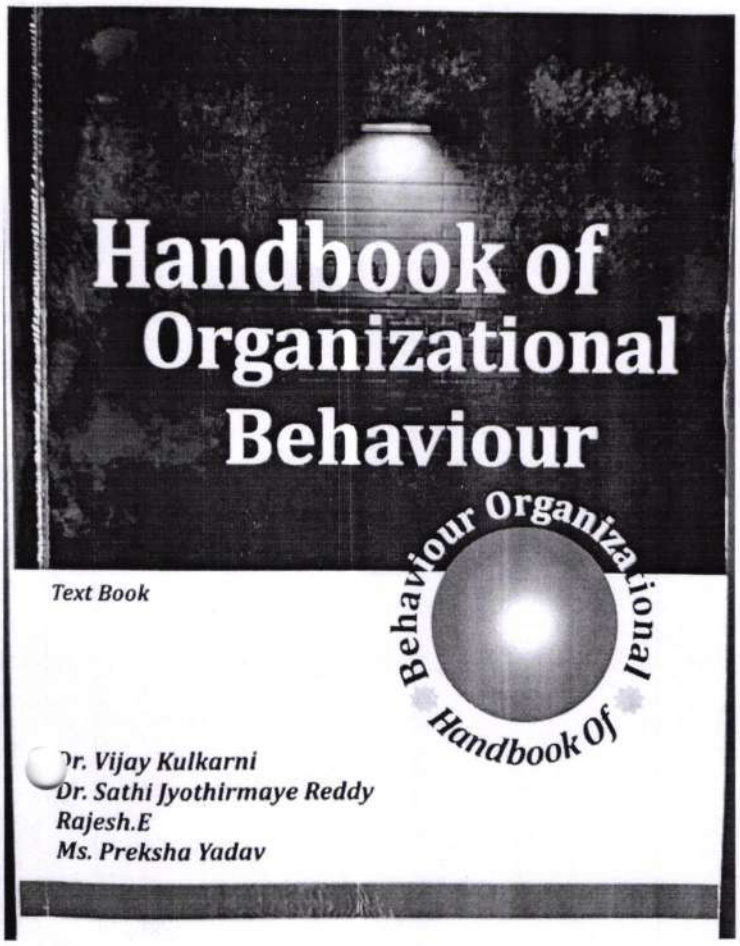

Published By:
KAAV PUBLICATIONS
Aggarwal Plaza, LSC- 1, Mayur Vihar Phase 3, DELHI- 110096
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Typesetting By: Ms. Shama
Kaav ID: KAAV/BP/A-287-22
Printed By: Amit Printers, Noida
First Edition: 2022
Print Copy: INR 581/- US$60
E-Book Price: INR 350/- US$10
E-ISBN: 978-93-91842-44-4
P-ISBN: 978-93-91842-36-9
DOI: https://doi.org/10.52458/9789391842369.2022.tb

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DOI: https://doi.org/10.52458/9789391842369.2022.tb

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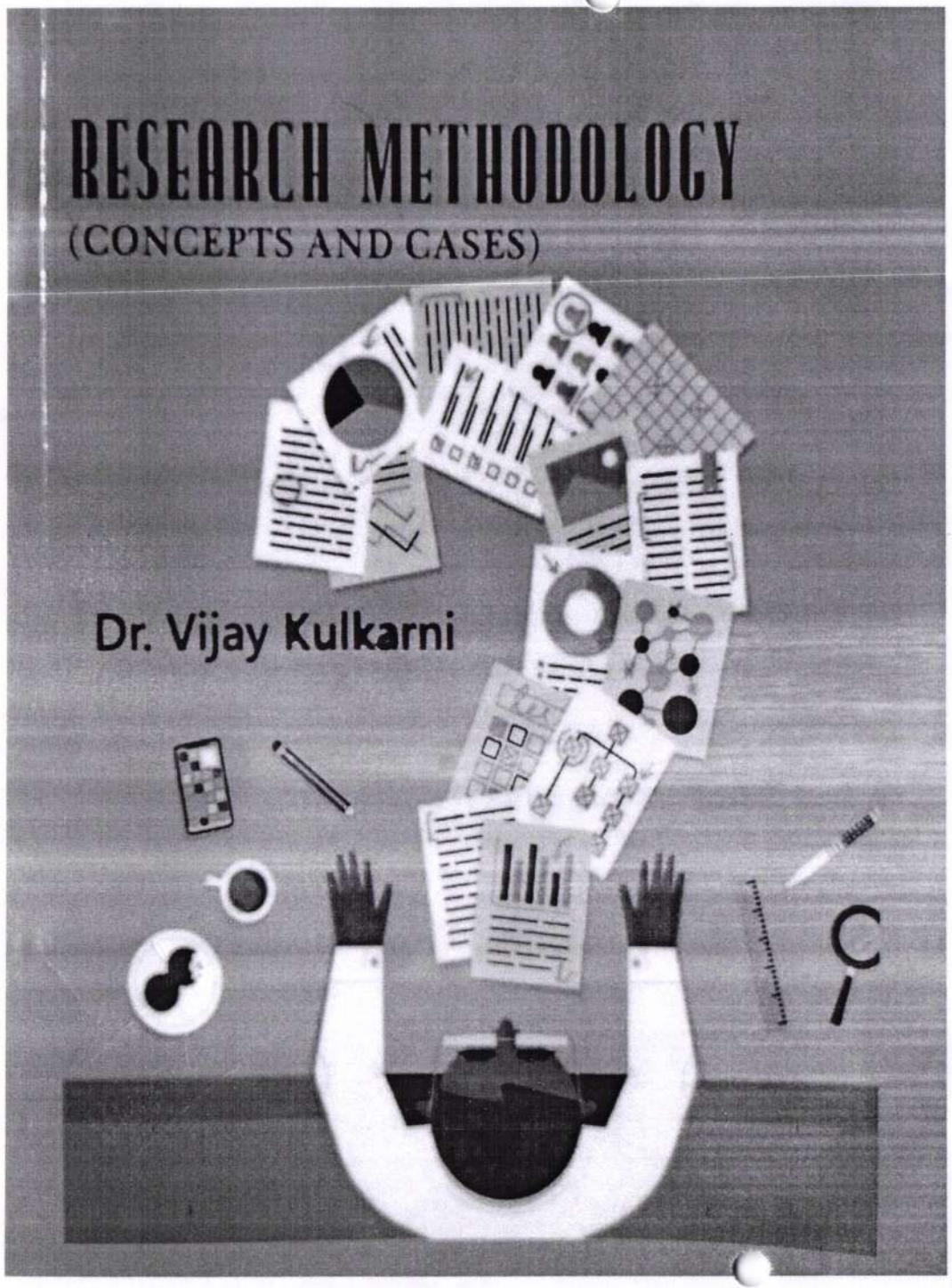

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Dr. Kavita Chordiya has an outstanding career. Her experience covers almost **[illegible]** years of teaching. She is currently working as **Sr. Faculty in Ajeenkya D. Y. Patil University**, Pune. She has completed her **M.Com** & **MBA** in **HR** specialization. She has attended various International & National level Seminars/Conferences. She also showed her presence/contribution in Paper Presentation at International & National level. & some of them got published/in UGC Care Listed Journals. She has attended several FDPs. She also holds the various other valuable positions like Chairperson for Labour Law, Member of the Syllabus Revision Committee of SPPU, Paper Setter, Examiner, Practical Exam Coordinator, Project Report Examiner etc. She is [illegible] of many books on the field of Commerce & Management. She also [illegible] Management, Science & Technology [illegible]

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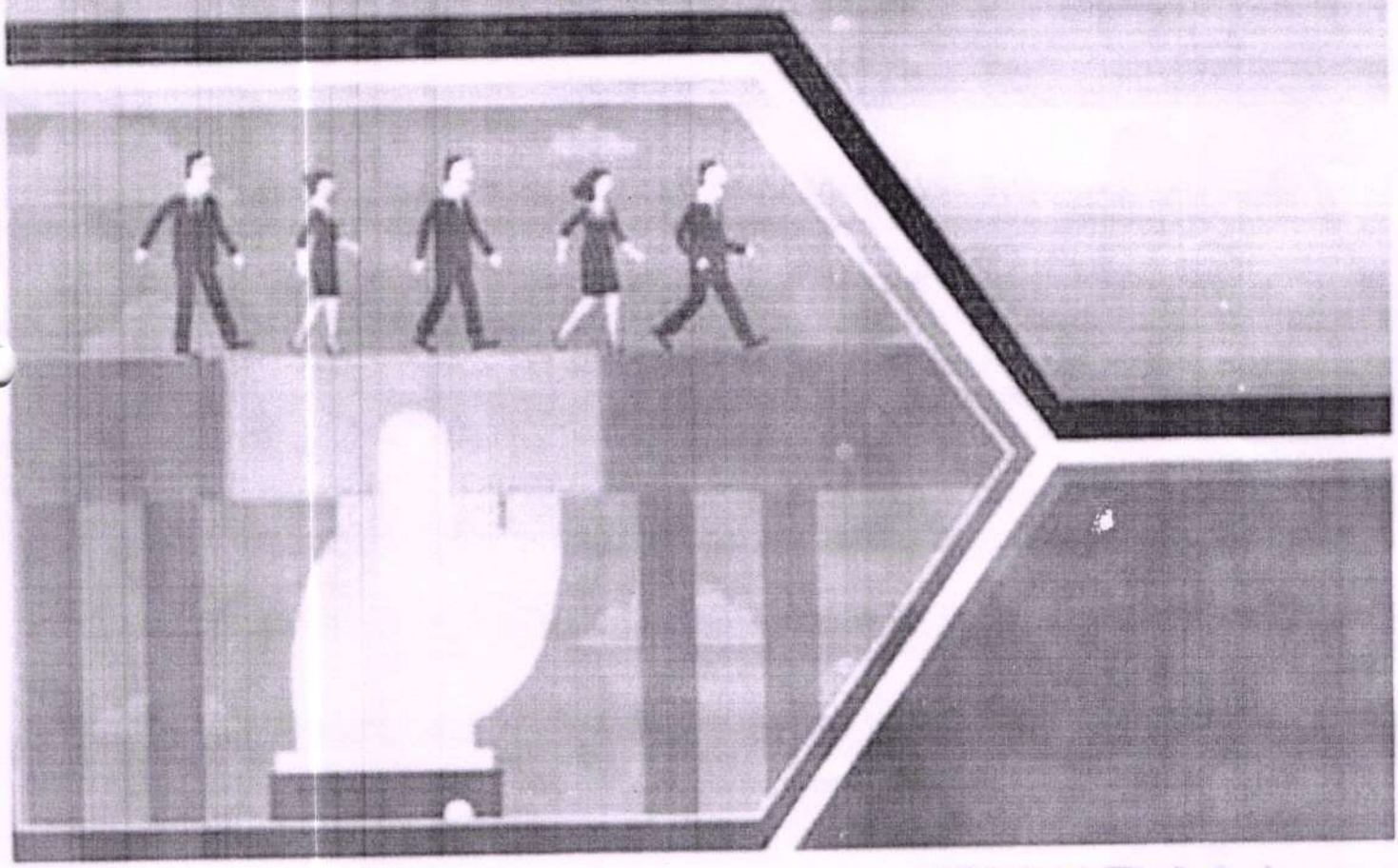

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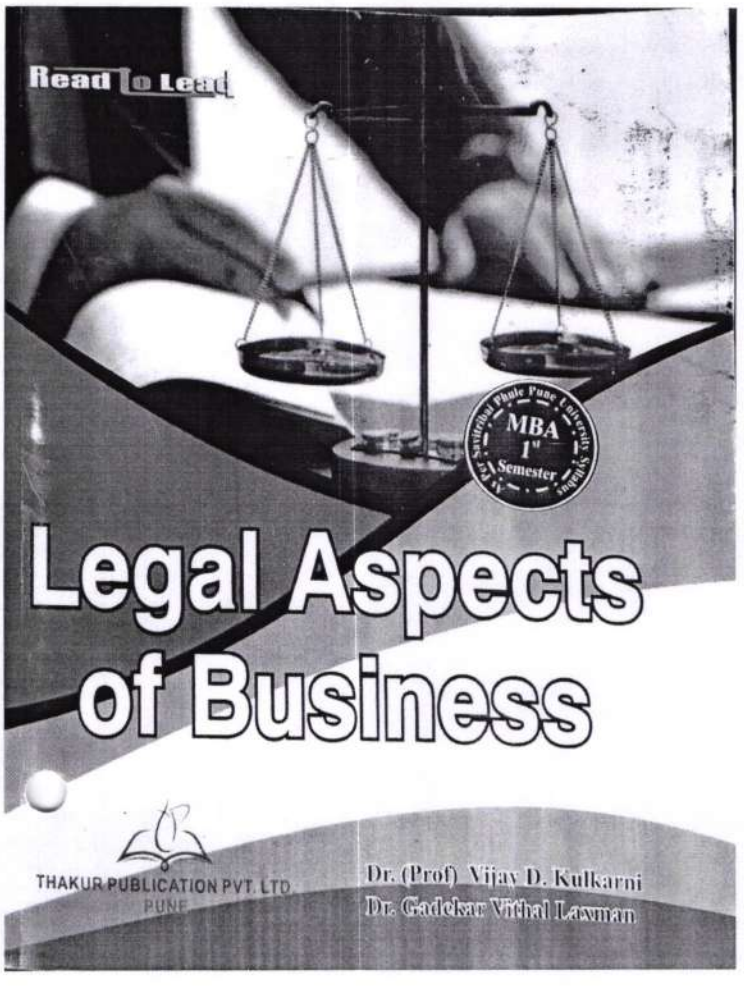

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About the Authors

Dr. (Prof) Vijay D. Kulkarni is masters in various disciplines such as MSW, PGDHRM, MBA, M.Com, MMM, Ph.D. He has three decades of experience blend of Industry and education. He has worked in the different capacities including **Director** in Management Institutes. He is Government Certified Internal Auditor for ISO- 9001-2015. He is Yellow and Green **Belt Holder** of six sigma.
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His expertise lies in the core subjects such as Legal Aspects of Business, Organizational Behavior, Business Research Methods, Marketing Management and Strategic Management. He has authored different books of management as per the university syllabus. He is recognized guide in various universities recognized by UGC. Under the guidance of him seven scholars have completed their Ph.D successfully. He has also worked as chairman on Ph.D committee for final viva-voce. He has organized international conference, editor of ISSN publication and also published research papers in various reputed journals.
He is recipients of various awards and to name few Radhikabai Apte Award from SPPU, Best Youth Leader Award from Ministry of HRD, Life Time Achievement Award in Management, Ideal teacher Award, We Care Award etc. Recently, he has joined Suryadatta Institute of Management and Mass Communication (SMMC) as Professor.

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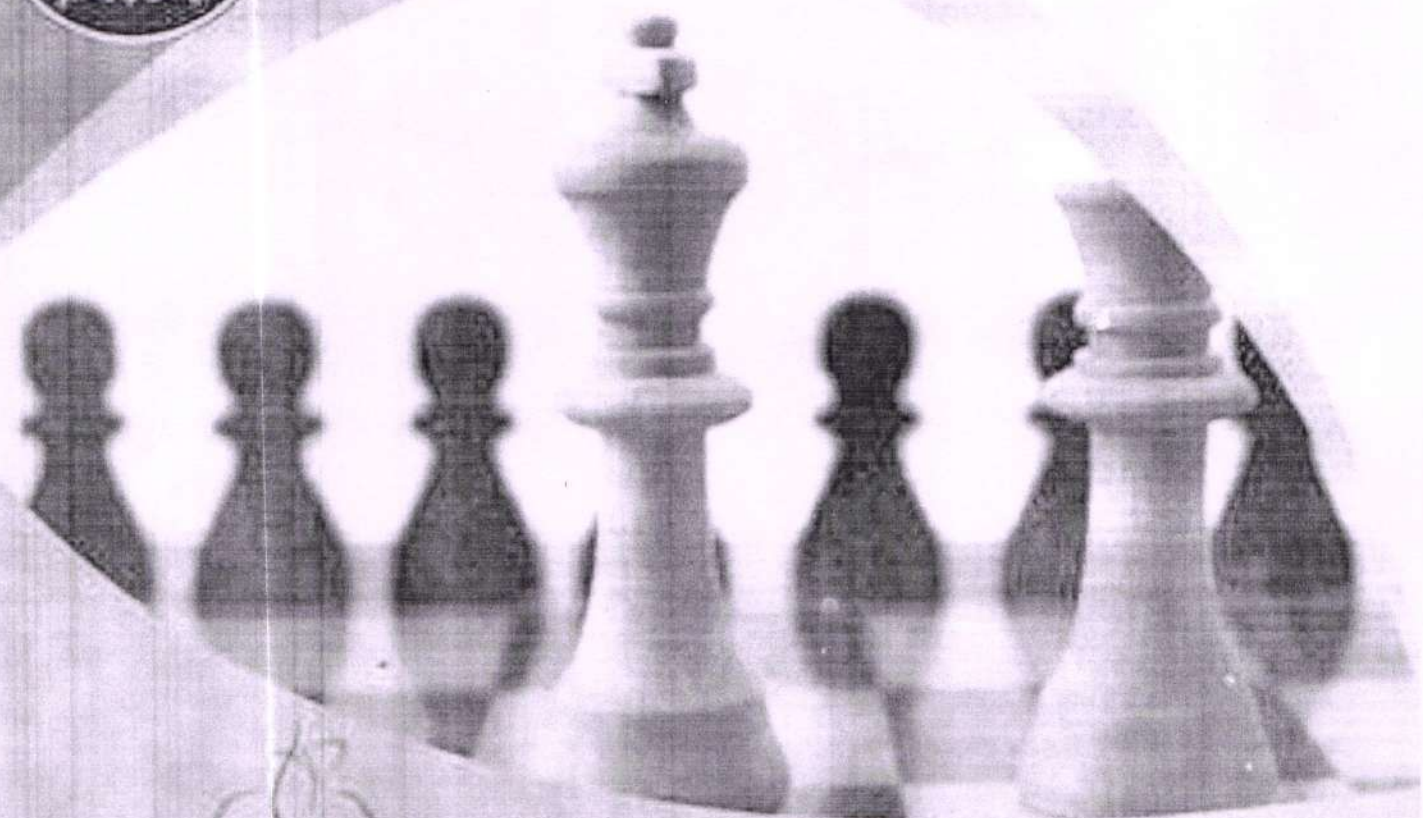

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