Clean Energy Production Technologies
Series Editors: Neha Srivastava · P. K. Mishra

Pradeep Verma *Editor*

Biorefineries: A Step Towards Renewable and Clean Energy

Springer

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Series Editors

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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 serve global readership on this theme.

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Biorefineries: A Step Towards Renewable and Clean Energy

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Preface

Post-industrialization, there was a rapid increase in the requirements of energy to run the machines and to manage the regular day-to-day activities of human beings. Industrialization has increased the dependence of human being on the machines that required energy to operate. The energy need was mostly met by fossil fuels such as coal and petroleum and thermal energy based electrical generation units. Attempts have been also made to harness the energy by using radioactive elements via nuclear power plants. However, these energy source have limitations such as finite availability, source of pollution and have several other negative environmental impacts as well. This has led to search for sustainable, renewable, and clean energy sources. Air, water, wind, and solar power are some alternative sources of renewable energy and are readily available. Biomass such as food crops, lignocellulosic, algal and hybrid energy crops are one such renewable resources that can be effectively converted to biofuels and various value-added chemicals. The petroleum-based biorefineries are also a source for several chemicals and the biomass-based biorefineries can also contribute to the generation of these essential chemicals. Also, the lignocellulosic biomass due to huge availability and less anthropogenic application are burnt or left-over which causes pollution. Thus, this has led to search for the methods to maximally utilize these available biomasses to meet the energy-chemical needs and minimize pollution. Therefore, in the past few decades attempts have been made to utilize the available biomass for energy generation and currently approximately 15–20% of global energy needs are met by biomass-derived energy.

This book is an attempt to provide an account of the past, present, and future of the biomass-based biorefineries. It gives an insight into the recent advancements in the technologies and methods used for the conversion of biomass to bioenergy and biochemicals. It also focuses on the limitations of already existing technologies and providing future prospects of different available technologies. Development of any technologies has a direct effect on the human being; therefore, the socioeconomic impact of the biomass-based biorefineries has also been included in the book. The biomass-based biorefinery can be broadly divided into first, second, third, and fourth generation. Thus, an approach will be suggested on how these different generations

can be consolidated in order to design an integrated self-sustainable biomass-based biorefinery.

The present book will be a very informative and valuable addition to the series "Clean Energy Production Technologies." This book is designed as such that it will be helpful as a reference material for students, academicians, and researchers while working in the area of biomass-based biorefineries. In addition, it should gain huge readership among environmental scientists, biotechnologists, enzymologists, and bioenergy researchers.

Ajmer, India Pradeep Verma

Acknowledgment

First of all, I would like to convey my gratitude to the series editor Dr. Neha Srivastava and Prof. P.K. Mishra for considering the submission of this book volume under the book series "*Clean Energy Production Technologies*." I am thankful to Springer Nature for accepting the proposal and myself as Editor for the current book volume. The current volume of the book series is only possible because of the support from all the researchers and academicians who have contributed to the book; therefore, the Editor is thankful for their contribution. I would also like to thank my PhD scholar Mr. Bikash Kumar for providing me with all the necessary technical support during the entire stage of book development. I am also thankful to Central University of Rajasthan (CURAJ), Ajmer, India for providing the infrastructural support and a suitable teaching and research environment. The teaching and research experience at CURAJ has provided me with 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 also thankful to the Department of Biotechnology for providing me the funds through sponsored projects (Grant No. BT/304/NE/TBP/2012 and BT/PR7333/PBD/26/373/2012), for setting up of my laboratory "Bioprocess and Bioenergy Laboratory."

I am always thankful to god and parents for their blessings. I also express my deep sense of gratitude to my wife and kids for their support during the development of the book and in life.

Contents

About the Editor

Pradeep Verma is the Group leader of the "Bioprocess and Bioenergy Laboratory" at the Department of Microbiology, School of Life Sciences, Central University of Rajasthan, Ajmer, Rajasthan, India. He is working extensively in the area of microwave/biological delignification, enzyme-mediated hydrolysis, and development of consolidated/integrated biorefineries. He has contributed significantly to the area of lignocellulosic biomass based biorefineries that is evident from his highly cited publication in peer-reviewed journals. He has contributed to several international patents. He is working as editor and reviewer to numerous high impact journals.

Chapter 1
Biorefinery for Agro-Industrial Waste Into Value-Added Biopolymers: Production and Applications

Sanjeet Mehariya, Tiziana Marino, Patrizia Casella, Angela Iovine, Gian Paolo Leone, Dino Musmarra, and Antonio Molino

Abstract Agro-industrial waste (AW) could be attractive carbon (C) source that have potential for production of high value-added biopolymers. AW can be derived from different sectors based on the compositional variation and transformed into biopolymers. Biopolymers are synthesized from diverse group of microbes and can be categorized into different groups. These biopolymers are a storing compound that is available in the cytoplasm of different group of microorganisms. This chapter describes the potential of AW for production of biopolymers, which allows the conversion of organic AW into biodegradable polymer production using the ecobiotechnological approach to reduce the overall cost. This will allow the development of the low-cost biopolymers, which can have different applications in various sectors. Therefore, it will increase demand of sustainable products with the rising its market demand.

Keywords Polyhydroxyalkanoate · Polyhydroxybutyrate · Biosynthesis · Organic waste · Sustainable development

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Abbreviations

2HA	2-hydroxyalkanoate
3HB	3-hydroxybutyrate
AD	Anaerobic Digestion
GHG	Greenhouse Gas
HHV	High Heating Value
PDLA	Poly(D-lactic acid)
PDLLA	Poly(L- and D-lactic acid)
PGA	Poly-γ-glutamic acid
PHA	Polyhydroxyalkanoate
PHB	Polyhydroxybutyrate
PLA	Polylactic Acid
PLLA	Poly(L-lactic acid)

1.1 Introduction

In the twenty-first century researchers and society are more concerned about the environment and adopting the sustainable to way of leaving, therefore searching for sustainable bioproducts (Mierzati et al. 2020). In the 1980s, biopolymers (Bioplastic) have emerged as an alternative potential candidate to synthetic plastics due to several environmental benefits such as reducing the carbon footprint and greenhouse gas (GHG) emissions (Rivero et al. 2017; Kumar 2020). The bioplastic can reduce the use of fossil resources because these polymers are synthesized by a several type of microbes (Kumar et al. 2014). In the last few years, biopolymers have raised research interest as an alternative plastic due to similar property like thermoplastic with synthetic plastic and faster degradation (Kovalcik et al. 2017). Several microbial species can synthesize biopolymers in different forms with varied amount due to their genetic metabolism. Different organisms such as *Bacillus* sp., *Pseudomonas* sp., and *Ralstonia eutropha* are commonly reported for synthesis of biopolymers during the cell growth (Kumar et al. 2015a; Ray et al. 2016; Kovalcik et al. 2017; Kumar and Kim 2018; Evangeline and Sridharan 2019; Kumar 2020). While genetic engineered strains of *Escherichia coli* are also capable to accumulate biopolymers (Seo and Choi 2020).

Biopolymers can be categorized as polyhydroxyalkanoate (PHA), polyhydroxybutyrate (PHB), Poly-γ-glutamic acid (PGA), and poly(lactic acid) (PLA) (Rivero et al. 2017; Kumar and Kim 2018). However, for the cell growth microorganisms required carbon source, which can be derived from different source. Although PHA, PHB, PLA, and PGA are biopolymers with higher industrial demand, but industrial production is expensive because of high operation cost (Kumar et al. 2015a; Rivero et al. 2017; Kumar and Kim 2018; Sharmila et al. 2020). Therefore, to overcome this issue inexpensive feedstock, i.e. AW, food waste,

lignocellulosic biomass, and other carbon rich waste can be used as substrate for microorganisms for biopolymers production (Kumar and Kim 2018). However, among the available different feedstocks, AW could be potential source due to huge amount of availability as well as its need to depose, which demand extra cost (Khardenavis et al. 2007; Anjali et al. 2014). Therefore, AW recognized as possible feedstock for biopolymers production with possibilities of large-scale production. Currently, several industries are producing biopolymer-based plastics for applications in different industrial sectors such as biomedical, packaging, textiles sector. Due to wide range application of bioplastic, research needs to carry out in-depth for production of high-quality bioplastics using the cheap AW to meet the growing demand.

Therefore, this chapter discusses the different type of bioplastics, i.e. PHA, PHB, PGA, and PLA for various applications.

1.2 Agro-Industrial Waste as Source for Biopolymer Production

Agro-industrial feedstock can be derived from the agriculture and food processing sector. In the last decades, environmental issue raised due to increasing waste, which cause several environmental issues such as greenhouse gas (GHG) emission; therefore, various AWs can be utilized for biopolymer production. The AW has high nutritional compositions and can be converted into biopolymers via different biotechnological routes. The biotechnological routes depend on the type or physiochemical composition of AW. Based on the physiochemical composition of AW, it can be derived from different source. AWs generated from various sectors and can be classified into different types as based on the source of residue as discussed in Fig. 1.1.

AWs are generally categorized as industrial and agricultural residues, while it can be subcategorized based on composition. Agricultural residues can be considered as farm biomass and processed biomass. Field residual biomass are left-over biomass after crop harvesting such as leaves, stems, stalks as well as seedpods of various crops. In the agricultural sector, volume of field residues is large, which need to be managed properly to avoid environmental issue and problems for next crop. Furthermore, after the crop processing, left-over residues known as process residue. For example, during the rice harvesting from paddy, rice husk is a left-over residual biomass. While further processing residual biomass includes seed particles, roots, bagasses, and molasses. Generally, process residual wastes are considered as animal feed, biofertilizer for soil improvement, raw material for fertilizer industry (Sadh et al. 2018). The main constituent of agricultural residues is cellulose, hemicellulose, and lignin; therefore, agricultural residues termed as lignocellulosic biomass. However, based on the physiochemical properties of agricultural residues, it can be

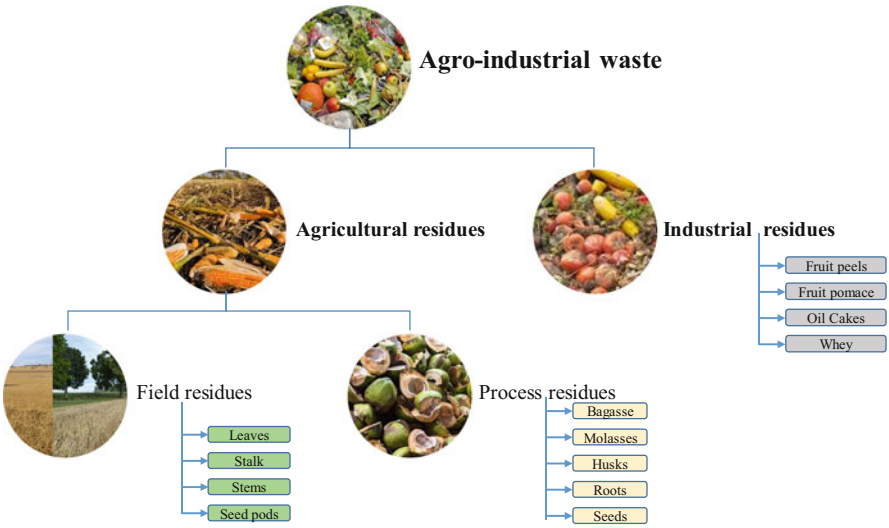

Fig. 1.1 Classification of AW based on various sources

Table 1.1 Approximate chemical compositions of various agricultural residues (Hiloidhari et al. 2020)

	Chemical composition (% w/w)		
Agro-industrial residue waste	Cellulose	Hemicellulose	Lignin
Sugarcane bagasse	30.0	57.0	13.0
Rice straw	39.0	23.0	36.0
Corn stalks	61.0	19.0	7.0
Sawdust	45.0	28.0	24.0
Sugar beet waste	26.0	19.0	3.0
Barley straw	34.0	22.0	14.0
Cotton stalks	59.0	14.0	22.0
Oat straw	39.0	27.0	18.0
Soya stalks	34.0	25.0	20.0
Sunflower stalks	42.0	30.0	13.0
Wheat straw	33.0	24.0	9.0
Tea waste	30.0	20.0	40.0

converted into biopolymers. The composition of various kinds of agricultural residual wastes reported in Table 1.1.

The chemical composition of agricultural residues contains its compositional value, calorific value (Heating Value), proximate analysis, and ultimate analysis. The compositional analysis of different agricultural residues reported in Table 1.2, which includes hemicelluloses, cellulose, and lignin contents. While the calorific value is a heating value which is majored as high heating value (HHV) of agricultural residues, which refers to the energy content and energy content unit expressed

Table 1.2 Heating value (HV), proximate and ultimate analysis of different agricultural residues (Gupta and Mondal 2020)

Agro-industrial residue	Analysis (wt.%)									HVV (MJ/kg)
	Ultimate					Proximate				
	C	H	N	S	O	MC	VMC	FC	AC	
Wheat straw	53.9	7.0	3.0	–	36.1	8.5	63.0	23.5	5.5–13.5	17.1
Corn cob	43.6	5.8	0.7	1.3	48.6	9.7	80.6	18.2	1.2–2.8	16.9
Sugarcane Bagasse	45.1	6.05	0.3		42.8	8.5	84.0	1.64	4.5–9.0	18.2
Corn Stover	–	–	–	–	–	10.6	78.7	17.6	3.7	17.8
Banana waste	43.5	6.2	0.86	0.95	42.3	7.8	78.2	15.6	11.4	17.1
Tea waste	–	–	–	–	–	6.5	85.0	13.6	1.4	17.1
Barley straw	41.4	6.2	0.63	0.01	51.7	6.9	78.5	4.8	5–9.8	15.7
Almond shell	54.7	7.5	0.3	0.3	37.4	8.7	79.7	4.9	2.3	20.2
Flax straw	43.1	6.2	0.7	0.1	49.9	7.9	80.3	8.8	3.0	17.0
Rice husk	36.9	5.0	0.4	–	37.9	–	81.6	–	18.4	15.3
Hazelnut shell	52.3	6.5	5.2	9.2	26.8	9.0	69.3	28.3	4.3	19.3
Babul seeds	54.1	6.12	5.2	–	34.5	12.5	69.1	11.0	7.3	–

Note- *C* Carbon, *H* Hydrogen, *N* Nitrogen, *S* Sulfur, *MC* Moisture concentration, *VMC* Volatile matter concentration, *HV* Heating value, *FCC* Fixed carbon concentration, *AC* Ash concentration

as Megajoule/kilogram (MJ/kg). The proximate analysis of agricultural residues determines the amount of ash, fixed carbon, moisture, and volatile matter in the dry biomass. Furthermore, ultimate analysis of agricultural residues analyzes the elemental concentrations such as carbon, hydrogen, nitrogen, and sulfur (CHNS) concentration in the dry residual organic waste.

Second major AW belongs to food processing sector, which generates huge amount of industrial residues during the canning and packaging of brewery, confectionery, dairy, juice, meat products. However, the potential of food industry waste has been widely investigated for production of several bioproducts such as biofuel (Karthikeyan et al. 2017; Mehariya et al. 2018). Moreover, industrial residues are rich sources of carbon, nitrogen, and different wastes have varied composition. In recent years, agro-industrial sector grows tremendously for fruit and vegetable processing to meet the consumers supply. While processing of fruit and vegetable generates huge quantity of residues such as pulp, pomace, seeds, etc. The processing of different type of fruit and vegetable generates varied quantity of residues in the range of 10–50% as reported in Table 1.3 (Ajila et al. 2012). For example, citrus processing units generate around 50% of residual waste, while onion processing industries generate around 10% residual biomass. Siciliano et al. (2019) reported that mixture of fruit and vegetable wastes (tomato, asparagus, kiwi, and potato) was used for biofuel production and showed significant recovery of bioproducts. Mehariya et al. (2020) reported that different AWs can be converted into bioproducts such as biofuels and biopolymers.

Table 1.3 Type of industrial residual waste derived during the processing of fruits and vegetables (Gupta and Mondal 2020; Hiloidhari et al. 2020)

Type of fruit and vegetable for industrial processing	Type of waste	Approximate waste production (%)
Fruit processing		
Apple	Peels, pomace, seeds	250.
Banana	Peels	35.0
Citrus	Peel, rag, seed	50.0
Grapes	Stem, seed, pulp	20.0
Guava	Peel, core, seed	10.0
Mango	Peels, stones	45.0
Pineapple	Peels, core	33.0
Vegetable processing		
Tomato	Skin, core, seed	20.0
Pea	Shells	40.0
Potato	Peels	15.0
Onion	Tunic, outer leaves, roots	10.0

1.3 Classification and Properties of Biopolymers

Biopolymers known as plastic, which are synthesized during biotechnological process. In the biotechnological process organic material is converted into different types of biopolymers through various microorganisms. Different types of microorganisms synthesize various types of biopolymers as shown in Fig. 1.2 and these polymers have diverse chemical properties as discussed in below subsections, and properties of Polypropylene and biopolymers are reported in Table 1.4.

1.3.1 Poly-3-Hydroxybutyrates (PHA)

Nowadays, many PHA polymers have been successfully synthesized from several microorganisms using different organic waste (Kumar et al. 2019). PHA is polyesters of hydroxy acids, which is organized in polymeric forms and has similar properties to petroleum-based plastics. PHAs are biocompatible, biodegradable and therefore considered as "bioplastic/green plastic." PHAs have different physiochemical properties such as crystalline hard and flexible in nature; these properties could be manipulated by tuning the type and structure of the hydroxy acids. However, the physiochemical properties of PHAs can be manipulated using different approaches such as bioengineering approach for pathway manipulation. Mierzati et al. (2020) conducted the study and synthesized a novel type of PHAs co-polymers containing different unit of 2-hydroxyalkanoate (2HA), [P(3HB-co-

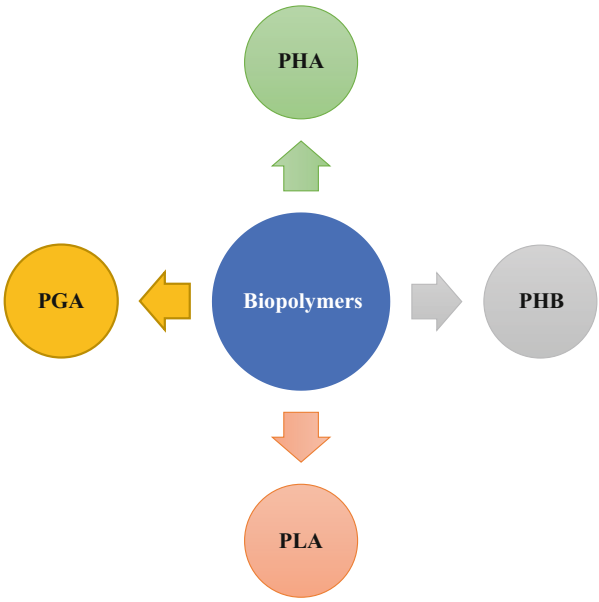

Fig. 1.2 Main types of biopolymers

Table 1.4 Properties of Polypropylene, PHB, and PLA (Rivero et al. 2017)

Property	Polypropylene	PHB	PLA
Crystallinity	60	60–70	na
Density (g/cc)	0.91–0.94	1.17–1.25	1.25
Elongation (%)	400	6–17	na
Tensile strength (MPa)	34.5	18–27	109.97
Tensile modulus (GPa)	1.4	na	3.3
Melting temperature (°C)	171–186	na	na
Estimated price ($/m^3)	1055	4320	na

Note: na: data not available

2HA)], and 3-hydroxybutyrate (3HB) and using the genetic engineering tool in a recombinant *Escherichia coli*. The synthesis of copolymer was attained by addition of leucine as precursor in the growth medium (Mierzati et al. 2020). Koller (2018) reported that the stiffness, elasticity, crystallinity, and degradability of PHAs could be manipulated by the monomeric structure, choice of bacterial species, type of substrates, operational conditions (Temperature and pH) during synthesis and processing after synthesis. The granular inclusion bodies of PHAs molecules showed in Fig. 1.3 in microbial cell and chemical structure of PHAs.

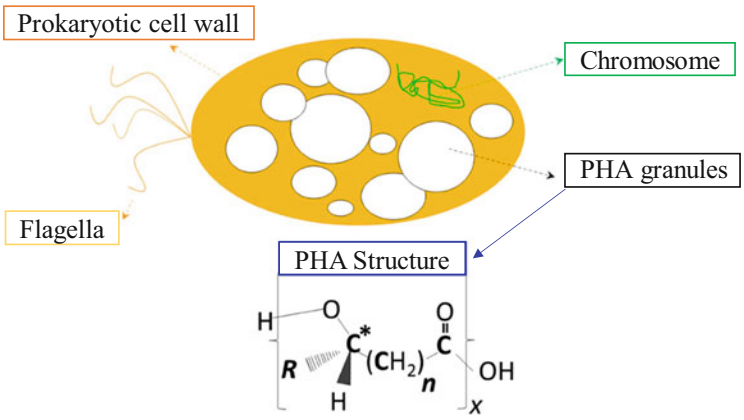

Fig. 1.3 PHA granules in microbial cell and chemical structure of PHA (Koller 2018)

1.3.2 Poly-3-Hydroxybutyrate (PHB)

PHB is a potential eco-friendly biopolymer with structural properties similar to polypropylene (Panaitescu et al. 2016, 2020). PHB has several benefits associated to biodegradable, biocompatible, high melting flow, better solubility, diffusion, permeability properties (Battegazzore et al. 2018; Yeo et al. 2018). PHB has remarkable mechanical properties comparable to synthetic polymers (Panaitescu et al. 2017). The chemical structure of PHB shown in Fig. 1.4. PHB has special stereo-chemical structure as shown in Fig. 1.4, which is responsible for higher crystallinity due to crystallized homopolymer. Moreover, PHB exhibited less ecological problem in relationships of ecotoxicity and human toxicity (Isola et al. 2017). However, PHB has lesser thermal durability due to small thermal handling window (Yeo et al. 2018). Therefore, several approaches developed to strengthen PHB properties via thermal and drawing process, blending, fiber reinforced composites, and chemical functionalization as shown in Fig. 1.4.

1.3.3 Poly-γ-Glutamic Acid (PGA)

PGA is an extracellular polymer produced by different bacterial spices. These polymers are a negatively charged ion, biocompatible, biodegradable, non-toxic and formed by D- and L-glutamic acid units, which are associated with γ-glutamyl bonds (Halmschlag et al. 2020; Yang et al. 2020). The chemical structure of γ-PGA showed that the D- and L-glutamic acid units connected with γ-glutamyl bonds by a γ-amide bond among α-amino and γ-carboxyl groups (Fig. 1.5). Literature showed that different *Bacillus* strains are capable to produce the significant amount of PGA (Xavier et al. 2019; Fang et al. 2020a; Halmschlag et al. 2020; Tang

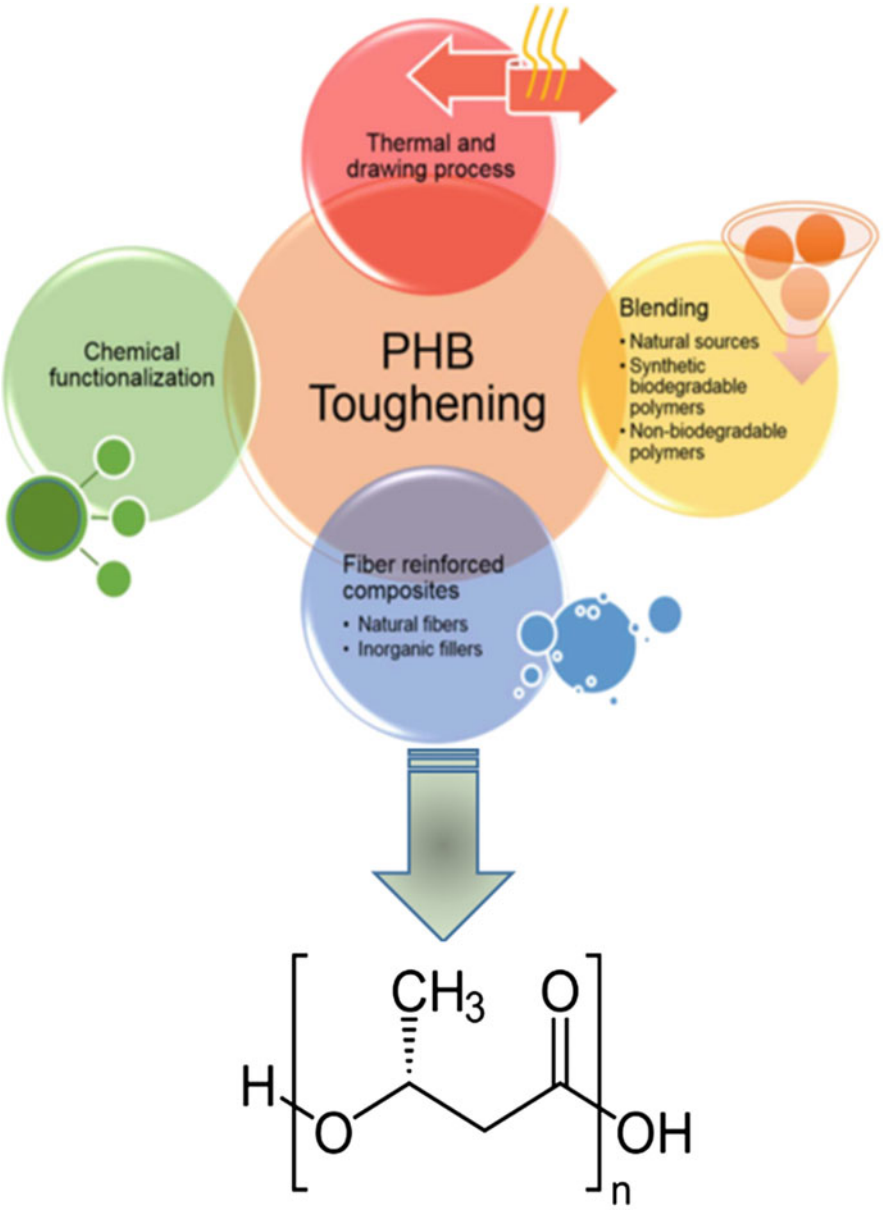

Fig. 1.4 Different PHB modification approaches and PHB chemical structure (Yeo et al. 2018)

et al. 2020). Fang et al. (2020b) carried out solid-state fermentation using AW (maize stalk and soybean meal waste) feedstock for PGA production under aseptic and non-aseptic conditions Furthermore, results indicated that AW can be converted into

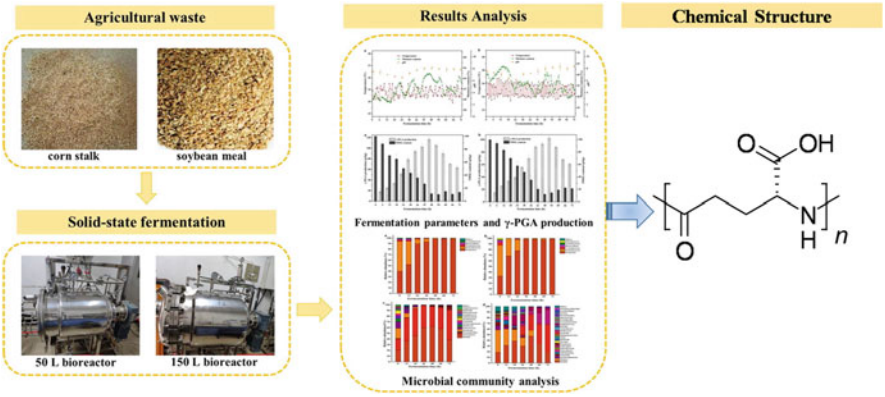

Fig. 1.5 Agro-industrial utilization for γ-PGA production up to 50 L to 150 L and chemical structure of γ-PGA (Fang et al. 2020a)

PGA using a solid-state bioreactor and unscalable up to 50 L. Recently, Fang et al. (2020a) demonstrated large-scale production of PGA using AW at 50 L–150 L.

1.3.4 Polylactic Acid (PLA)

PLA is a well-recognized thermo plastic polyester, which has the biological properties such as processable, biocompatible, and biodegradable. PLA is an aliphatic semi-crystalline polyester produced during lactic acid polymerization or monomer or ring-opening polymerization of cyclic lactide dimer (Nofar et al. 2019). Lactic acid has diverse enantiomeric forms as L-lactic acid, D-lactic acid, and meso-lactic acid (mixture of L- and D-lactic acid) (Rivero et al. 2017). Figure 1.6 showed that L-lactic acid, D-lactic acid, and their mixture lead to different PLAs, as poly(L-lactic acid) (PLLA), poly(D-lactic acid) (PDLA), and poly(L- and D-lactic acid) (PDLLA) (Inkinen et al. 2011). The numerous significant properties of PLA such as the degree of crystallization as well as thermal properties are influenced by percentage of D- to L-optical isomer of PLA. PLA shows attractive physicochemical properties; these are higher modulus of rigidity, great strength, transparency, and permeability properties (Nofar et al. 2019).

1.4 Biosynthesis and Production of Biopolymers by AWs

Biopolymers synthesized and produced from different types of microorganisms using various feedstocks such as pure substrate and organic matter. Different microorganism accumulate biopolymers as a storage granule of various sizes

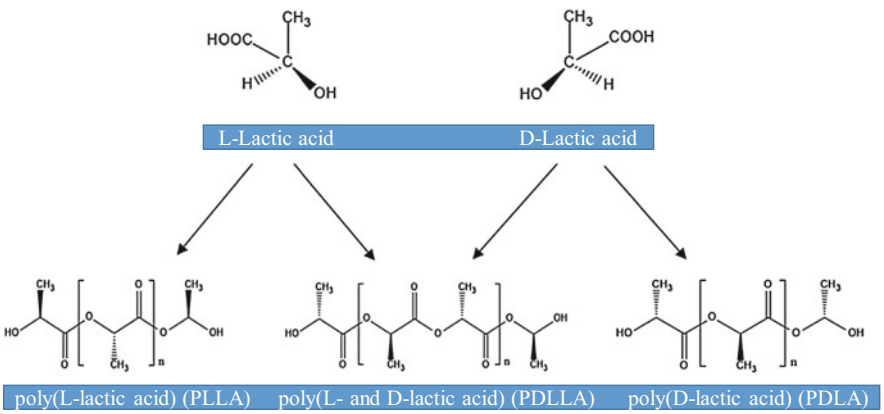

Fig. 1.6 Stereo forms of PLA (Rivero et al. 2017)

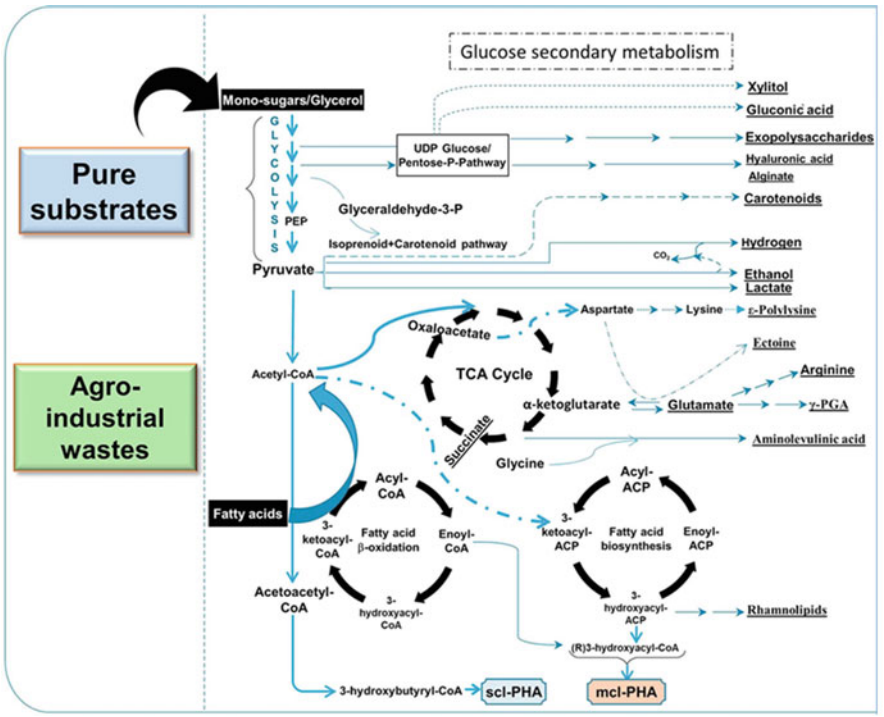

Fig. 1.7 Biosynthesis pathways for PHA production from pure substrate and AW

(0.2–0.5 mm diameter) that can be originate in the cytoplasm. These biopolymers are insoluble in water. The biosynthesis synthesis pathway for PHA production is reported in Fig. 1.7 using pure substrate and AW. Kumar and Kim (2018) reported

that fatty acids (FA) are main precursors for PHA biosynthesis that activates via β-oxidation and de novo synthesis pathway as shown in Fig. 1.7.

Furthermore, PHA biosynthetic pathway is related to carbon utilization pathways including acetyl-CoA, therefore it could be compatible for tuning other metabolic pathways for co-production of other valuable products. However, around 300 species reported for PHA production using different carbon sources under different operative conditions. Among, gram-negative bacteria, including *Azotobacter vinelandii*, or recombinant *Escherichia coli* and *Alcaligenes latus*, *Cupriavidus necator*, *Pseudomonas putida*, and *P. oleovorans*, are widely reported for PHA production. While gram positive bacteria belong to *Bacillus* genus including *B. cereus, B. megaterium, B. licheniformis*, and *B. subtilis* species, which are highly reported for PHA production.

Kumar and Kim (2018) reviewed literature for production of PHA with co-production of other bioproducts as shown in Table 1.5. Several types of bacterial strains can produce different biopolymers with co-production of other bioproducts. Biopolymers synthetic pathway of PHA production involves acetyl-CoA, which linking the pathways for co-synthesis of other high-value bioproducts. During the co-production of various bioproducts under optimized conditions, can reduce the cost of biopolymers production. Furthermore, several dark-fermentative and photosynthetic bacteria species, i.e. *Bacillus* sp., *Rhodospirillum rubrum*, *Rhodopseudomonas palustris*, *Rhodobacter sphaeroides* can produce biopolymers and biohydrogen under optimum conditions using different feedstocks (Arumugam et al. 2014; Kumar et al. 2014, 2015a, b; Montiel-Corona et al. 2015, 2017; Kumar and Kim 2018). While the photosynthetic organisms widely study for co-production of H_2 and PHA. Padovani et al. (2016) explored the potential of olive mill wastewater for co-synthesis of biopolymer and biohydrogen using *R. palustris*. Montiel-Corona et al. (2017) reported that *R. capsulatus* strain reduced biohydrogen production by 20% during light–dark cycles of 30:30 min, while PHB production increased by 3-folds (308 ± 2 mg PHB g dw^{-1}) as compared to 24 h light illumination and reduces the chemical oxygen demand. Patel and co-workers reported that several *Bacillus* sp. such as *B. cereus, B. thuringiensis, B. tequilensis* can produce bioH_2 and biopolymers using integrative bioprocesses using AW (Patel et al. 2015). Result showed that amalgamation of bioprocesses for producing bioH_2 followed by biopolymer could suggestively decrease the price of multi-step resource recovery approach. In addition, coupling with anaerobic digestion (AD) process could further reduce the cost of process and deliver a sustainable process.

Table 1.5 Production of biopolymers with co-production of various high-value products (Kumar and Kim 2018)

Microbial strain	Type of biopolymers	Co-product	Yield of PHA	Yield of co-product
A. beijerinckii	PHB	EPS	2.73 g/L	1.5 g/L
A.r chroococcum	PHB	EPS	0.75 g/L	0.6 g/L
Anabaena cylindrica	PHBV	EPS	0.01 g/L	0.32 g/L
Bacillus sp. CFR-67	PHBV	α-Amylase	5.9 g/L	2.0–40.0 U/mL/min
Bacillus sp. CFR-67	PHBV	α-Amylase	0.5 g/L	73.0 U/mL
B. sacchari DSM17165	PHB	Xylitol	22%	17 g/L
B. subtilis OK2 (recombinant)	PHB	Polyglutamic acid	1.0 g/L	0.4 g/L
B. thuringiensis IAM 12077	PHB	α-Amylase	29–51%	0.2–10 U/mL
B. licheniformis PL26	PHBV	ε-Polylysine	64.6%	0.2 g/L
Burkholderia thailandensis	PHB	Rhamnolipids	60.0%	2.2 g/L
Corynebacterium glutamicum	PHB	L-glutamate	7.0 g/L	18.0 g/L
C. crenatum SYPA5	PHB	L-arginine	15.7%	41.1 g/L
C. glutamicum 9114	PHB	L-glutamate	12.1%	17.2 g/L
Cupriavidus sp. USMAHM13	PHA	Yellow pigment	49% (2.94 g/L)	–
E. coli	PHB	Recombinant human tissue	23%	53.8 μg/g
E. coli	PHB	L-tryptophan	9.7%	14.4 g/L
E. coli	PHB	5-aminolevulinic acid	43.0%	1.6 g/L
E. coli	PHB	5-aminolevulinic acid	38.2%	3.2 g/L
E. coli	PHBV	5-aminolevulinic acid	38.9%	3.0 g/L
E. coli KNSP1	PHA	Succinate	5.62%	21.07 g/L
E. coli QZ1112	PHB	Succinate	4Table %	24.6 g/L
Halomonas TD01	PHB	5-aminolevulinic acid	22.0%	0.6 g/L
H. elongata	PHB	Ectoines	55.0%	14.0%
H. salina DSM 5928	PHB	Ectoines	6.0 g/L	2.9 g/L
H. salina DSM 5928	PHB	Ectoines	35.3 g/L	8.6 g/L
H. boliviensis LC1T	PHB	Ectoines	68.5%	4.3 g/L (7.2%)
Methylosinus trichosporium IMV3011	PHB	Methanol	38.6%	0.03 mmol/g dry cell
P. aeruginosa 7a	PHA	Rhamnolipids	50.4%	0.273 g/L
P. aeruginosa ATCC 10145	PHA	Rhamnolipids	7.5%	0.61 g/L

(continued)

Table 1.5 (continued)

Microbial strain	Type of biopolymers	Co-product	Yield of PHA	Yield of co-product
P. aeruginosa IFO3924	PHB	Rhamnolipids	55.0%	0.420 g/L
P. aeruginosa IFO3924	PHA	Rhamnolipids	36.0%	0.43 g/L
P. aeruginosa L2–1	PHA	Rhamnolipids	17.6%	0.3 g/L
P. aeruginosa L2–1	PHA	Rhamnolipids	39%	0.6 g/L
P. aeruginosa L2–1	PHA	Rhamnolipids	4.6%	0.248 g/L
P. aeruginosa PAO1	PHB	Rhamnolipids	0.15 g/L	0.454 g/L
P. aeruginosa UMTKB-5	PHB	Rhamnolipids	24.0%	0.05 g/L
P. mediterranea CFBP5447	PHA	Alginate	0.52 g/L	6.93 g/L
P. mendocina	PHA	Alginate	0.316 g/L	0.57 g/L
P. putida KT2440 (WT)	PHA	Gluconate	25.4% (0.90 g/L)	4.4 g/L
Paracoccus sp. LL1	PHBV	Astaxanthin rich carotenoids	39.3% (9.52 g/L)	7.14 mg/L
Paracoccus sp. LL1	PHBV	Astaxanthin rich carotenoids	40.64%	0.82 mg/L
R. eutropha	PHB	EPS	12.0 g/L	0.13 g/L
Rhodobacter sphaeroides O.U.001	PHB	Carotenoids	60 mg/L	40 mg carotenoids/L
Rummeliibacillus pycnus strain TS8	PHA	Lipids	46.9%	39.8%
Saccharomyces cerevisiae D603	PHB	Ethanol	3.5%	4.5 g/L
Sinorhizobium meliloti MTCC100	PHA	EPS	11.8 g/L	3.6 g/L
Sphingomonas sanxanigenens NX02	PHA	Sphingan	6.08 g/L	14.88 g/L
Thermus thermophilus HB8	PHA	Rhamnolipids	34.8%	0.2 g/L.

1.5 Market and Application of Biopolymers

Biopolymers can be considered as the strong candidate to replace petroleum-based polymers for extensive application in various sectors. Biopolymers have wide range application in different sectors based on mechanical properties of biopolymers as shown in Fig. 1.8 (Nofar et al. 2019). However, applications of biopolymers are limited due to great crystallinity and breakability; these properties can decrease its elasticity and ductility. However, the crystallinity and brittleness of biopolymers can be altered through blending or chemical alteration methods (Rodriguez-Contreras 2019). Commonly, biopolymers are used in packaging, textile and fiber, construction and automotive sector (Garlotta 2001; Auras et al. 2004; Nofar and Park 2014; Nofar et al. 2019). Furthermore, the biological properties such as non-toxicity, and biodegradability and biocompatibility of biopolymers make attractive for application

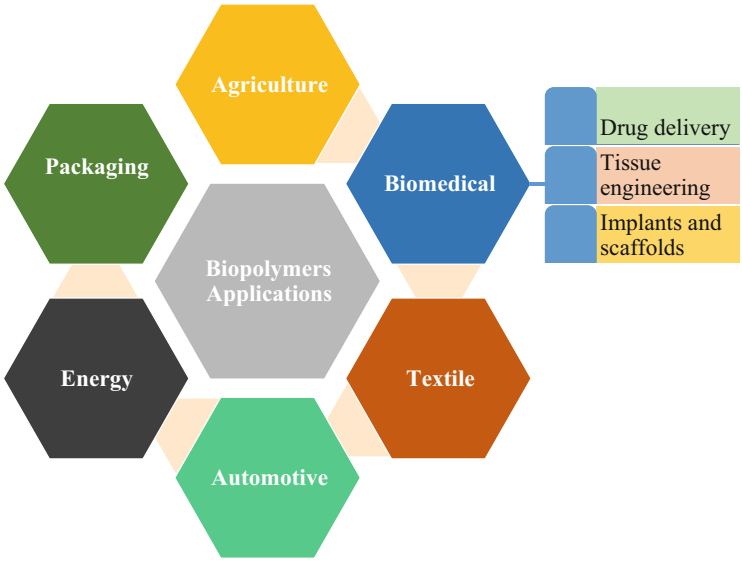

Fig. 1.8 Application of biopolymers in different sectors

in biomedical sector for drug delivery, blood vessels, tissue engineering, and scaffolds. Furthermore, the scaffolds are used for bone regeneration system, cardiovascular as well as cartilage support system and nerve regeneration system (Saini et al. 2016).

Biopolymers have several biomedical applications like absorbable nerve guides, surgical sutures, surgical meshes, thermogels for controlled-release drug delivery vehicle, wound dressing (Rodriguez-Contreras 2019). Rodriguez-Contreras (2019) summarizes the application of biopolymers (PHA) in biomedical sector and reported that PHAs are used for cardiovascular patches, cartilage, stents for nerve repair, sutures, valves for implants, bone graft substitutes for tissue engineering. PHA considered as potential material for drug carriers for delivery systems due to biodegradability without toxic effect.

Sadat-Shojai et al. (2016) attempted to develop a practical approach for development of ultrathin fibers for application in tissue engineering. Thus, innovative advance scaffolding structure using an electrospinning fiber production method through electric force with hydroxyapatite and biopolymer can be used in tissue engineering. In this system a protein-based hydrogel in a single three-layered scaffold was developed for various types of medical application as shown in Fig. 1.9.

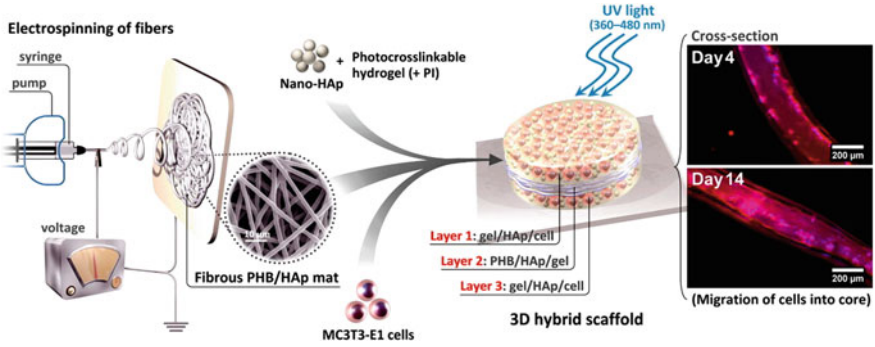

Fig. 1.9 Schematic overview of 3D cell-laden hybrid scaffolds for medical application (Sadat-Shojai et al. 2016)

1.6 Future Research Direction and Concluding Remark

AWs could be major feedstock for production of biopolymers and appropriate exploitation of AWs could lead to sustainable rural development under circular loop. The recent development for AW based biopolymers production can help in the green development of rural areas, which can stabilize the rural economy. The utilization of AWs as resources for biopolymer production can minimize the biopolymers production cost as well as slash the cost for waste treatment. The integration of biopolymer and biofuel production approach can increase the sustainability of process and co-synthesis of high-value products can cover the cost of biopolymer production as recommended by Kumar et al. (2019). Therefore, integration of biorefinery process can result in complete exploitation of AWs for production of several high-value products for profitable venture. In conclusion, this chapter summarizes the potential of AWs for different types of biopolymer production. Also, it summarizes the potential application of biopolymers in different sectors. The key application of biopolymers could be in the packaging and medical sector due to non-toxic, biodegradable, and biocompatible properties.

Competing Interests All the authors declare that they have no competing interests.

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Chapter 2
A Biorefinery Based Zero-Waste Utilization of Non-edible Oilseeds for Biodiesel and Biofuel Production Along with Chemicals and Biomaterials

Debashis Sut and Rupam Kataki

Abstract Non-edible oilseed yielding plant species comprises a huge volume of biota that produces non-edible oils of lesser economical importance. However, owing to the recent global thrust on renewable energy generation including bioenergy and biofuels, and in view of the emphasis on non-food resources for utilization as feedstock for biofuels, non-edible oilseeds have drawn the attention of the researchers all around the globe. Non-edible oilseed bearing tree species comprises a number of species mostly found to be scattered in different agro-ecological regions, viz. forests, non-forest areas, wasteland, deserts, and hilly areas throughout the world including India. Northeast India being one of the world's biodiversity hotspots has been reported to harbor a large number of the group. The extractable non-edible oil, depending on its chemical characteristics, can be utilized for biodiesel production following various standardized techniques. However, the biodiesel production process also produces a huge amount of biowastes in the form of seed covers and de-oiled seed cakes, which are lignocellulosic in nature. These wastes can be utilized as a feedstock for pyrolytic valorization to bio-oil and biochars. The de-oiled seed cakes can also be utilized for a number of other industrial uses like preparation of improved feed material, mosquito repellents, etc. Further, the biochar generated as a co-product of pyrolysis meets various uses like soil amendments, drinking water filtration, remediation of heavy metal contaminated wastewater, catalyst preparation, to name a few. It is imperative therefore, that using a cascade of approaches, a little used biomass of lesser economical importance could be utilized for a variety of products and services in a biorefinery mode. This chapter discusses in details about the status of non-edible oilseeds yielding plant species and various approaches for exploring the non-edible oilseed with zero wastes and envisions that non-edible oilseed could be utilized as a possible way-out in various useful purposes.

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Keywords Non-edible oilseeds · Biowastes · Biodiesel · Bio-oil · Biochar · Cascade of approaches

Abbreviations

GHGs	Greenhouse gases
FFA	Free fatty acid
NPB	National policy on biofuels

2.1 Introduction

Energy is one of the most essential building blocks for human development and as such acts as a crucial factor in determining the socio-economic as well as human welfare of a country. Assuring everyone has sufficient access to continuous energy to sustain a high living standard is an ongoing and pressing challenge for global development. Fossil fuels like natural gas, coal, and oil were a prime mover of the industrial revolution and the social, economic, and technological development that had followed. Modern transportation systems are almost entirely dependent on the use of fossil fuels. Recently a survey conducted on the global energy consumption emphasizes that more than 80% of the consumed primary energy is obtained from the combustion of fossil fuels, of which about 60% is consumed in the transportation sector (International Energy Agency 2019a). Thus, fossil fuels take a significant part in the progress of modern society. The vulnerabilities of the present energy system largely originated from its reliance mostly on fossil fuels: non-renewable, limited, and exhausting resources that, when burned, emit environment-impacting greenhouse gases. The dispute of environmental changes suggests a drastic change in global energy sources—a move that would be immensely demanding and remarkable and that would also involve a huge amount of funding. These two challenges, depletion of fossil fuel and climate change, necessitate action for the world to evade economic as well as environmental adversity. Thus, a move away from the existing dependence on fossil fuels has become essential and inexorable, and the shift towards renewable energy provides a realistic opportunity.

Renewable energy sources are the everlasting sources of energy that swiftly regenerates itself and does not emit noxious elements into the atmosphere that could result in pollution. Apart from geothermal and tidal energy, most of the renewable forms of energy are eventually derived from the sun, i.e. the solar energy. It is estimated that about 18.1 % of total global primary energy consumption has been contributed by renewable energy (REN21 2019). In spite of the advancement in renewables intake, energy access, and efficiency, the world is yet to achieve the Sustainable Development Goal 7 or the targets of the Paris agreement. The world CO_2 emissions based on energy consumption soared by about 1.7% in 2018 owing to an increase in fossil fuel consumption (Global Energy and CO_2 Status Report,

International Energy Agency 2019b). The changing environment threatens the ongoing global development driven by the imprudent exploitation of fossil fuels. The expeditious replenishment of fossil fuels by renewable energy is considered as the most suitable approach towards alleviating the changes in the environment.

The contribution of renewable energy sources to the total final energy consumption is a crucial aspect in ascertaining the progress of renewables worldwide. According to Global Bioenergy Statistics 2018, approximately 81% of the world's total primary energy in 2016 was supplied by natural gas, oil, and coal, whereas renewable sources contributed about 18%. Among the renewables, biomass is the largest contributor accounting for 13% of the world's primary energy supply, while hydropower contributed about 3% and the renewables such as solar, wind, tidal, geothermal, etc. accounted for about 2%. Moreover, biomass resources are the highest contributor to the total primary energy with approximately 56.5 EJ—accounting for about 70% of the total energy supplied by all the renewable sources, whereas hydropower contributed about 18% of the renewables energy share. In addition, the biomass resources also have a vital part in terms of primary energy supply in the continents. In Africa, among the renewable energy sources, biomass contributed about 90% of the total primary energy supply. In other continents also biomass (both traditional and modern) is the largest contributor of primary energy among the renewables (World Bioenergy Association 2019).

At present, biomass is one of the predominant renewable energy sources with various usages such as heat, power, and transportation fuels. It is the biological matter derived from living organisms. Every year, plants convert 120 gigatons of atmospheric carbon and 4500 EJ of solar energy into biomass which is approximately eight times the global energy demand (global biomass potential towards 2035, WBA Factsheet 2019). Animals and microorganisms disintegrate a major portion of the plant biomass into water and CO_2 as a part of the natural carbon cycle, while the remaining of the biomass can be utilized to fulfill the human demands. Biomass and biomass energy can provide a solution in terms of the diversity of products that can be produced by employing modern technologies and replace conventional sources such as coal and petroleum with large social and environmental benefits. Biomass resources and their utilization offer a new paradigm for research in solving the various problems emerging from the excessive use of fossil fuel. The term "biomass" includes forestry, plants and trees, agricultural crops, and wastes such as agricultural, organic, agro-industrial, domestic (solid and municipal) wastes. Biomass can be used directly (for cooking and heating) or indirectly by transforming it into other energy forms like gaseous or liquid fuel like biogas, biodiesel, bio-oil, bioethanol, etc.

In India, biomass resources have always been regarded as an essential source of energy considering the advantages it provides for the generation of power and cooking gas and other useful recycled products. India has an enormous potential of biomass which could be easily depended upon to satisfy most of the energy demands. In India about 0.05 billion metric tons of fossil fuels (liquid) are consumed every year (Bhuyan et al. 2019). However, India got the ability to produce nearly twice that quantity per year with the available biomass potential and its

comprehensive application. These estimates of biomass potential in the country include the existing crop residues only and are basically second-generation feedstock since the application of crops or first-generation feedstock is strictly prohibited in India. Apart from crop residue biomass, a huge amount of other biomass and biowastes generated in the country remain unutilized or underutilized. Among these, non-edible oilseed bearing tree species is one such resource which produces non-edible oils. Overall, the available biomass for energy production can be grouped into three classes: (1) lignocellulosic biomass which includes agricultural residues, forestry residues, agro-industrial wastes, weed biomass, etc., (2) starch/sugar based biomass like corn, sugarcane, and sweet sorghum, and (3) lipids or oil crops which include edible and non-edible oilseeds, algae, animal fat, etc.

Currently, there are various technological options to process the above mentioned biomasses and generate energy and other valuable products. Lignocellulosic biomass can be easily utilized as feedstocks for the thermochemical conversion process with suitable pretreatment. Starch/sugar crops can undergo saccharification to yield fermentable sugars for the production of ethanol and butanol, while lipid or oil crops are generally used for transesterification to produce biodiesel. All these biomass conversion processes are generally centered around the production of heat and or electricity and biofuel, and many of these technologies also produce wastes in the process. The wastes so generated in the conversion processes are left with numerous chemical groups that could be utilized for the production of numerous valuable products. In fact, transforming these wastes to different value added products can have multiple benefits, viz. (1) complete utilization of the feedstock leading to an economy of scale, (2) abatement of GHG emission from dumping, (3) contribution to circular bioeconomy. In this regard, the concept of biorefinery takes the center stage of bioeconomy as biorefinery intends to produce not only fuels, heat, and or power but also chemicals and materials.

2.1.1 The Biorefinery Approach

The quest to attain energy security, environmental sustainability, and to realize the never-ending demand of feed/fodder and chemicals has emerged as noteworthy factors for the biorefinery industry. The biorefinery concept is akin to the traditional petroleum refineries which include the assimilation of different processing techniques and biomass treatments into a single arrangement, and as such leading to the generation of several compounds from the same parent biomass. According to the bioenergy task document (No. 42) of the International Energy Agency (IEA), a biorefinery can be interpreted as the continuous treatment of biomass to energy and wide range of profitable assets (Heo et al. 2019). Therefore, the biorefinery concept envisions to extract maximum utility from a given kind of biomass for the reduction of waste generation and its environmental release. It also focuses on improving the economy of biowastes produced in the whole approach. In addition, the biorefineries have been visualized as one of the potential candidates for realizing the never-ending

demand for energy, fuel, chemicals, and materials and also as a medium for quelling the impacts of man-induced climate changes. This makes the whole chain of various processes more feasible economically besides minimizing the generation of biowastes. The products vary from low-energy but high-volume liquid fuels that could assist the requirements of the transport industry to the high-value yet low-volume chemicals that could be an addition to the viability of the project (Fernando et al. 2006). Heat and steam generated in the process could be applied to meet the heat requirements of the process. The generated by-products like polymers, chemicals, pharmaceuticals, fertilizers, etc. contribute further to the added revenue streams. Thus, biorefineries can bolster the maximum exploitation of organic wastes and as such may help to overcome the environmental concerns related to greenhouse gases (GHGs) emissions and waste management. Through appropriate conversion technologies wastes can be converted into gaseous or liquid fuels. Even though the technology is still in nascent stage, it is very much essential for the optimum utilization of natural resources and wastes which mankind have always endeavored to accomplish (Kumar and Singh 2019).

2.1.2 Classification of Biorefineries

Depending on various process parameters, the biorefineries can be classified as given below (de Jong and Jungmeier 2015):

- *Raw materials applied*: Lignocellulosic feedstock biorefineries, marine biorefineries, oleo-chemical biorefineries, whole-crop biorefineries, and green biorefineries.
- *Conversion methods adopted*: Two-platform concept biorefineries, biochemical biorefineries, and thermochemical biorefineries.
- *Technology adopted*: Advanced and conventional biorefineries; first-, second-, and third-generation biorefineries.
- *Intermediates generated*: Sugar and syngas platform biorefineries.

During the transesterification process using non-edible oilseeds, it also produces huge amounts of biowastes in the form of seed cover and de-oiled seed cake, which are lignocellulosic in nature. The biorefinery approach for biodiesel production from non-edible oilseed by integrating various processes to generate various high-value products from the biowastes can lower the present high production cost of biodiesel compared to petroleum fuels and the first-generation biofuels. It is projected that the biorefinery approach would (1) enhance the overall economy of biodiesel generation, (2) generate biofuel and chemical, (3) improve the sustainability and viability of the biodiesel production, (4) minimize the emissions of greenhouse gases, and (5) generate prospects of employment and (6) overall impact on bioeconomy. Thus, accommodating various biodiesel amenities into the biorefinery concept, the feasibility and sustainability are assured in addition to the efficient energy utilization, minimizing the waste generation and environmental benefits (Ahmad et al. 2019).

2.1.3 Biodiesel: A Potential Alternative Fuel

Biodiesel, regarded as a substitute to diesel, is derived from the biological renewable sources like animal fats or vegetable oils. Biodiesel is drawing increasing worldwide attention as a direct replacement or blending component for diesel due to its biodegradable, non-toxic, and environmentally benign nature. Generally, the methods used for producing biodiesel can be categorized into four classes, namely micro-emulsions, thermal cracking, direct use and blending, and transesterification (Demirbas 2009). However, transesterification is considered as the most distinguished method for biodiesel production as it reduces the viscosity of biodiesel to make it comparable with diesel and thus improving the combustion quality of biodiesel. In transesterification reaction, alcohol (preferably methanol and ethanol) reacts with triglycerides to form biodiesel and glycerol which is produced as a by-product. Owing to its lower cost, methanol is preferred over other alcohols (Ma and Hanna 1999).

Biodiesel is regarded as the most suitable substitute for conventional diesel in a CI engine. It is non-inflammable and non-explosive since the flash point is very high. Moreover, biodiesel is biodegradable and significantly decreases the noxious and other harmful emissions when used as fuel (Demirbas 2009). Biodiesel has better lubricating properties compared to the conventional diesel (approximately 66% more than diesel) and thus increases the lubricating properties of the biodiesel-diesel blend (Demirbas 2008). It also reduces the long term engine wear and tear of the diesel engine. In addition, biodiesel can present other advantages like reduction in greenhouse gas emissions, developing social and regional structures, particularly in the developing countries. Biodiesel, a renewable fuel, with lower harmful gas emissions is the demand of the current world energy structure. A pure biodiesel or 100 % biodiesel is indicated as B100, while a biodiesel blend denotes the biodiesel blended with conventional diesel and depicted as BXX. The amount of biodiesel present in the biodiesel-diesel blend (percentage composition) is denoted by XX (e.g. a B10 blend indicates 90% diesel and 10% biodiesel in the blend) (Demirbas 2007).

However, biodiesel has some limitations such as low-energy content or low heating value, problems of fuel pumping owing to high viscosity, cold start problems due to poorer cold flow properties and high copper strip corrosion, etc. But, the major hindrance in the widespread utilization of biodiesel is the price associated with its production that is 1.5–3 times more compared to the diesel fuel (Bala 2005). At present the global production of animal fats and vegetable oils is not sufficient enough to substitute the usage of fossil fuel. As a result, the availability of quality and low price raw materials is indispensable for the biodiesel production.

2.1.4 Biodiesel Feedstock

More than 400 oil-bearing tree species available worldwide are recognized as the prospective feedstocks for the production of biodiesel (Government of India, Ministry of Petroleum and Natural Gas 2019). The existence of feedstocks for biodiesel production relies on various factors like agricultural practices prevalent in the region, local soil conditions, geographical locations, and regional climate. It is reported that the raw material alone contributed about 60–80% of the overall cost of biodiesel production (Atadashi et al. 2012a, b; Shikha and Rita 2012). Hence, choosing the low-cost feedstock is crucial to assure the reduced price for biodiesel generation. Overall, biodiesel feedstocks can be generally categorized into four classes as given below:

1. Edible oil or first-generation feedstock: this includes coconut oil, palm, rapeseed, soybean, peanut, sunflower, etc.
2. Non-edible oil or second-generation feedstock: karanja, nahar, karabi, jatropha, etc.
3. Third-generation feedstock or algae.
4. Others include animal fats (e.g. chicken fat, tallow, etc.), by-products of fish oil, recycled or waste oil, yellow grease, etc.

2.2 Status of Non-edible Oilseed Bearing Trees

Depending on availability, numerous oils have been used as raw materials for biodiesel production in countries throughout the world. For example, in the US soybean oil is predominantly utilized as a feedstock for biodiesel production. Similarly, rapeseed oil in Europe, coconut and palm oils in Indonesia and Malaysia are used for biodiesel production. In Southeast Asia and India, several non-edible oilseeds of plant origin are utilized as feedstocks for the generation of biodiesel (Atabani et al. 2012). Recently algae, an aquatic plant, is gaining importance as a prospective raw material for the generation of biodiesel owing to high oil content. Biodiesel from waste cooking oil or recycled oil has been gaining prominence due to its low cost compared to fresh vegetable oil.

2.2.1 The Global Scenario

In order to minimize the reliance on edible oil for the production of biodiesel, non-edible oil has been explored comprehensively over the past few years. The utilization of non-edible oilseed for biodiesel production in the developing countries is notable owing to the immense demand of edible oils as foodstuff. Moreover, the cost of edible oil is very high in order to be used as feedstock for generating

biodiesel. In contrast, the non-edible oils are not fit for human intake owing to the presence of various noxious elements in them. Besides, these can be raised in arid or barren lands, and the cost of cultivation is very low compared to that of edible oil plants. Also, intensive care is not necessary to maintain a reasonably high yield (Atabani et al. 2013; Banković-Ilić et al. 2012). Globally, there exists quite a good number of non-edible oil yielding plants naturally which are explored for biodiesel production (Table 2.1).

2.2.2 An Indian Perspective with Special Reference to NE India

In an agriculture based economy like India, the use of non-edible oilseed for generating biodiesel could become a significant platform for mitigating poverty besides offering energy security and improving the rural non-agricultural sector. Moreover, the feedstocks have the potential to restore degraded lands and reduce CO_2 emissions while overcoming the food versus fuel crisis (Banković-Ilić et al. 2012). This is significant as these resources (non-edible oilseeds) are otherwise treated as low-value products or biowastes owing to their toxic nature. The north-eastern (NE) part of India is renowned for its high biological and ethnic diversity. It includes two geographical zones, namely northeast India which includes Assam, Nagaland, Manipur, Tripura, Meghalaya, Sikkim, and Mizoram and Eastern Himalayas embracing Arunachal Pradesh. The region is recently acknowledged as one of the world biodiversity hotspots and considered as a significant region of the Indo-Myanmar biodiversity hotspot (Chakraborty et al. 2012; Sut and Kataki 2014). The states of Arunachal Pradesh (91.20°/E to 97.30°E and 26.28°/N to 29.30°/N) and Assam (24°30/N to 28°10/N and 89°50/E to 96°10/E) are among the richest biodiversity regions of India. In Assam about one-third of its area (78,438 km^2) is designated as forest area. Apart from tropical rainforests there are numerous orchards, bamboo, revering grasslands, and wetland ecosystems available in Assam. Most of these areas have been preserved by establishing Reserve Forests, Wildlife Sanctuaries, and National Parks. The state is also home to various faunas and floras, and vast forest areas enrich its biological diversity. Likewise, Arunachal Pradesh earns a lion's share of its total revenue from its vast area of forest resources (about 51,540 km^2). There are about 700 wood species that grow naturally in these hilly terrains. The forests of Arunachal Pradesh are generally exploited for timber. However, destruction of the forest cover owing to various human activities such as excessive exploitation, urbanization, agriculture, etc. forest area is depleting and timber becomes limited. Consequently, the minor forest products such as non-edible tree borne oilseeds, medicinal plants, bamboos, cane, spices, gums, resins are gaining importance (Basumatary 2013).

It has been found that most of the forest trees produce a large amount of seeds, a bulk of which are wasted as unutilized. The left-over seeds if utilized suitably would

Table 2.1 Some of the typical non-edible oilseeds used for biodiesel production

Non-edible feedstocks Botanical name	Vernacular name	References
Annona diversifolia	Ilama	Reyes-Trejo et al. (2014)
Argemone mexicana	Mexican poppy	Azam et al. (2005)
Aphanamixis polystachya	Pithraj tree	Palash et al. (2015)
Azadirachta indica	Neem	Betiku et al. (2014)
Ailanthus altissima	Tree of heaven	Hoseini et al. (2018)
Brucea javanica	Kosam	Hasni et al. (2017)
Brassica carinata	Ethiopian mustard	Cardone et al. (2003)
Cascabela ovata	Lucky nut	Sánchez-Arreola et al. (2019)
Camelina sativa	Camelina	Ciubota-Rosie et al. (2013)
Citrullus colocynthis	Thumba	Chavan et al. (2014)
Euphorbia lathyris	Paper spurge	Wang et al. (2011)
Euonymus maackii	Himalayan spindle	Liu et al. (2019)
Guizotia abyssinica	Niger	Sarin et al. (2009)
Garcinia indica	Kokum	Hosamani et al. (2009)
Carica papaya	Papaya	Anwar et al. (2018)
Moringa oleifera	Moringa	Rashid et al. (2008)
Jatropha curcas	Jatropha	Wang et al. (2011)
Hevea brasiliensis	Rubber seed	Roschat et al. (2017)
Portulaca oleracea	Common purslane	Hoseini et al. (2019)
Gossypium hirsutum	Cottonseed	Karmakar et al. 2010
Nicotiana tabacum	Tobacco	García-Martínez et al. (2017)
Mesua ferrea	Nahor	Aslam et al. (2014)
Terminalia catappa	Indian almond	Dos Santos et al. (2008)
Ricinus communis	Castor	Özcanlı et al. (2010)
Raphanus sativus L	Forage turnip	Faria et al. (2018)
Simarouba glauca	Paradise tree	Jeyalakshmi (2019)
Schleichera triguga	Kusum	Sarve et al. (2015)
Simmondsia chinensis	Jojoba	Shah et al. (2010)
Terminalia bellirica	Bahera	Chakraborty et al. (2009)
Sterculia foetida	Wild almond	Ong et al. (2013)
Sapium sebiferum	Chinese tallow	Wang et al. (2011)
Calophyllum inophyllum	Indian laurel	Atabani and da Silva César (2014)
Shorea robusta	Sal	Pali and Kumar (2014)
Sapindus mukorossi	Soapnut	Chakraborty and Baruah (2013)
Madhuca indica	Mahua	Ghadge and Raheman (2005)
Cerbera odollam	Sea mango	Kansedo and Lee (2013)
Pongamia pinnata	Karanja	Kumar and Sharma (2011)
Thevetia peruviana	Yellow oleander	Deka and Basumatary (2011)
Melia azedarach	Indian lilac	Kumar and Sharma (2011)
Asclepias syriaca	Milkweed	Holser and Harry-O'Kuru (2006)
Putranjiva roxburghii	Putranjiva	Kumar and Sharma (2011)

generate significant revenues and help in alleviating poverty. The non-edible oilseeds can be used as prospective feedstocks for generating biodiesel. It is reported that in India about 400 non-edible oilseeds yielding plants are known that can be explored as raw materials for biodiesel production or in various industries such as varnish, candles, paints, cosmetics, lubricants, etc. Unfortunately, only a small portion of the total available oilseeds are exploited owing to numerous limitations related to transportation, collection, accessibility, extraction, etc. In addition, there exists some lacuna in awareness about the availability of various tree species in the NE region particularly in Arunachal Pradesh and Assam. Some of these trees have the prospects to emerge as a better feedstock for biodiesel production than the already known ones if properly utilized and hence need to be exploited as potential sources of bioenergy and biofuel. Some of the non-edible oilseeds easily available in NE region and having potential as raw materials for biodiesel production are presented in Table 2.2.

2.3 Chemical Constituents of the Non-edible Oils

The fuel quality is one of the major concerns in the area of biodiesel research. The most important compositional difference between diesel and biodiesel is the amount of oxygen present in the fuel. Biodiesel comprises of 10–12% (wt.%) oxygen that reduces its heating value as well as lowers the emission of particulate matters (Banković-Ilić et al. 2012). Biodiesel characteristics are controlled significantly by the fatty acid composition of the parent oil. A given fatty acid is characterized by the length of its chain and the saturation, i.e. the number of double and triple bonds. The properties that are influenced significantly by the fatty acid profile of the parent oil include cetane number, viscosity, density, calorific value, cold filter plugging point, cloud and pour point, etc. It has been reported that with the growth in saturation level and chain length, the density, viscosity, and calorific value also increases. Moreover, cetane number and heating value also rise with chain length and decreasing branching and unsaturation. The fatty acid profile of the parent oil also affected the cold flow properties like cloud point, pour point, cold filter plugging point, etc. Besides, the freezing point decreases with an increase in double bonds while increases with the increasing number of carbon atoms (Ashraful et al. 2014; Atabani et al. 2013, 2012).

A large number of non-edible oilseeds bearing plant species have been available globally and are utilized for generating biodiesel. Some of the promising non-edible oilseeds yielding plants utilized globally for the biodiesel production are listed in Table 2.3 with their fatty acid profile and oil content. The physico-chemical properties of the respective biodiesel obtained from the non-edible oilseeds are presented in Table 2.4.

Table 2.2 Potential non-edible oilseeds yielding plant species available in NE India (Mohan and Deori 1990; Kotoky et al. 2007)

Non-edible feedstock (botanical name)	Local name	Place of origin
Aphanamixis polystachya	Bogamari	Assam
Ailanthus grandis	Borpat, botpat	Arunachal Pradesh
Artocarpus chaplasha	Sam	Arunachal Pradesh
Artocarpus heterophyllus	Kathal	Arunachal Pradesh
Artocarpus lakoocha	Dewa-sali	Arunachal Pradesh
Bombax ceiba	Semul	Arunachal Pradesh
Bischofia javanica	Urium	Arunachal Pradesh
Baccaurea sapida	Leteku	Arunachal Pradesh
Bauhinia variegata	Boga katra	Arunachal Pradesh
Canarium strictum	Dhuna	Arunachal Pradesh
Cedrus deodara	Deodaru	Arunachal Pradesh
Chisocheton paniculatus	Bandordima	Assam, Arunachal Pradesh
Claoxylon khasianum	–	Arunachal Pradesh
Cinnamomum glaucescens	Gonsorai	Assam
Crateva nurvala	Barun	Arunachal Pradesh
Croton caudatus	Ghahe-lewa	Arunachal Pradesh
Croton oblongifolius	Mahunda	Arunachal Pradesh
Croton tiglium	Jamalgota	Arunachal Pradesh
Elaeocarpus aristatus	Gaharisopa	Assam, Arunachal Pradesh
Endospermum chinense	Phulgamari	Arunachal Pradesh
Garcinia stipulate	–	Arunachal Pradesh
Gironniera cuspidata	–	Arunachal Pradesh
Gmelina arborea	Gamari	Assam, Arunachal Pradesh
Gynocardia odorata	Chaulmugra	Arunachal Pradesh
Knema linifolia	Amool	Arunachal Pradesh
Knema angustifolia	Tezranga	Arunachal Pradesh
Litsea angustifolia	–	Assam, Arunachal Pradesh
Litsea confertiflora	–	Arunachal Pradesh
Litsea cubeba	Mejangkori	Assam, Arunachal Pradesh
Litsea glutinosa	Baghnola	Assam, Arunachal Pradesh
Litsea laeta	Bon sualu	Assam
Litsea lanuginosa	–	Assam, Arunachal Pradesh
Magnolia griffithii	Gahorisopa	Arunachal Pradesh
Mangium chinensis	Bogamarulia	Assam, Arunachal Pradesh
Mallotus albus	Morali	Arunachal Pradesh
Neolitsea cuipala	Phulsopa	Assam
Olia dioica	–	Arunachal Pradesh
Ostodes paniculata	Tasichangne	Arunachal Pradesh
Putranjiva roxburghii	Putranjiva	Arunachal Pradesh
Prunus jenkinsii	Thereju	Assam
Sterculia villosa	Udal	Arunachal Pradesh
Styrax serrulatum	Phulkat	Arunachal Pradesh

(continued)

Table 2.2 (continued)

Non-edible feedstock (botanical name)	Local name	Place of origin
Sapium baccatum	Saleng	Arunachal Pradesh
Talauma hodgsonii	Boranthuri	Arunachal Pradesh
Pterygota alata	Karibadam	Arunachal Pradesh
Vangueria spinosa	Ketkora	Arunachal Pradesh
Vatica lancaefolia	Morhal	Arunachal Pradesh

2.4 The Road Map for Complete Utilization of the Oilseeds

2.4.1 Extraction of Oil and Conversion to Biodiesel

The non-edible oilseeds contain kernel and seed cover. Vegetable oil can be extracted from the seed kernel adopting numerous methods such as Soxhlet extraction, mechanical extraction, supercritical fluid extraction, ultrasonic extraction, etc. However, the Soxhlet extraction method is generally used for obtaining vegetable oil from the seed kernel owing to its low cost, easy operability, higher rate of solvent recovery, and its efficiency. But, the method has some disadvantages such as longer period of extraction and requirement of higher amount of solvent. The oil extraction process can be presented in a flowchart as shown in Fig. 2.1. After extracting vegetable oil from the seed kernel, the available de-oiled seed cake is further explored for converting thermochemically into bio-oil, biochar, and gas through the pyrolysis process.

The vegetable oil obtained after extraction process undergoes esterification or pretreatment and transesterification method for generating biodiesel. The process of converting vegetable oil into biodiesel depends on the acid value or free fatty acid (FFA) of the parent oil. It is reported that oil with acid value less than 1% can be directly transformed to biodiesel by the transesterification method. However, if the parent oil has acid value more than 1%, then a two-step biodiesel production method is followed. In this method, pretreatment or esterification of the parent vegetable oil is done to reduce the acid value followed by transesterification reaction to finally convert the oil into biodiesel.

2.4.1.1 Biodiesel Production Process

Alcoholysis or transesterification reaction can be interpreted as the chemical reaction of triglycerides with alcohol to produce alkyl esters (biodiesel) and glycerol. Three consecutive and reversible reactions form the overall transesterification reaction where triglyceride is transformed into diglycerides, monoglycerides, and eventually biodiesel and glycerol. At each step a mole of ester is liberated (Ma and Hanna 1999; Demirbas 2009; Meher et al. 2006) (Fig. 2.2). Transesterification reaction is catalyzed by either homogenous catalyst (NaOH, KOH, H_2SO_4, HCl, etc.) or heterogeneous catalyst (anion exchange resins, titanium silicates, alkaline earth metal

Table 2.3 Fatty acid composition and oil content of some non-edible oil yielding plant seeds

Botanical name	Local name	Oil content (wt. %)		Fatty acid composition (wt. %)												References
		Seeds	Kernel	$C_{14:0}$	$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	$C_{20:0}$	$C_{20:1}$	$C_{22:0}$	$C_{24:0}$	Others	
Annona diversifolia	Ilama	21	–	–	16.40		5.22	70.42	7.97	–	–	–	–	–	–	Reyes-Trejo et al. (2014)
Aphanamixis polystachya	Pithraj tree	–	35	–	18.4	0.3	11.8	18.3	26.7	23.2	0.5	0.2	–	–	0.6 (C22:1)	Palash et al. (2015)
Argemone mexicana	Mexican poppy	–	–	0.8	14.5		3.8	18.5	61.4	–	1.0	–	–	–	–	Azam et al. (2005)
Azadirachta indica	Neem	20–30	25–45	–	18.1	–	18.1	44.5	18.3	0.2	0.8	–	–	–	–	Betiku et al. (2014)
Ailanthus altissima	Tree of heaven	38	–	–	2.01		0.88	25.53	37.36	0.17	–	–	–	–	34.05	Hoseini et al. (2018)
Brucea javanica	Kosam	–	–	–	6.20		46.93	9.59	23.94		2.09	2.78			2.81	Hasni et al. (2017)
Brassica carinata	Ethiopian mustard	42	2.2–10.8	–	3.1	–	1.0	9.7	16.8	16.6	0.7	–	–	–	42.5 (C22:1)	Cardone et al. (2003)
Calophyllum inophyllum	Indian laurel	65	22	–	14.7	0.3	13.2	46.1	24.7	0.2	0.8	–	–	–	–	Atabani and da Silva César (2014)
Madhuca indica	Mahua	35–50	50	–	16.0–28.2	–	20.0–25.1	41.0–51.0	8.9–13.7	–	0.0–3.3	–	–	–	–	Ghadge and Raheman (2005)
Cascabela ovata	Lucky nut	–	64	–	19.53	0.35	11.50	59.3	6.78	–	1.56	–	–	–	0.58 (C10:0)	Sánchez-Arreola et al. (2019)

(continued)

Table 2.3 (continued)

Botanical name	Local name	Oil content (wt. %)		Fatty acid composition (wt. %)												References
		Seeds	Kernel	$C_{14:0}$	$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	$C_{20:0}$	$C_{20:1}$	$C_{22:0}$	$C_{24:0}$	Others	
Camelina sativa	Camelina	–	–	0.05	5.16	0.04	2.68	15.21	17.90	34.64	1.44	15.14	0.3	0.14	2.17 (C20:2) 2.57 (C22:1) 0.62 (C22:6)	Ciubota-Rosie et al. (2013)
Citrullus colocynthis	Thumba	–	–	0.084	10.07	–	7.89	18.19	56.89	0.2	–	0.277	–	0.189	0.21 (C22:2)	Chavan et al. (2014)
Pongamia pinnata	Karanja	25–50	30–50	–	10.6	–	6.8	49.4	19.0	–	4.1	2.4	5.3	2.4	–	Kumar and Sharma (2011)
Euphorbia lathyris	Paper spurge	–	–		6.8	0.5	1.98	81.46	3.71	2.78		0.5	–	–	0.2 (C22:1)	Wang et al. (2011)
Euonymus maackii	Himalayan spindle	–	–	–	14.5	2.01	3.1	29.8	49.3	–	0.07	0.1	–	–	–	Liu et al. (2019)
Guizotia abyssinica	Niger	50–60		–	9.2	–	10.1	9.0	71.70	–	–	–	–	–	–	Sarin et al. (2009)
Garcinia indica	Kokum	–	–	–	8.0	–	5.5	50.2	26.0	–	–	–	–	–	10.3	Hosamani et al. (2009)
Gossypium hirsutum	Cottonseed	18–25	–	0.4	20	–	2	35	42	0.1–2.1	0.5	–	–	–	–	Karmakar et al. (2010)
Hevea brasiliensis	Rubber seed	40–60	40–50	–	9.1	–	5.6	24.0	46.2	14.2	–	–	–	–	0.9	Roschat et al. (2017)
Jatropha curcas	Jatropha	20–60	40–60	–	13.23	0.85	5.40	41.62	36.99	0.22	–	–	–	–	–	Wang et al. (2011)
Melia azedarach	Indian lilac	10–45	2.8	0.1	8.1	1.5	1.2	20.8	67.7	–	–	–	–	–	–	Kumar and Sharma (2011)

Mesua ferrea	Nahor	58–75	–	0.047	13.635	0.087	13.646	50.713	19.47	0.316	1.3	0.18	0.351	0.186	–	Aslam et al. (2014)
Carica papaya	Papaya	–	–	–	6.07	–	3.13	47.73	37.25	1.78	0.76	–	0.68	–	1.51 (C22:1) 1.09	Anwar et al. (2018)
Nicotiana tabacum	Tobacco	36–41	17	0.20	8.90	–	3.50	14.10	70.10	1.00	0.30	0.15	0.25	–	–	García-Martínez et al. (2017)
Portulaca oleracea	Common purslane	24	–	–	16.4	–	3.6	11.8	34.1	32.4	–	–	–	–	–	Hoseini et al. (2019)
Moringa oleifera	Moringa	33–41	–	–	6.5	–	6.0	72.2	1.0	–	4.0	2.0	7.1	–	1	Rashid et al. (2008)
Putranjiva roxburghii	Putranjiva	41–42	–	0.03	10.23	0.07	10.63	48.65	27.50	0.87	1.05	0.30	0.24	0.31	0.12	Kumar and Sharma (2011)
Ricinus communis	Castor	45–50	–	–	1.71	–	2.04	6.40	7.41	–	–	–	–	–	82.44	Özcanlı et al. (2010)
Raphanus sativus L	Forage turnip	–	–	–	–	3.5	–	31.1	9.2	6	7.8	–	42.4	–	–	Faria et al. (2018)
Simarouba glauca	Paradise tree	55–65	–	–	12.25	–	23.45	58.10	3.10	0.32	–	–	–	–	0.15 (C17:2) 1.37 (C20:2) 0.35 (C22:2)	Jeyalakshmi (2019)
Shorea robusta	Sal	–	–	37.23	21.69	3.26	–	0.59	0.15	–	–	–	–	–	0.58 (C12:0) 36.03 (C15:0) 0.43 (C17:1)	Pali and Kumar (2014)

(continued)

Table 2.3 (continued)

Botanical name	Local name	Oil content (wt. %)		Fatty acid composition (wt. %)												References
		Seeds	Kernel	$C_{14:0}$	$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	$C_{20:0}$	$C_{20:1}$	$C_{22:0}$	$C_{24:0}$	Others	
Cerbera odollam	Sea mango	54	6.4	–	24.86	0.75	5.79	52.82	13.65	0.08	1.09	0.19	0.37	0.16	–	Kansedo and Lee (2013)
Simmondsia chinensis	Jojoba	45–55	–	–	1.2	–	–	10.7	0.01	0.4	0.2	59.5	–	–	12.3 (C22:1) 1.7 (C24:1) 9.1 (C20:4) 3.7 (C22:4)	Shah et al. (2010)
Sapindus mukorossi	Soapnut	51.8	–	–	5.5	0.3	2.3	58.4	5.4	17.1	7.5	–	1	0.2	0.4 (C22:1)	Chakraborty and Baruah (2013)
Sterculia foetida	Wild almond	–	–	0.2	18.4	0.3	7.6	4.0	11.1	0.7	2.3	–	–	–	72.7	Ong et al. (2013)
Sapium sebiferum	Chinese tallow	13-32	53-64	–	8.0	3.71	2.13	13.78	30.71	38.87	–	0.59	–	–	3.21 (C10:2)	Wang et al. (2011)
Schleichera triguga	Kusum	–	55–70	0.31	8.0	–	2.3	42.6	4.5	–	21.3	–	1.5	–	0.31 (C12:0) 15.2 (C20:2) 1.9 (C22:1)	Sarve et al. (2015)
Terminalia bellirica	Bahera	–	43	–	32.8	0.5	6.4	31.3	28.8	–	0.3	–	–	–	–	Chakraborty et al. (2009)

Thevetia peruviana	Yellow oleander	–	60–65	–	23.28	–	10.71	43.72	19.85	–	2.41	–	–	–	–	Deka and Basumatary (2011)
Terminalia catappa	Indian almond	–	–	–	35.0	–	5.0	32.0	28.0	–	–	–	–	–	–	Dos Santos et al. (2008)
Asclepias syriaca	Milkweed	20–25	0.019	–	5.9	6.8	2.3	34.8	48.7	1.2	–	–	–	–		Holser and Harry-O'Kuru (2006)

Table 2.4 Physico-chemical properties of biodiesel produced from some non-edible oilseeds

Botanical name	Local name	Physico-chemical properties										References
		Density (kg/m^3)	Kinematic viscosity (mm^2/s)	Acid value (mgKOH/g)	Heating value (MJ/kg)	Cetane number	Pour pt. (°C)	Cloud pt. (°C)	Flash pt. (°C)	Oxidation stability (h)	Ash content (%)	
Annona diversifolia	Ilama	886	4.451	0.5	36.30	44.70	−9	0	–	–	–	Reyes-Trejo et al. (2014)
Camelina sativa	Camelina	888	4.3	0.15	–	42.76	–	0	152	1.3	0.0013	Ciubota-Rosie et al. (2013)
Argemone mexicana	Mexican poppy	860	11.1	0.95	41.5	–	−14	8	171	–	–	Rao et al. (2012)
Brucea javanica	*Kosam*	*871*	*3.556*	*0.027*	–	*51*	*1*	*2*	*164*	*3.0*	–	Hasni et al. (2017)
Azadirachta indica	Neem	899.1	5.50	0.22	–	56.78	12	20	168	–	–	Betiku et al. (2014)
Brassica carinata	Ethiopian mustard	879	4.5	0.08	–	52	–	–	>120	–	–	Cardone et al. (2003)
Cascabela ovata	Lucky nut	866.8	4.98	0.49	–	–	–	3	168	–	–	Sánchez-Arreola et al. (2019)
Asclepias syriaca	Milkweed	870	4.0	0.26	–	–	–	8	128	1.4	–	Phoo et al. (2014)
Ailanthus altissima	Tree of heaven	873	4.74	0.37	–	49	−4	2	169	–	0.01	Hoseini et al. (2018)
Calophyllum inophyllum	Indian laurel	877.6	5.5377	–	39.513	–	13	12	162.5	6.12	–	Atabani and da Silva César (2014)
Aphanamixis polystachya	Pithraj tree	893	4.7177	0.448	39.96	44	8	8	188.5	0.16	–	Palash et al. (2015)
Citrullus colocynthis	Thumba	870	4.78	0.42	37.0	41.7	−6	2	164	–	0.01	Chavan et al. (2014)

Euphorbia lathyris	Paper spurge	876.1	4.637	0.19	–	59.6	–	–	181	10.4	–	Wang et al. (2011)
Euonymus maackii	Himalayan spindle	880	4.1	0.42	–	56.9	–	–	–	–	0.01	Liu et al. (2019)
Guizotia abyssinica	Niger	–	4.30	0.15	–	57	–	4	157	1.02	0.0016	Sarin et al. (2009)
Garcinia indica	Kokum	862	5.8	0.3	39.12	–	9.5	12	135	–	–	Attal et al. (2014)
Gossypium hirsutum	Cottonseed	850	6.0	–	41.68	52	−4	−2	–	–	–	Nabi et al. (2009)
Hevea brasiliensis	Rubber seed	880	4.84	0.35	40.04	–	−8	4	184	9.82	0.01	Roschat et al. (2017)
Jatropha curcas	Jatropha	880.2	4.312	0.04	41.17	57.1	–	4	163	–	–	Wang et al. (2011)
Madhuca indica	Mahua	880	3.98	0.41	37	51	6	5	208	–	0.01	Ghadge and Raheman (2005)
Melia azedarach	Indian lilac	880	4.37	0.45	–	45	–	−10	–	4.8	–	Akhtar et al. (2011)
Mesua ferrea	Nahor	890	5.525	1.8	35	55	3	–	113	–	0.01	Aslam et al. (2014)
Raphanus sativus L	Forage turnip	877.8	3.23	0.2	38.75	50.0	−4	10	186	1.6	0.01	Shah et al. (2013), Fadhil et al. (2019)
Simarouba glauca	Paradise tree	862	3.1	0.4	40.33	56.80	14	17	178	–	0.01	Jeyalakshmi (2019)
Carica papaya	Papaya	840	3.53	0.42	38.49	48.29	–	–	112	5.61	–	Anwar et al. (2018)
Portulaca oleracea	Common purslane	878	4.91	0.41	40.5	62.9	−6	3	163	–	–	Hoseini et al. (2019)

(continued)

Table 2.4 (continued)

Botanical name	Local name	Physico-chemical properties										References
		Density (kg/m^3)	Kinematic viscosity (mm^2/s)	Acid value (mgKOH/g)	Heating value (MJ/kg)	Cetane number	Pour pt. (°C)	Cloud pt. (°C)	Flash pt. (°C)	Oxidation stability (h)	Ash content (%)	
Pongamia pinnata	Karanja	890	4.85	0.3	35.56	58	–	–	180	6	0.0004	Meher et al. (2008), Sureshkumar et al. (2008)
Putranjiva roxburghii	Putranjiva	–	–	0.42	39.58	54.99	−3	–	48	–	0.005	Atabani et al. (2013)
Ricinus communis	Castor	920	11.5	0.586	38.7	80	−30	−13.4	>130	1.1	<0.005	Özcanlı et al. (2010)
Nicotiana tabacum	Tobacco	882.0	5.2	0.66	39.81	51.6	–	–	165.4	0.8	0.0004	Veljković et al. (2006), Usta et al. (2011)
Moringa oleifera	Moringa	883	4.83	–	–	67.07	17	18	–	3.61	–	Rashid et al. (2008)
Shorea robusta	Sal	874	4.86	0.18	39.88	53	18	–	160	–	–	Hajra et al. (2015)
Cerbera odollam	Sea mango	847.9	3.1578	0.4	40.49	53.4	–	–	133	6.35	–	Khairil et al. (2018)
Simmondsia chinensis	Jojoba	865	6.783	–	–	69	−16	−13	154	0.35	–	Shah et al. (2010), Taiseer et al. (2019)
Sapindus mukorossi	Soapnut	876	4.63	0.14	40.02	56	−4	−1	140	–	0.003	Chakraborty and Baruah (2013)

Sterculia foetida	Wild almond	873	4.92	0.14	40.179	56.5	−3	1.2	160.5	3.44	0.003	Ong et al. (2013)
Sapium sebiferum	Chinese tallow	892.0	3.698	0.15	–	40.2	–	–	180	0.8	0.01	Wang et al. (2011)
Schleichera triguga	Kusum	857	5.34	0.42	37.595	42.47	−2	–	152	0.86	–	Sarve et al. (2015), Sarve et al. (2016)
Terminalia bellirica	Bahera	882.8	5.17	0.23	39.22	53	3	6	90	–	0.0005	Chakraborty et al. (2009)
Thevetia peruviana	Yellow oleander	875	4.33	0.057	44.986	61.5	3	12	75			Deka and Basumatary (2011)
Terminalia catappa	Indian almond	873	4.3	0.5	38.5	–	–	–	–	2.1	–	Dos Santos et al. (2008), Iha et al. (2014)

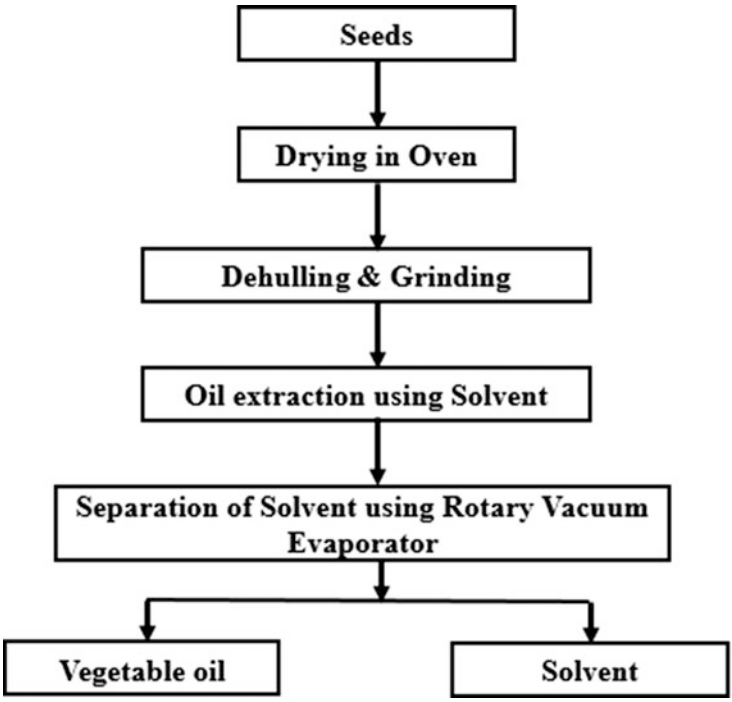

Fig. 2.1 Flowchart of oil extraction process

compounds, enzymes, etc.) (Atadashi et al. 2012a, b). At present, the primary chemical processes utilized for generating biodiesel comprise base-catalyzed transesterification, acid-catalyzed transesterification (simultaneous esterification and transesterification of FFA), ultrasound and microwave assisted transesterification, transesterification under supercritical alcohol conditions, and non-catalytic conversion by esterification (Demirbas 2009). Of late, owing to the significant advantages like catalyst reusability, product separation, and reaction conditions, the heterogeneous base-catalyzed transesterification is gaining much importance. However, each non-catalytic and catalytic process for biodiesel production has its own significance and advantages as the conversion efficiency is highly influenced by the moisture content and FFA composition of the feedstocks.

Theoretically, the transesterification reaction is an equilibrium reaction that needed an excess quantity of alcohol to move forward the reaction equilibrium and generate more methyl esters as product. Generally, triglycerides and alcohol do not react to form a single phase mixture. Therefore, the lesser surface contact between alcohol and triglycerides results in transesterification reaction to progress relatively slow. In order to solve the two-phase problem between alcohol and triglycerides, a catalyst is generally applied which enhances the surface contact and subsequently the reaction rate. As a result, the yield of biodiesel also increases (Ma and Hanna

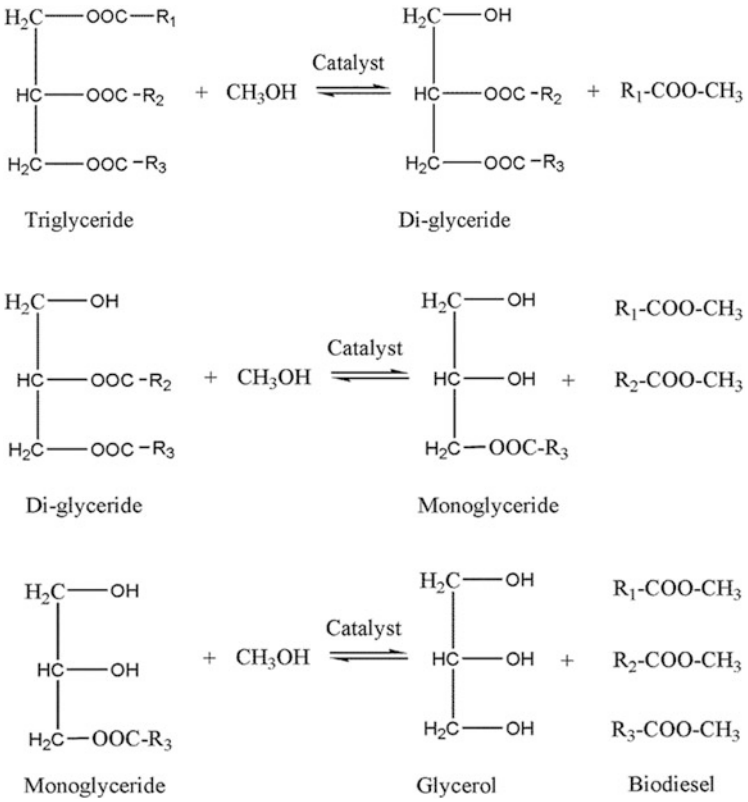

Fig. 2.2 Transesterification reaction showing the intermediate steps

1999; Demirbas 2009). The overall chemical reaction of the transesterification process can be summarized as given below (Fig. 2.3).

In the transesterification process, glycerol is also generated as by-product along with biodiesel. Pure glycerol has various industrial applications especially in cosmetic industries, pharmaceuticals, food and chemical industry (Chakraborty and Baruah 2013; Pali and Kumar 2014). Moreover, pure glycerol can also be utilized in several chemical reactions, such as glyceraldehydes and dihydroxyacetone production via catalytic oxidation reaction, as feedstocks in fabricating hyper-branched polyester, dendrimers, 1,3-propanediol, etc. (Bouaid et al. 2007; Naik et al. 2008; Haldar et al. 2009). In addition, acrolein can be produced by the dehydration of glycerol in the presence of a catalyst (Silitonga et al. 2016). Besides, glycerol is utilized in the production of numerous medicines such as plasticizers for medicine capsules, cough syrup, as a shipper for antiseptics and antibiotics and ear infection medicines (Chakraborty et al. 2009). Thus, it is observed that utilizing glycerol can improve the economic feasibility of the biodiesel generation process (Podkuiko et al. 2014; Vlysidis et al. 2011).

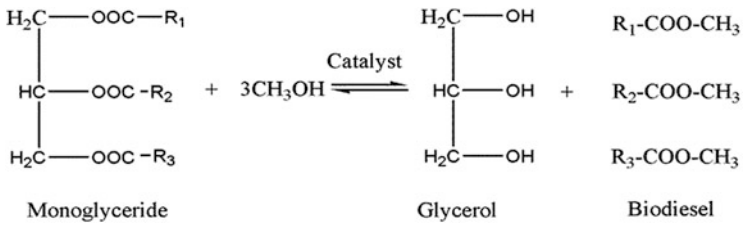

Fig. 2.3 Overall transesterification reaction

2.4.2 Utilization of By-products for Pyrolytic Valorization

Government of India's recent biofuel policy (National Policy on Biofuels (NPB) 2018) emphasizes on exploring non-edible oilseeds for biodiesel production. However, this bioenergy production process would also generate huge amount of biowastes, i.e. de-oiled seed cake and seed covers during the process (Government of India, Ministry of New, and Renewable Energy 2019). Proper utilization and management of these seed cakes and seed covers will not only improve the overall economy of biodiesel production but also mitigate environmental concern related to waste dumping (Cortés and Bridgwater 2015). These biowastes are rich sources of hydrocarbons owing to the presence of cellulose, hemicellulose, and lignin, yet they have been used only for low value applications such as heating or as solid fuel (Chutia et al. 2013). The valorization of lignocellulosic biowaste such as de-oiled cake, seed cover, agricultural waste, corn stalk, wheat stock, etc. has gained prominence as it serves the dual advantage of producing energy and biomaterials/chemicals as well as mitigating environmental concerns. Biofuel production from biowastes through thermochemical conversion technique promotes waste management as well as energy and chemical recovery. Thus, it will be a value addition to the national biofuel policy to investigate these biowastes for their feasibility in terms of sustainable energy and biomaterials/chemicals production.

Among the various thermochemical conversion technologies, pyrolysis is the most promising route to produce biofuel and chemicals (Kusdiana and Saka 2001). Pyrolysis is the process of thermochemical disintegration of organic materials at moderate temperatures (400–800 °C) in the absence of oxygen or with a limited oxygen supply that does not permit complete combustion. It is the initial stage of both gasification and combustion process. The pyrolysis process for organic material is very complex and comprises both successive and simultaneous reactions as the organic matter is heated in an inert environment. In the pyrolysis reaction, the organic elements of biomass degraded thermally at 350–550 °C and move up to 700–800 °C in a limited or air/oxygen free environment (Fisher et al. 2002). Under the pyrolytic conditions, the long chains of hydrogen, oxygen, and carbon present in the biomass decompose to smaller compounds of condensable vapors (oils and tars), gases, and solid charcoal. The rate and extent of decomposition vary with different

Table 2.5 Characteristic product yields (dry basis) obtained from the various pyrolysis methods of wood (Mohan et al. 2006; Jahirul et al. 2012)

Pyrolysis process	Operating conditions				Product yield (wt.%)		
	Temperature (°C)	Heating rate	Particle size (mm)	Residence time	Liquid	Solid (char)	Gas
Flash	<650	High	<0.2	<1 s	75	12	13
Fast	650	Very high	<1	0.5–5 s	75	12	13
Conventional (slow)	600	Low	5–50	5–30 min	30	35	35

process parameters like feedstock, heating rate, pyrolysis temperature, configuration of the reactor, pressure, etc. Depending on the various process parameters pyrolysis can be categorized primarily into three classes: slow or conventional, flash, and fast pyrolysis (Guedes et al. 2018). Moreover, the composition and proportion of the pyrolysis products also depend on operating conditions and type of pyrolysis reaction as shown in Table 2.5. Slow pyrolysis takes place at low heating rate, low temperature, and high residence time, which increases the char production. Fast pyrolysis occurs at moderate temperature, shorter vapor residence time, high heating rate, and enhances the production of bio-oil. During flash pyrolysis, the reaction time is very short (few seconds or less), the heating rate is very high and the particle size due to rapid heating should be small (Guedes et al. 2018).

2.4.3 Utilization of De-oiled Seed Cake

De-oiled seed cake is obtained from the seed kernel after the oil extraction process is performed for biodiesel production and termed as a biowaste. However, owing to its lignocellulosic nature the de-oiled seed cakes find application as raw materials for the pyrolysis process as discussed above. In addition, it can also be explored for fabricating value added product like mosquito repellent, as it contains some toxic component in it. Pant et al. (2016) investigated the utilization of de-oiled seed cakes of karanja (*Pongamia glabra*) and jatropha (*Jatropha curcas*) to prepare a novel and herbal mosquito repellent with the *Aedes aegypti* larvae. Apart from the biological active ingredients (karanja and jatropha) the repellent also contained inerts, burning agents, and preservatives. The percentage mortality obtained from the mosquito repellent with 20% concentration each for jatropha and karanja de-oiled seed cake powder was found to be 96%. The study also revealed that the coil prepared from the biodiesel by-products was cost-effective, less noxious, and environmentally benign in nature compared to the commercial coils having the chemically active ingredients. The study greatly emphasized on the efficient utilization of non-edible de-oiled seeds cakes having an insecticidal property which would otherwise create disposal problems.

2.4.4 *Utilization of Co-products of Pyrolysis*

Biochar is produced as the solid co-product of pyrolysis. A significant amount of mass loss occurred in pyrolysis in the form of volatiles due to the thermal degradation of lignin and hemicellulose, resulting in a rigid amorphous carbon structure as leftover and termed as biochar (Jahirul et al. 2012). Biochar is primarily carbon (~85%), also contains hydrogen and numerous inorganic compounds in two structures: amorphous aromatic structures ordered arbitrarily and fixed crystalline graphene sheets. The heteroatoms H, N, O, P, and S included in the aromatic rings have a great effect on the physical and chemical properties of biochar (Cetin et al. 2004; Dawei et al. 2006). Depending on various parameters like biomass feedstock and pyrolysis conditions, about 10–35% biochar is produced. The physical characteristics of biochar are significantly influenced by various pyrolytic parameters like type of biomass and drying treatment, biomass particle size, flow rate of inert gas, pyrolysis temperature, heating rate, residence time, pressure, chemical activation, reactor type and shape, etc. (Bourke et al. 2007).

Biochar can be used in numerous industrial processes depending on the physical properties and its composition. For example, biochar can be used for the production of carbon nanotubes, activated carbon and hydrogen rich gas, and also as a solid fuel in boilers. Besides, biochar can alter various soil properties, such as pH, water holding capacity, soil aggregation, bulk density, nutrient and organic carbon availability, etc. Moreover, biochar has remarkable potential for reducing the climate change as it can improve the stable soil carbon stocks and soil carbon sequestration while lowering the concentration of atmospheric CO_2 (Goyal et al. 2008). Recently, biochar has gained immense attention due to its valuable potential application in agriculture as well as environmental repercussions. Besides its various applications such as amendment of the soil (Meyer et al. 2011), carbon sequestration (Laird et al. 2010), noxious material removal (Cao et al. 2009), waste recycling (Field et al. 2013), generation of value added products (Azargohar and Dalai 2008), and pollution remediation (Mohan et al. 2014), biochar also showed great potential as a carbon based solid acid catalyst in the esterification and transesterification reactions (Shu et al. 2009; Zeng et al. 2014; Dehkhoda and Ellis 2013). Biochar contains a high amount of oxygen mostly in the form of acidic groups such as carboxylic and phenolic groups (Liu et al. 2011; Lehmann and Joseph 2009; Brewer et al. 2009), which can improve the catalytic activity by enhancing the acidity and increasing the adsorption of reactant molecules on the catalyst surface (Dehkhoda and Ellis 2013).

2.5 Cascading of Approaches for Zero Waste

Over the past few years non-edible oilseeds have emerged as a prospective feedstock for the generation of biodiesel due to the ready availability, low cost, higher production rate, and environment friendly nature (Demirbas et al. 2016;

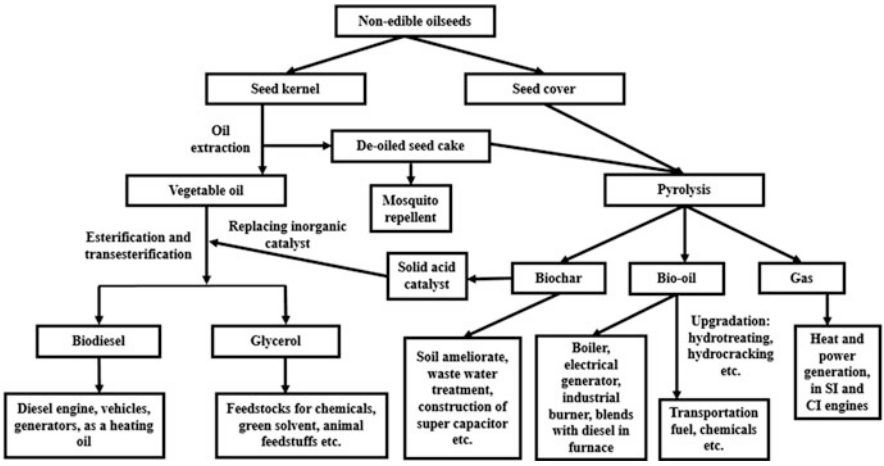

Fig. 2.4 A biorefinery approach for biodiesel and biofuel production along with chemicals and value added products for the zero-waste utilization of non-edible oilseeds

Banković-Ilić et al. 2012). But, the imposing price associated with biodiesel production acts as a major hindrance in commercialization of the biodiesel. It is reported that the raw materials alone contribute approximately 60–80% of the overall production cost of biodiesel (Borugadda and Goud 2012). Consequently, the researchers working on biodiesel have diverted their focus from the generation of a single product to the simultaneous formation of multiple products in a biorefinery approach that would exploit both the raw material (feedstock for biodiesel production) and biowastes generated therein (Demirbas et al. 2016; Bhowmick et al. 2019). Though various studies on biodiesel production in a biorefinery model are available, yet the report on the complete utilization of non-edible oilseeds in a systematic method to produce biodiesel and biofuel along with chemical and value added products in a "zero waste disposal" concept is limited. Considering the immense potential of non-edible oilseeds as feedstocks for biodiesel production, this chapter focuses on complete utilization of non-edible oilseeds in a biorefinery model through a cascade of approaches to produce biodiesel and biofuel along with value added products and chemicals with zero waste disposal (Fig. 2.4). Thus, biofuel production from low-cost, economical resources and simultaneously producing various value added products and chemicals would be a win–win scenario while reducing the environmental concerns.

2.6 Future Perspectives

The exploration of a low cost and abundantly available feedstock is both critical and indispensable as the price of feedstocks accounts for about 70–90% of overall cost of biodiesel production (Shikha and Rita 2012). The national policy for biofuel of the Government of India envisages biofuel production mostly from non-edible feedstocks in order to avoid possible food versus fuel crisis. Therefore, utmost emphasis has been given on the utilization of waste and degraded lands for biofuel production by growing non-edible oilseeds bearing shrubs and plants. However, this bioenergy production process would also generate a huge amount of biowastes, i.e. de-oiled seed cake and seed covers throughout the process (Government of India, Ministry of New, and Renewable Energy 2019). Proper utilization and management of these biowastes will not only improve the overall economy of biodiesel production but also mitigate environmental concern related to waste dumping (Cortés and Bridgwater 2015). These biowastes are rich sources of hydrocarbons owing to the presence of cellulose, hemicellulose, and lignin (Chutia et al. 2013), yet they have been used only for low value applications such as heating or as solid fuel. The thermochemical conversion, i.e. pyrolysis is an effective route for sustainable utilization of the organic carbon present in these bioresources. Therefore, valorization of the wastes generated during biodiesel production could be an attractive option in enhancing the overall economy of the biodiesel production process.

It has been reported that apart from producing biofuel, the non-edible seeds can also produce various significant products. *Jatropha curcas* seeds can be used to obtain activated carbon with excellent regeneration, surface, and thermal properties (Hsu et al. 2014; Karthikeyan et al. 2008; Xin-Hui et al. 2011; Ramakrishnan and Namasivayam 2009). The activated carbon can also be utilized for water treatment, purification, dye removals, etc. Similarly, castor seeds can be employed for producing both polymeric surfactant and surfactant (Babu et al. 2015). The adsorbents obtained from Karanja and *Moringa oleifera* seeds showed admirable surface area (Ganvir et al. 2012) and can remove 95% of dye (Reck et al. 2018). In addition, *Calophyllum inophyllum L.* and *Hevea brasiliensis* have the ability to synthesis paint, detergent, soap, and cosmetics (Leksono et al. 2018; Iyayi et al. 2008; Widyaningsih et al. 2018). Biochar, co-produced as by-product during the pyrolysis process, not only have some fuel applications but can also be used as a source for chemicals and value added products (Meyer et al. 2011; Azargohar and Dalai 2008). The agricultural, environmental, and industrial applications of biochars such as soil amendment, carbon sequestration, removal of toxic materials, etc. have drawn the attention of the researchers (Laird et al. 2010; Glaser et al. 2002). In recent years the carbon based solid acid catalysts have gained much attention in the esterification or pretreatment of the FFA as well as the transesterification of vegetable oils (Li et al. 2013). These reports show that the non-edible oilseeds can generate numerous essential products and chemicals besides producing the biofuel.

The cascade of approaches comprises "zero waste disposal" concept with process integration, wherein non-edible oilseeds are utilized for biodiesel production using

various standard techniques and the biowastes generated during the process were utilized for simultaneous production of biofuel (bio-oil and biochar) and value added products (mosquito repellent) and chemical. Furthermore, the biochar generated in the process can capture and sequester the atmospheric CO_2 which is beneficial in attaining carbon negativity, along with wastewater treatment and off-setting the energy requirements. Thus, the "zero waste discharge" concept endures the prospect of making the biorefinery approach a viable one, besides improving the carbon credits.

2.7 Conclusion

In this chapter, a biorefinery approach to zero-waste utilization for producing biofuel and biodiesel from non-edible oilseeds along with chemicals and biomaterials is discussed. The biorefinery approach can be considered as an improved and economically viable one compared to the conventional methods used so far for biofuel production. For the production of biodiesel and biofuel, many techniques and approaches have been adopted; however, a comprehensive method for complete utilization of non-edible oilseeds into valuable products is yet to be developed. This chapter endeavors to elucidate the zero waste generation from the non-edible oilseeds for biofuel and biodiesel production incorporating the biorefinery approach of generating wealth from biowaste which is economically fascinating. While biorefinery approach for generating value added products from biowastes has been utilized by various industries around the world, in India, it is still in emerging stage and yet to reach a fully operational and functional status. It is expected that biorefineries will play a prominent part in modeling the energy sector of India as the country is abundant in non-edible oil containing feedstocks for biodiesel and biofuel production.

Competing Interests All the authors declare that they have no competing interests.

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Chapter 3
Emerging Trends in Food Industry Waste Valorization for Bioethanol Production

Manpreet Kaur Mann and Balwinder Singh Sooch

Abstract A number of complex processes involved in food processing industries generate huge amount of waste. This waste is now becoming a global concern; therefore, sustainable interventions for its management are required. Traditional measures like animal feeding, anaerobic digestion, composting, incineration, land spreading, and land filling proved uneconomical with less environmental advantage in the past. In contrast, food waste material represents a lot of valuable nutrients; hence, their valorization for wide range of commercial applications is necessary. In this scenario, biorefineries have proven promising to combat with this persistent problem of waste, which can be utilized as a potential raw material in many biochemical processes like photosynthesis, fermentation, methanogenesis, acidogenesis, etc., for the development of various value added products such as biofuels, bioelectricity, biofertilizers, animal feed, and biochemicals. The tailored bioprocesses with enhanced efficiency and good productivity used in biorefineries maximize the waste value. The present chapter highlights various emerging bioprocesses for the utilization of waste generated from diverse types of food industries as a feedstock to produce bioethanol as a value added product.

Keywords Valorization · Biofuels · Bioethanol · Biorefinery · Dairy waste · Meat waste · Instant noodle waste

Abbreviations

SSF Solid state fermentation

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3.1 Introduction

Global population with exponential growth rate is causing a substantial hazard for available limited resources of energy. Presently, more than 90% demand for energy and other essential materials is catered by fossil-based reservoirs (Mohan et al. 2019). The available fossil fuel reserves may soon get exhausted with the present rate of consumption of fossil fuels. Therefore, an alternative sustainable resource to meet the present energy requirements is the need of the hour (Hemalatha et al. 2019). Sustainability has become a key consideration over the last decades, which is largely driven by increased awareness for cleaner environment and decreased reservoirs of fossil fuels. The utilization of industrial waste as a feedstock to generate higher value products will greatly help to achieve this sustainability. Several new technologies are derived from microbial processes for waste valorization in the recent past for biorefineries. The use of bio-waste has proven to be a core competency for waste valorization in biorefineries and therefore exploited magnificently in the recent years with the help of innovative biochemical strategies (Safari et al. 2017; Lugani et al. 2019). Various types of industrial waste have already been tested in biorefineries for their conversion into bioenergy and diverse range of biochemicals (Fava et al. 2015; Sooch et al. 2019; Lugani et al. 2020). The concept of biorefinery is aimed to generate zero waste like traditional petroleum refinery by utilizing biomass. The biofuel production from renewable biomass has been started during the nineteenth century. A program named "Proalcool" was initiated during 1975 in Brazil to produce ethanol from sugarcane with the aim to reduce oil import in the country (Amorim and Lopes 2005). Thereafter, the biomass based biorefineries appeared in the 1980s to suppress the methane production by encouraging the synthesis of other useful products like biofuels and biochemicals. Biofuels production has been specifically focused in last decade with the utilization of lignocellulosic biomass to meet the essential need to produce fuels from renewable energy sources. Amongst many fuel alcohols, bioethanol referred as ethyl alcohol, is a major fuel, having a heating value of 26.7 MJ/kg (Khuong et al. 2016). It is an essential organic solvent used in many industries and also used as antiseptic, disinfectant, and dye removal. Ethanol produced through biotechnological (fermentation) and chemical route is known as bioethanol and synthetic ethanol, respectively. Bioethanol is being produced from a variety of substrates like algae biomass, agro residues, lignocellulosic biomass, food grains, molasses, food waste, and waste from food processing industries (Singh and Sooch 2009; Sooch et al. 2016). In addition to agrowaste, food processing industries also generate a huge amount of waste that causes a major global threat related to its disposal. Around 1.3 billion tons of food is wasted every year in the world (Paritosh et al. 2017) and it is estimated to rise to 416 million tons in 2025 in Asian countries. Food industry waste includes waste from food industry, catering business, consumer and household waste, but, fruits and vegetables are on the top of all waste foods. The amount of waste generated from food industries can originate at different levels of processing, handling, storage, distribution, or consumption. The increased amount of food waste from food industries is raising serious issues with regard to its disposal,

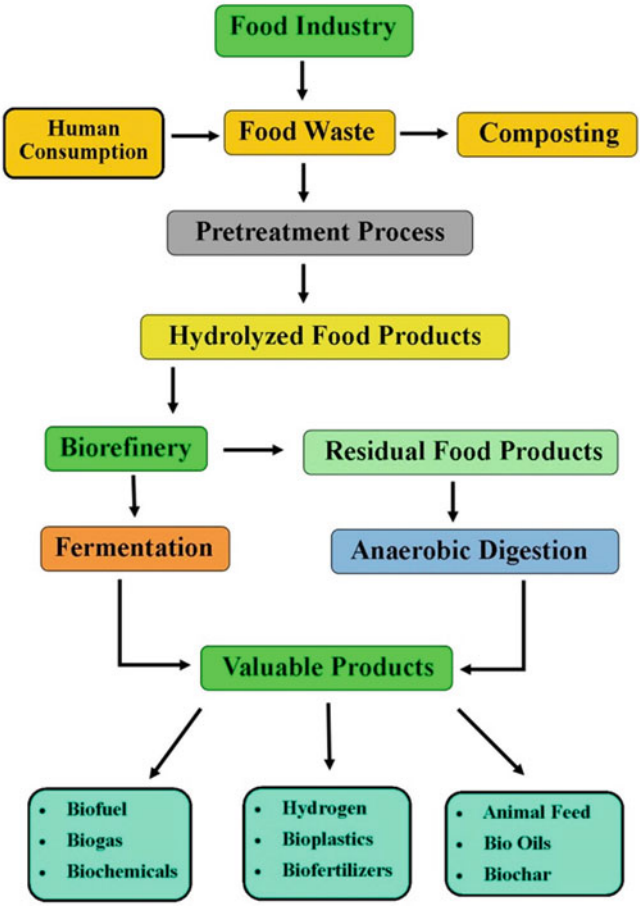

Fig. 3.1 Schematic representation of food industry waste valorization

pollution from landfills, and economics. Moreover, high moisture content in the food industry waste is also detrimental for its management. Food waste is mainly constituted of organic components such as proteins, carbohydrates, fats that can be easily disintegrated into simpler compounds like amino acids, pentose or hexose sugars, fatty acids, and many more. Hence, this nutrient rich food waste can be converted into a variety of bio-based products by employing a plethora of microbial processes (Dahiya and Joseph 2015).

Bioconversion of food waste into value added products may prove as an alternative renewable source to reduce the exploitation of natural fossil fuels. In addition to its utilization for biogas production, trend is also flowing toward the production of chemicals (Lee et al. 2014), hydrogen (Pasupuleti et al. 2014), bioelectricity, and biofuels (Karmee 2016). Figure 3.1 represents the schematic overview of food industry waste valorization into various products. Although the conversion of food

industry waste to biofuels is potentially an efficient route, but watchful evaluation of the waste pretreatment, process optimization, and technology economics is vital for its advancement towards industrial application. Hence, the present chapter aims to analyze various emerging bioprocesses for the utilization of waste generated from diverse food industries as a feedstock to produce bioethanol.

3.2 Types of Food Industry Waste and Byproducts for Biorefineries

Food waste valorization caters pollution problems caused by food waste disposal and its decay in exposed landfills, and in addition, it also provides monetary benefits in the form of various valuable products. Food industry waste is generated in three forms, namely solid form, liquid form, and semi-solid form. The liquid food waste is produced from several food processing operations such as cooking, temperature control, cleaning, transportation, sanitation, etc. The run-off from food industries consists of organic matter, suspended solids, nitrogenous compounds, fats, oils, and other inorganic substances. The major liquid effluent forms are generated in the form of whey from dairy industry, waste water from washing operations in bakery and soft drinks industry, brewery discharge from multiple operations, oil mill discharge, apple pomace sludge, and waste water from potato processing industries. This liquid waste contains a variety of valuable constituents and can be used in biorefineries for its valorization. The fruit and vegetables industry liquid waste contains about 80–90% water with 8–18% of total solids. The total volatile solids present in the effluent mainly contain sugars and hemicellulose (75%), cellulose (9%), and lignin (5%) (Ruynal et al. 1998). The starch rich potato processing industry wastewater generated during different industrial operations is a suitable feedstock for the production of alcohol (Wang et al. 2006). Whey, rich in lactose, causes many pollution problems if disposed in water or on land without treatment but this is a very good substrate for many lactose metabolizing microorganisms like *Kluyveromyces* for the production of ethanol (Singh and Singh 2001; Sooch and Singh 2002; Singh et al. 2002). Almost 160 million tons of whey is produced annually, which contains a very high biological oxygen demand (30,000–50,000 ppm) and chemical oxygen demand (60,000–80,000 ppm) load (Das et al. 2016). Whey retains around 55% of milk constituents including lactose (4.5–5% w/v), proteins (0.6–0.8% w/v), lipids (0.4–0.5% w/v), vitamins, minerals, and many other micronutrients (Casal et al. 2006).

Food waste valorization for biorefineries involves the knowledge of various environmental quality parameters to formulate appropriate waste treatment plans along with the understanding about novel products expected from biorefineries. Environmental quality parameters like chemical oxygen demand, biological oxygen demand, suspended solids, volatile solids, etc., indirectly measure the organic compounds present in the waste water. Other important constituents of the industrial

effluent such as total solids, nitrogen, and phosphorus are also estimated to know the exact composition of effluent for its further treatment. The wastewater generated from food processing industries is generally considered nontoxic because it mainly consists of dissolved compounds including mild cleaning chemicals.

Majority of solid waste materials includes the inedible dough, waste bread, tomato waste, potato waste, apple pomace, grape pomace from wineries, and soybean curd residue. The solid and semi-solid forms of food waste mainly contain celluloses, hemicelluloses, lignins, starch, and sugars, mainly glucose and fructose. Hence, both types of liquid and solid food waste materials have significant potential to be used in biorefineries for their conversion to alcohols and other types of renewable fuels. The requirements of feedstock to be used for fuel production in biorefineries are like it must be abundantly available and should not be a part of food material meant for direct consumption of livestock or human beings. Food industries waste is one such raw material that fulfills these criteria and is available in abundance with zero or low cost of procurement. Vegetables and fruits are the most sought source of significant nutrients and used in routine in every household. However, a lot of fruits and vegetable produce is wasted during various harvesting and post-harvesting operations. These damaged market rejected fruits and vegetables are commonly used as feed for animals or left for decomposition. Amongst vegetables, tomatoes, cabbage, and cauliflower bear serious postharvest loss ranging from 30.3–39.6%, 24.9–30.4%, and 28.6–35.1%, respectively, during several operations (Pal et al. 2002). This waste indeed can be utilized in biorefineries for its conversion to ethanol through bioprocesses by exploiting the biodegradation pathways of microbes. Although various valuable nutrients like starch, cellulose, and hemicelluloses are present in food waste but pretreatment of this waste with acids and/or enzymes is a prerequisite for fermentation processes (Singh et al. 2012). Hence, carbohydrates and other nutrients present in diverse type of food waste can be converted to assorted fuel alcohols by exploiting appropriate metabolic pathway of microbes. It is a fact that almost 40% (w/w) of processed tomatoes goes as waste in tomato processing industries (Chandrasekharan 2012). Surprisingly, this waste is very less explored for its valorization, whereas, it is rich in many fermentable sugars like dextrose, sucrose, and fructose (Del et al. 2006). Apple pomace, produced during apple processing includes core, peel, calyx, seeds, stem, and soft tissues, contains number of nutrients assimilable for microbes. In juice industry, 12–20% of original weight ends up as apple pomace (Kosmala et al. 2011). Similarly, grape pomace obtained from wineries and juice industries is rich in fermentable sugars and other nutrients (Zheng et al. 2012). Unfortunately, most of the fruit pomace is dumped in landfills or used for composting or processed for feed, but this nutrient rich waste is less explored for alcohol production.

A large amount of starch containing waste is generated from noodle industries, which is generally disposed without any further treatment and thus posing many environmental hazards. The liquid waste consisting of starch materials discharged into the environment by many food industries causes problems like ground water contamination and bad odor at the disposal site (Tan et al. 2009). The accumulation of solid waste containing lignocellulose, starch, and oil is also becoming problematic

due to many reasons at the site. Many research efforts has been made in the past to use this starch rich noodle waste for the production of bioethanol after some pretreatment (Siripattanakul-Ratpukdi 2012; Karmee and Lin 2014; Yang et al. 2014; Karmee 2016). A huge amount of husk, pulp, and wastewater is generated from coffee processing industries and all of these are environmental pollutants. The spent coffee pulp, when released into water bodies, destroys the flora and fauna of that ecosystem (Seboka et al. 2009). It is estimated that around half amount of the coffee pulp by weight is generated after processing of coffee cherries (Roussos et al. 1995). The coffee pulp, rich in fermentable sugars and nitrogenous compounds, has a good potential to be used as a substrate directly or after pretreatment for fermentation for the production of ethanol (Urbaneja et al. 1996; Gouvea et al. 2009; Kefale et al. 2012; Pereira et al. 2020). It is inferred from the discussion that most of the food waste materials are rich in fermentable sugars, nitrogenous compounds, and many other micronutrients to meet the nutritional requirements of microbes supposed to be employed in biorefineries. Therefore, the optimal use of these food processing industry waste materials may prove promising for biorefineries for the production of alcohol in future.

3.3 Major Pretreatment Approaches for Processing of Food Industry Waste for Biorefineries

Most of the food processing industry waste materials require specific type of pretreatment for their optimal utilization in bioconversion process to obtain desired valuable products. Pretreatment strategy of food waste material has a large influence on bioethanol production and overall economy of the process in biorefineries (Hafid et al. 2017). The major objective of technological progressions in biorefining is to deoxygenate and depolymerize the food industry waste to make it readily available for microbial conversion. There are several technological interventions also known as fractionation processes of feedstock that ensures the availability of sugars, carbohydrates, lipids for further refining. The residual sugarcane bagasse can be used as a boiler fuel after direct extraction of sugars from it, whereas corn waste has to be ground and hydrolyzed by enzymes for the conversion of its starch into sugars. Further, fibers, corn oil, and proteins can be recovered from food residual materials. Similarly, a specific type of pretreatment is necessary for cellulosic and hemicellulosic materials to obtain fermentable feed stock for ethanol production. This can be achieved by thermal, enzymatic, or acidic hydrolysis based pretreatments. A huge amount of food waste biomass was also generated from several more industrial processes which can be converted into assimilable sugars through these hydrolytic processes. Broadly, the technological processes required for pretreatment of food waste materials can be categorized into five following broad categories.

3.3.1 Thermochemical Approach

Direct combustion process is the primitive known thermochemical approach, in which biomass is incinerated in the presence of oxygen for heat generation. The ineffectiveness of this approach has shown a way for the development of some advanced tools. Gasification and pyrolysis are the other two major thermo chemical processes to generate energy and other byproducts from biomass. The biomass is heated in gasification process at elevated temperatures (approximately >700 °C) in the presence of low O_2 to obtain syngas and methane (Caputo et al. 2005). Syngas can be used as a suitable podium for synthesis of many fuels, chemicals or for generation of electricity. Pyrolysis degrades the organic matter at high temperature in the range of 300–600 °C without the presence of oxygen. The main products recovered after pyrolysis include solid charcoal, pyrolytic oil, and few light gases (Du et al. 2012). The product yield depends upon the processing conditions during pyrolysis. Bio-oil is the most sought product obtained from pyrolysis, which acts as a precursor for the formation of various fuels and chemicals (Cherubini 2010). Marculescu and Stan (2012) have used temperature in the range of 450–600 °C for the pyrolysis of feathers and bones to produce char, tar and gases. Abnisa et al. (2013) have also obtained bio-oil through pyrolysis at 500 °C with high oxygen and lower heating value. An oil yield of 40% has been obtained from cashew nut shells using vacuum pyrolysis (Das and Ganesh 2003). Several studies on mixed food waste pyrolysis have also been reported for conversion of food waste into useful forms of energy like bioethanol (Mahapatra et al. 2012; Liu et al. 2014; Grycova et al. 2016). Elbeshbishy et al. (2011) have used a food waste pretreatment method with mild heating at 70 °C with processing time of 30 min resulting in increase of protein content by 8%, carbohydrate by 10%, and COD by 20%.

3.3.2 Hydrothermal Approach

Hydrothermal approach is the most popular lower temperature biomass pretreatment approach in which materials are cooked in water under pressure (2 mPa) at temperature in the range of 80–100 °C for 10–50 min. The two most common types of hydrothermal processes are liquid hot water process and uncatalyzed steam explosion process (Ruiz et al. 2020). The aforementioned technique is generally applicable at industrial level; hence, prior size reduction is not required. But, it was observed that the said process can be 50% more energy efficient by adopting this additional step of size reduction (Hosseini and Shah 2009). The aforesaid treatment method acts by removing lignin fractions and changing their structure by coagulating, melting, and successively depolymerizing the cellulose fibers. This method does not yield the exact extract of lignin from solid waste in its functional form, but it increases the available surface area of cellulose for swift action of enzymes. Since, harsh steam action also leads to the greater degradation of sugar containing waste

and several inhibitors such as 5-hydroxyl-methyl-furfural and furfural are formed during this process. Some other byproducts like organic acids formed in traces during the process also act as inhibitors for the yeasts. This method does not involve steam explosion because biomass is heated and cooled gradually at the start and end of treatment process, respectively (Ruiz et al. 2020). This low temperature treatment operates under ambient conditions without any prerequisite for chemicals and hence this approach is attracting focus of bioethanol producing industry. Deniel et al. (2016) have used the hydrothermal liquefaction for blackcurrant pomace including seeds, peels, and pulp, and 24–31% bio-oil yield was achieved at temperature range of 290–335 °C. The high oil yield was achieved with hydrothermal treatment method from peanuts deoiled meal and shells when sodium hydroxide and potassium hydroxide are used as catalysts in place of sodium carbonate and potassium carbonate (Tu et al. 2016). Similarly, Akhtar et al. (2010) have also reported the better results with the use of potassium carbonate as catalyst than potassium hydroxide for the liquefaction treatment of empty palm fruit. The bio-oil yield of 28% was obtained when cornelian cherrystone was subjected to hydrothermal treatment at 300 °C (Akalin et al. 2012). Li et al. (2016) have obtained a biofuel yield of 15% from rice, 30% from potato, and 33% from sweet potato, during liquefaction treatment at temperatures of 300, 260, and 200 °C, respectively. Yang et al. (2016) have reported pretreatment of spent coffee grounds via hydrothermal liquefaction process with bio-oil yield of 47.3% under subcritical processing conditions. A sugar yield of 27.59 g/L was obtained after pretreatment of Malaysian food waste at 90 °C by Hafid et al. (2017) and they have observed that further rise in temperature does not result in any considerable increase in sugar concentration in the hydrolysate.

3.3.3 Biotechnological Approach

Several pretreatment approaches such as physical, chemical, or thermal are commonly used for the conversion of complex lignocellulosic materials into simple fermentable sugars for their bioconversion into ethanol, gas, or other products and these processes generally use severe conditions of treatment. However, biotechnological or biochemical approach employs microbes or enzymes and the processes are usually conducted at low temperatures as compared to other methods. Among many biochemical processes, enzymatic hydrolysis is one of the most popular methods used for pretreatment of complex materials. This method involves a variety of enzymes to degrade complex materials into simple assimilable forms, which are further subjected to bioconversion through fermentation by microorganisms into many valuable products. In spite of early acquaintance with the fermentation process, the bioconversion of sugars into biofuels is still a slow process. Recently, biochemical processes progressed to use genetically modified microorganisms for the degradation or production of targeted compounds. Amongst various sugars, hexoses have been considered as the best choice for microorganisms in many biochemical processes, while pentoses and other substrates need tailored microbes

for their bioconversion into ethanol and other products (Argyros et al. 2011). Biological pretreatment methods involve low energy for disintegrating the complex biomass into simpler form by employing enzymes (Kavitha et al. 2019), single cell organisms (Kavitha et al. 2013; Lakshmi et al. 2014; Gopikumar et al. 2016), or multicellular organisms (Pleissner et al. 2014). The bioagents employed for treatment of food waste must be capable of secreting hydrolytic enzymes such as cellulases, amylases, xylanases, proteases, lipases, etc. (Meng et al. 2017; Banu et al. 2018). Meng et al. (2017) have also reported a good hydrolytic efficiency for animal fat, vegetable oil, and floatable grease in selected microbes. Among many tested microorganisms, *Aspergillus* and *Candida* exhibited more than 70% hydrolytic efficiency within time period of 4 h against animal fat. Pleissner et al. (2014) have successfully treated the food waste by employing *A. awamori* and *Aspergillus oryzae*, and 143, 1.8, and 1.6 g/L of glucose, free amino nitrogen, and phosphate were obtained, respectively, after pretreatment. Similarly, cellulose was treated through acid hydrolysis (Dussan et al. 2014) or enzymatic hydrolysis using cellulase (Yamada et al. 2013) to release glucose for their conversion into bioethanol. A good yield of glucose from food waste including grains, fish and meat, fruits and vegetables was achieved by combining the enzyme activity of carbohydrase and amyloglucosidase by Moon et al. (2009). Hence, it can be concluded that the biotechnological approach is an easy, efficient, and economical approach of pretreatment to degrade complex carbohydrates into fermentable sugars.

3.3.4 Size Reduction Approach

Ultrasonic and disperser pretreatments are mechanical methods widely used for size reduction of waste materials. The size reduction orientated mechanical operations only involve in the size reduction or ingredient separation processes, thereby, not changing the structural framework of biomass. This is generally considered as typical first step in any biorefinery set-up due to the requirement of tiny particles with more surface area accessible for the action of microorganisms and enzymes to achieve the significant bioconversion rate and yield (Taherzadeh and Karimi 2008). The size reduction of large or bulky materials is accomplished by the use of different types of mechanical forces that changes the shape, size, density, and flowable characteristics of the material. The end product of size reduction process depends upon the nature of biomass, type of force employed, and application time. Various constituents of the substrates are sorted through different separation techniques depending upon their color, size, shape, or other characteristics, whereas extraction processes concentrate and extract the value added components from heterogeneous and massive substrates. The formation of vapor bubbles and their collapsing is causing biomass breakdown during ultrasonic treatment method. John et al. (2019) reported the amalgamation of thermal and ultrasonication treatment for hydrolysis of sweet lime peel. Gadhe et al. (2014) have used the ultrasonic impact on food waste to enhance the productivity of anaerobic reactor and around 42% chemical oxygen

demand was solubilized with consumption of 16,875 kJ/kg energy. Shanthi et al. (2018) have also enhanced the process output by utilizing 5400 kJ/kg input energy, where, nearly 10% of suspended solids was reduced and 16% of organic matter was solubilized. In another study by Elbeshbishy et al. (2011), 25% of chemical oxygen demand of waste material was solubilized with an input energy of 79 kJ/kg. Ma et al. (2011) have advocated the use of high pressure in pretreatment of food waste and they have achieved 12–67% chemical oxygen demand solubilization by implementing this strategy. Falls et al. (2019) have observed the effect of sudden shock wave on a biomass during enzymatic hydrolysis and recorded that shock treatments help to release more sugars from lignocellulosic materials.

3.3.5 Chemical Approach

Chemical pretreatment approach is generally performed by the action of strong acids (HCl, H_2SO_4, HNO_3), salts ($MgCl_2$, $CaCl_2$, NaCl, NaOH, KOH, $Ca(OH)_2$), cation-binding substances, chemical surfactants, and oxidizing agents (Kavitha et al. 2013, 2015a, b, 2016; Kavitha 2014; Gayathri et al. 2015; Packyam et al. 2015; Eswari et al. 2017; Kannah et al. 2017; Ushani et al. 2017; Kumar et al. 2018; Solarte-Toro et al. 2019; Banu et al. 2020). Amongst these chemical agents, dilute acid is generally preferred for the pretreatment of food waste for bioethanol production. Chemical processes may change the chemical state of the biomass when it interacts with other amalgams in catalyzed reactions. Enzyme assisted hydrolysis and transesterification processes are the popular chemical pretreatment methods employed for the degradation of complex materials. Complex food waste materials are decomposed into simple fermentable sugars in hydrolysis treatment with the help of strong acids or alkalis or enzymes. This process also generates a byproduct like glycerin, which has diverse commercial utility (Demirbas 2010). Dufour and Iribarren (2012) have used transesterification method for the treatment of beef tallow and poultry waste for the production of biodiesel and glycerol by the reaction of fats or oils with methanol in the presence of potassium hydroxide or sodium hydroxide under specific conditions. Ebner et al. (2014) have reported that high free fatty acid containing food waste can be treated with the help of acid esterification technique using H_2SO_4. Hafid et al. (2017) have successfully employed a combination of hydrothermal treatment and acid hydrolysis using dilute acid to obtain simple sugars from complex food waste. Several workers have also demonstrated that sodium hydroxide (NaOH) is a best and economical salt for food waste pretreatment. The food waste was treated with strong alkali by employing sodium hydroxide at pH 11 and 28% chemical oxygen demand was solubilized within 24 h (Elbeshbishy et al. 2011). Shanthi et al. (2018) have also obtained around 11% chemical oxygen demand solubilization in 60 mins using sodium dodecyl sulfate based pretreatment method.

3.4 Food Industry Waste Valorization for Bioethanol Production

The food processing industries in the world generate vast amount of waste materials in the form of whey, discarded fruit and vegetables and their residues obtained after processing, molasses, bagasse, bones, flesh, blood, etc. (Arora et al. 2002). These waste materials represent a plethora of potentially reusable materials and energy. Therefore, these waste materials can act as a good feedstock or substrate for a variety of biotechnological processes to produce a number of valuable products. But, there is an urgent need to develop innovative methods for biorefineries to achieve more efficiency with minimum energy consumption to produce value added products from this type of waste. Ethanol is such a product that can be produced from sugar based raw materials by adopting proper pretreatment process and employing appropriate microorganisms. *Saccharomyces cerevisiae* is a most suitable organism for the fermentation process to produce ethanol because of its high osmotic tolerance and ability to grow well in the presence of alcohol and CO_2. This process of ethanol production from food waste can be accomplished in three separate phases (1) suitable pretreatment of waste to prepare broth with assimilable sugars, (2) fermentation process involving efficient microorganisms for ethanol production, and (3) recovery of ethanol. Various critical variables including composition of feedstock, pretreatment process, bioprocess conditions, type of microbial culture along with many process parameters affect the productivity, efficiency, and economy of this bioprocess. In addition, the active pure cultures require extra protection with notable precautions against contamination by wild cultures. Appropriate strategies are also required for extraction and pretreatment of complex carbohydrates present in typical type of food waste to obtain fermentable sugars. Further, batch, fed-batch, or continuous type of fermentation strategies are adopted for the bioconversion of food waste into ethanol. In batch system, the feed substrate along with inoculum culture is added simultaneously into the fermentation vessel at the start of bioprocess and then harvesting of the product is carried out after predetermined time. However, nutrients are supplied constantly at certain intervals of time during the fermentation till the attainment of particular stage in fed-batch system and the whole batch is harvested at once after the expiry of predetermined incubation time to recover products. On the other hand, continuous system involves the regular addition of ingredients in to the fermentation system at one end and simultaneous recovery of products from the system at other end. The high cell density is desired to enhance the efficiency of the fermentation process in continuous system, which can be attained through recycle strategy or immobilization of microorganisms. The fermentative processes for bioethanol production are usually carried at temperature of 25–30 °C and fermentation time of 6–72 h, and ethanol production in the range of 8–14% is generally obtained in this bioprocess. In these bioprocesses, once the fermentation stage is over, the ethanol is recovered from fermentation broth through distillation. Some innovative and alternative approaches to traditional distillation process during recovery of alcohol from harvested broth have proven useful to save energy. Various

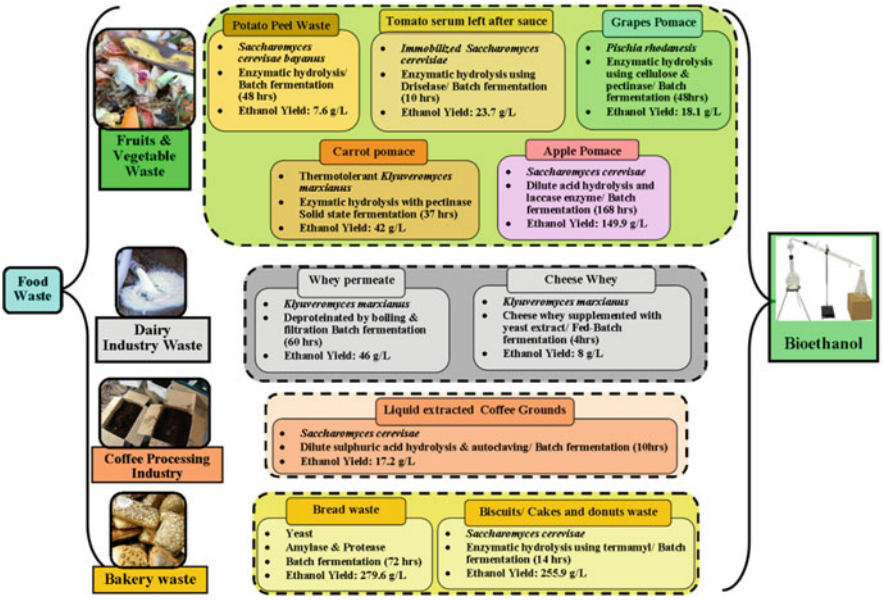

Fig. 3.2 An overview of food industry waste valorization for bioethanol production

previous studies reveal that these upcoming tools will help to cut the processing cost and also lessen the environmental impacts. But, the practical application of many newly developed tools to produce bioethanol is still a big question to answer for the biorefineries. However, few emerging distillation techniques such as membrane-assisted distillation, ohmic-assisted hydro-distillation, etc., have proven beneficial to save energy. Hence, these emerging distillation solutions along with other better up-streaming and down-streaming practices can be adopted to develop an integrated ecofriendly approach to produce ethanol in the upcoming era. An overview of food industry waste valorization for bioethanol production is represented in Fig. 3.2 and discussed in further sections.

3.4.1 Valorization of Fruit and Vegetable Industry Waste

Fruit and vegetable waste is rich in pectin, anti-oxidants, fibers, flavors and colors, carbohydrates, and mineral salts. These substances are considered appropriate to be utilized in biorefineries for their conversion into many useful products like ethanol, gas, and biochemicals, through fermentation. These waste materials can be easily used in bioprocesses by adopting some pretreatment strategies having capability for the conversion of complex materials to available sugars. The environment related parameters have to be kept in consideration before the use or disposal of these waste materials because this type of waste of variable pH also contains elevated values of

biological oxygen demand and chemical oxygen demand along with huge amount of suspended solids.

Amongst food processing industries, potato processing industry is growing at a very fast pace and produces massive waste. Potato waste is characterized by having huge amount of many complex carbohydrates like starch, cellulose, hemicellulose, etc., and these materials serve as a good feedstock for ethanol production (Arapoglou et al. 2010). Wastewater generated during different processing operations in potato industry is opulent in starch, and is also a good substrate for alcohol production (Sanusi et al. 2019). Chohan et al. (2020) have used the potato peel waste of pH 5.78 with 12.25% (w/v) solid loading for ethanol production and 22.54 g/L ethanol with 0.32 g/g yield was obtained at 40 °C. Although the dried tomato pomace has many assimilable sugars but major chunk of tomato pomace produced by industries is still utilized only as feed for livestock. The feasibility of utilizing tomato pomace generated from food processing industry for the production of different products has been investigated in the past, but convincing results could not be obtained in terms of productivity and economics. It has been observed that tomato pomace cannot be used directly to produce bioethanol and typical type of pretreatment is required for its utilization for bioethanol conversion. Hence, tomato pomace extract obtained after supercritical extraction was used for ethanol production and 46.8 g/L ethanol yield was achieved (Lenucci et al. 2013). Carrot pomace was also tested for bioethanol production using solid state fermentation by employing *Kluyveromyces marxianus* and yielded 0.18 g/g of bioethanol production (Yu et al. 2013). Ethanol (0.79 mg/mL) from sweet potato waste was also produced through fermentation by *Saccharomyces cerevisiae* (Gosavi et al. 2017).

Apple juice industry produces 12–20% of apple pomace including seeds, peels, calyx, cores, soft tissues, and stems during processing. Grape pomace is a type of solid waste obtained after extraction of juice in juice and wine industry (Zheng et al. 2012). The fate of pomace from different fruits processing ends up into feed for livestock or sometimes oil is extracted from seeds of few specific fruits. But, the utilization of these waste materials for alcohol production could be an ideal option due to their availability in abundance. Ebner et al. (2014) have obtained 295 L/dry ton yield of ethanol from processed food waste. It was observed that less amount of greenhouse gases are produced in the aforementioned process of ethanol conversion as compared to the process of its production from corn. Apple pomace as a feedstock for ethanol production has also been explored by many workers (Hang et al. 2006), but more research work is required to support its application at commercial scale. Some investigations have also pointed towards its use as a substrate in solid state fermentation for ethanol production (Afifi 2011). The maximum ethanol production of 190 g/kg has been obtained from enzyme treated apple pomace adopting vacuum extraction based recovery process (Parmar and Rupasinghe 2013). The co-fermentation approach using *Saccharomyces coreanus* and *Pichia stipitis* has been implemented for ethanol production using apple pomace based feedstock. This approach is a good example to achieve a synergistic effect in which more than one microorganisms are employed in fermentation process for effective utilization of all sugars present in the feed stock. This synergism between two microbes has enhanced

the bioethanol bioconversion rate by 8.0 g/L (Jeong et al. 2012). This approach was also validated by Kumar et al. (2014) in one study by employing both bacteria (for degradation of starch) and yeast (for ethanol production) cultures for bioethanol production from cassava sago waste. Hence, the strategy of co-fermentation has made this alcohol production process more energy efficient because it has eliminated the requirement of any prior treatment for fruit waste based substrate. In another approach, 190 g of alcohol is produced from 1 kg of pretreated apple pomace using enzymes through solid state fermentation. Hegde et al. (2018) have reported the ethanol production from grape pomace (18–50 g/kg), tomato serum (47 g/kg), carrot pomace (37 g/kg), and household food waste (108 g/kg) through solid state fermentation process.

Banana fruit waste in the form of rotten pulp and peels were also utilized as feedstock for the bioethanol production by many researchers (Guo et al. 2018; Alonso-Gómez et al. 2019). The critical aspect for the use of banana waste in this process is the requirement of suitable pretreatment strategy for the hydrolysis of lignin that acts as a big hurdle in ethanol bioconversion process. Hossain et al. (2011) have used the banana waste after saccharification to obtain bioethanol through fermentation using *Saccharomyces cerevisiae* under optimized conditions and 4.1–7.1% alcohol yield was obtained. Jahid et al. (2018) have also obtained the maximum bioethanol production of 6.3 g/L from banana peels used for fermentation after pretreatment through enzymatic hydrolysis followed by acid hydrolysis. The effect of pH on acidification and fermentation process using fruit and vegetable based waste was also explored by Zheng et al. (2015). Stable ethanol yield of 30% has been obtained at a pH range of 3.0–4.0 and it has been concluded that higher ethanol is produced if higher amount of water is present in the food waste substrate. Sarkar et al. (2019) have also obtained an ethanol yield of 23.6% from dried banana peel waste under optimized conditions of temperature and pH using isolated ethanol tolerant bacteria *Enterobacter* sp. *EtK3*. Hence, it can be concluded that banana waste is one of the potential candidate to be used for ethanol production in modern biorefinery.

Citrus fruits are one among the most consumed fruits in the world. Globally, 50.2 million tons of oranges are produced every year (Cypriano et al. 2018). Orange juice production gives many types of waste materials such as orange peels, floater from citrus pulp, internal sections of tissues and fibers, etc. The citrus waste possesses high levels of sugars, celluloses, hemicelluloses, pectins, and proteins. Rotten citrus fruits and allied waste materials are suitable for production of second-generation fuels. These waste products otherwise clog the floatation tank if dumped untreated and also create havoc due to the growth of deadly fungus and bacteria. Citrus fruit waste with plentiful sugars is a suitable candidate for the production of ethanol through fermentation. The fruit waste from most grown citrus fruit varieties (*Citrus limetta* and *Citrus sinensis*) contains 17–21 mg/mL of glucose (Girish et al. 2014). The co-fermentation technique is the most competent approach due to the conversion of unavailable sugars like pentoses by the newly developed strain and thereby promoting the ease of fermentation process. Girish et al. (2014) have used the citrus waste after pretreatment with enzymes (pectinases, cellulases, and

hemicellulases) for the production of bioethanol through fermentation at 35 °C using single culture of yeast and aerobic conditions are maintained for initial 5 days and followed by anaerobic environment from sixth to ninth day. The produced bioethanol was purified by distillation process. Boluda-Aguilar and López-Gómez (2013) obtained highest ethanol yield of 60 L/1000 kg from fresh lemon peel biomass with prior pretreatment with steam explosion and enzymatic hydrolysis. The valorization of citrus peel waste into bioethanol has also been carried out by Zema et al. (2018).

Another major fruit waste comprises *Ananas comosus* (pineapple) and about 50% of its total produce is wasted due to poor and inadequate storage facilities and post-harvesting damages. Most of the pineapple waste consists of leafy shoots, which is used as feedstock to produce bioethanol. Common pretreatment strategies are used to break the complex materials of this waste into simple assimilable forms for yeast fermentations to produce ethanol and other products. It is advocated that pineapple waste based biorefineries can produce more bioethanol due to the presence of higher sugar content (Hossain and Fazliny 2010). Gosavi et al. (2017) obtained an ethanol yield of 0.090% (0.90 mg/mL) from pineapple waste via fermentation process using *Saccharomyces cerevisiae*. Conesa et al. (2017) have produced ethanol from pineapple waste by pretreating with enzymes like celluloses, hemicelluloses, and pectinases followed by fermentation of hydrolysate with yeast culture for 11 days. Chintagunta et al. (2017) have also used leaf waste from pineapple for its bioconversion in to ethanol, and obtained a maximum yield of 7.12% (v/v) under optimized conditions. In another study, solid pineapple waste was also used for ethanol production and highest ethanol production of 5.4%, (v/v) was obtained by employing saccharification and fermentation process simultaneously as compared to other processes, where, only 4.7% (v/v) of ethanol was obtained through direct fermentation, and 4.9% (v/v) of ethanol was recovered when saccharification was followed by fermentation process (Gil and Maupoey 2018). Jahid et al. (2018) have achieved 3.59 g/L of bioethanol after 20 h of fermentation using *Saccharomyces cerevisiae* from pineapple peels hydrolysate obtained through enzymatic pretreatment with xylanase.

Pomegranate (*Punica granatum*) is another popular fruit due to its high nutritive value. The major load of its waste is generated by peels which are rich in sugar content, thus this waste can be used for the production of ethanol. Demiray et al. (2018) have used two yeast stains, namely *Saccharomyces cerevisiae* and *Pichia stipitis* under co-fermenting strategy to produce ethanol from the sugars present in pomegranate waste hydrolysate obtained after pretreatment with acid hydrolysis. Demiray et al. (2019) have recommended in his study that *Kluyveromyces marxianus* is a microbe of choice for bioethanol production from pomegranate peels with maximum ethanol concentration of 7.20 g/L. Mango fruits, being prone to rapid spoilage, deteriorate easily during storage. The waste generated from spoiled or rejected mangoes possess around 18–20% of fermentable sugars, and this waste is also a suitable substrate for its bioconversion to ethanol. *Saccharomyces cerevisiae* and *Kluyveromyces marxianus* were successfully used for the fermentative

production of ethanol (49%) from mango waste with 80% of process efficiency (Buenrostro-Figueroa et al. 2018).

Jackfruit (*Artocarpus heterophyllus*) is the sweetest tropical fruit having fructose, sucrose, and glucose in every part. The seeds of this fruit contain about 12.78% starch and hence these seeds can be a good feedstock for bioethanol bioconversion after appropriate pretreatment strategy (Nuriana and Wuryantoro 2015). The jackfruit waste and its seeds were used for ethanol production by Gosavi et al. (2017) by fermenting its hydrolysate obtained after pretreatment through acid or enzymatic hydrolysis, and 0.045% ethanol yield was obtained in this process using yeast culture (Gosavi et al. 2017). Dharamveer et al. (2019) testified the pretreatment of jack fruit peel waste through chemical and enzymatic saccharification and yielded 31 g/L of sugars. The pretreated fruit waste was used as substrate in fermentation involving *Saccharomyces cerevisiae* and highest bioethanol (3 g/L) was obtained under optimized conditions.

The waste from date palm is a rich source of fermentable sugars and can be exploited for ethanol production through suitable bioprocesses (Boulal et al. 2016). The ethanol bioconversion was carried out using pretreated hydrolysate of palm waste through yeast fermentations after 72 h of incubation at 32 °C (Sivakumar 2012). Besides the aforementioned fruit waste, the waste of papaya and grapes has also been used for ethanol production by some workers (Janani et al. 2013; Jahid et al. 2018). Indian water chestnut waste was also used for production of ethanol (0.045%) through fermentation by *Saccharomyces cerevisiae* (Gosavi et al. 2017). Papaya waste was also used for its conversion into bioethanol through saccharification and fermentation approach by Bhuvaneswari and Sivakumar (2020). A detailed insight into food industry waste valorization into bioethanol has been shown in Table 3.1.

3.4.2 Valorization of Dairy Industry Waste

Whey, one of the main waste products of cheese and dairy industry, is rich in lactose and proteins. Its disposal is a major problem for dairy industry because it causes environmental hazards due to its high biological oxygen demand and chemical oxygen demand. However, its valorization for the production of bioethanol by employing lactose utilizing microorganisms is a good alternative to address the pollution problems caused by its disposal.

The yeast strains, namely *Kluyveromyces* sp., and *Candida* sp. are generally employed for ethanol production from cheese whey. These microbial strains, which are capable of hydrolyzing lactose, are beneficial in direct fermentation of whey. However, sometimes the co-fermentation approach using two or more organisms becomes mandatory for efficient bioconversion of sugars into bioethanol. Some yeast strains like *Candida* and *Kluyveromyces* are proficient for direct lactose metabolism, but *Saccharomyces cerevisiae* is not able to utilize lactose. Hence, lactose is required to be hydrolyzed into glucose and galactose if efficient

Table 3.1 An insight into food industry waste valorization for bioethanol production

S. no.	Type of food waste	Pretreatment methods	Microorganisms	Type of fermentation/ process conditions	Ethanol yield	References
1.	*Fruit and vegetable industry waste*					
	Potato starch	Enzymatic hydrolysis	*Saccharomyces cerevisiae*	Batch/aerobic, 30 °C, 200 rpm	19 g/L	Rani et al. (2010)
	Potato peel waste	Enzymatic hydrolysis	*S. cerevisiae var. bayanus*	Batch/30 °C, pH 5.0,100 rpm	7.6 g/L	Hossain et al. (2018)
	Potato processing wastewater	Enzymatic hydrolysis	*S. cerevisiae*	Batch	3 g/L	Mironescu (2011)
	Grape pomace	Enzymatic hydrolysis	*Pichia rhodanensis*	Batch, 30 °C	18.1 g/L	Korkiel and Janse (2002)
	Carrot pomace	Enzymatic hydrolysis	*Kluyveromyces marxianus*	Solid state/ 42 °C, pH 5.0, 680 rpm	37 g/L	Yu et al. (2013)
	Kitchen waste	Enzymatic hydrolysis	*S. cerevisiae*	Batch/30 °C, 150 rpm	32.2 g/L	Hafid et al. (2015)
	Household food waste	Enzymatic and microwave heat treatment	*S. cerevisiae*	Batch/30 °C, 100 rpm	42.8 g/L	Matsakas et al. (2014)
	Potato chips	Enzymatic hydrolysis	Commercial yeast *Super Camellia*	Continuous/ 32 °C, 5 rpm	32 g/L	Moukamnerd et al. (2013)
	Apple pomace	Dilute acid hydrolysis and enzymatic hydrolysis	*S. cerevisiae*	Batch/25 °C	149.9 g/L	Parmar and Rupasinghe (2013)
	Pineapple and banana peels	*A. niger* assisted hydrolysis of oven dried and ground waste material	Consortium of *Aspergillus niger* and *S. cerevisiae*	Batch/28 °C, pH 5.5	32–49 g/L	Shilpa and Malhotra (2013)

(continued)

Table 3.1 (continued)

S. no.	Type of food waste	Pretreatment methods	Microorganisms	Type of fermentation/ process conditions	Ethanol yield	References
	Banana peels from a processing plant	Drying, grinding, steam treatment, and acid hydrolysis	*S. cerevisiae*	Batch/30 °C, pH 5–5.5, 200 rpm	0.45 g/g (wet food waste)	Gebregergs et al. (2016)
	Sweet potato waste	Enzymatic hydrolysis	*S. cerevisiae*	Batch/30 °C, 72 h	79 g/L	Wang et al. (2016)
	Tomato serum left after sauce production	Enzymatic hydrolysis	*S. cerevisiae*	Batch/10 h	23.7 g/L	Lenucci et al. (2013)
2.	*Dairy industry waste*					
	Whey permeate	Deproteinated through boiling and filtration and concentrated with vacuum evaporation	*Escherichia coli W*	Batch/30 °C, pH 4.6	44 g/L	Pasotti et al. (2020)
	Cheese whey	Cheese whey supplemented with yeast extract	*K. marxianus*	Fed-batch/4 h	8 g/L	Hadiyanto et al. (2014)
3.	*Beverage industry waste*					
	Cola-based sweet beverage	The sweet beverage medium degassed before use	*S. cerevisiae var. Windsor*	Batch/30 °C, 8 h	55 g/L	Isla et al. (2013)
4.	*Spent coffee grounds*					
	Lipid extracted coffee ground waste	Dilute sulfuric acid hydrolysis along with autoclaving	*S. cerevisiae*	Batch/30 °C	17.2 g/L	Rocha et al. (2014)

5.	*Bakery waste*					
	Bread waste	Enzymatic hydrolysis	Yeasts (name not available)	Batch/35 °C, 150 rpm, 72 h	279.6 g/L	Pietrzak and Kawa-Rygielska (2014)
	Bread crust	Enzymatic hydrolysis	Commercial yeast *Super Camellia*	Fed-batch/ 32 °C, 5 rpm, 30 h	0.27 g/g (wet food waste)	Moukamnerd et al. (2013)
	Bakery waste (bread, biscuits, buns, cakes, flour, etc.)	Dilute acid hydrolysis and amylolactic enzymatic hydrolysis	*S. cerevisiae*	Batch/35 °C, 150 rpm, 96 h	248 g/kg dry bread basis (dilute acid hydrolysis) and 313 g/kg dry bread basis (enzymatic hydrolysis)	Torabi et al. (2020)

microorganisms like *S. cerevisiae* are used for ethanol fermentation (Cot et al. 2007). Murari et al. (2019) have demonstrated the fermentative production of ethanol from dairy byproducts (cheese whey and whey permeate) by exploiting the yeast *Kluyveromyces marxianus* URM 7404 and obtained greater ethanol yield (10 g/L after 18 h) from whey permeate under anaerobic conditions than cheese whey (8 g/L after 10 h) under same process conditions. Several molecular biology related methodologies have been used in the past to construct genetically modified strains of *S. cerevisiae* and other microorganisms capable of metabolizing lactose (Farahnak et al. 1986). These approaches include fusion of *S. cerevisiae* with some lactose metabolizing strain *or* cloning of genes of β-galactosidase and lactose permease from *K. lactis* into *S. cerevisiae* (Silva et al. 2010; Risner et al. 2020; Álvarez-Cao et al. 2020; Mervat et al. 2020) or creating *S. cerevisiae* cells capable of releasing β-galactosidase into the culture medium or lysis of recombinant cells by over expression GAL4 factor (Kumar et al. 1992). But, these processes involving modified *S. cerevisiae* cells exhibited lower yield of bioethanol. However, an efficient approach employing a tailored strain of *S. cerevisiae* having expression of gene LAC4 and LAC12 for β-galactosidase and lactose permease, respectively, has proven very fruitful in the past for production of ethanol from lactose in a continuous culture process. A high productivity of ethanol with bioconversion theoretical yield of 60% was achieved in this process. In other research advancement, Shen et al. (2019) have produced ethanol from delactosed whey permeate by developing a novel bacterial strain (*Corynebacterium glutamicum* JS95) through the introduction of lacSZ operon from *Streptococcus thermophilus*. This constructed and adapted strain of *Corynebacterium glutamicum* JS95 grew recklessly in lactose enriched medium under anaerobic conditions and its resting cells were able to convert 100 g/L lactose into 46.1 g/L of ethanol with a theoretical yield of 88%. These types of developments provide new perceptions for building new large-scale effective systems using efficient strains of microorganisms for ethanol production from whey without any pretreatment. Silva et al. (2010) have used a recombinant strain of *S. cerevisiae* having activity of lactose permease and ß-galactosidase for alcoholic fermentation using media having deproteinized cheese whey and corn steep liquor. In this process, 7.4% (v/v) of bioethanol with 1.2 g/L/h productivity was obtained using lactose (150 g/L). This investigation has also proved the robustness and stability of the culture with high viable rate of 97%. Some other workers have also used yeast strain of *Kluyveromyces marxianus* for ethanol bioconversion from whey (Sooch and Singh 2002; Singh et al. 2002; Krishnan et al. 2020; Pendón et al. 2020). The genetically engineered bacterium *Escherichia coli W* was also exploited for ethanol fermentation and 30–40 g/L yield was obtained using concentrated whey permeate as feedstock in this bioprocess (Pasotti et al. 2020).

3.4.3 Valorization of Instant Noodle Waste

Instant noodles are one of the most consumed ready to eat food with huge demand. The first instant noodle brand "Chikin Ramen" was brought into market in 1958 after its invention by Mr. Momofuku Ando from Japan. Thereafter, Nissin Foods (a company started by him), invented the first "Cup Noodle" in 1971 and today, through many revolutionary technological developments, a wide variety of noodles are available with different flavors and textures in every corner of the world. A lot of liquid and solid waste generated from noodle processing plants during different operations is dumped without any further utilization in many countries and this action causes many pollution problems. In addition, noodle serving spots also generate huge amount of solid and liquid waste. Various strategies can be beneficial for the valorization of this nutrient rich organic waste to synthesize value added products like biofuels, and research efforts in this direction are undertaken by some researchers (Karmee and Lin 2014; Yang et al. 2014; Karmee 2016). Karmee (2017) demonstrated the conversion of residual lignocellulose and starch waste material into bioethanol through fermentation process after treating through enzymatic hydrolysis. Instant noodle waste is generally concentrated into fat and starch. The fat content from noodle waste is processed for biodiesel production, and the residual starch content is subjected to enzymatic hydrolysis using starch degrading enzymes to release fermentable sugars. The starch hydrolysate was then used for ethanol production using *Saccharomyces cerevisiae* K35 through fermentation process (Yang et al. 2014). More than 96% ethanol conversion rate was achieved using noodle waste in one study. Approximately 150 tons of udon noodle waste has been annually generated in Takamatsu city of Japan. In this regard, a company named "Chiyoda" in alliance with noodle producers and restaurant owners is able to transform this waste material into bioethanol and methane gas. In this process, 10% of noodle waste was transformed into bioethanol and residual was used for methane production.

Huge amount of waste water containing organic compounds and starch residues is also generated during production of fermented rice noodles. This waste water has also been exploited for bioethanol production by Siripattanakul-Ratpukdi (2012). The alcohol fermentation of waste water was carried out using immobilized *S. cerevisiae* after pretreating the starch present in water with acid hydrolysis to release sugars. Yang et al. (2014) have also reported the production of bioethanol after converting the starch residues into glucose by α-amylase and glucoamylase. The optimal pretreatment conditions were determined for the saccharification process for starch residues and the bioethanol conversion was carried out using *Saccharomyces cerevisiae* K35 in solid state fermentation mode. The highest ethanol concentration of 61.1 g/L with 1.7 g/L h productivity was obtained in this process. The oil extracted during the pretreatment process was also utilized further as feedstock for the conversion of high quality biodiesel via chemical route using KOH and H_2SO_4. It has been concluded from various research studies that liquid or solid waste from noodle industry can be a promising substrate for biorefineries for the production of bioethanol.

3.4.4 Valorization of Spent Coffee Ground Waste

Coffee is among the largest agricultural produce used for beverages. According to one estimate, the global production of coffee is around 16.34 billion pounds per annum (Pumphrey 2007). The coffee ground waste can be used as fuel pellets, garden fertilizer, and feedstock for ethanol production. The coffee pulp is a rich source of proteins (8.25%) and sugars (23–27%). The coffee pulp hydrolysate obtained through acid hydrolysis using diluted sulfuric acid contains a variety of pentose and hexose sugars (xylose, arabinose, fructose, glucose, sucrose, and maltose) in variable concentration ranging from 3.23 g/L to 11.26 g/L (Urbaneja et al. 1996). Choi et al. (2012) evaluated the production of bioethanol from residual coffee waste. They demonstrated that the carbohydrates from coffee processing waste can be fermented by *Saccharomyces cerevisiae* after hydrolysis treatment for efficient ethanol production (15.3 g/L) with final yield of 87.2%. Gouvea et al. (2009) have reported that coffee husks in various forms (whole, ground, or their aqueous extract) are suitable feed stocks for ethanol bioconversions using *S. cerevisiae* and a maximum yield of 13.6 g/L ethanol was obtained in the process. The left over coffee grounds on average contain approximately 15% oil that can be converted to biodiesel through transesterification technique (Gui et al. 2008). The solid residual waste can also be used as compost or substrate to produce fuel pellets and ethanol (Sendzikiene et al. 2004). Dadi et al. (2017) have successfully undertaken the conversion of various coffee co-products into bioethanol after pretreating with enzymatic and acid hydrolysis. They have adopted a co-fermentation strategy by employing two yeast strains, namely baker's yeast and lignocellulosic yeast in fermentation process for maximum utilization of waste. The superior quality ethanol was recovered by pervaporation and maximum yield of 52 g/L from residual coffee waste and 132 g/L from coffee husk was obtained in the fermentation processes. Kefale et al. (2012) have tested the possibility of ethanol production from coffee pulp hydrolysate obtained by acid hydrolysis using *S. cerevisiae*. In this process, 7.4 g/L of bioethanol was obtained after incubating the hydrolysate at 30 °C for 24 h. Coffee spent grounds after pretreatment through acid or enzymatic hydrolysis yielded a higher concentration of ethanol (50 g/L) by anaerobic digestion (Batista et al. 2020). In another process, ethanol yield of 23 g/L was obtained through fermentation by microalgae *Scenedesmus acutus* using pretreated spent coffee amalgams (Pereira et al. 2020).

3.4.5 Valorization of Other Types of Food Industry Waste

There are many other types of food waste and agro-based waste materials which possess a good potential to be used as feedstock for ethanol production like sugarcane bagasse, corncobs, rice straw, meat and fish industry waste, etc. Sugarcane bagasse, a residual product obtained after the sugar processing, was used for

bioethanol conversion and an optimized method using enzyme loading of 100 U/g along with yeast *S. cerevisiae* was developed to carry simultaneous saccharification and fermentation process. The maximum ethanol production of 4.88 g/L was obtained at 39 °C in this bioprocess (Jugwanth et al. 2020).

A lot of wheat is also damaged every year during storage due to one or the other reason, and this waste can be used for bioethanol bioconversion using appropriate methods. It has been suggested that agrowaste biomass from barley can also be utilized for ethanol conversion (Kim and Dale 2004). Globally, rice straw has been considered as the most plentiful lignocellulosic waste material and it was reported that this enormous waste possesses ability to produce 205 billion liters of ethanol each year (Karimi et al. 2006). The bioethanol concentrations of 31.5 g/L with 0.42 g/g yield was obtained from rice straw hydrolysate by *Saccharomyces cerevisiae* in a continuous bioprocess coupled with a membrane fermenter (Zahed et al. 2016). The utilization of maize corn for its bioconversion into bioethanol is a common practice mainly in the USA and around 50% of its mass is lost as waste in the form of cobs, shells, and stovers. Since, this corn waste contains all the required nutrients, hence, this can be used as a feedstock for the production of bioethanol after adopting efficient pretreatment strategies (Panahi et al. 2020). Torabi et al. (2020) have investigated the production of ethanol from waste wheat bread by hydrolyzing it with enzymes and dilute hydrochloric acid. The theoretical ethanol yield of 86.9% and 83.0% was obtained from these dry bread residues treated through dilute acid hydrolysis and enzymatic hydrolysis, respectively.

The olive oil industry generates a huge amount of solid residues that can act as a promising lignocellulose based feedstock for bioethanol production (Abu Tayeh et al. 2014 2016). Georgieva and Ahring's (2007) have obtained an ethanol yield ranging from 0.49 to 0.51 g/g from olive mill solid waste following a two-step strategy using enzymatic hydrolysis and fermentation process. Olive stones were also used to obtain 0.25 g/g of ethanol yield by Cuevas et al. (2009). Ballesteros et al. (2001) have obtained a theoretical solid state fermentation yield of 65% under optimal treatment temperature in solid state fed-batch fermentation system. Senkevich et al. (2012) have used the thermochemical pretreatment method for treatment of olive mill solid waste and obtained the maximum ethanol yield of 49.59 mL per kg during fermentation process. Abu Tayeh et al. (2014) have used the pulp from olive oil industry for solid state fermentation and the highest bioethanol yield of 3 g/100 g of dry olive mill waste was attained. Herreo et al. (2016) have adopted the acid based treatment method followed by enzymatic hydrolysis for olive mill solid waste pretreatment for obtaining an ethanol yield of 7.34 g/kg olive mill solid waste. Battista et al. (2016) have achieved the maximum ethanol concentration of 9 g/L using both olive mill waste water and solid waste after following basic pretreatment techniques. The ethanol yield of 0.46 g/g was attained after pre-saccharification of extracted olive pomace followed by SSF (solid state fermentation) strategy (Fernandes et al. 2016). Recently, Azaizeh et al. (2020) summarized an extensive overview on valorization of solid waste from olive oil industry for the extraction of bioethanol and other value added chemicals.

In meat and fish processing industry, the main processed products are chops, fillets, and mince. The industrial processing operations in meat industry produce huge quantities of protein enriched residuals in the form of heads, carcasses, bones, skin, blood, viscera, feathers, and hooves. The silage and rendering process along with enzyme hydrolysis is used for the treatment of fish and meat residuals to prepare a suitable feedstock for biorefineries for ethanol production. Shamsul et al. (2016) have examined the potential use of animal waste for ethanol production after its co-digestion with agricultural waste under anaerobic conditions.

3.5 Patent Status on Valorization of Food Industry Waste for Ethanol Production

The bioconversion of food industry waste in to ethanol has been successfully achieved through various innovative strategies by researchers and many patents have been granted on the same throughout the world. Rim et al. (2007) have disclosed an efficient method for preparing ethanol from food waste through pretreatment using enzymatic hydrolysis followed by fermentation using *Saccharomyces cerevisiae*. Offerman et al. (2009) have invented a new method, apparatus, and kits for producing ethanol and other alcohols from food waste involving another microorganism in addition to yeasts that reduce ferric iron to ferrous iron to enhance the efficiency of the fermentation and the yield of alcohol. Widmer et al. (2011) have developed a new method of pretreatment of citrus waste for ethanol production by breaking the cell structure and removal of inhibitory peel oil components like limonene. This method also demonstrates the saccharification of waste with enzymes before its fermentation to produce ethanol. Stewart (2012) has disclosed a process in his invention for the fermentative production of ethanol from various cellulosic feedstock materials. This invention also provides method for ethanol recovery from highly viscous fermented citrus waste biomass. Stewart et al. (2013) has also designed a method for production of bioethanol using heat and enzyme hydrolyzed citrus fruit waste. The limonene content from the fruit waste was also reduced to facilitate the bioprocess. Song (2014) has developed a process for fermentative production of ethanol by utilizing pretreated watermelon seeds with high production yield under anaerobic conditions. The fine ground seeds were pretreated for the removal of linoleic acid and used for ethanol production by employing *Saccharomyces cerevisiae*, at 25–35 °C for 5–15 days under agitation conditions. In another invention, valorization of food waste materials or their mixture from different industries was carried out to produce ethanol using saccharification treatment before fermentation and obtained a good ethanol yield of 95% (Lehr 2015). Grillo et al. (2018) have developed a system for producing food-grade ethanol from food waste procured from restaurants, bakery, grocery stores, commercial kitchens, food vendors, etc. The hydrated food waste was processed into flowable slurry before fermentation and then alcohol was recovered by distillation. A list of selected

patented processes on food industry waste valorization for bioethanol is given in Table 3.2. It can be clearly depicted from the aforementioned data on patents for conversion of food industrial waste into bioethanol that there is a vast scope of food industrial waste valorization into bioethanol. Therefore, the food industry waste can be converted into useful products through various novel waste valorization strategies, thus rendering the environment free from pollutants generated from industrial waste.

3.6 Conclusions and Future Prospects

Food waste disposal without its valorization leads to a loss of many resources utilized for food production like water, land, material, and power in addition to the loss of many nutrients present in it. Its discharge or disposal without treatment also causes many environmental and societal problems. Food waste generated through several operations in different food industries possesses many prospective for its conversion into a plethora of valuable goods. Food waste valorization offers many possibilities for the amalgamation of chemicals and intermediates like alcohols, which are able to replace fossil-based products. Thus, valorization of food industry waste is necessary in the present era to render the environment free from threats caused by disposal of solid or liquid waste. Several integrated biorefineries working in this direction still need efficient and economical methods for pretreatment, fermentation, and downstreaming according to the industry requirements. Development of appropriate technical advancements involving interdisciplinary approach may help in building a cost-effective solution for food waste biorefinery by spanning the existing gaps. Recovery of various bio-based feedstocks from fruits and vegetable waste industry, dairy industry, instant noodle waste industry, and spent coffee ground has proven beneficial for the conversion of waste into ethanol by employing efficient microorganisms through several bioprocesses in biorefineries. Despite the fact that food waste is of low cost or of no cost, but the labor and transport costs are of greater concern to fetch them to biorefineries. In addition to it, the time-consuming bioprocesses with low productivity are another hindrance on the way to success for biorefineries. To encounter these challenges, the present day research should be focused to obtain different value added products from biorefineries through efficient production and recovery processes. To achieve this, some innovative strategies are required to construct specific microorganisms or enzymes with tailor made properties using advanced molecular and bioinformatics tools. Hence, the multidisciplinary approach through biorefineries involving efficient microorganisms may prove beneficial to achieve the desired cost-effective and sustainable food waste management system.

Research efforts on food industry waste valorization originating from laboratories to scale-up should be strongly supported and encouraged by industries and government for the success of biorefineries, and this should also be adopted with same zeal by industries.

Table 3.2 Summary of patents on food industry waste valorization for bioethanol

S. no.	Patent/application number	Title of patent	Year of filling/ grant	References
1.	KR20090001116A	Method for producing ethanol by using kitchen refuse	2007	Rim et al. (2007)
2.	US 20050153410 A1	Integrated process for producing "clean beef" (or milk), ethanol, cattle feed, and biogas/bio-fertilizer	2008	Hallberg et al. (2008)
3.	US 20080026442 A1	Ethanol production from biological waste	2010	Hogen et al. (2010)
4.	US 7,879,379 B1	Method of pretreating citrus waste	2011	Widmer et al. (2011)
5.	WO 2011/147032	Integrated method of biological valorization of organic waste in an agricultural environment	2011	Dufour et al. (2011)
6.	US 20090291482 A1	Ethanol production from citrus waste through limonene reduction	2012	Hillyer (2012)
7.	US 20100167367 A1	Ethanol recovery system for cellulosic feedstocks	2012	Stewart (2012)
8.	US 20080213849 A1	Ethanol production from solid citrus processing waste	2013	Stewart et al. (2013)
9.	US 8,367,378 B2	Process for producing sugars and ethanol using corn stillage	2013	Balan et al. (2013)
10.	US 20120034667 A1	Method for recovering and producing ethanol and oil	2014	Kiuchi et al. (2014)
11.	US 20120045810 A1	Method for production of bioethanol using watermelon seeds	2014	Song (2014)
12.	FR2987842A1	Optimized process for the valorisation of bio-oils to hydrocarbon fuels	2014	Daudin et al. (2014)
13.	US 2014/023591.0 A1	Integrated process for the production of biofuels from solid urban waste	2014	Bosetti et al. (2014)
14.	US 20110039318 A1	Method and apparatus for transforming waste into fuel ethanol	2015	Lehr (2015)
15.	US20120071697A1	Method for producing ethanol	2015	Ichikawa (2015)
16.	US 20140308715 A1	Microbial conversion of sugar acids and means therein	2016	Hilditch et al. (2016)
17.	US 20090291481 A1	Removal of fermentation inhibiting compounds from citrus waste using solvent extraction and production of ethanol from citrus waste	2016	Hillyer (2016)

(continued)

Table 3.2 (continued)

S. no.	Patent/application number	Title of patent	Year of filling/ grant	References
18.	US 20170142994 A1	Process for obtaining honey and/or flour or coffee from the pulp or husk and the mucilage of the coffee bean products	2016	Velez et al. (2016)
19.	US 20140273106 A1	Efficient process for producing saccharides and ethanol from a biomass feedstock products	2017	Okeke et al. (2017)
20.	US2018305648A1	Systems and methods for distilling food-grade ethanol from food waste	2018	Grillo et al. (2018)
21.	US 20180273569 A1	Processing biomass products	2018	Medoff et al. (2018)

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Competing Interests All the authors declare that they have no competing interests.

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Chapter 4
Development of Pretreatment of Lignocellulose for Bioenergy

Jian Liu

Abstract The excessive use of fossil fuels has caused serious shortages and environmental problems. Lignocellulosic materials are abundant, cheap, and carbon-neutral renewable biological resources used to produce biofuels and bio-based products. In this review, various pretreatment studies that enhance the enzymatic hydrolysis of lignocellulosic materials are introduced. Different pretreatment process and their synergistic effect on the enzymatic breakdown of lignocellulosic materials are also explained. The studies indicate that a combination of effective pretreatments is a potential strategy for an improved enzymatic breakdown of biomass for further conversion to biofuel and value-added compounds.

Keywords Lignocellulose · Pretreatment · Bioenergy · Saccharification

Abbreviations

5-HMF	5-hydroxymethylfurfural
AP	Alkaline pretreatment
BM	Ball milling
BP	Biological pretreatment
CS	Combined severity
LCB	Lignocellulosic biomass
LHW	Liquid hot water
SE	Steam explosion
SEM	Scanning electron microscopy

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4.1 Introduction

Lignocellulose is mainly composed of cellulose, hemicellulose, and lignin, as well as a small amount of protein, pectin, extracts, and ashes (Pereira and Costa 2017). The conversion of lignocellulosic biomass (LCB) to biofuels and different bio-chemicals typically involves three steps, i.e. pretreatment of biomass to remove recalcitrance, enzymatic hydrolysis for the generation of fermentable sugars followed by fermentation for the generation of biofuels such as ethanol and biomethane (Mohapatra et al. 2017). The closely associated structural packaging of hemicellulose and cellulose with lignin is one of the prime causes of low enzymatic saccharification (Alvarez-Vasco and Zhang 2017). Thus there is need of a pretreatment step to breakdown the biomass recalcitrance. The ideal biomass pretreatment process can destroy the stubborn lignin and make cellulose and hemicellulose more accessible to hydrolyzing enzymes (Liu et al. 2011). Several pretreatment methods have been developed for efficient delignification of the lignocellulosic materials, thus enhancing the reactivity of cellulose and hemicelluloses molecules with cellulases and hemicellulases, respectively, to improve enzymatic saccharification (Martin et al. 2008). The operating parameters and pretreatment intensity of different pretreatment technology are key to the breakdown and removal of hemicellulose and lignin from the biomass. In general, the depolymerization of polysaccharides and lignin is closely associated. Pretreatment includes physical (grinding, shredding, and milling), chemical (dilute or concentrated alkali, dilute/strong acid, organic solvent, and oxidants), physical chemistry (steam pretreatment/automatic hydrolysis, hydropyrolysis, and wet oxidation), biological process, or a combination of them (Millett et al. 1976). Some of the pretreatment methods along with their limitations and advantages are discussed below.

4.2 Chemical Pretreatment

4.2.1 Ozonolysis

Naturally the LCB has a very complex structure consisting of interlocked complex network of lignin, hemicellulose, and cellulose providing it in-built recalcitrance toward natural hazardous and to enzymatic hydrolysis. Ozone (O_3) based pretreatment is capable of efficient delignification and hemicellulose removal from lignocellulosic materials. It significantly reduces the lignin content. However, hemicellulose in biomass can be slightly removed. The major advantage associated with ozone based pretreatment is that it can be performed at standard room temperature and pressure (Quesada et al. 1999). As one of the disadvantages, ozone is very costly and during the ozone based pretreatment large amount of O_3 is needed that makes the overall process cost very high, thus limits its application at large scale (Shi et al. 2015).

4.2.2 Acid Pretreatment

Inorganic acids are commonly used catalysts for biomass pretreatment (Yan et al. 2009). The concentrated acid pretreatment can result in high sugar yields even at very low temperature (Rabemanolontsoa and Saka 2016). After one-step hydrolysis with concentrated acid, the pentose and hexose are often degraded easily into furfural and 5-hydroxymethylfurfural (5-HMF). These furfural compounds are potent fermentation inhibitor; thus, generation of these compounds during pretreatment can potentially inhibit the subsequent fermentation step. Some of the other disadvantages of concentrated acid pretreatment include high acid consumption and high energy consumption during unused acid recovery. Further the concentrated acid based methods are corrosive to equipment, thus require sophisticated equipment and also the washout released is toxic to the environment. The major advantage associated with dilute acid pretreatment as compared to the concentrated acid is the low consumption of acid.

It was reported that eight acids with different *pKa* were applied in the pretreatment, as shown in Fig. 4.1 (Liu et al. 2010). Among the acid catalysts, HCl gave the highest sugar yield at 0.1% acid concentration. The rate of acid hydrolysis of amorphous hemicellulose is faster than that of crystalline cellulose because of their inherent characteristics. It is reported that specific surface area and pore volume of rice straw significantly reduced after acid pretreatment (Chen et al. 2011), as shown in Table 4.1.

How do the acids work in the pretreatment process? There is a report on scanning electron microscopy (SEM) analysis indicating the surface properties of native sugarcane bagasse, acid pretreated sample, and enzymatically digested biomass (Fig. 4.2). The acid pretreated bagasse fiber showed small pores on the surface and fiber disruption, which revealed the efficacy of the acid pretreatment process (Fig. 4.2b). After the enzyme treatment, the analysis showed maximum disintegration and numerous holes in the cell wall (Fig. 4.2c). The exposure of cellulose through structural alteration by acid is the crucial factor in the hydrolysis of the remained cellulosic fraction.

4.2.3 Alkaline Pretreatment

In biorefineries, alkaline pretreatment (AP) is mainly used to reduce the alkaline pretreatment causes, lignin removal, swelling of LCB fiber, and reduction in crystallinity as well as degree of polymerization. Overall, the accessibility of enzyme to polysaccharide is improved (Chong et al. 2018). The alkaline pretreatment of biomass is derived from soda pulp. $Ca(OH)_2$, KOH, NaOH, NH_3, and oxidizing alkali are some of the major alkaline compounds used for alkaline pretreatment of biomass (Bali et al. 2015). The major advantage associated with the alkaline pretreatment is that it can be carried out at lower temperature under normal pressure,

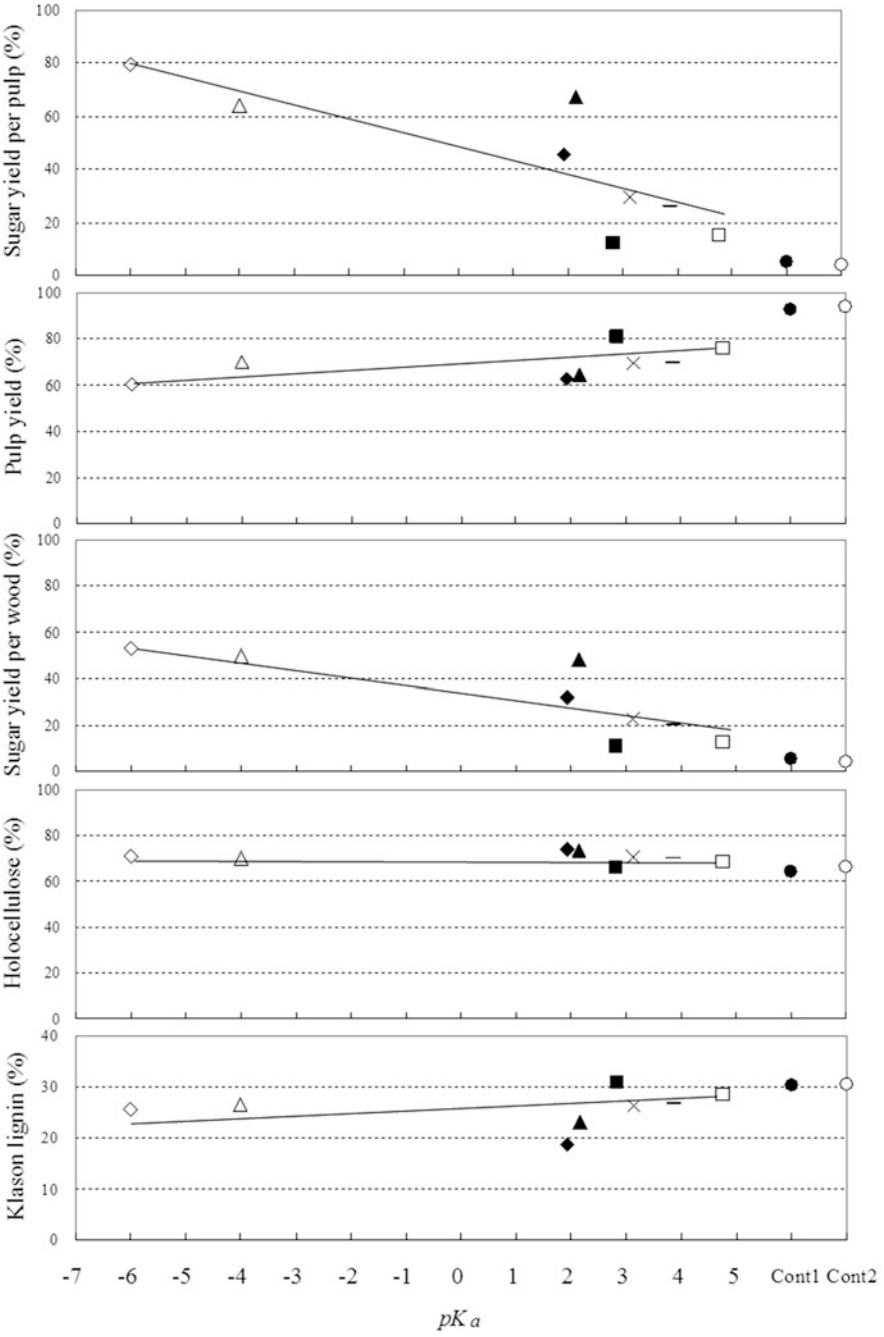

Fig. 4.1 Saccharification yield per pulp, pulp yield, sugar yield per wood, holocellulose and Klason lignin content of pretreated softwood by acids with various pK_a. HCl, ◇; H_2SO_4, △; Maleic acid, ◆; H_3PO_4, ▲; Malonic acid, ■; Citric acid ×; Lactic acid, −; Acetic acid, □; Control 1, ●; Control 2, ○. All the reactions except for HCl and H_2SO_4 were carried out at 200 °C for 12 min. Reactions with HCl and H_2SO_4 were performed at 180 °C for 6 min due to their high acidic effects.

Table 4.1 Specific surface area and pore volume before and after pretreatment for rice straw

Acid pretreatment conditions	Specific surface area (m^2/g)	Pore volume (mL/g)
Untreated	1.33	0.004
130 °C, 2% H_2SO_4, 15 min	4.48	0.012
150 °C, 2% H_2SO_4, 4 min	5.35	0.020
160 °C, 2% H_2SO_4, 2 min	5.76	0.022
170 °C, 2% H_2SO_4, 1 min	8.94	0.027

thus avoid application of sophisticated equipment and also the sugar recovery rate is higher as compared to acid pretreatment.

4.3 Physical Pretreatment

4.3.1 Mechanical Pretreatment

Some of the available mechanical pretreatment processes are chipping, milling, and grinding. The main purpose of this pretreatment is to decompose the biomass solid particles into smaller fragments and improve permeability to lignocellulose biomass structure. It was found that vibratory ball milling is economical than ball milling (BM). Ball milling causes a reduction in cellulose crystallinity of the biomass and thus causing enhanced saccharification. Due to the shear force generated during grinding, the particle size and cellulose crystallinity can be effectively reduced, while debris can considerably reduce the precincts of heat and mass transfer (Kumar and Sharma 2017).

4.3.2 Pyrolysis

Pyrolysis is a high temperature controlled degradation of lignocellulosic materials into its constituent components during biorefining. Pyrolysis is thermal decomposition of biomass in the absence of oxygen. It results in the conversion of lignocellulosic materials into carbon-rich liquids or solids. According to the residence time and heating rate, pyrolysis pretreatment can be broadly classified as slow, fast/flash pyrolysis, and intermediate [17]. Slow pyrolysis at lower temperatures for longer duration in the presence of oxygen (Case et al. 2015) can be more effective. In addition, it is flexible in terms of production and sales.

Fig. 4.1 (continued) Two corresponding control experiments without acids (Control 1: 200 °C for 12 min; Control 2: 180 °C for 6 min) were carried out

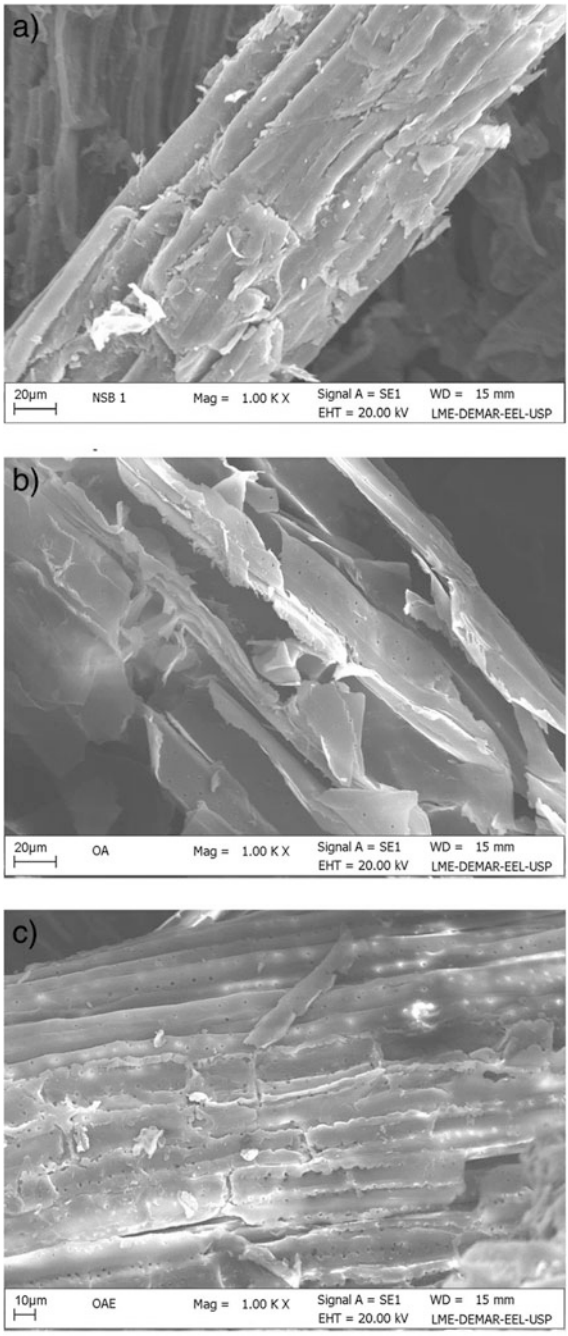

Fig. 4.2 Scanning electron microscopy (SEM) surface images of the sugarcane bagasse. (**a**) Native, (**b**) acid pretreated, (**c**) enzymatic hydrolyzed

4.3.3 Irradiation

In the reports, various irradiation pretreatments under ultrasonic, electron beam, ultraviolet, and microwave heating help in pretreating the LCB causing delignification and improving enzymatic accessibility to polysaccharides enhancing its saccharification (Kapoor et al. 2017). Microwave radiation is highly specific and simple process consuming low energy with high heating capacity, requires very short pretreatment time with minimal or no fermentation inhibitor production (Kumar and Sharma 2017). Ultrasound can change the shape and structure integrity of lignocellulosic material. Ultrasounds (10–100 kHz) pretreatment can rupture the biomass and causes polymer degradation, thus can be used efficiently as a biomass pretreatment system (Gogate et al. 2011). The characteristic properties of slurry and the biomass are used as parameters for optimization of duration and power of ultrasonic waves based to achieve the required pretreatment goals.

4.4 Physical–Chemical Pretreatment

4.4.1 Steam Explosion

Steam explosion (SE) is typically a combination of mechanical deconstruction of biomass under influence of chemicals and is one of the most preferred methods. During SE the lignocellulosic biomass are targeted with saturated steam temperatures (160–260 °C) and high pressure (0.7–4.8 MPa). The steam at such pressure penetrates the LCB causing expansion of the cell wall fibers resulting in partial hydrolysis and improvement of cellulase accessibility to cellulose component of the biomass (Rabemanolontsoa and Saka 2016). SE results in the removal of hemicelluloses and improving the enzymatic hydrolysis of the cellulosic content (Kumar and Sharma 2017). Chip size, moisture content, residence time, and temperature are important factors that need to be regulated to control SE pretreatment. Longer residence time at even lower pretreatment temperature results in more improved enzyme accessibility and low inhibitor formation (Rosgaard et al. 2007).

4.4.2 Liquid Hot Water Pretreatment

Liquid hot water (LHW) is a promising pretreatment method due to its associated advantages of no need for additional chemicals. LHW is also known as aqueous fractionation, aquasolv, solvent decomposition, hydro-pyrolysis, and hydrothermal pretreatment (Agbor et al. 2011). Even though LHW can be operated at low temperatures using low-cost solvents, the major disadvantage associated with it is

the need to recover large amount of water during downstream processing (Singh et al. 2016).

One of the main research objectives of LHW pretreatment is to develop a relationship between severity factors and the hydrolysis. The severity factors are used to characterize LHW pretreatment methods currently. The representative factors affecting the efficiency of pretreatment enable an easy comparison of experimental results to facilitate process design and operation. They include the temperature, residence time, and pH in the processes, as well as the size and shape of biomass particles. In some studies, the models based on the factors were developed (Hosseini and Shah 2009). With the aid of developed model, an improvement in the yield of biomass conversion process can be achieved. The combined severity factor (CS) is reported as a function of cooking time (t), temperature (T), and pH of the cooking mixture (Chum et al. 1999). The CS is suggested to be calculated as the following Eq. (4.1):

$$\mathrm{CS} = \log\left[t \exp\left(\frac{T-100}{14.75}\right)\right] - \mathrm{pH} \tag{4.1}$$

4.4.3 Organosolv Pretreatment

Organosolvolysis is a potential delignification process for the pretreatment of woody biomass. It promises high yield recovery of pulp and co-products, such as fermentable sugar, lignin, and extractives (Amiri and Karimi 2015). However, there are several drawbacks, including the risks associated with high pressure operations, use of volatile flammable solvents and energy loss in evaporation of organosolv pretreatment with low-boiling-point solvents, showing that it is not feasible for the industrial bioethanol production. An organosolv pulping process with high-boiling-point solvents is potentially applicable to pretreatment (Liu et al. 2010). The usual high-boiling-point organic solvents include ionic liquid and higher alcohols (glycerol, butanol, and glycol).

4.5 Biological Pretreatment

Lignin, hemicelluloses, and polyphenols present in the LCB can be utilized selectively by the microbes such as bacteria and fungi. The microorganisms or microbe based product assisted biomass deconstruction can be termed as biological pretreatment (BP). The two major modes of the biological pretreatment are direct microbe assisted pretreatment or application of delignifying enzyme cocktail for biomass deconstruction. The biological pretreatment has several advantages such as high substrate specificity, lesser energy consumption, no requirement of toxic

Table 4.2 Some researches on lignocellulosic biomass pretreated with organosolv and fungus

Feedstock	Time (min)	Temperature (°C)	pH	CS	Catalyst	References
Pinus Radiate	18	185	2	1.76	0.13% H_2SO_4	(Monrroy et al. 2010)
Cedar	10–60	190–220	–	3.65–5.31	–	(Baba et al. 2011)
Pinus Radiate	5–100	150	–	2.17–3.47	–	(Ferraz et al. 2000b)
Eucalyptus Grandis	5–100	180	–	3.05–4.36	25 mM $CaCl_2$ and $MgSO_4$	(Ferraz et al. 2000a)
Beech wood	120	180–200	2.5	1.93–2.52	–	(Itoh et al. 2003)
Pine wood	60	170	0.96	2.88	1% H_2SO_4	(Kandhola et al. 2017b)
Pinus radiata and *Acacia Dealbata*	60	200	2.5	2.22	–	(Muñoz et al. 2007)

chemicals with no inhibitory compound formation (Sindhu et al. 2016). Fungi are candidates for efficient degradation of biomass and play a vital role in the global carbon cycle and ecology (Kandhola et al. 2017a). Fungal assisted pretreatment is suggested to be an efficient method for selectively removing lignin by the action of white rot and brown rot fungus (selective lignin degrader) and enhances the enzymatic saccharification of lignocellulosic materials for the generation of biofuels or bio-based products (Mäkelä et al. 2014).

Furthermore, the combined fungal-organosolv pretreatment has been also applied to reduce the CS of reaction, as shown in Table 4.2. In the pretreatment on beechwood, Itoh observed an improvement of ethanol yield when carrying out the organosolv pretreatment at 180 °C concerning the non-fungal treated biomass (Itoh et al. 2003). Kandhola et al. (2017b) investigated the synergistic effects of fungal pretreatment combined with organosolv pretreatment on lignin recovery and quality. It is observed that the fungal treatment reduces CS significantly and shows an effect on the saccharification yield and the structural modification of lignin in the organosolv precipitates.

4.6 Summary

Several lignocellulosic materials have been chosen as inexpensive preliminary materials for the generation of biofuels. A pretreatment technique that helps to quickly and efficiently treat one lignocellulosic material may not be suitable for the pretreatment of another material. The main advantages and disadvantages of

pretreatment of lignocellulosic materials with these common techniques are summarized. The biomass composition and the targeted products and anticipated by-products dictate the choice of pretreatment technology to be used for the efficient conversion of specific LCB. These factors notably regulate the costs associated with pretreatment methods. There have been several reports comparing various pretreatment methods of biomass for deducing and suggesting a best possible pretreatment. Also, the cost-effective pretreatment to enhance enzymatic saccharification is worthy of in-depth research.

Competing Interests The authors do not have any competing interests to declare.

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Chapter 5
Thermal Pre/Treatment of Organic Fraction of Municipal Solid Waste

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Abstract OFMSW consists mainly of food waste, with small proportions of the yard and paper waste, composed of bio-polymers-lignin, cellulose, and hemicellulose, whose cross-linkage leads to difficulty in enzyme degradation thus reduce the overall process efficiency. Thermal pretreatment helps in the breakage of the lignin bonds and breaks down the crystalline structure of cellulose, leading to solubilization of COD, proteins, carbohydrates of OFMSW, higher biogas yield, and volatile solid removal during anaerobic digestion. This chapter concentrates on the temperature phase treatment and thermal pretreatment (conventional, microwave, and thermo-chemical), their principle, and their effect on process efficiency. Along with it, the merits, demerits of thermal pre/treatment, production of inhibitory compounds, and their effect on AD have been discussed. Furthermore, the commercial application, economic and environmental feasibility of thermal pretreatment of OFMSW has also been covered in this chapter.

Keywords Organic fraction of municipal solid waste · Temperature phased treatment · Thermal pretreatment · Biogas · Solubilization · Recalcitrant

Abbreviations

AD	Anaerobic digestion
CHP	Combined heat and power systems
FVW	Fruit and vegetable waste
HHV	High heating value
HMF	5-Hydroxy methyl furfural

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LCFA	Long-chain fatty acids
MSW	Municipal solid waste
OFMSW	Organic fraction of municipal solid waste
POPs	Persistent organic pollutants
SRT	Solid retention time
THP	Thermal hydrolysis process
TPAD	Temperature phased anaerobic digestion
TSS	Total suspended solids

5.1 Introduction

Municipal solid waste (MSW) is generally defined as a mix of wastes generated from households, commercial, and institutional establishments. World bank report estimates that around 2.1 billion metric tons of MSW are produced per year, which is about 0.74 kg/capita/day and projected to increase to 3.4 billion metric tons by 2050 (Kaza et al. 2018). High-income countries like the USA, Canada, and EU members comprise 16% of the world's population but 34% of the global waste generation. Detailed region-wise waste production is given in Fig. 5.1.

Solid waste management is a serious challenge, especially in low-income countries, where 93% of the waste is mismanaged compared to only 2% by developed

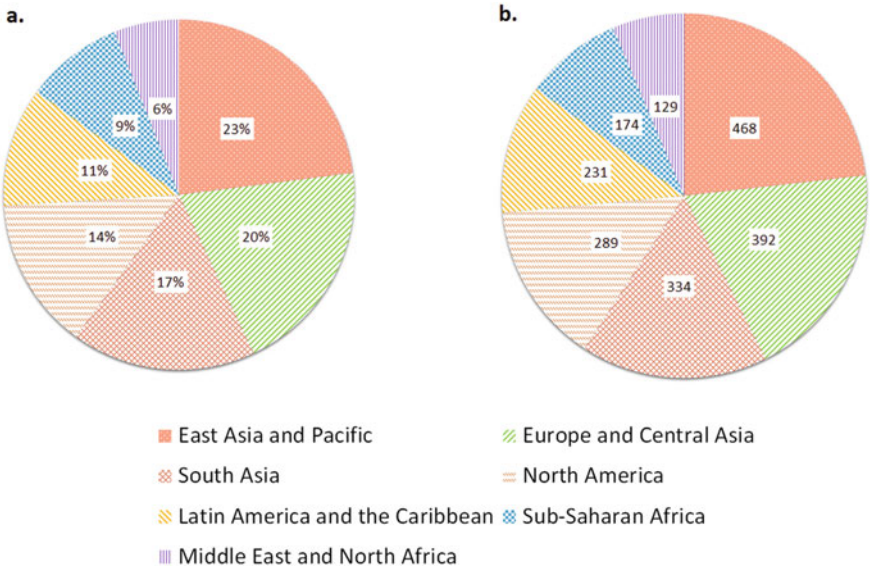

Fig. 5.1 Region-wise waste production (data adjusted to 2016) (**a**) Region's share in waste produced globally (**b**) Quantity of waste produced by each region (million tons per year). (Modified from Kaza et al. (2018))

nations (Kaza et al. 2018). The conventional methods of MSW disposal are landfilling, incineration, composting, and open dumping. While incineration is prominent in developed countries, in poorer countries, open dumping is more common. About 33–40% of global waste production is dumped or burned openly (Kaza et al. 2018). It creates large garbage heaps, which is prone to collapsing and fires, thus causing health hazards and loss of precious lives. Open dumping and improper landfilling can also contaminate nearby soil and water sources (surface and sub-surface) with leachate having heavy metals, pathogens, and persistent organic pollutants (POPs). Inefficient landfills create an odor, emit greenhouse gases like methane, attract rodents and vectors of infectious diseases, and make the surroundings aesthetically unpleasing. Many countries are also moving away from incineration because of the emission of pollutant gases (dioxins, furans, CO_2, N_2O, POPs). Besides, it has high capital and operating costs and generates ash, which has its disposal problem. Other thermal treatment methods like pyrolysis and gasification have questionable techno-economic feasibility and need high input power for operation (Tyagi et al. 2018). Composting has its challenges like low waste volume reduction and poor commercial value of the end product, that is, compost (Tyagi et al. 2018). The share of various treatment and disposal options is given in Fig. 5.2a.

Waste generation worldwide constituted about 5% of the global emissions in 2016 (Kaza et al. 2018). It is around 1.6 billion metric tons of carbon dioxide equivalent (CO2-equivalent) and expected to increase to 2.6 billion metric tons by 2050 (Kaza et al. 2018). There is an increasing focus on waste reduction and recycling, as well as the recovery of useful materials from it. It is essential to discuss the composition of MSW (Fig. 5.2b) as it influences the waste treatment and disposal. Developed countries produce more recyclable waste like paper, cardboard, metal, and plastic, while middle and lower-income countries produce more food and

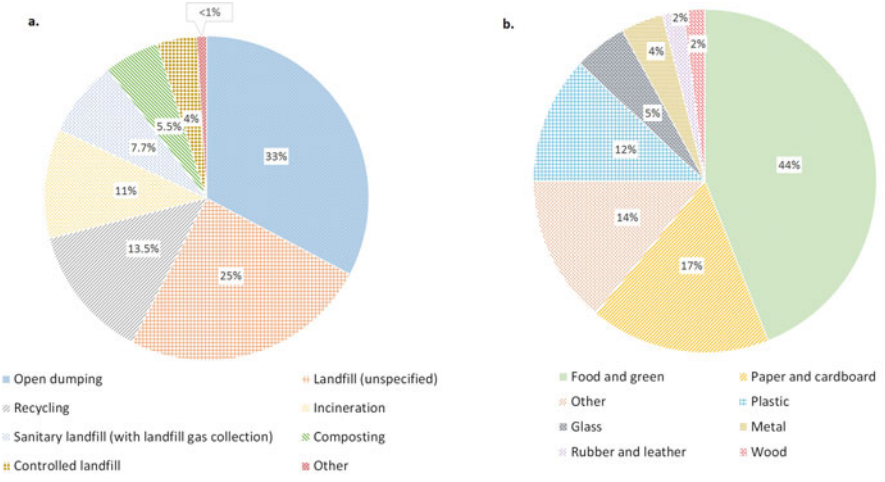

Fig. 5.2 (**a**) Share of various waste treatment and disposal methods worldwide (**b**) Composition of waste globally (Modified from Kaza et al. (2018))

green waste (Kaza et al. 2018). Overall, organic fraction of MSW (OFMSW), that is, food and green waste worldwide, dominates the waste composition. Thus, anaerobic digestion (AD) could be a prudent approach to waste treatment.

AD offers two key advantages: production of biogas as renewable and clean energy and stable and nutrient-rich digestate or manure, which can be used directly or composted before use. AD can produce up to 200 m^3 of biogas per ton of OFMSW (approximately 400 kWh of power) digested (Bolzonella et al. 2006) or 330 L CH_4/kgVS (Hartmann et al. 2002). If biogas is used instead of fossil fuel, CO_2 emissions can be reduced by 200–300 kg CO_2 per ton of OFMSW. Additionally, 30–40 kg CO_2 can be saved per ton of biowaste if the digestate is used in place of mineral fertilizer (Hartmann and Ahring 2005). Thus, AD of OFMSW can contribute to fulfil increasing energy demand in the world. Nevertheless, hydrolysis of OFMSW is a rate limiting step due to hard to degradable lignocellulosic fractions (yard waste, paper, and raw vegetable waste). At this juncture, thermal pre/treatment proved to be a robust method to enhance the substrate solubilization and the process performance. This chapter comprehensively reviews and comprised the available studies conducted globally to realize the effects of thermal pre/treatment on AD of ODMSW.

5.2 Characterization of OFMSW

Methane yield from AD of OFMSW depends on some important characteristics of the feedstock like pH, temperature, C/N ratio, moisture content, and nature of organics in it. These parameters have to be maintained in the optimum range for the most efficient biogas yield. For example, C/N ratio of about 25–30 is regarded optimal for AD of OFMSW (Kayhanian and Hardy 1994). A compiled characterization of OFMSW has been shown in Table 5.1.

5.3 Temperature Phased Anaerobic Digestion of OFMSW

5.3.1 Comparison of AD at Various Temperatures

The syntrophic interaction between bacteria and archaea plays an essential role in the AD process. They are involved in four interlinked stages of the AD process: hydrolysis, acidogenesis, acetogenesis, and methanogenesis. Anaerobic digesters are operated under three different types of temperature regimes: psychrophilic ($T < 20$ °C), mesophilic (33–37 °C), and thermophilic ($T > 45$–55 °C). The mesophilic AD is most common at the industrial scale (Fernández-Rodriguez et al. 2016). A comparative assessment of mesophilic and thermophilic digestion has been summarized in Table 5.2.

Table 5.1 Physical-chemical, elemental, and bromatological characterization of OFMSW (Source: Tyagi et al. (2018), Copyright with permission from Elsevier)

Parameters	Range	Average (SD)
Physical characteristics		
Density, kg/m^3	328–1052	722
Chemical characteristics[a]		
pH	3.9–7.9	5.2 ± 0.95
Humidity, %	49.8–85.0	72.8 ± 7.6
Total solids (TS), %	15.0–50.2	27.2 ± 7.6
Total volatile solids (TVS), %	7.4–36.1	22.9 ± 6.3
TVS/TS, %	43.0–94.9	84.6 ± 9.9
COD, g/kg	140–575	332 ± 122
Total Kjeldahl nitrogen (TKN), g/kg	1.0–28.0	7.9 ± 5.4
Total phosphorous (TP), g/kg	0.5–13.0	1.7 ± 2.5
CH_4 NL/kg$_{TVS}$	177–580	415 ± 137.7
Elemental composition[b]		
C, %	37.6–51.3	46.6 ± 4.4
H, %	5.6–7.3	6.6 ± 0.62
N, %	1.5–3.8	2.9 ± 0.6
S, %	0.1–0.9	0.3 ± 0.26
Bromatological characteristics[c]		
Fat, oil, grease, %TVS	6.09–35.0	17.5 ± 6.6
Protein, %TVS	7.7–30.0	17.7 ± 5.5
Raw fiber, %TVS	13.6–71.9	29.2 ± 15.0
Lignin, %TVS	3.8–18.5	9.7 ± 5.3
Carbohydrates, %TVS	35.0–63.2	55.5 ± 10.1

[a]Wet weight based values
[b]Dry weight based values
[c]Values in %TVS

Kuo and Cheng (2007) reported the 14% and 27% COD solubilization at 50 °C and 60 °C operating temperature in comparison with 5% COD solubilization at 37 °C. The total biogas produced at 50 °C and 60 °C were 2020 mL and 2054 mL, respectively, i.e., significantly higher than those observed at 37 °C. Komemoto et al. (2009) studied the AD of OFMSW at variable temperatures ranges from 15 to 65 °C. The highest substrate solubilization in terms of total suspended solids (TSS) removal was observed at temperature 45 °C followed by the highest biogas production of 64.7 mL/gVS and 62.7 mL/gVS under 35 °C and 45 °C, respectively.

Temperature phased anaerobic digestion (TPAD) uses two temperature ranges for the operation to utilize the advantages of both the ranges. Many studies have been carried out over the years using the combination of mesophilic (35 °C)-thermophilic (55 °C) or thermophilic (55 °C)-hyper-thermophilic (70 °C) (Bolzonella et al. 2007; Lee et al. 2008). Generally, two digesters are operating at different temperature ranges, e.g., thermophilic in the first stage and mesophilic in the second stage. As

Table 5.2 Comparison of mesophilic and thermophilic AD

Mesophilic AD	Thermophilic AD
Advantages	
• Relatively stable process • Less energy requirement • Operates at low organic loading • Lower ammonia and volatile fatty acids (VFAs) inhibition • Operating temperature is around ambient temperature, especially in tropical countries	• Faster hydrolysis and acidogenesis • Higher organic loading rate (OLR), lower retention time, and dry AD possible • Smaller digester possible due to above reasons, leading to lower capital cost • Faster substrate solubilization, consequently less moisture addition required • Higher biogas production and yield • Better pathogen removal
Disadvantages	
• Longer retention time leading to construction of bigger digesters thus higher capital cost required • Biogas production and yield are lower	• May need external energy source for maintaining operating temperature • Relatively less stable process • Sensitivity to temperature fluctuation; need precise temperature regulation

hydrolysis is the rate-limiting step in the AD of OFMSW, the first stage will increase the hydrolysis rate and thus reduce the time. The second stage will provide stability as the mesophilic range is less prone to inhibition due to ammonia and VFAs accumulation. Fernández-Rodriguez et al. (2016) found that TPAD processes had higher methane yields (26–60%) and organic matter removal of 16%, 10%, and 30% for DOC, $COD_{soluble}$, and VS, respectively, compared to the single stage digesters (mesophilic and thermophilic) at similar solid retention time (SRT). Bolzonella et al. (2012) did a pilot scale study at mesophilic, thermophilic, and TPAD (65 + 55 °C) and found that specific biogas production were 0.33, 0.45, and 0.49 m^3/kgVS (fed), respectively. TPAD has been operationalized at the industrial scale by Infilco Degremont, Inc. (ODI 2 PAD™ System). However, TPAD has higher operating and capital costs than conventional digestion (Neczaj and Grosser 2019).

5.4 Thermal Pre-Treatment of OFMSW

5.4.1 Conventional Heating (<100 °C, >100 °C)

Under high temperature and pressure treatment of OFMSW, sugars are solubilizes by improved hydrolysis, and the heterogeneity decreases which in turn acts as a hydrolyzed substrate for AD (Karthikeyan et al. 2018). The structure of the insoluble part of the substrate gets altered by the application of high temperature. As the rate of hydrolysis improved by thermal pre-treatment, shortening of HRT for solubilization and reduction of the total volume of digesters follow (Cesaro and Belgiorno 2014). A wide range of temperature has been studied showing the enhancement of anaerobic digestion of OFMSW. Bougrier et al. (2006) suggested that at temperature >70 °C

may cause formation of chemical bonds leading to particle agglomeration. Some studies have shown that thermal pre-treatment <100 °C did not disintegrate complex molecules, instead it induces the deflocculating of macro-molecules (Barjenbruch and Kopplow 2003; Prorot et al. 2011). Thermal pre-treatment results in solubilization of proteins and increases the removal of carbohydrates (Neyens and Baeyens 2003). Liu et al. (2012) observed that OFMSW pre-treatment at 175 °C for 60 min led to an increase in solubilization of particulate matter from 96.6 to 116.5 g/kg in terms of volatile dissolved solid concentration. Ma et al. (2011) observed that food waste pre-treatment at 120 °C for 30 min, the COD solubilization and biogas production were improved by 19% and 11%, respectively. Thermal pre-treatment of food waste at 80 °C leads to 52% enhancement in methane yield (Ariunbaatar et al. 2014).

Thermal pre-treatment of OFMSW at 80 °C, 100 °C, 120 °C for 2 h and at 140 °C for 1 h was carried out in order to study the methane yield and solubilization efficiency. At the optimum temperature of 80 °C, the maximum methane yield of 442 mL/gVS_{added} was reported. It showed an enhancement of 28% with respect to control. Yeshanew et al. (2016) observed that with an increasing treatment temperature from 80 °C up to 140 °C, the tCOD decreased by 4% at 80 °C to 8% at 140 °C. The highest carbohydrate and protein solubilization of 30% and 20% were achieved at 80 °C and 140 °C, respectively. Liu et al. (2012) observed that OFMSW pre-treatment at 175 °C for 60 min solubilized the organic matter by 60% and a soluble sugar concentration increased by 60.5 g/kg, however, the methane yield was decreased by 8% than control due to melanoid formation. Qiao et al. (2011) observed that pre-treating fruit and vegetable waste (FVW) and food waste at 170 °C for 1 h leads to a methane yield of 525 mL/gVS (18% higher than control) and 754 mL/gVS (3% less from control), respectively. Wang et al. (2010) observed that OFMSW pre-treatment at 175 °C for 60 min leads to 13% higher methane yield over control owing to higher concentration of dissolved volatile solids in medium.

In terms of best conventional thermal pre-treatment temperature range, contradictory results were reported by the researchers. As per Ma et al. (2011), and Yin et al. (2014), the best temperature for thermal pre-treatment of OFMSW ranges between 120 and 160 °C, while Wang et al. (2010) and Zhou et al. (2013) reported the 170–175 °C as best range of temperature. On the other hand, the optimum temperature range of thermal pretreatment of OFMSW has been reported to be between 80 and 100 °C (Ariunbaatar et al. 2014).

5.4.2 Microwave Pre-Treatment

When OFMSW is subjected to electromagnetic radiations, which uses a wide range of frequency and wavelength energy, the kinetic energy of the water dipoles increases and reaches its boiling point soon, leading to cleavage of bonds. It causes an increase in temperature and subsequently pressures, which destroys complex organics bonds and resulting in the transfer of organics from particulate to soluble

phase. Microwave heating stimulates polar molecules with the electromagnetic field by influencing the dielectric properties of a given substrate. Therefore, the heating process is faster, more uniform, and has a lower energy demand than conventional thermal pre-treatment methods. With microwave pre-treatment, complex structures in substrates are broken down into small and uniform components, which increase the accessibility and degradability of the substrate (Quitain et al. 2013).

Ismail et al. (2019) studied the effect of microwave pre-treatment on the solubilization of OFMSW. Microwave heating power of 240 W for 5 min was considered optimum, followed by torrefaction at 320 °C for 30 min. A high heating value (HHV) of 23.82 MJ/kg and an energy yield of 95.54% and a mass yield of 84.28% were obtained from the process. The heating of OFMSW by microwave pre-treatment increased the surface area of the substrate by creating pores, which increased the thermal degradation during torrefaction. Deepanraj et al. (2017) compared the conventional thermal pre-treatment (120 °C, 10 bar, 30 min) and microwave pre-treatment (1460 W, 2450 MHz, 12.2 cm wavelength, cavity size 470) of OFMSW. The conventional method showed a slightly higher VS removal efficiency of 62% over microwave heating (59% VS removal). However, the COD removal efficiency of thermally and microwave pretreated substrates was 52%. Bundhoo (2017) studied the microwave pre-treatment of OFMSW at varying power intensities and pre-treatment times from 0 to 30 min to obtain different specific energy ranging from 0 to 6946 kJ/kg TS. The highest TS solubilization was observed as 3.5% and 8.0% for the microwave at a specific energy of 4210 and 6946 kJ/kg TS, respectively. The maximum COD solubilization was observed for the substrate with specific energy 6946 kJ/kg TS with the sCOD concentration of 75% higher than the control. Marin et al. (2010) observed that when OFMSW was subjected to microwave pre-treatment at a heating rate 7.8, 3.9 and 1.9 °C/min starting from room temperature up to 175 °C at 1 min heating time, the highest solubilization of COD was observed at a heating rate of 1.9 °C/min. Yet, the enhanced methane yield was observed at a heating rate of 7.8 °C/min. An increase in biodegradability was found to be ranging from 5 to 16%. Shahriari et al. (2012) investigated the effect of microwave irradiation (at the temperatures ranging from 115 °C to 175 °C) on OFMSW in the absence as well as in the presence of hydrogen peroxide addition. At temperatures of 115 °C and 145 °C, an improvement of 4–7% in biogas production was observed, with a decrease at 175 °C due to the formation of refractory compounds.

5.4.3 Thermo-Chemical Pre-Treatment

Chemical treatment of OFMSW in conjugation with thermal pre-treatment involves either acid or alkaline pre-treatments. Acids like sulfuric acid, nitric acid, hydrochloric acid, and phosphoric acid are primarily used for thermo-acid pre-treatment. Hydrolysis of unstable hemicellulose (e.g., Xylan) is the main reaction that occurs during thermo-chemical pre-treatment. HMF and furfurals also get

generated, followed by formic and levulinic acid. Although solubilization of lignin is difficult yet its disruption is high enough to increase the susceptibility of cellulose to enzymes. Alkalis like NaOH, $Ca(OH)_2$, and ammonia are the most commonly used thermo-alkaline pre-treatment. Breakdown of lignin is more effective by these alkalis, which causes depolymerization and breaking of carbohydrate–lignin bonds. Although less than thermo-acid pre-treatment, yet the hemicellulose solubilization is also enhanced into oligomers. The structure of cellulose is less affected by thermos-alkali treatment. Thus, comparatively, thermos-alkali pre-treatment is more beneficial than thermo-acid pre-treatment of OFMSW. Güelfo et al. (2011) optimized the working parameters for thermo-chemical pre-treatment of OFMSW at 180 °C, 5 bar pressure, and 3 g/L NaOH, which showed an increase in sCOD by 246%. Although improvement in solubilization of organic matter improves due to thermo-chemical treatment but improvement in methane production does not occur due to the production of inhibitory compounds, structure of difficult-to-degrade molecules, and toxicity issues due to usage of chemicals. Ma et al. (2011) studied the thermo-acid pre-treatment of OFMSW with 10 N HCl (pH 2) at 120 °C and observed a COD solubilization of 32%, which was the highest among other pre-treatment studied (acid, thermal, pressure, and freeze-thaw). Miljic et al. (2014) studied the effect of different chemicals (HCl, H_2SO_4, NaOH, H_2SO_3) at variable concentrations (0.7%, 1.5%, 3%), temperature (50, 75, 100, and 120 °C), and residence time (15, 30, 60 and 120 min) for the pretreatment for OFMSW. Compared to untreated OFMSW, the soluble sugar increased by 120% when pre-treated by 1.12% HCl for 94 min at 100 °C or 1.17% HCl for 86 min at 100 °C. This improvement was mainly due to the production of mono-sugars: glucose and fructose. Junoh et al. (2015) studied the thermo-chemical pre-treatment of OFMSW at different temperatures (50–90 °C), reaction time (30–120 min), and NaOH concentration (0.7–15 g/L). The maximum COD solubilization of 32% was obtained for treatment conditions of OFMSW at 90 °C, 15 g/L NaOH for 2 h reaction time. Table 5.3 summarizes the comparison among conventional heating, microwave pre-treatment, and thermo-chemical pre-treatment.

5.5 Inhibitory Compounds Formation During Thermal Pre-Treatment

When organic wastes are subjected to thermal and thermo-chemical pre-treatments (under extreme treatment conditions of higher chemical dosage and treatment temperature), the lignin-derived by-products are produced, which inhibits the microbial and enzymatic activities in digesters. At temperature >140 °C, as the complex structure of OFMSW is degraded, amino acids react with sugars forming melanoidins, which are difficult to degrade anaerobically and reduce the biogas yield. The soluble proteins get denaturated to ammonia at a faster rate and resulting in higher ammonia into the medium. It adversely affects the biogas yield and inhibits

Table 5.3 Comparison between different thermal pre-treatment techniques

Parameter	Conventional heating	Microwave	Thermo-chemical
Equipment	Autoclave, pressure-vessel	Microwave	Autoclave, microwave
Chemical used	No	No	Acid/alkali
Energy requirement	High	Less	Less
Working	High temperature and pressure dissociates the cell membrane leading to improved hydrolysis for anaerobic digestion (AD)	Electromagnetic radiation utilizes a wide frequency range (300 MHz–300 GHz) and wave-length (1 mm to 1 m), the kinetic energy of the water dipoles increases and reaches its boiling point soon, leading to cleavage of bonds	The substrate is subject to chemicals (acids and alkalis) at high temperature causing hydrolysis of unstable hemicellulose and breakdown of lignin
Capital cost	Less	Cannot say. No full scale operation	High

anaerobic activities. Long-chain fatty acids (LCFA) are also formed during thermal pre-treatment and AD of OFMSW. LCFA produced from the degradation of lipids, causing major lag in digestion. However, this inhibition is temporary (Cirne et al. 2007). Besides, the volatilization of short-chain organics also causes lower methane production in the initial days. OFMSW being highly biodegradable, the high carbohydrates content and high treatment temperature cause a loss in degradable sugars, thus necessitating a balance between lipids, proteins, and carbohydrates degradation.

Thermal and thermo-chemical pre-treatment of OFMSW produces recalcitrant such as 5-Hydroxy-Methyl Furfural (HMF) and furfurals. Cellulose, hexose, and their polymers are responsible for the production of 5-HMF, and pentose (obtained from hemicellulose) produces furfurals. 5-HydroxyMethyl Furfural (HMF) and 2-furaldehyde (hereafter referred to as furfurals) are the most important members of furfurals (Ahmed et al. 2019). Under thermo-acid pre-treatment of OFMSW, the uronic acids and pentose formed as a result of hydrolysis of hemicellulose, dehydrates leading to the formation of 2-furaldehyde, and hexose formed due to hydrolysis of cellulose dehydrates leading to the formation of 5-HMF. Under high temperature and acid dose for long reaction time, HMF is degraded to levulinic acid and formic acid (Fengel and Wegener 2011). Furfurals are also further degraded to formic acid and resins. Formation of both 5-HMF and furfurals leads to a decrease in the specific growth rate of methanogens and less production of biogas.

5.6 Commercial Application of Thermal Pre-Treatment

The commercial application of the thermal hydrolysis process (THP) began more than two decades ago. Cambi AS, a Norwegian company, developed the Cambi process (thermal pre-treatment), which was operationalized in Hamar (Norway) in 1995 (Kepp et al. 2000). Later, some other commercialized thermal pre-treatment technologies were implemented like Biothelys by Veolia in 2006, Exelys by Kruger-Veolia in 2010, Turbotec by Sustec in 2011 (one pilot plant), CTH by Aqualogy in 2012 (industrial prototype), Lysotherm by Eliquo in 2012, and Biorefinex by Biorefinex Canada in 2013 (Neczaj and Grosser 2019). These technologies are used for sludge pre-treatment but have the potential for OFMSW as well. Some of the prominent technologies are discussed as follows:

5.6.1 Cambi Process

It consists three batch digesters sequentially treating the sludge (Fig. 5.3), Cambi THP offers many advantages over conventional mesophilic AD (Wang et al. 2017) (Table 5.4).

Besides offering significant sludge volume reduction, smaller digester volume, and higher biogas yield, Cambi THP also led to net increase in energy production by 20% (Kepp et al. 2000). But there are few drawbacks like relatively complex process (three batch digesters), odor, and recalcitrant compound formation (Neczaj and Grosser 2019).

5.6.2 Biothelys Process

It was first implemented at Saumur in France in 2006. It consists of a single batch digester with treatment at 150–180 °C at 8–10 bar pressure using steam injection for 30–60 min retention time (Nazari et al. 2018). There were positive outcomes from Saumur like TS removal increased from 25% to 45%, sludge cake TS increased from 22% to 30% and 46% sludge volume reduction compared to the conventional digester (Chauzy et al. 2008). Though there are advantages with this process (pathogens are inactivated, higher biodegradability of treated sludge and greater dewatering), there are the disadvantage, i.e., concentrated return flow recirculated to the wastewater processing units after dewatering (Foladori et al. 2010).

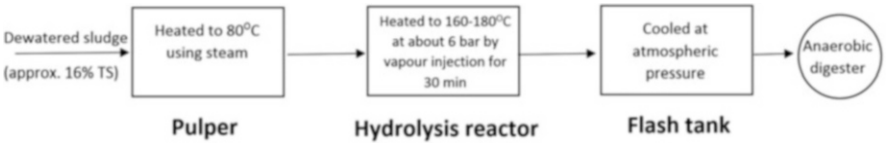

Fig. 5.3 Simplified process flow of Cambi THP (Nazari et al. 2018)

Table 5.4 Comparison of Cambi digester with conventional mesophilic anaerobic digester (Reproduced from Neczaj and Grosser (2019), Copyright with permission from Elsevier)

Parameters	Unit	Cambi reactor	Conventional digester
SRT	days	<15	>20
Volume of reactor	–	1/3 of the volume of a conventional digester	1
VS	%	12	4–6
OLR	$kg/m^3/d$	5	2–3
pH	–	7.5–8.0	6.8–7.5
Temperature	°C	38–42	35–37
VFAs/alkalinity	–	0.1–0.5	0.1–0.5
Ammonia	mg/L	2500–3200	600–1000
Composition of biogas	%	65–70% CH_4 Low concentration of H_2S	60–65% CH_4 High concentration of H_2S
VS removal	%	70	50–55
Biogas yield	m^3/mgVS	510	350

The biggest challenge to thermal pre-treatment is the production of recalcitrant at higher than 140 °C, which hinders the anaerobic digestion process. pH adjustment by vacuum evaporation, sulfite, alkali can remove volatile inhibitors. Application of activated charcoal and resins are the detoxification procedures that can be used to adsorb the recalcitrant compounds. Evolutionary engineering is one of the detoxification techniques, which mitigate the inhibitors. Inhibitors like furans and phenolics can be prevented by *S.cerevisiae* (microorganism producing ethanol). HMF is reduced to 2,5-bis HMF and the furfurals to furfural alcohols. Additionally, genetically or metabolically engineered strains of yeast can be used to improve microbial performance. *S. cerevisiae* resists furfurals, hence helping in better production of biogas (Ahmed et al. 2019).

5.7 Environmental Feasibility and Sustainability

Thermal pre-treatment has been studied as one of the major pre-treatment techniques for highly heterogeneous substrates like OFMSW. Although it leads to a great deal of substrate solubilization, yet its environmental feasibility needs to be paid heed. As discussed above, there has been a positive influence on the degradation kinetics of sugars, carbohydrates, volatile matter, and VFAs. Also, it is observed that at high temperatures, the solubilization of organics may increase. Still, it does not necessitate the increase in methane production due to the formation of inhibitory compounds.

Additionally, combining the thermal pre-treatment with chemical treatment may enhance the methane yield. When the environmental feasibility of thermal pre-treatment is concerned, the thermal energy required to ease up hydrolysis is combatted from the energy produced from the AD of OFMSW, thus making the

process a self-reliant process (Pérez-Elvira et al. 2006). The digestate of AD can also be used as a nutrient-rich fertilizer for agricultural purposes and land-remediation (Sargalski 2008). The Cambi industrial process can be adopted for determining the energy feasibility of thermal pre-treatment of OFMSW. Heat is recovered from steam saturated at 105 °C, followed by pre-heating the substrate, making the process energy-economical. In other scenarios, the biogas can be burnt in combined heat and power systems (CHP), providing electrical energy, green technology for electricity production, which can provide net benefits when sold. From the CHPs, the hot exhaust gas can be used to produce steam for thermal pre-treatment. Additionally, hot water can also be used to heat the digester if required. When energy considerations are taken into account during pre-treatment by thermal hydrolysis, factors like tank insulation, heat recovery, substrate heating need to be implemented. The solubilization of organics, its fermentation rate, and methane production rate is improved by thermal pre-treatment, leading to a reduction in the HRT. The recycling of OFMSW can be enhanced by AD as thermal pre-treatment leads to a lesser generation of digestate. Sterilization, stabilization, and enhanced sanitation are the net outcomes of thermal pre-treatments of OFMSW. Thus energy saving is quite feasible by thermal pre-treatment, but extra costs like capital cost and equipment maintenance are notably the obstacles to plant-scale production.

5.8 Conclusion

Thermal pre-treatment of OFMSW can be performed via the conventional method, microwave treatment, and thermo-chemical pre-treatment, each having its principle and mechanism. The common aspect in all these three methods is the production of recalcitrant at high treatment temperature, i.e., inhibitors to AD. The rate-limiting stage, hydrolysis, is accelerated by thermal pre-treatment leading to better production of biogas. Subsequently, solubilization also improves in terms of COD, proteins, carbohydrates, and lignocellulosic characters. Cambi and Bioethyl processes are the two major commercial thermal processes and could be applied for thermal pre-treatment of OFMSW.

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Competing Interests All the authors declare that they have no competing interests.

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Chapter 6
Biotechnological Aspects of Microbial Pretreatment of Lignocellulosic Biomass

Jefferson Poles Felipuci, Caroline de Freitas, Hernan Dario Zamora Zamora, Derlene Attili Angelis, and Michel Brienzo

Abstract In several areas, products are obtained from lignocellulosic biomass, such as bioethanol and personal items. Notwithstanding, it features high recalcitrance, hence its use often demands pretreatment and hydrolysis stages to reach bio-based final products. Industrially, the most common method is the chemical pretreatment which, as the name implies, involves chemical components with potential environmental risks. This procedure is responsible to increase biomass accessibility and to enhance polysaccharides achieving in subsequent stages. Biological pretreatment presents a new perspective to replace or cooperate with its chemical counterpart, once microorganisms can modify the lignocellulosic structure and facilitate accessibility to macromolecules of interest. According to the above, this chapter covers the potential of biological pretreatment as well as the mechanisms of microbial degradation, their enzymes, and the impacts on the economy worldwide.

Keywords Microbial enzymes · Wood decay · Rot microorganisms · Recalcitrance · Biological delignification

Abbreviations

GHs Glycosyl hydrolases
LiP Lignin peroxidases

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6.1 Introduction

Recent technological, social, and environmental changes have brought new needs in both science and industry for developing alternative technologies that make it possible to achieve similar products, than those obtained from petroleum sources (Ruan et al. 2019). Since the last years of the nineteenth century, the world energy matrix has been based on fossil fuels (British Petroleum 2019). Among the possibilities to replace oil, biomass has become the most important resource, able to generate several products by different routes, with the great advantage of being environmentally friendly (Guedes et al. 2019). In this perspective, bio-based products are currently part of everyday life, with applications in sectors such as engines, packaging, medicines, and many others. With or without slight treatment/modifications, vegetal biomass like crops, vegetable oils, forest, agricultural waste, and also the municipal and industrial ones are used to produce bioproducts (Sorokina et al. 2017; Rosales-Calderon and Arantes 2019). However, turning vegetal biomass into bioproducts may become a challenge, since the raw material needs to be undergone to different types of stages during the conversion process until reaching suitable yields (Holwerda et al. 2019). Pretreatment has a huge importance in the steps of value-added products generated from biomass systems, where complex structure presented in plants must be conditioned for subsequent stages (Antunes et al. 2019).

The most used methods of biomass pretreatments, such as chemical and physical procedures, have in common the demand for plenty of chemical reagents and/or energy inputs in its process. Such chemicals are widely used in industries to separate biomass components in order to manufacture all kinds of (bio-) products, but in consequence, those reagents are found polluters for the environment. Nowadays, facing an economic and global warm crisis, it is essential and recommended looking for alternatives to low-cost, less oil-dependent, and non-polluting manufacturing methods.

Biological pretreatment of biomass is already known as an option to conventional methods used in industries. This method does not generate toxic and inhibitory compounds and need low quantity of chemical and energy input, which makes it an economically and eco-friendly feasible process. Biological pretreatment also can be used before a chemical or physical pretreatment: the biological stage can provide a better decrease of the recalcitrance while the chemical stage provides the separation of the macromolecules. This combination can reduce the costs and chemicals in the whole process (Sindhu et al. 2016; Singh 2018; Agbor et al. 2011; Felipuci 2020).

In this chapter will be discussed biological pretreatment characteristics, including the enzymes and microorganisms involved in the biomass structure modification. Moreover, the benefits and disadvantages of this method are discussed, as well as value-added and commodity products, mainly on large scale.

6.2 Biological Pretreatment

Biological pretreatment of lignocellulosic biomass became a fundamental research topic since it is clear that a near-term economy will depend on the supply of biomass to produce bioproducts and bioenergy. It is related to the use of microorganisms, aiming to degrade or modify vegetal biomass structure employing their special enzymatic complexes (Agbor et al. 2011; Sindhu et al. 2016). Among the vast variety of species in the world, fungi and bacteria are well known to produce specific enzymes for lignocellulose deconstruction, called cellulases, hemicellulases, and ligninases. These enzymes are capable to degrade natural macromolecules found in the plant cell wall, such as cellulose, hemicelluloses, and lignin. Cellulose and hemicelluloses, for instance, are hydrolyzed into smaller molecules (the monomeric sugars) (Sharma et al. 2019).

Among the numerous enzymes produced by fungi that degrade cellulose, hemicellulose and lignin, the most studied are: endoglucanases, exoglucanases, and β-glucosidases that hydrolyze cellulose; endoxylanases, β-xylosidases, acetyl xylan esterases and others that degrade xylan and laccases, manganese peroxidases and lignin peroxidases that degrade lignin (Pamidipati and Ahmed 2019; Gautam et al. 2019; Malgas et al. 2019).

The species of fungi that degrade lignin are known as white-rot. The ones that depolymerize cellulose and hemicelluloses are named brown-rot because the wood degraded takes a brownish appearance, due to the loss of polysaccharides (cellulose and hemicellulose) remaining high amounts of lignin (Hatakka and Hammel 2011).

Biological pretreatment does not generate toxic compound (degradation products, inhibitors) during its process and it is ecologically promising, which is an advantage comparing to other usual methods. Moreover, results can be optimized when the strains are pre-selected (Sindhu et al. 2016; Van Kuijk et al. 2015). In the biodegradation, variable microbial communities are important to the quality of the final results due to its vast amount of enzymes. However, in addition to the microorganism itself, biomass composition, temperature, humidity, pH, aeration rate, incubation time, and biomass particle size are elements that can also affect the result and the quality of the pretreatment (Sindhu et al. 2016; Fang et al. 2012; Li et al. 2012; Iqbal et al. 2013; Fatokun et al. 2016).

Usually, biological pretreatment needs long-time requirements (10–14 days), space, and careful growth conditions to work. In industrial scale it may be less attractive but the biological pretreatment can be used together with chemicals and physical pretreatment. The potential of delignification by microorganisms combining with chemical and physical methods is inviting because of the complete degradation of lignocellulosic biomass components, mainly lignin, that can take a long time to reach significant results (Agbor et al. 2011; Hatakka 1994; Hatakka et al. 1993).

Recalcitrance is the capacity of a biomass resist to a pretreatment or to enzyme action. The quantity and organization of the components into the cell wall such as cellulose crystallinity are factors that may change the recalcitrance level of biomass

(Naidu et al. 2018; Melati et al. 2019; Park et al. 2010). Lignin contributes to the material recalcitrance due to its resistance against pathogens and insects, and its removal influences the access to the polysaccharides (Shimizu et al. 2020; Schmatz et al. 2020; Zhao et al. 2012; Phitsuwan et al. 2013).

High recalcitrance is a challenge in the search for better methods of macromolecules isolation from biomass. Accordingly, different pretreatment methods have been developed, aspiring to circumvent this problem in order to separate its components. One method to work around the recalcitrance problem is to select varieties with low lignin content (Brienzo et al. 2015) or delignify biomass decreasing lignin content, considering that lignin is a barrier in carbohydrate extraction (Shimizu et al. 2020; Brienzo et al. 2017). A usual pretreatment focuses on improving the formation or capability to form fermentable sugars by hydrolysis; to prevent loss of carbohydrates; avoid by-product formation that may prevent subsequent processes and be a good cost-benefit ratio method (Melati et al. 2019). Thus, biological pretreatment is an option to replace or co-work with other methods of pretreatment by attending such ideal requirements.

Other way to degrade lignocellulosic biomass is using co-culture, which use more than one microorganism. This method is based in to use fungus or/and bacteria to degrade the lignocellulosic biomass. However, competition between microorganisms for the substrate is not recommended, and it can be used one after other. This technique is useful due to microorganisms encompass large quantities of enzymes, which can completely degrade the lignocellulosic material. This process can be used in different areas such as agronomy (degrade pesticides) and industry (carpet decolorization) (Yoon et al. 2014; Sariwati et al. 2017; Wang et al. 2017; Kumari and Naraian 2016).

6.2.1 Lignocellulosic Biomass Structure

Lignocellulosic biomass englobes all organic matter directly from plant sources. It is the largest source of carbohydrates in nature, with a great variety, abundance, and availability, involving wood, agro-industrial waste, municipal waste, and plants. What draws attention to these materials is that they are renewable resources with energy potential. This presents them as possible substitutes for fossil fuels, generating sustainable energy through bioethanol and co-generation of electric energy (by a burning process) (Nanda et al. 2015). Consequently, interest in research, both in scientific and industrial fields, grows constantly (Bilgili et al. 2017; Mao 2015; Aslan 2016; Toklu 2017; Sharma et al. 2019).

One of the most used lignocellulosic biomass is the sugarcane bagasse (*Saccharum* spp). Currently, the bagasse is used in the production of electrical and thermal energy through its combustion in high-pressure boilers in plants (Fernandes 2018). Another application aims to obtain second-generation ethanol (cellulosic ethanol), serving as an alternative to replace fossil fuels and charcoal.

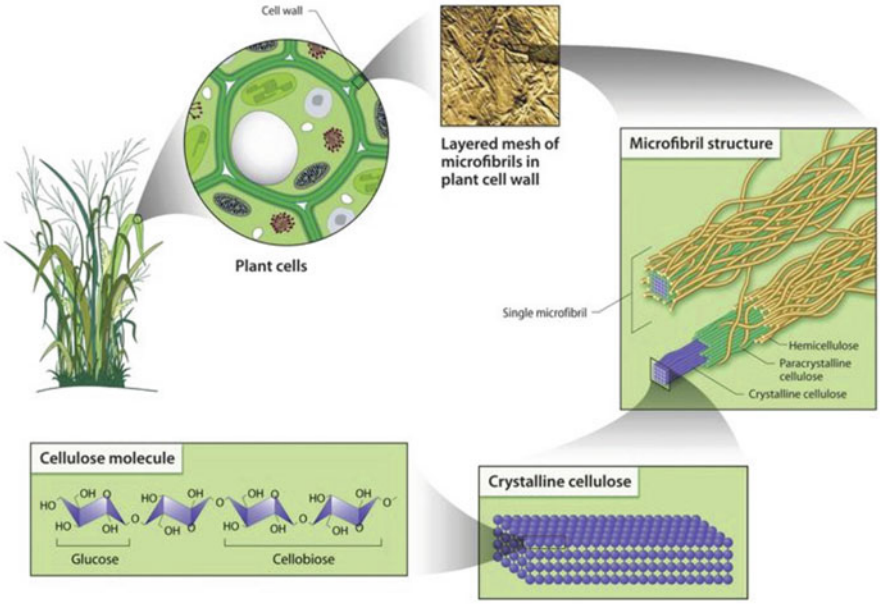

Fig. 6.1 Schematic representation of lignocellulosic biomass emphasizing the cellulose macromolecule (Jasmania and Thielemans 2018)

Lignocellulosic biomass is also used in the production of clothing, artificial skin, paper, and other products in common use (Mizuhashi et al. 2015; Kim et al. 2014). More specifically, in biotechnology and biomass conversion, it is possible to produce briquettes, carbon adsorbents, and biofilms. The production of these items depends on the treatment that those biomasses will be undergone. For separation of each macromolecule, there is one or a series of treatments to be based on biological routes.

The main characteristic of vegetal biomass is its lignocellulosic structure existing into the cell wall, presented in all plant forms. Its composition is mainly cellulose, hemicelluloses, and lignin, with less quantities pectins, proteins, and extractives (Naidu et al. 2018). Quantities of each component change according to biomass and soil types, geographic localization, and other factors (De Vasconcelos 2015). The three main components (cellulose, hemicelluloses, and lignin) in the cell wall are organized in a way that recalcitrance is increased, making its separation harder in biotechnological processes. Cellulose and hemicelluloses are strongly connected by hydrogen bonds. Hemicelluloses can be located between cellulose fibers, while lignin is connected to the carbohydrates forming a complex interaction network (Schmatz et al. 2020; Busse-Wicher et al. 2016).

Cellulose is the major macromolecule in the plant cell wall (Fig. 6.1). The quantity varies according to biomass type: rice toasts showed 28.7–34.7%; cotton presented around 95%, and sugarcane bagasse showed 25–45% (Naidu et al. 2018). It is also considered most abundant organic polymer found on the planet Cellulose is

an arrangement constituted by cellobiose unities (glucose dimers) joined by β-1,4 glycosidic chains. In the cellulose structure, there are amorphous regions which are organized regions demined crystalline and non-crystalline zones (Ioelovich 2016). Cellulose is widely sought in the industry as raw material for common use products, such as varnish, films, paper, among others. Due to several industrial interests, cellulose isolation from biomass is widely studied. Cellulose can be separated from other carbohydrates by alkaline treatment or broken by acid treatment. In the case of alkaline treatment, ester linkages break down, resulting in structural modification of the cell wall and facilitating separation from hemicelluloses (Galletti and Antonetti 2012).

Hemicelluloses, different from cellulose, are composed of more than one monosaccharide: pentoses, hexoses, and uronic acids. In pentoses group is found xylose and arabinose; in hexoses group is found mannose, glucose and galactose and in uronic acids is found glucuronic and galacturonic acids. Those monosaccharides can also be subdivided into three main groups: xyloglucans, xylans, and mannans, that are formed by subunits of mannose. The monosaccharides are connected by β and α glycosidic bonds and can have between 80 and 200 units. Hemicelluloses have amorphous characteristics and a lower degree of polymerization than cellulose. It makes up 15–35% of lignocellulosic biomass and it is associated to the integrity of the plant cell wall, having great importance in its shape and resistance. Hemicelluloses correspond to one-third of all renewable carbon on the planet. Hemicellulose has been studied for several applications, with a feature for oligomers such as xylooligosaccharides and manooligosaccharides (De Freitas et al. 2019; Chiyanzu 2014).

Lignin is a biomass macromolecule composed of phenylpropane units of p-hydroxyphenyl (H), syringyl (S), and guaiacyl (G). This polyphenolic structure is organized irregularly and has an amorphous structure. Depending on species, lignin comprehends between 10 and 20% of lignocellulosic biomass, being the third most abundant macromolecule in the plant cell wall. For plants, lignin helps in protection against insects and fungi and also contributes to growth development and mechanical strength. This protection is one of the reasons to the biosynthesis, once infections, metabolic stress, and disturbances in cell wall structure are starters to the plant initiate the process (Vanholme et al. 2010). It is arranged mainly on the secondary wall, making it rigid and waterproof. Lignin organization is to be linked with hemicelluloses, together with its irregular structure and a gigantic number of possibilities for connections between its forming units, which suggests that there is a low chance of existing two similar lignin molecules (Ralph et al. 2004). This favors the increasing recalcitrance of its biomass (Schmatz et al. 2020). Lignin is an obstacle for a process dedicated to macromolecule separations as it remains as residual content/contaminant (Felipuci 2020).

6.2.2 *Microorganisms in Biological Pretreatments*

Microorganisms are considered of key function in biological pretreatments of lignocellulosic biomass. Degradation capacity of microorganisms is widely known, mainly because of the degradative potential of its enzymes, which are produced during its growth. Biological pretreatment technology has generated results in several areas involving biotechnology, bioremediation, bio-pulping among others.

The most common microorganisms applied in biological pretreatment are white-rot, brown-rot, and soft-rot fungi, besides bacteria. These microorganisms are capable to consume all components in lignocellulosic biomass, mainly lignin, and the capacity to mineralize lignin into carbon dioxide and water. Brown-rot fungi are known to degrade polysaccharides more efficiently, and only slightly modifies the lignin, while white-rot fungi can degrade lignin with more facility (Kirk and Moore 1972; Kirk and Highley 1973). Holocelluloses/lignin ratio presented in biomass after degradation can be used to measure the fungal effect on the biomass decomposition. The effect on the biomass components can be classified at different ratios: Class 1 (corresponds to brown decomposition agents): ratio less than one; Class 2: whose process has a low amount of residual lignin; Class 3: holocelluloses content is two to five times higher than lignin content; both classes 2 and 3 correspond to white decomposition agents (Trojanowski 2001).

6.2.2.1 White-Rot Fungi

Industrially white-rot fungi are well known as lignin consumers, found in Basidiomycota phylum. Those comprehend over than 90% of all Basidiomycetes that rot woods. (Riley et al. 2014). This phylum has been studied in several areas, including medicine (Madhanraj et al. 2019), agriculture (Duplessis et al. 2011), and forestry (Martin et al. 2008). This phylum also includes mushrooms (Morin et al. 2012), and pathogens of plants, animals, and other fungi (Duplessis et al. 2011; Dawson and Thomas 2007).

White-rot fungi have great potential to degrade lignocellulosic biomass (Fig. 6.2). Although those fungi also can degrade polysaccharides, they are known as a well specific lignin degrader (Rudakiya and Gupte 2017). Syringyl (S) units of lignin usually are preferred instead of guaiacyl (G) units, due to its less resistance to degradation. In certain conditions, white-rot fungi are lignin-selective depending on several factors, like cultivation time, temperature, wood species, and other variables (Hatakka and Hammel 2011; Hakala et al. 2004). The degradation ability of these fungi has been quite studied not only in lignocellulosic biomass researches, but also in other areas, such as bioremediation, food, pharma, and other industries. These abilities allow the fungi grow in restrictive conditions, such as lignocellulosic wastes. In the last decade, several studies focused on these group showed results to degrade pesticides (Kaur et al. 2016; Gouma et al. 2019), to increase productivity,

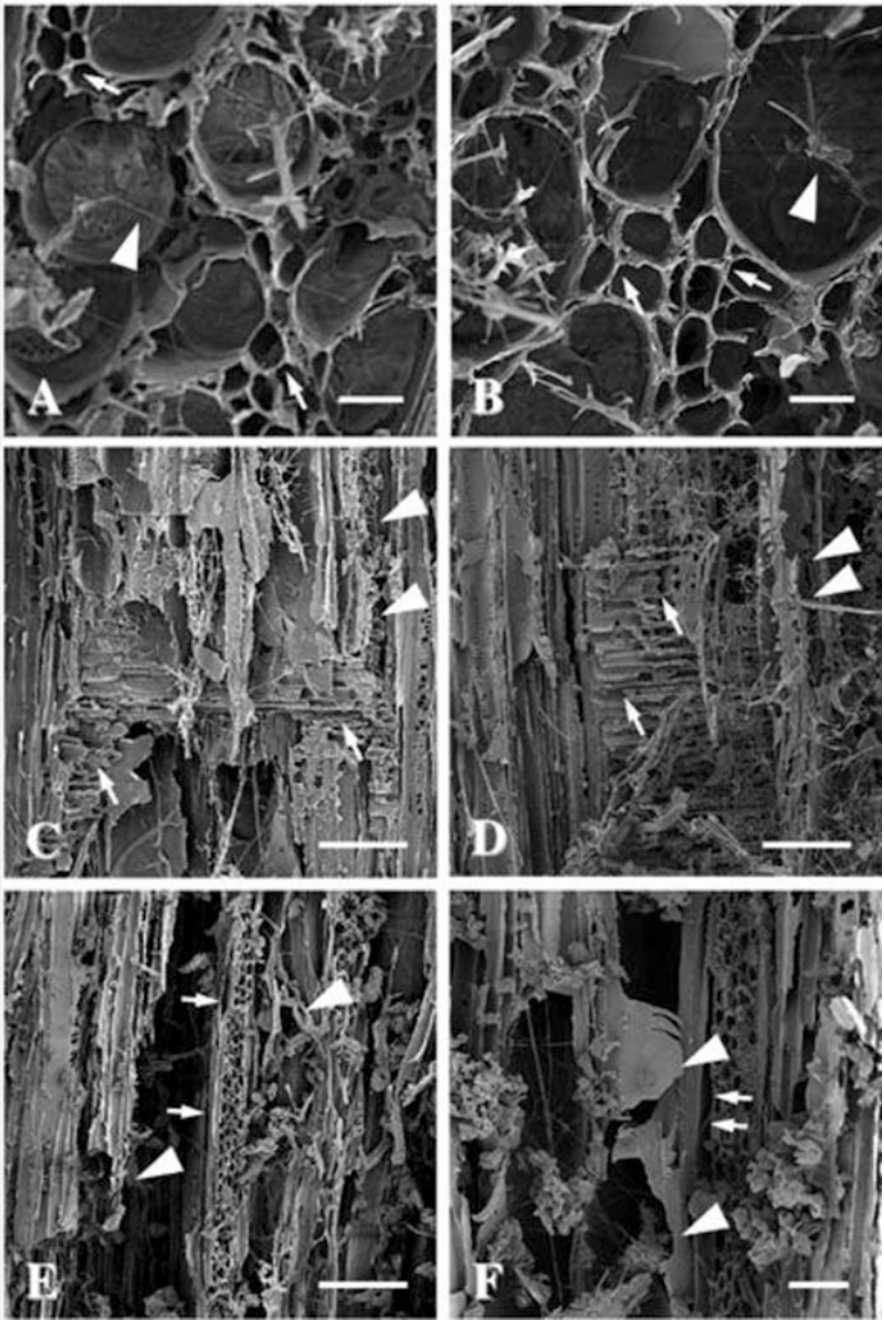

Fig. 6.2 Scanning electron micrographs of beech wood degradation by white-rot fungi after 120 days; (**a**, **c**, and **e**) *Pleurotus ostreatus*; (**b**, **d**, and **f**) *Trametes versicolor*. (**a**) and (**b**) show cross-sections (bar 20 μm): the arrows point cell walls already degraded and arrowheads point colonization of hyphae in the cell lumina; (**c**) and (**d**) show radial sections (bar 100 μm): the arrows point an entire decomposition of ray parenchyma and arrowheads point deconstruction of cell walls

efficiency, and quality of several products (Kushwaha et al. 2018) and applied in pulp and paper industry (Singh 2018).

6.2.2.2 Brown-Rot Fungi

Brown-rot fungi are also found in the Basidiomycota group, representing nearly 7% of this phylum (Hatakka and Hammel 2011; Goodell 2003). Evolutionarily, most of this group are derived from white-rot fungi, probably by losing of decay capability and biodegradative mechanisms (Hibbett and Thorn 2001). Otherwise, white-rot and brown-rot classification are discussed, since new genetic studies suggest continuum rather than a dichotomy between these two groups. In this case, authors suggest that the "white-rot fungi" term would be restricted to fungi that consume all the cell wall macromolecules through activity of lignin-degrading peroxidases (Riley et al. 2014).

The brown color of brown-rot fungi is due to residual lignin left after degradation. It is caused by fungi enzymatic arsenal that degrades polysaccharides: cellulose and hemicellulose contents decrease, and lignin percentage increases in the pretreated material (Felipuci 2020). Hemicellulose degradation is faster and polysaccharide depolymerization involves oxidative components and hydrolytic enzymes (Hatakka and Hammel 2011).

Degradation capacity is widely known in the bio-pulping area. Bio-pulping is a process where wood chips are treated by microorganisms to improve quality and make stronger paper produced. This method removes wood extractives and lignin, reducing toxicity and pitch content (Gupta 2019). Using some species of brown-rot fungi with worms to degrade paper mill sludge is a useful strategy to enhance cellulose decomposition (Negi and Suthar 2018).

6.2.2.3 Bacteria

Bacteria are known to produce cellulolytic, hemicellulolytic, and ligninolytic enzymes that can also be used in biological pretreatment (Sharma et al. 2019). An advantage in comparison to fungal pretreatment is that some bacteria can grow faster than fungi besides degrade lignin into small particles. Those small particles can be recovered to be used as value-added products as well being faster and low cost since it does not need high temperature and many processes after hydrolysis (Hatakka 2005; Kurakake et al. 2007).

Although bacteria can properly degrade lignocellulosic biomass, its sole use as biological pretreatment has not proved efficient. However, it can improve the enzymatic digestion of lignocellulose after applying another pretreatment, such as

Fig. 6.2 (continued) and vessels; (**e**) and (**f**) show tangential sections (bar 100 μm): the arrows point the separation of ray wall with vessels lumina, while arrowhead point disintegration of woody structure (Bari et al. 2018)

physicochemical method (Zhuo et al. 2018). Co-culture using bacteria and/or fungi can degrade lignocellulosic biomass almost completely due to high enzymatic activity. Selecting the best strains that can produce necessary enzymes is essential for an efficient biological pretreatment in order to produce biofuels and bioproducts (Sharma et al. 2019).

6.2.3 *Enzymes Involved in Biological Pretreatment*

The effectiveness of a biological pretreatment depends on enzymes ability to address biochemical and physical barriers to hydrolysis. Therefore, a mix of enzymes can co-work to increase biomass access by expanding small pores and open the cell wall matrix (Amin et al. 2017).

Lignocellulose degradation by microorganisms is mainly accomplished by a system of extracellular enzymes that hydrolyze and oxidize the biomass component (Fig. 6.3). Hydrolases (cellulases and hemicellulases) are produced by hydrolytic system to degrade polysaccharides and oxidative catalytic system to degrade lignin by the production of ligninases (Sajith et al. 2016).

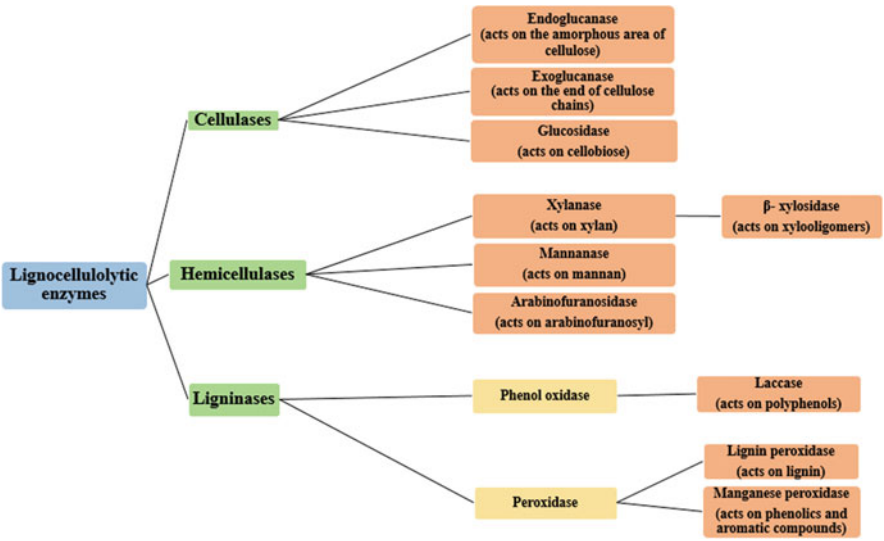

Fig. 6.3 Simplified representation of lignocellulolytic enzymes and their action mode (Sajith et al. 2016)

6.2.3.1 Cellulases

Cellulases are glycosyl hydrolases (GHs) produced by microorganisms while they grow on lignocellulosic materials. They hydrolyze cellulose into shorter chain polysaccharides by breaking down β-1,4-glycosidic bonds. In their structure, they usually have a catalytic domain at the N-terminal and a carbohydrate-binding module at the C-terminal. The catalytic domain cleaves the glycosidic linkage and the carbohydrate-binding module destiny the catalytic domain to the polysaccharide substrate (Jayasekara and Ratnayake 2019; Obeng et al. 2017).

Three main enzymes comprise cellulases enzyme system, endoglucanases (endo-β-1,4-D-glucanases; EC 3.2.1.4), exoglucanases (exo-β-1,4-D-glucanases; EC 3.2.1.91), and glucosidases (β-D-glucoside glucan hydrolases, EC 3.2.1.21). These enzymes are categorized as per their structure and function; however, their collaborative work is essential for complete hydrolysis of the complex cellulose fibers (Sajith et al. 2016).

Endoglucanases generate oligosaccharides with free chain ends by hydrolyzing internal β-1,4-glycosidic bonds and acting randomly on amorphous areas of cellulose. These enzymes can convert cellodextrin (intermediate product of cellulose hydrolysis) into cellobiose and glucose (Singh et al. 2016). Endoglucanases has rapid dissociation, can reduce chain length and viscosity by acting on cellulose but exhibit no activity against crystalline cellulose such as avicel (De Moraes Akamine et al. 2018; Obeng et al. 2017; Sajith et al. 2016).

Exoglucanases act on the crystalline region of cellulose and release cellobiose as product from reducing (EC 3.2.1.91) or non-reducing ends (EC 3.2.1.176). The oligosaccharide chain portion that each enzyme attacks are related to its classification. However, the actions of the enzymes are unidirectional in a long-chain oligomer (Obeng et al. 2017; Singh et al. 2016). These enzymes are more active against crystalline cellulose substrates such as avicel and cellooligosaccharides but do not hydrolyze soluble resultants of cellulose like carboxymethyl cellulose (Jayasekara and Ratnayake 2019; Sajith et al. 2016).

β-glucosidases present rigid structure with an active site that favors disaccharides entry, however, they also can hydrolyze low degree of polymerization soluble cellodextrins. These enzymes act on cellobiose to complete the hydrolysis process of cellulose. As result, glucose with a free hydroxyl group at C^4 from the non-reducing end of oligosaccharides are released (Obeng et al. 2017; Sajith et al. 2016).

Retention and reversion are catalytic mechanisms that lead to successful cellulose hydrolysis. This is performed by two catalytic amino acid residues of the enzymes, a proton donor and a nucleophile. Both of them stereochemically modifies the anomeric carbon configuration, facilitating enzymatic cleavage of the glycosidic bonds (Garvey et al. 2013).

Cellulolytic enzyme multisystem can suffer inhibition by its products. For this reason, β-glucosidases and exoglucanases are essential to alleviate exo- and endoglucanases, respectively, from feedback inhibition. In the same way,

β-glucosidase is also inhibited by glucose, therefore is necessary a search for glucose tolerant β-glucosidases (Obeng et al. 2017). Complementary action of these cellulases is crucial for efficient hydrolysis in order to obtain glucose residues, which can be used for several applications such as the production of biofuel and chemicals. Among microorganisms, fungi are responsible for approximately 80% of cellulose hydrolysis and therefore, considered great cellulase producers (Singh et al. 2016).

6.2.3.2 Hemicellulases

Efficient hemicellulose hydrolysis of lignocellulosic biomass improves hydrolysis yield and consequently reduces enzyme costs and dosages, which makes crucial the use of hemicellulases. They are most often glycoside hydrolases and are usually produced by microorganisms together with cellulases. The hemicellulose backbone of a lignocellulosic biomass can be composed by different polysaccharides, depending on the source (Sindhu et al. 2016; Singh et al. 2016).

Mannan and xylan are the most common hemicelluloses found in nature. Xylan is the main hemicellulose in lignocellulosic biomass from agriculture residues, comprised of xylose units in the backbone chain that are usually linked to acetyl and ferulic groups, arabinofuranosyl or glucuronic acid residues. Therefore, multiple enzymes are necessary to decompose xylan, including endoxylanase (EC 3.2.1.8), β-xylosidase (EC 3.2.1.37) that act on the main chain of xylan. The enzymes that work on the pending groups are α-arabinofuranosidase (EC 3.2.1.55) and α-glucuronidases (EC 3.2.1.139) (Ábrego et al. 2017). In addition, acetyl xylan esterases (EC 3.1.1.72), ferulic acid esterases (EC 3.1.1.73), and p-coumaric acid esterases (EC 3.1.1.x) are also requested for the complete deconstruction of xylan (Chadha et al. 2019). Hemicellulases structures are consisted by a catalytic domain to perform enzyme functions. They can be glycosyl hydrolases that cleave glycosidic bonds or can be carbohydrate esterases that hydrolyze ester bonds, between xylan and acetic acid or ferulic acid substitutions (Juturu and Wu 2013).

Xylanases hydrolyze β-1,4 linkages in xylan backbone chain, producing xylooligosaccharides. Most of them belong to glycoside hydrolase (GH) families 10 and 11, however, enzymes that are exclusively active on D-xylose-containing substrates, known as "true xylanases," are only on family 11 (Tyagi et al. 2019). β-xylosidases hydrolyze a low degree of polymerization xylooligomers, produced by xylan hydrolysis, into xylose. Xylanases action is inhibited by xylooligomers produced in the hydrolysis, therefore β-xylosidases action removes end-product inhibition increasing the efficiency of xylanases (Chadha et al. 2019).

β-mannanases hydrolyze mannan-based hemicelluloses. As result, short β-1,4-mannooligomers are released that can be hydrolyzed into mannose by β-mannosidases. Arabinofuranosidases catalyze the removal of arabinosyl substituents and facilitate an increase in access points of xylanase to xylan Both β-mannanases and arabinofuranosidases are required for mannan or arabinofuranosyl containing hemicelluloses (Terrone et al. 2020). The

α-1,2-glycosidic bond can be broken down by α-D-glucuronidases releasing glucuronic acid from the xylan chain (Chadha et al. 2019; Singh et al. 2016).

Acetyl xylan esterases are enzymes responsible to remove acetyl groups linked to β-D-xylopyranosyl residues by hydrolyzing the ester bonds. The accessibility of enzymes that break the backbone by steric hindrance can be interfered by acetyl side-groups, therefore their removal makes the xylanases action easier. Ferulic acid esterases and p-coumaric acid esterases also catalyze ester bonds on xylan. The first enzymes are recognized to break down ester linkages between ferulic acid and arabinose substitutions on xylan, and the second acts on the bond between arabinose and p-coumaric acid (Chadha et al. 2019; Bajpai 2014).

Hemicelluloses are chemical structure complex, its hydrolysis into its constituent monomers requires catalytic action of versatile enzymes that work synergistically. Hemicellulolytic enzymes can be produced by different fungi and bacteria, however, the source of most commercially important hemicellulases is fungi (Manju and Chadha 2011). They have biotechnological potential and several industrial applications, like hemicelluloses hydrolysis of lignocellulosic biomass, improving cellulose saccharification (Chadha et al. 2019).

6.2.3.3 Ligninases

Lignin is one of the main responsible for recalcitrance in lignocellulosic biomass because its complex structure, protecting polysaccharides (Schmatz et al. 2020). To break down the lignin structure, microorganisms developed some specific extracellular enzymes based on oxidative reactions. In nature, lignin degradation is important to the biogeochemical carbon cycle (Ruiz-Dueñas and Martínez 2009). Those enzymes are also used in the bioremediation process and its action is an important step for lignin removal in industries that work with cellulosic biomass (Jha 2019).

Ligninases are, generally, separated in two different types: phenol oxidases and peroxidases. Laccases are an example of phenol oxidases enzymes. Lignin degradation by laccases (EC 1.10.3.2) is normally by oxidation of phenolic compounds, yielding quinines and phenoxy radicals. Peroxidases make part of oxidoreductases family. This group of enzymes catalyzes lignin depolymerization utilizing H_2O_2 (Sajith et al. 2016).

Laccase enzymes are observed in plants, insects, bacteria, and fungi, mainly in the white-rot group. In fungi, these enzymes are involved not just in lignin degradation but also in sporulation, pigmentation of the fungus, detoxification, and fruiting body (Clutterbuck 1990; Thurston 1994). The molecular weight of laccase is around 50–100 kDa and they are classified as multicopper oxidases, which can be monomeric, dimeric, or tetrameric. Laccase use molecular oxygen to oxidize phenolic rings to phenolic radicals. Laccase can cleave Cα–Cβ cleavage, aryl-alkyl cleavage, and Cα-oxidation. Products may be submitted through non-enzymatic reaction, like polymerization, hydration, or dismutation, or a second enzyme-catalyzed oxidation (Madhavi and Lele 2009; Sajith et al. 2016). With a redox mediators present,

laccases can also catalyze the breakdown of non-phenolic lignin structures, and cleave β-O-4 linkages.

Lignin peroxidase (EC 1.11.10.14) is considered one of the key enzymes in plant cell wall degradation due to its ability to oxidize non-phenol lignin structures. This reaction can cleavage Cα–Cβ bonds, mediating ring-opening reactions. Lignin peroxidases are oxidized by hydrogen peroxide, and, this catalysis results in the creation of intermediate radicals such as phenoxy and veratryl alcohol (Wong 2009; Ruiz-Dueñas and Martínez 2009). Lignin peroxidase and laccase are considered "partners" enzymes in certain conditions, due to substrate provided by lignin peroxidase after lignin degradation (Boominathan and Reddy 1992).

Manganese peroxidase (EC 1.11.1.13) attacks both phenolic and non-phenolic lignin units. This enzyme works as a mediator in enzymatic activity, once it is converted from Mn^{2+} into Mn^{3+}. Several monomeric phenols are oxidized by Mn^{3+} cation, including dyes and phenolic lignin model compounds (Datta et al. 2017).

6.2.4 Enzymatic Hydrolysis of Biological Pretreated Material

In a biorefinery system, lignocellulosic biomass hydrolysis is an essential phase in the whole process, since through hydrolysis intermediate products are obtained by breaking up of macromolecules existent in pretreated biomass (Bichot et al. 2018; Pocan et al. 2018). The intermediate denomination is because these products will be used at a subsequent stage of conversion, the main intermediate products are monomers such as hexoses and pentoses coming from cellulose and hemicelluloses (Loow et al. 2016). Hydrolysis or saccharification can be performed by acid, enzymatic or combined procedures, among the aforementioned, the biological process is possibly the most researched in the last years (Pocan et al. 2018). Hydrolysis by biological routes shows benefits associated to mild temperature in operation, high ratio (quantitative) between obtained product and precursors (monomers), minimal corrosion problems and in enzymatic hydrolysis does not produce inhibitory chemicals that can modify enzymes activities (Amezcua-Allieri et al. 2017; Jahnavi et al. 2017).

The key to the biological hydrolysis of pretreated lignocellulosic biomass is the hydrolytic enzymes; cellulose saccharification happens by deed of cellulolytic enzymes (cellulases), and hemicelluloses splitting befalls by action of hemicellulolytic enzymes (hemicellulases) (Bhardwaj et al. 2019; Barbosa et al. 2020). These enzymes can be synthesized mainly by fungi, bacteria, yeast, or algae through its controlled growth in solid or submersed fermentations (Dotsenko et al. 2018; Aruwajoye et al. 2020). Instead of producing hydrolytic enzymes, there is the alternative to purchase commercial enzymes prepared by different industries dedicated to synthesize and purify enzymatic cocktails that act according to specific conditions in hydrolysis (Flores-Gómez et al. 2018). Table 6.1 shows a summary of some characteristics related to hydrolytic enzymes, their mode of action, product formation, and inhibitory aspects.

Table 6.1 Properties of cellulases and hemicellulases action on lignocellulosic biomass

Macromolecule	Enzyme	EC/Synonym	Act on	Product	Compounds that cause inhibition	References
Cellulose	Endoglycanases	3.2.1.4/ Carboxylmethylcellulases (CMCases)	β-(1 → 4) bonds at non-crystalline sections	Reducing and non-reducing new parts	Cellobiose	Murphy et al. (2013), Park et al. (2019)
	Exoglycanases	3.2.1.91/ Cellobiohydrolases (CBHs) or Avicelases	β-(1 → 4) bonds at cellulose ends and new reducing parts and non-reducing parts	Cellobiose and other glycooligomers	Glycose	Fry (2003), Vianna Bernardi et al. (2019)
	β-glycosidases	3.2.1.21/Cellobiases	β-(1 → 4) bonds at cellobiose	Glycose	Glycose, mannose and galactose	Teter et al. (2014), Hsieh et al. (2014)
Xylan	Endoxylanases	3.2.1.8	β-(1 → 4) bonds at xylan backbone	Xylobiose and other xylooligomers	Xylobiose and Xylotriose	Puchart et al. (2018), Fu et al. (2019)
	β-xylosidases	3.2.1.37	β-(1 → 4) bonds at xylobiose	Xylose	Xylose	Yeoman et al. (2010), Bosetto et al. (2016)
	α-arabinofuranosidases	3.2.1.55	α-(1 → 2), α-(1 → 3) and α-(1 → 5) bonds at xylose-arabinose linkages	Xylose and arabinose	Glycose and galactose	Numan and Bhosle (2006), Yeoman et al. (2010), Malgas et al. (2019)
	α-glucuronidases	3.2.1.139	α-(1 → 2) bonds at glucuronic acid-xylose linkages	Methyl glucuronic acid and xylose	–	Dashnyam et al. (2018), Malgas et al. (2019)
	β-galactosidases	3.2.1.23/lactases	β-(1 → 4) bonds at galactose-xylose linkages	Galactose and xylose	Galactose	Husain (2010) Khosravi et al.

(continued)

Table 6.1 (continued)

Macromolecule	Enzyme	EC/Synonym	Act on	Product	Compounds that cause inhibition	References
						(2015), Malgas et al. (2019)
	Acetyl xylan esterases	3.1.1.6	Acetyl groups located at xylan branches	Acetyl groups	[a]Organophosphate compounds	Montoro-García et al. (2011), Pawar et al. (2016), Razeq et al. (2018)
	Ferulic acid esterases	3.1.1.73	Ester bonds in arabinose-ferulic acid linkages	Arabinose, Ferulic acid and p-coumaric acid (phenolic acids[b])	–	Szwajgier et al. (2010), Lopes et al. (2018)
	p-coumaric acid esterases	3.1.1.73	Ester bonds in arabinose-p-coumaric acid linkages	Arabinose and p-coumaric acid (phenolic acid[b])	–	Lopes et al. (2018)
Linear mannan	Endomannanases	3.2.178	β-(1 → 4) bonds at mannan backbone	Non-reducing new parts and mannobiose and mannotriose	Glycose and galactose	Vries et al. (2005), Da Cruz (2013), Lopes et al. (2018)
	β-Mannosidases	3.2.1.25	Non-reducing new parts and other mannosaccharides	Mannose	Sucrose	McCabe et al. (1990), Da Cruz (2013), Lopes et al. (2018)
	α-Galactosidases	3.2.1.22	α-(1 → 6) bonds at galactose-mannose linkages	Galactose and mannose	[a]Ag^{2+} and Hg^{+}	Da Cruz (2013), Sirisha et al.

						(2015), Lopes et al. (2018)
Galactan	Endogalactanases	3.2.1.89	α-(1 → 3) bonds at galactanan backbone	Galacto-disaccharide Galacto-trisaccharide and Galcto-tetrasaccharide	–	González-Ayón et al. (2019)
	β-galactosidases	3.2.1.23/lactases	β-(1 → 4) bonds at galactose-xylose linkages	Galactose and xylose	Galactose	Husain (2010), Khosravi et al. (2015), Malgas et al. (2019)

[a]Product did not generate in pretreatment and/or hydrolysis of lignocellulosic biomass
[b]Cause inhibition in most of hydrolytic enzymes

Finally, it should be taken into account that hydrolytic enzymes can suffer deactivation by temperature, pH, reaction time, stirring intensity, enzymatic loads, and mixing modes (Balan 2014; Hu et al. 2016; Singhvi and Gokhale 2019). Substrate characteristics and modifications over the enzymatic hydrolysis can increase the material recalcitrance (Wallace et al. 2016). Therefore, it is recommended to develop new researches with new conditions that exploit novel tolerance levels for increasing pretreatment and hydrolysis yields.

6.2.5 *Mechanisms of Cell Wall Degradation by Microorganisms*

During periods of fungal growth, cell wall undergoes structural modifications that allow access to inside components (Riley et al. 2014). Although degrading enzymes are known and studied, degradation can occur in a different manner according to situations: chemical structure and composition of the cell wall are different among woody materials (or non-wood) and enzymatic arsenals of microorganisms are different among them (Fig. 6.4). These factors determine the degradation level of the material and make it difficult to fully understand how biomass is consumed and how the degradation process occurs. Thus enzymes involved in the degradation process must be suitable to each substrate. Furthermore, it is important to evaluate which microorganism and its respective strain are most adequate for each kind of substrate.

Degradation efficiency by microorganisms depends, in many cases, on the chemical structure of molecules and on the presence of efficient enzymes in degrading compounds, which are specific for most substrates (Pereira and De Freitas 2012). Biomass chemical structure can influence the metabolism of the microorganisms, especially regarding rates and extent of biodegradation. In the case of catabolic enzymes that have low specificity for its substrate, xenobiotics with a chemical structure similar to natural compounds can be recognized by an active enzyme system and, consequently, used by microorganisms as a source of nutrients and energy (Pereira and De Freitas 2012).

Carbon sources can influence fungi growth, which can affect growing patterns (Mannaa and Kim 2017). Hyphae development allows better colonization of lignocellulosic material and also penetrate easily to plant cell walls than bacteria, reaching macromolecules unavailable for those microorganisms (Pereira and De Freitas 2012). Enzymes are a crucial tool for the degradation of lignocellulosic biomass. Microorganisms release those enzymes which work in a synergistic and independently action, such as peroxidases, laccases, xylanases, and the other enzymes.

An example of cell wall degradation is proposed in Fig. 6.5 (Zeng et al. 2014). In this degradation proposal, the plant cell wall is degraded by *Phanerochaete chrysosporium*, which is capable to degrade all components of the lignocellulosic biomass. Fungal hyphae attach inside the cell wall, secreting enzymes. Manganese

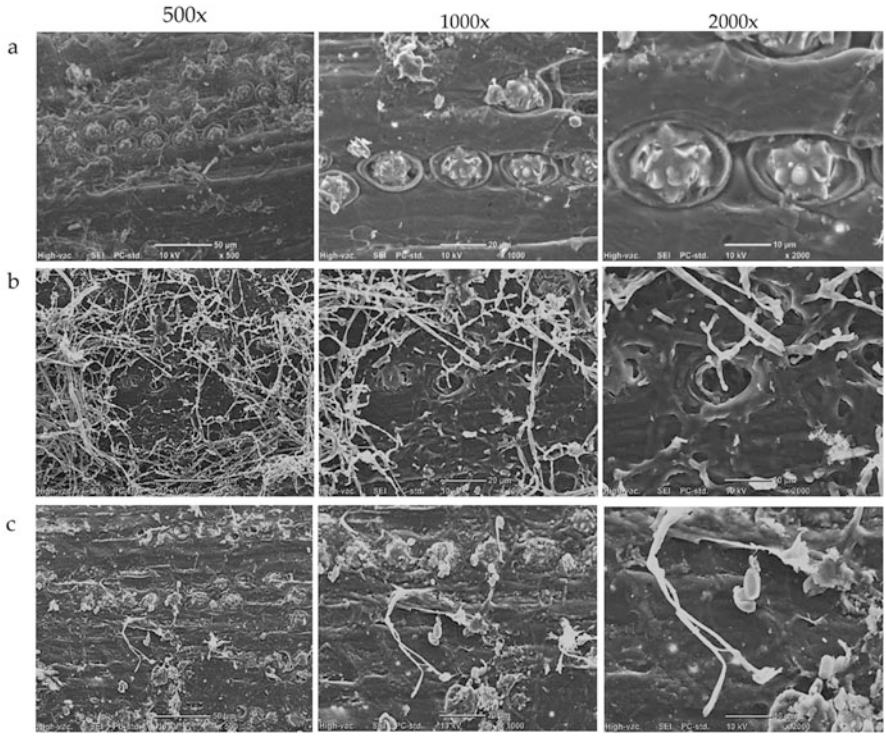

Fig. 6.4 Scanning electron microscope images on the surface of the Oil palm Empty Fruit Bunch. (**a**) Untreated; (**b**) biologically pretreated using *Schizophyllum commune* (ENN1); (**c**) biologically pretreated using *Phanerochaete chrysosporium* (Arbaain 2019)

peroxidases (MnP) oxidize Mn^{2+} to Mn^{3+} and break the phenolic and non-phenolic lignin units (Datta et al. 2017; Wong 2009). Lignin peroxidases (LiP) oxidize non-phenolic structures to mineralized lignin, cleaving Cα–Cβ bonds, mediating ring-opening reactions (Wong 2009; Ruiz-Dueñas and Martínez 2009). This process occurs in the secondary cell wall, in which are located structural carbohydrates as well as aromatic backbone. Cellulases hydrolyze β-1,4-glycosidic bonds and act on the microcrystalline region in cellulose chain to break the cellulose into monomers of cellobiose and D-glucose. Cellobiose dehydrogenases co-work with cellulases to break cellulose chains into small saccharides, generating hydroxyl radicals, H_2O_2, and Fe^{3+}.

Although the process of degradation could be different from all microorganisms, the enzymes work similarly but secreted at a different amount, and one characteristic that can be noticed is the variety of the lignocellulosic structure/composition. In wheat lignin degradation using analytical pyrolysis was revealed that Cα–Cβ bonds and free phenolic units are preferred than non-phenolic units by *Pleurotus eryngii* and *Phanerochaete chrysosporium*. This preferential is due to the redox potential that is lower in comparison with the etherified ones, permitting easier oxidation by

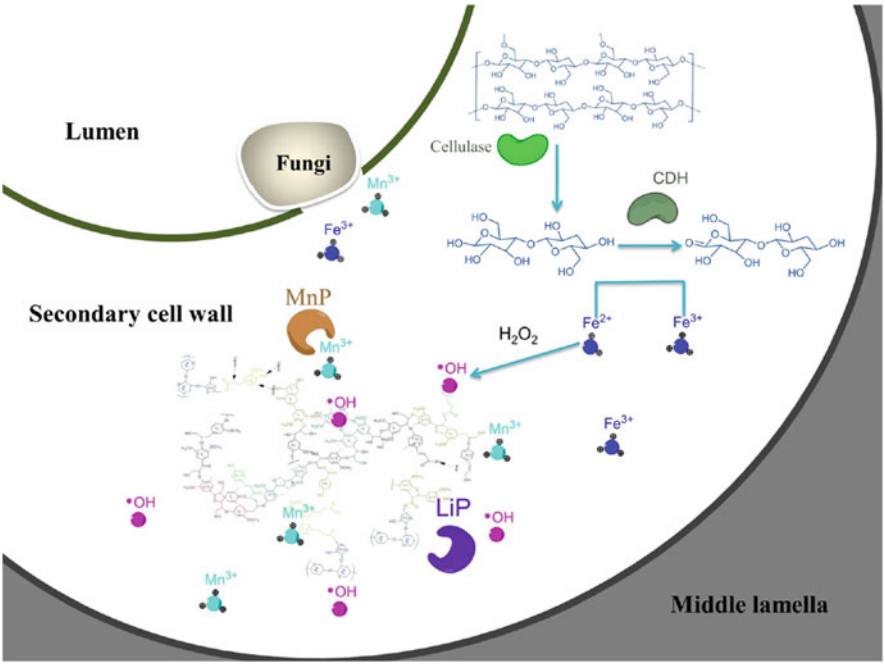

Fig. 6.5 Proposed process of degradation of the wheat straw cell wall by *Phanerochaete chrysosporium* (Zeng et al. 2014)

ligninolytic peroxidases and laccases produced by the fungi. In vitro, applying enzyme in lignocellulosic biomass, *P. eryngii* is capable to reduce the phenolic content of lignin, evidencing its capacity of modifying lignocellulosic materials (Martínez et al. 2001; Camarero et al. 2001). Another example of lignocellulosic biomass deconstruction is with the brown-rot fungi *Penicillium echinulatum*. In this case, using different carbon sources was grown wild-type (2HH) and a mutant strain (S1M29). It was realized that the mutant was more capable to produce cellulases and hemicellulases, showing that the variety of microorganisms can differentiate by the quantity of enzymes produced (Schneider et al. 2016).

6.3 Economic Impacts and Challenges on Industrial Scale Involving Biological Pretreatment

Studies involving biological pretreatments are needed today for several reasons, including environmental friendly process, chemical reduction, and energy savings. There is a growing number of items produced from fossil derivatives such as plastics and tires that are not renewable, in addition to remaining in nature indefinitely. Nevertheless, it is important to mention that a biotechnological route should concern

about energy and chemical reagents applied, aiming to be more advantageous than traditional processes.

For biofuels, specifically, greenhouse gases bring concern and it is on the part of governments. Gas derived from fossil is already being replaced by biofuels, which draws attention to new processes of production and ways to reduce costs. The type of biomass, process complexity, and value of by-product influence the choice of pretreatment (Bajpai 2016). Despite chemical pretreatments holding the main focus on these procedures, biological pretreatments are able to optimize those processes in several levels, for instance: reduce the water, chemicals, and energy spent, generate less inhibitor and toxic compounds, reduce the costs, and improve performance and yield.

In the food industry, one of the most worrying problems is waste since all economic classes in society have a certain degree of waste generation (McCarthy and Liu 2017). This food that is not used can be turned into energy by the biological or thermochemical process. Biological pretreatment in food waste has advantages in comparison with conventional methods of pretreatment such as low cost and simplicity (Pham et al. 2015). Lignocellulosic biomass products can be a source of material and energy in order to support a more sustainable society. Products of direct consumption or second value-added are already present in human life such as paper, fibers and textiles, nanocellulose, organic acids, furfural, and others (Zamani 2015). Food and biofuels are examples where biological pretreatment can be used to improve the productivity and reduce costs. Moreover, several million tons of lignocellulosic are produced annually, and the biological pretreatment can makes this biomass even more useful.

Biological pretreatment can be economical. The extensive number of products that can be produced with lignocellulosic biomass after a biological pretreatment makes harder this count, considering the production cost and sell value of each one. An example, the xylan extraction using biological pretreatment before chemical (H_2O_2) pretreatment reduced the need for the chemical reagent to reach the same results, which means less cost in the process (Felipuci 2020). On the other hand, the production of fermentable sugar by biological pretreatment of corn stover using posterior enzymatic hydrolysis showed to be more expensive (1.41 $/kg) than steam explosion (0.43 $/kg), dilute sulfuric acid (0.42 $/kg), and ammonia fiber explosion (0.65 $/kg) methods (Baral and Shah 2017). In this case, there was no need of detoxification using biological pretreatment. However, this method investigated required reactors, mainly due to long pretreatment time. Biological pretreatment could considerer an option of process outside not using any reactor, but face other problems such as contamination.

Although the advantages of an experimental scale, the use of biological pretreatment in the industry is still a challenge. Recent studies showed the potential of microorganisms in biofuels productions using biological pretreatment (Yahmed et al. 2017; Zabed et al. 2019). However, it is a common view of all the difficulties involved in biological pretreatment on a large scale. Microorganism utilization in biotechnological processes requires certain precautions, which needs to add one or

more steps in the process: contamination and sterilization of growth site are some examples. Furthermore, microorganism growth is slow, while sugars are fundamental as an energy source (Vasco-Correa et al. 2016; Ummalyma et al. 2019). An option to improve the process and pass through those problems is the genetic engineering as well as co-culture of suitable microbial consortium (Sharma et al. 2019).

6.4 Concluding Remarks

Biological pretreatment has several advantages over traditional biomass separation methods. Application of microorganisms and their enzymes, in addition to enhancing the breakdown of lignocellulosic structure, makes the process cheaper and less aggressive to nature. An important advantage is no by-products generation, improving the fermentable sugars production by enzymatic hydrolysis of cellulose, with appreciable cost-benefit, among other benefits.

Microorganisms present great potential for industrial use. Employment of microorganisms in pretreatments, or just their enzymes, can provide a reduction of energy and chemical reagents consumption in the separation process of lignocellulosic biomass macromolecules. Microorganism co-cultivation is a valid technique option with biotechnological potential, once the enzymes produced by the microorganisms can complement each other, achieving a greater degree of degradation. Mechanism degradation of plant cell wall depends on the microorganism in question and, mainly, on its enzyme production and action on lignocellulosic biomass. Even though the use of the micro in the industrial scale requires greater cultivation assistance, it still offers important advantages: there are cost reduction and yield improvement for the biorefinery area and also less chemical residues in the environment.

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Chapter 7
Enhanced Biofuel Production from Lignocellulosic Biomass: An Overview of Advanced Physico-Chemical and Biological Technologies

Dolly Kumari and Yogita Jain

Abstract Lignocellulosic biomass has become popular as an untapped source for biofuel production which plays a dual role, i.e., sustainable development and renewable energy production. Biofuels are the alternatives to fossil fuel which are cheap and environmental friendly and also have the capability to reduce energy crisis along with waste management. But, there are some hurdles in direct conversion of lignocellulosic biomass (composed of cellulose, hemicellulose, and lignin mainly) to biofuels (i.e., biohydrogen, biogas, bioethanol, biobutanol, etc.). The main problem occurs during the renovation of the lignocellulosic substrate to biofuel with due to the composite nature of lignin. Lignin also prohibits cellulose and hemicellulose to expose to digestion easily by microbial activity. Pretreatment of lignocellulosic biomass is a pre-requisite to alter the compositional and the structural obstruction of the biomass resulting in an enhanced yield of biofuel. This chapter provides an overview of various lignocellulosic biomass resources along with biofuel production technologies with special reference to physico-chemical and biological technologies.

Keywords Lignocellulosic biomass · Pretreatment · Biofuel · Organic waste · Biohydrogen · Anaerobic digestion

Abbreviations

AD	Anaerobic Digestion
MSW	Municipal Solid Waste
MW	Microwave

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7.1 Introduction

There are various challenges in the present world like climate change, depletion of resources, and increase in demand for fuel, etc. for which we humans are fighting. A robust increase in industrialization leads to an increase in demand for fuels which provides benefits to some countries that are capable to fluctuate the price of fuels. As these issues grow over the years, research has also been increased to find some alternative sources of fuel (Aftab et al. 2019). These alternative sources usage can help in controlling the environmental pollution and have the potential to fulfill the global energy demand to some extent.

While finding the alternative solution, biomass-derived energy sources came out as boon to the world. These biomass-derived fuels have the potential to replace conventional energy sources and are also non-polluting and environmentally benign (Mahapatra and Kumar 2017). This book chapter thus designed to have some in-depth knowledge about the biomass-derived fuels, their advantages, and different ways through which yield can be enhanced.

7.1.1 History and Progression of Biofuel Production

For a very long time period, we (humans) depended on fossils to meet our energy demands. Plant biomass was the first source of fuel utilized by human beings. With increase demand of industrialization, fertile lands and biomass were destroyed. The negative impact of traditional fuels on circumstances and increase demand lead researchers to shift their focus towards biomass again. Fuels generated from this biomass were then classified into first, second, third, and fourth generation which will be discussed later in this chapter. A huge variety of biomass has been utilized and explored till now for biofuel production. Currently biomass fulfills more than 10% global energy demands which make it a viable source of fuel for the present and future generations (Miao and Wu 2004; Saidur et al. 2011; Heidenreich and Foscolo 2015; Sikarwar et al. 2017).

7.1.2 Current Scenario

Increase in population resulted in the increased demand for food and fuel both. Lignocellulosic waste generated during the production of crops can act as a good energy source which also leads to the sustainable development of the society. The present era has lots of technologies that can help in extracting the energy from the lignocellulosic biomass and the continuous development also helps in finding the more suitable way to increase the yield of biofuels (Mari 2016). Various modifications have been done in different countries to increase the utilization of

the biomass-derived fuels. As there are many pros of biomass-derived fuels, there are also some negative aspects which have to solve like pilot-scale modifications, higher yield, storage, transport, and the modifications which have to be done to utilize these fuels in efficient manner. Apart from all these technological issues, awareness regarding biofuels is also very important. In the present conditions, there are various pretreatment methods that are being explored to increase the yield of biofuels like physical, chemical, physico-chemical, biological, etc. (Kumari and Singh 2018) which will be discussed in detail further.

7.1.3 Different Types of Biofuels

Now-a-days there are several types of biofuel that can be extracted from lignocellulosic biomass such as hydrogen, methane, ethanol, etc. (Mahapatra and Kumar 2017). These renewable energy sources have greater potential in comparison to the conventional fuels in relations to energy security, financial issues, and ecological stability (Demirbas 2009; Nigam and Singh 2011; Zabed et al. 2016; Zabed et al. 2019). Some of these fuels are:

7.1.3.1 Bioethanol

At present time bioethanol is majorly produced from corn and sugarcane at the industrial level and can be blended with gasoline in specific proportions (RFA 2015). A huge variety of waste can be exploited to produce bioethanol, e.g., citrus peels, leaves, straw, corn stock, sugarcane bagasse, etc. (Kumari and Singh 2018). Fermentation of molasses and starch for bioethanol production is emerging out as a mature technology and different cellulolytic clostridia can be used for this fermentation process. (Antoni et al. 2007).

7.1.3.2 Biobutanol

Biobutanol is considered as an excellent fuel as it can be used directly in engines without modifying them. Production of biobutanol is mainly done by solventogenic *Clostridium sp*. bacteria during the fermentation process which help in the breakdown of the complex sugars like pentose, hexose (Mahapatra and Kumar 2017). Fermentation pathway used for the production of biobutanol is termed as the ABE pathway (Kumar and Gayen 2011). There are various feedstocks which can be utilized for the production of biobutanol like sugarcane juice, Napier grass, corn stover, willow biomass, rice straw, etc. (Cheng et al. 2012, Ranjan et al. 2013, Guo-Chao et al. 2016, Chi-Ruei et al. 2017, Jianzhi et al. 2017). There are various kinds of usage of biobutanol like in cosmetics, drugs, antibiotics, hormones, etc. (Cheng et al. 2012).

7.1.3.3 Biomethane

Biomethane is another viable fuel that can be formed from biomass through anaerobic digestion (AD). Production of biomethane is considered as the simplest and favorable method due to less capital investment and economic benefits (Rao et al. 2010; Chandra et al. 2012). Basically biomethane can be produced through two methodologies, i.e., chemical and biological, out of which biological process operates at a slower rate (Michailos et al. 2020). In agricultural driven countries there is a huge feedstock which can be utilized for the biomethane production like sugarcane, food waste, petha wastewater, etc. (Bansal et al. 2012; Kumar and Lin 2013; Bruno et al. 2016).

7.1.3.4 Biohydrogen

Hydrogen is regarded as the most excellent fuel due to its clean, non-polluting nature and high calorific value. All these properties make hydrogen as a future fuel drawing more and more attention (Valdez-Vazquez et al. 2005). Numerous methods have been used for the production of hydrogen-like steam remodeling, hydrocarbon's partial oxidation, coal gasification, photolysis of H_2O, biological method, etc. In comparison to the other methods, the biological hydrogen production method is an energy extensive process that can be performed using various feedstocks both solid and liquid-like food waste, petha wastewater, rice straw, vegetable waste, etc. (Singhal and Singh 2014; Kumari and Singh 2018).

7.1.4 Advantages of Biofuels

Till now we have an outlook of biofuel, its types and capabilities in the present time. Worldwide researchers are focused to develop some efficient technology and method for the production of biofuels which can meet global energy demands (Zabed et al. 2019). Some of the advantages of biofuels are listed below:

- Reduced the use of fossil fuels
- Higher reliability and renewability
- Reduced air pollution and landfill sites
- Waste stabilization
- Reduced Greenhouse gases
- Carbon sequestration
- Sustainability
- Employment generation and rural development
- New industrial development, etc.

7.1.5 Environmental Challenges and Remediation

Global, economic and social changes are mainly related to the energy potential of a country. Presently, petroleum and coal are used to fulfill 80% of the energy demands worldwide which is expected to grow by 37% till 2040 (Joshi et al. 2017; Kour et al. 2019). A large number of methods are available and can be expended for the production of biofuels (Tomes et al. 2010). Plant biomass, agricultural wastes, food waste, etc. has the potential and are being studied over the years for the production of biofuels. There are various advantages of biofuels but still some challenges have to be faced to make biofuels worldwide acceptable in an efficient manner (Dragone et al. 2010; Rodionova et al. 2017).

Presently, the challenges some of which have to be confronted for mercantile extraction of biofuel to meet the global energy demands are (Scott et al. 2010; Nigam and Singh 2011; Wang et al. 2018):

- Enzyme production
- Technology development and cost
- Pretreatment methods for enhanced yield of biofuels
- Storage and transport facilities
- Food-fuel competition, etc.

To overcome most of these challenges, various biofuel production technologies have developed and some are under the developing stage. For the production of second-, third-, and fourth-generation biofuels by pretreatment of lignocellulosic biomass and technologies utilized is discussed in the following sections.

7.2 Biofuel Production Technologies

A number of biofuel production technologies are used in the last decades for biofuel generation. Generally, biofuels are derived from a large variety of biomass, microorganisms, and different plants or animal wastes (Mari 2016). Biofuels are classified into four main categories according to the feedstock used and the production technology applied for the production process. Figure 7.1 represents the four categories of biofuels (Kumari and Singh 2018).

7.2.1 First-Generation Biofuel Production

Production of "first-generation" biofuels takes place by utilization of agricultural food crops like wheat, rice, maize, sugarcane, barley, soybean oil, sugar beet, sunflower seed, palm oil, cellulose, etc. But, utilization of these food crops is not advantageous as it can result in problems like food scarcity in developing countries

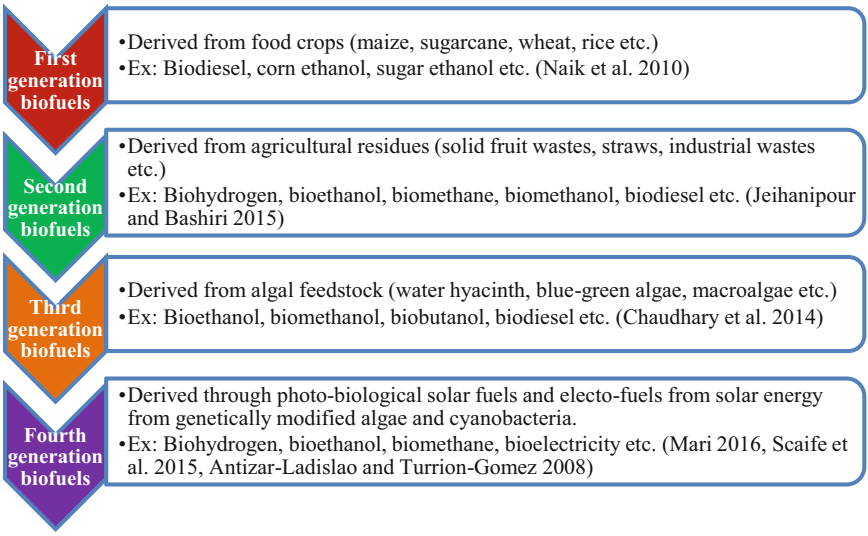

Fig. 7.1 Classification of biofuels. *Source*: Kumari and Singh 2018

like India which is already facing food supply problems due to the enhancement of the human population (Naik et al. 2010). Some of technologies provide a limited yield of specific biofuel and also negatively impact the food security (Mari 2016). The rise in food prices is an undesirable effect of first-generation biofuel production. These biofuels also have some negative impression on surroundings and carbon balance which confines the intensification of first generation biofuel production (Laursen 2006). Thus there is an urgent need to develop an advanced technology for biofuel production which could be capable to produce biofuels from non-food renewable sources of biomass. This necessity attracted researchers to switch to produce second and third generation biofuels.

7.2.2 Second-Generation Biofuel Production

"second generation" biofuels are derived from the agricultural biomass (also called lignocellulosic biomass) rather than food crops by use of advanced technologies. The main objective of second-generation biofuel production is to sustainably produce a higher amount of biofuels from non-food parts of crops, agricultural residues, municipal solid wastes, and industrial wastes (Antizar-Ladislao and Turrion-Gomez 2008). Production of these biofuels is also environmental friendly because these are carbon deficient or carbon neutral and hence do not contribute to CO_2 concentrations like fossil fuels. Production of second generation biofuels is expensive as these are produced from cheap, abundant, and easily available non-food biomass (Naik et al. 2010). second generation fuels have the potential to demote net carbon discharge,

upsurge energy proficiency, and undermine the drawbacks of first generation biofuels (Antizar-Ladislao and Turrion-Gomez 2008). These fuels can be obtained by AD and fermentation after the pretreatment of lignocellulosic biomass (Kumari and Singh 2020a).

7.2.3 Third-Generation Biofuel Production

Production of "third generation" biofuels is now under massive research to develop a cost-effective method to upgrade the metabolic production of biofuels from algal biomass. The separation process in bio-oil production of third-generation biofuels to eliminate non-fuel part to lower the production cost is in practice correspondingly (Mari 2016). Hence specific extraction of biofuels like biodiesel, ethanol, and biogas from algal biomass is of great interest to enhance biofuel production from renewable sources that would able to compete with fossil fuels in the future (Panbdey et al. 2014). This expertise is still not commercial and sustainable due to low Photon to fuel transition proficiency of biodiesel production. Recent improvements in metabolically engineered algal biomass to enhance lipid production without cooperating progress can be considered likely an imperative breakthrough regarding the ecological production of biodiesel (Trentacoste et al. 2013). The combined production of algal biofuel and high-value chemicals, using wastewater (and or seawater) as cultivation modes along with the elaboration of supplementary economical bio-reactors are some expertise, which will force to produce algal biofuel more advantageous in the immediate future. It is the matter of immediate concern to modify a genetically engineered cost-efficient algal biofuel to restrain the problem of agronomy, harvesting, and handling (Medipally et al. 2015).

7.2.4 Fourth-Generation Biofuel Production

Production of "fourth-generation" biofuels takes place by the use of photosynthetic biology of algae and cyanobacteria (Scaife et al. 2015) being a juvenile but highly progressing field for renewable energy production. Production of first-, second-, and third-generation biofuels is dependent whichever upon biomass or organic wastes those are the result of former time photosynthesis of plants (different from contemporary resources). Even though these fuels are very useful but their production is often limited to the productivity and availability of the consequent raw material which bounds their global applications. Conversely production of fourth-generation biofuels would be centered on widely available, cheap, and principally everlasting raw materials (Mari 2016). Photosynthetic or photocatalytic water splitting into its elements through sunlight can be fitted a huge supplier of global scale fuel production. This can be achieved either by unnatural photosynthesis (Inganäs and Sundström 2016) or and through uninterrupted solar biofuel fabrication expertise.

Not only the hydrogen generation (Sharma et al. 2019) but also the manufacture of carbon deficient fuels is promising via simultaneous boosted preoccupation of atmospheric carbon dioxide and inventive strategy of artificial metabolic routes to produce biofuels. Hence fourth-generation biofuels can be produced by designer photosynthetic bacteria, electro-biofuels, or specifically couturier synthetic organelles designed to produce highly esteemed products (Mari 2016).

7.3 Lignocellulosic Biomass Resources

Mainly two types of biomass resources are available in the world which can be obtained from the agriculture sector, aquatic plants, forest, and industries as depicted in Fig. 7.2. Plants, trees, grasses, forests, agricultural crop residues, and municipal solid wastes are the versatile renewable sources of biofuel production for a long time because these are everlasting and renew through the photosynthesis process by use of environmental CO_2, water, and sunlight. Due to the presence of their constituents (namely cellulose, hemicellulose, and lignin) these are called lignocellulosic biomass. Primary metabolites like carbohydrates and lignin are present in abundance in lignocellulosic biomass as compared to secondary metabolites (i.e., gum, resins, rubber, waxes, alkaloids, tannins, etc.) being lower amounts present (Clark 2007;

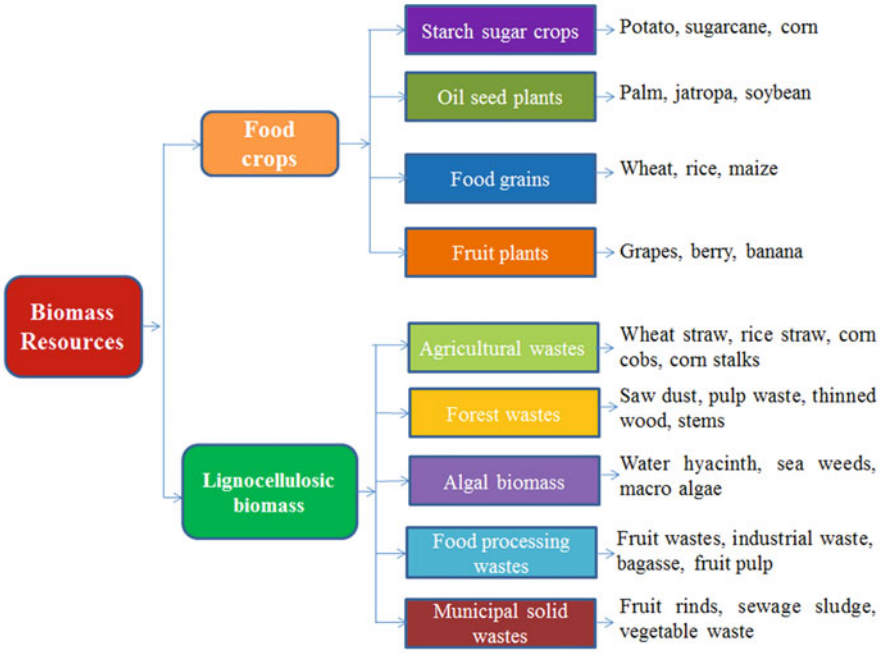

Fig. 7.2 Schematic representations of various kinds of biomass resources. *Source*: Naik et al. 2010

Naik et al. 2010). These low volume secondary metabolites are applied to produce prominent chemicals (i.e., medicines, cosmetics, food flavors, etc.) using assimilated techniques. Whereas the lignocellulosic portion of plant biomass can be exploited for a large variety of biofuel production (i.e., biogas, biohydrogen, bioethanol, biomethanol, biodiesel, etc.) after suitable pretreatment of biomass (Yadav and Vivekanand 2020; Kumari and Singh 2018).

7.3.1 Agricultural Crops and Their Waste by-Products

The agriculture sector produces two types of crops mainly; food crops and grasses. Food crops include wheat, maize, rice, sugarcane, sugar beet, sweet sorghum, vegetables, fruits, oilseed rape, etc. and grasses include switch grass, rye, alfalfa, Miscanthus, etc. But the use of these crops for biofuel production creates conflict between food and energy (Fenning et al. 2008) so crop residues are the best alternatives of food crops that can be used for biofuel production. Rice straw, wheat straw, barley straw, corn cobs, corn stover, citrus waste, switch grass, etc. are the good option of agro-industrial wastes (Jeihanipour and Bashiri 2015). Production of lignocellulosic ethanol and the use of rice straw for this purpose is now in demand because other agricultural residues (i.e., wheat straw, barley straw, and corn stalks) are used as cattle fodder. But high silica content makes rice straw unfit to use as cattle feed and confirms its high availability for biofuel production (Kumari and Singh 2020a, 2020b; Singh and Kumar 2019; Hans et al. 2019).

7.3.2 Municipal Solid Waste (MSW)

MSW comprises residues from household and industry (i.e., fruits and vegetable rinds, food waste, rotten fruits and vegetables, fruit pulp from juice industries, paper waste, kitchen waste, etc.) which is a virtuous raw material for biofuel production. A variety of MSW had been recycled for biogas production (Bolzonella et al. 2019; Kader et al. 2015; Bansal et al. 2013), biohythane (Gottardo et al. 2017; Yeshanew et al. 2016; Giuliano et al. 2014), biohydrogen (Singh et al. 2017; Ghimire et al. 2015; Singhal and Singh 2014; Bansal et al. 2012; Elsayed et al. 2011), bioethanol (Kumari et al. 2018), volatile fatty acids (Kumari and Singh 2020a) and other high-value commodities. The great advantage of MSW is its biodegradability (about 65%) which eases its utilization to produce biogas and bioethanol (Li et al. 2007). The use of biodegradable MSW is more suitable if it treated on site for waste to energy purpose because this practice will reduce the extra load of landfills (McIvor and Evans 2008). The government of India has encouraged people to segregation of biodegradable and non-biodegradable fraction of their household waste and door to door collection of both kinds of waste is also managed separately for proper utilization of biodegradable fraction by energy as well as manure production plants.

For this purpose, "Swachh Bharat Abhiyan" campaign was launched in India on October 2, 2014, on the occasion of the birthday of Mahatma Gandhi.

7.3.3 Wood and its Wastes

Wood residues like sawdust and wood chips are another option of lignocellulosic biomass which can be obtained from a number of trees (e.g., eucalyptus, poplar, Salix, bamboo, pine, willow, etc.) (Salehian et al. 2013; Tomé and Verwijst 1996) and provide hypothetically high calorific values than agronomic wastes for biofuel production. A large advantage of cultivating these woody plants is growth in the marginal of agronomic land without compete with food harvests for space (Fenning et al. 2008). Pine forests in India (Uttarakhand) produce around 20.58 lakhs tones biomass per year on dry basis (e.g., pine needles, bark, etc.) (Bisht et al. 2014) which can be a good option for biofuel production prior suitable pretreatment. The use of poplar leaves was found effective after acid and alkaline enzymatic pretreatment for biohydrogen production (Cui et al. 2010). Currently, just a minor fraction of liquid fuels is derived from forests which can be enhanced by the development of a viable and economic technique for liquid biofuel production from woods for widespread use in the transportation segment.

7.3.4 Algal Biomass

The use of algal biomass for biotechnological biodiesel production is a new emerging field of research (Kim et al. 2013). Bioenergy production using algal biomass (microalgae and macroalgae both) attracted investors for funding projects to produce biofuels using this sustainable biomass even the cultivation of some species of algae is also in practice to achieve good biofuel yields. Biofuels derived from algal stuff are generally called third- and fourth-generation biofuels (Fig. 7.1). Cultivation of these algae can be done in natural ponds, man-made ponds, or in photo-bioreactors. Thousands of algal species are available with varying sizes from microscopic to about 60 meter long. In sympathetic environments, algal commodity grows very swiftly and attains its total mass with 50% oil (Algae base 2020). Various microalgal (Feng et al. 2019; Fabiana et al. 2016; Costa et al. 2015) and macroalgal biomass (Montingelli et al. 2016; Xue et al. 2016; Yann et al. 2015) were consumed to produce biohydrogen, biogas, and other biofuels. Application of a synergistic approach for sustainable production of third- and fourth-generation biodiesel from algal biomass can reduce the cost of biofuel (Raslavičius et al. 2014).

7.3.5 *Food Processing Wastes*

A large variety of food processing wastes has been utilized for the production of various kinds of biofuels. Being produced from different industries, these wastes can also be called the industrial wastes and includes; palm oil mill effluent (Seengenyoung et al. 2018, Mamimin et al. 2015), dairy wastewater (Kumari et al. 2018), sugarcane bagasse (Bruno et al. 2016), wastewater from pulp and paper industry (Antizar-Ladislao and Turrion-Gomez 2008), olive mill wastewater (Anish et al. 2016), de-oiled jatropha waste (Kumar and Lin 2013), wastewater sludge (Guo et al. 2008), rambutan fruit waste (Kandari and Gupta 2012), used cooking oil, etc. are used for the production of biofuels. These food processing wastes were used solely or with some other lignocellulosic biomass for ethanol and biogas production (Kumari and Singh 2020a; Kumari et al. 2018). Besides from these wastes, various industrial wastewater forms are also rich source of organic substrates and energy can be recovered from these wastewaters in the form of biofuels and electricity (Kumari and Singh 2019; Singhal and Singh 2015).

7.3.6 *Garden Waste*

Tree pruning, leaves, stem, grasses, bark, nutshells, etc. are some types of garden wastes and can be utilized for producing biofuels after collection at the time of seasonal or manual cutting and shaping of garden plants and hedges. This is also a kind of lignocellulose rich biomass but cannot be used without prior pretreatment for biofuel extraction. Agricultural residues contribute only 5% of lignocellulosic biomass production (similar to landfill gases) followed by MSW (24%) and woods and wood residues (65%) (Deshmukh et al. 2008).

7.4 Advanced Pretreatment Technologies and Their Effects

Pretreatment is a process of converting complex lignocellulosic biomass into its simple components (cellulose, hemicellulose, and lignin). Removal of lignin, hemicellulose preservation with enhancement in the porosity of the material is the main objective of the pretreatment process. An effectively economic pretreatment should reduce the crystallinity of cellulose with improvement in released sugar and produce the least amount of inhibitors (Chiaramonti et al. 2012). For this purpose, a number of pretreatment technologies (namely, physical, chemical, physico-chemical, biological, and combined) are available, shown in Fig. 7.3.

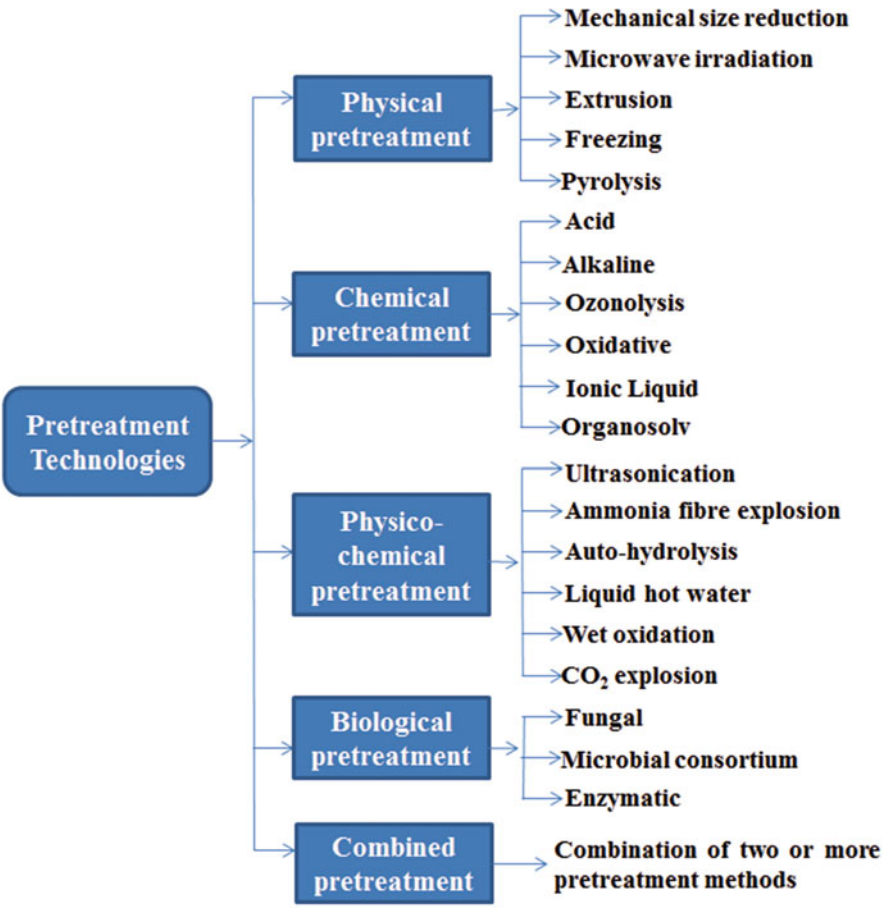

Fig. 7.3 Schematic representation showing various types of pretreatment technologies

7.4.1 Chemical Pretreatment

Figure 7.3 shows various types of chemical pretreatment methods of lignocellulosic biomass for enhanced biofuel production. Different kind of lignocellulosic biomass was pretreated chemically for the production of biofuels. Olive mill residue was pretreated with alkaline H_2O_2 for biomethane production (Siciliano et al. 2016). Free nitrous acid was used by Xue et al. (2016) for the pretreatment of algal biomass for methane production. Ghimire et al. (2015) used Sodium 2–bromoethane sulfonic acid for pretreatment of potato and pumpkin waste for biohydrogen production. NaOH was used for alkaline pretreatment of Teff straw (Akiber et al. 2015), pine wood (Salehian et al. 2013), rice straw (Chandra et al. 2012), and poplar leaves (Cui et al. 2010). Alkaline petha wastewater and acidic dairy wastewater pretreatment were applied for pretreating rice straw for bioethanol and methane production

(Kumari and Singh 2020a, Kumari et al. 2018). Olive mill wastewater and olive pomace were pretreated with H_2SO_4, NaOH, and $CaCO_3$ for bioethanol production (Sen et al. 2016). Gamba grass (Bagudo et al. 2014), willow biomass (Han et al. 2013), and rice straw (Ranjan et al. 2013) were pretreated with H_2SO_4 for ethanol and biobutanol production, respectively. Corn stover was pretreated with deep eutectic solvents for biobutanol production (Guo-Chao et al. 2016).

7.4.1.1 Effect of Chemical Pretreatment Methods

The higher concentration of acid in acid pretreatment produces many inhibitors which inhibits the growth of microbes and reduces biofuel production (Taherzadeh and Karimi 2008). Strong oxidizing agents like H_2O_2 oxidizes lignin to produce inhibitors (soluble aromatic compounds), more than 4% H_2O_2 inhibits the anaerobic digestion (AD) process (Song et al. 2012). Alkaline pretreatment also inhibits AD particularly methanogenesis (Chandra et al. 2012). Ozonolysis is an expensive technique and utilization of large amount of ozone limits its extensive use (Appels et al. 2012). Ionic liquid pretreatment is a new technique for the pretreatment of lignocellulosic biomass but the high cost is major drawback of this technique due to the use of expensive solvents (Kumari and Singh 2018). In organosolv pretreatment organic solvents (ethanol, methanol, acetone, ethyl glycol, etc.) with acid and alkalis as catalysts are used as de-lignifying agents (Guo-Chao et al. 2016).

7.4.2 Physical Pretreatment

These pretreatments include milling, grinding, freezing, pyrolysis, and biomass irradiation with various rays (Fig. 7.3). Many of these pretreatment technologies were used on a large variety of biomass by various researchers for biofuel production. Microalgal biomass was pretreated by beating, ball milling, and microwave (MW) for biomethane production (Montingelli et al. 2016). Milling was applied to a large variety of biomass (e.g., Sawdust, Japanese cedar, rice bran, rice straw, and husk) for enhanced biomethanol production (Hitoshi et al. 2005). MW irradiation was applied to wastewater sludge (Guo et al. 2008), mixed microbial culture (Singhal and Singh 2014), and microalgal biomass (Feng et al. 2019) for biohythane, biohydrogen, and biomethane production, respectively. Wheat straw was MW irradiates at 150 °C for enhancement in methane yield (28%) than untreated straw (Jackowiak et al. 2011). Extrusion of cassava was done for the maximum conversion of starch to ethanol (Chang and El-Dash 2003). Catalytic pyrolysis was applied by Bu et al. (2016) for biofuel and other chemical production.

7.4.2.1 Effects of Physical Pretreatment Methods

Size reduction enhanced biofuel production but De la Rubia et al. (2011) found that milling should be done in such a way so that it will not negatively affect the biofuel production process because excessive milling may result in reduced biofuel yield with extreme amount of inhibitory products (volatile fatty acids). A great advantage of milling is that fermentative inhibitors like furfural and hydroxymethyl furfural are not produced in milling (Ramos 2003). According to Gabhane et al. (2011) MW pretreatment collapses the cellulosic component of biomass as a result of dielectric polarization by molecular conflict. Li et al. (2012) reported that MW irradiation of *Pennisetum hybrid* at 260 °C adversely affected methane production. Extrusion is thermo-physical pretreatment which is more advantageous because fiber shortening occurs due to disruption of biomass by application of shear forces (Senturk-Ozer et al. 2011). Freezing is an eco-friendly but comparatively expensive pretreatment technique because of applying less amount of harmful chemicals but less research has been done. Pyrolysis is done at more than 300 °C for the disintegration of biomass cellulose into gases (CO and H_2) and char which is finally converted into glucose for the biofuel production process.

7.4.3 Physico-Chemical Pretreatment

Various physico-chemical pretreatment methods are also listed in Fig. 7.3 which was used for the lignocellulosic biomass pretreatment. Use of ultrasonication was done to pretreat rice straw (Kumari and Singh 2020a; Kumari et al. 2018) and food wastes (Elsayed et al. 2011) for methane, ethanol, and biohydrogen production. Coconut husk and cactus (Fabiana et al. 2016), sugarcane bagasse were auto-hydrolyzed (Bruno et al. 2016), beach-macro-algae was pretreated with thermo-acidic (Yann et al. 2015; Akiber et al. 2015), *Miscanthus lutarioriparius* was treated with a steam explosion (Ivo et al. 2016). Household food wastes were pretreated with ammonia for biohythane production (Gottardo et al. 2017). Microalgae were pretreated with chemical assisted ultrasonication for biomethane production (Caporgno et al. 2016).

7.4.3.1 Effect of Physico-Chemical Pretreatment Methods

These pretreatment methods are more advantageous over solely physical and chemical pretreatment methods because most of the methods are cost-effective. Ammonia fiber explosion does not necessitate reduced grain size and the formation of inhibitors does not take place (Kumar et al. 2009). Ultrasonication can able to disrupt the cell wall configuration via cavitation with intensification in specific surface areas and also reduce the degree of polymerization with an increase in biomass solubility (Sen et al. 2016). Autohydrolysis is more beneficial because it disrupts biomass to makes

it more operative without the use of any chemical for the enzymatic attack but low saccharification rate and production of inhibitory products is a major drawback (Taherzadeh and Karimi 2008). Liquid hot water pretreatment does not demand any exclusive and expensive corrosive-opposing objects for biofuel producing reactor setup (Laser et al. 2002). CO_2 explosion is more effective but costly as compared to lower inhibition formation (Sindhu et al. 2016).

7.4.4 Biological Pretreatment

These methods are extra eco-friendly than other methods for lignin removal which are namely fungal, microbial consortium, and enzymatic. MSW was pretreated biologically for biohydrogen and methane production (Bolzonella et al. 2019). Lignocellulosic biomass was pretreated biologically with *Curvularia lunata* (Yadav and Vivekanand 2020) for methane production, microalgae (Fabiana et al. 2016), poplar leaves (Cui et al. 2010), and rice straw (Sen et al. 2016) were enzymatically pretreated for biohydrogen production. *Cassia fistula L.* fruit pulp was pretreated with *Rhodosporidium kratochvilovae* (Alok et al. 2015) for biodiesel production. Sugarcane bagasse was pretreated with by *Thermoascus aurantiacus* for biobutanol production (Zong-Wen et al. 2016). Microalgae were enzymatically pretreated with cellulase and enzyme mixture for biomethane production (Fabiana et al. 2016). Pretreatment of corn stover via co-culture of two fungi (*Clostridium cellulolyticum* and *Citrobacter amalonaticus*) was used for biohydrogen production (Shou-Chi et al. 2018).

7.4.4.1 Effect of Biological Pretreatment Methods

A wide variety of white rot-fungi have been used for biological pretreatment, among which *Phanerochaete chrysosporium* has highest proficiency in lignin disruption (Sindhu et al. 2016). In case of pure fungal strains, sterilization of lignocellulosic biomass is required to avoid the contamination of fungi to escape the reduced biofuel yields which is not pre-requisite for most of the microbial consortium pretreatment. Nowadays, lignocellulosic biomass pretreatment in assistance with genetically modified fungal or microbial species have gained more attention because of high biofuel yield as compared to natural species. Microbial consortium pretreatment was found to be best in all three biological pretreatments because less care is required for maintaining the mixed microbial source to prevent from being contaminated which also resulted in reduced pretreatment cost. But biological pretreatment is not as much effective as chemical pretreatment due to long retention time, high microbial selectivity, and being overpriced, and further research is required (Kumari and Singh 2018).

Apart from the above pretreatment methods, a long list of research is additionally available for the application of combined pretreatment technologies for enhancement

of biofuel yields. Combined pretreatments have many advantages over single pretreatments (e.g., more lignin removal and high cellulose solubility). Singhal and Singh (2015) applied combined heat with acid and alkali on petha wastewater for biohydrogen generation. Rice straw was pretreated with ultrasonic-assisted alkaline petha wastewater and acidic dairy wastewater for bioethanol and methane production (Kumari and Singh 2020a, Kumari et al. 2018). Ivo et al. (2016) applied a steam explosion with NaOH and size reduction and Montingelli et al. (2016) used beating and ball milling with MW for enhanced biomethane production.

7.5 Future Research Directions

There is a great scope in biofuel production as these are capable of eliminating various problems such as fuel security, depletion of resources, environmental challenges, etc. Apart from this biofuel production can also led to the waste minimization which will reduce the load on landfill sites (McIvor and Evans 2008). A lot of research has been done and it is still going further to find out the suitable method which can enhance the production. There are various methods of biofuel production which have been explored at the lab scale but the main challenge is to imply that at large pilot scale. There is no doubt in accepting that biofuel has the potential to fulfill the world energy demand and future research will be focused on the applications of the technology at pilot scale. After the implementation of biofuel technology, there will be various effects which have to be monitored. Lignocellulosic biomass will play an effective role in biofuel production as the amount of this biomass will increase with enhanced energy demand as the population increases.

Pretreatment methods are also explored which are less energy intensive, chemical free, and result in better yield production. Coupling of various pretreatment methods can also affect the yield either in positive or negative manner (Kumari and Singh 2018). Treatment of mixed lignocellulosic waste can also be carried out such that there should be some symbiotic relationship between the two biomass and help in the degradation of each other. This can reduce the pretreatment cost and make the process more viable (Kumari and Singh 2020b) which can be easily adapted anywhere. Climatic conditions also affect the efficiency of bacterial degradation which reduced biofuel production for some favorable time period during the year. This problem should also be focused so that the continuous production of biofuel can be done at a pilot scale.

Future research should explore some easy growing enzymes which can be adapted by a farmer so that they can treat their agricultural waste and energy produced from this can be utilized. Technology should be made adaptive to use biofuels for transportation and conversion of it into electricity which can help in reducing the loads on conventional fossil fuels.

7.6 Conclusion

Keeping the present situation of fulfilling energy demand in mind, the production of biofuels from cheap and alternative biomass sources has been highly encouraged in the last decades. The complexity of lignocellulosic biomass restricts to utilize the biomass resources directly for energy generation. Pretreatment is the pre-requisite to produce biofuels from lignocellulosic biomass. A large variety of pretreatment processes are available but most of these processes are chemical based and harmful to environment. To find a cheap, energy-efficient and eco-friendly (green) pretreatment process is a challenge that would be able to reduce or totally remove the hurdles of available pretreatment technologies. Two or more pretreatment processes when applied in combination can solve some part of the problem but still further research is required to tackle the rest. Utmost all combinations of pretreatments utilize high energy for further energy generation in the form of biofuels which enhances the cost of the pretreatment process. This energy consumption should be balanced in such a way so that biorefineries become a profitable sector for the developers and workers of this field.

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Chapter 8
A Kinetic Framework for Microwave-Irradiated Catalytic Conversion of Lignocelluloses to Biofuel Precursors by Employing Protic and Aprotic Ionic Liquids

Subhrajit Roy and Saikat Chakraborty

Abstract In this work, we present a kinetic framework for microwave-irradiated catalytic conversion of lignocelluloses such as Sunn hemp fibre (75.6% cellulose content, Crystallinity Index (CI) 80.1%) and June grass (82.3% cellulose, CI 54%) to biofuel precursors such as glucose, 5-Hydroxmethylfurfural, Levulinic, and Formic acids by employing protic (PIL) and aprotic (APIL) ionic liquids. While the APIL forms a large supramolecular complex, the PIL rapidly ionises to form a Lewis acid catalyst with metal chloride and water and creates a metal-aqua complex. Since the APIL and the PIL follow different reaction mechanisms for microwave-irradiated catalytic conversion, catalyst-substrate loading, IL-substrate loading, water concentration, temperature, and time are optimised to regulate the product distribution. The APIL functions better for the higher crystalline substrates (with CI $>$ 70%) to produce glucose, whereas the PIL, which is much cheaper than the APIL, produces more high-value products such as 5-Hydroxmethylfurfural. Sunn hemp fibres produce a maximum glucose yield of 78.7% and 75.6% (using the APIL and PIL, respectively), while June grass produces a maximum glucose yield of 88.2% and 84.2%, and a maximum 5-Hydroxmethylfurfural yield of 23.4% and 34.9% (using the APIL and PIL, respectively). This work also explores the economic viability and the scale-up potential of the above processes.

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Keywords Sunn hemp · June grass · Catalytic hydrolysis · Ionic liquid · Microwave-irradiated process · Biofuel precursors

Abbreviations

APIL	Aprotic ionic liquid
BET	Brunauer-Emmett-Teller
BJH	Barrett-Joyner-Halenda
BMIMCl	1-butyl-3-methylimidazolium chloride
CI	Crystallinity index
CMF	5-Chloromethylfurfural
DEF	2, 5-Diformylfuran
DF	Dilution factor
DHMF	Dihydroxymethylfuran
DLS	Dynamic light scattering
DMF	2, 5-Dimethylfuran
DP	Degree of polymerization
EMF	5-Ethoxymethylfurfural
FA	Formic acid
FDCA	2, 5-Furandicarboxylic acid
GOD	Glucose oxidase
HMF	Hydroxymethylfurfural
ILs	Ionic liquids
LA	Levulinic acid
MF	5-Methylfuran
ORR	Oxygen reduction reaction
PIL	Protic ionic liquids
POD	Peroxidase
XRD	X-ray diffraction

8.1 Introduction

8.1.1 Impact of Climate Change

Relentless consumption of fossil fuels such as coal, petroleum, and natural gas across the world for the last two centuries have led to global air pollution (Prat et al. 2016) through emissions of particulate matter and is also the primary cause of anthropogenic global warming through greenhouse gas emissions (Schleussner et al. 2016). Both of these are the biggest threats to our planet and its ecosystem, which have led to catastrophic consequences such as health hazards and dramatic global climate change crises such as glacial melting, tornadoes, cyclones, floods, droughts, wildfires, and famines. The Paris Climate Agreement (2015) has triggered various

research and deployment programs aimed at providing affordable and reliable clean energy for everyone, which is critical for limiting the global temperature rise 'well below 2°C' above the pre-industrial levels—as agreed in the Paris Accord—combating climate change, and promoting energy security by significantly reducing our dependence on fossil fuels (Rogelj et al. 2016). To reduce the carbon dioxide emission from fossil fuels and global warming, researchers have suggested the use of biofuels produced from various biomass sources to push the earth's carbon cycle towards carbon neutrality (Harmsen et al. 2010).

8.1.2 Biomass as a Potential Source of Fuel

Biofuels—depending on the biomass source they are produced from—can be categorised into four generations. First-generation biofuels are produced from edible food sources such as wheat, maize, corn, sugarcane, and animal fat. However, the production of the first generation of biofuels is not feasible in developing nations due to their high population density and various other socio-economic factors that lead to the food-versus-fuel debate. Non-food crops such as straw, bagasse, forest residues, etc. have been identified globally to avoid this debate, and second-generation biofuels are now being produced from such non-edible lignocellulosic biomass (Yang et al. 2013a). Third generation biofuels are produced from algal biomass, whereby the lipid fraction of the algae is converted into biodiesel via transesterification, and the carbohydrate fraction is converted to bioethanol and bio-hydrogen. However, several challenges involved in the cultivation, harvesting, and downstream processing of the algal biomass cause significant bottlenecks for this technology (Yang et al. 2013b). Metabolic engineering is being employed to produce genetically engineered algae with high CO_2 sequestration rate to overcome some of these challenges (Leung et al. 2014). However, these fourth-generation biofuels require substantial initial investment compared to the other three generations, one of the reasons being that genetically engineered algae are not allowed to be cultivated outdoor in open raceway ponds, and must be produced in closed photobioreactors.

8.1.3 Second Generation Lignocellulosic Biofuel

The use of non-edible lignocellulosic biomass is necessary for the production of second-generation biofuels in order to circumnavigate the food-versus-fuel debate. Lignocelluloses are primarily composed of three different polymers, namely, cellulose, Hemicellulose, and lignin, which are linked to each other inside the plant cell walls through hydrogen and covalent bonds (Dutta and Chakraborty 2016). Lignin and Hemicellulose form a protective structure around the cellulose polymer, making it difficult for enzymes and catalysts to access the latter and release the glucose

monomers by rupturing the β-1-4-glycosidic bonds linking the monomer molecules (Dutta and Chakraborty 2015). Among such bioenergy crops, Switchgrass, Napier grass, Miscanthus, etc. have been used for second-generation biofuel production (Naik et al. 2010), and lignocelluloses from non-food crops are now considered as the main feedstock for future biofuels (Sheridan 2013).

There are two major steps in converting raw biomass to commercial fuels. The first one is breaking down biomass from complex and intertwined molecules to an intermediate product consisting of simpler and more separable substances (Silveira et al. 2013). The second step is to process this intermediate product into a commercial end-product with uniform and desirable properties (Bozell et al. 2000).

Sunn hemp and June grass—two cellulose-rich crystalline non-food energy crops, containing high cellulose percentage, low Hemicellulose, moderate lignin, with high crystallinity and high Degree of Polymerization—have been identified as non-food substrates for second-generation lignocellulosic biofuel production (Paul and Chakraborty 2018; Roy and Chakraborty 2019). While the high cellulose content in these non-edible lignocelluloses makes them potential candidates for large-scale biofuel production, their high Degree of Polymerization, high crystallinity and low porosity make them recalcitrant and not readily amenable to enzymatic treatment (Kimon et al. 2011). Enzymatic hydrolysis of these cellulose-rich lignocelluloses requires pretreatment by acids or alkalis for lignin removal; it is a slow process (with timescales ranging from 1 to 3 days) (Paul and Chakraborty 2019), and usually produces low glucose yields (50-65%). To overcome this drawback, microwave irradiation is employed to rapidly rupture the glycosidic bonds in the raw mixture and produce biofuel precursors such as glucose and hydroxymethylfurfural (HMF), as well as levulinic acid (LA) and formic acid (FA), in less than an hour (Paul and Chakraborty 2018). The reactants—long-chain cellulose in untreated biomass mixture, ionic liquid, transition metal catalyst, and water—form a polar supramolecular complex that rotates under the microwave's alternating polarity and rapidly dissipates the electromagnetic energy through molecular collisions, thus accelerating glycosidic bond breakage and biofuel production (Tagliazucchi et al. 2014).

Ionic Liquids (ILs) are salts, which have melting points near or below ambient temperature (Marcus 2016). As ILs are less volatile and less hazardous to handle than acids and bases, they can be used as green solvents and were initially considered to be the most effective pretreatment agent (Doherty et al. 2010). It has now been discovered that ILs, with metal chloride catalysts and water, can depolymerize cellulose to glucose. Therefore, instead of using ionic liquids as the only pretreatment agent, they are also used as preferred solvents for hydrolysis (Chakraborty and Gaikwad 2013). Furthermore, in this reaction medium itself, glucose can be dehydrated to form HMF (Peleteiro et al. 2016). The ionic liquid also serves as a heating medium and can be recycled readily for photo-catalysis (Gaikwad and Chakraborty 2014). The depolymerization of lignocellulosic materials in ionic liquid medium is accelerated by the reduction of viscosity of the ionic liquid at temperatures above 100 °C (Yoon et al. 2012). As the reaction temperature increases from 180 °C to 200 °C, glucose and HMF yields decrease and the yields

of levulinic and formic acids increase (Peng et al. 2010). However, the major bottleneck in commercializing this technology is the prohibitive cost of ionic liquids, despite their recyclability.

Sunn hemp (*Crotalaria juncea*) is a widely grown as a non-edible lignocellulosic cover crop in the subtropics of Bangladesh, Brazil, India, Pakistan, Russia, Sri Lanka, USA, Uganda, etc. Sunn hemp fibre and June grass (*Koeleria macrantha*), with their low lignin and hemicellulose contents, high cellulose content, and high energy content, have the potential of being converted to transportation biofuels rather than being merely used as bast fibres (Cantrell et al. 2010). The main obstacle to their large-scale deployment as future fuel sources in our fight against climate change is their low porosity, high crystallinity and long-chain length, which together render the cleaving of the glycosidic bonds between its glucose monomers rather challenging.

This work presents a kinetic framework for microwave-irradiated ionic liquid mediated catalytic conversion of lignocelluloses to biofuel precursors by optimizing the reaction parameters that maximize the yield of a target product from a particular lignocellulose.

8.2 Ionic Liquids: Classification and Properties

Ionic liquids (ILs) are generally defined as liquid compounds, composed entirely of ions that display ionic-covalent crystalline structures (Yoon et al. 2012). This class of liquid materials displays various ionic properties such as Columbic interactions, hydrogen bonding, and Van-der-Waals interactions of their ions (Sowmiah et al. 2009). They are also called 'designer solvents' due to their ability to vary the ions, thereby modifying and optimising the properties of IL for a specific task (Vekariya 2016). Today, ILs are extensively used as solvents in organic synthesis, electrochemistry, catalysis, chemical separation, nanoparticle formation, etc. Moreover, ILs possess intrinsic ionic conductivity at room temperature and exhibiting excellent electrochemical stability (Swatloski et al. 2002). Among solvents, ILs are categorised as environmentally friendly or 'green solvents'. These ILs are also non-volatile and can be used in low-pressure environments.

8.2.1 Generations of Ionic Liquids

Based on their occurrence and development, Ionic Liquids (ILs) are broadly categorised into three generations (Fig. 8.1a). The first generation of ILs came in the 1980s in the form of chloroaluminate ILs (Gilbert et al. 2007), and are mixtures of anhydrous $AlCl_3$ and an organic pyridinium or imidazolium chloride or any chloride quaternary ammonium salt. The second generation of ILs came in the 1990s as Air and Moisture Stable ILs (Fig. 8.1a). Previously, the first generation

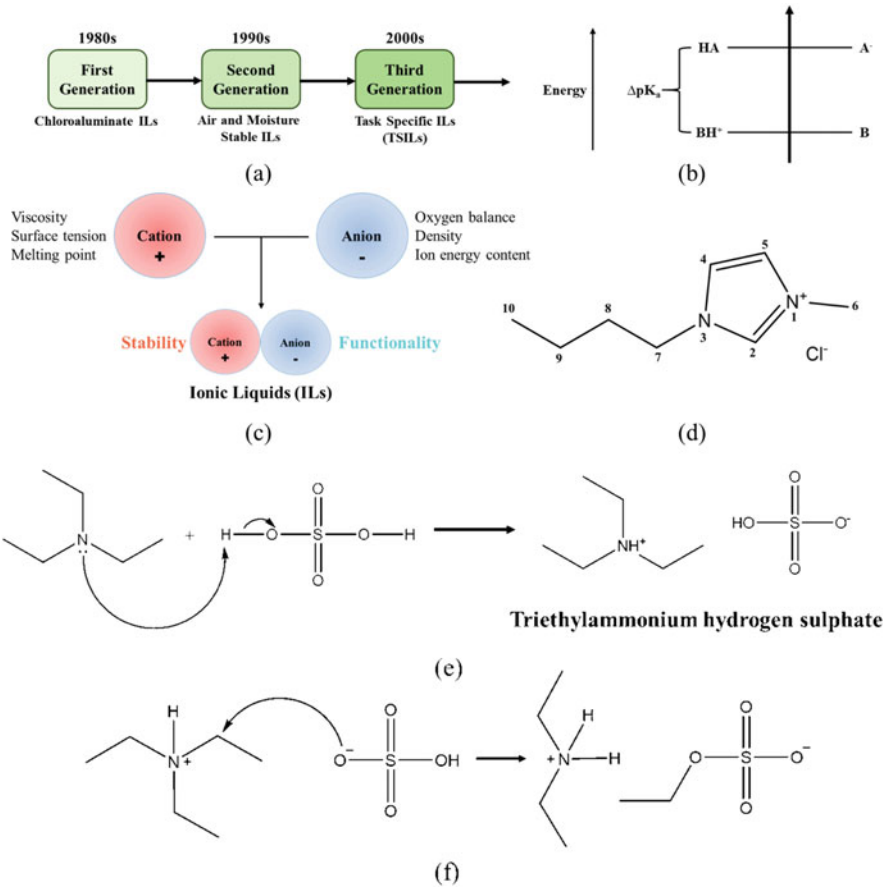

Fig. 8.1 (**a**) Generations of ILs, (**b**) Bronsted acid (HA) + Bronsted base (B) → $BH^+ + A^-$, (**c**) Properties of ILs, (**d**) Structure of BMIMCl, (**e**) Synthesis of $[Et_3N][HSO_4]$, and (**f**) Alkylation of $[Et_3N][HSO_4]$

of ILs was very hygroscopic, which delayed their progress of implementation in various fields (Endres and El Abedin 2006). Unlike the first-generation ILs, the second generation could be prepared and stored without an inert atmosphere. The most recent and third generations of ILs came in the 2000s as Task-Specific ILs (Doherty et al. 2010), which are designed to perform a specific set of tasks in various chemical reactions.

8.2.2 Classification of ILs

The various types ILs such as bio-ILs, amphiphile ILs, supported ILs, metal salts ILs, and polarisable ILs (Panda et al. 2017) can be broadly categorised into two groups namely Protic Ionic Liquid (PIL) and Aprotic Ionic Liquid (APIL). PILs are prepared by mixing Bronsted acid and a Bronsted base (Hossain et al. 2019) and can transfer proton from the acid to base due to the presence of proton-donor and -acceptor sites, which also facilitate in the build-up of the Hydrogen bond network (Angell et al. 2007).

In aqueous solutions, the pKa values of the acid and the base indicate the strength of PIL (Fig. 8.1b), as the driving force for proton transfer depends on the strength of the acid and the base (Ejigu and Walsh 2015). On the other hand, APILs contain a typical alkyl group at the site which is occupied by a proton in the analogous PILs. These groups of ILs are much more common and more hydrophobic (Chen et al. 2014). They contain no acid protons, unlike the PIL counterpart.

8.2.3 Properties of ILs

In recent times, ILs have gained much attention due to some of their unique and useful properties in various fields. They are non-flammable and non-volatile due to their negligible vapour pressure, which decreases the chances of fugitive emissions (Kolbeck et al. 2010). They show high thermal stability (decomposition temperatures around 300–500 °C), and high polarity, yet are non-coordinating with good intrinsic conductivity and are extremely redox-robust.

8.2.3.1 Viscosity

ILs are entirely composed of ions, and their properties such as viscosity and conductivity depend on the structure and the composition of the ILs (Pinkert et al. 2011). Generally, ILs are viscous liquids, and the viscosity depends on the nature of the anion. Other essential parameters affecting viscosity are their ability to form H-bond and temperature. The viscosity of the ILs is inversely related to temperature and can be described by the Vogel-Tammann-Fulchers equation (Eq. 8.1).

$$\ln \eta = \ln \eta_{\infty} + \frac{E_{\eta}}{\mathrm{RT}}, \tag{8.1}$$

where η_{∞} is the viscosity at infinitely high temperatures.

8.2.3.2 Conductivity

Conductivity plays a significant role in any electrochemical process. Since ILs are composed of ions, the number of charge carriers per unit volume is very high. Viscosity is linked inversely to the conductivity of the ILs (Every et al. 2004). Apart from viscosity, other factors influencing the conductivity of the ILs are ion size and aggregation, anionic charge delocalisation, and co-related ionic motions (Zhang et al. 2006). The anionic part of the ILs dictates the oxygen balance, density, and ion energy content, thereby influencing the functionality of the ILs (Fig. 8.1c), whereas the cationic part controls the stability of the ILs by regulating physical properties such as viscosity, surface tension, and melting point (Dong et al. 2014).

The presence of water can greatly influence the quality, solubility, and other properties of the ILs. Anions such as $[BF_4]^-$, $[NO_3]^-$, $[ClO_4]^-$ are hydrophilic, while ILs with anions such as $[PF_6]^-$, $[OSO_2CF_3]^-$, $[SbF_6]^-$ are hydrophobic (Reid et al. 2017). These hydrophobic ILs are also very hygroscopic and can easily absorb moisture from the air.

8.2.3.3 Polarity

ILs are highly polar. Generally, PILs have more significant polarity compared to APILs. PILs form H-bond networks and incorporate metal salts much more efficiently than APILs due to their hydrophobic character (Guan et al. 2017). APILs have a higher preference towards ion-water interactions at low concentrations, while the PILs exhibit a greater preference towards ion-water interactions at higher water concentrations (Ab Rani et al. 2011).

ILs have widespread applications in various processes, such as solvents, electrolytes, liquid crystals, lubricants, liquid crystals, lubricant additives, analytics, and electro-elastic materials (Greaves and Drummond 2015). In this study, we will focus on the role of both PIL and APIL as bio-polymer solvents (George et al. 2014). ILs are very effective in the dissolution of biomass containing cellulose, Hemicellulose, and lignin, without emitting any volatile organic solvents. Since ILs exhibit a wide range of polarities, their capacity to dissolve various types of biomass is another significant advantage that can be exploited.

8.3 Comparison of Protic and Aprotic Ionic Liquids

Protic (PIL) and Aprotic (APIL) ionic liquids follow different pathways in biomass dissolution (Roy and Chakraborty 2019). Their distinctive properties drive them to take separate routes to produce hexose and pentose sugars by breaking the polymeric cellulose chain in lignocellulosic biomass (French and Finch 1999). In this study, we have used both PIL and APIL with metal chloride catalyst and water to breakdown

cellulose to produce glucose monomers and other high-value biofuel precursors (Parker 1969). As discussed in the earlier section, PILs are easier to synthesize than APILs. For our study, we have selected Triethylammonium hydrogen sulphate ($[Et_3N][HSO_4]$) as the PIL (Goswami et al. 2016), which we have synthesised in the lab and 1-butyl-3-methylimidazolium chloride (BMIMCl) as the APIL (Paul and Chakraborty 2018). We have analysed the kinetics and reaction mechanism of these two types of ILs in hydrolysing cellulose polymer to glucose and furanic fuel precursors.

8.3.1 Properties of BMIMCl and $[Et_3N][HSO_4]$

In order to optimise the use of PILs and APILs, we must investigate their structural and physical properties thoroughly (Panda et al. 2017) so that we can expand the use of these ILs as efficient solvents for synthesis and formation of new products.

8.3.1.1 BMIMCl (1-Butyl-3-Methylimidazolium Chloride)

The imidazolium-based ILs are the most common, and have been thoroughly studied, both experimentally (Wang et al. 2014) and computationally (Mushrif et al. 2012). BMIMCl forms crystalline structures, either in a monoclinic or orthorhombic lattice. Thermodynamical studies on BMIMCl reveals that solid-state structures may inhibit the crystallisation of ILs and result in complex melting behaviour (Dharaskar et al. 2013). From the Raman spectra, it was found that the imidazolium rings are planar and the butyl chain in the monoclinic crystals shows an anti-anti (AA) conformation around C7–C8 and C8–C9 bonds (Fig. 8.1d), while in the orthorhombic crystals the butyl chain exhibits Gauche-anti (GA) conformation (Dong et al. 2014). Hence, at least two rotational isomers of the BMIM cation can co-exist in the liquid state.

The imidazolium-based ILs are highly hygroscopic (Yong et al. 2005), and therefore, must be preserved in a moisture-free condition. The anions readily react with the water molecules and form H-bond. Thus, water plays an essential role in the structural and functional properties of imidazolium-based ILs. It is also reported that the acidic proton of the imidazolium ring does not take part in water absorption (Tian et al. 2012). In BMIMCl, water takes away the chloride anion from the cation, and thus, is responsible for a change of conformation in the cation.

8.3.1.2 $[Et_3N][HSO_4]$ (Triethylammonium Hydrogen Sulphate)

PILs are multi-ion ILs synthesized by the simple neutralisation reaction of a Bronsted acid and a Bronsted base (Greaves and Drummond 2008). The preparation and preservation of PILs are much easier and are more accessible compared to

Table 8.1 Properties of APIL (BMIMCl) and PIL ([Et_3N][HSO_4])

Properties	BMIMCl	[Et_3NH][HSO_4]
Chemical formula	$C_8H_{15}ClN_2$	$C_{12}H_{30}N_2O_4S^{2-}$
Molecular weight	174.67 g/mol	298.45 g/mol
Boiling point	192 °C	90.5 °C
Melting point	−70 °C	−114.7 °C
Density	1.086 g/cc	0.75 g/cc

APILs. PILs do not require additional purification after production. In our study, [Et_3N][HSO_4] is prepared by mixing Triethylamine and sulphuric acid (Fig. 8.1e) in a stoichiometric ratio (Karimi-Jaberi et al. 2017). This PIL is thermodynamically stable and can work under hydrous conditions.

[Et_3N][HSO_4] is equally effective in bio-polymer dissolution compared to BMIMCl, and gives a high rate of delignification (Roy and Chakraborty 2019). The anion $[HSO_4]^-$ is responsible for breaking the β-1,4-glycosidic bonds of cellulose polymers and forms glucose monomers from it. It also dissolves the lignin present and easily separates it from the insoluble cellulose. The synthesis process of [Et_3N][HSO_4] is straightforward, and comprises of two steps: firstly, the mixing of 5 M H_2SO_4 solution with Triethylamine (mole ratio of N_{222}: H_2SO_4 is 1:1), and secondly, the removal of excess moisture by a rotary vacuum evaporator if required (Brandt-Talbot et al. 2017). The 5 M H_2SO_4 solution is added drop-wise in Triethylamine, and this reaction is performed in an ice bath as the reaction is highly exothermic (Fig. 8.1f).

The neutralisation reaction proceeds via proton transfer because of the high ΔpK_a (>10) value between N_{222}($pK_a = 18$) and H_2SO_4($pK_a = -3$). After complete proton transfer has taken place, PIL is formed consisting of $[N_{222}]^+$ cation and $[HSO_4]^-$ anion, which is soluble in water (Table 8.1).

8.3.2 Oxygen Reduction Reaction (ORR) in PIL and APIL

The Oxygen Reduction Reaction (ORR) plays a vital role in energy conversion and IL chemistry (Doblinger et al. 2019). PIL and APIL follow different pathways for ORR due to their differences in structural and functional properties. APILs do not have active protons in their structure and show excellent stability against the attack of superoxide (Zhang and Pozo-Gonzalo 2018), while PILs contain active protons that give them thermal stability.

In APILs, O_2 undergoes one-electron reduction to form superoxide ($O_2^{\bullet-}$) which is unstable and can further undergo one-electron reduction to peroxide dianion (O_2^{2-}) electrochemically (Eq. 8.3). Now, this O_2^{2-} is extremely reactive and reacts with O_2 again to form $O_2^{\bullet-}$. This superoxide is a strong nucleophile that attracts water molecules to form H_2O^- through irreversible disproportionation reaction.

$$O_2 + e^- \rightarrow {O_2}^{\S-} \tag{8.2}$$

$${O_2}^{\S-} + e^- \rightarrow {O_2}^{2-} \tag{8.3}$$

$${O_2}^{\S-} + H_2O \rightarrow {HO_2}^{\S} + HO^- \tag{8.4}$$

$${O_2}^- + {HO_2}^{\S} \rightarrow O_2 + {HO_2}^- \tag{8.5}$$

$$O_2 + H_2O + 2e^- \rightarrow {HO_2}^- + HO^- \tag{8.6}$$

In PIL medium, O_2 undergoes a four-electron reduction pathway (Eq. 8.7) to form H_2O (Khan et al. 2017). The proton activity of the PIL exhibits a significant influence on the ORR. Hence, PILs are less hygroscopic than the APIs, and the presence of H_2O in the reaction does not cause any effect (Khan et al. 2013).

$$O_2 + 4e^- + 4H^+ \rightarrow 2H_2O \tag{8.7}$$

8.3.3 Reaction Kinetics of BMIMCl and [Et_3N][HSO_4]

This study illustrates the effect of both BMIMCl and [Et_3N][HSO_4] on lignocellulosic biomass to produce biofuel precursors. Along with the ILs, we have used $CuCl_2$ as catalyst and water as cross-catalyst to perform the hydrolysis reaction followed by a dehydration reaction to produce furanic fuel precursor and other value-added products.

8.3.3.1 BMIMCl

The APIL (BMIMCl) rapidly ionises to form a Lewis acid catalyst in the presence of $CuCl_2$ and water, which then forms a supramolecular structure, $(C_m(\text{Bmim})^+(CuCl_3(H_2O)_n)^-)$, after interaction with the cellulose (Paul and Chakraborty 2018). Water hydrolyses the supramolecule to give α-glucose that isomerises to a more stable β-glucose (Eq. 8.12). This β-glucose is again dehydrated to produce 5-Hydroxymethylfurfural (5-HMF) and three molecules of water (Guan et al. 2011). Two of the three water molecules react with 5-HMF to form an equimolar amount of Levulinic Acid (LA) and Formic Acid (FA), while the extra water molecule is available to hydrolyse the cellulose and performs the role of a cross-catalyst (Prigogine and Lefever 1967).

$$\text{BmimCl} + CuCl_2 + (H_2O)_n \rightleftharpoons (\text{Bmim})^+ + \left(CuCl_3(H_2O)_n\right)^- \tag{8.8}$$

$$\text{Cellulose}_m + \text{Bmim}^+ + \left(\text{CuCl}_3(\text{H}_2\text{O})_n\right)^- \rightarrow \text{Cellulose}_m(\text{Bmim})^+\left(\text{CuCl}_3(\text{H}_2\text{O})_n\right)^- \quad (8.9)$$

$$\text{Cellulose}_m(\text{Bmim})^+\left(\text{CuCl}_3(\text{H}_2\text{O})_n\right)^- + \text{H}_2\text{O} \rightarrow \alpha\text{-Glucose} + \text{Bmim}^+ + \left(\text{CuCl}_3(\text{H}_2\text{O})_n\right)^- + \text{Cellulose}_p + \text{Cellulose}_{m\text{-}p\text{-}1} \quad (8.10)$$

$$\alpha\text{-Glucose} + \text{Bmim}^+ + \text{CuCl}_3{}^- \rightleftharpoons \alpha\text{-Glucose}(\text{Bmim})^+(\text{CuCl}_3)^- \quad (8.11)$$

$$\alpha\text{-Glucose}(\text{Bmim})^+(\text{CuCl}_3)^- \rightleftharpoons \beta\text{-Glucose} + \text{Bmim}^+ + \text{CuCl}_3{}^- \quad (8.12)$$

$$\beta\text{-Glucose} + \text{Bmim}^+ + \text{CuCl}_3{}^- \rightleftharpoons \beta\text{-Glucose}(\text{Bmim})^+(\text{CuCl}_3)^- \quad (8.13)$$

$$\beta\text{-Glucose}(\text{Bmim})^+(\text{CuCl}_3)^- + \text{H}_2\text{O} \rightleftharpoons \text{Fructose} + \text{Bmim}^+ + \text{CuCl}_3{}^- \quad (8.14)$$

$$\text{Fructose} \rightleftharpoons \text{HMF} + 3\text{H}_2\text{O} \quad (8.15)$$

$$\text{HMF} + 2\text{H}_2\text{O} \rightarrow \text{LA} + \text{FA} \quad (8.16)$$

Due to chlorine's higher electron affinity, the Lewis acid catalyst tends to accept an electron from the Cu-metal centre and form the supramolecular structure when reacted with the cellulose polymer (Carraher et al. 2015). The β-1,4-glycosidic bond present in the cellulose chain donates the electron to the Lewis acid and weakens the C_1–O bond due to the partial positive charge on oxygen (Su et al. 2009). Water molecules act as a nucleophile and attack the positive charge C1 atom to form an adduct (Zhou et al. 2011). The glycosidic oxygen attaches with the hydrogen atom by transferring its electrons away from the Lewis acid. This leads to the breaking of β-1,4-glycosidic bond and the formation of the free α-glucose molecule. The bulk cation present in the APIL provides a steric hindrance effect and further weakens the Lewis acid-oxygen bond. After the formation of β-glucose, fructose is produced by a series of irreversible proton exchanges (da Silva Lacerda et al. 2015). Then, 5-HMF is formed by removing three water molecules, and subsequently, LA and FA are formed by an irreversible rehydration reaction (Peng et al. 2010).

8.3.3.2 $[Et_3N][HSO_4]$

$[Et_3N][HSO_4]$ reacts with water much faster than BMIMCl, as it follows the S_N1 pathway, where BMIMCl follows the S_N2 pathway (Joshua et al. 2017). This is because PILs are generally much more polar than APILs. PIL forms a metal-aquo complex when reacting with a halide catalyst and water (Ogden and Beer 2006). This is the fundamental difference between BMIMCl and $[Et_3N][HSO_4]$ reaction pathways. $[Et_3N][HSO_4]$ is also an adduct salt and forms an octahedral ($t_{2g}{}^6\ e_g{}^3$) hexa-aquo complex $(Cu(H_2O)_6)^{2+}$, when reacting with $CuCl_2$ and water (Housecroft and Sharpe 2012). This complex, like the one with APIL, actively takes part in breaking the glycosidic bond of the cellulose polymer chain. Copper, with d^9e^- electronic configuration, plays an important role in the formation of the polar octahedral

complex (Sham et al. 1980). Its M-O distance ranges between 1.97 Å and 2.30 Å and exhibits a high water-exchange rate ($5.7 \times 10^9\ s^{-1}$). Cu^{2+} has a good affinity for -O- or anomeric –OH and is more prone to forming a complex (Helm and Merbach 2005). In the reaction, the cleavage is facilitated by the in situ HCl, which also helps in breaking the ethereal bond and producing β-glucose (Wang et al. 2014). Besides, β-glucose undergoes isomerisation via Lobry de Bruyn–Alberda–van Ekenstein (LdB-AvE) mechanism (Angyal 2001) to form fructose, followed by a dehydration reaction to give 5-HMF and subsequently rehydrates again to give LA and FA.

$$[Et_3NH]^+[HSO_4]^- + CuCl_2 + 6H_2O \rightarrow \left[Cu(H_2O)_6\right]^{2+} + SO_4 + Et_3N + 2HCl \tag{8.17}$$

$$\left[Cu(H_2O)_6\right]^{2+} + H_2O* \rightarrow \left[Cu(H_2O)_5(H_2O)*\right]^{2+} + H_2O \tag{8.18}$$

$$\text{Cellulose } (C_n) + \left[Cu(H_2O)_5(H_2O)*\right]^{2+} + Et_3N + 2HCl \rightarrow C_n\left[Cu(H_2O)_5\right]^{2+} + Et_3N + 2HCl + H_2O \tag{8.19}$$

$$C_n\left[Cu(H_2O)_5\right]^{2+} + Et_3N + 2HCl + H_2O \rightarrow \beta\text{-Glucose}\left(C_\beta\right) + \left[Cu(H_2O)_6\right]^{2+} + Et_3N + 2HCl + C_{n\text{-}1} \tag{8.20}$$

$$\beta\text{-Glucose}\left(C_\beta\right) + H_2O \rightleftharpoons \text{Fructose} + H_2O \tag{8.21}$$

8.4 Composition and Properties of Sunn Hemp and June Grass

In this study, we focus on lignocellulosic biomass as the feedstock for second-generation biofuels. It is composed of three major polymeric components: Cellulose, Hemicellulose, and Lignin (Harmsen et al. 2010). The composition of lignocellulose varies from plant to plant depending on its genetic conditions and its environmental habitat. Cellulose, Hemicellulose, and Lignin form microfibril structures that give structural stability to the plant cell wall (Dutta and Chakraborty 2016).

Cellulose is the most abundant natural polymer on earth and the most significant constituent in the lignocellulosic biomass. It consists of glucose molecules linked by the strong β-1, 4-glycosidic bond and is arranged in a micro-crystalline structure (Dutta and Chakraborty 2015). It is also a linear homopolymer of repeated units of cellobiose, and its molecular weight can be very high depending upon the chain length. Cellulose exists in two forms, namely crystalline and amorphous, in the lignocellulose. The crystalline part of the cellulose is exceptionally resistant to chemicals and is tough to hydrolyse (Yang et al. 2013b).

Hemicellulose is the second most abundant natural polymer on earth. It is composed of pentose (5-carbon) and hexose (6-carbon) sugar units (Silveira et al.

Table 8.2 Composition of lignocellulosic feedstock (Garrote et al. 1999)

Biomass	Cellulose (%)	Hemicellulose (%)	Lignin (%)
Corn cobs	33.7–41.2	31.9–36	6.1–15.9
Wheat straw	32.9–50	24–35.5	8.9–17.3
Rice straw	36.2–47	19–24.5	9.9–24.1
White birch	41–42	35.2–36.2	17.5–18.9
Poplar	41.7–42.4	20.2–21.5	29.3–30.25
Miscanthus	43.2–50.1	20–22	9–10
Switch grass	32.9–50	24–35.5	8.9–17.3

2013). It has lower molecular weight and low crystallinity than cellulose. The monomeric sugar molecules in Hemicellulose are not as tightly packed as cellulose, making the former more amorphous.

The third component, lignin, is composed of three major phenolic compounds, namely, p-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol (Bozell et al. 2000). Lignin links covalently with Hemicellulose and they together form a hydrophobic structure. It gives structural strength to the plant tissues by providing stiffness to the cell wall, in turn, making them recalcitrant (Isikgora and Becer 2015). Lignin is often termed as 'green coal', due to its fuel value.

8.4.1 Advantages and Disadvantages of Lignocellulose

The major advantages of using lignocellulose for biofuel production are its wide availability across the globe, its renewability as an energy source, and the fact that it does not conflict with food sources. The compositional analyses of a few lignocelluloses are listed in Table 8.2.

The main disadvantage of using lignocellulosic feedstock for second-generation biofuel is its recalcitrant property. Generally, lignocellulose contains a high amount of cellulose, which increases the crystallinity of overall biomass and makes it difficult to hydrolyse the polymeric chain. Apart from its high crystallinity, a high Degree of Polymerisation (DP), low porosity, and less surface area also contribute to the recalcitrant property of the biomass. β-1, 4-glycosidic bond is one of the strongest bonds and breaking it to form glucose is the slowest and the rate-limiting step in the biofuel production process (Chakraborty et al. 2014).

8.4.2 Physical and Chemical Properties of Lignocellulose

The physicochemical characterisation of lignocellulosic biomass is necessary to understand the structure and the composition of particular lignocelluloses, which helps us decide the technological approach needed to be implemented to get the

desired product. In this section, we shall discuss the various methods to determine critical physical properties such as crystallinity, pore volume, degree of polymerisation, surface area, and particle size as well as the composition of the lignocellulose.

8.4.2.1 Determination of Cellulose and Lignin Content

Five-hundred milligram of the lignocellulosic substrate is mixed with 10 mL of 0.275 M H_2SO_4 solution in a flask and is stirred for 24 h at 150 rpm and 25 °C. Subsequently, the mixture is heated to 118 °C in an autoclave (Toribio-Cuaya et al. 2014). Once the required temperature is attained, the flask is kept in the reactor for 30 min, after which, the reactor is cooled by releasing the pressure, and the flask is taken out from the autoclave. The solution is then filtered to recover the solid which is washed with 60 mL of deionised water, and the filtrated volume is measured in a 100 mL measuring cylinder. The treated solid is dried at 40 °C for 8 h. The filtrate is centrifuged at 3000 rpm for 15 min, and the supernatant is filtered using a filter paper. Acid soluble lignin concentration is determined by UV–Vis spectrophotometer at 280 nm wavelength.

The next stage is the delignification process using an alkaline media catalysed with H_2O_2 (Cao et al. 2012). The oven-dried solid is taken in a 50 mL flask to which 6 mL of 0.375 M NaOH and 90 μL of 12.8 M of H_2O_2 are added. The temperature of the flask is maintained at 50 °C, and the stirring speed is 150 rpm for 3 h. When the reaction is complete, the solid is recovered by filtration and washed with 30 mL of water to remove the NaOH. The solid is then dried at 80 °C for 10 h and weighed to measure the cellulose content. The filtered liquor is acidified with acetic acid (50% v/v) to precipitate the alkaline lignin. The solution is centrifuged at 3000 rpm for 20 min, the supernatant removed and the precipitate dried at 80 °C for 8 h, after which, the weight of the alkaline lignin is determined.

8.4.2.2 Determination of Hemicellulose Content

Five-hundred milligram of lignocellulosic material is washed 8–10 times using filter paper, in a 250 mL beaker with 5 mL of boiling water. The material is then washed with 5 mL of anhydride ethanol, and the solid is recovered by filtration and dried at 80 °C in a hot air oven for 8 h to remove the ethanol from the sample. After the drying process, an alkaline extraction is performed to solubilise the two fractions (A and B), which comprise the hemicelluloses (Geng et al. 2018). The alkaline extraction is performed by taking the dried material in a 50 mL flask and adding 10 mL of 1 M NaOH solution. The solution is stirred at 100 rpm at room temperature for 24 h, and the insoluble material is separated by filtration and washed three times with 10 mL of deionised water. The filtrate pH is acidified from 9 to 4 by using acetic acid 50% (v/v), and the resultant suspension is then stirred for 15 min at room temperature and centrifuged at 3000 rpm for 20 min. Hemicellulose A is recovered by removing the supernatant, and the solid is dried at 90 °C for 8 h, after which, the

weight of the product is determined. The evaporation process concentrates the supernatant obtained from the first part. The supernatant is added drop by drop to a methanol solution up to a ratio of 1:3 by volume to precipitate the Hemicellulose B. The mixture is centrifuged at 3000 rpm for 20 min. Hemicellulose B is recovered by removing the methanol supernatant and drying the wet solid at 90 °C, after which, its weight is determined. The total amount of hemicellulose present in the lignocelluloses material is obtained from the sum of hemicellulose fractions A and B.

8.4.2.3 Determination of Moisture and Ash Content

In order to determine the moisture content, 1gm of lignocellulosic material is kept in a hot air oven at 105 °C for 5 h (Carrier et al. 2011). The moisture percentage is determined by the weight difference between the initial lignocellulosic biomass and the oven-dried lignocellulosic biomass.

One gram of lignocellulosic biomass is kept in a muffle furnace at 550 °C up to 5 h for the complete combustion of the material (Carrier et al. 2011). After combustion, the material is weighed, and the ash percentage is calculated by dividing the ash weight by the initial dry lignocellulosic material weight.

8.4.2.4 XRD, BET-BJH, and Particle Size Analysis

Powder X-ray diffraction (XRD) patterns of unreacted and reacted Sunn hemp fibres are obtained using a diffractometer, using Cu-Kα radiation at 40 kV and 30 mA with 2θ angle scanning from 10° to 90°. The relative amount of crystalline material in each sample is calculated using the peak height method (Park et al. 2010; Thygesen et al. 2005).

$$\mathrm{CI} = \frac{I_{2\theta=20.4^{o}} - I_{2\theta=14.9^{o}}}{I_{2\theta=20.4^{o}}} \times 100 \tag{8.22}$$

Here, CI is the crystallinity index, $I_{2\theta} = {}_{20.4^{\circ}}$ is the crystalline peak, and $I_{2\theta} = {}_{14.9^{\circ}}$ is the amorphous peak (Eq. 8.22).

The surface area and the pore size of the lignocellulose are determined by using a Brunauer-Emmett-Teller (BET) surface area analyser. The samples are degassed at 110 °C for 8 h under high vacuum. The specific surface area is calculated by the BET method, while the pore surface area, pore volume and average pore diameter are determined using the Barrett-Joyner-Halenda (BJH) method (Dutta and Chakraborty 2016).

The particle size of the substrate is measured by estimating the average particle size, and the particle size distribution by Dynamic Light Scattering (DLS) technique using a zeta sizer nanoparticle analyser with the laser at a wavelength of 633 nm and a constant temperature of 25 °C. A sample of 1.5 mL is taken in a cuvette, and the

scattering intensity of the laser is measured at 90° angle with a counting time of 80 s (Tuoriniemi et al. 2014).

8.4.2.5 Determination of Degree of Polymerisation (DP)

The Degree of Polymerization (DP) can be determined using various experimental techniques such as vapour pressure osmometry, membrane osmometry, ebullioscopy, size-exclusion chromatography, reducing end concentration and glycosyl monomer concentration methods (Ryu and Lee 1986; Zhang and Lynd 2005). DP measurement through reducing end concentration method requires less time for sample preparation and smaller sample volume than other methods. Therefore, in our experiments, we measure the DP of cellulose in the lignocellulosic substrate by the reducing end concentration method. DP is calculated as the ratio of glycosyl monomer concentration measured by the phenol-sulphuric acid method divided by the reducing end concentration determined by the modified 2, 2′ bicinchoninate (BCA) method.

The BCA working solution is prepared by mixing solution A and solution B in 1:1 ratio (by volume). For solution A, 0.971 g of disodium 2, 2-bicinchoninate, 27.14 g of Na_2CO_3, and 12.1 g of $NaHCO_3$ are dissolved in 500 mL of distilled water, and for solution B, 0.624 g of $CuSO_4.5H_2O$ and 0.631 g of L-serine are dissolved in 500 mL of distilled water. 1 mL of 5 mg/mL sample is added with 1 mL of BCA working solution in a 5 mL vial and incubated at 75 °C for 30 min with moderate shaking to avoid the precipitation of cellulose. After the vials are cooled to room temperature, the reaction mixture containing cellulose particles in the 5 mL vial is transferred into a 2 mL centrifuge tube and centrifuged for 5 min at 5000 rpm. The absorbance of the supernatant is then measured at 560 nm, and the glucose concentration is measured using a standard glucose calibration plot.

One millilitre of the diluted sample is taken into a 5 mL vial, and 1 mL of phenol reagent (5% v/v) is added to it followed by 3 mL of concentrated sulphuric acid. The solution is then mixed and incubated for 5 min at 90 °C in a water bath. The resulting solution is cooled to room temperature, and its absorbance is measured at 490 nm.

8.4.3 Sunn Hemp and June Grass

In this study, we have employed two lignocellulosic biomasses: one fibre (Sunn hemp) and one grass (June grass). Sunn hemp fibre (*Crotalaria juncea*) is a non-edible lignocellulosic substrate widely used as a cover crop. It is an annual shrub cultivated as a legume, especially for its fine fibres (Paul and Chakraborty 2018). The crop is cultivated in tropical and sub-tropical countries such as India, China, Korea, Japan, Brazil, and Bangladesh. Sunn hemp is a fast-growing crop and also very useful in smothering out weeds. It is grown on relatively light well-drained soil having sandy textured that retains moisture. The Sunn hemp fibre used in our

Table 8.3 Classification and habitat, composition, and physical properties of Sunn hemp and June grass (Paul and Chakraborty 2018; Roy and Chakraborty 2019)

Specification		Sunn hemp	June grass
Classification and habitat	Family	Fabaceae	Poaceae
	Size	100–1000 cm tall	Medium plant, 12–24 in.
	Soil preference	Moist	Dry and sandy
	Climate	Tropical and sub-tropical	Temperate
	Tolerance	High temperature	Dry sites, draught
Composition	Cellulose (%)	75.6	82.3
	Hemicellulose (%)	10.5	1.4
	Lignin (%)	10.3	9.5
	Moisture (%)	3.6	4.5
	Ash (%)	0.43	1.9
Physical properties	Crystallinity index	80.17	47.6
	Surface area	6.12 m^2/g	0.53 m^2/g
	Pore surface area	6.85 m^2/g	0.52 m^2/g
	Pore diameter	4.31 nm	2.46 nm
	Particle size	682.3 nm	120 nm
	Degree of polymerisation	650	1200

study is obtained from the ICAR—Central Research Institute for Jute and Allied Fibres, India (Table 8.3).

June grass (*Koeleria macrantha*) is a perennial grass that mostly grows in temperate regions during spring and early summer (Roy and Chakraborty 2019). This June grass is a conservative species and typically found in dry habitats with sparse vegetation. This grass is shorter than prairie grass and rarely forms dense stands. It grows best under full sun and in well-drained soils. It is very tolerant of dry and infertile soil. Sample of June grass is collected from the local campus fields of the IIT Kharagpur campus.

Both Sunn hemp and June grass are rich in cellulose and low on Hemicellulose. However, the fibre is much more crystalline than the grass, which indicates that it will require harsher treatment to breakdown the cellulose chain. Except for crystallinity, all other physical properties are comparable and suited for the production of second-generation biofuels.

8.5 Microwave-Irradiated Catalytic Conversion of Lignocellulose

Green chemistry has emerged as one of the multifaceted fields, which permeates all aspects of chemistry. Its core principle is to protect the environment by discovering new chemical pathways and technologies that reduce pollution. Solvents, along with technology, play an essential role in deciding cost, safety, health issues, and environmental impact of a chemical process (Klein-Marcuschamer et al. 2011). Conventional chemical synthesis is generally slow and is thus unable to satisfy the growing demands for the production of compounds. Rapid and environmentally benign methods for chemical synthesis are in high demand. Microwave technology has the potential to meet these demands. It can reduce reaction times—from days to minutes—that are potentially significant for the generation of high-value chemicals (Dąbrowska et al. 2018). Microwave-irradiated technology is based on the interaction of molecules, in a reaction mixture, with the generated electromagnetic waves. This technology depends on the polarity of the molecules and broadly follows two fundamental reaction mechanisms: dipolar rotation and ionic conduction of polar molecules (Thawarkar et al. 2016). Microwave results in the irradiation of a reaction mixture by dipole rotation of the polar molecules and reactants in the electromagnetic field (Grewal et al. 2013).

8.5.1 Microwave and Ionic Liquids (ILs)

Microwave heating develops the tendency of dipole inversions in alternating electromagnetic fields and induces distribution of heat through molecular friction and dielectric loss, which allows for better energy dissipation compared to conventional heating methods (Kappe 2019). The microwave also enhances the diffusion of an active species by controlling the molecular transport. Molecular shakeup and movement also contribute to the microwave effect. The exposure of polar molecules to microwave causes rapid rotation of the molecules (Fig. 8.2). This increases the probability of contact between the reactant molecules and generates heat due to friction, thus enhancing the reaction rate by reducing the activation energy. Microwave increases the molecular mobility to a great extent and also increases the rate of reaction due to an increase in the Arrhenius pre-exponential factor (Eq. 8.23).

$$K = Ae^{-\Delta G/\mathrm{RT}}, A = \gamma\lambda^2\Gamma, \tag{8.23}$$

where γ = number of neighbour jump sites, λ = jump distance, and Γ = jump frequency.

Microwave irradiation affects the frequency of vibration of the atoms at the reaction surface, and therefore, it has a significant impact on the Arrhenius pre-exponential factor.

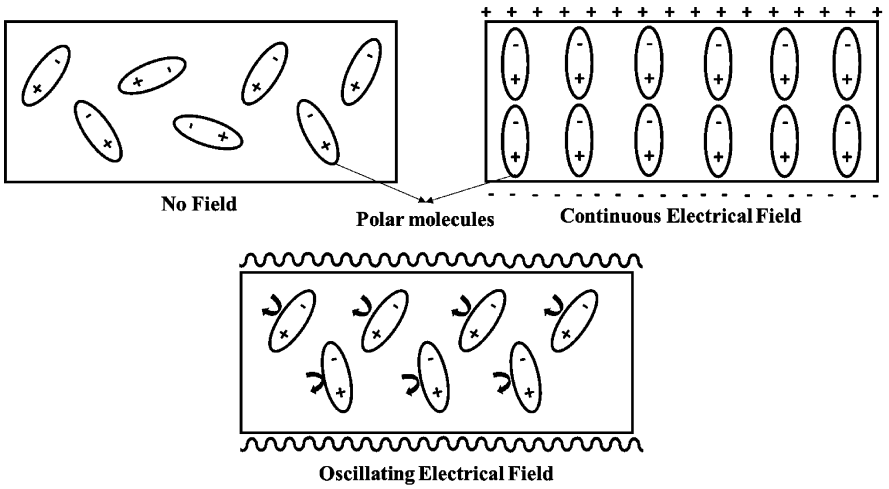

Fig. 8.2 Effect of microwave on the polar molecules

Microwave heating depends on the composition and the structure of molecules present in the reactor. This property enables the superheating of the polar molecules such as Ionic Liquids, while the non-polar molecules do not absorb the microwave and do not take part in the heating process (Li et al. 2010). Researchers have found that the selective heating property of the microwave leads to rapid and high yields of products when compared to other traditional heating processes. Since microwave reactions are rapid, the reaction time at such high temperature is low. Hence, the catalysts are protected from deactivation and decomposition due to shorter reaction times.

The superheating of solvents is one of the key advantages in the microwave-irradiation process (Lu et al. 2012). Control of the heating process is also an essential aspect of organic synthesis. Conventional single-mode microwave reactors (Kitchen microwave) do not allow us to control and operate the temperature by regulating the output power of the system. This shortcoming is overcome in the dual-mode or multimode microwave reactors. These are very sophisticated instruments designed to regulate and programme temperature, power output, and pressure through a software interface. Hence, most of the current technologies are rapidly evolving around dual-mode microwave reactors, not only in chemical synthesis but also in various other fields, such as food processing and pharmaceutical industries (Hoz et al. 2016).

Over the past few decades, several methods for cellulose hydrolysis have been developed, including biological, physical, physio-chemical, and chemical processes. Each of the above processes contains certain drawbacks: (1) the biological methods require long reaction time; (2) the physical processes are too energy-intensive and expensive for scale-up; (3) the physio-chemical processes require extreme reaction conditions; (4) the chemical processes involve highly volatile and toxic reagents that are harmful to the environment. Thus, to overcome the drawbacks of the usual

methods, we have implemented a dual-mode microwave heating process along with ILs and catalyst to breakdown the lignocellulose to biofuel precursors. As discussed earlier, the microwave works best with polar molecules, and ILs being highly polar perfectly match the required criteria. Both APIL and PIL reaction pathways are investigated in the dual-mode microwave reactor by optimising reaction parameters, such as catalyst-substrate loading, IL-substrate loading, water content, temperature, and time.

The ionic property of the ILs allows them to absorb microwave irradiation very efficiently and facilitate the quick transmission of energy by ionic conduction (Priecel and Lopez-Sanchez 2019). Therefore, ILs can reach a very high temperature in a very short time. Ionic conduction contributes to the superheating process by generating an electric field. However, the superheating in the microwave reactors may sometimes cause problems in the organic synthesis process due to their non-volatility. In this study, we have experimented with both APIL and PIL with $CuCl_2$ and water to breakdown lignocellulose in a dual-mode microwave reactor. As discussed in the earlier section, PIL and APIL form hexa-aqua and supramolecular complexes, respectively, and align themselves in the electromagnetic field generated by the microwaves. These polar complexes with electric dipole moments absorb microwave energy and undergo molecular dipole rotation, which leads to the collision of complexes with the adjacent molecules. The electromagnetic field, along with the aligned polar complexes, rotates millions of times per second due to changing polarity (Paul and Chakraborty 2018). The frequent dipole rotation causes friction between the complex and the molecule, which, in turn, breaks the polymeric chain of cellulose by breaking the β-1, 4-glycosidic bonds. Thus, the combined effect of the IL complexes, along with the electromagnetic field of the microwave enhances the hydrolysis of lignocellulose to glucose monomers significantly as compared to conventional processes such as the oil bath or enzymatic treatment.

8.5.2 Process Parameter Optimisation

Catalytic hydrolysis of both Sunn hemp fibre and June grass are performed in a dual-mode microwave reactor with PIL ($[Et_3NH][HSO_4]$) and APIL (BMIMCl) separately, $CuCl_2$ as the catalyst, and water as cross-catalyst (Chakraborty and Paul 2016). Both the substrates are dried in the open air and shredded down to a fine powder (100 mesh size approx.) before putting it in the microwave reactor. Five reaction parameters, namely catalyst-substrate loading, IL-substrate loading, water content, temperature, and time, are optimised based on the yield of glucose and 5-HMF.

The glucose concentration in the solution is measured by using Glucose oxidase (GOD) and peroxidase (POD) methods (Trinder 1969). Glucose is estimated using UV–Vis spectrophotometer (Agilent Technologies, USA) at 505 nm wavelength. An HPLC system (Perkin Elmer, USA) with a Wakosil II 5C18 column (5 μm,

4.6 × 150 mm^2) is used to measure the concentrations of HMF, LA, and FA. A solution of 90:10 (v/v) methanol: water is used as the mobile phase at 30 °C with a flow rate of 1 mL/min, at 282 nm wavelength for the measurement of HMF concentration. Hydrolysed lignocellulose sample is diluted with distilled water to achieve a certain Dilution Factor (DF). The yield of glucose is calculated based on the total amount of cellulose content in the lignocellulose. The yields of the samples are calculated using the formula: yield (%) = (product concentration (mg/mL)/ cellulose concentration in raw lignocellulose (mg/mL)) × 100.

8.5.2.1 Catalyst-Substrate Loading

Catalyst-loading is optimised with respect to lignocellulose loading. Figure 8.3 shows the variation of glucose and 5-HMF for catalyst-substrate loadings (w/w) of 6%, 7%, 8%, 10%, 12%, 14%, 16%, and 20%, for all the four possible combinations of Sunn hemp fibre and June grass with PIL and APIL, respectively. It is observed from Fig. 8.3a, c that at 160 °C with water addition of 40%, the maximum glucose yield increased as catalyst-substrate loadings varied from 6% to 16% for all four combinations. Similarly, Fig. 8.3b, d shows the maximum yields of HMF at the temperature of 180 °C with catalyst-substrate loading of 14% for Sunn hemp-PIL, 10% for June grass-APIL (Roy and Chakraborty 2019), and 12% for both PIL cases. As the catalyst-substrate loading is increased from 16% to 20%, the yield of the glucose decreases for the Sunn hemp-APIL case. This is attributed to the fact that high catalyst-loading promotes side reactions (Eminov et al. 2016). As the maximum yields of glucose and 5-HMF decrease beyond 16% for Sunn hemp-APIL, 20% and 12% for both PIL cases, and 16% and 10% for June grass-APIL, respectively, this can be designated as the optimum for each case.

8.5.2.2 Reaction Temperature

The reaction temperature is found to have a significant effect on product yields. The glucose yield decreases with increasing temperature, but it is much higher than the HMF yield (Fig. 8.4). Figure 8.4b, d show the optimum temperature for maximum HMF yield to be 180 °C for all the four combinations. If the reaction temperature is further increased to 200 °C, HMF readily converts to LA and FA, thereby decreasing the HMF yield. Thus, the reaction temperature is found to be an essential parameter, which can be regulated to obtain the desired product distribution that maximises either the yield of glucose (at 120 °C for June grass-PIL, 140 °C for Sunn hemp-PIL, 160 °C for Sunn hemp-APIL, 180 °C for June grass-APIL) or that of HMF (at 180 °C).

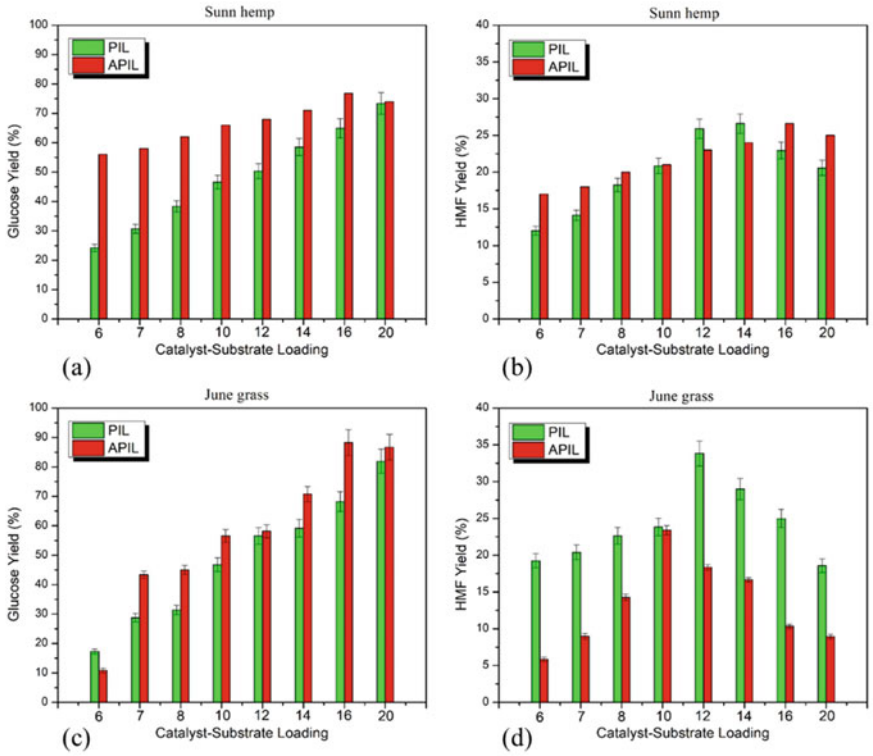

Fig. 8.3 Effect of catalyst-substrate loading in the microwave reactor on the yields of (**a**) Glucose from Sunn hemp at 160 °C, 40% water, 2.5 wt.% IL-loading using APIL for 46 min and 140 °C, 15% water, 4 wt.% IL-loading using PIL for 36 min; (**b**) HMF from Sunn hemp at 180 °C, 25% water, 2.5 wt.% IL-loading using APIL for 46 min and 180 °C, 0% water, 4 wt.% IL-loading using PIL for 36 min; (**c**) Glucose from June grass at 180 °C, 40% water, 4 wt.% IL-loading using APIL for 36 min and 120 °C, 15% water, 4 wt.% IL-loading using PIL for 36 min; (**d**) HMF from June grass at 180 °C, 30% water, 4 wt.% IL-loading using APIL for 36 min and 180 °C, 0% water, 4 wt.% IL-loading using PIL for 36 min (Paul and Chakraborty 2018; Roy and Chakraborty 2019)

8.5.2.3 Water Content

The most crucial component of product distribution is the water concentration in the microwave reactor. Water combines with the transition metal catalyst and ionic liquid to form complexes that help in the rapid absorption and dissipation of microwave energy and accelerates glycosidic bond rupture. Water also acts as a cross-catalyst that first hydrolyses the cellulose polymer to glucose, which is subsequently dehydrated to HMF. Figure 8.5 shows that the maximum yield of glucose was obtained at 40% water addition for APIL and at 15% water addition for PIL with both substrates, whereas the maximum yield of HMF was obtained at 25% and 30% water addition for APIL case for both substrates, and 0% for the PIL cases. HMF

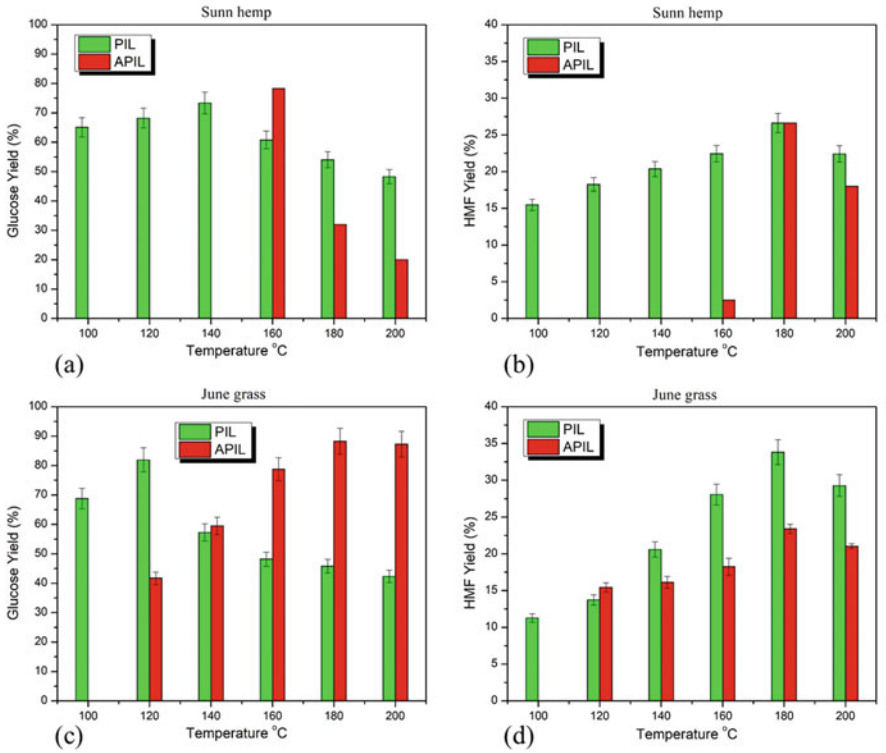

Fig. 8.4 Effect of the reaction temperature in the microwave reactor on the yields of (**a**) Glucose from Sunn hemp at 16 wt.% catalyst-loading, 40% water, 2.5 wt.% IL-loading using APIL for 46 min and 20 wt.% catalyst-loading, 15% water, 4 wt.% IL-loading using PIL for 36 min; (**b**) HMF from Sunn hemp at 16 wt.% catalyst-loading, 25% water, 2.5 wt.% IL-loading using APIL for 46 min and 14 wt.% catalyst-loading, 0% water, 4 wt.% IL-loading using PIL for 36 min; (**c**) Glucose from June grass at 16 wt.% catalyst-loading, 40% water, 4 wt% IL-loading using APIL for 36 min and 20 wt.% catalyst-loading, 15% water, 4 wt.% IL-loading using PIL for 36 min; (**d**) HMF from June grass at 10 wt.% catalyst-loading, 30% water, 4 wt.% IL-loading using APIL for 36 min and 12 wt.% catalyst-loading, 0% water, 4 wt.% IL-loading using PIL for 36 min (Paul and Chakraborty 2018; Roy and Chakraborty 2019)

production maximises at a lower water concentration since the conversion of glucose to HMF is a dehydration reaction (Zhang and Zhao 2010; da Silva Lacerda et al. 2015), whereas the conversion of lignocellulose to glucose is a hydrolysis reaction that requires the presence of water in the system. Excessive water addition is seen to precipitate the cellulose, thus reducing product yields. Thus, we observe that the optimum amount of water addition depends on the target product (glucose versus HMF) and the type of ionic liquid used (APIL versus PIL) but is independent of the lignocellulosic substrate.

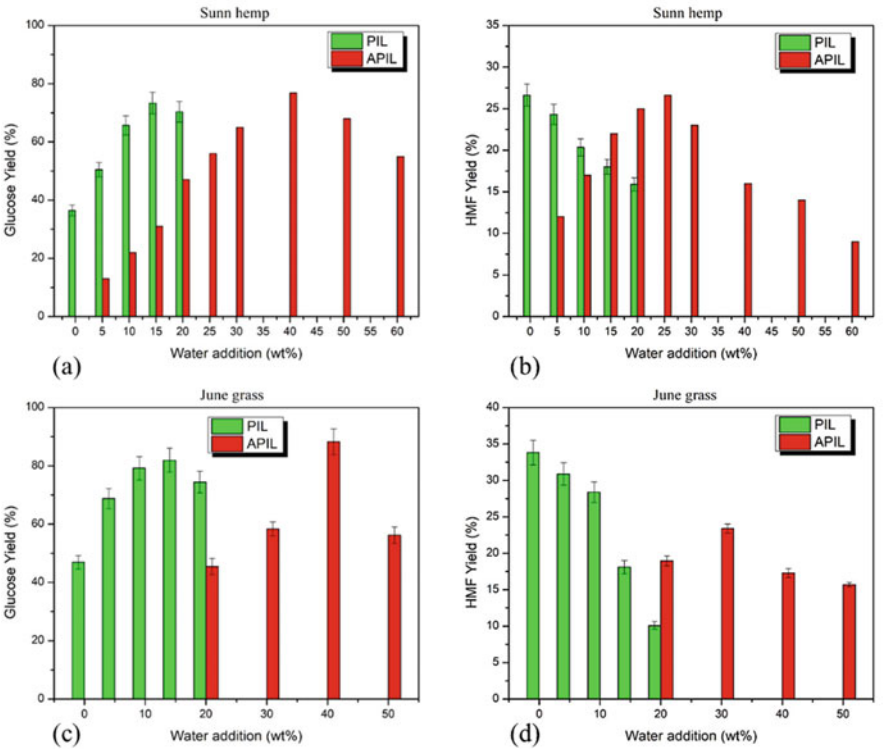

Fig. 8.5 Effect of water concentration in the microwave reactor on the yields of (**a**) Glucose from Sunn hemp at 16 wt.% Catalyst-loading, 160 °C, 2.5 wt.% IL-loading using APIL for 46 min and 20 wt.% Catalyst-loading, 140 °C, 4 wt.% IL-loading using PIL for 36 min; (**b**) HMF from Sunn hemp at 16 wt.% Catalyst-loading, 180 °C, 2.5 wt.% IL-loading using APIL for 46 min and 14 wt.% Catalyst-loading, 180 °C, 4 wt.% IL-loading using PIL for 36 min; (**c**) Glucose from June grass at 16 wt.% Catalyst-loading, 180 °C, 4 wt.% IL-loading using APIL for 36 min and 20 wt.% Catalyst-loading, 120 °C, 4 wt.% IL-loading using PIL for 36 min; (**d**) HMF from June grass at 10 wt.% Catalyst-loading, 180 °C, 4 wt.% IL-loading using APIL for 36 min and 12 wt.% Catalyst-loading, 180 °C, 4 wt.% IL-loading using PIL for 36 min (Paul and Chakraborty 2018; Roy and Chakraborty 2019)

8.5.2.4 IL-Substrate Loading

The ionic liquid loading is also optimised with respect to lignocellulose loading. IL-substrate loading (wt%) ratios are varied as 1.25%, 2%, 2.5%, 4%, 5%, 6%, 7.5%, 8%, and 10% in the reaction. Figure 8.6 shows the effect of IL-substrate loading on the yields of glucose and HMF at their respective optimum temperature, water addition and catalyst-loading. The yields of glucose and 5-HMF are observed to be optimum at 2.5% for Sunn hemp-APIL, 4% for June grass-APIL, and 6% for PIL cases for both the substrates and decrease gradually as the substrate to ionic liquid loading increases. This decrease may be attributed to the fact that low

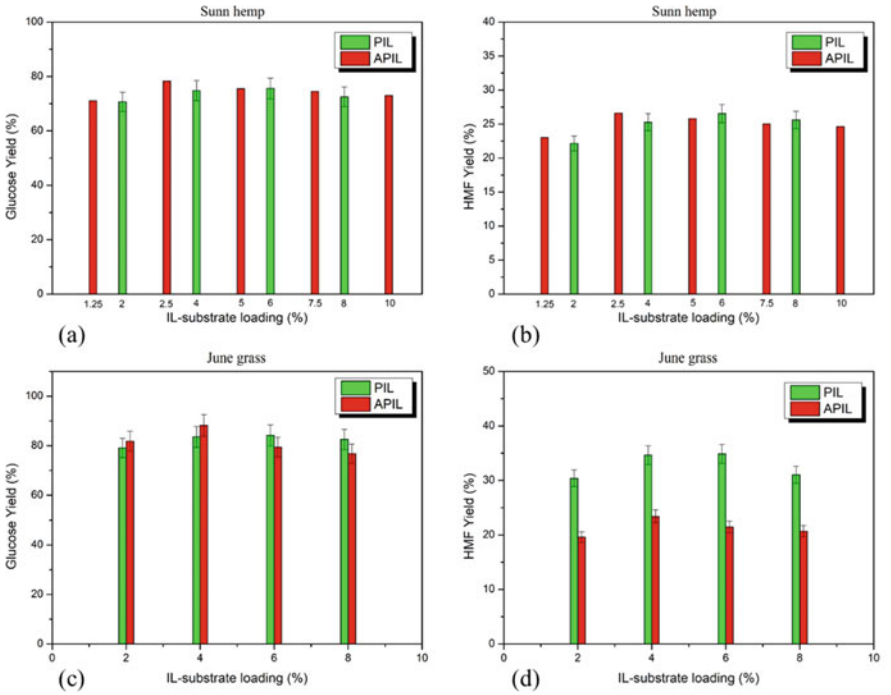

Fig. 8.6 Effect of IL-substrate ratio in the microwave reactor on the yields of (**a**) Glucose from Sunn hemp at 16 wt.% catalyst-loading, 160 °C, 40% water using APIL for 46 min and 20 wt.% catalyst-loading, 140 °C, 15% water using PIL for 36 min; (**b**) HMF from Sunn hemp at 16 wt.% catalyst-loading, 180 °C, 25% water using APIL for 46 min and 14 wt.% catalyst-loading, 180 °C, 0% water using PIL for 36 min; (**c**) Glucose from June grass at 16 wt.% catalyst-loading, 180 °C, 40% water using APIL for 36 min and 20 wt.% catalyst-loading, 120 °C, 15% water using PIL for 36 min; (**d**) HMF from June grass at 10 wt.% catalyst-loading, 180 °C, 30% water using APIL for 36 min and 12 wt.% catalyst-loading, 180 °C, 0% water using PIL for 36 min (Paul and Chakraborty 2018; Roy and Chakraborty 2019)

IL-substrate loading favours the dispersion of molecules in the solution and leads to a high dissolution rate. On the other hand, high IL-substrate loading causes heat and mass transfer limitations in the reaction mixture and decreases the accessibility of the ionic liquid to the biomass (da Costa Lopes et al. 2013).

8.5.2.5 Reaction Time

The effect of optimal reaction time is determined for various IL-substrate loadings at their respective optimum temperature, water concentration, and catalyst-substrate loading. The total reaction times of the process are varied as 31 min, 36 min, 41 min, 46 min and 51 min. Figure 8.7 shows that the yields of Glucose and 5-HMF were optimum at 36 min (June grass-APIL), 41 min (June grass-PIL), 46 min (Sunn

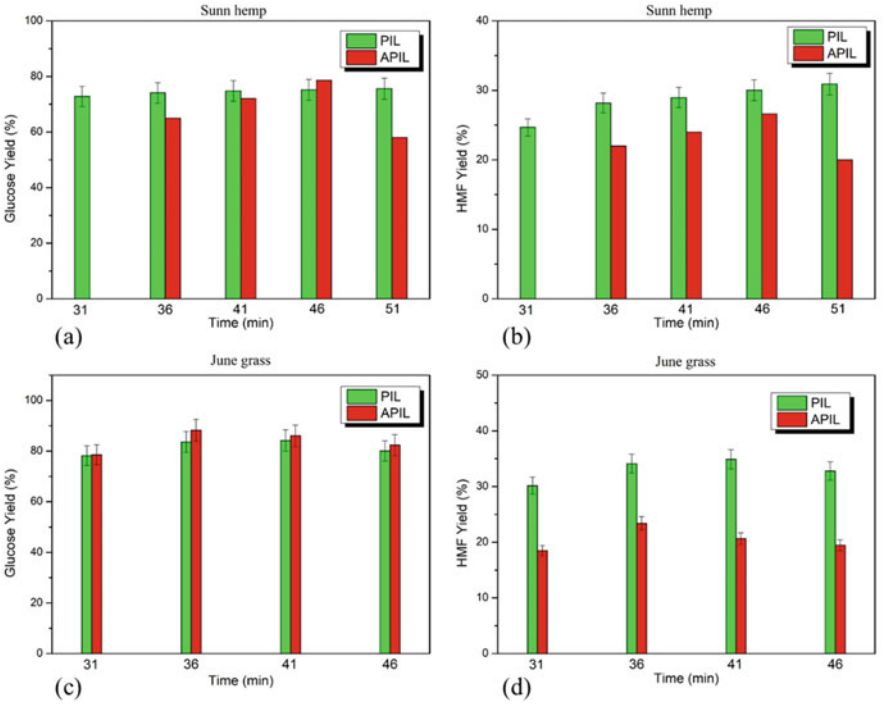

Fig. 8.7 Effect of reaction time in the microwave reactor on the yields of (**a**) Glucose from Sunn hemp at 16 wt.% catalyst-loading, 160 °C, 40% water, 2.5 wt.% IL-loading using APIL and 20 wt.% catalyst-loading, 140 °C, 15% water, 6 wt.% IL-loading using PIL; (**b**) HMF from Sunn hemp at 16 wt.% catalyst-loading, 180 °C, 25% water, 2.5 wt.% IL-loading using APIL and 14 wt.% Catalyst-loading, 180 °C, 0% water, 6 wt.% IL-loading using PIL; (**c**) Glucose from June grass at 16 wt.% Catalyst-loading, 180 °C, 40% water, 4 wt.% IL-loading using APIL and 20 wt.% Catalyst-loading, 120 °C, 15% water, 6 wt.% IL-loading using PIL; (**d**) HMF from June grass at 10 wt.% Catalyst-loading, 180 °C, 30% water, 4 wt.% IL-loading using APIL and 12 wt.% Catalyst-loading, 180 °C, 0% water, 6 wt.% IL-loading using PIL (Paul and Chakraborty 2018; Roy and Chakraborty 2019)

hemp-APIL), and 51 min (Sunn hemp-PIL) and they decrease if the reaction is continued beyond that time, which may be caused by the formation of undesired by-products such as humin (Peng et al. 2010).

8.5.3 Optimum Product Yields

The optimisation experiments reveal that glucose is formed at relatively lower temperatures compared to 5-HMF. APIL gives higher glucose yield with June grass, whereas PIL gives a higher 5-HMF yield with June grass (Choudhary et al. 2012). 5-HMF prefers low water concentrations in the reaction media for higher

Table 8.4 Optimised process parameter for Glucose and 5-HMF production (Roy and Chakraborty 2019; Paul and Chakraborty 2018)

Lignocellulose type	IL type	Target precursor	CS loading	IL-loading	Water content	Temp. (°C)	Time (min)	Yield (%)
Sunn hemp	PIL	Glucose	20	6	15	140	51	75.6
		5-HMF	14	6	0	180	51	30.8
	APIL	Glucose	16	2.5	40	160	46	78.7
		5-HMF	16	2.5	25	180	46	26.8
June grass	PIL	Glucose	20	6	15	120	41	84.2
		5-HMF	12	6	0	180	41	34.9
	APIL	Glucose	16	4	40	180	36	88.2
		5-HMF	10	4	30	180	36	23.4

yields. All the optimised reaction parameters for the four combinations are given below in Table 8.4.

8.6 A Kinetic Framework for Microwave-Irradiated Catalytic Conversion by Employing Ionic Liquids

We have investigated the biofuel precursor production process from lignocellulose by applying the dual-mode microwave-irradiation technology. The reaction parameter optimisation study reveals that APIL tends to produce more glucose from the cellulose-rich highly crystalline substrate as compared to PIL. The experimental results also indicate that PIL works better with a moderately crystalline substrate to give a high yield of 5-HMF at low water concentrations. In order to understand and establish a working procedure, we have proposed a kinetic framework that will provide us with the necessary information to achieve high yields of alcoholic and furanic fuel precursors such as glucose and 5-HMF.

In this study, we have conducted our experiments with two cellulose-rich (>75%) lignocellulosic substrates, Sunn hemp and June grass. The XRD analysis shows that Sunn hemp is highly crystalline with Crystallinity Index of 80%, whereas June grass is moderately crystalline with Crystallinity Index of 47%. Thereby, we have tested one highly crystalline and one moderately crystalline cellulose-rich substrate. Other physio-chemical properties and composition are very much comparable. After characterisation, the substrate was treated with both PIL and APIL separately, with $CuCl_2$ as catalyst and water as cross-catalyst in a dual-mode microwave reactor. Experimental results show that the combination of Sunn hemp fibre and APIL generates higher yields of glucose as compared to Sunn hemp and PIL. Therefore, we came to an inference that APIL works better with highly crystalline substrates to produce glucose. On the other hand, PIL works better with a moderately crystalline substrate to generate a high yield of 5-HMF. Based on our experimental results, we have drafted a kinetic framework which is presented in Fig. 8.8.

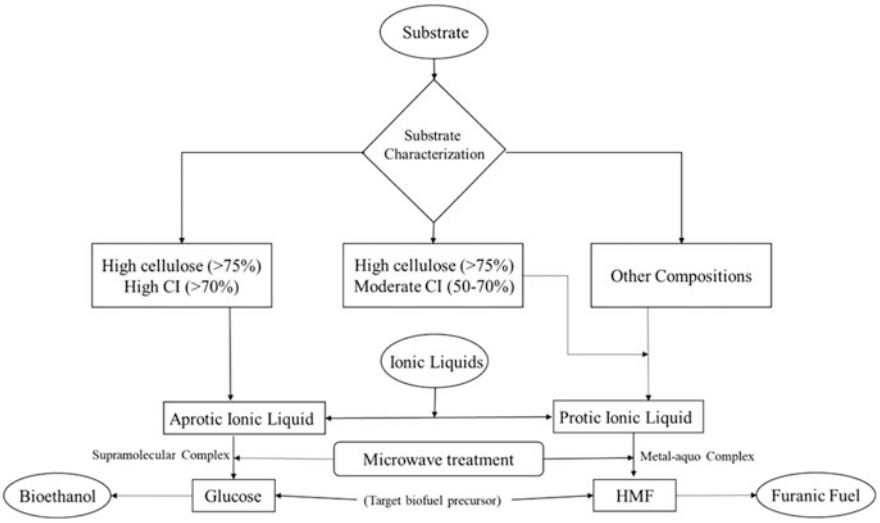

Fig. 8.8 Proposed kinetic framework for microwave-irradiated catalytic conversion of lignocellulose by employing ionic liquids

According to the proposed framework presented in Fig. 8.8, the supramolecular formation in the APIL chemistry plays a significant role in breaking the highly crystalline polymeric cellulose of Sunn hemp fibre to glucose monomers. However, PIL breaks down the moderately crystalline cellulose in June grass in low water concentrations to generate a high yield of 5-HMF. PIL with $CuCl_2$ and water forms a hexa-aquo complex which favours dehydration reaction more in the presence of in situ HCl in the reaction mixture. Now depending upon the characteristic, composition of biomass and the desired product, we can choose the respective pathway from the proposed kinetic framework shown in Fig. 8.8.

8.7 Biofuel Precursors for Alcoholic and Furanic Fuels

Feasible and efficient conversion of biomass to platform chemicals has become one of the significant research domains. The consensus is that we need to develop various green technologies based on renewable energy (Li and Yang 2014). The process involving the conversion of biomass to platform chemicals is capable of reserving all carbon atoms, and thus can be the most efficient pathway from the atom-economy viewpoint. Glucose and 5-HMF are outstanding platform chemicals (Guo et al. 2020) and are considered to be the bridge between biomasses and valuable biochemical. Although several platform chemicals and biochemical can be generated through various technologies, our primary focus is on glucose and 5-HMF (Fig. 8.9a). Both these precursors, especially 5-HMF, have enormous potential to

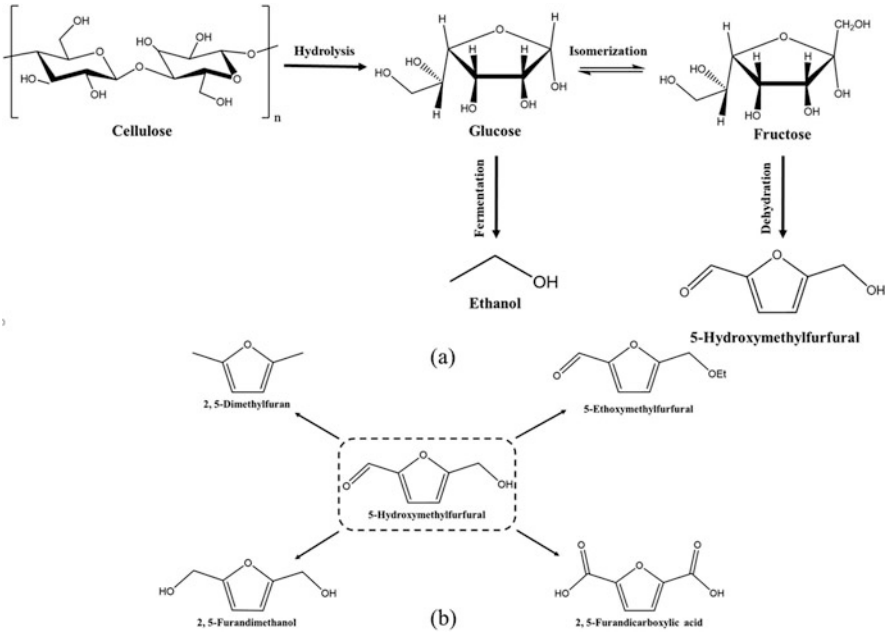

Fig. 8.9 (**a**) Cellulose to ethanolic and furanic fuel pathway, and (**b**) Value-added products from 5-HMF

generate a variety of value-added chemicals. Glucose can be fermented to bioethanol while 5-HMF plays the role of the principal component in the synthesis of various high-value chemicals such as 2, 5-Dimethylfuran (DMF), 5-Ethoxymethylfurfural (EMF), 2, 5-Furandicarboxylic acid (FDCA) and 5-Methylfuran (MF) (Wang et al. 2019).

8.7.1 Fermentation of Glucose to Bioethanol

The glucose produced from catalytic hydrolysis of lignocelluloses can be fermented to ethanol by using various microbes. Microorganisms such as yeasts and bacteria can ferment the monosaccharides into ethanol. The fermentation of sucrose or glucose derived from lignocellulosic biomasses typically uses yeasts such as *Saccharomyces cerevisiae* (Holzberg et al. 1967), and bacteria such as *Zymomonas mobilis* (Swings and Ley 1977), and *Escherichia coli* (Ingram et al. 1998). *Saccharomyces cerevisiae* is the most commonly used yeast for the commercial production of ethanol because of its ability to tolerate high temperature, high ethanol concentration, and a wide range of pH (Lin et al. 2012). Other microorganisms, such as *Pichia stipitis* (Kosaric and Velikonja 1995) and *Kluyveromyces fagilis* (Mussatto et al. 2012), are also used to produce bioethanol from monomeric sugars. Fungi that

show high tolerance to ethanol for some strains and media are also suitable for the fermentation of glucose (Olsson and Hahn-Hagerdal 1993) to ethanol. The conversion of glucose to ethanol involves several metabolic pathways. Glucose is converted to ethanol via pyruvate in two steps.

$$CH_3COCOO^- + H^+ \rightarrow CH_3CHO + CO_2 \uparrow \quad (8.24)$$

$$CH_3CHO + NADH^+ + H^+ \rightarrow C_2H_5OH + NAD^+ \quad (8.25)$$

The first step involves the conversion of glucose to acetaldehyde, which is a decarboxylation process that generates CO_2 (Eq. 8.24). In the next step, acetaldehyde is converted to ethanol, which is a dehydrogenase reaction (Ingram et al. 1998). Microorganisms under anaerobic growth conditions can utilise glucose by Embden–Mereyhof–Parnas (EMF) pathway (Bailey and Ollis 1985). The phosphorylation of carbohydrates is carried out through the metabolic pathway, and the end products are 2 mol of ethanol and carbon dioxide (Eq. 8.25) (Harden and Young 1908). Certain factors in yeast and bacterial fermentation affect the production of ethanol, which include the fermentation temperature, the ability of strains to ferment sugars, and the tolerance of the yeast strain towards the inhibitors present in the fermentation broth. Yeasts and bacteria cannot directly ferment lignocellulosic feedstock unless pretreatment and hydrolysis precede the fermentation process. The reaction conditions must be varied depending on the nature of the microorganism used in the fermentation process. It is necessary to run the fermentation reaction at optimum process conditions to produce a high yield of ethanol.

The production of bioethanol during fermentation is influenced by several process parameters such as temperature, initial sugar concentration, initial cell concentration, mixing, and fermentation time. Thus, the effects of these process parameters on the growth of yeast cells (*Saccharomyces cerevisiae*), glucose consumption and ethanol formation are observed through fermentation experiments after catalytic and enzymatic hydrolysis of lignocellulose.

8.7.2 Furanic Fuels and Other Value-Added Chemicals from 5-HMF

5-HMF serves as the precursor to high-value furanic compounds such as FDCA, DMF, EMF, and MF (Fig. 8.9b). Some of the compounds can be used as a primary material for new products as well as replacement of fossil-fuel derived chemicals and polymers (Yan and Chen 2014).

DMF has an energy density compared with that of gasoline and can act as a biofuel. The hydroxygenation of 5-HMF is the main route for DMF production (Kazi et al. 2011). A bimetallic catalyst such as Pd-Au, Pd-Au/C, Pd/C could catalyse the conversion of 5-HMF to DMF in the presence of HCl under atmospheric pressure. These catalysts have effectively hydroxygenated 5-HMF to DMF with high yield

(>85%). More recently, it is reported that copper and cobalt bimetallic catalyst on nanoparticles could also catalyse the conversion of 5-HMF to DMF with a higher yield (of about 99%) (Leshkov et al. 2007).

2, 5-Diformylfuran (DEF) is another crucial derivative obtained from 5-HMF. It can be employed in the synthesis of pharmaceutical products, poly-Schiff bases, and many other functional materials (Zhang et al. 2015). Hollow Fe-Co bimetallic Nanoparticles could catalyse the selective oxidation of 5-HMF to DEF with high yield under mild conditions. Graphene oxide can catalyse the selective oxidation process and shows high activity even after several cycles (Chen et al. 2014).

FDCA is another essential building block of green plastic that can be synthesised from 5-HMF. FDCA can be prepared via either catalytic or biological pathways (Ouyang et al. 2019). Au-Pd alloy nanoparticles could catalyse the aerobic oxidation of 5-HMF to FDCA (Xia et al. 2018). Functionalised carbon nanotubes could also effectively catalyse the conversion of 5-HMF to FDCA in a base-free condition (Sajid et al. 2018).

EMF is another important furan derivative that can be used as an additive for regular diesel. It has a high boiling point (508 K), high energy density (30.3 MJ/L), low toxicity, high cetane number, and a low SO_2 emission rate (Lewkowski 2001). 5-HMF undergoes etherification reaction with ethanol in the presence of an acid catalyst to produce EMF. A multi-step method can be followed to prepare EMF, where the biomass along with HCl leads to the production of 5-Chloromethylfurfural (CMF), followed by nucleophilic substitution of CMF with ethanol.

8.8 Process Economics

The proposed framework in the earlier section suggests that the hydrolysis of lignocellulose primarily produces glucose, 5-HMF, LA, and FA. Out of these four, our primary focus is on glucose and 5-HMF production. The four possible combinations of two lignocelluloses and two ILs have been studied thoroughly for the production of biofuel precursors. From the proposed framework, it is inferred that the combination of June grass and APIL gives maximum glucose yield, whereas the combination of PIL and June grass gives maximum HMF yield. In this section, we discuss the economic aspects of the two routes and also comment on the profitability of the routes for scale-up and commercial production.

Bioethanol will be produced from glucose via fermentation, while 5-HMF is the precursor to various furanic fuels such as 2, 5-Dimethylfuran (DMF), 5-Methylfuran (MF) and 5-Hydroxymethylfurfural (EMF) (Sitthisa et al. 2011). 5-HMF is also the precursor for various other high-value products such as 2,5-Furandicarboxylic acid (FDCA) and Dihydroxymethylfuran (DHMF) (Zhang et al. 2018). Although APIL gives 4% more glucose than PIL, 5-HMF yield is higher with the PIL. Since the difference in product yield is not that significant, it is challenging to decide the best route based on product yields alone. Hence, we calculate the product-reactant cost ratio (product market value/reactant market value) to determine the economic

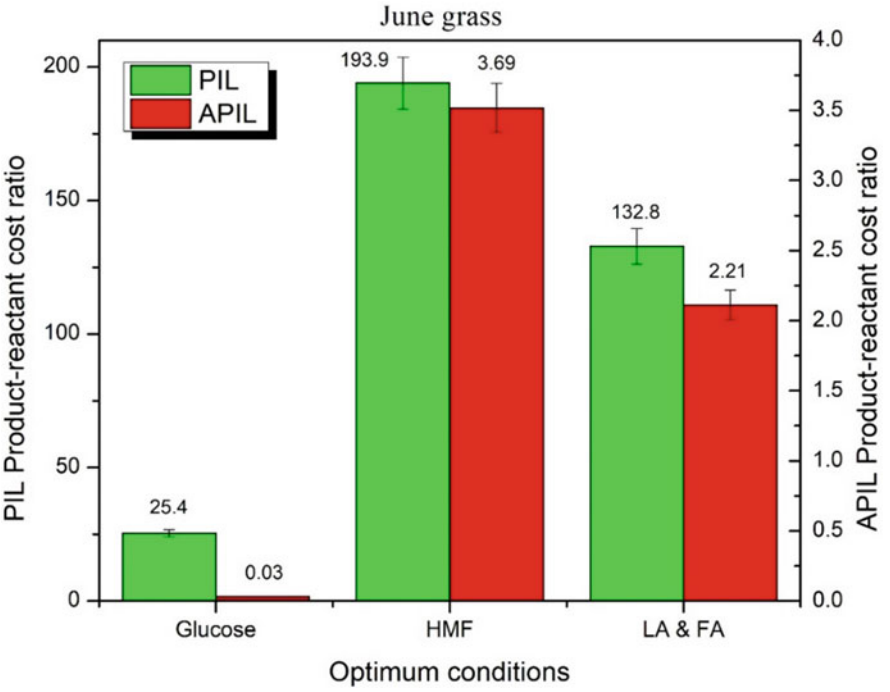

Fig. 8.10 Product-reactant cost ratio at optimum for Glucose and HMF from June grass using PIL and APIL (Roy and Chakraborty 2019)

feasibility of the process. Only the reactant and the product costs are considered in these calculations, and no other costs or expenditures. A comparison of the market values of the two ionic liquids and the two target molecules in the two processes would convince us of the cost-effectiveness of the proposed process. The market price of bioethanol is INR 75/kg, while that of HMF and LA are INR 147,000/kg, and INR 8500/kg, respectively; the market price of APIL is INR 240,000/kg, while that of PIL is INR 88/kg.

For the APIL process with June grass, the product-reactant cost ratio for optimum glucose condition is 0.03, and that for the 5-HMF optimum condition is 3.7 (Fig. 8.10). The <1 value of product-reactant cost ratio indicates that the route is economically viable for commercial production. On the other hand, the PIL-based process has product-reactant cost ratio of 25.4 when optimizing the glucose yield, and 194 when optimizing the HMF yield. Thus, the PIL route to produce HMF is far more economically viable from the scale-up viewpoint. Therefore, it is clear from the cost analysis that the PIL route is not only beneficial for biofuel production but also opens a full window of opportunity to explore other aspects of this technology to develop various other high-value chemicals and products.

8.9 Process Scale-Up and Integration into Bio-Refinery

In this work, we talked about the nature of the ILs and catalyst, along with the type of lignocellulose substrates for the production of ethanolic and furanic fuel precursors. The experiments were conducted in a lab-scale dual-mode microwave reactor, and it is designed in such a way that the data generated from them will provide us with a clear idea about scaling up the whole process. The proposed framework and the cost analysis show us that the cost of ILs and the product market value play significant roles from an economic viewpoint. Although we cannot directly control the product market value, it is possible to recycle the ILs to reduce production and further increase the economic feasibility of the entire process.

In literature, there are various methods available (Fig. 8.11) for the efficient recycling of ILs such as phase-separation, extraction, distillation, adsorption, membrane separation and crystallization (Oliveira et al. 2011). All the above processes have some pros and cons depending upon the nature of IL and the target product. In this study, phase-separation and extraction techniques were implemented to separate IL from the products (Sklavounos et al. 2016).

To recycle the Ionic Liquid, 5 mL of 40 wt% K_3PO_4 solution was added to the reaction mixture, and the solution was stirred at 100 rpm for 15 min with its temperature maintained at 70 °C (Shill et al. 2011). The samples were cooled to room temperature and centrifuged. Centrifugation produces three different phases in the solution: the upper portion is the ionic liquid-rich phase, the middle part contains the unreacted solid substrate, and the bottom portion is the K_3PO_4 salt-rich phase. The ionic liquid phase is separated and evaporated at 120 °C for 20 min to remove water. The solid substrate is washed with water and then dried.

An Ionic Liquid immiscible organic solvent ($V_{1\text{-Butanol}}$: $V_{MIBK} = 3$: 7, $M_{organic\ solvent}$: $M_{IL} = 3{:}1$) is added to the reaction mixture, following which the aqueous

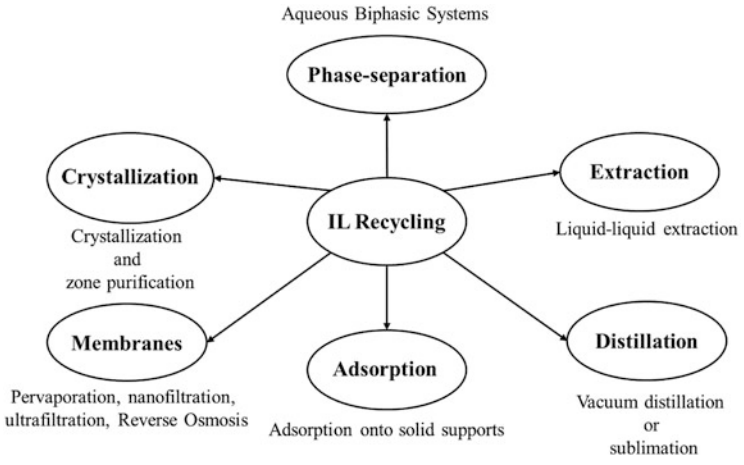

Fig. 8.11 Various methods for IL recovery and recycling

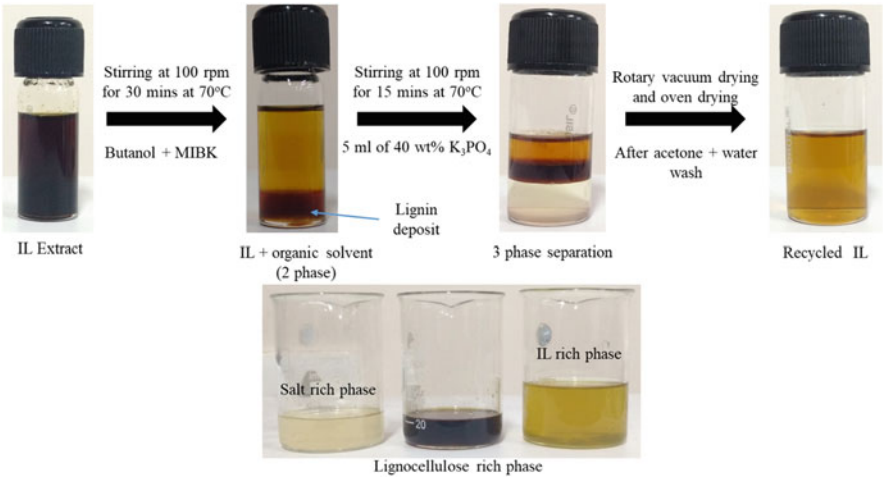

Fig. 8.12 Phase-separation and extraction of IL after hydrolysis

phase (ionic liquid phase) and the organic phase are separated (Saha and Abu-Omar 2013). The upper layer of the organic phase is removed for analysis of HMF, while glucose (Fig. 8.12), which is insoluble in the organic phase, is left in the ionic liquid phase.

Apart from the recycling of the ionic liquid, the other significant bottlenecks in the scale-up and commercialization of the proposed technology are capital investment and power consumption. The capital cost to run the microwave mediated catalytic process is higher than the conventional processes. However, the total cost of energy consumption is low for microwave mediated process than that of the conventional oil bath mediated catalytic one. So, the total energy cost to produce biofuel precursors is much higher in oil bath mediated catalytic hydrolysis compared to microwave-assisted catalytic hydrolysis. Controlled heating by microwave irradiation leads to lesser heat dissipation and better energy utilization as compared to conventional oil bath heating. Thus, less time is required to complete the reaction for microwave mediated process, and hence the total cost of energy consumption is reduced significantly. The net energy production from the process can be further increased by integrating solar panels with the dual-mode microwave reactor (Mohsenian et al. 2019). The electricity generated by the solar panels will reduce the conventional electricity consumption and make the whole process much more sustainable and environmentally friendly. The integration of solar panels with the microwave reactor will be a big leap towards carbon neutrality.

8.10 Conclusion

This work presents a kinetic framework for microwave-irradiated ionic liquid mediated catalytic conversion of lignocelluloses to biofuel precursors. We have earlier identified two non-food lignocellulosic substrates, namely, Sunn hemp fibre and June grass, on which this kinetic framework is now employed to produce biofuel precursors. Contrary to first-generation biofuels that use starch and sugars as the source of lignocellulose, the present method uses non-food cover crops like local grass and fibre which are readily available in tropical/sub-tropical countries, has a low lignin content (thus eliminating the need for delignification) and a high cellulose content. This highlights of our process are that: it is very rapid, requiring less than an hour to complete; it is very cost-effective and produces very high-value products such as HMF using very low-cost Protic Ionic Liquids; it does not require any pretreatment and can catalytically convert raw lignocelluloses to biofuel precursors in a one-pot synthesis system; being feedstock agnostic, it converts a broad spectrum of recalcitrant lignocellulosic bioenergy crops—with high cellulose content, DP and crystallinity, and low porosity—to biofuel precursors. The proposed kinetic framework provides a cost-effective pathway to produce biofuel precursors from untreated, non-edible lignocellulose in less than an hour. The high molecular weight, long-chain cellulose polymers in the lignocellulose facilitate the formation of a polar complex in the presence of a metal chloride catalyst and water, which help in the rapid absorption of microwave energy at the reactor scale, and its subsequent dissipation at the molecular scale.

The design and synthesis of a cheap recyclable ionic liquid is an essential step in scale-up and commercialization of this technology. The ionic liquid can be designed to meet our specific needs of high recycling rate and high yields of biofuel precursors. APIL performs better with higher crystalline substrates to produce high yields of glucose, while PIL works effectively with moderately crystalline substrates to produce more 5-HMF. The recovery efficiency of the ionic liquid needs to be high for cost-effective scale-up and commercialization. Attaining high recovery efficiency would require the use of other ionic liquids with molecular structures that facilitate easier separation from biofuel precursors. The integration of solar panels with the microwave reactor will further increase the energy efficiency of the commercial process.

Competing Interests All the authors declare that they have no competing interests.

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Chapter 9
Biomass Fractionation Based on Enzymatic Hydrolysis for Biorefinery Systems

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Abstract A biorefinery system scheme has developed great interest in researchers and industries to obtaining products. A biorefinery is based on efficient use of different types of raw material, such as biomass, for integrated or simultaneous obtaining of several kinds of value-added products. Among used biomass, it is highlighted the vegetable sources, which can come from agro-industrial crops or from waste of biomass processing. There is an extensive variety of biomass that implicates diverse conversion process. Lignocellulosic biomass, in particular, can be transformed into biobased products by different conversion stages. Biofuels, biopolymers, and others bioproducts come from several types of biomass and various processes. One of the most important phases in the transformation process is the hydrolysis, which can be performed by conventional (chemical) and non-conventional (biological) routes. Enzymatic hydrolysis process allows to breakdown chemical structures into smaller molecules, which can be took in advantage for generation of value-added products at subsequent stages in biorefineries.

Keywords Biomass disruption · Renewable energy · Bioresources refinery

Abbreviations

BRF	Biorefinery
CL	Cellulose
DEAE	Diethylaminoethyl
EDTA	Ethylenediamine tetraacetic acid
HC	Hemicelluloses
LC	Lignocellulosic
LG	Lignin

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VB Vegetal biomass
XOS Xylooligosaccharides

9.1 Introduction to a Biorefinery

In 2018, non-renewable fossil sources share 84.7% to sustain the world energy consumption matrix; among those, oil was the main contributor with 33.6% (British Petroleum 2019). Thanks to its chemical composition, oil is considered as a versatile raw material that allows to obtain different chemical products through refinery process (U.S. Energy Information Administration 2012). Despite this, crude oil systems for refining are great CO_2 producers and are generally placed in water-stress sites (Guedes et al. 2019). Besides this, oil has serious problems related to its depletion, non-uniform distribution of reserves, price fluctuations and various negative impacts in human health and environment caused mostly by combustion of its derivatives (Hoegh-Guldberg et al. 2018; Statista 2020). According to this context, world and local researches have been developed to replace oil with renewable sources (biological material) that can be used in a similar process route than oil (refinery). Refinery operating with biological raw material (biomass) and basing on integrated or simultaneous generation of different types of products is named "biorefinery" (BRF).

BRF system can be described through the basic process scheme shown in Fig. 9.1, where vegetal biomass (VB) is turned into value-added products by four main stages: pretreatment, hydrolysis, co-production process (conversion or transformation stage), and adjustment process (separation or purification stage). Raw VB generally is dried before get in BRF system in order to eliminate water content avoiding microorganism activity that causes modifications in physical-chemical properties and thus can be stored (Rentizelas 2016). In some cases, when VB is waste from any type of productive chain like fruits, flowers, or another type of vegetal species, an extraction step into a BRF can be considered important. Some fruit peels, barks, leaves, and others vegetal waste (petals, seeds, bagasse, etc.) have special substances in their chemical structure that can be extracted (McCabe et al. 2007; Seader et al. 2011). According to some researches, from fruit peels, vegetal barks, and leaves it is possible to obtain essential oils, antioxidants, or repellent substances, which have a high interest for some industrial sectors (Asha et al. 2015; Ortiz et al. 2017; Asadollahi et al. 2019).

After extracting stage, BRF process obtains its first value-added products (extracts in liquid fraction 1) and solid biomass, which can be either directly sent to transformation (third stage) or be transported to pretreatment process (first stage) where biomass chemical structure is modified. Pretreatment outflows are solid and liquid fractions of chemically modified biomass.

The main characteristic of pretreated biomass is that access to compounds of its chemical structure is enhanced, which is really helpful for subsequent procedures in BRF (Shimizu et al. 2020; Coral Medina et al. 2015). Liquid fraction generated after

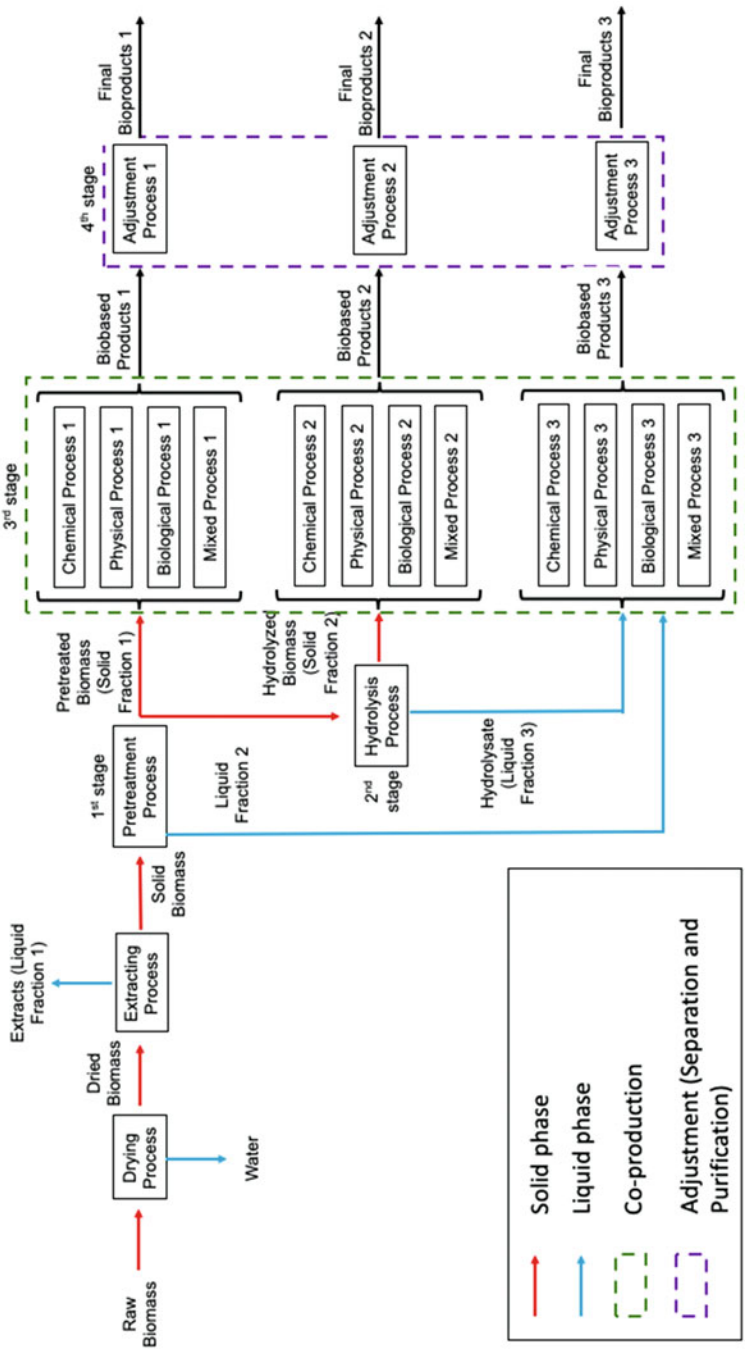

Fig. 9.1 Basic representation of a biorefinery general system

pretreatment (identified with number 2) incorporates soluble substances such as oligosaccharides and others that can be sent to the third stage for transformation (Silva et al. 2018). Pretreated biomass can be directly sent to conversion and obtaining of new products (biobased products 1) or be transported to hydrolysis process (second stage), where chemical structure is broken into smaller molecules such as monosaccharides (Loow et al. 2016). After hydrolysis, a hydrolyzed biomass (solid fraction 2) and hydrolysate (liquid fraction 3) are generated.

Depending on applied pretreatment and hydrolysis procedures, hydrolyzed solid fraction and liquid hydrolysate will have specific chemical compositions, which will determine the conversion route at co-production stage for product obtaining. From hydrolyzed solid fraction can be obtained products (biobased products 2) such as active carbon, lignin, biofilms, adsorption material, micro-fibrillated cellulose, biochar, bio-oil, electricity, etc. (Nanda et al. 2014; Coral Medina et al. 2015; Li and Kumar 2016; Vassileva et al. 2016; Virtanen et al. 2017; Rosales-Calderon and Arantes 2019). From liquid hydrolysate that contains soluble substances such as monosaccharides can be obtained a huge variety of products (biobased products 3); among them are bioethanol, furfural, hydroxymethylfurfural, carboxylic acids (itaconic, glutamic, lactic, and succinic), biopolymers, etc. (Sorokina et al. 2017; Steinbach et al. 2017; Rosales-Calderon and Arantes 2019; Dulie et al. 2020). After any kind of transformation process (chemical, physical, biological, or mixed) each crude outcome generally is sent to an adjustment stage, where separation operations occur for product quality improvement (Geankoplis 1993; Seader et al. 2011).

Aside to known processes involved in BRF, it is essential to find the best type of raw material (biomass) to employ in the process. Raw VB can come from agro-industrial crops or from waste of biomass processing; the last one usually is burnt in situ at rural fields as practice for solving accumulation problem (Almsatar 2020). This burning practice caused by human activities has generated significant concerns as a result of greenhouse gases and others chemical substances emitted into the atmosphere, both considered as pollutants (Zhang et al. 2016). Although burning raw VB waste solves accumulation problem in situ, this condition enlarges negative effects on environment (Onoja et al. 2018). To avoid this disadvantage, it is convenient to use mainly VB waste for transformation into value-added products by a BRF process.

9.2 Biomass Composition and Recalcitrance

Biomass is defined as any biological resource that can be turned into value-added products (including those energy producers). Biomass can be used in a direct way, when biological matter is consumed in its natural form; such as wood for firewood, and indirectly, when biological material undergoes some conversion process for product obtaining, such as biodiesel from oilseeds, carboxylic acids and biopolymers from lignocellulosic (LC), and bioethanol from starchy sources (Pervez et al. 2014; Sorokina et al. 2017; Rosales-Calderon and Arantes 2019). In addition, any kind of

biomass is originated from spontaneous or induced biochemical processes occurring in biological sources that belong to vegetal, animal, and algae kingdoms (Allen et al. 2018; Jha and Kumar 2019; Sharma et al. 2019). Due to huge availability of biomass, chemical compositions are vast and different from one another, depending on source.

In the biosphere, VB is the largest biological biomass; around 450 gigatons of carbon are distributed over the Earth, meaning that 81.82% of the world biomass belongs to dominant kingdom of (mostly terrestrial) plants. Besides this, it is estimated that its renewal rate rises 3% per year. Bacteria biomass shares 12.73%, fungi 2.18%, archaea 1.27%, protists 0.73%, animal (predominantly oceanic) 0.36%, and others 0.90% (Gandini and Belgacem 2008; Bar-On et al. 2018). Thus, biomass from plants has caused great interest for scientific and industrial sectors. All plants have the common characteristic of being constituted by millions of cells, which are considered as the basic functional unit and the fundamental unit of life. These have enormous variety in shape, structure, and functions allowing existence of huge diversity of plant species (Revilla and Zarra 2013). Plants are multicellular organism constituted by eukaryotic cells, which have a nucleus, other organelles, a plasma membrane and a cell wall.

The plant cell wall encloses three main layers; the middle lamella, the primary wall, and the secondary wall (together with plasma membrane), respectively, from external to internal side. The first one is plentiful in pectic compounds and protein. The primary wall is subsequent to middle lamella and comprises cellulose microfibrils inserted in a matrix made of pectic compounds, hemicelluloses, and glucoproteins. The secondary wall is the thickest layer made of cellulose (CL), hemicelluloses (HC), and lignin (LG) (Manavalan et al. 2015). This three-layer structure is responsible for allowing the transit of benefit substances into cell and for protection against different kinds of chemical, thermal, physical, or biological attacks that can negatively affect the cell functions (Alberts et al. 2014). As the thickest layer makes up most of cell wall, then, the CL, HC, and LG contents are a determinant factor for using it as raw material in a BRF. Considering the latest, Table 9.1. shows different mass compositions (dry basis) of CL, HC, and LG of several VB.

The three-layer structure is rigid in order to act as protection system, which is very important when it comes to cell preserving; however, this cell wall characteristic is not useful when it is intended to use VB waste as raw material in a BRF (Bichot et al. 2018). This drawback is named recalcitrance, which is generated by complex interactions among LC fibers made of CL, HC, and LG. Besides, it is well-known that recalcitrance is a crucial issue when using plant biomass (preferably waste) in a BRF system (Holwerda et al. 2019). With the aim of overcoming the described impedement caused by reclacitrance, BRF has the pretreatment stage, which allows to disrupt rigid LC fibers into a looser material and to improve component (CL, HC, and LG) accessibility for its utilization in subsequent steps (Melati et al. 2019; Antunes et al. 2019).

Table 9.1 Cellulose (CL), hemicelluloses (HC), and lignin (LG) composition in some vegetal biomass (VB)

Biomass	CL (%)	HC (%)	LG (%)	Reference
Tropical corn bagasse	25.6	34.6	20.5	Chen et al. (2020)
Sweet sorghum bagasse	30.0	17.3	21.4	Chen et al. (2020)
Sugarcane bagasse	14.8–42.8	15.5–27.7	16.3–24.28	Silva et al. (2018), Hirunsupachote and Chavalparit (2019), Chen et al. (2020)
Wheat bran	32	26	20	Annamalai and Sivakumar (2016)
Sunflower stalk	38.5	33.5	21.9	Kim et al. (2016)
Waste triticale	31.9	25.4	18.4	Kucharska et al. (2020)
Meadow grass	27.1	22.6	16.8	Kucharska et al. (2020)
Beech	38.6	19.9	28.3	Kucharska et al. (2020)
Mixed municipal trimming	49.5	23.0	18.4	Rosen et al. (2019)
Corn cobs	38.8	44.4	11.9	Pocan et al. (2018)
Pomegranate peels	26.2	10.8	5.7	Pocan et al. (2018)
Walnut shell	32.2	26.2	36.9	Özsin et al. (2019)
Orange bagasse	19.8	6.4	4.2	Cypriano et al. (2017)
Napier grass	26.7	37.9	3.8	Hirunsupachote and Chavalparit (2019)
Acacia leaves	18.8	14.3	17.4	Hirunsupachote and Chavalparit (2019)
Empty fruit bunches	26.9–28	22.7–24.1	14.5–20	Medina et al. (2016), Hirunsupachote and Chavalparit (2019)

9.3 Biomass Pretreatment to Improve Cellulose Accessibility

Many kinds of processes can be employed before pretreatments, such as milling, extraction, and drying. Material can be milled in order to reduce particle size and increase the exposed surface area, and thus raising effectiveness of pretreatment and subsequent enzymatic hydrolysis (Da Silva et al. 2013). The most commonly employed mills are ball mill (crushes material using spheres) (Lin et al. 2010) and discs mill (employs serrated plates to pulverize biomass) (Hideno et al. 2009). However, this milling step is expensive and hard to achieve in large scale. Some factors such as dry material and milling coupled with pretreatments affect hydrolysis performance. Milling process is pivotal to enable efficient pretreatments.

Pretreatments employing acid are known for solubilizing HC. These processes commonly use sulfuric acid at high temperature and pressure conditions (Brienzo et al. 2017). This pretreatment is sectioned roughly in these events (Herrera et al. 2003):

Table 9.2 Acid, alkaline, and oxidative pretreatment effect on chemical composition after enzymatic hydrolysis of sugarcane biomass (node)

Pretreatment	Glucan (%)		Hemicelluloses (%)		Lignin (%)	
Untreated	38.61	(± 0.91)	29.47	(± 1.53)	30.46	(± 2.61)
Acid 5%	38.47	(± 2.82)	9.65	(± 0.87)	33.59	(± 2.14)
Acid 10%	42.20	(± 2.69)	7.56	(± 0.32)	41.96	(± 13.39)
Acid 20%	41.96	(± 3.67)	5.20	(± 0.17)	42.67	(± 0.72)
Alkaline 5%	39.61	(± 5.29)	13.72	(± 1.50)	27.28	(± 1.53)
Alkaline 10%	40.75	(± 2.00)	11.18	(± 0.93)	17.33	(± 0.14)
Alkaline 20%	53.59	(± 6.65)	14.08	(± 0.73)	12.59	(± 0.21)
Alkaline 30%	42.55	(± 10.55)	13.11	(± 1.70)	7.63	(± 0.04)
Oxidative 0.5 h	38.20	(± 1.95)	29.02	(± 1.95)	34.21	(± 4.26)
Oxidative 1 h	38.50	(± 0.03)	28.47	(± 1.56)	28.90	(± 5.70
Oxidative 2 h	39.38	(± 0.26)	28.49	(± 2.80)	23.40	(± 1.82)
Oxidative 3 h	41.08	(± 3.55)	28.45	(± 1.64)	11.65	(± 5.21)

Lignin = soluble + insoluble lignin; Oxidative pretreatment was executed by adding new charges of acetic acid and sodium chlorite at longer reaction times

- Proton scattering through damp LC material;
- Protonation of ether-oxygen binds chaining monomeric sugars;
- Rupture of glycosidic bond;
- Generation of intermediate carbocation;
- Solvation of carbocation in liquid;
- Reconstruction of protons and sugars;
- Distribution of products in liquid phase.

Looking at CL, this pretreatment can remove up to 20% of its content in biomass (Trajano and Wyman 2013). Acid process enhances acid hydrolysis accomplishment, but generates degradation products such as furfural and hydroxymethylfurfural (Alonso et al. 2013). Enzymatic hydrolysis is considerably influenced by HC and LG contents (Meng et al. 2017); lowering these contents is an essential step of biomass transformation. HC does not possess a linear structure, making it more exposed to acid pretreatments than CL. Therefore, HC content can be totally removed with mild pretreatments that will not degrade CL within biomass (Aguilar et al. 2002). Steam explosion pretreatment uses SO_2 at high temperatures to reduce HC content in corn straw up to 65%, being an alternative to acid pretreatments (Öhgren et al. 2005). The kind of acid employed can also enhance HC removal. LC materials pretreated with sulfuric acid showed complete HC removal while steam explosion using SO_2 showed minor promising results. Even though it removed most of HC, acid pretreatment produces more degradation products due to its severity, damaging obtained sugars and producing furfural (Martín et al. 2002). Sugars present in HC are solubilized into oligomers or monomers (influenced by reagent concentration, temperatures, and reaction times) (Trajano and Wyman 2013). Higher temperatures (Excoffier et al. 1991) and acid concentrations improve removal of HC content (Table 9.2) (Kumar et al. 2009).

Acid pretreatments are much less effective concerning LG removal regardless of acid employed (Kumar et al. 2009). Normally, severe conditions cause lower LG solubilization, resulting in condensation in fiber surface (Schmatz et al. 2020). During the process of LG removing (via acid route), usually occurs generation of aromatic molecules and different types of phenolic compounds (Du et al. 2010). Solid fraction microscopy shows considerable change in LG structure and content, presenting LG droplets within plant cells (Schmatz et al. 2020). These characteristics suggest a trend of rupture and aggregation that could cause less efficiency in LG content removal (Selig et al. 2007).

Many substances can be employed during alkaline pretreatments (NaOH, $Ca(OH)_2$, KOH, etc.). During these pretreatments, solvation and saponification processes happen first, resulting in swelling and higher accessibility for enzymes to act within LC material. Aggressive alkaline pretreatments can cause dissolution, peeling, decomposition, and degradation of polysaccharides (Hendriks and Zeeman 2009). The focus of alkaline pretreatments is LG content reduction, clearing a path for enzymatic hydrolysis and enhancing polysaccharide reactivity (Table 9.2). Studies show that this trend is related to intermolecular saponification of ester links among HC and LG, increasing pore density (Da Silva et al. 2013). Peroxide pretreatments are also quite capable of HC and LG removal, generating a pretreated biomass with improved enzymatic digestibility (Monte et al. 2011). This pretreatment can successfully solubilize HC and leaves only residual LG (Brienzo et al. 2009).

9.4 Lignocellulosic Biomass Hydrolysis

Many variables are involved in LC biomass conversion into sugars through enzymatic hydrolysis. For example, accessibility issues and costs of employed technologies are obstacles often encountered. CL accessibility is crucial since it is closely tied to enzymatic hydrolysis (Crowe et al. 2017). Shortly, accessibility is a way to measure how much CL can be reached by enzymes during hydrolysis. Biomass on its own has an impact on accessibility since it is a heterogenic material, has variable crystallinity, polymerization degree, and HC and LG contents creating a physicochemical barrier. Actually, one of the hardest obstacles to jump is the difficulty to reach a large portion of CL covered by robust and organized microfibrils.

It is safe to say that there is a close connection between internal surface area and enzymatic hydrolysis (Cosgrove 2005), being one of the obstacles in the way of biomass hydrolysis (Huang et al. 2010). Accessibility is also heavily tied to pore size distribution, significantly more than what is observed with external surface area (Wang et al. 2012). With that in mind, enzymes are able to reach internal surface through big enough pores (Harmoko et al. 2016). Pores smaller than 5.1 nm are not wide enough for enzymes to hydrolyze LC material. It is important to note that most studies use dried biomass, which shows lower digestibility due to shrinkage of pores (Luo and Zhu 2011).

LG and HC contents have a close relation to accessibility, influencing pore distribution, pretreatment efficiency, and enzymatic hydrolysis (Zhao et al. 2017). LC biomasses are heterogeneous, possessing its area sectioned into external and internal areas, presenting different characteristics. Regarding internal surface, accessibility can be inferred by looking at its rifts, voids, and openings generated by HC and LG removal by employing many kinds of pretreatments (steam explosion, peroxide, diluted acid, alkaline, and others) (Karimi and Taherzadeh 2016). It has been reported that HC removal could be a more important asset to accessibility than delignification (Leu and Zhu 2013). HC present barriers to enzymatic hydrolysis as it is placed between and around fibers within cell walls (Zhu et al. 2010).

LG inherently hinders enzymes by forming a physicochemical wall that adsorbs enzymes (Ximenes et al. 2011). By employing correct pretreatments, lower contents of LG and HC may diminish or stop enzymatic hydrolysis hindrance (Chen et al. 2015). While a complete removal of LG would be too expensive in large scale (Yang et al. 2015), mild delignification increases accessibility and can increase enzymatic hydrolysis yield dramatically (Shimizu et al. 2020).

Digestibility can also be influenced by particle size. Quantifying particle size is a problem since they have variable shapes and commonly agglomerate (Ek et al. 1994). Particle size can be visualized by employing microscopy, image analysis, or mechanized particle analyzers. On the down side, these techniques do not discriminate between material's topology and ruptures that can increase exposed surface areas. However, it is expected that small particles provide higher digestibility by presenting a wider exposed surface area for enzyme action. In sum, HC and LG removal is related to accessibility just as much as physicochemical properties of biomass (Shimizu et al. 2020).

Lower degrees of polymerization present an increase in the number of linkage sites for enzymes, enhancing biomass hydrolysis. Regardless, CL needs to be exposed to be reached by enzymes. Altering of polymerization degree usually involves meddling with porosity and crystallinity, as the milling process rips fibers and increases pore size distribution (Zhao et al. 2012). Depolymerization happens when polysaccharides are broken into monomers (Goufo and Mugisha 2018). Depolymerization of CL chains within LC biomasses is a step of enzymatic hydrolysis process. For example, cellulases are capable of depolymerizing CL in order to obtain glucose (a specific monomer) (Yücel and Göycıncık 2015). Enzymes used and substrate employed are also important. The spread of enzymes through pores, the binding sites occupied during hydrolysis, inhibition products and other enzymes used to attack cell wall are properties related to accessibility (Zhao et al. 2017).

9.5 Enzymatic Hydrolysis

Cellulases are an enzymatic cocktail of various enzymes that depolymerize CL into sugars by breaking its glycosidic links. Cellulases are actually three enzymes: exo-β-1,4-glucanases, endo-β-1,4-glucanases, and β-glucosidases. These enzymes

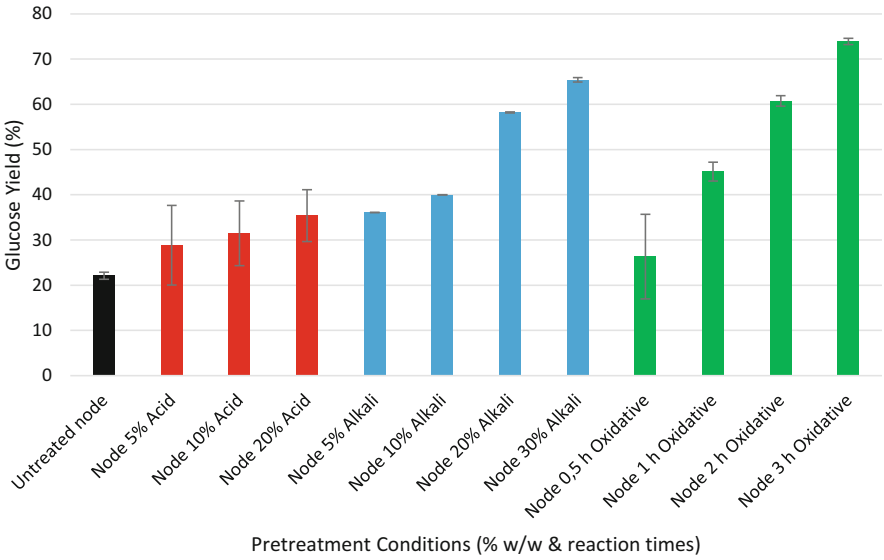

Fig. 9.2 Increased cellulose accessibility by pretreatment allows an efficient enzymatic hydrolysis (sugarcane bagasse node)

work in synergic manner, where their efficiency combined is higher than if they were used separately (Yücel and Göycıncık 2015). Among these enzymes, endoglucanases are capable of breaking chains through interchain, exo-β-1,4-glucanases attack free ends of chains created by endo-β-1,4-glucanases deploying water-soluble cellobiose, and β-glucosidases finish the process by hydrolyzing cellobiose into monomers. Difficulty is faced if LC materials possess HC and LG, which makes enzymes unable to reach and hydrolyze CL. Endo-β-1,4-glucanases role is most likely affected during hydrolysis since it starts the action on CL. The exposed surface area by pretreatments accessible to enzymes is closely related to glucose yield (Fig. 9.2) (Brienzo et al. 2017). Enzymatic hydrolysis of CL chain can be sectioned in these parts (Walker and Wilson 1991): Moving enzymes from liquid medium to LC material; Adsorption and generation of enzyme–substrate complex; Hydrolysis of CL chain; Moving of sugars obtained from biomass surface to the liquid medium; Hydrolysis of cellobiose and oligomers into monomers in liquid medium.

Many factors influence the enzymatic hydrolysis process, such as HC and LG contents present in LC material, generating a physicochemical barrier against enzymes (Fig. 9.3). Furthermore, during hydrolysis LG content increases, slowing down the process. While enzymatic hydrolysis process occurs, the material loses CL content, increasing LG/CL ratio and hindering hydrolysis (Wallace et al. 2016).

Mild delignification of VB enhances glucose yield, more so when LG content reaches 10% (Mooney et al. 1989). Other than that, it increases hydrolysis efficiency due to larger quantity of enzymes retrieved, caused by less unproductive binding to

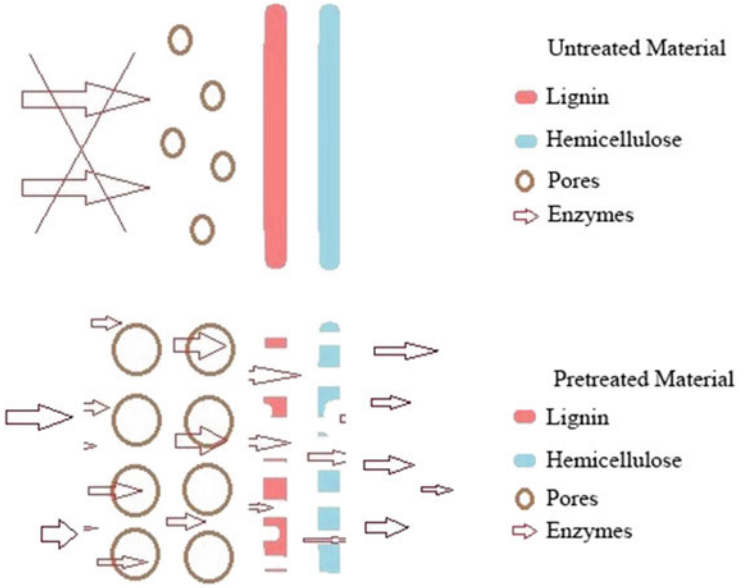

Fig. 9.3 Representation of hemicelluloses–lignin barrier before and after of pretreatment

LG. High LG contents can cause redepositing, occupying pores that could be penetrated by enzymes. Enzymes are unproductively adsorbed by LG within biomass, severely hindering enzymatic hydrolysis (Schmatz et al. 2020). In addition, LG amounts in LC material can be used as a discrimination parameter for enzymatic hydrolysis (Brienzo et al. 2014).

9.5.1 Cellulases Performance

Enzymes that play a synergistic important role on CL depolymerization into cellobiose and glucose molecules at saccharification step are denominated cellulases or cellulolytic enzymes, which can be synthesized during growth of some microorganism (fungi or bacteria) in LC substrates through solid-state or submerged fermentations (Aruwajoye et al. 2020; Barbosa et al. 2020). CL hydrolysis requires participation of three types of cellulolytic enzymes, all of them characterized by cleaving glycosidic linkages β-(1→4).

Cellulases performance is synergistic, which means that if one of them does not act, then hydrolysis stage will have low yield and therefore BRF process will not be technically feasible. These drawbacks can occur by different factors such as temperature, pH, chemicals (that can come from pretreatment), or products generated by own cellulases action (Hsieh et al. 2014; Teixeira da Silva et al. 2016). Recent studies have confirmed inhibitory effects on cellulases by cellobiose and some

monosaccharides like glucose, mannose, and galactose (Hsieh et al. 2014). Although inhibitory compounds concentration can vary, there is evidence of a generality; CMCases activity can be inhibited by cellobiose, CBHs can be deactivated by glucose, and β-glucosidases sensibility can be greater in glucose presence than mannose and galactose (Murphy et al. 2013; Hsieh et al. 2014; Vianna Bernardi et al. 2019). Nevertheless, one of the principal challenges is focus on researching minimum level of inhibitory concentration that can affect negatively the cellulases activities and that inhibitory effects can be reversible or irreversible.

In addition to mentioned inhibitors, others chemical compounds can affect cellulohydrolytic system performance, among them are ionic liquids, some salt and metal ions, phenolics and furans (Teixeira da Silva et al. 2016; Zhai et al. 2016; Summers et al. 2017).

9.5.2 Xylanases Performance

Because HC is a branched heteropolysaccharide constituted by several kinds of molecules, a multienzyme system that works synergistically is required for its depolymerization. These enzymes are denominated xylanases or xylanolytic enzymes, which can be synthesized by some microorganism (fungi, bacteria, yeast, or algae) using xylan-containing substrates through solid or submersed fermentations (Bhardwaj et al. 2019). Xylan hydrolysis needs contribution of various types of xylanolytic enzymes, which acted both on xylan main chain cleaving chemical bonds β-(1→4) and on main chain sides cleaving linkages α-(1→2) and α-(1→3) principally (Chakdar et al. 2016). In main chain, endoxylanases (EC 3.2.1.8) are responsible for depolymerizing the xylan backbone (β-(1→4) bonds) to obtain xylobiose and shorter xylooligomers. β-xylosidases (EC 3.2.1.37) breakdown xylobiose and other soluble xylooligomers into xylose molecules (Bosetto et al. 2016; Puchart et al. 2018). Accessory enzymes act on xylan side chains, among them are α-arabinofuranosidases (EC 3.2.1.55), α-glucuronidases (EC 3.2.1.131), β-galactosidases (EC 3.2.1.23), acetyl-xylan esterases (EC 3.1.1.6), ferulic acid esterases (EC 3.1.1.73), and p-coumaric acid esterases (EC 3.1.1.73) (Malgas et al. 2019).

The first one, also called α-arabinosidases, break α-(1→2), α-(1→3), and α-(1→5) bonds, and release arabinose from xylose–arabinose linkage (Terrone et al. 2020; Yeoman et al. 2010). α-glucuronidases disrupt α-(1→2) bonds and split off methyl glucuronic acid from acid-xylose linkages (Dashnyam et al. 2018). β-galactosidases are responsible to cleave β-(1→4) linkages, which allows galactose to unlink from galactose–xylose bonds (Khosravi et al. 2015). Acetyl-xylan esterases attack acetyl groups located at xylan branches, these type of enzymes release acetyl groups allowing endoxylanases to work on the main chain (Pawar et al. 2016; Razeq et al. 2018). Feruloyl and p-coumaroyl esterases are capable to cleave ester bonds in arabinose–ferulic acid linkages and in arabinose-p-coumaric acid linkages, respectively (Lopes et al. 2018).

The complex system of xylanolytic enzymes act to breakdown xylan (as is mentioned above), the process can develop some difficulties related to inhibitory effects in xylanases activities. Among factors that can negatively modify their activities are temperature, pH, chemicals (that can come from pretreatment), or products generated by own xylanases action (Bajpai 2014a, b). Although inhibitory substance concentration can differ, it exists an overview; endoxylanases activity can be susceptible to xylobiose and xylotriose presence, and β-xylosidases can be deactivated by xylose (Yeoman et al. 2010; Fu et al. 2019). It is necessary to expand and deepen studies of inhibitory effects on accessory enzymes; these could include analysis of dimmers and monomers produced in pretreatment stage.

Although xylanases and cellulases can be synthesized extracellularly by different fungi or bacteria species, few microorganisms are able to produce the whole set of hydrolytic enzymes. For this reason, it is important to continue with researches involving analysis of microorganisms, methods, and variables for hydrolytic enzymes production.

9.5.3 *Cellulolytic Enzymes Synthesized by Fungi and Bacteria*

CL is the main constituent of plant cell wall, in addition to being the most abundant natural polymer available (George and Sabapathi 2015). CL is chemically composed of linear chains of 7000 to 15,000 glucose residues joined by β-1,4 glucosidic bonds, without branches. β-type bond causes a 180° rotation alternating glucose unit, resulting in a linear chain. CL is hydrolyzed by the action of microbial enzymes, such as endoglucanases, exoglucanases, and β-glucosidases (Pino et al. 2018; Bagewadi et al. 2017; Sorensen et al. 2014).

Cellulases are produced by several species of fungi (Narra et al. 2014). Endoglucanases can be produced by *Myceliophthora sp.* (Zanelato et al. 2012), *Streptomyces sp.* (Chellapandi and Jani 2008), *Trichoderma viride* (Irfan et al. 2012), *Trichoderma harzianum* (Bagewadi et al. 2017), and *Aspergillus oryzae* (Kotaka et al. 2008). Exoglucanases, as well as endoglucanases, are also produced by a wide variety of fungal species, such as *Rhizopus oryzae* (Mukherjee et al. 2011), *Aspergillus niger* (Chandra et al. 2008), *Aspergillus fumigatus* (Mahmood et al. 2013), *Trichoderma reesei* (Elshafei et al. 2014), *Trichoderma viride*, and *Ganoderma lucidum* (Shahzadi et al. 2014).

In addition to endo and exoglucanases, β-glucosidases can be produced by *Trichoderma reesei* (Juhász et al. 2005*), Penicillium funiculosum* (Ramani et al. 2012), *Aspergillus niger* (García-Kirchner et al. 2005), *Debaryomyces pseudopolymorphus* (Barbosa et al. 2010), *Pichia pastoris*, (Batra et al. 2014) and *Aspergillus saccharolyticus* (Sorensen et al. 2014). Besides fungi, bacteria also produce cellulases of industrial importance. Endoglucanases can be produced by *Bacillus agaradhaerens* (Hirasawa et al. 2006), *Clostridium thermocellum*

(Romaniec et al. 1992), *Clostridium thermocellum* (Fauth et al. 1991). Exoglucanases have also been identified in bacteria of species *Clostridium stercorarium* (Creuzet et al. 1983), *Cellulomonas fimi* (Duedu and French 2016), and *Xanthomonas oryzae* pv. *oryzae* (Tayi et al. 2018). Additionally, β-glucosidases can be produced by bacteria of species *Dyella koreensis sp. nov.* (An et al. 2005), *Burkholderia ginsengisoli sp. nov.* (Kim et al. 2006), *Microbacterium ginsengisoli sp. nov.* (Park et al. 2008), *Lactobacillus kimchicus sp. nov.* (Lian et al. 2011), *Brevibacillus panacihumi* (Kim et al. 2009), most of which are species that had never been described before.

Cellulases, in general, are enzymes that are of great use in several industrial sectors. Endoglucanases, exoglucanases, and β-glucosidases are enzymes widely used in bioethanol production (Kotaka et al. 2017; Singhania et al. 2017). Endoglucanases are used in textile and food industries (Narra et al. 2014). Exoglucanases are used mainly in textile and in pulp and paper industries (Castro and Pereira 2010).

Beside from cellulases, other enzymes play fundamental roles in the development of products with high added value, such as hemicellulases, which for industrial sector may be essential in obtaining medicines, packaging and products of relevance in the medical field. Hemicellulases, like cellulases, can also be produced by fungi (Liu et al. 2010) and bacteria (Walia et al. 2014).

9.5.4 Hemicellulolytic Enzymes Synthesized by Fungi and Bacteria

Basically, xylan hydrolysis, the most abundant compound of HC in nature, occurs through endo-enzymes action, which act internally on main chain, and exo-enzymes or auxiliary enzymes that hydrolyze oligosaccharides and produce monosaccharides (Kalogeris et al. 2001). Some enzymes produced by microorganisms hydrolyze xylan in specific regions of its main chain and side chains, leading to monosaccharides production that are used by microorganisms themselves as carbon source for cellular metabolism.

Among microbial enzymes that hydrolyze xylan into monosaccharides, it can be highlighted endo-β-1,4-D-xylanases (EC 3.2.1.8), which acts on xylan main chain and generates low polymerization xylooligosaccharides that are substrates for exo-β1,4-xylanases (EC 3.2.1.37), that act through non-reducing terminals generating D-xylose (Beg et al. 2001; Wong et al. 1988).

In addition to the enzymes that hydrolyze xylan main chain, there are still some enzymes that hydrolyze the pendant-side groups. Among these accessory enzymes, α-arabinofuranosidases (EC 3.2.1.55) stands out, which removes arabinose, α-glucuronidases (EC 3.2.1.131) which removes glucuronic acids, acetyl-xylan esterases (EC 3.1.1.72), which removes acetyl groups (Juhász et al. 2005), and feruloyl esterases which remove ester bonds between hydroxycinnamic acids and

sugars (Mathew and Abraham 2004). The consequent action of accessory enzymes produces debranched xylan, which is of great interest for industrial applications.

α-glucuronidases are classified into two families: GH 67, which cleaves uronic acids linked to non-reducing terminals of xylosyl residues of small xylooligosaccharides, and GH 115, which hydrolyze 4-O-D-methylgluvuronic acids linked to non-reducing terminals of xylopropanosyl residues and also those linked to internal xylosyl residues. They are produced by bacteria (about 70 kDa) and fungi (about 90 kDa) (Yeoman et al. 2010; Dimaragona and Topakas 2016).

Acetyl-xylan esterases are enzymes produced by bacteria and fungi. They are responsible for cleaving acetyl groups from heteroxylans. Microorganisms that produce these enzymes become capable of degrading acetylated xylans. Feruloyl esterases cleave bonds between esterified hydroxycinnamic acids for arabinoxylans. They are also produced by fungi and bacteria, and its production can be induced according to presence of certain substrates in microbial growth (Wong et al. 1988).

Complete hydrolysis of xylan requires joint action of enzymes that hydrolyze its main chain (xylanases and β-xylosidases) and accessory enzymes responsible for hydrolyzing its branches (α-arabinofuranosidases, acetyl-xylan esterases, α-glucuronosidases, and feruloyl esterases) (Yang et al. 2017). Several microorganisms have the capacity to produce xylanolytic enzymes, which in turn have great potential for industrial uses. Below, more details of some xylanase-producing microorganisms are presented.

Xylanolytic enzymes are usually best induced by xylan-containing substrate (Espinar et al. 1994). However, some authors show inducing effect with xylan fragments (Aro et al. 2005) and its repression by readily assimilable sugars such as glucose, lactose, and xylose (Gaspar et al. 1997). LC substrates are used to obtain xylanolytic enzymes on a large scale and at low cost in submerged or solid culture (Mishra et al. 1990).

Biochemical properties of endoxylanases from bacteria and fungi vary in terms of molecule size (8.5–85 KDa) and isoelectric point (4.0–10.3 pI) (Chakdar et al. 2016). Table 9.3 shows some xylanase-producing microorganisms found in specialized literature (Chakdar et al. 2016; Goswami and Pathak 2013; Walia et al. 2017), showing optimal conditions for action of such hemicellulases and then, its respective molecular sizes.

Among the products of high added value obtained by hemicellulases use, it can be highlighted: xylooligosaccharides (XOS), xylitol, hydrogel, dressings and substitutes for human skin, films for biodegradable packaging, medicine encapsulation, substitute for fat in cheeses, additives in paper production, substitute for gelatin and in food gums, additives in textile and cosmetic industries, production of new biomaterials such as glucagel, etc. (Ebringerová 2006).

The study of HC applications is constant, such as XOS with high purity (Reddy and Krishnan 2016), XOS for fermentation (Chen and Liu 2017), mucus adhesive

Table 9.3 Hemicellulases from bacteria and fungi

Microorganism	Optimal conditions		Molecular Weight (kDa)	Reference
	pH	Temperatue (°C)		
Bacteria				
Bacillus pumilus SSP 35	6	50	20	Subramaniyan (2012)
Bacillus halodurans	9	78	40	Kumar and Satyanarayana (2013)
Cellulosimicrobium cellulans CKMX1	8	60	58	Walia et al. (2014)
Paenibacillus macerans IIPSP3	4.5	60		Dheeran et al. (2012)
Paenibacillus sp. NF1	6	60	37	Zheng et al. (2014)
Fungi				
Aspergillus niger ANL-3019	5.5	45	13.5–14	Okafor et al. (2007)
Geotrichum candidum	4	50	60–67	Radionova et al. (2000)
Trichoderma reesei	5–5.4, 4–4.5	45.4	20, 19	Tenkanen et al. (1992)
Penicillium capsulatum	5–5.5, 4–4.5	48	22	Ryan et al. (2003)
Penicillium sp. CGMCC 1669	3.8	40	21	Liu et al. (2010)

film (Hanif and Zaman 2017), xylan-based thermoplastic-lactate copolymer (Zhang et al. 2017) and also desirable for biomedical applications (Jungles et al. 2017).

9.5.5 Enzymes Purification Systems

Over the past five decades, it has been a great development of techniques and methods for separation and purification of biological macromolecules such as enzymes. As a result, advances in biosciences and biotechnology have become increasingly important (Ersson et al. 2011; Yimer and Tilahun 2018).

In order to acquire knowledge about structural and functional properties of enzymes, the purification process is an essential step. Enzyme purification often requires the use of multiple purification steps applying different methods in suitable order to obtain the highest possible yield. However, it should be kept in mind that the more steps of purification, the lower the yield. Therefore, knowledge of enzyme's properties is of upmost importance to reduce purification steps (General Electric Healthcare 2010).

The most common method for enzyme purification is chromatography (General Electric Healthcare 2010). Enzyme characteristics that should be taken into

Table 9.4 Purification methods, fold, and yield of cellulases from different microorganisms

Microorganism	Purification methods	Yield (%)	Purification fold	Reference
Aspergillus oryzae	DEAE-cellulose Sephadex G-75	65.7 27.4	15.0 41.4	Begun and Absar (2009)
Bacillus vallismortis	Ammonium sulfate Q-Sepharose Sephadex G-75	83.8 75.4 28.8	4.7 11.6 39.1	Gaur and Tiwari (2015)
Aspergillus terreus	Ammonium sulfate DEAE-cellulose	96.2 42.6	16.0 135.5	Megha et al. (2015)
Anoxybacillus gonensis	Ammonium sulfate DEAE-sepharose	30.7 20.7	55.1 74.4	Genc et al. (2014)
Paenibacillus sp.	Ammonium sulfate DEAE-cellulose CM-cellulose	91.8 78.9 35.7	1.7 5.9 9.7	Islam and Roy (2018)
Trichoderma longibrachiatum	Ammonium sulfate DEAE-cellulose Sephadex G-200	95.8 78.1 68.8	1.1 7.8 9.7	Hamdan and Jasim (2018)
Pseudomonas sp.	Ammonium sulfate DEAE-cellulose	37.7 33.4	4.1 14.5	Goel et al. (2019)

consideration when choosing a purification method are solubility, size, charge, and specific binding affinity (Berg et al. 2002; Yimer and Tilahun 2018).

Cellulases purification, an enzyme group that plays an important role in hydrolysis of renewable LC materials, can be carried out using different methods. Purification efficiency is generally analyzed in terms of yield and purification fold, as is presented in Table 9.4, along with purification methods used for cellulases reported in specialized literature.

Salting out and dialysis are methods that are usually used as first steps. Protein solubility usually decreases at high salt concentrations, causing its precipitation. Different proteins precipitate in different concentrations of salt; therefore, salting out can be used to fractionate proteins (Wingfield 2016; Duong-Ly and Gabelli 2014). Ammonium sulfate is commonly used as a precipitate agent to separate other proteins from cellulase (Farinas et al. 2011). Dialysis is usually used after salting out to remove salt or other small molecules through a semipermeable membrane, which can come in a variety of pore sizes (Berg et al. 2002; Yimer and Tilahun 2018).

Different types of chromatography can be used for cellulases purification. Ion exchange chromatography (Fig. 9.4) separates proteins based on their charge.

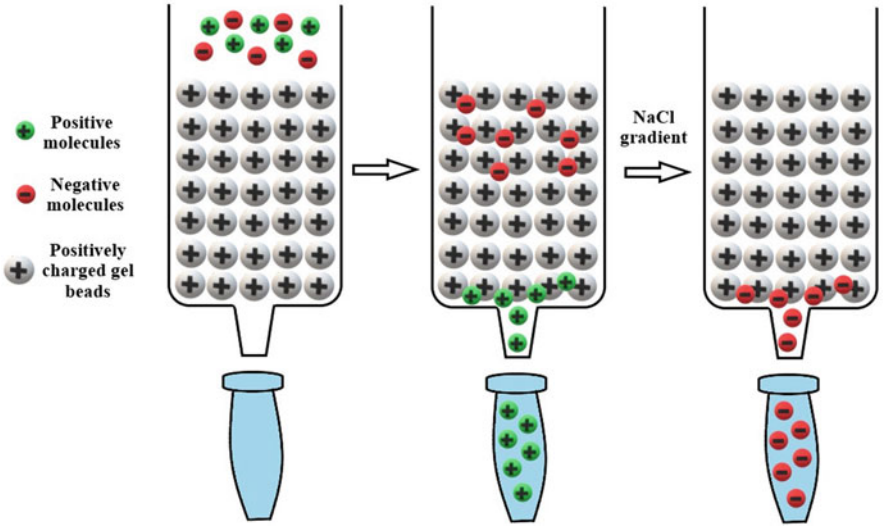

Fig. 9.4 Ion exchange chromatography

Protein with positive charge will usually bind to a column negatively charged with sulfopropyl (SP) or carboxymethyl (CM). Likewise, negatively charged proteins will bind to a positively charged column containing quaternary amines (Q) or diethylaminoethyl (DEAE) (Berg et al. 2002). When above its isoelectric point (pI), an enzyme tends to bind to a positively charged anion exchanger, and when below its pI, a cation exchanger negatively charged matrix is where the enzyme will bind (Bauer and Schnapp 2007; General Electric Healthcare 2010).

Depending on pH, cellulases can be negatively or positively charged; however, several researches show that most of them present better purification when using anion exchange chromatography (Begun and Absar 2009; Genc et al. 2014; Gaur and Tiwari 2015; Megha et al. 2015; Goel et al. 2019). DEAE is a positively charged matrix commonly used as the stationary phase, where cellulases will bind if negatively charged. In order for cellulases to be eluted from the matrix, a linear sodium chloride gradient from zero to 1 mol/L is generally used. Chloride ions will compete with negatively charged groups on cellulases for binding to the column (Bauer and Schnapp 2007).

Size exclusion chromatography (Fig. 9.5) allows separations of substances with different molecular sizes. Large molecules that cannot enter into pores of the chromatography beads will elute first, while smaller molecules will penetrate the pores and therefore will take longer to elute (General Electric Healthcare 2010). Sephadex is the most popular matrix for gel exclusion chromatography. Molecular weight of cellulases produced by different microorganisms can vary from 12 to 126 kDa; therefore, column packing material with different beads sizes can be used for purification (Tao et al. 2010; Chandra and Madakka 2019).

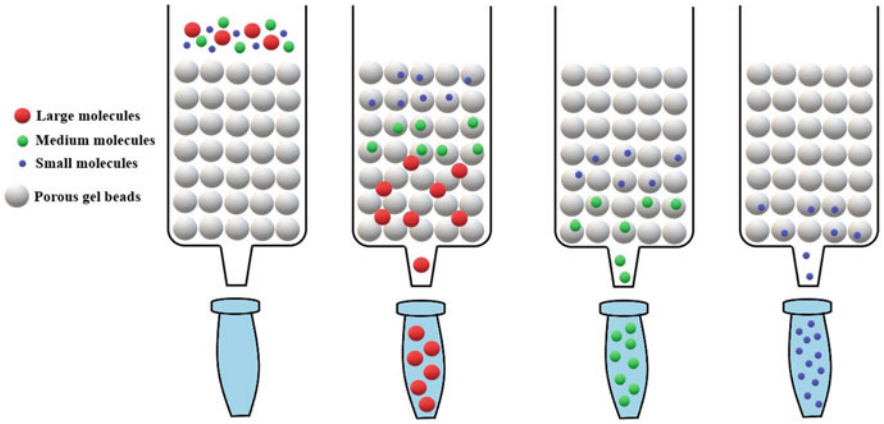

Fig. 9.5 Size exclusion chromatography

Specific activity of cellulases is usually twofold lower than of other hydrolytic enzymes. For industrial purposes, large quantity of cellulases is needed and this production becomes a "metabolic burden" even for native cellulolytic organisms. Therefore, heterologous expression of cellulases in industrial microorganisms, such as *E. coli*, *Pichia pastoris*, and *Saccharomyces cerevisiae*, enables robust and cost-effective production (Vinuselvi and Lee 2012). Several researches with this goal have been carried out in the past years, mainly focusing on heterologous expression of cellulases produced by different microorganisms and termites in *E. coli* or other model microorganisms (Wei et al. 2015; Zeng et al. 2016; Chahed et al. 2018; Trollope et al. 2018; Zeeshan et al. 2018; Zhang et al. 2018; Raza et al. 2020).

Purification of recombinant proteins can be influenced by molecular biology of gene isolation and expression. For an economical production, efficient implementation of purification methods to maintain a high yield of the heterologous protein is of great importance. Affinity chromatography is one of the most efficient methods for heterologous proteins purification due to its high recovery yield and purity achieved (Evangelista and Suttnar 1997; Pina et al. 2014; Mahmoodi et al. 2019).

Affinity chromatography (Fig. 9.6) separates molecules based on a reversible interaction between the protein of interest and a specific ligand attached to a chromatography matrix. In this method, specific tag sequence is attached to the protein of interest at DNA level, generally fused to N- or C-terminal, which leads to expression of a tagged protein. Besides facilitating the purification process, theses tags also enhance protein stability and increase expression levels. Metal ions such as zinc, copper, and nickel have been found to bind favorably with histidine residues in proteins (Evangelista and Suttnar 1997; General Electric Healthcare 2010; Pina et al. 2014; Mahmoodi et al. 2019).

For purification of heterologous cellulases, the use of a nickel–nitrilotriacetic acid matrix, in which metal ions interact with histidine residues in the affinity tag, is commonly found in literature. Several methods can be used for cellulases elution

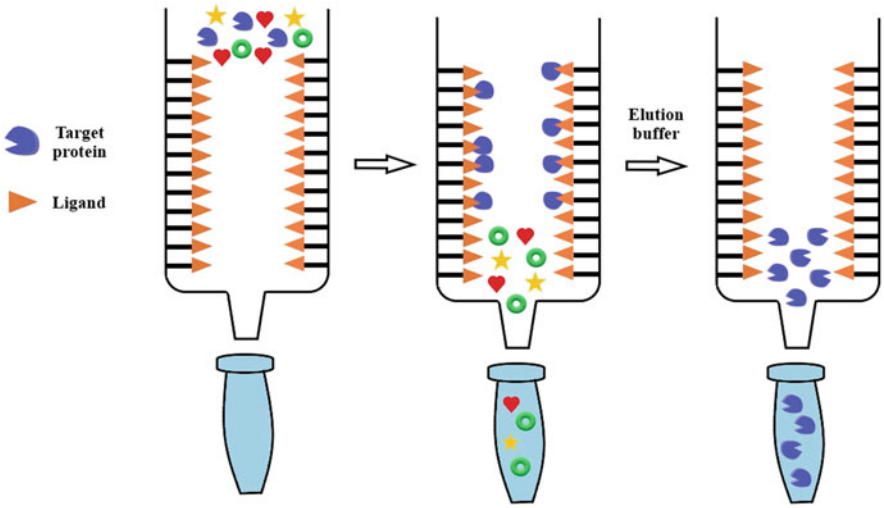

Fig. 9.6 Affinity chromatography

from the matrix. High concentration of imidazole buffer, use of strong chelating agents such as ethylenediamine tetraacetic acid (EDTA) and lowering pH in order to protonate histidines are conditions that decrease cellulase affinity to the nickel resin, thus enabling enzyme recovery (Reverbel-Leroy et al. 1997; Campen et al. 2017; Ni et al. 2005; Shi et al. 2013; Zeng et al. 2016).

There are several strategies for purifying cellulases. It must be taken into consideration the best system to obtain the highest yield with the highest catalytic activity of the enzymes. Purity degree will depend on its end use; however, most of purification systems used in laboratory researches can be scaled up to industrial processes (Bajpai 2014a, b).

9.5.6 Interference Among Enzymes

Conversion process of polysaccharides on LC biomass into simple sugars through enzymatic hydrolysis is a crucial and limiting step for biofuel production (Sheldon 2014). Enzymatic hydrolysis is a heterogeneous process that involves close contact between enzyme and substrate, enzyme activity and reaction conditions such as temperature and pH (Saini et al. 2016).

Enzymatic hydrolysis of lignocellulose can be affected by several factors, mainly substrate-related since the presence of LG and HC in biomass can decrease accessible area to cellulases (Shimizu et al. 2020). Polymerization and crystallinity degrees of CL also play a role on enzymatic hydrolysis yield. Interaction between CL and cellulases can be inhibited by LG in different ways depending on its structural properties (Ying et al. 2018).

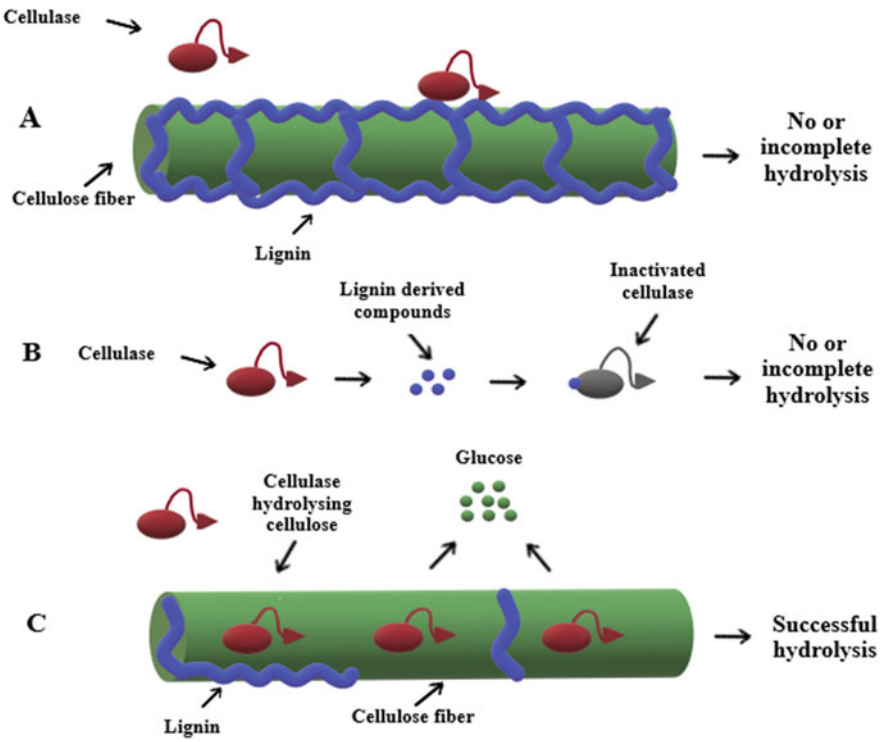

Fig. 9.7 Cellulase inhibition by LG. (**a**) Physical barrier of cellulase progress on CL fibers and non-production binding of cellulase to LG. (**b**) Inhibition due to exposure to LG derived compounds. (**c**) Normal hydrolysis where cellulase binds to CL releasing glucose

Inhibition of cellulases by LG can occur by three major factors: exposure to soluble lignin-derived compounds, non-productive binding of cellulase into LG, and physical barrier of cellulases progress (Fig. 9.7). Enzyme inhibition due to LG is a complex process and will depend on biomass botanical origin and pretreatments applied to it, since both affect localization and chemical properties of LG (Saini et al. 2016).

Phenolic compounds can cause loss of cellulases activity as the length of exposure to them increases. This occurs due to cellulases precipitation and can vary depending on microbial source of enzymes, type of cellulases, and type of phenolic compound (Saini et al. 2016). In addition to act as a shield preventing enzymes to reach CL, LG can also adsorb cellulases irreversibly by hydrophobic, electrostatic, or hydrogen-bonding interactions (Kumar et al. 2012).

All of these factors led to decrease of glucose release in enzymatic hydrolysis process. Therefore, a way to reduce cellulases–lignin interaction is widely studied. Some of the strategies used are chemical modification of LG or its complete removal, cellulases modification, and addition of LG blocking additives. Pretreatment of LC

biomass can remove or modify LG and HC contents, which can raise accessibility for cellulases (Shimizu et al. 2020; Melati et al. 2019; Saini et al. 2016).

The rate of LC biomass enzymatic hydrolysis usually decreases during hydrolysis. Steam pretreated sugarcane bagasse was enzymatic hydrolyzed for 72 h, showing an initial phase with fast conversion of 61.7% glucose and a final conversion of 86%. As hydrolysis progressed, rate of conversion decreases as well as glucan content while LG, phenolic compounds, and ash increased. In addition, enzyme activity was lost during the first 24 h of hydrolysis. Recalcitrance increasing during hydrolysis and deactivation of enzymes due to thermal stability are factors that contribute for slowdown and incomplete hydrolysis (Wallace et al. 2016).

After different pretreatments, sugarcane biomass (external fraction, node, internode, and leaf) presented improve on CL conversion into glucose after enzymatic hydrolysis. A complete conversion of CL into glucose was possible after oxidative pretreatment of the internode fraction. LG removal of 15% and 10%, respectively, resulted in at least 60% of glucose yield (Shimizu et al. 2020). Addition of a lignin-blocking agent to sugarcane bagasse can increase hydrolysis yield by 40% or even 60%, depending on agent concentration. They prevent non-productive adsorption of cellulases by blocking exposed LG surfaces (Àzar et al. 2020).

Steam explosion and alkaline sulfate pretreatments on sugarcane bagasse resulted in more accessible substrate, with approximately 90% of CL hydrolyzed using high enzyme loadings. Besides, after pretreatment with sodium sulfate and sodium hydroxide hydrolysis using low loading of a modern enzyme preparation used in industrial processes, Cellic CTec3, resulted in 70% conversion into glucose (Siqueira et al. 2017). In contrast, the presence of vanillin, a lignin-derived phenolic, can cause cellulases deactivation and precipitation, resulting in a decrease of 20% in CL conversion with a concentration of 5 mg/mL vanillin and 50% with 10 mg/mL. (Qin et al. 2016).

The majority of β-glucosidases from commercial cellulases (Cellic CTec 2), derived from *T. reesei*, adsorb to LG, whereas the same enzyme derived from *A. niger* presents less inhibition. Therefore, engineering robust cellulases that are highly active and stable is a strategy to mitigate non-productive adsorption. Modification of cellulase from *T. reesei* surface charge by succinylation, converting positively charged primary amine groups to negatively charged acid and neutral acetyl groups, resulted in greater than twofold increase in Avicel conversion after 170 h (Nordwald et al. 2014).

Several techniques can be used to improve enzymatic hydrolysis of LC biomass; however, for economic feasible process a multi-disciplinary research involving areas of plant biotechnology, enzyme engineering, lignin-cellulase interactions, and process development is important (Sipponen et al. 2017).

9.5.7 Assistance Elements for Improving Enzyme Activity

For industrial applications, knowledge about enzymes activators and inhibitors is relevant. Many compounds can influence on enzymes activities and may be present in water and/other reagents employed in industrial processes or can be a result of equipment corrosion. Metal ions can activate or inhibit enzyme activity by interacting with amine or carboxylic acid group of amino acids. They can act like electron donors or acceptors by forming complexes with other molecules linked to enzymes, besides help reduce non-productive adsorption of enzymes. Ionic charge and ion radius size are properties that can influence activity and stability of enzymes (Pereira et al. 2017).

Effects of each metal ion can differ from cellulases, depending on its type and source. Addition of Cu^{2+}, Fe^{2+}, Co^{2+}, and Mn^{2+} at 2 mmol/L in the reaction medium increased activity of endoglucanases from *A. niger*, especially manganese, which improved activity by 57%. However, β-glucosidases from the same microorganism showed increase of activity by 33% after Co^{2+} addition. Glucose releasing after enzymatic hydrolysis of acid pretreated bagasse increased 34% after addition of 10 mM Mn^{2+} (Vasconcellos et al. 2012).

Some metal ions can be cellulases inhibitors. Chelating agents such as EDTA (ethylene diamine tetra acetic acid) can be used to enhance cellulases activity since they act by sequestering inhibitors metal ions and as protease inhibitor. EDTA forms a complex with metal ions in the reaction medium, allowing the enzyme site to react with the substrate. After an addition of 2 mmol/L EDTA, endoglucanases from *A. aculeatus* presented increasing of 1.5-fold in its enzymatic activity and enzyme stability also increased (Naika and Tiku 2011).

The high crystallinity of CL structure makes cellulases penetration difficult. Surfactants tend to decrease surface tension of aqueous systems and their addition during enzymatic hydrolysis can modify cellulases surface and improve CL conversion. CL conversion increased 38.4% after addition of 0.5% (v/v) Triton X-100 and 60% after addition of azobenzene-based surfactant (Nurul Adela et al. 2015; Seidel and Lee 2020).

Although there are few studies about the effect of ultrasound on enzymes performance, the use of ultrasonic treatment at appropriate frequencies and intensity levels can increase enzymatic activity and favorably change enzymes without altering its structural integrity. After a 17.33 W/cm2 intensity and time of 30 min, ultrasonic treatment was able to increase cellulases activity by 25% (Subhedar and Gogate 2014).

Some HC, mostly xylan, can still remain in LC biomass after mild pretreatment conditions and this can influence cellulases accessibility. Xylanases supplementation along with cellulases can potentially improve glucose yield. The partial combination of cellulases with xylanases in enzymatic hydrolysis contribute to increase CL conversion to glucose from 70% to 86%. This improvement shows that there is a synergistic interaction between xylanases and cellulases, since xylanases can hydrolyze the remaining xylan improving CL accessibility (Hu et al. 2011).

9.6 Challenges on Industrial Scale of Cellulose Hydrolysis Via Enzymatic

Comparing biological hydrolysis with its chemical homologous process, it can be observed that the former presents more favorable characteristics, among them are: slight temperature conditions (up to 50–55 °C), possibility to raise sugar yields (especially when commercial enzymes are used), low operation costs, low energy supply, no use of corrosive chemicals (like inorganic acids), and no production of inhibitory substances (such as furfural and hydroxymethylfurfural principally) that contribute with conversion, low yields (Amezcua-Allieri et al. 2017; Jahnavi et al. 2017; Steinbach et al. 2017; Chen and Liu 2017). Nevertheless, enzyme hydrolysis has difficulties related to elevated process time, high cost of enzyme (in synthesis and purification, or purchasing), enzymatic loads, pH and temperature sensibility, mixing modes, stirring intensity, low degree of synergy, recovery system of used enzymes and some good experiences obtained in laboratory have not been tested in large scale (Balan 2014; Hu et al. 2016; Kadić 2017; Kumar et al. 2018; Singhvi and Gokhale 2019).

In the particular case of CL hydrolysis by biological route, it can be enlisted several strains found mainly in pilot-scale experiences. Elevated viscosity, unsuitable mass and heat transference, and significant inhibitory concentrations are reasons for decreasing in enzyme hydrolysis yields specially when high solid biomass loads are used (Agrawal et al. 2018). According to this, the research principle challenges are to focus on different solutions to design non-conventional units that let a continuous or fed-batch process; free-fall horizontal devices and helical impellers could be promising (Da Silva et al. 2020). Also regarding reactor design, it could be researched a reaction-separation system that allows a simultaneous extraction of intermediate-products while these are obtained, avoiding inhibitory effects (Kiss 2014). However, if conventional BRF process (hydrolysis and adjustment stages separately) is carried on, a recovery system of enzymes and how many times they could be used again as a recycling strategy into the BRF would be a great approach (Mesa et al. 2016).

On the other hand, physical issues associated with surface tension and viscosity are also treated. Currently, to correct surface tension problems in the system of solubilized enzymes in aqueous medium—pretreated biomass, different surfactant agents that do not destabilize enzymatic activity and reduce superficial tension in the aqueous medium can be added to improve enzymes access into biomass (Wang et al. 2018; Zhou et al. 2019; Yusuf and Kresnowati 2019). Taking viscosity into account, an intermittent or split feed of biomass and aqueous medium enzymes can improve hydrolysis yield since system viscosity decreases and enzyme contact to inhibition chemicals is reduced (Agrawal et al. 2018).

In addition to above, techno-economic studies are necessary to analyze the best configuration of BRF systems; considering variety, availability, and quality of raw material (biomass), desired outflows (biobased products), type and yield of pretreatments, and co-production and adjustment stage category (separation and

purification process). Finally, it should reminded that enzymes are sensible to temperature and pH; therefore, it is suggested to conserve constant thermal and pH ranges in pretreatment and hydrolysis stages or to continue investigating thermal and pH tolerance in both processes working in sequential way.

9.7 Concluding Remarks

In each stage of proposed BRF, there is flexibility to use several types of processes (chemical, physical, biological, or combined). However, biological pretreatment and hydrolysis presents a special interest for new researches due to its mild conditions and lesser energy inputs compared with chemical route. These factors could be taken into account as potential for large-scale production. Different stages of configurations (extraction, pretreatment, hydrolysis, co-production, and adjustment) and types of processes (chemical, physical, biological, and combined) in the proposed BRF approach should be further researched and investigated in order to analyze limitations for BRF efficiency improvement.

Despite its recalcitrance behavior, LC biomass remains as a significant alternative to generate biobased products such as biofuels, biopolymers, and others biochemicals. Breaking CL and HC to obtain smaller molecules (monomers) is not a stress-free activity, the specific chemical bonds in both macromolecules and its curious configuration in the cell wall leads to continuous scientific developments in this area. The LC biomass is the most abundant resource in the world and is found in several categories; agro-industrial and industrial residues are attractive to take advantage and obtain different biobased products.

Cellulases purification can be carried out by different methods; however, all of them should aim for high yield and catalytic activity. Although addition of pure cellulases in LC biomass enzymatic hydrolysis process is an important factor to obtain great conversion to glucose, LG can negatively interfere in this process.

LG creates a barrier that undermines cellulases action by non-productive binding and its derived compounds can inhibit their activity; therefore, its removal increases enzymatic hydrolysis yield. Some other strategies such as cellulases modification and addition of LG blocking additives are also known to minimize this problem. On the other hand, some compounds can enhance cellulases activities such as few metal ions, surfactants, and auxiliary enzymes such as xylanases.

The co-work multisystem of hydrolytic enzymes in pilot-scale units for hydrolysis should be more explored in order to analyze how to increase mass and heat transfer efficiency, how to reduce viscosity effects (rheology), how to decrease surface tension effects and thus improve hydrolysis performance.

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Chapter 10
Sustainability of Biorefineries: Challenges Associated with Hydrolysis Methods for Biomass Valorization

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Abstract Biorefineries are mechanisms for adapting modern technologies toward the goals of recovery and aggregation of biomass value by creating processes that are renewable and environmentally friendly. The transformation of biorefineries to industrial realities is an area of intense study worldwide. Creation of more efficient systems requires hydrolysis processes that maximize the fractionation of biomass structure to produce high yields of several products. Scientific studies search to fill gaps in previously developed hydrolysis methods that may be coupled to biorefinery processes. There is great interest in advances in biomass enzymatic hydrolysis technologies that aim to convert complex compounds efficiently in innovative clean technology. This review will explore current scientific works that search integration of multiple processes through efficient hydrolysis methods for converting biomass, finally realizing the biorefinery concepts.

Keywords Enzymes · Bioprocess · Biofuel · Integrated bioprocess · Enzymatic technologies

Abbreviations

AFEX	Ammonia fiber expansion
HMF	5-hydroxymethyl-2-furaldehyde

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10.1 Biorefinery Backgrounds: Current Scenario of Hydrolysis Methods Aimed at the Valorization of Biomass

Biorefineries are processes based on biomass conversion to generate bio-based products, acting as a promising linkage approach in the sustainable production of biofuels, renewable materials and chemicals, to ensure the use of no-food resources (Aslanzadeh et al. 2014; Mongkhonsiri et al. 2018; Sailwal et al. 2020). Biorefinery allows industry standards with linear economy concept to change to a circular economy concept, which is environmentally and economically sustainable (Mohan et al. 2016). These concepts consider that all waste has inherent positive net energy, that can be recovered and reused for bio-based reactions, allowing the implementation with closed biotechnological routes (Mohan et al. 2016).

Development of biorefinery on a commercial scale is in full expansion worldwide. Most of the industries already implemented have used lignocellulosic matrix compounds as raw material to generate biofuels as the main product, and the conversion strategies are diverse, varying according to the desired products (Table 10.1).

Biorefineries with emphasis on biofuels depend on an efficient biomass fractionation process to make it possible to maximize the recovery and selective conversion of structural components. The process of fractionating biomass is usually carried out employing pre-treatment followed by the conversion of polysaccharides (fibers of cellulose, hemicellulose, and pectin, starch) in to simple sugar, carried out by hydrolysis methods. These processes make available the component fractions of the raw material used and allowing them to be applied in other production routes. However, hydrolysis methods are not trivial due to the complexity of the chains that make up the raw materials used, and each biomass with different compositions must be evaluated especially when applied to different biomasses in the same plant (Binder and Raines 2010; Den et al. 2018; Islam et al. 2020).

Two routes frequently used in hydrolysis methods are the application of acids or the use of enzymes (Fig. 10.1), with enzymatic hydrolysis being the most used in commercial scales of the biochemical route. Both processes vary in the mode of action and in the parameters depending on the composition of the biomass and mainly on the result of the pre-treatment that precedes the hydrolysis (dos Santos et al. 2019). The application of enzymes in biorefineries is an essential step to produce fermentable sugars, with structural and chemical polysaccharides modification for the biofuel and chemical production. Different strategies for the effective use of enzymes in these processes are of great biotechnological interest (Lara et al. 2020).

In the scenario of biorefineries, there is a great interest in enzymatic hydrolysis methods, and the determination of the appropriate biocatalysts for biomass and the process used are the main bottlenecks of these methods. The most relevant enzymes for biofuel biorefineries are amylases, cellulases, and xylanases, in addition to pectinases and ligninases, which act with high specificity on the substrate and

Table 10.1 Some biorefinery that operate on a commercial scale (own elaboration with information collected on the companies' official website)

Biorefinery	Location	Biomass	Technological Routes	Products
GranBio	Alagoas, Brazil Atlanta, USA	Agricultural residue (corn straw, empty fruit bunch, sugarcane—straw and bagasse, tobacco stalks) Wood	Biochemical	Ethanol Nanocellulose Biochemicals
Enerkem	Edmonton, Canada Westbury, Canada	Biomass residues Solid waste Mixed plastic waste	Thermochemical	Syngas Ethanol Methanol
COFCO Biochemical	China	Corn stover (main)	Biochemical	Ethanol Starch Fructose, etc.
Zeachen	Boardman, USA	Corn stover Poplar chips	Hybrid process: Biochemical and thermochemical	Ethanol Chemical products (lignin, acids, acetates)
Fulcrum BioEnergy	Story City, USA	Municipal solid waste	Combination of gasification and Fischer–Tropsch process	Jet fuel Diesel
Raizen	São Paulo, Brazil	Sugarcane Sugarcane bagasse Vinasse Wastes from purification of sugarcane juice	Biochemical	Sugar Ethanol Biogas Electricity

must be chosen based on biomass used and the applied process (Ríos-Fránquez et al. 2019).

In comparison with chemical hydrolysis, it is believed that biological processes that apply enzymes or enzyme excretory microorganisms are more advantageous because they present: high specificity in different substrates, are environmentally safer, produce less microbial inhibitors, operate in lower temperature conditions, require installation and operation of simpler reactors with lower energy consumption (Zabed et al. 2019). However, when applied on a commercial scale, enzymatic methods face technical obstacles, such as loss of enzyme activity, reduced efficiency, longer operating time, and cost associated with obtaining commercial enzymes (Ma et al. 2020).

Considering that biorefinery processes depend on large, efficient, simple, and direct systems, operating with different biomasses, hydrolysis methods still need to be extensively studied. In this scenario, achieving the technical and economic feasibility of large-scale enzymatic hydrolysis processes that are inexpensive,

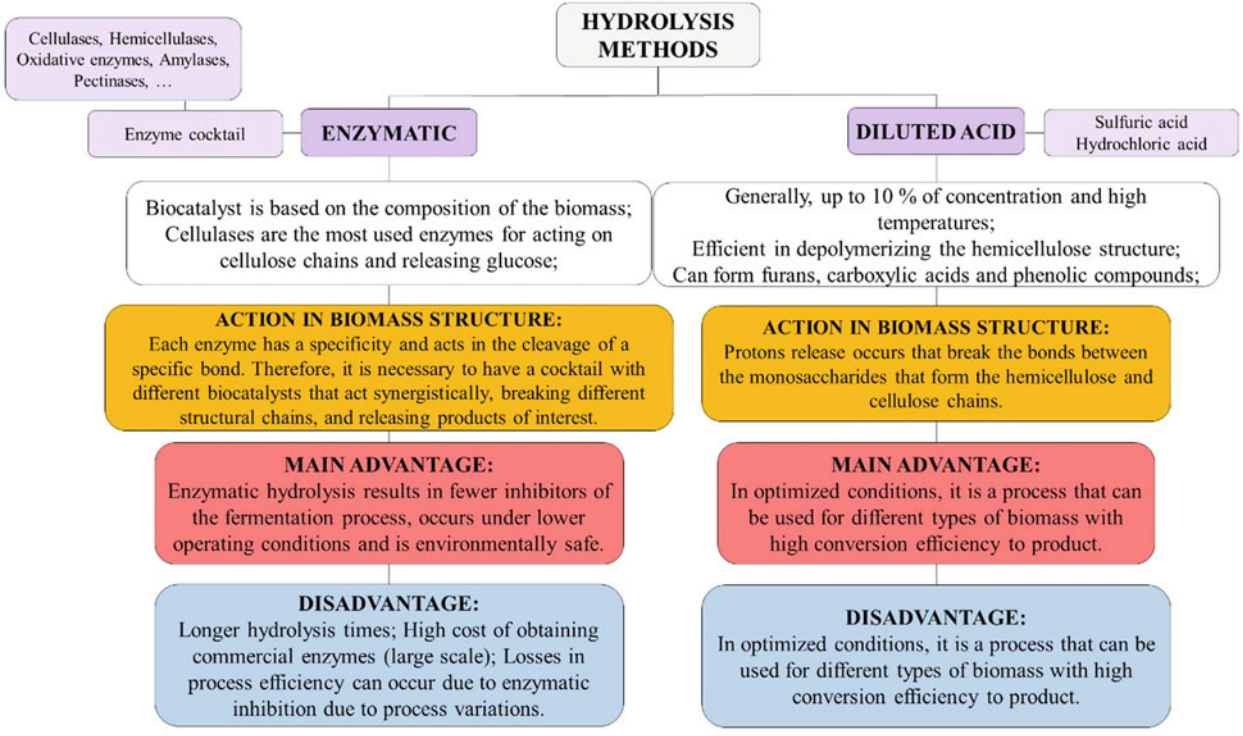

Fig. 10.1 Hydrolysis processes applied on a commercial scale

efficient, and do not depend on commercial enzymes is in the process of exploration, and substantial research efforts are still needed.

10.2 Hydrolysis Methods in the Biorefinery Concept

Biomass hydrolysis in biorefineries is a challenging scenario considering the recalcitrance of the raw materials used, which make necessary unitary operations that directly influence the efficiency of the system to obtain the products of interest. Current research and biorefineries on a commercial scale focus on the use of lignocellulosic matrices. This factor is linked to the abundant availability of these raw materials, and because are a promising alternative for the biofuel and chemicals production based on the use of non-food biomass (Dutta and Pal 2014). Other systems still on a laboratory scale are evaluated using algae and urban waste using biochemical routes (Ansari et al. 2017; Wu et al. 2019; Qarri and Israel 2020).

Hydrolysis processes applied on a commercial scale follow two routes (Fig. 10.1): chemical, with the application of diluted acids; or enzymatic, being carried out by means of enzymatic cocktails or by direct use of the microorganism that produces the enzymes of interest (Nguyen et al. 2014; Jiang et al. 2015).

Chemical hydrolysis using diluted acid is a consolidated technique on commercial scale biorefineries because it is the most favorable method to be applied in various types of lignocellulosic biomass because promoting the hydrolysis of hemicellulose in pentoses and facilitates the decomposition of cellulose into hexoses (Alvira et al. 2010; de Medeiros et al. 2018). Acid hydrolysis process generally uses sulfuric acid (H_2SO_4) or hydrochloric acid (HCl) in concentrations of 1–10% and elevated temperatures and pressure as catalysts (Wingren et al. 2003; Lenihan et al. 2010; Treichel et al. 2020). The hydrolysis reaction using acids is efficient in depolymerizing the hemicellulose structure because they release protons that break the bonds between the monosaccharides that form the hemicellulose and cellulose chains, resulting in the release of several compounds, main sugars such as glucose, xylose, and arabinose (Aguilar et al. 2002).

The use of high temperatures in the diluted acid hydrolysis process can hydrolyze monosaccharides into other compounds, such as furfural, 5-hydroxymethyl-2-furaldehyde (HMF), and formic acid (Jin et al. 2011; Nguyen et al. 2014; de Medeiros et al. 2018). The generation of these products may or may not be desired in the context of biorefineries, depending on the used routes and the products of interest to be generated. In the production of biofuels, such as ethanol, the formation of these compounds is not desired due to the potential for microbial inhibition in the fermentation process. The generation of furans, carboxylic acids, and phenolic, if not desired in the process, can be minimized by reducing the hydrolysis temperature, and using higher concentrations of catalyst (Nguyen et al. 1999). However, by increasing acid concentrations, a unitary chemical recovery operation is necessary, which increases the economic cost of the process (Wingren et al. 2003).

Enzymatic hydrolysis is considered an effective and economic methodology with the potential to return results close to the theoretical in sugar conversion (Lee et al. 2009; Liu et al. 2018). With the enzymes application as catalysts for the biomass hydrolysis process, the decomposition of structures occurs efficiently. There is less formation of inhibitors of fermentation processes, as evidenced in acid-catalyzed hydrolysis, the reaction conditions are lower, and with the advances in enzymatic biotechnology, the economic costs of the process are being reduced. In addition, enzymes are natural, biodegradable, and environmentally safe compounds (Wyman 1994).

In the current scenario, where a large part of the biomasses used in biorefineries are agricultural residues of lignocellulosic matrix and with homogeneous characteristics, standardized commercial enzymes are used, mainly by American and European companies, which develop enzymatic cocktails such as Spirizyme® and Cellic®Ctec3 (Dragone et al. 2020). Although substantial efforts and advances in technology are made to reduce costs, it is still one of the largest economic investments required in processes, which may vary according to the production scale, fermentation yield, and enzymatic cocktail complexity (Pellis et al. 2018).

In biorefineries, the enzymatic hydrolysis is dependent on an enzymatic cocktail that acts synergistically with cellulases, hemicellulases, and other auxiliary proteins, such as amylases, pectinases, and oxidative enzymes (Pellis et al. 2018).

The use of combined hydrolysis methods, such as diluted acid hydrolysis associated with enzymatic hydrolysis, is a promising technology already developed in technological routes of biorefineries on commercial scale (Hasan Ba Hamid and Ku Ismail 2020). The result of the combined hydrolysis reaction may be favorable to increase the hydrolysis yield of biomasses with a high degree of polymerization, such as wood chips. The sequential reaction with acid followed by enzymes can result in the reduction of the lignin structure, which can act as an inhibitor of enzymatic hydrolysis. In addition, chemical hydrolysis increases the pores of the structure, facilitating the access of enzymes increasing the overall process yield (Jiang et al. 2015; Liu et al. 2018).

The challenge of operating biorefineries with a wide diversity of biomass in the same plant, it is important to develop efficient technologies, environmentally safe and with low cost, making these systems competitive compared to conventional refineries. In this scenario, advances in enzymatic biotechnology are relevant, aiming to develop sustainable and economically viable systems, to positively impact the circular economy for the recovery of various products.

10.3 Advances in Enzymatic Hydrolysis Technologies

Enzymatic hydrolysis is responsible for breaking long carbohydrate chains, in the presence of water and biocatalysts (enzymes). Generally, this process is performed by numerous enzymes with different specificities, denominated an enzymatic cocktail, that acts synergistically in the biomass structure, and allows the sugars

liberation. The enzymatic cocktail is composed mainly by cellulases that are considered the basis of hydrolysis, acting on the cellulose chains, resulting in glucose; and xylanases that convert hemicellulose, which is an amorphous structure and composed of xylan, into xylose (Aditiya et al. 2016).

Synergistically, the ligninolytic enzymes will influence the lignin–carbohydrate complex by removing it. The main enzymes acting on this route are laccases, ligninases, and manganese peroxidase, acting by the cleavage of bonds between carbons (C-C) due to the generation of reactive free radicals. This synergic action is essential because the biomasses used are usually formed by heterogeneous fiber networks, an example is the pectinase enzyme, which is necessary for the removal of pectin present in some biomasses, like in fruit waste (Champreda et al. 2019).

The enzymes involved in the biomass hydrolysis are in great demand and have a significant cost linked to the process. Based on ethanol, about 18–43% of the sale price of this biofuel refer to the commercial enzymes used in the production (values may vary according to the biofuel analyzed). In view of this, enzymes produced *on site* have a relevant logistics to process operation with a more cost-effective approach. Enzyme-producing microorganisms found in the environment can serve as an enzymatic source for the hydrolysis process, making the process more economic (Chandel et al. 2018; Singh et al. 2019).

In biorefineries, the sugar production rate, concentration, and conversion yield are critical factors that, if improved in the enzymatic hydrolysis, can favor the technical-economic viability of operations (Wingren et al. 2003; Geng et al. 2015). According to the operational mode, the enzymatic hydrolysis can be applied in stages of (1) separate hydrolysis and fermentation (SHF); (2) simultaneous (SSF); (3) semi-simultaneous (SSSF); (4) consolidated bioprocessing (CBP); and (5) simultaneous saccharification and co-fermentation (SSCF) (Aguilar et al. 2018).

CBP is potentially considered the most economical because it uses a single microorganism or microbial consortium to produce enzymes and perform hydrolysis and fermentation (Xu et al. 2009) without requiring a separate process dedicated to enzyme production (Lynd et al. 2002). In SHF process, higher temperatures (50 °C) can be used for hydrolysis. In SSF process the temperature of hydrolysis is lower (30–32 °C) to satisfy the ideal temperature of the microorganism in fermentation (Andrić et al. 2010; Van Dyk and Pletschke 2012). SSF process is interesting in biorefinery systems and is a promising path in the development of bioprocesses with microorganisms with high enzymatic production. The application in synergistic systems of enzymatic production or enzymatic cocktail for hydrolysis of biomass structure and fermentation for the biofuels production can present a promising and efficient alternative. Considering that the enzymes produced by these microorganisms can perform well at temperature of process, and would make systems more flexible, allowing the hydrolysis of different biomasses in the same reactor.

The improvement of enzymatic hydrolysis through pre-treatments was recently reported that for sugar cane bagasse, pre-treatments based on diluted acid subjected to delignification (de Godoy et al. 2019), hydrogen peroxide (de Guilherme et al. 2017), microwave irradiation/acid glycerol solutions (de Moretti et al. 2016), and more recently, gradual alkaline pre-treatment followed by hydroxymethylation (Jin

et al. 2020), enabled an increase in enzymatic reactions hydrolysis and higher glucose yields. In corn straw, the delignification and de-crystallization of cellulose favored the hydrolysis process using combined pre-treatment of ozonolysis and planetary ball milling (Shi et al. 2015).

Overall, for different types of lignocellulosic biomass, the most widespread techniques in terms of pre-treatment are steam explosion, ammonia fiber expansion (AFEX), diluted acid and pre-treatment with ionic liquid, which the last one appears to be the most promising and with the least generation of inhibitors (Wi et al. 2015). Mechanical refining is another technology that can also be used to overcome the recalcitrance of biomass and modify its properties (crystallinity, morphology, particle size, and porosity). This technology is interest because is independent to chemical composition of the raw material, it exposes several carbohydrate sites that were previously hidden in lignocellulosic biomass, increasing the accessibility of enzymes, and allowing faster hydrolysis (de Assis et al. 2018).

Some studies search innovations in the sense of the improvement of enzymatic hydrolysis and the pre-treatment used in different biomasses. A summary of techniques recently applied is presented in Table 10.2.

The enzymatic hydrolysis is still limited to largely used commercial enzymes, usually cellulases. In contrast, the use of different biomasses is rapidly expanding, as demonstrated in Table 10.2. To ensure the energy source increase through industries with a circular economy concept, it is necessary to extend the process until the commercial level, with established and optimized systems, high yields of products of interest, and microorganisms and enzymes highly adapted to the environment (Bonatto et al. 2020). In this context, the study of different biomasses is relevant considering the interest in expanding production systems and decentralizing processes using food biomass. However, there are still several challenges in the face of enzymatic hydrolysis, especially the necessity for enzymatic cocktails with high efficiency and low economic cost.

Enzymatic production by different microorganisms aiming at the hydrolysis of biomass is a little-explored field, with the potential to expand the vision of biorefinery aiming at the integration of different processes and products. Enzymatic production can reduce the hydrolysis' costs, and recent studies demonstrate the relevance to improve enzymatic cocktails by optimizing or creating new projects using new enzymes or improving the engineering of existing ones (Chylenski et al. 2017; Oamen et al. 2019; Du et al. 2020; Adsul et al. 2020; Tramontina et al. 2020; Prajapati et al. 2020; Jain et al. 2020; Maleki et al. 2020).

Linked to the development of enzymatic cocktails, additives appear as another strategy to improve the interface accessibility and enzymatic activity. One of the principles is related to the hydrophobic nature of these agents and linkage with post-treatment residual lignin, preventing the unproductive linkage of hydrolytic enzymes, consequently increasing conversion efficiency (Eriksson et al. 2002; Yang and Wyman 2006). Additives such as non-catalytic proteins, non-ionic surfactants and polymers are the most studied additives in recent years to improve the enzymatic hydrolysis process by reducing enzyme dosage and reaction time (Zhang et al. 2017; Rocha-Martín et al. 2017; Wei et al. 2019; Mukasekuru et al. 2020).

Table 10.2 Summary of studies focusing on advances in enzymatic hydrolysis technologies (own elaboration)

Hydrolysis target	Raw material	Pre-treatment	Hydrolysis methods	Results	Reference
Cellulose	Corn stover	Two stage: dilute hydrochloric acid/aqueous ammonia wet oxidation	Enzymatic: 3 FPU g^{-1} Cellulase and 20 CBU β-glucosidase (Novozymes™) at pH 4.8, 50 °C, 120 rpm, 72 h	Two-stage pre-treatment enabled the biomass fractionation by solubilizing most hemicellulose and lignin Glucose yield high with a low enzyme loading Yield: 71.5% of glucan	An et al. (2019)
Hemicellulose	Corn stalk (pith, leaf, and bark)	Alkaline extraction of hemicellulose Sodium hydroxide 10% (w v^{-1})	Enzymatic: 1% (w v^{-1}) xylanase (Shandong Sukahan Bio-Technology Co.) at pH 8.0, 50 °C, 200 rpm, 24 h	Enzymatic hydrolysis ratio was highest in pith (40.09%), followed by leaf (37.57%), and bark (20%)	Zhang et al. (2018)
Cellulose	Sugarcane bagasse and Norway spruce	Acid sulfite with calcium and sodium	Enzymatic: cocktail purified of cellulases from *Trichoderma reesei* and commercial glucosidase from *Aspergillus niger* and in-house expressed lytic polysaccharide monooxygenases, at pH 5.0, 50 °C, 48 h	Enzymatic hydrolysis resulting in a yield of 80% (glucan conversion) The performance was as good as the yield results found using commercial cellulases	Chylenski et al. (2017)
Cellulose	Corncob residue	Bisulfite	Enzymatic:18 FPU g^{-1} cellulase and 27 CBU g^{-1} β-glucosidase (Nozymes™) at pH 4.8, 50 °C, 150 rpm, 72 h	Highest glucose yield of 99.45% with a solid loading of 12.5%, without detoxification of hydrolysate	Xing et al. (2016)
Cellulose Proteins Lipids Phospholipids	Green microalgae (*Chlorella vulgaris*)	Ball mills	Enzymatic: commercial enzymes, applied separately in solution of 2% (v w^{-1}) cellulase, protease, lipase, phospholipase A1 (Sigma-Aldrich) Process conditions varied according to the enzyme applied	Resulting in 93% of the initial lipid content of the dry biomass recovered in the solid phase, about 80% of the initial carbohydrate content and 54% of the initial protein	Alavijeh et al. (2020)

(continued)

Table 10.2 (continued)

Hydrolysis target	Raw material	Pre-treatment	Hydrolysis methods	Results	Reference
				content were recovered in the liquid phase	
Cellulose Starch	Food waste and microalga *Auxenochlorella protothecoides*	Without pre-treatment	Enzymatic: cocktail with Cellic Ctec2 (Novozymes) and 20 AGU g^{-1} α-amylase (Sigma-Aldrich), at pH 5.0, 180 rpm, 50 °C, 24 h	Proposed a biorefinery system using microalgae and food waste Glucose concentration of 78.34 g L^{-1}, with a simultaneous total conversion of sucrose Fructose concentration of 24.96 g L^{-1} 14.54% of crude oil and 20 g L^{-1} of glycerol was obtained from food waste Cultivation of microalgae resulted in a biomass yield of 0.346 g g^{-1} of sugars and a lipid of 0.216 g g^{-1} of sugars	Patel et al. (2019)

Challenges of the use of enzymes in hydrolysis process are related to inhibition by the excess of sugar and other products that can lead to low mass transfer, problems with viscosity and to handle the fermenters, in addition to high economic costs to obtain different enzymes that act synergistically for different biomasses in the concept of biorefinery. Some bottlenecks can be solved by using robust and concentrated enzymes, with high performance (Larnaudie et al. 2019). Thus, optimizing the enzymatic hydrolysis is essential to the process since it can intensify productivity, reduce operating costs.

Another technology that is exploited for hydrolysis methods is enzymatic immobilization techniques, being a strategy of molecules reuse aiming to reduce the costs of commercial enzymes. The performance of an immobilized system depends on factors such as support and immobilization techniques. Porous supports with a wide contact surface are interesting because they allow a more efficient interaction between enzyme and substrate, since the enzymatic load will be greater than in non-porous supports. Examples of efficient supports for enzymatic immobilization are alginate, chitosan, polymers, biochar, and agricultural and food residues (Girelli et al. 2020; Kyriakou et al. 2020).

Among the immobilization techniques between the support and the enzyme, those that use the principles of covalent bonds present relevant results, but they trap the enzyme, being able to change its conformation and active site, resulting on its inactivation. Adsorption approach uses weaker bonds such as van der Waals, ion exchanges, and hydrogen bonds; these interactions are light but sufficient to keep the enzyme adhered to the support in a stable manner. These approaches would allow the development of an immobilized enzyme system, optimizing catalytic processes yields, overcoming the bottleneck of free enzymes stability in extreme conditions (high temperature, presence of inhibitors and solvents) (Sheldon and van Pelt 2013; Ahmed et al. 2018). However, the challenge of enzymatic immobilization is the need for an extra unitary operation and obtaining supports, increasing the cost of the process. In addition, it is necessary to develop processes with high performance, with the ability to recycle enzymes without significant loss of activity, and with the application of environmentally safe immobilization supports.

Even with the increasing number of researches about enzymatic hydrolysis, the results are still limited to ensure a successful commercial scale. The diversity of biomass, combined with the different pre-treatment methods, hydrolysis strategies, and reaction operations, are approaches that have been extensively studied to overcome the main challenges of the enzymatic hydrolysis.

Considering the proportion of enzymes that biorefineries need to the hydrolysis, some alternatives should be considered regarding costs. In this perspective, the bioprospecting of new enzymes and potential microorganisms that produce enzyme cocktails are opportunities for biotechnological development, with the aim of improving enzyme performance and stability, possibly they will shape the process advances in biorefineries (Dragone et al. 2020).

10.4 Challenges Perspectives: Advances in Hydrolysis Strategies and Scale-Up Feasibility

Traditional biorefineries' focus used to be on biofuel production and, because of these, a huge amount of demonstration plants have been constructed all over the world (Galbe and Wallberg 2019). In the last decades, the focus changed thus to the fact that during the processes, valuable product could be generated and recovered, however, the extensive implementation of full-scale plants still needs to improve issues before make these processes successful projects. Some of the problems are related to technical operation, transport of biomass, types of raw material, pre-treatment processes, generation of inhibitors; but the biggest challenge is the high economic cost and efficient techniques to act in different raw materials associated with pre-treatment and hydrolysis steps (Ragauskas et al. 2014; Galbe and Wallberg 2019). After the optimization of the process in a full-scale, there will be the need for clear regimentation, capital incentive, and investment (Sanford et al. 2016).

The biomass fractionation is also an important step, considering that with a more fragmented biomass, higher are the contact surface to react during the enzymatic step (Elliott et al. 2015). Ionic liquid has emerged as potential "green" and powerful pre-treatment, however, the challenge is about the development of a method to recover the ionic liquid to be reused and, consequently decrease the cost (Elgharbawy et al. 2016).

The enzymatic hydrolysis is probably the most worrying part when considering the full-scale biorefinery, because in this step occurs the carbohydrates solubilization by a cocktail of commercial cellulase enzyme (Abraham and Puri 2020). In a biorefinery process, with the application of biomasses with different compositions, enzymatic hydrolysis requires a cocktail with wide specificity. The solution could be the use of microorganisms that promote the enzymatic hydrolysis and fermentation simultaneously or in co-fermentation processes (Siqueira et al. 2020). For that, researchers recommend microorganisms able to use pentose and hexose for fermentation yield (Houfani et al. 2020; Yamakawa et al. 2020). However, is difficult to find native microorganism with this profile and to produce high amounts of fermentation products. To solve this gap, geneticists are looking for engineered microorganisms combining these characteristics (Hu and Zhu 2019; Sohn et al. 2020). Some estimative revealed that the enzymatic process cost contributes up to 40% of total processing cost (Chandel et al. 2018), however, the hydrolysis and the fermentation cost depends on the processes used, and these are the crucial steps when optimizing the process cost.

After the fermentation, there is the recovery processing, which means to use methods such as distillation or centrifugation, purification, concentration, or crystallization. The cost of these processes depends on the type of product recovered, and the processes used (Chandel et al. 2018). After recovery of all products, the waste biomass needs to be disposed or destinated to other use. One of the possibilities is to use it on boilers for energy generation, closing this way the circular process. The

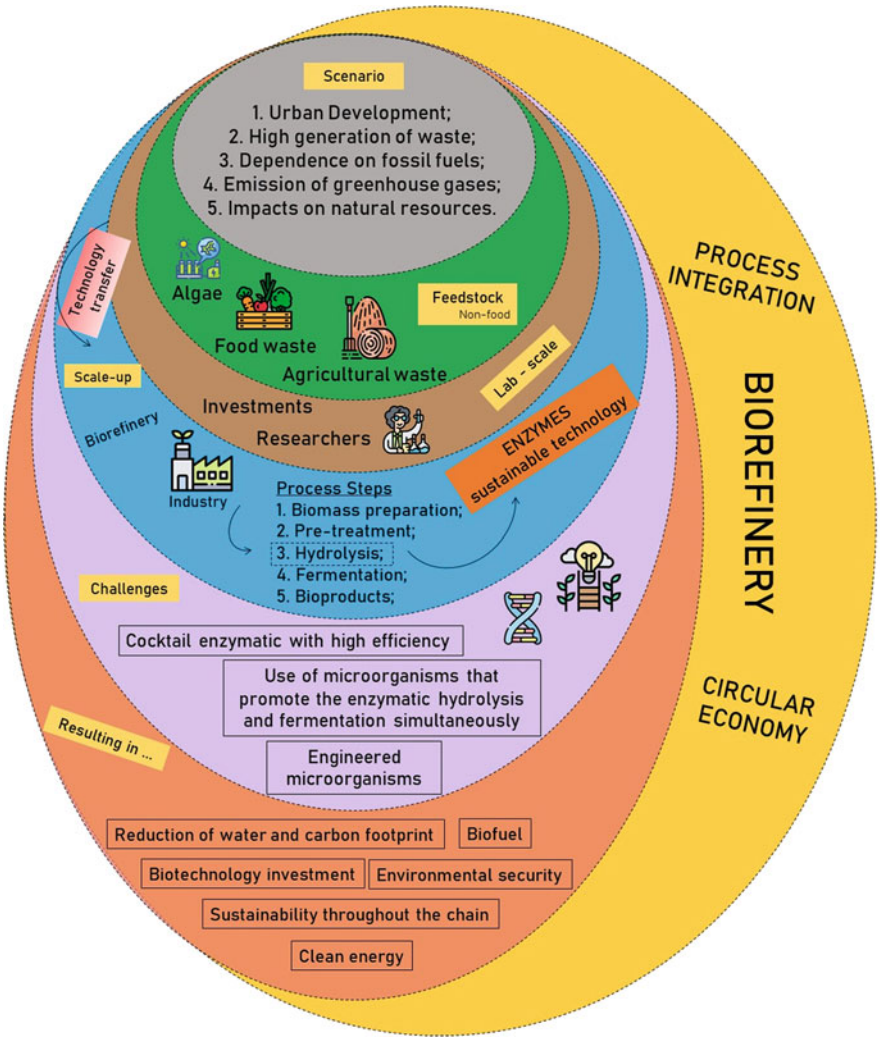

Fig. 10.2 A brief biorefinery approach as a large integrated system, challenges and expected results (Own elaboration)

reuse of this "biomass cake" has been pointed as a significant input of value on biorefineries processes (Ragauskas et al. 2014).

Considering the huge amount of studies applied on biorefinery improvement and aiming the lab-to-industry transfer, it would be important to add economic analysis when performing these researches (Kapanji et al. 2019), so that, it will be shown the hot spots of cost that should be redesigned. Figure 10.2 schematically presents the approach proposed by this chapter, in which it considers the inputs and outputs of a biorefinery as a large integrated system, operating in a sustainable manner, based on

the bioeconomy and with the capacity to produce relevant bioproducts for the world biotechnological scenario. It is necessary to emphasize that this approach still needs impulses to structure and expand its horizons.

Competing Interests All the authors declare that they have no competing interests.

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Chapter 11
Efficient Utilization of Lignocellulosic Biomass: Hydrolysis Methods for Biorefineries

Shritama Aich and Supratim Datta

Abstract The environmental impact, as well as the limited stock of fossil fuels, has led to significant interest in lignocellulosic biomass for the generation of biofuels. The pre-treatment of the recalcitrant and complex lignocellulosic biomass enables the enzyme-catalyzed hydrolysis to produce monosaccharides. The sugars are then fermented by microbes to produce fuels. Such fuels face steep competition from the easily extractable and cheaper fossil-based fuels. Biorefineries constitute a broad class of processes that sustainably convert the biomass into one or many products or novel products efficiently. Here we discuss the processes that are considered to be the vital elements of biorefining systems. In particular, we detail the recent development in the conversion of biomass to sugar and the enzyme technologies for cost-effective biorefinery strategies.

Keywords Lignocellulosic biomass · Biorefinery · Microbes · Pre-treatment · Enzymatic hydrolysis · Biofuel · Fermentation

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Abbreviations

AFEX	Ammonia Fiber Expansion
BCL	*Burkholderia cepacia* lipase
CBD	Cellulose-Binding Domains
CBM	Carbohydrate-Binding Modules
CELF	Co-Solvent Enhanced Lignocellulosic Fraction
CWDE	Cell Wall Degrading Enzymes
GH	Glycoside Hydrolase
ILs	Ionic Liquids
LCA	Life Cycle Assessment
LPMO	Lytic Polysaccharide Monooxygenase
MNNG	N-methyl-N-nitro-N-nitrosoguanidine

11.1 What Is a Biorefinery?

The International Energy Agency Bioenergy Task 42, in its report, first defined the biorefinery as "the sustainable processing of biomass into a spectrum of bio-based products (food, feed, chemicals, materials) and bioenergy (biofuels, power and/or heat)" (de Jong et al. 2012). Thus, the biorefinery is a facility for the efficient and economical conversion of biomass to bioenergy, chemicals, and other products in a sustainable manner. The depletion of fossil fuel and the climatic changes due to the emission of harmful gases and chemicals from the burning of fossil fuel has led to the need for an alternative source of energy (Clauser et al. 2016). Biomass is a sustainable resource because its use leads to the lowering of greenhouse gas emissions and other pollutants. The generation of bio-products helps in the recycling of biomass and reduces the overall environmental impact. Thus, biorefineries have been identified as the cornerstone of a bio-based economy where enzymatic, non-enzymatic, biological, thermochemical, and biochemical technologies would be used together to convert the organic molecules of the biomass to bio-products (Vanholme et al. 2013). The intermediates generated by the initial hydrolysis of the biomass in such a biorefining process, like carbohydrates, proteins, and triglycerides, can be further processed for the generation of value-added products (Cherubini 2010). The pulp and paper industry is considered to be one of the first industrialized biorefinery systems.

The common goals of any biorefining system include converting the industrial and agricultural wastes to useful materials, maintaining a constant supply of fuels and chemicals, and the generation of new chemicals for use as building blocks for novel materials. All of these would be expected to expand the manufacturing economy of the region where the biorefinery might be located and improve the lives of people, particularly in the developing economies of the world. For example, India's first integrated biorefinery for fuel and chemical production was established

in Pune in 2017. The biorefinery produces one million liters of ethanol per annum from biomass like rice and wheat straw, sunflower seeds, sugar beet, soybean, sugarcane bagasse, corn cobs, and cotton stalk. India has the sixth-highest energy demand in the world, the highest being the USA, and consumes 3.5% of the worlds' commercial energy. The demand for transportation fuels in India may increase by 2.2-fold by 2030. To reduce dependence on fuel imports and meet this energy demand, India has set a target to replace 20% of the fuel requirement from oil extracted from renewable sources such as Jatropha and Pongamia (Sankar et al. 2013). Such a target would only become economically feasible through a biorefinery-based approach.

11.2 Classification of the Biorefinery System

The biorefinery system is defined by classifying into four groups based on the essential features, namely, platform, type of feedstock, the process, and the products generated.

11.2.1 Classification Based on Platforms

The platform refers to all the intermediate products that are formed from the raw materials or the feedstocks to the final product generated. These platforms can again be converted and processed further to usable products by different processes. Some of the essential platforms of a biorefinery are listed in Table 11.1.

11.2.2 Classification Based on Feedstock

In the biorefinery, the feedstock refers to renewable biomass and can be categorized as primary, secondary, and tertiary feedstocks. The primary feedstock is typically obtained from forest and agricultural land. The secondary feedstock refers to the waste products of pulp and paper mills, sawmills, forest wood industries, etc. Finally, the human-derived wastes like solid and liquid wastes are referred to as tertiary feedstocks. In the biorefinery, the primary sources of biomass are the biomass crops and crop residues from the agricultural lands, forest products like wood and wood residues, process residues and waste from industries, domestic wastes, and seaweed and algae from aquacultures. The amounts of cellulose, hemicellulose, lignin, and starch across these varied biomass crops are different.

Another classification divides feedstock into dedicated crops from agricultural land and aquacultures and the crop waste residues from agriculture, forestry, and human activities, including industry. The dedicated feedstocks includes sugar crops

Table 11.1 Biorefinery platforms

Platforms	Components of the platform	Source of intermediates/platforms
Biogas	A mixture of mainly CH_4 and CO_2	Anaerobic digestion
Syngas	A mixture of CO and H_2	Gasification
C5 sugars	Xylose, arabinose ($C_5H_{10}O_5$)	Hydrolysis of hemicellulose
C6 sugars	Glucose, galactose, fructose ($C_6H_{12}O_6$)	Hydrolysis of sucrose, starch, cellulose, and hemicellulose
Lignin	Phenylpropane building blocks, $C_9H_{10}O_2(OCH_3)_n$	Processing of lignocellulosic biomass
Oil	Triglycerides	Processing of oilseed crops, algal, and other residues (oil-based)
Pyrolysis liquid	A mixture of different molecules of different sizes	Thermal biomass decomposition in the absence of oxygen
Hydrogen	H_2 gas	Electrolysis of water, water-gas shift reaction, steam reforming, and fermentation
Organic juice	Different chemicals	Liquid phase extracted from wet biomass
Electricity and heat	Energy	To meet biorefinery energy needs

like sugarcane, and starch-based crops like wheat, corn, sweet sorghum, lignocellulosic crops like switchgrass, oil-based crops like rapeseed, and jatropha, and marine biomass like microalgae and macroalgae. The residual feedstocks include animal fat from food industries or cooking oil from commercial and domestic sources, crop residues, and human-derived waste like urban waste. Urban waste includes the municipal solid waste (MSW) and residues from fruit and vegetable-based industries. Thus, urban waste is heterogeneous, and the physical and chemical characteristics of the biomass in the waste vary. The high moisture contents in sewage sludge, farm manure, and residues from food processing industries make it more suitable for biogas generation by an anaerobic digestion process. The heavy metals contaminated waste may also present an opportunity to recover energy. Thus, the differences in biomass waste properties and characteristics dictate the choice of conversion processes.

11.2.3 Classification Based on Processes

The underlying technology of the biorefinery process is the depolymerization and deoxygenation of the biomass components and subsequent conversion through different processes into valuable products. The processes can be thermochemical, biochemical, mechanical/physical, and chemical, and are based on the final product choices such as biofuels, biochemicals, biomaterials, food, and other products. The

Table 11.2 Processes of the biorefining system

Processes	Conditions of processing	Examples
Thermochemical	Combustion, pyrolysis, hydrothermal upgrading, gasification, supercritical	High temperature and pressure, with or without a catalyst
Biochemical	Enzymatic hydrolysis, fermentation, anaerobic digestion, aerobic conversion	Under mild condition by natural microorganisms that secrete enzymes or model organisms that produce recombinant enzymes
Chemical	Esterification, hydrogenation, hydrolysis, electrolysis, methanization, steam explosion, water-gas shift, catalytic processes, pulping	A chemical change in the substrate backbone
Mechanical/ physical	Pre-treatment, mechanical fractionation, separation, disruption, pressing, extraction	No change in the chemical structure of the biomass but decreases the complexity by breaking into smaller units or removal of specific components.

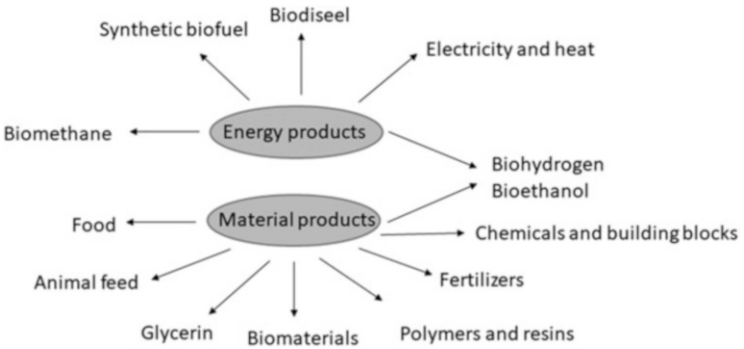

Fig. 11.1 The energy and the material-based products generated by biorefinery system

different processes of the biorefining system, conditions of processing, and a few examples of each are included in Table 11.2.

11.2.4 Classification Based on Products

Biorefineries broadly produce two types of products from biomass. The products could be in the form of energy, in the form of transportation biofuels, heat, or other bio-products to reduce the cost of the biomass supply chain as well as make it energy efficient. The physical products may be in the form of bio-based products like lubricants, chemicals, food, and the reprocessing of intermediates for energy production. The types of products generated by biorefineries are shown in Fig. 11.1. In

the rest of this chapter, we will concentrate on biofuel as the product generated by a biorefinery system.

11.3 Environmental Impact of Biorefinery Systems

The generation of fossil fuels required for transportation and the burning of fossil fuels to produce carbon dioxide as a by-product is one of the significant contributors to environmental pollution in the twenty-first century. Carbon dioxide is a greenhouse gas and a leading contributor to global climate change. Biofuels generated by biorefineries can be an alternative to fossil fuel and reduce emissions as the plants and crops that are feedstocks for biofuels absorb carbon dioxide to grow. Water pollution can also be reduced by replacing the use of fossil fuels with biofuels. The environmental impacts of groundwater contamination from fossil fuel extraction are huge. Replacement of fossil fuels by biofuels derived from lignocellulosic biomass also helps in the recycling of crop waste and thus decreases the burning of waste and environmental pollution. Thus, biorefineries contribute towards a more sustainable environment by reducing the emission of greenhouse gases and other pollutants and by conservation and recycling of resources.

Life cycle assessment or LCA is a method that is used to evaluate the total environmental impact from the extraction of raw material to the generation of the final product and any waste (Curran 2008). LCA is useful for determining the benefit of the biorefinery system over conventional alternatives. Such a study involves the use of specialized software and simulations of the involved processes. LCA increases the efficiency of material and energy use and reduces waste. In a lignocellulosic biorefinery, the LCA analyses are essential to understand the effects of different biomass, products, processes, and the impact of each on environmental sustainability. The cradle to a grave type of LCA is the assessment of the cycle, starting with the use of the resource to the final user and the waste generated. A simpler model is the cradle to gate LCA analyses that evaluate the cycle from a source of extraction (cradle) to the factory (gate), without any consideration of the application and waste disposal in the model. The cradle to cradle assessment is similar to the cradle to grave, with the difference being in minimization of environmental impacts by recycling the waste generated towards zero waste emissions.

In biorefinery, LCA is critical for the development of new bio-based substrates as raw materials for the production of bio-products. LCA is also applied to research on new product development. In biofuel production, LCA studies enable the comparison to fossil fuels by calculating the emission of greenhouse gases (GHG) and energy consumption. The primary source of environmental impact in biofuel generation is the feedstock. Among the feedstocks, agricultural residues have the lowest environmental impact, followed by lignocellulosic residues. The biomass feedstock leads to lesser emission of greenhouse gases, saving of non-renewable energy, and generation of chemicals from biomass-based feedstocks. Biofuel from second-generation crops and lignocellulosic residues is called second-generation biofuels

and shows up to 80% less impact on the environment than the non-renewable fossil fuel (Junqueira et al. 2017). Fuel from lignocellulosic feedstock shows up to 41% lesser environmental impact. However, LCAs of variants of the biorefinery system show that there are some disadvantages to a biorefinery system. The disadvantages include health effects from by-products if released without treatment, the use of land for the cultivation of dedicated crops for biofuel generation, and soil pollution from pesticides used for growing the crops (Uihlein and Schebek 2009). The lignocellulosic biorefinery could be a better alternative to fossil fuel if the process technologies can reduce or treat any effluents that are generated.

11.4 Lignocellulosic Biomass and Biorefineries

The three main constituents of the plant cell wall are the carbon-based polymers of cellulose, hemicellulose, and lignin. Lignocellulosic biomass is mainly made up of 20–50% cellulose, 25–35% hemicellulose, and 10–25% lignin with the remaining percentage in protein, simple sugars, and minerals. This biomass composition varies depending on the type, species, and the location from where the biomass is harvested.

Lignocellulosic biomass is derived from forests, agricultural lands, and agro-industrial wastes. The conversion of lignocellulosic biomass to biofuels and other products is one of the most promising strategies to replace the depleting and non-renewable fossil fuels. The biofuels generated can be categorized into three generations depending on the source. First-generation biofuels are produced from the sugars extracted from sugarcane, corn, sugar beet, etc. and directly compete with food production. For this reason, development of biofuels from second-generation substrates like the agricultural residues generated from corn stover, wheat straw, rice straw, and sugarcane bagasse, as well as non-food energy crops that are grown in lands unsuitable for food crop has gained more interest. The third-generation biofuels are generated from algal sources. Microalgae have a rapid growth rate, fixes greenhouse gas, and high capacity to produce lipids to be an ideal biofuel feedstock. Another advantage is that algae can be grown on non-arable land as well as saline water.

One of the main bottlenecks in the production of biofuels is the initial conversion of lignocellulosic biomass to sugars. The recalcitrant lignocellulosic biomass requires aggressive pre-treatment to yield a substrate that can be readily hydrolyzed by the commercially available cellulolytic enzymes or microorganisms that secretes the enzymes, to release sugars for further fermentation to fuels. Effective pre-treatment of lignocellulosic biomass requires a process that reduces the biomass crystallinity and ensures the highest recovery of the pre-treated biomass. The pre-treated biomass should contain minimal amounts of inhibitory products so as not to affect enzymatic hydrolysis or the microbial fermentation to produce fuels. The ideal pre-treatment method must apply to a wide range of biomass, be scalable, and have low capital and energy cost. The generation of co-products from lignin may

be beneficial to the process economics. Similarly, the generation of sugars from hemicellulose may eliminate the requirement for hemicellulase during the enzymatic hydrolysis of the pre-treated biomass (Galbe and Zacchi 2007). When cellulose is the primary substrate, a pre-treatment process that removes lignin and hemicellulose, aside from decreasing the cellulose crystallinity and increasing the accessibility to enzymes, is often desired. The four main categories of pre-treatment methods are: physical (ultrasound, pyrolysis, milling, and grinding of the biomass), physicochemical (steam explosion, ammonia treatment, wet oxidation), chemical (alkali, dilute acid, oxidizing agents, catalyst, and ionic liquids), biological (microorganisms and enzymatic hydrolysis), and sometimes a combination of two or more pre-treatment processes (Fig. 11.2).

Chipping, milling, and grinding are common physical pre-treatments that reduce the cellulose particle size, crystallinity index, and the degree of polymerization in an environmentally friendly manner to increase the specific surface area of cellulose. A potential drawback of this process is the energy-intensive nature of the method. Extrusion is another physical pre-treatment method based on the simultaneous heating, mixing, and shearing of the biomass into the extruder to physically and chemically modify the biomass (Zheng and Rehmann 2014). Concentrated as well as dilute acids have been used to pre-treat biomass. In particular, dilute acid shows greater promise as the acid does not need to be recovered, and there is less equipment

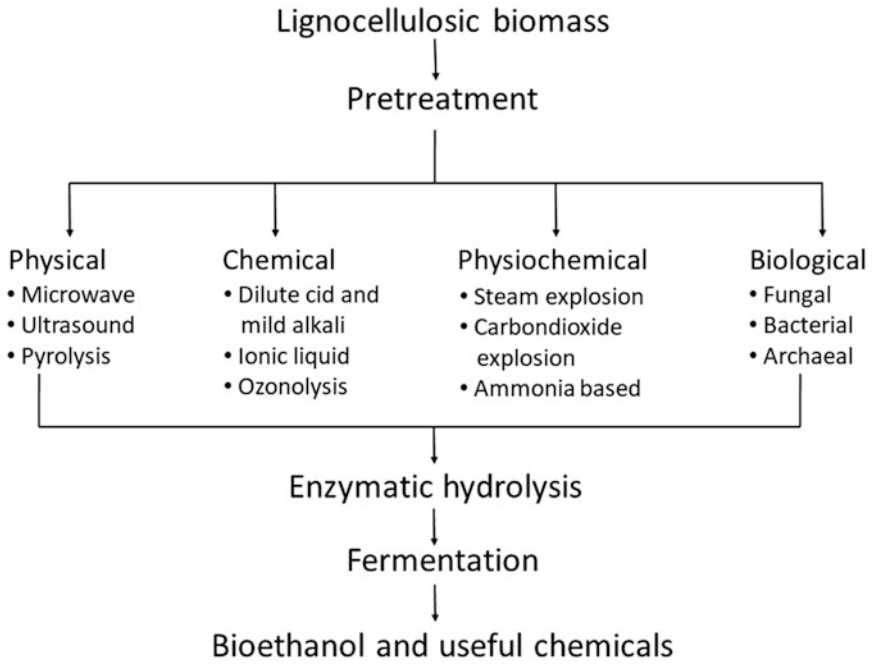

Fig. 11.2 The process to convert lignocellulosic biomass to biofuel and useful chemicals

corrosion. One advantage of acid pre-treatments is the solubilization of hemicellulose derived sugars leading to a reduction of biomass heterogeneity. However, the degradation of the biomass by the acid also releases compounds that inhibit the downstream hydrolysis and fermentation. Recently, the discovery of lower corrosivity and the generation of less number of inhibitory compounds upon phosphoric acid pre-treatment offers an alternative to dilute acid (Castro et al. 2014). Alkaline pre-treatment confers the added advantage of lignin solubilization and removal. In particular, lime or calcium hydroxide cleaves the acetyl groups in hemicellulose and improves the efficiency of cellulose hydrolysis. Organosolv pre-treatment by organic or aqueous solvents like ethanol, ethylene glycol, acetone, etc. helps in extracting lignin and enables more efficient enzymatic hydrolysis. A newer organosolv pre-treatment process called co-solvent enhanced lignocellulosic fraction (CELF) uses a tetrahydrofuran solution for the extraction of lignin polymer and yields pre-treated biomass (Nguyen et al. 2015). Ionic liquids (ILs) have emerged to be a promising green solvent for efficient biomass pre-treatment (Socha et al. 2014). The ILs are molten salts at temperatures below 100 °C and made up of large organic cations and small inorganic anions. ILs can fractionate different types of biomass into cellulose, hemicellulose, and lignin and produce minimal inhibitory compounds to downstream processes (Ouellet et al. 2011). Ammonia fiber expansion (AFEX) pre-treatment combines high temperature and pressure and ammonia to effectively pre-treat biomass and enables the subsequent recovery and recycling of ammonia (Wyman et al. 2005). The AFEX pre-treated biomass containing cellulose and hemicellulose is amenable to efficient enzymatic hydrolysis. Steam explosion is a popular hydrothermal pre-treatment method in which the biomass is treated by saturated steam at temperatures higher than 160 °C and high pressures (5–30 bar) up to a few minutes, followed by a quick depressurization. The solubilization of acetyl groups to form acetic acid contributes to hemicellulose hydrolysis by hemicellulases. Some solubilization of lignin also occurs in this method. Hydrothermal pre-treatment usually occurs at the temperature range of 160–240 °C since temperature above 240 °C degrades cellulose. It is another popular pre-treatment method due to the relatively lower costs and the generation of very low concentrations of inhibitors.

Compared to chemical and physical pre-treatment methods, biological pre-treatment is an environmentally friendly and low energy consuming process. There are a vast number of cellulolytic and hemicellulolytic microbes that are effective for biomass pre-treatment. For instance, pre-treatment of wheat straw for 10 days by a fungus generated fermentable sugars and did not produce any other inhibitors to affect the fermentation process (Kuhar et al. 2008). The biological pre-treatment is not yet a potential pre-treatment method for consideration at an industrial scale because of the time required and a lack of understanding of the exact mechanism (Sun and Cheng 2002). To realize the full potential of biological pre-treatment, more biomass-degrading fungi need to be identified, and the mechanisms studied to increase the rate of biomass delignification. Initial studies have shown that biological pre-treatment, as an addition to another pre-treatment process, may be more effective as compared to a single method. For example, the rate of

saccharification of wood increased when biological pre-treatment with *P. chrysosporium* for 28 days was combined with the steam explosion at 215 °C for 60–65 min (Sawada et al. 1995). Microbes like a variety of bacteria, fungi, and actinomycetes have potential cellulolytic abilities that have critical industrial applications (Kuhad et al. 2011).

Thus, efficiently fractionating the biomass is essential for the development of a bio-based economy. In the biorefinery context, techno-economic analyses of biomass processing technologies are critical for identifying energy expenditure, the biomass of choice, catalyst loadings, and the tolerable moisture content. The development of industrial pre-treatment processes requires digestible pre-treated cellulosic biomass after the recovery of hemicellulose and lignin by a process requiring minimal energy, chemical, and water. The choice of the process will be dependent on the feedstocks available in the area and a design that takes into account all such available feedstock.

11.5 Enzymatic Hydrolysis of Lignocellulosic Biomass

In the biorefinery process, enzymes play a crucial role not only in the generation of fuels but also in the other products that may be produced. Enzymes catalyze the hydrolysis of lignocellulosic biomass to monomeric sugars for further microbial conversion to fuels. The advantages of enzymatic hydrolysis over non-enzymatic catalysis include high selectivity under the mild reaction conditions of enzymatic reactions, less number of inhibitory by-products, and the possibility of combining the hydrolysis step with further downstream fermentation. While the cellulose in lignocellulose is abundantly available on earth, it is also very recalcitrant to enzymatic hydrolysis. Cellulose is made up of β-D glucose linked by the β-1,4-glycosidic bonds with a reducing and non-reducing end. The reducing end can be converted from the ring open form to an aldehyde. Each cellulose chain runs in parallel to each other to form cellulose microfibrils, and the chains in the microfibrils are hydrogen bonded to each other, forming a microfibrillar structure. Many naturally occurring organisms have evolved to convert the lignocellulose to soluble sugars for subsequent use as a food and energy source. These organisms usually produce different enzymes that synergistically break down the recalcitrant polysaccharides. The type and relative ratios of the different enzymes vary between species. All the enzymes that take part in degradation, formation, or modification of glycosidic bonds are listed in the CAZy database (Cantarel et al. 2009). The enzymes that are involved in breaking cellulose units are known as glycoside hydrolase (GH) family. The enzymes that depolymerize the cellulose chains are called cellulases, while those that hydrolyze hemicellulose are known as hemicellulases. Cellulases are the primary group of cell wall degrading enzymes (CWDE) and are a cocktail of many enzymes. The main constituents of cellulase are endoglucanases, exoglucanases or cellobiohydrolases, and β-glucosidases (Datta 2016). Endoglucanases (EC 3.2.1.4) randomly hydrolyze the cellulose glycosidic bonds and generate shorter units to

increase the concentration of reducing sugar. Cellobiohydrolases (EC 3.2.1.91) hydrolyze the shorter cellulose chains by removing cellobiose from either end. β-Glucosidases (EC 3.2.1.21) hydrolyze the cellobiose to glucose. The cellulases act synergistically to convert the cellulose into β-glucose.

Lignocellulolytic fungi can be found across terrestrial and marine environments and are an efficient source of biomass-degrading enzymes, and fungal cellulases perform the majority of biomass degradation in nature. The brown-rot fungi initiate the attack on plant cell walls through oxidative chemistry by free radicals. Filamentous fungi, on the other hand, employ enzymes to break down biomass. The filamentous ascomycete *Trichoderma reesei* was isolated in the 1940s and was the first filamentous fungi known to degrade biomass. Since then, many such fungi have been discovered and found to produce large amounts of cell wall degrading enzymes. The fungal cellulases are distributed mostly across GH 1, 3, 5, 6, 7, 9, 12, and 45 (http://www.cazy.org/Glycoside-Hydrolases.html) (Cantarel et al. 2009).

In general, microorganisms degrade the plant cellulose through two systems. Some organisms produce cellulases, which are a set of free enzymes not complexed to each other. One of the best-known examples of this free enzyme paradigm is the cellulase cocktail secreted by saprophytic *Trichoderma reesei* (Durand et al. 1988). Many other *Trichoderma* species are known to be cellulolytic and survive by degrading the plant cell walls for energy. These strains include *T. reesei, T. lignorum, T. koningii, T. pseudokoningii, T. virens, T. harzianum, T. longibrachiatum*. The *T. reesei* QM6a species are reported to express more than 193 GH's and 41 CBMs (Kubicek et al. 2011). Another system that is often found in anaerobic microorganisms is when these cell wall degrading enzymes are not free but organized into enzyme complexes called a cellulosome. These stable cellulolytic complexes may vary in size between 2.0 and 16.0 MDa, with polycellulosome molecular weights going up to 100.0 MDa. The cellulosomes contain a large non-catalytic scaffolding protein with cohesins that contain the enzyme binding sites. Cellulosomes also contain dockerins which bind to the cohesin modules. The non-covalent cohesion–dockerin interactions enable the formation of the cellulosome complex and ensure a scaffold that enables the enzyme–enzyme interactions. While *Clostridium thermocellum* was the first anaerobic rumen bacteria that were found to contain cellulosomes, many other anaerobic bacteria were subsequently discovered also to contain cellulosomes (Bayer et al. 1998, 2004; Doi and Kosugi 2004; Fontes and Gilbert 2010).

A new group of enzymes that are beginning to be characterized for their role in cellulose depolymerization is the lytic polysaccharide monooxygenase (LPMO). These copper-containing enzymes employ oxygen and a reducing agent for the oxidative cleavage of cellulose. The LPMOs act synergistically with the GH enzymes and help in increasing the efficiency of biomass hydrolysis by acting on the crystalline substrate surfaces, which are otherwise inaccessible to the endoglucanase (Harris et al. 2010; Vaaje-Kolstad et al. 2005). Thus, the LPMOs can act as an endo-acting enzyme directly on the crystalline cellulose surface, in contrast to the endoglucanases, which primarily act on the amorphous cellulose. LPMOs were initially annotated as family 33 carbohydrate-binding module (CBM)

when isolated from bacteria and GH61 cellulase when isolated from the fungus. Subsequently, the catalytic roles of LPMOs began to be discovered and thus re-annotated. LPMOs are upregulated during biomass hydrolysis in biomass-degrading-organisms and show broad diversity (Eastwood et al. 2011; Horn et al. 2012; Wymelenberg et al. 2010). Thus far, the structures of 36 fungal and non-fungal LPMOs have been solved. The LPMO contains the immunoglobulin-like distorted β-sandwich fold made up of antiparallel β-strands with α-helix inserted through flexible loops.

The carbohydrate-binding modules (CBM) were previously known as cellulose-binding domains (CBD) as they were found in cellulases and used to bound cellulose. Subsequently, the modules were discovered in other enzymes that target carbohydrate ligands and thus were referred to CBM. These modules play an essential role in binding to the substrate and enabling substrate accessibility to the catalytic domain of the enzyme. The role of CBMs was first verified by limited proteolysis experiments on the CBHI and CBHII, from *Trichoderma reesei* and cellulases CenA and CexA from *Cellulomonas fimi*, all of which contained the CBM module (Gilkes et al. 1988; Tilbeurgh et al. 1986; Tomme et al. 1988). Upon truncation, the specific activities of the enzymes on cellulose were severely reduced or lost. Amino acid mapping experiments correlated this loss of activity to the truncation of around 100 amino acid at the C-terminal domain. Thus, CBM is another vital protein class found in a wide range of CWDE that helps in crystalline cellulose hydrolysis. CBMs are also found in other plant cell wall degrading enzymes (Ferreira et al. 1990, 1993; Kellett et al. 1990).

Hemicelluloses are a heteropolymer with sidechains made up of pentoses (xylan) and alternating units of mannans or glucomannans, or galactans. Hemicelluloses make up 20–30% of the lignocellulosic biomass, depending on the biomass type. Grasses, for example, typically contain a high proportion of hemicellulose. In the biorefinery, the synergistic action of cellulases and hemicellulases enables the saccharification and further fermentation of both lignocellulosic constituents. Enzymes that degrade hemicellulose hydrolyze the hemicellulose backbone β-1,4 bonds. Hemicellulases include endo β-1,4-xylanases (EC 3.2.1.8), β-1,4 mannosidases (EC 3.2.1.25), xylan 1,4-β-xylosidases, (EC 3.2.1.37), and endo-1,4-β-mannanases (EC 3.2.1.78). Hemicellulases also perform de-branching of the hemicellulose through *p*-coumaric acid esterase (EC 3.1.1.1), α-L-arabinofuranosidase (EC 3.2.1.55), acetylxylan esterase (EC 3.1.1.72), ferulic acid esterase (EC 3.1.1.73), and β-glucuronidase (EC 3.2.1.139). The esterases are involved in the hydrolysis of ester bonds. Feruloyl esterase breaks up the hemicellulose and lignin linkages and increases the amounts of free saccharides, for subsequent hydrolysis by the carbohydrate-degrading enzymes.

11.6 Engineering Enzymes and Recent Developments in Biomass Hydrolysis for Cost-Effective Product Generation

The cost of enzyme production is a significant bottleneck in the economical production of biofuels. That is because the enzymes produced naturally either have a low catalytic activity under industrial conditions or often the amount of enzyme produced is little. For cost-effective enzymes to work synergistically and within the identified process parameters, the naturally occurring enzymes often need to be genetically or chemically modified to be better biocatalysts. Chemical modification is often through immobilization of the enzyme to a matrix or a nanoparticle for stability, reusability, and higher activity. Genetic modification is usually done either by rationally redesigning the enzyme through insights derived from prior knowledge of the protein structure or a model or by evolving the enzyme by directed evolution. Directed evolution seeks to simulate natural selection in the laboratory by mutations, deletions, insertions, etc. to generate different protein variants that are then screened to discover improved traits. In the next parts of this section, some of the recent progress in improving enzymes for biomass conversion will be discussed through a few chosen examples.

Engineering cellulase Activity: Some of the ways to improve cellulase activity are by increasing substrate specificity by constructing a multifunction enzyme, sequence alteration of the catalytic domain, the addition of extra modules to the catalytic domain, increasing enzyme secretion, etc.

The surface-exposed loops of the TIM-barrel fold structure in GH5 cellulase that surround the catalytic pocket interact with solvent and ligand molecules. Zhang et al. reported the enhancement in the specific activity of a *Gloeophyllum trabeum* CBS 900.73 GH5 enzyme by targeting a loop region to improve the flexibility of the loop and enhance the enzyme–substrate interactions (Zheng et al. 2018). Kim et al. targeted a bifunctional glycoside hydrolase CelA containing a family 9 endoglucanase and a family 48 exoglucanase. CelA is secreted by the thermophilic *Caldicellulosiruptor bescii*, and implicated to play a role in the organism's ability to grow on untreated lignocellulosic biomass. To increase the secretion efficiency, they inserted aspartic acid residues into the N-terminus of GH9A or GH48. They showed that the modification mainly increased the activity of the endoglucanase domain, and the exoglucanase domain on Avicel, probably due to changes in protein packing (Kim et al. 2017). Saavedra et al. used site-directed mutagenesis to understand the role of active site flexibility in enzyme activity based on a comparative in silico analysis of the alkaliphilic Cel5A from *Bacillus agaradherans* with two other structurally homologous cellulases, GH5-2 from the mesophilic *Erwinia chrysanthemi* and Cel5G from the psychrophilic *Pseudoalteromonas haloplanktis*. The authors identified three positions N141L, A137Y, and I102A/A137Y near the active site, which upon mutation increased the catalytic activity at low temperatures and decreased activation energy and activation enthalpy. The authors hypothesized that the mutations disrupted a hydrogen bond network to increase local flexibility near the active

site tunnel (Saavedra et al. 2018). Torktaz et al. rationally engineered Cel5E, a bifunctional xylanase/cellulase from *Clostridium thermocellum,* by targeting a pocket with many hydrophobic residues lying near the Cel5E active site. Instead of initial experiments, they first used molecular dynamic simulations to screen the variants for higher thermal stability (Torktaz et al. 2018). They then verified the results experimentally and verified the increased CMCase and β-glucanase activity.

Matsuzawa et al. used X-ray crystallography to improve the thermostability of a metagenomic β-glycosidase, MeBglD2 (Matsuzawa et al. 2017). They identified specific residues and generated variants that were tolerant to higher temperatures, organic solvents, and metal ions. Sinha et al. rationally enhanced the activity of a β-glycosidase from *Halothermothrix orenii* by identifying single mutations, V169C and I246A, that enhanced the already high WT enzyme activity by 1.7 and 1.2 times (Sinha et al. 2017). Upon combination of the two mutations to generate V169C/I246A, the specific activity of the double mutant increased nearly twofold over the WT, along with a fivefold higher half-life. They probed the non-conserved residues at the aglycone-binding part of the active site pocket as well as at the gatekeeper region to show that hydrophobicity as well as the steric at the pocket entrance improve activity and increase glucose tolerance (Sinha et al. 2020). Goswami et al. used rational design to increase the glucose tolerance of a mesophilic bacterium, *Agrobacterium tumefaciens 5A,* by nearly twofold by tuning the hydrophobicity and steric in the active site tunnel (Goswami et al. 2016, 2017). Sinha et al. reported a very high glucose tolerant β-glucosidase from the archaeon *Thermococcus* sp. (Sinha and Datta 2016). They further reported that glucose tolerance might be due to specific residues at the entrance to the active site tunnel instead of the gatekeeper residues near the tunnel entrance (Sinha et al. 2019).

Site-directed mutagenesis to shift the pH optimum of a GH7 CBH to a more alkaline pH is an example of pH engineering (Boer and Koivula 2003). Voutilainen et al. improved the thermostability of a GH7 CBH, *Te*Cel7A, through the use of computational prediction tools to create disulfide crosslinks around the active site tunnel (Voutilainen et al. 2010). The newly introduced disulfide bonds increased the T_m up to 5.0 °C, and the variant containing all the three disulfide bonds increased the T_m by 9 °C. Structure-guided recombination methods are another way of improving an enzyme's activity and stability. The same strategy of engineering was used on the GH6 CBH, *Tf* Cel6B (Zhang et al. 1995). Aich et al. reported thermostable GH7 endoglucanase from the pathogen *Bipolaris sorokiniana* and rationally designed variants with higher activity (Aich and Datta 2020; Aich et al. 2017). Structure-guided enzyme recombination and generation of chimeras have also been another method of increasing protein activity and thermostability (Heinzelman et al. 2009).

Almost all of the cellulosic enzyme cocktails for biomass hydrolysis are produced by fermentation of the ascomycete *Trichoderma reesei*. Understanding the structure and mechanism of the *T. reesei* enzymes, as well as the regulation of expression and secretion, is thus an essential area of study since there are many *T. reesei* enzymes that may be improved. Chokhawala et al. increased the thermostability of the Cel7B endoglucanase of *T. reesei* by a structure-guided evolution approach (Chokhawala et al. 2015). The authors used the structure derived amino acid B-factor's to shortlist

20 amino acids that could play a role in protein flexibility. They then used site-directed mutagenesis to discover a variant, G230A/D113S/D115T, with a 3 °C higher melting temperature (T_m) and a nearly fourfold higher specific activity compared to the WT. Another *T. reesei* endoglucanase, Cel5A was compared to a thermophilic Cel5A from *Thermoascus aurantiacus*, and two disulfide bonds were eliminated through Cys mutations to increase enzyme thermostability (Akbarzadeh et al. 2018). Indeed, the thermal stability of C99V and C323H increased by more than twofold. Like the endoglucanase, the *T. reesei* cellobiohydrolase Cel6A and Cel7A have also been targeted for improvement. Smith et al. used SCHEMA to identify structural blocks among homologous proteins for shuffling and generation of chimeric protein with improved thermostability (Smith et al. 2013). Goedegebuur et al. improved the thermal stability of the mesophilic *Hypocrea jecorina* cellobiohydrolase I, Cel7A, to enable usage at higher temperatures (Goedegebuur et al. 2017). They used directed evolution to increase the melting temperature and half-life through a variant containing 16 mutations and then through molecular dynamics simulations hypothesized the possible stabilizing effects.

While natural proteins typically have single-activity, engineering artificial multiple domain enzymes by combining two or more catalytic domains has recently evoked some interest since such proteins may be more active, stability, and lower production costs. Taylor et al. combinatorially permuted across two GH7 cellobiohydrolases, TrCel7A from *T. reesei* and PfCel7A from *Penicillium funiculosum*. Both enzymes contain a catalytic domain linked to a CBM via a linker, allowing them to test various combinations across the two enzymes. They discovered that the CBM and linker of PfCel7A on TrCel7A improved the *T. reesei* enzyme activity (Taylor et al. 2018). Bifunctional enzymes created by combining two different genes have often been a desired trait. Rizk et al. fused two thermostable proteins, endoglucanase (cel5A) and an endoxylanase (xylT) by a linker sequence to create

Two bifunctional chimeras (Cel5A–XylT and XylT–Cel5A) (Rizk et al. 2015). The fused proteins were not only active on the respective substrates, β-glucan, and beechwood xylan, but the enzyme activities improved. Ribeiro et al. also engineered two bifunctional chimeras between a laccase and an engineered xylanase from *B. subtilis* (Ribeiro et al. 2011). They inserted the xylanase into a surface loop of the laccase such that the xylanase was surrounded on both sides by the laccase N-terminal and C-terminal regions. Both constructs had higher specific activity than the wild-type laccase catalytic. In yet another example, Ribeiro et al. inserted a *B. subtilis* GH11 xylanase into *E. coli* xylose-binding protein (XBP) rationally and randomly (Ribeiro et al. 2015, 2016). The screening of the chimeric XynA–XBP libraries generated allosteric variants with xylanase activity modulated by xylose and higher thermostability than the XynA parent.

Carbohydrate-binding modules (CBMs) bind carbohydrate substrates to direct the substrate towards the active site of the enzymes. Duan et al. engineered chimeras that showed higher activity than the metagenomic cellulase GH9 (Umcel9A), by fusing CBMs from different families, using native linkers (Duan et al. 2017). The catalytic efficiency (k_{cat}/K_M) of the chimeric CBM4-Umcel9A on insoluble substrates like

phosphoric acid pre-treated cellulose, alkali-pre-treated sugarcane bagasse, and Avicel was higher than the wild-type enzyme. To find the best CBM for a *Bacillus subtilis* endo-β-1,4-glucanase (BsCel5A) containing a CBM3 that hydrolyzes the β-1,3-1,4-linked glucan, Fonseca-Maldonado et al. exchanged the CBM3 with a CBM11 from *Ruminiclostridium thermocellum* celH (RtCBM11) that also had a β-1,3–1,4 glucan affinity (Walker et al. 2015). The chimeric BsCel5A-RtCBM11 showed improved specific activity and catalytic efficiency. Strobel et al. also targeted the CBM to improve cellulose binding. They engineered the *Trichoderma reesei* Cel7A CBM and linker by interrogating a library of CBM variants. They obtained a mutant with higher cellulose affinity to pre-treated Miscanthus biomass and a higher rate of glucose generation (Strobel et al. 2015).

Furtado et al. adopted a slightly different approach to increase the xyloglucan binding of a CBM from *R. thermocellum*. They used directed evolution to generate a random library of the CBM and screened for improvement in xyloglucan binding (Furtado et al. 2018). They reported a redesigned CBM containing four mutations linked to a xyloglucanase from *Aspergillus niveus* that increased xyloglucan.

Romero et al. used a microfluidic-based assay to interrogate a library generated by the random mutagenesis of a glycosidase from *Streptomyces sp* and enriched for thermal stability (Romero et al. 2015). After adding a droplet of a fluorogenic substrate to the cell extract, the active clones were sorted by a nanodrop sorter and then sent for illumina sequencing. The sequencing allowed the authors to comprehensively map the mutations generated to the enzyme activity and facilitated the understanding of all the interactions in the enzyme function landscape. Previously, the use of microfluidic-based high-throughput assays for insoluble substrates has also been shown to be possible (Bharadwaj et al. 2010). The development of such high-throughput techniques may be especially suitable for enzyme engineering in an industrial setting.

Engineering efficient protein secretion in prokaryotic hosts is an essential area of research. One way to direct the expressed protein extracellularly is to attach a signal peptide to the target protein. For example, Camarero et al. included an α-factor prepro-leader sequence to enable secretion of laccase in yeast (Camarero et al. 2012). Subsequently, they used directed evolution to improve secretion by 40-fold. Yildirim et al. targeted the native secretion signal of *E. coli* for the efficient periplasmic and extracellular secretion of Cel5A endoglucanase from *Fusarium graminearum* and showed that the yields of the extracellular enzyme were nearly twofold higher than yields of periplasmic Cel5A (Yıldırım and Çelik 2017). Lee et al. used protein-specific translational fusion partners (TFP) from yeast and fused to cellulases to express these enzymes in *Saccharomyces cerevisiae*. The enzymes were expressed in mixed cultures, and the synergistic effect of the mixed yeast culture was demonstrated by the threefold higher yields than the wild type (Lee et al. 2017). The success of the biorefinery and the final aim of engineering better enzymes are to reduce enzyme loading through an optimized combination of enzymes to hydrolyze the biomass synergistically.

11.7 Other Enzymes in the Biorefinery

Biodiesel is a renewable fuel and may be manufactured from vegetable oils, animal fats, or recycled restaurant grease. Biodiesel can also be synthesized by microbes through the transesterification of triglycerides to produce the methyl (or ethyl) esters of fatty acid of chain length C14 to C22 (Demirbas 2009). There are a few companies already commercially producing the fuel. Lipase and phospholipase are necessary enzymes used for biodiesel production. Lipase is a triacylglycerol acyl hydrolase that esterifies the free fatty acids and triacylglycerol to fatty acid methyl esters. These enzymes are produced by many microorganisms. While the chemical transesterification of methanol is an easy reaction in chemistry, the chemical process requires the removal of the FFAs and the phospholipids before the transesterification reaction and, thus, wasted. Phospholipase converts phospholipids to diacylglycerol, and thus the enzyme can utilize these as substrates and increase the efficiency of the process. As per the literature reports, *Burkholderia cepacia* lipase (BCL) is one of the most widely reported bacterial enzyme used in biodiesel production. The immobilized form of the *Burkholderia cepacia* lipase produces biodiesel in isooctane (Yan et al. 2014). The bacterial lipase from *Pseudomonas fluorescens*, *Pseudomonas cepacia*, and *Chromobacterium viscosum* is also known to catalyze biodiesel production (Ranganathan et al. 2008; Tan et al. 2010). In fungi, while the lipase from *Thermomyces lanuginosus*, *Rhizopus oryzae*, *Penicillium expansum*, and *Geotrichum sp*. is catalytically active and well known, Novozyme 435 from *Candida antarctica* is the most widely used yeast lipase (Ortiz et al. 2019; Ranganathan et al. 2008).

Among the other types of biofuels generated, like gasoline and bioethanol, the advantages of using biobutanol over bioethanol are the higher blending capacities, the higher energy content, and lower moisture affinity. Biobutanol and other bio-product production are primarily based on the anaerobic fermentation of lignocellulosic biomasses by organisms such as *Clostridium sp.* (Jang et al. 2012; Poehlein et al. 2017; Tracy et al. 2012). Biobutanol, like other biofuels, may be categorized into three different generations depending upon the biomass hydrolyzed for its production. First-generation biobutanol is produced from food crops, the second-generation from non-food crops, and the third-generation from microalgae. The second- and third-generation biobutanol is more sustainable as it does not compete with the food crops and is produced from cheaper renewable sources. Butanol is also used in industries like rubber, dye, printing ink, textile, and pharmaceutical industries. The production of biobutanol by *Clostridium sp.* is, however, limited by substrate inhibition, toxicity, and production of other end products like acetone and ethanol, which can act as inhibitors to the hydrolysis process. One way of overcoming this limitation in biobutanol yield is by the development of efficient microbial strains by metabolic and genetic engineering (Bankar et al. 2015; Joseph et al. 2018). The most commonly used microorganisms for biobutanol production are *C. beijerinckii* and *C. acetobutylicum* (George et al. 1983; Nakayama et al.

2011). Mutagenic strategies like UV exposure or N-methyl-N-nitro-N-nitrosoguanidine (MNNG) can increase the product yield of these microbes.

11.8 Conclusions

The biorefinery enables as much usage of the biomass as possible towards the production of chemicals, fuels, and various by-products such that the process is sustainable. The heterogeneity in biomass and the resultant molecular properties along with biomass diversity make finding a suitable pre-treatment technique a challenge. A low-cost pre-treatment method with well-characterized inhibitory products that does not impede the conversion to sugars or further microbial action is an essential requirement in an integrated biorefinery. The current progress in gene synthesis, deep sequencing, molecular biology, throughput screening methods has enabled progress towards discovery and engineering of novel biocatalysts. Understanding the diversity and mechanisms of the biomass-degrading enzymes will enable the search for more efficient biomass conversions. Catalytically efficient enzymes are another crucial requirement for an economically viable product, and thus identifying microorganisms that synthesize such enzymes or are amenable to genetic manipulations to increase the secretion of native or engineered enzymes is a crucial part of the biorefining system. Finally, techno-economic evaluation is critical to facilitate the transition from laboratory scale to industry for an integrated biorefinery. Biomass-based biorefinery towards the utilization of cellulosic biomass for the production of advanced biofuels, biochemicals, and biomaterials is critical to mitigating the current environmental crisis.

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Chapter 12
Lytic Polysaccharide Monooxygenases-Driven Degradation of Biorefinery Lignocellulose

Saikat Dutta

Abstract Bacterial and fungal lytic polysaccharide monooxygenase (LPMO) play an important role for cleavage and conversion of recalcitrant polysaccharides. Notwithstanding, their modes of action has persisted largely obscure till the high-resolution structure was revealed. Alongside, modality of binding of LPMO active site for insoluble substrates and redox site were revealed. The LPMOs disrupt the crystalline packing of cellulose microfibrils to create new chain ends for hydrolase enzymes. LPMOs contain a mononuclear Cu(II) center which is being responsible for C-H hydroxylation. In LPMOs action mechanism, the role of co-substrates (O_2 or H_2O_2) is to oxidize polysaccharide with core of cellobiohydrgenase, endoglucanases, and β-glucosidases. Therefore, LPMOs reduces O_2 in the coupled hydroxylation of substrate and utilizes H_2O_2 generates from the coupled reduction of O_2. The co-substrates and copper-active site dependent mechanism are linked with a Cu reduction to Cu(I) with non-specific oxidation by H_2O_2 and regiospecific substrate oxidation. This article focuses on structural and reactivity of LPMOs for wood biomass degradation by the reduction of active site copper prior to the O_2 activation. The major areas that are focused: (1) spectroscopic analysis of three-dimensional structures of LPMOs (AA9 to AA13), (2) conformation of polysaccharides, (3) catalytic domains, (4) role in cellulose accessibility, (5) penetration and movement on cellulose ribbons. Additionally, structural evolution of the LPMOs, their role in oxidative mechanisms and redox site determination are reviewed. The choice of redox partner and evolution is linked with biorefinery applications for starch, cellulose, and chitin that possess tight organization of polymers with low hydration levels. Mysterious substrate dependence of the LPMO reactivity with H_2O_2 was reviewed to emphasize how the power of LPMO improved further. Prospects of current biorefinery applications of LPMOs were discussed with an emphasis of fungal species.

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Keywords Polysaccharide monooxygenases · Oxidative degradation · Biorefining · Xylan conformation · Active sites · Enzyme cocktail

Abbreviations

CBMs	Carbohydrate-binding modules
CDH	Cellobiose dehydrogenase
DQ	Double-quantum
EPR	Electron paramagnetic resonance
LPMO	Lytic polysaccharide monooxygenase
MAS	Magic-angle spinning
MD	Molecular Dynamics
NMBU	Norwegian University of Life Sciences
NMR	Nuclear magnetic resonance
SHF	Superhyperfine

12.1 Introduction

Wood biomass is the most abundant renewable source of organics with an annual production range of about 5.6×10^{10} tons of carbon. To overcome the recalcitrance of lignocellulosic matrix of woods, biorefineries have been relying on the energy-incentive pretreatment processes to solubilize inaccessible biomass components prior to enzymatic saccharification of the recalcitrant fraction (Biely et al. 2016). The recalcitrant fraction of biomass contains heteroxylans which is highly resistant to xylanases enzymes due to the extensive decoration and adapted flat conformation with xylan chain strongly adhere via hydrogen bonds to the surface of cellulose fibrils (Simmons et al. 2016; Biely et al. 2016; Couturier et al. 2018). In nature, fungi drive the wood decomposition by natural degradation of cellulose and hemicellulose through a library of carbohydrate-active enzymes. The realization of cellulose-based cell wall deconstruction has been overturned by the discovery of fungal LPMO that operates through an oxidative mechanism as opposed to already known hydrolytic mechanism of hydrolase enzymes (Vaaje-Kolstad et al. 2010; Kracher et al. 2016). Since then the discovery of LPMO by the researchers at the Norwegian University of Life Sciences (NMBU) (Agger et al. 2014; Bissaro et al. 2017; Vaaje-Kolstad et al. 2010) a change in biorefinery processes towards sustainability has been witnessed such that now the industrial enzyme mixtures for the conversion of agricultural residues to biofuels include cellulose-active LPMOs. These LPMOs drive the desired transformation of biorefineries to sustainable biocatalytic strategies by banking on the powerful oxidative chemistry of biopolymer degradation.

Beside the LPMOs classification in the CAZy database as auxiliary activities (AA) families AA9, A100, AA11, and AA13, (Quinlan et al. 2011; Harris et al. 2010), discovery of oxidative mode of LPMOs is linked to the amino terminus

nitrogen atom by N-terminal histidine and additional histidine in a structural motif (Phillips et al. 2011; Quinlan et al. 2011). Fungal LPMO families (AA9, AA10, AA11, AA13) are identified based on genome sequence by their modular structure (Aachmann et al. 2012) where from the catalytic domain appended to carbohydrate-binding modules (CBMs). Active site of LPMO is located in the center of extended flat face unlike other polysaccharide enzymes (hydrolases) with grooves or tunnels (Davies and Henrissat 1995) In LPMOs, CBM is connected to the catalytic domain featuring length, sequence, and flexibility driven linkers for attachment (Courtade et al. 2018; Kurašin and Väljamäe 2011). The major focus of current fundamental research on the structural side of LPMOs include (1) distance between CBM and the catalytic domain, (2) movement of CBM, (3) substrate binding affinity, (4) substrate binding zone. By this far, the origin of LPMO-substrate specificity remain scarcely realized; therefore, a complex combination of structural and electronic factors can be anticipated. The NMR analysis and docking studies on AA9 LPMO (found in fungi and preferentially act on cellulose) in contact with oligosaccharides revealed that more extended substrates (long-chain) may exhibit significantly higher binding affinity. In this regard, post-genomic approaches on fungal enzymes, existence of unknown family of LPMOs, a new family AA14 in the CAZy classification differs structurally from the family AA9 to AA13. Distinctly, AA14 enzymes act on xylan bound to cellulose which differentiates AA14 from other LPMOs (Couturier et al. 2018).

The interaction between substrates and LPMO can be scanned from crystal structures of LPMO:oligosaccharide complexes (Frandsen et al. 2016). This reveals that cellulose binds at the subsite via interactions with aromatic residues, N-terminal His and conserved Tyr by hydrogen bonds as additional residues in a contour binding surface on the LPMO. Additionally, how the substrate is bound with an exogenous ligand on the copper ion which is determine by the oxidative mechanism adopted by LPMO. In this case, positioning of the substrate on the LPMO surface is a determinant. The molecular and electronic insights of the LPMO-substrate interaction have been explored by X-ray diffraction (XRD) and electron paramagnetic resonance (EPR) spectroscopic studies.

Generally, O_2 activation via a reducing agent-dependent route on an exposed Cu-active site on the surface of LPMOs is responsible for cellulose cleavage (Frandsen et al. 2016). The copper-dependent LPMOs utilize O_2 and an electron donor to catalyze the oxidative O_2-depedent cleavage of insoluble polysaccharides through depolymerization of C1 and C4 position of the scissile glycosidic bonds to generate oxidized glucose units such as aldonic acid and 4-ketoaldose (gem diols), respectively (Villares et al. 2017). Thus, the effect of LPMO actions on structural disruption of the cellulose insoluble residue is a fundamental issue which paves the way to better understanding of LPMOs mechanism. In this context, the spatial position of the aromatic residues involving perpendicular orientation across trajectory of the polymer determines the binding at the cellulose microfibrils. LPMO activity on the insoluble fraction of cellulose needs more attention as they are even more complex and realistic substrates (Villares et al. 2017).

The chemical biology of lignocellulose deconstruction is an intricate and complex process. This process depends on the microorganisms which rely on simple to complex arrangements of glycoside hydrolases to deconstruct most of the polysaccharide via an oxidative mechanism catalyzed by active sites of LPMOs (Himmel et al. 2007). Traditionally, the biopolymer degradation requires hydrolytic enzymes and thermal treatment to overcome remarkable recalcitrance due to hydrogen bonding of the cellulosic microfibrils (Simmons et al. 2016). Aldonic acid or 4-keto sugar products are resulted at the oxidized ends of LPMOs due to cleavage of glycosidic bond from a subsequent elimination process. The LPMO's oxidative action cleaves glycosidic bonds in polysaccharides which is inaccessible for hydrolytic enzymes such as endoglucanases and cellobiohydrogenase (Aachmann et al. 2012). LPMO includes a flat substrate binding surface and the solvent-exposed active site containing two histidines that coordinates a copper ion in a histidine brace. Therefore, generally LPMOs boost the overall efficiency of enzyme for the insoluble polysaccharides degradation (Cannella and Jørgensen 2014). The role of copper ion is to reduce the level of dioxygen that needs electron supply from external sources. In this process, the reduced O_2 captures hydrogen from a suitable substrate for cleaving β-1,4 glycosidic linkages by resulting oxidation of glycosidic bond (Beeson et al. 2012). An exclusive oxidation of C1 by some LPMOs and the same for C4 are known including some LPMOs that oxidize either C1 or C4 including double-oxidized products wherein C1 and C4 oxidation takes place in combination. The abundance of LPMOs in the genomes of biomass-degrading organisms suggest that in-depth structural studies of these enzymes highly desired. At the same time, fungal LPMO9s have been relatively better known for oxidizing C1, C4, or both in contrast to bacterial cellulose-degrading LPMO10s. In total, 34 reducing agents were resourced from plant-derived flavonoids and lignin-derived blocks for the LPMOs from fungus *Myceliophthora thermophile* C1 and others with varied substrate preferences (Frommhagen et al. 2016). It was found that 1,2-benzenedion or 1,2,3-benzenetriol moiety are the source of most of the oxidized as well as non-oxidized gluco-oligosaccharides for different types of *Mt*LPMOs derived from *Myceliophthora thermophile* C1. LPMOs not only vary in their C1-/C4-regioselectivity and substrate specificity, but also in their reducing agent specificity. For example, ascorbic acid donates electron to the LPMOs with similar efficiency as intrinsic mediators in biorefinery processes. When using higher refractory xylan-coated cellulose fibers as substrate, two LPMO members AA14 (*Pc*AA14A) originated from the white-rot fungi *Pycnoporus coccineus* that significantly pronounced the wood saccharification (Couturier et al. 2018). The xylans are susceptible to AA14 oxidative cleavage only when absorbed onto crystalline cellulose. Therefore, in addition to *Pc*AA14A to a GH11 xylanase significantly increased the release of xylo-oligomers from birchwood cellulose fibers.

Electron donors control the oxygen activation event such as cellobiose dehydrogenase (CDH), small-molecule reductants and photosynthetic pigments (Kracher et al. 2016). Various LPMO-activating mechanisms redox enzyme-based election systems with small-molecule reductants (e.g. ascorbate) were compared by biochemical and electrochemical methods. This extracellular electron transfer of LPMOs

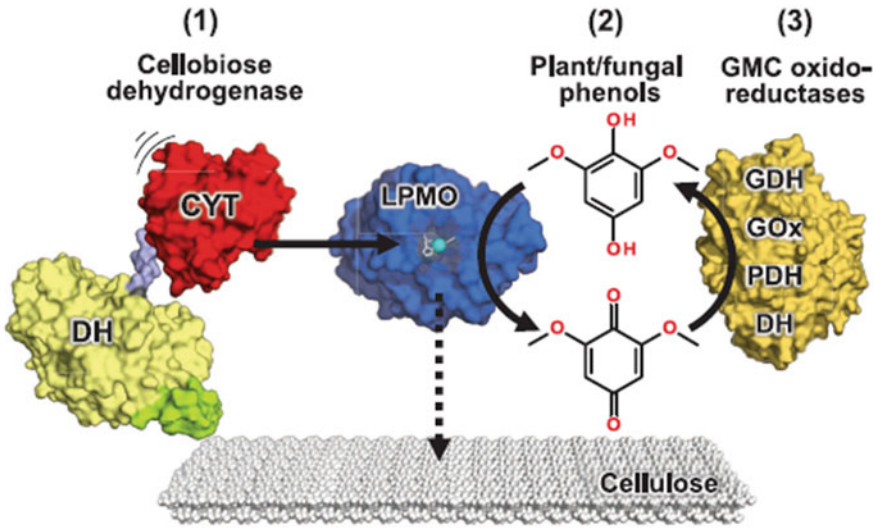


Fig. 12.1 Oxidative and hydrolytic mechanism. (1) CDH transfer electron, (2) fungal phenol as source of electrons, (3) GMC oxidoreductases (GDH) reduce LPMO active site (Adapted from (Kracher et al. 2016), copyright AAAS, 2016)

activation initiates the cellulose degradation via three-electron transfer systems that reduce the LPMO (blue) active site copper (cyan) to attack on crystalline cellulose (Fig. 12.1). The carbohydrate-binding modules (CBMs) usually target known substrates when the CBM is affixed with the fungal LPMO site.

Therefore, we aim to review origin of biomass recalcitrance in the context of binding of crystalline substrates with LPMO including catalytic domain. Activation of redox partner enzymes of LPMOs at the redox site determines the mode of bindings of crystalline substrates. It is evident that mode of bindings of crystalline substrates on LPMOs are not fully realized while the high-resolution structure of catalytic domain appears as convincing. Our goal is not limited within selective LPMO studies, but we focus on stimulating research on the LPMO active site which includes the role of catalytic domain in xylan and cellulose microfibril deconstruction mechanism. While reviewing the specific cases including overall realization of structure-reactivity relationships of LPMOs, we tend to magnify the role LPMO active sites for creating links between biocatalytic processes and practical biorefineries for selective enzymatic upgradation for high-value fuels.

12.2 Origin of Biomass Recalcitrance

The inherent recalcitrance of lignocellulose a one of the major barrier to degradation, is a consequence of both the heterogeneous composition and semi-crystalline association of the polymer (Marriott et al. 2016). Certain molecular interactions contribute to the recalcitrance to enzymatic digestion. Cell wall recalcitrance originates from the route by which the sugars are obtained from lignocellulose. Therefore, it is important to recognize the molecular basis of recalcitrance. What is meant by the secondary molecular structure of cell wall is that the molecular arrangements of the polysaccharides units and similar components based on their three-dimensional conformations. The semi-crystalline cellulose microfibrils consist of β-(1 → 4)-D-glucan in twofold helical screw conformation (one 360° twist per 2 glycosidic bonds) and cellulose microfibrils binds with xylan and glucomannan. The interaction of cellulose and xylan is expected to contribute most profound effect on cell wall recalcitrance. In this case, multiple layers of xylan could envelop and crosslink microfibrils, however, no clear evidence has been found.

Xylan can be linked to hydrophilic surfaces of cellulose micro fibrils by folding the xylan-chain conformation as twofold helical screw based on the alternate xylosyl residues (Simmons et al. 2016). Consequently, xylan forms a threefold helical screw (one 360° twist per 3 glycosidic bonds) in solution. To overcome the recalcitrance of woody biomass, biorefineries primarily utilize energy-intensive pretreatment process to solubilize the inaccessible biomass components before enzyme cocktail-driven saccharification. The recalcitrance is enhanced due to extensive decoration of cellulose fibrils and xylans in the secondary cell wall and an adapted flat conformation of xylans with their chains solidly adhering via hydrogen bonds to the surface of cellulose microfibrils (Fig. 12.2a) (Simmons et al. 2016; Biely et al. 2016). Cellulose-xylan interactions in the secondary cell wall (Fig. 12.2b) in which non-cellulosic polysaccharides binds to cellulose microfibrils where major domain of xylan interacts on the hydrophilic cellulose surface. Xylans are induced to adopt twofold screw conformation upon binding to cellulose hydrophilic face of cellulose fibrils (Busse-Wicher et al. 2016a) with a crosslink by minor domain of xylose.

^{13}C solid-state magic-angle spinning (MAS) nuclear magnetic resonance (NMR) spectroscopic analysis has revealed that xylans adopt distinct two- and threefold screw conformations (*Arabidosis stems*). Double-quantum (DQ) chemical shift of CP-INADEQUATE ^{13}C NMR suggests that change in xylan conformation from threefold to twofold screw is cellulose-dependent of which twofold screw conformation is cellulose-bound and relatively immobile (Wang et al. 2015). Xylan is induced to assemble on cellulose fibrils as twofold helical screw in secondary cell walls and such interactions on the hydrophilic surface of cellulose fibrils influence the cell wall recalcitrance (Busse-Wicher et al. 2016a; Busse-Wicher et al. 2014). Theoretical model based on molecular dynamics (MD) simulation demonstrate that hydrogen bonds can link the hydrophilic surfaces of cellulose and the unsubstituted face of xylan at its twofold helical screw conformation (Busse-Wicher et al. 2016b). Xylans folded on the cellulose surfaces is likely to be resistant to the action of

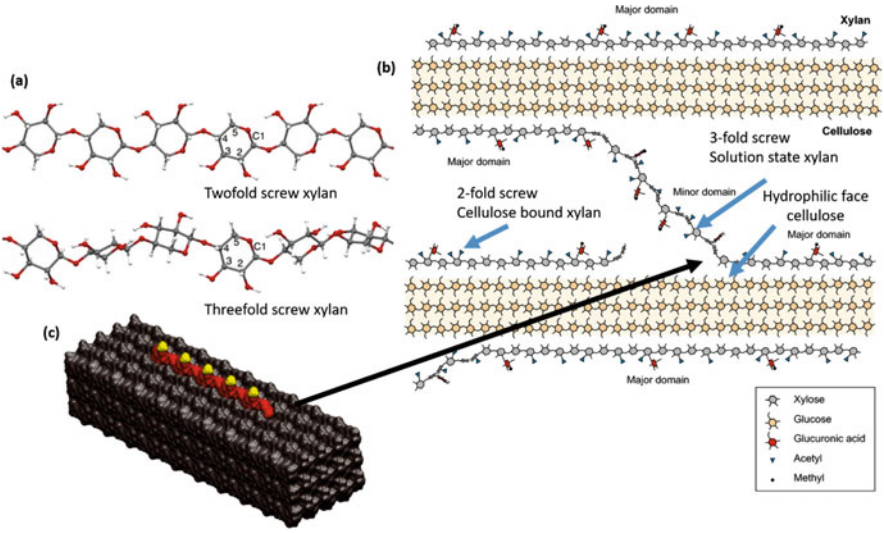


Fig. 12.2 In silico DFT-optimized xylan structures with xylan flat conformation (**a**), cellulose–xylan interactions in the secondary cell walls (**b**) (Adapted from (Biely et al. 2016), copyright Nature Journals, 2016). Docked model of acetylxylan chains interaction with a 24-chain cellulose microfibril on hydrophilic face (010) and (020) correlating the spot of such interaction with arrow sign to hydrophilic surface in (**c**) (Adapted from (Simmons et al. 2016), copyright Plant Journal, 2016)

microbial hydrolases. A confined docking condition of xylan is suitable on hydrophilic surface of cellulose which occurs for xylan decorated residue that retains a twofolded screw conformation (Marta et al. 2014). Xylan decoration accommodated on one side (a) or on either side of a ribbon (b) in case of acetylated xylan, however, steric factor prevents interactions between acetylated xylan and the hydrophilic face of cellulose irrespective of arrangement of cross sections (Busse-Wicher et al. 2014). Therefore, the ability of decorated xylan for interacting with cellulose microfibril surfaces depends on the pattern of xylan acetylation and xylosyl residue, which contributes in secondary cell wall strength by twofold helical screw (Manabe et al. 2013). Therefore, first atomic-scale arrangement of two distinct polymers (cellulose, xylans) discloses remarkable specificity of polysaccharide interactions on the basis of mechanical function of cell wall recalcitrance (Simmons et al. 2016). LPMOs initially deconstruct cellulose via the route of hydroxylation at C1 and C4 centers of the scissile glycosidic bonds followed by subsequent elimination to results cleavage of the glycosidic bond and formation of aldonic acids or 4-keto sugars on oxidized ends of cellulose surface (Hemsworth et al. 2013a; Walton and Davies 2016). The LPMO enhances degradation of all major polysaccharides including cellulose, hemicellulose, and starch by providing new chain ends as starting point for hydrolases. The LPMOs use a single active site copper ion to activate O_2 and hydroxylate the polysaccharide backbone which leads to chain break (Beeson et al. 2015). It is

also relevant to emphasize on how disruption of crystalline cellulose opens new binding sites for cellulases (Villares et al. 2017).

12.3 Mode of Binding of Soluble Substrates

The nuclear magnetic resonance spectroscopy (NMR) and crystallographic characterization are the key techniques which reveal the various interactions between the catalytic sites of fungal LPMO and soluble cello-oligosaccharides (Lo Leggio et al. 2015). The atomistic details of the interaction between the catalytic domain of the LPMO and a single sugar chain can be derived by crystallographic technique which offers insight of the effect of substrate binding on the LPMO copper site (Biely et al. 2016). The effect of substrate binding of an LPMO was first described by Borisova et al. (2015) by using electron paramagnetic resonance (EPR) spectroscopy. The EPR technique is capable of determining whether the unique features of *Nc*PMO9C reflects in the electronic structure of the active site copper ion that designated as type 2 copper active site. By the addition of soluble substrates (cellohexanose or tamarind seed xyloglucan) to Cu^{+2}-loaded *Nc*PMO9C led to the changes in EPR spectra which acts as an evidence of substrate binding effects on the copper site. The EPR spectral superhyperfine splitting reflects the interaction between the unpaired electron and nitrogen nuclei adjacent to the Cu^{2+} gets greatly enhanced.

Nevertheless, nature of interactions between an LPMO active site and a soluble substrate differs from interactions with a substrate embedded in a crystalline lattice despite similarities. Currently, computational calculations, based on certain theoretical interpretation of interactions and bonding, allow simultaneous atomic-scale visualization of enzyme active site and a crystalline substrate (Dutta and Wu 2014; Dutta et al. 2014). The combined analysis of structural modelling and electron paramagnetic resonance (EPR) spectroscopy pattern determines structure of LPMO-oligosaccharide complex and interaction parameters of LPMOs and saccharide substrates (Frandsen et al. 2016). The interaction between *Ls*(AA9)A and oligosaccharide cellohexaose (G6) in solution phase was determined by using continuous wave (cw) X-band EPR spectra of the Cu(II)-site under low- and high concentration of chloride ions of which under low-chloride conditions, a substrate-induced perturbation of the copper site with a small increase in $|A_z|$ (458–515 MHz) including decrease in g_y and g_z with the appearance of superhyperfine (SHF) coupling results due to an increase in magnetically coupled two to three N nuclei. This suggests addition of substrates result in larger contribution of the N terminus to the singly occupied molecular orbitals (SOMO) on Cu(II) (Frandsen et al. 2016). For example, in the low-dose X-ray structure of *Ls*(AA9) A, a configuration of Cu (II) center linked to multiple His-N atoms in addition to a water molecule linked with Cu-N(His1) (1.9 Å) units in a square planar geometry (Fig. 12.3a). Association of structures with unambiguous oligosaccharide density can be linked to the putative binding surface of *Ls*(AA9)A (Fig. 12.3b). The terminal glucosyl unit at subsite +2 is

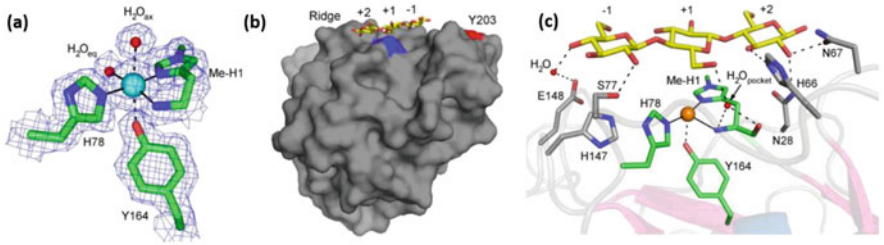


Fig. 12.3 (**a**) Electron density of Cu(II)center with low X-ray dose; Cu(II) blue sphere; (**b**) Ls (AA9)A-G3 (low) with Tyr203 is shown in red; (**c**) Interactions between G3 and the binding surface of Ls(AA9)A in Ls(AA9)A-G3. (Adapted from (Frandsen et al. 2016), Copyright Nature Publishing, 2016)

held within a hydrogen-bonding network with protein residues Asn28, His66, and Asn67 (Fig. 12.3c).

Most residue of LPMOs form hydrogen bonding with oligosaccharides. The substrate binding orientation of *Ls*(AA9)A determines close approximation at the copper site on which C1-H subsite −1 and the C4-H subsite +1 appears in near proximity to copper site through ligands. The oxidized C4-H bond is closer to the exogenic ligand on copper as opposed to C1-H which reflects from the exclusive oxidation of C4-H of subunit +1. However, a small shift in substrate positioning could easily alter the balance between C1 and C4 oxidation. Soaked crystals of *Ls* (AA9)A with cellotriose (G3) or cellohexose (G6) at lower pH (5.5) generate new structures with unambiguous density associated with oligosaccharide in contact with the putative binding surface of Ls(AA9)A (Quinlan et al. 2011). The terminal glucosyl at the active site with subunit +2 finds within a hydrogen bond networks that feature protein residues such as Asn28, His66, and Asn67 by anchoring the unit at a fixed position. The glycosyl unit at subsite +1 stacked directly on top of His1 via a lone pair-aromatic interaction. At subsites +1 and − 1 over the O_2 binding site on Cu ion which bridges the C4-H and C1-H linkage in +1 and the −1 respectively. These interactions are combined contributions of hydrogen bonds, lone pair-aromatic interactions, and CH-π interaction with protein residues at fixed aspect of active Cu site. This lack of distortion supports that LPMO catalysis is mediated largely by the exceptional chemical environment of copper histidine brace active site as compared to influence of enzyme-mediated distortion of the substrates.

Based on the orientation of enzyme on a single-chain substrate by molecular dynamics (MD) simulations interactions of LPMO and three different topologies of crystalline chitin for polysaccharide chain cleaving (Bissaro et al. 2018a). This features a constrained active site geometry including tunnel connecting bulk solvents to copper active sites (Fig. 12.4a) for diffusing small molecules such as H_2O, O_2, and H_2O_2. In this case, rearrangement of Cu-interacting with water molecules is essential when binding substrate for C1 oxidative regiospecificity. This interaction within LPMO active site and substrate chitin involves confined active sites which effectively determines the chemical oxidative process of the enzyme through its active

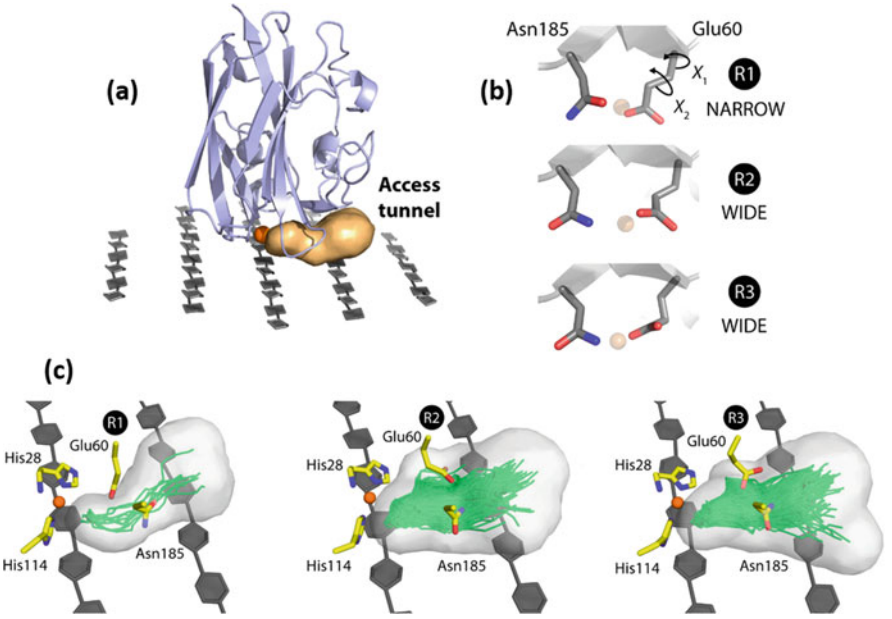


Fig. 12.4 (**a**) An access tunnel (beige) by binding of SmAA10A to the polysaccharide by connecting copper site (orange sphere) with the bulk solvent. (**b**) Glu60 displays three main rotamer populations R1 − R3 influenced by shaping of the tunnel. (**c**) Population of R1 (left), R2 (middle), and R3 (right). (Reproduced from (Quinlan et al. 2011) with permission, Copyright American Chemical Society 2010)

site. It was found that Glu60 residue substrate interactions exhibit multiple rotamers (R1-R3) that possibly influence on the access to active site (Fig. 12.4b). β-chitin model and R1 state that resemble a "closed" tunnel (Fig. 12.4c). Glu60 residue side chain develops a conformer that rotates away from the crystalline surface which affects the access to Cu-active site.

Amino acids on the LPMO surface preferably interact with the polysaccharide chain such as SmAA10A is selective for cleaving chitin chains at crystalline edges with very narrow substrate surfaces interaction. The LPMO-substrate interaction features were reinvestigated that includes a certain constrained associated with catalysis center that directs restricted oxidative chemistry by LPMOs (Bissaro et al. 2018a). This study on monocopper active site modelling magnifies the fact that, for the C1 oxidative process, the confinement of catalytic center with water molecules are prerequisite for binding the crystalline chitin to form the LPMO-chitin complex. In this process, computations based on the EPR spectral Hamiltonian parameters were adapted to calculate hyperfine spilling which accommodates contribution from the (1) magnetic spin–dipole interactions and (2) second order spin–orbit coupling.

NMR and isothermal titration calorimetry (ITC) results revealed that the copper ion alone hardly contributes to the substrate binding affinity as revealed from

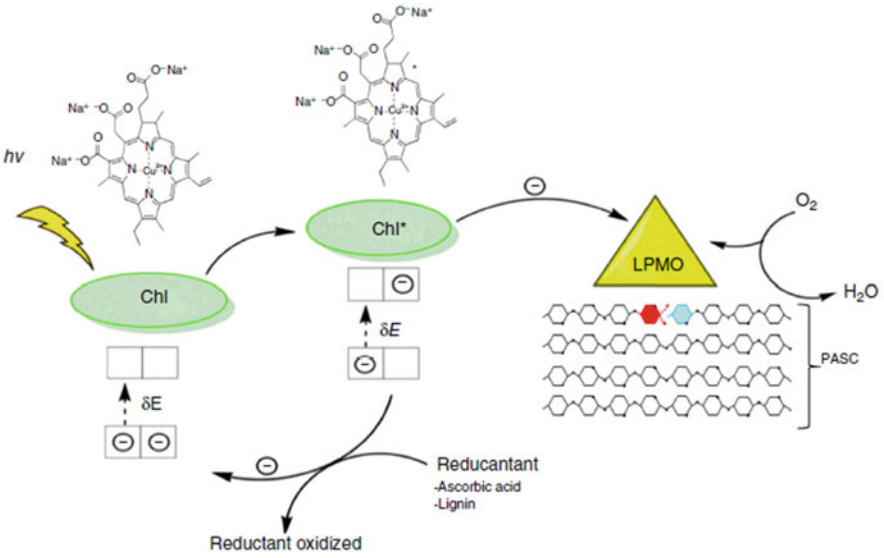


Fig. 12.5 Proposed mechanism for light-induced electron transfer to LPMO with reduction of copper ion that activates oxygen to oxidize polysaccharides. The oxidized pigment undergoes reduction with electron from a reductant (Adapted from (Cannella et al. 2016), Copyright Nature Publishing, 2016)

interaction with cellohexaose around copper site (Courtade et al. 2016). Studies with cellobiose hydrogenase (CDH) including its isolated cytochrome domain (heme *b*) that was found as unambiguous on interaction of cytochrome domain of CDH with the copper site of the LPMO. Such substrate binding forbids any interaction with CDH. The LPMO surface interacts with the varying substrates which results in electron transfer before substrate binding. Requirement of an extracellular electron donor for oxidizing recalcitrant polysaccharides using LPMOs with the use of a photosynthetic pigment as electron donors in the presence of light which results a 100-fold enhancement (Cannella et al. 2016). A strategy of a light excitation of the pigments generate a strong reductant for reducing copper ion in the LPMO active site (Fig. 12.5). In this process, oxidized form of the pigments is reduced by a reductant with lower redox potential (ascorbic acid, lignin). Such biotic mechanism of light-driven degradation within the natural environments of plant-degrading organisms form a highly reactive system that leverages from high-energy to boost the degradation of plant cell walls.

The reduced redox state of the active site copper is responsible for an increased affinity towards cellulose, however, the lower affinity of oxidized LPMO results desorption after catalysis event which paves the way for hydrolases action on the cleavage site. The copper reduction is not necessarily occurred in the substrate-bound state of LPMO. A stabilizing effect of cellulose and xyloglucan on active enzyme was evident from using differential scanning fluorimetry technique applied to Cu-saturated bacterial LPMO which showed oxidative auto-inactivation and

destabilization in the absence of a substrate (Kracher et al. 2017). The major difference of considering the reducing state of enzyme which reduces deleterious uncoupling reactions of reduced Cu which limit H_2O_2 formation.

The LPMO crystal structure was determined from fungus by using an x-ray absorption fine structure scanning which analyzes metal binding with a focus on conservation of active site residues and surface as a structural alignment of *Pch*GH61D and with other LPMOs (Wu et al. 2013). The recently reported crystal structure of the catalytic domain of NcLPMO9C displays a typical core LPMO structure: two β-sheets forming a β-sandwich fold with multiple loops protruding from the β-sandwich (Borisova et al. 2015). This enzyme contains a flat surface containing copper site which is coordinated by histidine brace consisting of N-terminal amino groups (His1), the side chain of His-1 and the side chain of His83, Tyr166 also tunes the copper site. Therefore, formidable challenges for large-scale applications for cellulose, starch, and chitin which give rise to tightly packed organizations of polymer chains with minimum hydration levels that originates recalcitrance to enzymatic degradation. Thus, oxygen activation by the Cu site of LPMOs while interacting with the recalcitrant polysaccharides is a crucial phenomenon to fully realize the major role of Cu-active sites (Meier et al. 2018).

Generally, an extended highly polar substrate binding surface interacts with a variety of sugar substrates which enables LPMO-substrate to act as soluble substrates. The EPR technique helps in finding that Cu^{+2} active site environment senses chemical and physical changes upon substrate binding. Isothermal titration calorimetry (ITC) technique helps in revealing the binding affinities in the low micromolar range for polymeric substrates that remains a part of the carbohydrate-binding module (CBM1). Regioselectivity either C1 or C4 of LPMO9s for oxidation contains distinct environment for Cu-center. Access to the solvent-facing axial side is restricted by a conserved tyrosine residue in a C1 oxidizing LPMOs in which the same face remains unrestricted for C4 oxidizing LPMO9s. The differential scanning fluorimetry was also used for determining the stabilizing effect of the cellulose xyloglucans on the apparent transition midpoint temperature of the reduced LPMO. However, limited knowledge on identifying differences of LPMOs interacting with substrates embedded in crystalline lattice (heterogeneous substrate) and soluble substrates have set certain limitations. The orientation of LPMO on the cellulose chain was determined by analogy to molecular dynamics studies performed in CBMs which lacks sufficient experimental probe.

Few major lessons learned from both experimental and molecular modelling studies are as follows: (1) copper reduction is not necessarily performed in the substrate-bound state and desorption of oxidized LPMO occur due to the lower affinity for cellulose substrates. (2) Effective extracellular electron donor pigments when combined with LPMO and a reducing agent. This results in increase in electron flow by exposing into light that enhances LPMO oxidative activity significantly which offers a highly reactive and stable light-driven system. (3) Experimental confirmation of computational results can be done by using EPR spectroscopy which confirms binding-induced effects on LPMO active site such as orientation of LPMO and regioselective mode of crystalline substrate binding. One of the major

voids in interactions of substrates and LPMOs is the correlations between surface architecture and substrate specificity which originates from the diversity of loops and surface topologies displayed by LPMOs which may reflect the diversity of substrate structure (topologies and decorations). Limited knowledge in fine-tuning the copper active site of LPMOs such as (1) increase of cellulose affinity, (2) reduced form of the copper active site, and (3) subsequent formation of the activated oxygen unable to enhance the affinity for cellulose. Beside insights into LPMO-substrate interactions, possible mechanisms for electron supply for LPMO action is an open question.

Based on the sequence characteristics, LPMOs activity have broadened by its cleavage of soluble cellodextrins, hemocelluloses with β(1 → 4)-glucan backbones, and starch α(1 → 4)-D-glucan. Determining the O_2 binding mode for the regiospecific oxidation (C4 or C1) is essential for the modern biorefinery. The geometry of the enzyme-substrate complex is influenced by the structural variation at the exposed axial site; therefore, causing a different oxidative feature and directly relates with the selectivity of the product. Starch holds an α(1 → 4) and another α(1 → 6) linkages in higher order structures as opposed to other biopolymer (chitin, cellulose). A redox partner of cellulose-active LPMOs serves as the electron donor to the active site containing one copper atom per protein molecule coordinated by two histidines as confirmed by X-ray absorption spectroscopy and sequence analysis (Vu et al. 2014). The discovery of starch-active enzyme reveals the oxidative mechanism of glycosidic bond cleavage over cellulose and chitin (Vu et al. 2014).

12.4 High-Resolution Structure and Catalytic Domain

Tertiary protein structure of LPMO families' share a core immunoglobulin-like *β*-sandwich topology in which *β*-strands are linked by loops of variable length and structure. A relatively flat surface containing the catalytic site with a mononuclear copper center which is bound to two conserved histidine residues (N-terminal residue and amine) that are referred as the histidine brace (Meier et al. 2018). The first discovered structure CBM33 (AA10) was of SmLPMO10A (CBP21) which contains a fibronectine type III fold which was named after the third β-sandwich domain of glycoprotein fibronectin (Vaaje-Kolstad et al. 2005). First structure and early years of discovery of selected AA10 structures (2008–2010) which includes metal site, oxidation state by electron density mapping, identification of substrate binding surface, regiospecificity of substrate binding are the key features that address a family of LPMOs (Frandsen and Lo Leggio 2016). The copper based LPMO enzymes undergo a mechanism involving a divalent metal ion (copper), molecular oxygen, and an electron donor (Lombard et al. 2014). The AA9 LPMOs found in the exoproteome of *Podospora anserine* (Pa) which harbors a CBM1 domain to release oxidized oligosaccharides (Bennati-Granier et al. 2015). Fungal and bacterial origin AA9 and AA10 families of which AA9 is non-selective for oxidizing carbon positions (C1, C4, C6) in the presence of reducing equivalents and AA10 acts on

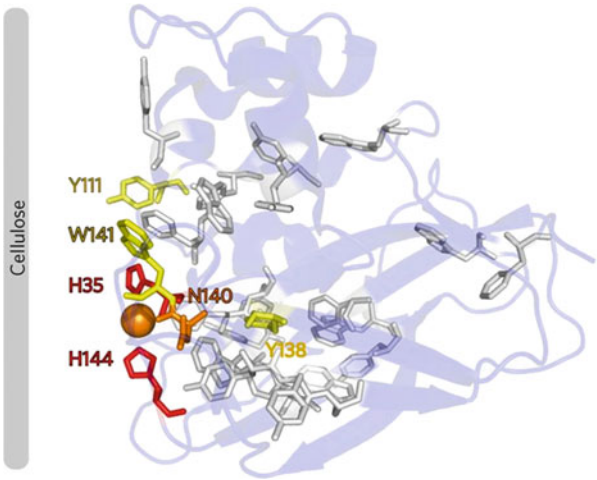


Fig. 12.6 Location of oxidative modifications in ScLPMO10C catalytic domain where oxidative damaged site was determined near the Cu on the catalytic domain histidine (H35 and H144). (Adapted from (Bomble et al. 2017), Copyright Nature Publishing, 2017)

the C1 positions of cellulose and chitin (Bomble et al. 2017). In addition, PaLPMO9H acts on the mixed-linkage glucans, xyloglucans, and glucomannan via C4 oxidative cleavage of mixed-linkage glucans (Fanuel et al. 2017). LPMOs derived from *Thermoascus aurantiacus* when included with commercial cellulase, a significant enhancement in cellulose solubilization under O_2 was found (Müller et al. 2015).

The 3D structure of AA13 holds a histidine brace active site and extra conserved histidine where the active site resides within a shallow groove unlike the binding surfaces of other LPMOs (Lo Leggio et al. 2015). The location of oxidation site and oxidative damage on the inactivated LPMO can be determined by mapping of modified residues on the catalytic domain of a cellulose-active LPMO from *Streptomyces coelicolor* (ScLOMO10C) with H_2O_2 as co-substrate for polysaccharide oxidation (Bissaro et al. 2017). It is disclosed from mapping of modified residues that N-terminal residues sensitive to oxidative modification (Fig. 12.6). Despite many high-resolution LPMO structures, ultrahigh-resolution structure of a catalytically competent LPMO for unambiguous identification of H atoms is yet to be found. A neutron diffraction data have been collected from a *Jd*AA10_A enzyme crystal (Bacik et al. 2015), which claimed as first high-quality room-temperature structure of a completely non-photo-reduced LPMO. The AA9 LPMOs are associated with the typically cellulose-binding CBM1 (Book et al. 2014). AA10 enzymes are often connected with cellulose-binding CBM2 or CBM3 or chitin-binding CBM5 or CBM12 cumulatively (Book et al. 2014). Recently, a new family of chitin-binding CBM73 was revealed from characterization of a module of unknown function of LPMO from *C. japonicus* (Forsberg et al. 2016). Non-catalytic CBMs appended or substituted in LPMOs binds tightly to the cellulose in which case the appended CBMs retains the LPMOs on the substrate surface, however, effect of CBM is substrate- and enzyme-specific (Crouch et al. 2016). The interplay of CBMs and LPMOs is far beyond the enzyme-substrate proximity influenced by the

LPMOs. From the sequence of developments in understanding the high-resolution structure and catalytic domain. Some general features set the guidelines for LPMO catalytic domain. (1) polysaccharides and dioxygen onto the LPMO is synergistic, (2) superoxide generation is controlled by substrates, (3) substrate bindings by forming cavity at the active sites to accommodate other reactive species, (4) changes in coordinating power of N terminus that stabilize Cu(II) state.

12.5 Activation and Redox Partners of LPMOs

LPMOs are electronically activated by cellobiose dehydrogenases (CDHs) in combination with other recycling enzymes (Lee et al. 2014). The AA3_2 flavoenzymes are secreted to trigger the action of AA9 LPMOs for oxidative cellulose degradation. Glucose dehydrogenase and aryl-alcohol quinone oxidoreductases are efficient electron donors among the flavoenzymes with redox potentials compatible with electron transfer between their partners (Garajova et al. 2016). The O_2 affinity of the LPMO's and its partners is key feature that determines the extent of their cooperation. In flavoenzymes, cofactor environment and pH determine the redox potential. The redox potentials of the flavoenzyme AAQO1 (+86 mV) is in the same range of the cytochrome domain of *Neurospora crassa* CDH (+99 and + 93 mV) and *Phanerochaete chrysosporium* CDH (+130 mV) but lower than LPMOs responsible for electron transfer from AAQO1 to LPMOs. The LPMOs may rely on a series of electron donors among CDHs, GDHs, and AAQOs. These enzymes are co-secreted by fungi as found for cellobiohydrolases and β-glucosidases which also release CDH and GDH substrates (cellobiose and glucose, respectively) under hydrolysis condition. This forms a basis for extending the fungal redox partners by tuning up oxidative degradation of cellulose. LPMO-driven lignocellulose degradation can further be boosted with polyphenol oxidase enzyme (*Mt*PPO7) as redox partner that stimulates the methoxylated phenolic moiety predominant in lignin building block (Frommhagen et al. 2017). A thioether bridge within a cysteine and histidine residues resembles as the monophenolase and diphenolase activity. This ancillary redox partner (*Mt*PPO7) to LPMO boosts the release of oxidized gluco-oligosaccharides in cellulose degradation including a strong correlation between genes encoding *Mt*PPO7 like proteins and AA9 LPMOs.

12.6 Molecular Mechanism

It was found in LPMOs driven oxidative cleavage of polysaccharide on the C1 and/or C4 of glycosidic bonds. The site-directed mutagenesis and enzyme assay for screening are the two-general approach with a direct correlation with LPMO functionality. In structural determination of C1 and C4 oxidation, a small shift in substrate position can alter the balance between C1 and C4 oxidation. The copper

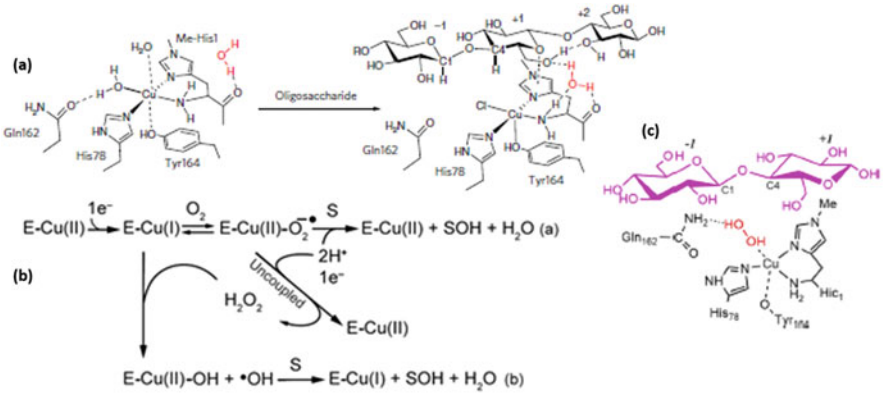
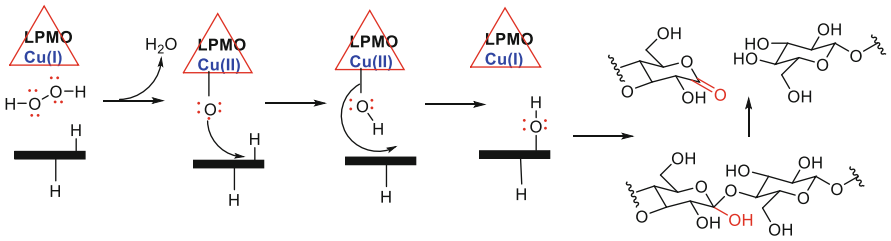


Fig. 12.7 (**a**) Drawn active site of LPMO embraced with histidine residues upon oligosaccharide binding, (**b**) O_2 binds to the Cu(I) active site in the presence of a polysaccharide substrate where O_2 reduction leads to substrate hydroxylation (pathway a). When the polysaccharide substrate is bound, substrate oxidation will occur (pathway b). E, PMO; S, polysaccharide substrate. (**c**) Cu site with ligating His ring atoms of LPMO-Cu(I) site

centers in *Ls*(AA9)A are complemented by a hydrogen-bonding network between water and the N terminus on substrate binding side as drawn in Fig. 12.7a for the embraced active site of LPMO with histidine residues (Lee and Karlin 2015; Frandsen et al. 2016). Therefore, the H-binding network facilitates to overpower the basicity of the N terminus.

It was found that the aromatic surface residues influence the C1/C4 oxidation ratio in LPMO9A of *Hypocrea jecorina* which comes from a regioselectivity indicator based on time-course of high-performance anion-exchange chromatography coupled with pulsed electrochemical detection (HPZEC-PAD) signals. The detection of C1-oxidized sugars confirm the differences in regioselectivity of LPMOs for which aromatic surface exposed residues play a key role in binding of the substrate. Moses et al. reported that the surface aromatic residue composition determines LPMO regioselectivity with C1 mode (Moses et al. 2016). A homology model based on 3ZUD as a template was used for identifying all surface exposed aromatic residues of *Hj*LPMO9A (Danneels et al. 2017). This is as per the hypothesis of orienting the oxidative force of the copper ion on C1 or C4 glycosidic position in which aromatic residues determine oxidation site (Hemsworth et al. 2013a).

An investigation on the co-substrate dependence on the oxidative degradation of cellulosic substrates by a C4-oxidizing LPMO depending on co-substrates (O_2 and H_2O_2) and solubility. The mechanistic study of O_2 versus H_2O_2 reactivity with LPMOs leads to uncoupling of O_2 activation (reduction) from substrate oxidation in which distinguishing oxygenase activity from peroxygenase results from in situ generation of H_2O_2 (Hangasky et al. 2018). The glycosidic bond connecting the second and third glycosyl units is positioned over the Cu-active site, such that regionselective oxidation would generate Glc2ox [β-D-xylo-hexos-4-ulopyranosyl-(1 → 4)-β-D-glucopyranosyl] which would subsequently hydrate in a nonenzymatic



Scheme 12.1 Putative LPMO-guided H_2O_2 splitting mechanism for oxidative cleavage of polysaccharides

chemical step to form a germinal diol [Glc2gem, 4-hydroxy-β-D-xylo-hexopyranosyl-(1 → 4)-β-D-glucopyranosyl] as the only products. The reaction schemes for PMOs (Fig. 12.7b) in which O_2 binds to the Cu(I) active site in the presence of polysaccharide substrate and O_2 reduction lead to substrate hydroxylation.

QM/MM (quantum mechanical/molecular mechanical) calculations suggest that O-O bond of H_2O_2 breaks via one-electron transfer from LPMO-Cu(I) site to form an HO* radical which is stabilized by hydrogen-bonding network of the enzyme that directs HO* radical for abstracting an H atom from the Cu(II)-OH via caged-radical reaction mechanism (Wang et al. 2018a). A small enzyme model consisting of high-resolution crystal structure and Cu site with ligating His ring atoms in which case the resulting structure (Fig. 12.7c) contains a Cu(I) state of Ls(AA9)A and cellotriose. The H_2O_2 binds 3.5 Å distance from the Cu(I)-site which forms hydrogen-bonding interactions with Gin162 and the C3-OH at the subsite +1. The difference in H_2O_2 binding pattern reveals key contribution of secondary sphere hydrogen-bonding interactions in aiming H_2O_2 on the active site pocket. In this case, enzyme H-bonding capability directs the HO* radical which abstract hydrogen from Cu (II)-OH. This inspires engineering of LPMOs via H_2O_2-depedent pathways of the O_2 activation processes.

By controlling H_2O_2 supply, stable reaction kinetics are achieved so that the LPMOs turns on in the absence of O_2 with the fueling of reductant and offers new perspective on the mode of copper enzyme action (Bissaro et al. 2017). This investigation on LPMO-guided H_2O_2 splitting mechanism reveals that the LPMO-Cu(II) unit is the first to be reduced to LPMO-Cu(I), with a subsequent interaction with H_2O_2 when the substrate sitting on active site. By eliminating a water molecule, a Cu(II)-oxyl or Cu(III)-oxo species generate which can abstract H atom by merging of Cu(II)-OH with the substrate radical through a mechanism that regenerates Cu (I) via hydroxylation (Scheme 12.1). This is followed by the polysaccharide undergoing molecular rearrangement for lactone formation.

A recent experimental investigation on the kinetics of chitin degradation by LPMO with co-substrate H_2O_2 revealed that the catalytic efficiency of LPMOs is similar to those of polyoxygenases with an effective inactivation of bacterial LPMO-chitin binding protein (CBP21) (Kuusk et al. 2018). Beside ^{14}C-labelled chitin

nanoshiskers (CNWs) to confirm ternary-complex mechanism of degradation of chitin, primed enzyme combines with H_2O_2 which lead to enzyme inactivation. In biological system, H_2O_2 is produced by strict oxidase enzymes whose genes co-occur with LPMO genes in fungal genomes which lacks in cellulose dehydrogenases. Multiple type active species capable of abstracting a H atom from the substrate was proposed based on recent crystallographic results on a substrate–LPMO complex with a superoxo $[CuO_2]^+$ unit which can be protonated by a nearby histidine residue and provides an electron that promotes the formation of an oxyl complex resulting from O-O cleavage (Hedegård and Ryde 2018). The oxyl complex may react with the substrate or further protonate to a hydroxyl complex. The superoxide state can be achieved by binding of O_2 to the reduced active site. The abstraction of the C_4–H atom from the polysaccharide substrate is catalyzed by the $[CuO_2]^+$ intermediate with an energy 140 kJ mol^{-1} and the activation barrier $>$150 kJ mol^{-1}. The current calculations show a new route to LPMO activity through both Cu–oxyl and Cu–hydroxyl intermediates.

Employing either only molecular mechanism (MM) that can only describe the active site with quantum mechanism (QM) without the effect of protein, a hybrid QM and MM methods were employed that reveal the first step of LPMO mechanism (reduction of Cu^{II} to Cu^{I}) with the formation of Cu^{II}-superoxide complex (Hedegård and Ryde 2018). This process of elucidating structure of superoxide complex are not only markedly different from previous studies, but also show that the equatorial isomer of the super oxide complex is thermodynamically more stable over the axial isomer due to additional stabilization via second-coordination sphere of protein residue. The chemical cluster calculations provide an estimate of the bond dissociation energy for a series of possible LPMO intermediates that effectively bridge the gap between model and the real LPMO active site (Hedegård and Ryde 2017). This QM calculation suggests that the reactive intermediate is either Cu^{II}-oxyl, a Cu^{III}-oxyl or a Cu^{III}-hydroxide which suggest that O-O bond breaking occurs before the C-H bond activation. This process as the calculation is dominated by the oxidation/reduction and proton/deprotonation reactions generating intermediates. However, argument for the best plausible intermediates derived from the QM calculation is continued, therefore requires further advanced methods. In a separate study, it was shown that the removal of CBM in post-translation mode with hydrolysis by papain and at the genetic level, activity of HjLPMO9A-ΔCBM decreases relative to the full-length enzyme as reflected in the Cu^{II} active site features (Hansson et al. 2017). A decreased affinity for cellulose results in no change in C-1 and C-4 specificity and approximately 21 amino acid residues connecting the catalytic domain to the non-catalytic CBM and extensively glycosylated linker organizes the crystal structure of the cellulose-active enzyme.

The Catalytic pathways in LPMOs can be a blue print of knowledge obtained from a series of small molecule and copper monooxygenase enzyme activity which involves the binding of O_2 and dissociation from the substrate between catalytic events. It is the Cu^{II}-superoxide complex which act as the key for rapid reaction with O_2 via inner sphere electron transfer as revealed from stopped-flow and EPR studies (Walton and Davies 2016). The possibility that exogenous superoxide, as generated

by LPMOs, is the active polysaccharide cleavage agent. Apart from the protective catalase, superoxide generation occur when an AA9 LPMO do not form a pair with a natural substrate or in high concentration of LPMO such that a substrate surfaces is saturated. The superoxide release mechanism in chitin-active AA10 LPMOs is limited due to the differences in active site configuration as compared to AA9 LPMOs. In AA10 LPMOs the axial "release" site on copper is blocked by the side chain of an alanine residue. In addition to these, (1) superoxyl rebound, (2) copper-oxyl was also revealed.

12.7 Redox Site Determination

The nature of active sites and detailed mechanism of action of LPMOs remain elusive despite that the active site contains a mononuclear copper center ligated by two His residues (methylated His1 and His 78) among which Cu(II)-superoxide, Cu (III)-OH, and Cu(II)-oxyl were the proposed active forms (Beeson et al. 2015; Lee and Karlin 2015; Kim et al. 2014). Cu(II)-superoxide is a relatively week C-H oxidant as compared to Cu(II)-oxyl, however, can be influenced by protein environments of Cu(II)-superoxide species. Therefore, dual dependence of LPMOs on both the co-substrates O_2 and H_2O_2 motivates H_2O_2-depedent activation. In this regard, high-resolution crystal structure of a LPMO from *Lentinus similis* bound to a polysaccharide with Michaelis–Menten kinetics helps to realize the site-specific oxidation of the C4-H bond of the oligosaccharide (Frandsen et al. 2016). An activation of H_2O_2 by the Cu(I) site in LPMOs leads to substrate oxidation by capturing all of the hydrogen-bonding interactions in the active site pocket wherein, the effect of protein environment and related hydrogen-bonding networks are the key factors that determine the structure of solvated enzyme complex. Oxidative force of the copper ion for the C1 or C4 position gives rise to debate in determining the exact site of oxidation. 3D structure of LPMO of both bacterial AA10 (formerly CBM33) and fungal AA9 (formerly GH61) origin revealed that β-sandwich folds with metal active site coordinated by an N-terminal histidine (Hemsworth et al. 2013a). We compare between known small molecules studied on copper–oxygen complexes and with copper methane monooxygenase. By using the technique of sequence conservation analysis of the amino acids attached to the binding face, an AA9 sub-classification evolved by admitting three subgroups LPMO1 (Fig. 12.8a), LPMO2 (Fig. 12.8b), and LPMO3 (Fig. 12.8c) which were determined as per the number of aromatic amino acid residues that can interact with the polysaccharide (Asensio et al. 2013). The positioning of the enzyme on the substrate surface side determines the site of oxidation as revealed by correlation studies with glucosidic unit oxidation site (Fig. 12.8d). Consequently, AA10s find their closest structural match with the LPMO3 class of AA9 enzymes. They mediate their interaction with substrate through direct hydrogen-bonding interactions (e.g. glutamine, threonine, glutamate) (Fig. 12.8d) (Hemsworth et al. 2013a, 2013b).

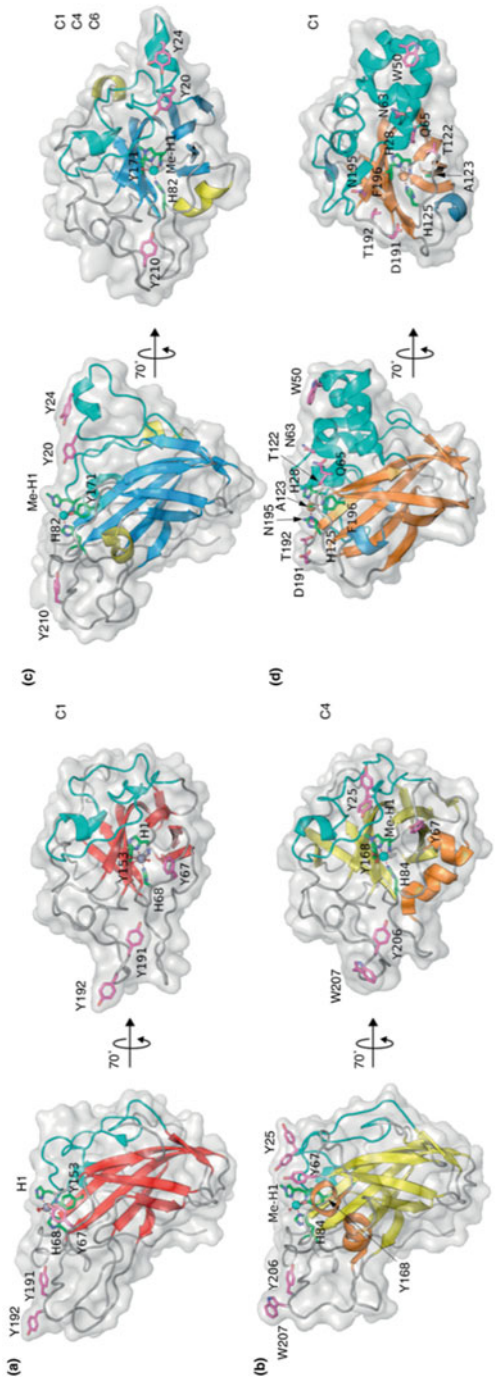


Fig. 12.8 Structures of AA9 representatives (**a**) LPMO1 from Thielavia terrestris PDB:3eii, (**b**) LPMO2 from Neurospora crassa PDB:4eir, and (**c**) LPMO3 from Neurospora crassa PDB:4eis at an orthogonal angle and of (**d**) AA10 from Bacillus amyloliquefaciens PDB:2yox. carbon atoms (magenta) and the alpha helical region of LPMO3 (**c**) and AA10 (**d**) (cyan). (Adapted from (Asensio et al. 2013), Copyright Elsevier Publishing, 2013)

It was revealed that due to a phenylalanine when replacing tyrosine/ate in AA9 (Fig. 12.8a with Fig. 12.8b–d) and in AA10, a uniquely conserved alanine residue edits the copper coordination site (superoxide site in AA9) and reactivity of any copper–oxygen species. The hydrophobic residues of the Cu-active site may dictate the ligand binding in AA10 (Hemsworth et al. 2013a). Predictable structural studies on AA9 and AA10 discloses that Cu(II) is linked to imidazole and amino of N-terminal histidine which point towards a histidine brace (Fig. 12.3). AA9s contain a copper ion associated with a distant (~2.9 Å, Cu····O distance) tyrosinate where the presence of a secondary coordination sphere glutamine has been crucial for enhanced activity. This glutamine is involved in hydrogen bonding to a substrate that is bound to the remaining equatorial coordination direction of Cu site. This also involves X-ray structural analysis to reveal the photoreduction of the copper ion in the histidine brace (Sommerhalter et al. 2005).

12.8 Cellulose Ultrastructure Disruption

How the LPMO action enhances cellulose hydrolysis by increasing the accessible surface area of cellulose substrate is a crucial topic to further investigate for advanced mechanistic understanding. In this context, enzymatic assays and real-time imaging using atomic force microscopy (AFM) jointly reveal molecular interactions of an LPMO *Tr*AA9A, formerly known as *Tr*Cel61A) from *Trichoderma reesei*] and a cellobiohydrogenase with I (*Tl*Cel7A from *T. longibrachiatum*) with bacterial microcrystalline cellulose (BMCC) as a substrate. AFM imaging studies support that *Tr*AA9A undergoes random movement along, across, and penetrates into the ribbon-like microfibrils of BMCC which results release of small oxidized sugars and also results splitting of large cellulose ribbons to smaller fibrils (Song et al. 2018). How the addition of cellobiohydrogenase with LPMO creates a dividing effect of cellulose microfibril more faster as compared with individual enzymes can offer more scope on finding the role of both enzymes. It is likely that LPMO attacks only the amorphous proportion of cellulose and increases the surface accessibility of cellulose elementary fibrils (CEFs) for cellulase enzymes. The way LPMO can oxidize the sugars in different amorphous polysaccharides which exposes the cellulose microfibrils to cellulases-driven hydrolysis. Herein, height profile analysis from AFM images of microfibrils shows that how TrAA9A moves in and out of the cellulose ribbon surface by height measurement during the incubation length time. Evaluation of mechanical resistance of the cellulose fiber is essential after being treated with LPMO. Therefore, experiments with LPMO-treated pulp material when subjected to mechanical and ultrasound treatment, a delamination into thinner and shorter structure resulted as emerged from optical microscopic studies at different enzyme concentrations before and after dispersion. An investigation on the distribution of fiber dimensions upon LPMO action reveals the fibrillation at micro and nanoscale from the entangled network of long fibers (Villares et al. 2017). This can be revealed from the height profile distribution analysis from AFM tomography of

LPMO-treated cellulose. From mechanistic standpoint, cellulose fiber gets weakens by cohesion of the fibers which results new entry point of attack by the other hydrolytic enzymes such as endoglucanases or cellobiohydrogenase which enhances nanofiber production with high crystallinity. This brings a starting point to further transformation into novel materials from post-LPMO action on cellulose.

12.8.1 Role of Real-Time Imaging for Realizing LPMO Actions

The need of a real-time visual tool for elucidating the molecular interaction between LPMO or cellobiodehydrogenase and microcrystalline cellulose was realized by tracking the random movement of enzyme on the surface of microfibrils and release of sugar. The most important characteristic of the molecular interaction between LPMO and microcrystalline cellulose (MC) or rather a cocktail of LPMO-cellobiohydrogenase with MC that results a dividing effect of cellulose microfibril by splitting a large cellulose ribbon into small diameter fibrils. The question is how this small diameter fibrils generate from the dividing effect of the cellulose microfibrils, whereas LPMO alone causes no separation into fibrils.

In a study of enzyme movement on the MC surface by AFM imaging, an unconventional three pattern movement was revealed that consists of (a) along, (b) across cellulose ribbon, and (c) moving from one ribbon to another with higher order of magnitude as compared to cellobiohydrogenase (CBHI) as found in case of Valonia microcrystalline cellulose as substrate (Fig. 12.9a–c) (Igarashi et al. 2011). Conclusive evidences from AFM imaging suggest the difference in shape of the binding face of LPMO and CBHs with flat surface LPMO, whereas CBH1 contains a tunnel-shape site to hold glucose residue of cellulose chain which results an obstruction for linear movement (Karkehabadi et al. 2008). More obvious edges appeared on the cellulose surface after 2-h incubation with *Tr*AA9A which resulted surface roughness significantly with obvious grooves and edges. The ability of LOMO (*Tr*AA9A) to penetrate inside the cellulose ribbon leads to a dividing of large cellulose ribbon into multiple smaller microfibrils. Possibly *Tr*AA9A oxidation disrupts the glucan chains between these CEFs.

LPMO reacts specifically to disintegrate large cellulose ribbons into small microfibrils with oxidized sugar products (Fig. 12.9b). Bacterial microcrystalline cellulose (BMCC) ribbons contain amorphous cellulose and cellulose elementary fibril (CEFs) that are composed of well-organized cellulose glucan chains. LPMO attacks only the amorphous proportion of cellulose and increases the surface accessibility of CEFs for cellulase enzymes. LPMO oxidizes sugars in different amorphous polysaccharides, for example, insoluble xylan degradation can be enhanced. TrAA9A cause a slight decrease of cellulose ribbon from 101.8 + 14.9 nm to 89.8 + 16.7 nm (about 11–15 nm) with an increase if surface roughness (edges and grooves). Eventual splitting of the ribbon-like cellulose microfibrils occurs along its axis

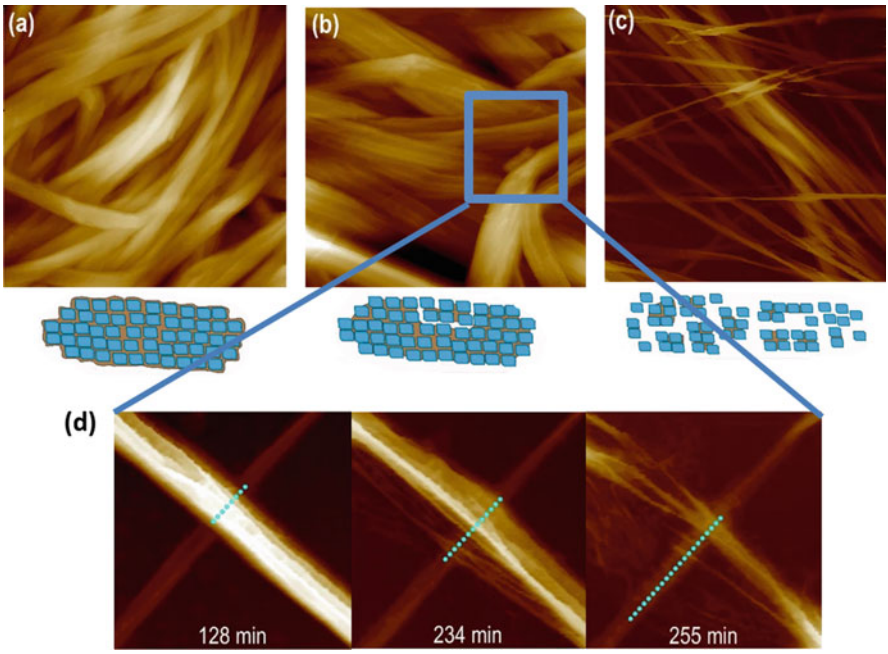


Fig. 12.9 TrAA9A enzyme molecule diffusion randomly on BMCC including moving across (**a**), along (**b**), and diffusing (**c**) between cellulose ribbons including their graphical representation below. (**d**) Enlargement in nanoscale (50 nm) for the time frame of 128 min to 255 min incubation resulting height of BMCC ribbon decrease while width increased concomitant with the disintegration of the large cellulose ribbon into microfibrils. (AFM images are Adapted from reference (Martínez-Sanz et al. 2016) with permission, copyright BMC Springer Nature 2018)

into smaller microfibrils which results approximately 52% of original ribbon width. Therefore, cellulose ribbon divides into multiple smaller microfibrils with an anticipation that TrAA9A oxidatively disrupts the glucan chains between these CEFs (Fig. 12.9c). AFM imaging measures the average height and width of the cellulose ribbon in which a significant decrease in height and increase in width was evident upon incubation as a result of disintegration of large ribbon to smaller microfibrils (~4 nm) which may contain few fundamental CEFs (Fig. 12.9d) (Martínez-Sanz et al. 2016).

A change in distribution of fiber dimension due to LPMO action upon applying a varied concentration of LPMO results in fibrillation at micro an nanoscale (Villares et al. 2017). Upon increase in LPMO concentration, reduce down of the non-fibrillar structures into elementary fibers of 5 nm diameter similar to enzymatic pretreatment and mechanical delamination. Height distribution is bread at the low LPMO concentration by eventually resulting mean height shift to lower range (75 nm to 30 nm shift) confirming an LPMO induced disruption trimming the fiber to nanoscale. In a recent report, height difference of LPMO and CBH I was meaningfully evidenced while using both the enzymes at a time over pyrolytic graphite as grid in AFM

(Eibinger et al. 2017). About 10–30% of the adsorbed CBH I molecules move on the surface of cellulose mostly on the side walls of the nanocrystals. Although LPMO is only about half the size of CBH I yet, it stays at the place of its adsorption on the cellulose over several minutes without none of it moved on the surface until it became eventually desorbed again. Therefore, it was revealed by AFM real-time imaging that the C4′-oxidizing LPMO additionally equipped with CBM is completely immobile on the cellulose surface unlike only CBH I.

Overlapping specificity for adsorption to crystalline cellulose surfaces is therefore suggested for the two enzymes. The cellulose nanocrystals used represent cellulose polymorph Iβ, the main crystalline form of cellulose in the plant cell wall. Therefore, it is not confirm if the localization of LPMO and CBH I is crystalline form of cellulose substrate. Orientation of such, ideally shaped, cellulose nanocrystals on the AFM grid with their broader surface in contact with the graphite surface would expose their presumably relevant hydrophobic faces on the side walls which is helpful for diffusion of LPMO or other degrading enzymes to adsorb predominantly to the side walls. This correlation of cellulose morphology and propensity for the diffusion site of LPMO is not revealed in details through AFM studies, however, exploring this direction of LPMO activity on cellulose should not be restricted to only AFM imaging, however, can be extended for other high-resolution imaging studies to track the mobilization of LPMO on the substrate surface in general.

12.9 Evolution of LPMOs

Based on similarities in their amino acid sequences through bioinformatics analyses (Levasseur et al. 2013) they have been classified into 7 groups (Table 12.1). LPMOs are carbohydrate-active enzymes or CAZymes (Levasseur et al. 2013) with the most variable number of genes and occur in various taxonomic groups with diverse nutritional capabilities.

In a recent study on in-depth characterization of Thermobia's LPMOs, it is proposed that diversification of these enzymes towards cellulose digestion occur by co-opting endogenous AA15 LPMOs to boost asymbiotic cellulose digestion (Sabbadin et al. 2018). Digestive enzymes from *T. domestica* include a previously uncharacterized family of endogenous LPMOs which is crucial in arthropod and food digestion with a new range of enzyme cocktail. This efficient digestion of crystalline cellulose without microbial assistance makes endogenous proteins responsible for evolutionary entomology. Protein sequence analysis showed that LPMOs carry a signal peptide that if removed, which allows the exposure of conserved N-terminal catalytic histidine-derived from secreted protein. There is also evolutionary relation of AA9 and AA10 from the amino acid sequences and protein structures in which case the conserved sequence and structural features were compared with potential substrate interactions and surfaces by electron donors. In this case, the phylogenetic analysis suggests that cellulose and chitin specific enzymes are distributed into different subclades within bacterial AA10 as in the

Table 12.1 Bioinformatics analysis results of LPMO classification

Auxiliary activities (AA) families	Substrates	taxonomic group	GenBank accession no.s (www.cazy.org)	References
AA9	Cellulose, cello-oligosaccharides, xyloglucans, glucomannans and β-glucans	Several	Q1K8B6, Q5BCX8	Bennati-Granier et al. (2015), Isaksen et al. (2014), Fanuel et al. (2017)
AA10	Chitin and cellulose	Bacteria and some viruses	Q9RJY2_STRCO, Q62YN7_BACLD	Vaaje-Kolstad et al. (2011)
AA11	Chitin	Fungi	BAE61530.1, 4MAI	Hemsworth et al. (2014)
AA13	Starch	Fungi	Q5B1W7_EMENI, Q7SCE9_NEUCR	Lo Leggio et al. (2015)
AA14	Recalcitrant xylan coating cellulose fibers	Fungi	A0A060SRI5, ALO60293.1	Vu and Marletta (2016)
AA15	Active on both cellulose and chitin	Animal origin (invertebrates)	SIW61372.1, AIG55964.1	Couturier et al. (2018)
AA16	Cellulose	Fungal	XP_020060743.1, CEF84218.1	Sabbadin et al. (2018)

case of fungal AA9 (Book et al. 2014). Amino acid sites analysis and structural homology modelling suggest that AA10 subclade contains diverse selection at the different surfaces and therefore this diverse AA10 are used for cellulose-binding and protein–protein interactions.

There is an unresolved issue such as gene number expansions in some of the species (Morgenstern et al. 2014). Considering the uncertainties in the dynamics of the early evolution in the AA9 gene family, white rot-fungi have a higher number of AA9-encoding genes because of expansions of AA9 homologs. The AA9 proteins can be divided into many subfamilies of which more than one-third of the sequences were too diverged to fall into one of the 16 subfamilies which reveals a high level of evolutionary rate change. Interestingly, the few enzymes with known regioselectivity result separate subfamilies which is the basis for this classification. It is argued that the sequence similarities between AA9 and AA10 are on the lower side that evolutionary relationship should not be assumed before significant functional and structural similarities are evident (Ekwe et al. 2013). However, recent discovery of a LPMO and taxonomic divide between fungal AA9 and bacterial/viral AA10 enzyme families suggest that the evolutionary history and homology among the LPMO families should be revisited.

Lytic polysaccharide monooxygenases (LPMOs) are novel enzymes possessing the ability to boost the hydrolytic cleavage of glycosidic bonds in lignocellulosic biomass via oxidation. The mechanism involves the supply for oxygen and an extracellular electron donor (Vaaje-Kolstad et al. 2010; Harris et al. 2010; Bissaro et al. 2018b; Bissaro et al. 2017). Based on similarities in their amino acid sequences

through bioinformatics analyses (Levasseur et al. 2013) they have been classified into seven groups (Table 12.1). LPMOs are carbohydrate-active enzymes or CAZymes (http://www.cazy.org/Auxiliary-Activities.html) with the most variable number of genes and occur in various taxonomic groups with diverse nutritional capabilities.

The origin of LPMO enzymes have been tracked back in Devonian Period (420 million years ago) when insects (arthropods) such as firebrat and silver fish appeared, having the innate capability of digesting cellulose with speed as higher as mammals (cows) and other detritivorous insects (termites). Digestive enzymes from firebrat (*Thermobia domestica*) when sequenced and phylogenetically analyzed revealed similarity with other LPMOs from diverse phyla, including algae, oomucetes, and complex animals which was also more active on both cellulose and chitin. It is also evident that ancient LPMOs played significant participation in metabolism of food and overall development of these insects. Sabbadin et al. (2018) proposed that initially the enzymes might have been used by the arthropods for chitin-structure remodelling and metamorphosis. Later on, due to evolution the enzymes diverged and participated in cellulose degradation.

Plant pathogens (e.g. barley powdery mildew, *Fusarium*, *Botrytis*), human pathogens (e.g. listeria and cholera) have been shown to use LPMOs as virulence factors. LPMOs might have dual role here, one as generator of reactive oxygen species (that kills host tissue) and for cleaving cell walls. Origin of LPMOs in fungi has been proposed towards the end of Carboniferous period, where coal deposition decreased (Floudas et al. 2012; Kohler et al. 2015).

Although the theory of coal formation and fungal lignin synthesis has been contested, yet lignocellulosic degradation and LPMOs have a close relation. Fungal decomposers such as *Podospora anserine* have been shown to possess potential genes of family AA9 and are induced during growth on lignocellulosic substances. Fungal root symbionts (ectomycorrizae) that participate in lignocellulosics degradation have also been proposed as oxidizers and degraders of soil organic matter for assimilating organic nitrogenous compounds. Light enhances the enzymatic activity of these enzymes, in the presence of photosynthetic pigment and therefore have been proposed as cleaners of forest-floor litter (Cannella et al. 2016). LPMOs show variability in copy number of genes in various plant pathogens and saprophytes or coprophilous fungi. Wood-decaying ancestors of certain mycorrhizae showed reduction in the number of cell wall degrading enzymes (Johansen 2016b). Various factors, e.g. electron donor availability, pH optima, salt tolerance, LPMO-inhibitors, etc. affect the amplification of the LPMO genes (Johansen 2016b).

12.10 Prospect of LPMO in Biorefinery

12.10.1 Current Status

Within a few years of discovery, LPMOs become a component of the commercial biomass-deconstructing enzyme cocktail which advances the bioethanol production and insoluble polysaccharide including cellulose biorefining processes (Harris et al. 2014). LPMO's industrial applications largely depend on starch and starch-containing crops which shares a global market of ~51 billion per annum (de Souza and de Oliveira Magalhães 2010). Therefore, it was evident that some starch-binding module in LPMOs can boost starch degradation as found in corn starch, amylose, and amylopectin (Horn et al. 2012). However, the most prevalent challenge for its application for biorefining is that the enzymes tend to be unstable under process conditions and requires co-substrate assistance (e.g. H_2O_2). For the purpose of practical biorefining, recombinant production of LPMOs in a bioreactor and protein purification in the batch phase was considered as a standard method for exploring for self-responsive LPMOs derived from mangrove fungus *Pestalotiopsis sp.* NCi6 (Patel et al. 2016). In this process, cultivations were performed according to the Invitrogen's *Pichia* fermentation process. Fractions containing recombinant enzymes were pooled, concentrated, and dialyzed. A study on the activity of *Pa*LPMO9H on mixed-ligand glucans, xyloglucan, and glucomannan by using tandem mass spectroscopy and ion-mobility mass spectroscopy reveals that a C4 oxidative cleavage of mixed-ligand glucans and mixed C1/C4 oxidative cleavage of glucomannan and xyloglucan occur at the non-reducing end when aldonic acids were produced (Fanuel et al. 2017). For the development of optimized enzyme cocktails in biorefineries, this approach of utilizing capability of *Pa*LPMO9H to target polysaccharides, differing from cellulose, may be advantageous for highly variable polysaccharides in biorefineries.

Desire to find the link between LPMO active sites and lignocellulosic degradation process prompts us to review the current status of implementing newly discovered LPMOs for lignocellulose bioprocessing (Chen et al. 2016) and further via degradation of cellulose fibers. The xylo-oligomers released from cellulosic fibers by xylanase activity which can be boosted by enhancing the activity of GH11 xylanase. However, xylanase combination with cellulose-active A9LPMO has not been effective for improving xylan conversion. GH11 xylanase on birchwood fiber offers xylobiose and xylotriose with a synergy between AA14A and GH11 xylanase (Couturier et al. 2018). C1 oxidized species with an aldonic acid at the reducing end produce C1 oxidize xylotriose. Such processes were performed at 45 °C after a prolong hours of incubation and by denaturing enzyme by heating at ~100 °C to freeze the activity (Garajova et al. 2016). The fungal synergies play a significant role in oxidative degradation of cellulose. The enzyme-based saccharification with H_2O_2 fed in a bioreactor using Avicel as substrate in which cellulose cocktail are composed of LPMO (MW 30,000 g mol^{-1}) (Bissaro et al. 2017). Hydroxylation of substrate in lignocellulose degradation proceeds via reaction of Cu(I) with H_2O_2

which eventually results a Cu(III)-oxo and Cu(III)-associated hydroxide leading to the hydroxylation of polysaccharide with molecular rearrangements for lactone formation. Supply of electrons were the major challenges for biorefining processes in the presence of redox active compounds (Bissaro et al. 2017). As the O_2 activation has been the initial step thereby H_2O_2 based activation of LPMO involves H_2O elimination with a Cu(II)-oxyl species for hydroxylation of substrate through reduction of C-O bonds. Commercial xylo-oligosaccharides (DP15) and β-(1 → 4) linked gluco-oligosaccharides (DP1–5) have been used as substrates ranging with medium viscosity. These substrates were incubated with LPMOs (e.g. MtLPMO9A) (50 °C, 24 h) with or without ascorbic acid and resultant mixture can be analyzed by HPAEC (high-performance anion-exchange chromatography) and MALDI-TOF-MS (matrix-assisted laser desorption ionization-time of flight mass spectrometry) (Frommhagen et al. 2015). The quantification of the ionic binding of cationic products to carboxyl groups formed by LPMOs action on polysaccharides depends on a colorimetric cation-determining method (Wang et al. 2018b). LPMOs act as peroxygenase or oxygenase in which case the polysaccharide substrates (e.g. Avicel cellulose) interact with the C4 position over the Cu-active site (Hangasky et al. 2018). Therefore, hydroxylation of polysaccharides using O_2 as co-substrate forms the basis for the O_2 activated glycosidase oxidation.

Therefore, LPMO active sites are linked with sustainability of polysaccharide degradation via oxidative protocol wherein, H_2O_2 and O_2 act as additional components of the system to realize their role in mechanism. This emphasizes the scope beyond Cu-active site mediated O-atom based reactions for intensive study of Cu-metalloenzymes.

12.10.2 Technological Limitation

In biorefinery, the activity of type-1 (C1-oxidizing) LPMOs can be determined by quantifying the ionic binding cations to carboxyl groups on polysaccharides (Wang et al. 2018b). Such rapid determination of activity is extremely path defining for the biorefinery applications. For certain substrates such as mixed-linkage glucan (MLG) and lichenan, oxidation at the non-reducing end may be localized at C3 or C4 for which a possible structure containing internal β(1 → 3) bonds at the backbone position can be assumed. This is a vital role of LPMOs in modern agricultural pests and diverse vectors which offers new opportunities to tackle global challenges of biorefinery (Dutta and Pal 2014). Therefore, a combined approach of revealing new LPMO families, the distinctive metal sites of action and oxidative degradation potentials which will set to push all boundaries of advanced biorefinery processes for economic shifts. Based on the refined knowledge of LPMOs on their substrate specificity, oxidative regioselectivity, and stability, advanced biorefinery application of LPMOs. A wide distribution, diverse sets, and isolation of potent LPMOs from fungal sources may provide an avenue for increasing efficiency of cellulases with LPMO as booster, thereby decreasing ethanol production costs. This would also

Table 12.2 Specificity of substrate for fungal LPMO

CAZy Family	Fungal species	Specific substrate
AA9	*T. Crustaceus*	Avicel, treated corn
AA13	*Aspergillus nidulans*	Starch
AA9	*T. terrestris*	Lignocellulose
AA11	*Aspergillus oryzae*	Chitin
AA9	*H. Jecorina*	CELLULOSE
AA9	*Phanerochaete chrysosporium*	PASC spruce
AA9	*Neurospora crassa*	Lignocellulosic biomass

need a simplified screening and detection method which are not widely available yet. Therefore, nonhydrolytic activity enhancement of LPMO is a challenging task for modern biorefinery (Table 12.2).

The amount of lignocellulosic biomass obtained from different agricultural resources and other industries are nearly 180 million tons per year. To overcome this issue a biotech company Novozyme successfully filed a patent against conversion of plant residues into sugar components. These patents explain the different classes of LPMOs and their synergistic effects with other enzymes. When these plant materials are pretreated by the hydrolysis process and some acid to degrade the cell wall and further introduced to feasible yeast strain, it leads to the production of ethanol from the released glucose compound.

Depending on the synergistic feature of LPMO which is evident only in the presence of molecular oxygen. In other words, activity of the LPMO ceases when the ethanol-fermenting yeast is added to the slurry of free sugars and partly degraded polymeric cell wall material (Johansen 2016a). Therefore, a competition for oxygen by LPMO and yeast for saccharification brings large extent of change in bioethanol production.

12.10.3 Advantages and Future Prospect

Few of the future prospects of application LPMO involves the following areas. (1) oxidative decomposition of organic matters in nature in biodegradable ways which will be enhanced by rapidly growing number of sequenced microbial genomes. Therefore, certain wood-degrading fungus may be source of such LPMOs that enable degrading organic matters with extension of this techniques for utilizing secondary agriculture products. This apart, (2) LPMO is deeply involved in the pathogenicity of bacteria including their presence in insect viruses in certain proteins which determines the pathogenicity. It was also found that cellulose degradation offers a potential therapeutic strategy as found in a comprehensive study on the structure of cellulose and cellulases, however, LPMO may also play significant role in cellulose degradation for effectively targeting a free-living

amoeba that cause hiding in keratitics by enclosing itself within a shell which makes it chemotherapeutic resistant (Lakhundi et al. 2015).

12.11 Challenges and Future Directions

The recalcitrant fraction known to be particularly resistant to xylanases due to extensive decoration and also due to xylan's adoptability to a flat conformation with their chains solidly adhering via hydrogen bonds to the surface of cellulose microfibrils. To overcome this resistance to degradation needs multidisciplinary approach to identify fungal LPMOs and understand their mode of action on degrading xylans. In contrast to bacterial LPMO10s, fungal LPMO9s accumulates multiple aromatic amino acid on the substrate binding surface of which at least one or two in the LC loop and in the L2 loop (Figs. 12.3 and 12.4). Such arrangements are mostly similar to carbohydrate-binding proteins; however, interaction between the substrate and the protein is arbitrated by CH-π interactions. The spacing within the aromatic residues on substrate binding face corresponds to the distance that separates monosaccharides units. Therefore, structural basis of substrate specificity, which is milestone of LPMO research, remain challenging until molecular determinants are fully revealed. A lack in engineered LPMOs with changes in substrate specificity is responsible for a void for conclusive role of CBMs. Surface topological features could discriminate between chitin and cellulose in LPMO10s. Despite recent progresses, the structural determinants of LPMO-substrate specificity remain largely unclear.

It was recently shown that the CBM and linker of modular LPMO promotes the localized cellulose oxidation (Courtade et al. 2018). The NMR spectroscopic study confirms for *Sc*LPMO10C from *Streptomyces coelicolor* that distance between CBM and the catalytic domain allows the domains to move independently. It was revealed that most of the substrate binding affinity of full-length *Sc*LPMO10C resides in the CBM. At a lower substrate concentration, CBM is beneficial for LPMO activity and it promotes localized and repeated oxidation of the substrate. This enables in designing a mechanical basis of the interplay between catalytic domains linked to CBMs in LPMOs. It was also found that replacement of natural CBMs by introducing non-catalytic CBMs from *Clostridium* family destroys the catalytic activity. This implies that the CBMs can influence the non-oxidized products and modulate the activity of LPMOs. Therefore, engineered LPMO-CBM hybrids can enhance the oxygenation of substrates.

The basis of plant recalcitrance to digestibility and deconstruction for biofuel production, any new finding on LPMOs will be important for improving existing biocatalytic processes. Therefore, boosting research directions of LPMOs and cell wall interactions on the basis of molecular architecture provides a new regime of plant biomass-oxidative enzyme research for production of high-value renewable fuels. Among the major challenges for advanced LPMO biorefinery, proper delivery of electrons and aeration at a commercial scale will play significant role. In this case,

a balance between activation-inactivation from the combined effect of H_2O_2 and reductants are crucial. More complicated will be with full-redox active lignin and metals to optimally reveal the potentials of LPMOs. In this process, effect of the other enzymes on LPMOs in a cellulolytic enzyme cocktail by stripping off the LPMO-disrupted chains from the hydrophilic surface of substrate may unlock new binding sites for LPMO. A major focus on the substrate specific (cellulose, starch, chitin) research on LPMO will also be desired in future.

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Chapter 13
Algal Bioeconomy: A Platform for Clean Energy and Fuel

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Abstract Global industrial operations and commodity productions rely on fossil fuels that are non-renewable resources, projected to be exhausted by 2050. To mitigate and compensate for the high-end use of fossil fuel consumption, the use of renewable energy resources, i.e., biomass, wind, and solar energy is getting attention recently. Using photosynthetic organisms, algae is a viable alternative to produce food, feed, and fuels as algae are considered as one of the most robust species and commercially exploited for CO_2 capturing or for water clean-up (i.e., remove pollutants and heavy metals at industrial settings). Very few produce high-value products using algae, e.g., carotenoids, vitamins, and pharmaceuticals that required extensive process integration and clean operations, but biohydrogen

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production using the algal platform was very scarce. Considering the future clean, green, and bio-circular economy, using algal technology to produce clean fuel hydrogen will be attractive and scalable. This chapter has summarized and culminated the literature about algal microbiology, mechanisms involved in the production of biohydrogen techniques, and the various secondary metabolites co-produced during the process of the algal hydrogen production platform. The chapter also listed and discussed the effect of various parameters on improving the biohydrogen yield and knowledge gaps to address shortly.

Keywords Biohydrogen · Algae · Metabolic pathways · Enzymes · Secondary products

Abbreviations

ATP	Adenosine triphosphate
C/N	Carbon/nitrogen
Cyt. b6f	Cytochrome b6f complex
EPBR	Electrochemical photobioreactor
FBR	Flat-panel bioreactor
FCEVs	Fuel cell electric vehicles
Fd	Ferredoxin
GHG	Greenhouse gases
H^+	Protons
HEVs	Hybrid electric vehicles
NAD^+	Nicotinamide adenine dinucleotide
NADP	Nicotinamide adenine dinucleotide phosphate
NADPH	Reduced form of NADP+
PBR	Packed-bed bioreactor
PC	Plastocyanin
pH	Power of hydrogen or potential for hydrogen
PQ	Plastoquinone
PS I	Photosystem I
PS II	Photosystem II
PS	Photosystem
PUFA	Polyunsaturated fatty acids
STBR	Stirred-tank bioreactor
TBR	Tubular bioreactor

13.1 Introduction

Undoubtedly, the fossil fuel crisis and exponential demand in the energy sector forced mankind to seek alternative options, to meet the continuous energy demand without disturbing nature (Ritchie and Roser 2020). The energy demand of the world is forecasted to approach $16.7 trillion by 2040, which is twofold higher than the

present need, and the fossil fuel demand is expected to compress by 2030 (IEA 2019). The environmental and socio-economical concerns of finding a substitute for petroleum-based fuels that should be equally efficient to reduce monopolies of oil-rich countries are growing (Jones and Mayfield 2012). Also, the questions are raised after the sudden surge of carbon dioxide emissions worldwide observed in 2017 (Christiansen et al. 2018). The ideal candidates to tackle this global energy problem are the prokaryotes and eukaryotes; where among all biological entities, algae seem to be the most prominent and renewable feedstock as a potential bio-refinery. Algae emerged as a substantial source of sustainable energy carrier, nutraceuticals, pharmaceutical, and cosmetic industries. It is proved to be a boon for humankind as value-added agricultural products that partly substitute the food and feed. Also, their multifaceted economic potential as biomass, especially bio-ethanol, biodiesel, biogas, and biohydrogen is explored worldwide (Chisti 2007). The algal biomass obtained is utilized as third-generation feedstock for liquid and gaseous fuels. They are functional at the lab scale and showed promising results with hybrid systems (Huber et al. 2006). Thus, the main challenge lies in its efficient role in pilot-scale production with respect to other competitors (bacteria, cyanobacteria, fungi, and their consortia).

Though the concept of biological hydrogen, i.e., biohydrogen production from the interaction of microbes and enzymes has come a long way yet the commercial success of hybrid combination to produce H_2 still required validation and optimization. The H_2 gas is heralded in many industrial applications such as methanol and ethanol production, refinery fuel, spacecraft fuel and to generate electricity on a mass scale as well domestic use (Venkata Mohan and Pandey 2019). Even some developed countries have already incubated the idea of encouraging vehicles run by H_2 fuel cells to displace petroleum. All these uses make H_2 an important versatile fuel option that is present abundantly and needs limited natural resources. To decrease the dependence on fossil fuels, biohydrogen from the living microbes under standard conditions provides promising results. For example, algal bioreactors can harness greenhouse gas (CO_2) present in the atmosphere when exposed to sunlight to produce H_2 gas along with secondary products. This H_2 gas can be used as a green and clean energy carrier to produce electricity (Al-Shorgani et al. 2013). Though the road ahead might have several technical challenges, many researchers and companies are working towards the next generation biohydrogen fuel being evolved from the living organisms. The commercialization of biohydrogen is the biggest barrier to get the best output and efficient source of energy security. To understand the fundamental question of biohydrogen economical technologies, we will discuss in detail the various sources of biohydrogen, the types and mechanisms involved, the systematic role of algae and enzymes in biohydrogen production, the algal bioenergy market opportunities and the challenges (Box 13.1).

Box 13.1 Biohydrogen Present Scenario and Future Scope

- The world is focusing on carbon-neutral hydrogen energy to fulfil the energy needs in various sectors of the economy like petrochemical refining industries, food processing, metal processing and fabrication, methanol production.
- Hydrogen generation market size was about $135.5 billion in the year 2018 and it is projected to reach $199.1 billion by 2023. Asia Pacific region is expected to be the largest market to a hydrogen-based electrochemical device. For example, fuel cell electric vehicles (FCEVs) and hybrid electric vehicles (HEVs) in the transportation segment. The concept of building hydrogen-highways is booming, where hydrogen refuelling stations will be made available, fitted with fuel cells that recombine hydrogen and oxygen to generate electricity.
- The prominent market players functioning on large scale production of clean energy are Air Liquide (France), Iwatani (Japan), Hydrogenics (Canada), Praxair (USA), and Linde (Germany).
- As an industrial gas, the existing method of hydrogen production is from methane, mainly obtained from gasification of coal and natural gas.
- The emerging sectors to derive hydrogen from gasification of fossil and biomass feedstocks which work on hydrogen conversion technology.
- Biohydrogen from biomass pathways via thermochemical technologies like gasification, grid electrolysis, and microbial conversion is the sustainable and economical algal-based technologies to meet hydrogen economy demands.

13.2 Hydrogen

As we know, hydrogen is a viable natural energy carrier and derived as a transitory by-product of various microbial-driven biochemical reactions. So, we can generate H_2 gas in biological machinery such as algal biomass, with zero emissions (Balat and Kırtay 2010; Kirtay 2011; Rosen 2017). Indeed, strict implementation is required to slow down the impact of global warming and replenish depleting fossil reserves. The hydrogen gas has emerged as a fascinating alternative fuel to conventional fuels because of only water as a reactant involved in the combustion process (Sindhu et al. 2019). Among all available biofuels, H_2 gas has the highest gravimetric energy mass at 141 MJ/kg. But at the normal temperature and pressure conditions, its volumetric energy mass is low, i.e., 12 MJ/m^3, which makes it an ideal transportation fuel (Singh et al. 2015). There is a global estimate that the current consumption of hydrogen is about 55 million tons, which are surging ahead by the rate of 6% per year (Kalamaras and Efstathiou 2013). The GHG contributors to meet this global demand are processed mainly through steam reforming of methane (natural gas) production, next to the line are chemical refineries, coal gaseous emissions, and other

sources (Muradov and Veziroglu 2005) polluting the environment. The other technologies like electrolytic and plasma processes have demonstrated high efficiency for hydrogen production, but unfortunately, they are considered as energy-intensive processes (Holladay et al. 2009). Thus, biohydrogen is considered as one of the clean, affordable, non-toxic, and renewable energy for future bioeconomy.

13.2.1 Definition

At this junction, humans are facing extinction challenges of conventional fuel resources due to their ever-increasing commercial greed. The development and advancement of humanity are looking for bio-based processes and sustainable biomass hydrogen sources to meet their daily energy consumption. The microorganisms or the biomass treated with biological routes to liberate hydrogen gas can be defined as 'biohydrogen' (Oncel 2015). This biohydrogen must be useful and safe as fuel in terms of production, storage, and energy conversion to electricity without any technical barrier (Hosseini and Wahid 2016). Photosynthetic microorganisms have a versatile role in sustainable energy as they can be genetically engineered to produce a variety of chemicals of interest for future fuel. Among all types of algae, especially blue-green algae are undergoing different commercial trials to produce hydrogen and support biohydrogen economy. The production of H_2 by biophotolysis of the water is the ultimate purpose regarding 'clean' technology, as far as just hydrogen and oxygen are produced by utilizing only solar energy. The interdisciplinary contributions of bio-inspired chemistry and genetic engineering of enzymes will help to better understand the coupling between the physiology of microorganisms and the production process to reach the expected yields (Kose and Oncel 2017).

13.2.2 Biological Sources of Biohydrogen

As per the photochemical evolution, different microorganisms produce biohydrogen in multiple ways like dark and photo-fermentation, direct and indirect biophotolysis, enzymatic hydrogenesis, and microbial electrolysis (Kumar and Das 2000; Hallenbeck 2014; Kadier et al. 2016). The biomass to hydrogen conversion route also involves thermochemical processes where thermal degradation of biomass occurs in the presence of oxygen. Methods like pyrolysis and liquefaction are used to produce biofuel and other chemicals after different thermochemical treatments (Box 13.2).

Box 13.2 Thermochemical Treatments

Thermochemical process: A traditional method of treating biomass residues at a higher temperature to decompose/to produce fuels and chemicals. The main thermochemical routes are pyrolysis and gasification. The composition of the gaseous fuel is dependent on temperature, type of substrate, availability of O_2, and the type of the reactor.

Pyrolysis: The thermal degradation of biomass feedstock occurs in the absence of oxygen to produce a mixture of synthetic gas, bio-oil (condensable gaseous mixture), and char residues. The purified biohydrogen can be obtained only after a gaseous mixture passes through steam methane reformer and water-gas shift reactor.

Gasification: The combustion of biomass feedstock (solid/liquid carbonaceous material) takes place in the presence of oxygen with some gasifying agent to produce syngas (gaseous fuel).

The biggest challenge faced is to effectively separate the mixture of products produced for the removal of char and tar residues, difficult via physical methods and time-consuming. Also, the hydrogen yield from biomass is comparatively low as 15% of the dry weight of biomass.

These processes generate a lot of heat, which can be converted into electricity by generator (Attard et al. 2020). But in this chapter, our focus is on the microbiological sources of hydrogen production (Fig. 13.1).

The microalgae such as Chlorella sp., Scenedesmus sp., and Saccharina sp. (Wang and Yin 2018) usually follow these biological routes to produce biological hydrogen by operating under mild conditions without relying on fossil fuels or electricity. They just require free natural resources like solar radiation, aquatic environment algal density that discharges zero carbon emission. But certain limitations in biomass process such as low hydrogen yield of 17.1% (Wang 1999), less substrate transfer competence, and removal of reaction wastes make biological hydrogen production a difficult task; however, it is still promising because of the advantages as a renewable form of bioenergy and clean production strategy. An overview of biohydrogen production technology involving various biological processes with their benefits and drawbacks is given in Table 13.1.

Also, under specific conditions, both prokaryotic and eukaryotic bacteria use enzymes like hydrogenase and nitrogenase involved in the biological pathway to release biohydrogen (Mohan and Pandey 2013; Kuppam et al. 2017). Hydrogenase is a metal enzyme present in all three domains of life, viz. archaebacteria, eubacteria, and eukaryotes, whereas nitrogenase enzyme is present in prokaryotes (Mona et al. 2020). All these microorganisms are ubiquitous in nature and early inhabitants of the earth since the evolution of life, surpassing extreme climatic conditions to survive. Experimental evidence of hydrogen production using anaerobic bacteria was first reported by Vatsala and Seshadri (1985), later on supported in the 1990s by the experimental trials for biohydrogen mass production using microorganisms as

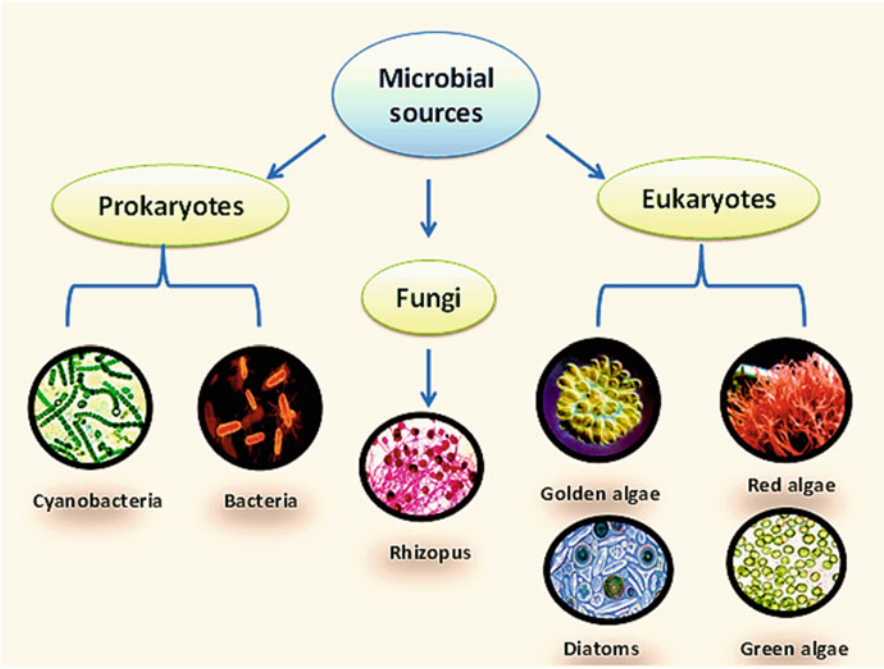


Fig. 13.1 Schematic illustration representing microbial sources of biohydrogen

investigated by many researchers (Tanisho et al. 1983; Benemann 1997; Nandi and Sengupta 1998; Kumar and Das 2000). The consortium of axenic and assorted cultures of these microorganisms was studied to generate novel biomass for biohydrogen extraction (Chandrasekhar et al. 2015). These hydrogen-producing microorganisms require low energy setup if given optimum pH, ambient temperature, specific nutrient concentration, and light intensity that can evolve hydrogen practically scalable to mass cultivation (Dasgupta et al. 2010).

13.2.2.1 Bacteria

Since the chemical evolution on the earth, diversified bacteria are the major hydrogen producers because of their continuous energy by certain metabolic mechanisms. All hydrogen-producing bacteria are broadly categorized into four groups (Gray and Gest 1965):

a. Closed anaerobic heterotrophs without a cytochrome system, e.g., *Clostridia* sp. *Micrococci* sp., *Chlamydomonas* sp., *Euglena* sp., etc.
b. Heterotrophic facultative anaerobes with cytochromes and lyses convert formates to produce H_2, e.g., methylotrophs like *Methylomonas albus* and *Methylosinus trichosporium*.

Table 13.1 Biological hydrogen generation via different routes, their benefits and drawbacks

	Biological sources of biohydrogen					
	Fermentation		Biophotolysis		Electro-hydrogenesis	In vitro hydrogenesis
Routes	Dark	Photo	Direct	Indirect	Microbial electrolysis cell (MEC)	Synthetic enzymes
Microorganisms	Mesophilous and thermophilous bacteria	Sulphur and non-sulphur bacteria	Algae and cyanobacteria	Algae and cyanobacteria	Exo-electrogens	Bacteria: *Pyrococcus furiosus* and *Thermoplasma acidophilum*
Benefits	Independent of solar energy	Majorly dependent on solar energy	Dependent on solar energy	Independent of solar energy	Independent of solar energy	Independent of solar energy
	Grown on many substrates in anaerobic process and produce several metabolites	Grown on the broad range of substrates like organic waste/ wastewater	Good H_2 conversion energy as compared to forests and farmlands	Comparatively higher H_2 yield than direct photolysis. Capable to fix N_2 from the atmosphere. Metabolites can be efficiently converted to H_2	By using bio-electrochemical reactions in the microbial system, the recovery of H_2 gas and substrate degradation is highest as compared to dark fermentation	Artificial enzymatic pathway created at the cellular level to achieve high H_2 yield at the fastest rate as compared to other processes
Drawbacks	The temperature-sensitive unstable process at higher H_2 yield	Light conversion efficiency is low	Requires high intensity of light, photochemical efficiency is low	Continuous supply of solar energy is required, thus unfeasible for large scale processes	Practically require external voltage for the specific growth of microbes on the anode	Prerequisite of multiple purified enzymes makes it costly
	The end product is a mixture of gases, where carbon dioxide has to be separated out	O_2 is inhibitory on nitrogenase enzyme	O_2 is inhibitory	Removal of uptake hydrogenase to stop the degradation of H_2	Dependent on various physico-chemical and microbial factors	Lab studies are going on to achieve full-scale commercial production

c. A strict anaerobe with a cytochrome system—facultative anaerobes (*Enterobacter* sp., *Escherichia coli*, *Klebsiella* sp., etc.).
d. Photosynthetic bacteria with light-dependent evolution of H_2 from reduced NADH—e.g., aerobes (*Bacillus* sp. and *Alcaligenes eutrophus)*, photosynthetic (*Rhodospirillum* sp. and Cyanobacteria *like Anabaena* sp., *Synechococcus* sp., *Oscillatoria* sp., *Nostoc* sp.) as well their consortia using enzymatic (*Thermoplasma acidophilum* and *Pyrococcus furiosus*) and electrogenic mechanisms (*Klebsiella pneumonia* and *Clostridium butyricum*). They are of paramount importance in terms of H_2 production on different raw substrates (Sasikala et al. 1994; Woodward et al. 2000; Tsygankov et al. 1999; Nandi and Sengupta 1998; Saratale et al. 2013).

A lot of research is undergone in the past decade on fermentative biohydrogen from these microbes to understand the process design and engineered mechanism. This led to optimize the structure of biohydrogen producing communities and improve it for a stable hydrogen economy (Venkata Mohan et al. 2019). The usual source of carbohydrate is glucose (raw substrate), which upon fermentation gives acetic acid and butyric acid together with hydrogen gas. But some technical issues need to be resolved to get an economically viable technology for mass production (Table 13.2). The problems that need to be tackled are poor conversation ratio of fermentative biohydrogen from the organic substrates, continuous light efficiency, oxygen sensitivity of hydrogenase enzyme, and separation of hydrogen from the gaseous mixture (Chen et al. 2008). Also, recent advances in genetic engineering, improved process design of bioreactors have opened a gateway for higher hydrogen production using microflora under aseptic conditions (Liu et al. 2008; Saratale et al. 2013). A brief discussion on the axenic culture systems and assorted culture systems of cultivation of these hydrogen-producing microorganisms is as follows.

Axenic Culture Systems

Many species of *Clostridium* and *Enterobacter* are the most widely studied in pure culture systems. They were grown on a variety of substrates like starch (Liu and Shen 2004), sweet potato starch (Yokoi et al. 2001), chitinous waste (Evvyernie et al. 2001), rice husk (Lo et al. 2009), and wastewater sludge (Wang et al. 2003) to check the exponential growth phase of hydrogen gas. The study by Shin (2004) revealed that the mesophilic acidogenic cultures were dominant species in hydrogen production. Further studies by Wu et al. (2008) showed *Klebsiella* sp. ability to produce some valuable products, such as gaseous H_2 and CO_2, a mixture of acids, ethanol, and 2,3-butanediol. Moreover, the successful trial of growing hyperthermophilic bacteria in the continuous growth setup showed promising results to produce good hydrogen yield resistant to high hydrogen partial pressure and without undesired by-products (Nguyen et al. 2010).

Table 13.2 Hydrogen photoproduction by various microalgae

S. No	Microalgae	Carbon source	Conditions tested			H_2 rate (ml L^{-1} h^{-1})	References
			pH	Temp. (°C)	L.I. (μE/m^2/s)		
1	*Chlamydomonas* MGA 161	CO_2: 5%, water: 95%	8	30	115	4.48	Ohta et al. (1987)
2	*Anabaena azollae*	2% CO_2 (v/v) in air	–	–	140	13.0	Tsygankov et al. (1998)
3	*Anabaena variabilis* PK84	Na_3VO_4 instead of Na_2MoO_4, N-free, modified BG-11	7.0	30	140	20.1	Liu et al. (2006)
4	*Chlamydomonas reinhardtii*	Sulphur-deprived; TAP medium	7.4	28	20	6.95	Kosourov et al. (2007)
5	*Clostridium pasteurianum*	Sucrose (20 g COD/l)	7.0	32	70	*7.1*	Chen et al. (2008)
6	*Chlorella sorokiniana*	Sulphur-deprived acetate (TAP-S medium)	7.2	30	120	1.4	Chader et al. (2009)
7	*Synechococcus* sp. H-1	CO_2: 6%, water: 94%	8–8.5	55	100	0.9	Asami et al. (2011)
8	*Arthrospira* sp. PCC 8005	Fe^{2+}: β-mercaptoethanol	7	30	40	5.91	Raksajit et al. (2012)
9	*Chlorella* sp. IOAC707S	Phosphorus-deprived; TAP/NaCl medium	7.2	28	25	40	Khorcheska et al. (2015)
10	*Chlorella pyrenoidosa* IOAC707S	Nitrogen-deprived L1 medium + 1 mL/L acetic acid	7.8	25 ± 1	25–30	26.26	Li et al. (2015)
11	*Tetraspora* sp. CU2551	TAP medium	7.2	36	*29*	24	Maneeruttanarungroj et al. (2010)

Assorted Culture Systems

The innovative experimentation to grow different microbes on the same substrate, to achieve the full-scale application is studied worldwide. The consortium can vary according to the chosen substrate with complex chemical compositions. The selection of different species works efficiently to degrade complex organic matter in an aseptic acidic environment. The self-regulating microbial consortium is capable of generating a renewable biohydrogen with good yield (Valdezvazquez et al. 2005). This mixed consortium can be derived from different substrates like sludge (Noike and Mizuno 2000), wastewater (Antonopoulou et al. 2008a, b), soil (Logan et al. 2002), compost (Khanal 2003), animal dung (Lay 2003), and agricultural waste (Wang et al. 2003). Despite the benefits of the mixed consortium, it has failed on commercial upscale because of the predominance of non-H_2 producing methanogenic bacteria over the hydrogen-producing bacteria. To overcome this situation, the pre-treatment of the substrate is carried out to remove methanogens (Shizas and Bagley 2005; Valdez-Vazquez and Poggi-Varaldo 2009). The seed cultures are exposed to thermal shock which kills the non-spore forming methanogenic bacteria (Wang et al. 2007), with different incubation periods between 15 min and 2 h (Mu et al. 2007). Some researchers even tried acid or base treatment to encourage the growth of hydrogen-producing bacteria (Lee et al. 2007). All these procedures showed promising results as methanogens cannot survive in highly acidic conditions.

13.2.2.2 Fungi

Mushrooms, the common edible fungi, anaerobic fungi, and fungal mycelium treated microbial consortia are the emerging trend in biohydrogen production. Fungi degrade the organic matter (carbon sources) and release acetic acid, molecular hydrogen, and carbon dioxide. The fungi treated lingo-cellulosic biomass and agricultural residue are economically suitable options for bioenergy generation (Singh et al. 2015). Recent studies on high hydrogen yield by the edible fungus; *Gymnopus contraries* supports biological hydrogen production process from lingo-cellulosic biomass (Sheng et al. 2018). Sometimes fungi accumulate certain lipids and fatty acids compositions by enzymatic action as oleaginous organisms (Fakas et al. 2009). That is why fungi are an ideal candidate for biofuel production as it has a short life span, rapid growth for mass production, require limited time and space. The mycelium biomass can be further optimized and easily scaled up in the fermentation process using integrated bacteria–fungus interaction to generate hydrogen and biodiesel in a continuous setup (Ghosh and Roy 2019). The filamentous fungi like *Rhizopus* sp. and *Aspergillus* sp., in combination with other microbial systems using enzymatic hydrolysis produce value-added products (Han et al. 2016; Benabda et al. 2019).

13.2.2.3 Algae

These are the most primitive photosynthetic microorganisms, found in all ecosystems, and they can flourish in diversified environmental conditions. They can be unicellular, multicellular, and filamentous algae and perform photosynthesis because of chlorophyll pigment. However, some algae are heterotrophic in nature, performing different biochemical routes to produce food. The biophotolysis of water facilitates algae to store chemical energy by capturing solar energy. The mechanism of biophotolysis can be classified as direct or indirect, depending on whether or not the flow of electrons through photosystem I and II directly to hydrogen-producing enzymes (Benemann et al. 1973).

Direct Biophotolysis System

This is the most studied method, where algae utilize sunlight as a source of energy by dividing the water molecules into hydrogen and oxygen (Mathews and Wang 2009). Recognised H_2-producing green algae-like *Chlamydomonas reinhardtii*, *Scenedesmus obliquus, Chlorococcum littorale*, and *Chlorella fusca* use the O2 sensitive hydrogenase enzyme in PS-I and PS-II where the electrons flow from water through to ferredoxin (Ding et al. 2016). In some green algae where the enzyme activity inhibits oxygen sensitivity causing sulphur deprivation conditions as proposed by Melis et al. (2000). In such a case, only limited electron acceptors are available in PS II, which activates the hydrogenases enzyme to generate electrons to protons to produce free hydrogen (Weber et al. 2014). This scenario clearly depicts the transition of direct biophotolysis to indirect biophotolysis, where the hydrogen produced is from the fixed carbon reserves in green algae (Hallenbeck 2013), whereas few algae-like *Dunaliella salina* and *Chlorella vulgaris* do not have hydrogenase activity.

Indirect Biophotolysis System

This mainly occurs in blue-green algae (*Anabaena* sp., *Nostoc* sp, *Synechococcus* sp., *Microcystis* sp., *Oscillatoria* sp., etc.) where the problem of enzyme inhibition by oxygen is removed through the temporal and spatial separation of oxygen and hydrogen evolution (Hallenbeck 2012). In the chronological phase, the normal growth of algae is by using light as the driving energy source to produce total carbohydrate and O_2 as a by-product. In the spatial phase, algae are reproduced photo-synthetically catalysing carbohydrate substrate by the nitrogenase enzyme and produce hydrogen via anaerobic fermentation (Noth et al. 2013). This is generally observed in cyanobacteria, where the nitrogenase enzyme is localized in the heterocysts that capture nitrogen in the air and fix it into the soil (Lopespinto 2002).

Integrated System

This system is employed as neither direct nor indirect biophotolysis algal system worked out significantly to achieve the sustainable and commercial biohydrogen production. The technical issues like low hydrogen yield, O_2 sensitivity of hydrogenase enzymes, high cost of cultivation, photobioreactor design, and harvesting methods have prompted for integrated approach studies (Khanna and Das 2013; Kuppam et al. 2017). This is focused on microalgal starch as a substrate for fermenting bacteria to produce biohydrogen during dark fermentation. Few recent studies in combination with hyper-thermophilic bacteria utilize starch accumulated cells of *Chlamydomonas reinhardtii* (Nguyen et al. 2010); *Chlorella vulgaris* (Sun et al. 2014), and *Clostridium acetobutylicum* (Efremenko et al. 2012) to yield hydrogen. The evolution of hydrogen was dramatically increased when the microalgal cells are exposed to enzymatic hydrolysis. Also, the microalgal biomass is subjected to two important pre-treatment steps: (1) disruption of the microalgae cell wall to release the starch and (2) hydrolysing the starch macromolecule into simple reducing sugar to enhance its utilization (Chandrasekhar and Venkata Mohan 2014a, b; Enamala et al. 2018). This system is exploring diversified biofuel production from the algal resources by optimizing the overall life cycle energy balance and economic feasibility of biohydrogen in industrial applications.

13.2.3 Types and Mechanisms of Biohydrogen Generation

Biological hydrogen generation is primarily dependent on the occurrence of a hydrogen-producing enzyme. The metabolic activity of enzymes works on complicated active sites made up of metalloclusters, and detailed molecular insights are going on to enhance the low production and slow energy conversion rates. To overcome such practical problems in a closed biological closed system, certain mechanisms are studied in detail along side advancements in hydrogen production technologies via the following different routes (Fig. 13.2).

13.2.3.1 Direct Biophotolysis

Direct biophotolysis includes photosynthetic production of hydrogen by dissociating water molecules into hydrogen and oxygen in the existence of solar radiation. Green microalgae can utilize H_2 as an electron donor in the CO_2 fixation process or evolve H_2 under anaerobic conditions (Ni et al. 2006). The general reaction is

$$2H_2O + \text{Light Energy} \rightarrow 2H_2 + O_2 \tag{13.1}$$

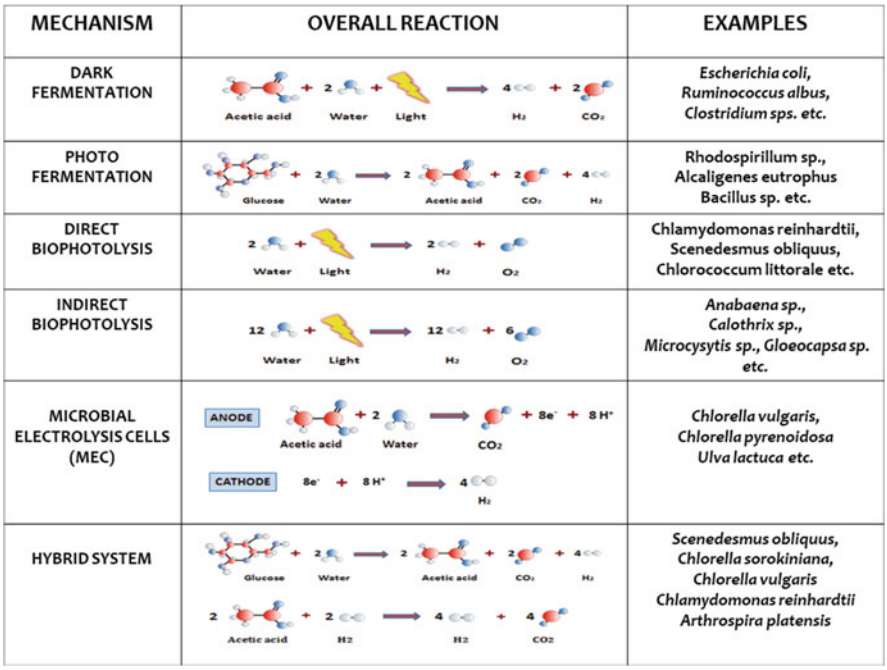


Fig. 13.2 Different mechanisms of hydrogen production technology

13.2.3.2 Indirect Biophotolysis

Indirect biophotolysis involves two step processes, firstly water molecules are split in the presence of light and hydrogen and oxygen molecules are released. Secondly, carbon dioxide fixation takes place by reducing carbohydrates by hydrogenase enzyme and producing hydrogen gas. Several species of blue-green algae-like *Anabaena*, *Oscillatoria, Calothrix*, and *Gloeocapsa* use this mechanism to produce H_2 (Sen et al. 2008).

$$12H_2O + 6CO_2 + \text{Light Energy} \rightarrow C_6H_{12}O_2 + 6O_2 \tag{13.2}$$

$$C_6H_{12}O_6 + 12H_2O + \text{Light Energy} \rightarrow 12H_2 + 6CO_2 \tag{13.3}$$

13.2.3.3 Biological Water-Gas Shift Reaction

Biological water-gas shift reaction includes only photo-heterotrophic and gram-positive bacteria, which can survive in the dark, utilizing CO as the one and only source of carbon to generate H_2 and CO_2 along with ATP (Levin 2004). By the

combination of steam reforming, pure H_2 is obtained following the H_2O-gas shift reaction, mainly used extensively to produce industrial ammonia.

$$CO + H_2O \rightarrow CO_2 + H_2 \qquad (13.4)$$
$$\Delta G^0 = 20\ (\mathrm{KJ/mol})$$

13.2.3.4 Photo-fermentation

Photo-fermentation is based on the decomposition of organic substrate to hydrogen in the absence of both O_2 and N_2, but in the presence of photosynthetic bacteria such as purple non-sulphur bacteria that have potential to convert light energy into H_2 (Sen et al. 2008).

$$C_6H_{12}O_6 + 12H_2O + \text{Light Energy} \rightarrow 12H_2 + 6CO_2 \qquad (13.5)$$

13.2.3.5 Dark fermentation

Dark fermentation takes place in a dark environment without light, air, and water. Fermentative microorganisms such as hydrogen-producing bacteria breakdown the complex organic polymers to monomers by hydrolysing them releasing a mixture of organic acids and alcohols. The bacteria used in dark fermentation can be either mesophilic (25–40 °C), thermophilic (40–65 °C), extreme thermophilic (65–80 °C), or hyper-thermophilic (more than 80 °C) temperatures (Levin 2004). Depending on the substrate used and the reaction process, it produces mixed biogas comprising of H_2 and CO_2, but sometimes it can release CH_4, CO, and H_2S. With glucose substrate, a maximum of 4 molecules H_2 is produced per mole of glucose when the end product is acetic acid.

$$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 4H_2 + 2CO_2 \qquad (13.6)$$

13.2.3.6 The Hybrid System

The hybrid system comprises of two stages, the first one is dark fermentation and the second is light fermentation using anaerobic and photosynthetic bacteria so that the hydrogen yield can be maximized.

$$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 4H_2 + 2CO_2 \quad (13.7)$$

$$2CH_3COOH + 4H_2 \rightarrow 8H_2 + 4CO_2 \quad (13.8)$$

In this hybrid system, the anaerobic fermentation of carbohydrate (or organic wastes) produces intermediates, which contains low molecular weight organic acids, that are later converted into hydrogen by photosynthetic bacteria in the second step. A total of twelve mol hydrogen could be expected in a combined process from 1 mol of glucose (Chandrasekhar et al. 2015, 2020; Kumar et al. 2017).

13.2.3.7 Microbial Electrolysis Cells (MECs)

Microbial electrolysis cell (MEC) is a novel system to generate hydrogen gas from a broad range of substrates, using small input of external voltage to produce hydrogen. It works on the thermodynamic principle of the redox potential of H^+ to H_2 and converts the electrically driven protons to microbial fuel cells. Since it is a new concept, the performance of the microbial electrolysis cell is based on the design. The functional design depends on the type of microorganism, the membrane used, electrode materials, voltage range, composition and concentration of the substrate (Kadier et al. 2018).

13.2.4 Developments in Biohydrogen Research

The last decade had witnessed a very extensive effort towards the exploration of alternative fuels. So, we have taken into consideration the year-wise data of the last 10 years, to sum up, the trending research interest for circular bioeconomy. In this direction, a lot of research work was dedicated to renewable sources of energy to meet the basic domestic and industrial demands. Also, the main objective was to look after the environment-friendly options of fuel and be the reason for cleaning nature rather than polluting it more. The production of biohydrogen (an emerging technology) with zero greenhouse gas emissions makes it an ideal candidate towards greener energy. This has created a huge turnout of research activities completely based on biomass to produce as much as H_2 as possible utilizing minimum resources (Chandrasekhar et al. 2020). A detailed statistical analysis has found the sum of 5149 peer-reviewed articles published in Frontiers journals on biohydrogen production studied different microorganisms, including algae that yield H_2 under specific conditions.

To understand the hydrogen energy development trend better a brief account of the year-wise graphical presentation is given in Fig. 13.3. For instance, Fig. 13.3a represents scientometric work published in ScienceDirect year-wise since its inception. There is a significant rise in a publication from 2010 onwards. The red bar

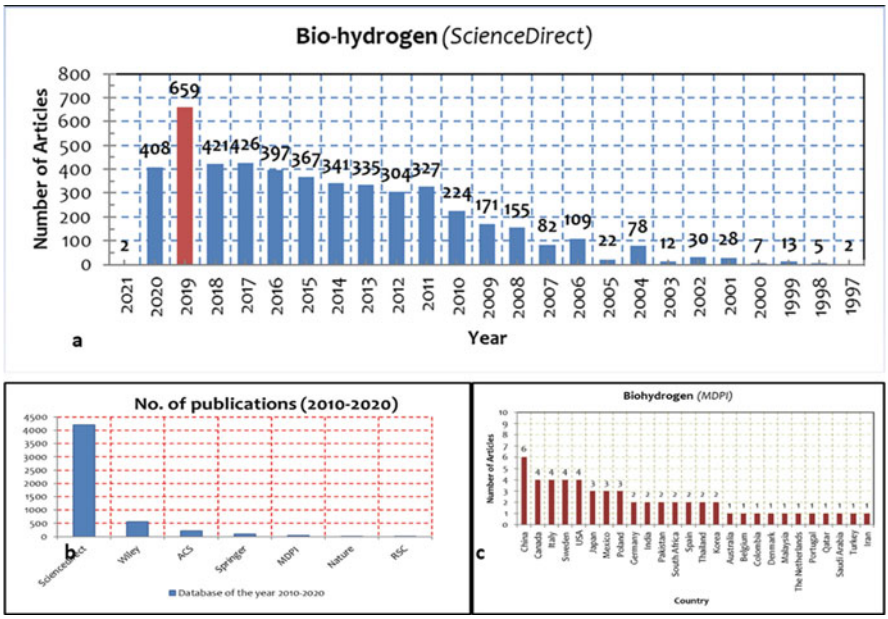


Fig. 13.3 (**a**) Scientometric analysis of biohydrogen research from the year 1997 to 2021; (**b**) Frontier research on biohydrogen from the year 2010 to present; (**c**) Statistical analysis of country-wise publications on biohydrogen

indicates the highest number of articles on biohydrogen in the year 2019, and it is still gaining momentum to outreach in 2020.

Figure 13.3b provides categorical details about articles on renewable sources of energy after 2010. The scientific publications were shortlisted and systematically reviewed on the basis of online information available on books, book chapters, research articles, short communications, review articles, conference proceedings, encyclopaedia, editorials, and other sources of information. Thomas Reuters database further supported with increased citations in this promising field of algal biohydrogen, where the continuous efforts are going on to make this novel technology visible and viable on a commercial scale.

a. The ScienceDirect database (2010–2021) represented a total of 4211 research articles, with the highest publications in the international journal of hydrogen energy.
b. ACS journals seen a total of 209 research articles published on biohydrogen until now and with the highest number, 51 publications were reported from the journal of energy and fuel.
c. There are a total of 555 publications in the Wiley journals focused on biohydrogen accounts for the highest number of online book publications, following research articles in the subject of chemistry and chemical and biochemical engineering.

d. About 103 publications were reported in Springer journals, where the renewable and green energy journal published 24 research articles, followed by 11 in the environmental engineering and biotechnology and 9 in the journal of microbiology.
e. MDPI research journals have published 45 research articles on the theme of biohydrogen. The prominent journals are Energies, International Journal of Molecular Sciences, Processes, and Sustainability.
f. In top-rated journal Nature, had a total of 14 publications, out of which 4 were the scientific reports and others include reviews and research highlights.
g. Presently, there are a total of 8 research articles in RSC journals and 4 books on biohydrogen.

Figure 13.3c represents the research publications country wise in the sector of bioenergy for the future hydrogen economy. So far, based on journal publications on biohydrogen; China is leading, followed by Canada, Italy, Sweden, and the USA.

This analysis provided a great insight into the past and ongoing research on biohydrogen production all over the world. The interest in green energy has developed a great competition in this field. The developed as well developing nations are putting a lot of funds towards the scientific community. The gained thrusts in this field especially after 2010 highlight its importance. The applications of microbial fuel cells, fermentation techniques, microbial electrolysis, and assorted consortium as biocatalyst for the hydrogen production showcased the trending publications of bacterial, fungal, and green algae in citations and H-index (Mohan and Pandey 2013). Most of the databases are published as research articles, conference proceedings papers, posters, short communication, review articles, and books.

13.3 Biohydrogen Generation by Algae

The algal biomass liberates the hydrogen in its metabolic reaction. The main challenge is to amplify the produced hydrogen and harness it by practical approaches. At present, many investigations are implemented all over the world to achieve viable biomass productivity. The volumetric rates are dependent on the nutrient dissolution, pH, temperature, salinity, inorganic carbon, oxygen, carbon dioxide, and light penetration that vary species to species to evolve optimum hydrogen in an efficient manner. In the coming topic, various algae utilized on upscale production of algal biomass and the manipulation of several factors that play a significant role in the growth and development of algal culture. The anticipated barriers in their light to hydrogen conversion efficiencies should be discussed.

13.3.1 Systematic Role of Different Algae

Algae are found universally in diverse aquatic environments, sharing common characteristics of the morphological and physiological features such as the capacity to perform photoautotrophic metabolic processes. They are able to remove ambient carbon dioxide from the environment and thus are the optimal sources regarding ease of production of biomass. More than 40,000 algae species were identified, which is possibly a minute portion of the overall amount of existing species. The Aquatic Species Program of the U.S. Department of Energy has evaluated over 3000 unique species of microalgae, which are potentially utilized as feedstocks for the production of various biofuels. Table 13.2 lists some microalgae species which have been utilized as feedstocks for biohydrogen production.

13.3.2 Factors Affecting Biohydrogen Production

There are several abiotic and biotic factors involved in the production of hydrogen from algae. For efficient growth, it requires certain nutrients: light, CO_2, water, and carbon source (Kakarla et al. 2017). Here, we will discuss the role of some factors, which play a critical role in the growth and development of algal biomass with respect to biohydrogen production.

13.3.2.1 Role of Temperature

The operating temperature is one of the main parameters of the biochemical procedures. The temperature controls the morphological, physiological, and cellular behaviour of microalgae. A higher temperature leads to the augmentation of the metabolic activities of microalgae and also affects the rate of hydrogen production (Saifuddin and Priatharsini 2016). In general, a specific operating temperature is a crucial factor in the formation of biohydrogen, which usually depends on the individual species of the microorganism selected for the production of biohydrogen along with the variety of substrate used.

13.3.2.2 Role of pH

pH is one of the most crucial factors which influence the production of hydrogen. There is a major impact of pH on the production of fermented hydrogen as it is a determining parameter for acidic and alkaline conditions. The production rate of hydrogen depends on the inner pH of the cells because the pH decides the proton concentration, which can affect the metabolism process resulting in the effect on the production rate of hydrogen (Manish and Banerjee 2008). Different strains of

microalgae have various optimum pH. Most of the microalgae species prefer neutral pH for the production of hydrogen. The appropriate pH to enhance the yield of hydrogen is largely dependent on the individual species of microorganisms and materials used (Nath et al. 2006). Ma et al. (2015) reported that the favourable pH range for hydrogen production is 5.5–6.5, and the highest production of gas occurs at this pH. Liu et al. (2008) and Ngo et al. (2012) reported the important aspect of the pH is that it influences the function of (Fe–Fe) hydrogenase enzyme because lower pH alters the function of this enzyme and reduces the production of hydrogen. Kosourov et al. (2003) noticed that *C. reinhardtii* has produced the highest hydrogen at pH 7.7, and the lowest production of hydrogen was achieved at pH 6.5 or pH 8.2. The pH of 7.7 was appropriate for the optimal evolution of hydrogen when compared to other sets of studied redox microenvironments.

13.3.2.3 Role of Nutrients

For the proper functioning of algae, various inorganic nutrients like carbon, nitrogen, phosphorus, sulphur, and potassium play an important role. The presence of sufficient nutrient and changes in nutrient load may affect algal growth. The study of Lin and Lay (2005) demonstrated that the carbon/nitrogen (C/N) ratio plays an important role in the productivity and the production rate of hydrogen. They reported that pure algal culture having a carbon–nitrogen ratio of 47 yields 4.8 mol H_2/mol sucrose that acts as a carbon source and releases 270 mmol H_2/L/day. Nitrogen is required as an exogenous source of ammonia, usually preferred by blue-green algae as a prime nutrient source (Berman-Frank et al. 2003). However, a high amount of nitrogen inactivates the nitrogenase enzymatic activities. To sustain in adverse conditions, algae require phosphorus element that accounts for 0.03–0.06% of total algal biomass (Hannon et al. 2010). It is essential for energy generation and other cellular metabolisms and its high concentration leads to low production of hydrogen (Chandrasekhar et al. 2015). Sulphur plays an important role in the electron transport chain, protein synthesis, and lipid metabolism. In case it is present in fewer amount may result in stunted growth and low density (Yildiz et al. 1994). Potassium, as a micronutrient, cannot be overlooked for its role in the growth of algae and other metabolic activities (Maathuis 2009).

13.3.2.4 Role of Trace Metals

The acquisitions of limiting metal ions are essential in the production of algal biohydrogen. Algae mostly prefer the chelated form of iron as its oxidized form is not readily absorbed by the algae. Iron is found as a bimetallic cluster in the hydrogenase enzyme and as an iron-sulphur cluster in the photosynthetic proteins. Iron ions act as an active site for the ferredoxin, which transports the electrons to the hydrogenase enzyme (Lee et al. 2007). Besides this, magnesium, zinc, and sodium

are important metal ions required in trace amounts for the optimum production of hydrogen in the blue-green algae (Srikanth and Mohan 2012).

13.3.2.5 Role of Enzymes

The main enzymes found in the green algae are called as algal hydrogenases, which are sensitive towards the oxygen (Vignais 2008). As discussed in Sect. 13.2.2.3 the FeFe-hydrogenases, NiFe-hydrogenase, and nitrogenase enzymes are responsible for hydrogen production in the green algae and blue-green algae (Cyanobacteria) where they catalyse high starch (carbohydrate) reserves in anaerobic conditions (Stripp and Happe 2009). In the green algae, the FeFe-hydrogenase is the most common enzyme consisting of a small peptide chain of 15 kDa. The active part of this enzyme is H-cluster which is located at the 'C' terminal. It is an active receptor site for electrons released from ferredoxin compound (4Fe-4S) which binds to the H-cluster. FeFe-hydrogenases in algae receive reducing equivalents at the end of the photosynthetic electron transfer chain via ferredoxin (Melis and Happe 2005).

Photosynthetic Hydrogenases

The [FeFe]- and [NiFe]-hydrogenase enzymes enhance the hydrogen production in the photosynthetic apparatus by partial inhibition of oxygen produced by photosystem II and also maximize the photosynthetic metabolism in the chloroplast. Therefore, they are also called as 'photosynthetic hydrogenases' (Hemschemeier et al. 2009). The study done by Ghirardi et al. (2009) emphasized the production of these enzymes to produce renewable 'biohydrogen' using sunlight as the source of energy. Another approach is to design electrical hydrogen battery cells separated by two gas-sealed cells to produce electricity. The exchange of electrons between the anode site (photosystem II) and cathode site (photosystem I) on illumination liberates oxygen and hydrogen (Esper et al. 2006). The uptake of multiple electrons in hydrogenases is because of large starch macromolecules that carry catalytic centres of iron and sulphur atoms. The positive charge of the proton compensates for the negative charge of the electron, which accelerates the conversion of hydrogen ions (protons) to hydrogen gas with high efficiency. Thus, the light is an important determining factor for the hydrogenases to absorb excess electrons that are generated during photosynthesis and release hydrogen gas as a by-product.

Nitrogenase

In the case of cyanobacteria (blue-green algae) the nitrogenase is located in the heterocysts which shield it from the oxidation process. ATP is required by the nitrogenase enzyme to reduce nitrogen to ammonia. In the enzymatic process, hydrogen gas is evolved in the absence of nitrogen gas in the heterocystous cells

later transported via cellular metabolic activities to vegetative cells (Asami et al. 2011; Chandrasekhar et al. 2015).

All three enzymes can work together or solely based on the environmental factors required for the growth. The hydrogenase enzyme is categorized into two metal forms, FeFe-hydrogenase and NiFe-hydrogenase, whereas the nitrogenase enzyme is found in association with Mo, V, and Fe-metal contents. Because of this closed association, the hydrogen uptake or evolution is very difficult to observe, and more studies are needed to understand this mechanism further (Benemann 1999).

13.3.3 Metabolic Pathways Involved in Hydrogen Generation

It is a well-known fact that in green algae and blue-green algae, hydrogen can be generated by biophotolysis, which includes water-splitting and oxygen-evolving system. The charged electrons are carried by ferredoxin, Fd through the photosystem II and photosystem I, and by using enzyme hydrogenase converted into hydrogen gas (Fig. 13.4).

In this coupled photosystem process, the starch (glucose) fermentation involves three metabolic pathways, first by the oxidative decarboxylation of pyruvic acid to acetyl-CoA, followed by oxidation of NADH to NAD+, and lastly acetogenesis by hydrogen-producing acetogens. These mechanisms can be aerobic and anaerobic based on the availability of light, the catabolic fermentation of endogenous substrate is done via oxidative carbon metabolism, whereas during unavailability of light, the heterotrophic fermentation takes place in an anaerobic environment. The hydrogen evolution rates are high when switching of normal photosynthesis with the dark anaerobic condition takes place. The hydrogenase enzyme plays an important role in hydrogen production by glucose fermentation (Woodward et al. 2000).

Generally, the oxygen produced by direct biophotolysis generates a low amount of hydrogen because of oxygen inhibition with the hydrogenase enzyme. Also, the

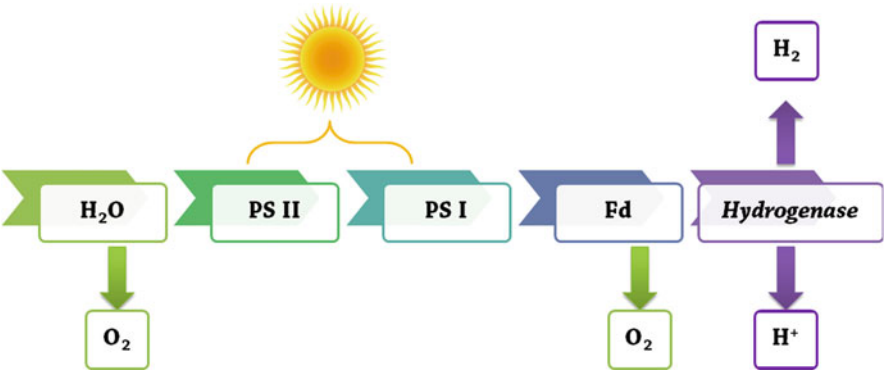


Fig. 13.4 Systematic metabolic pathway in the green algae

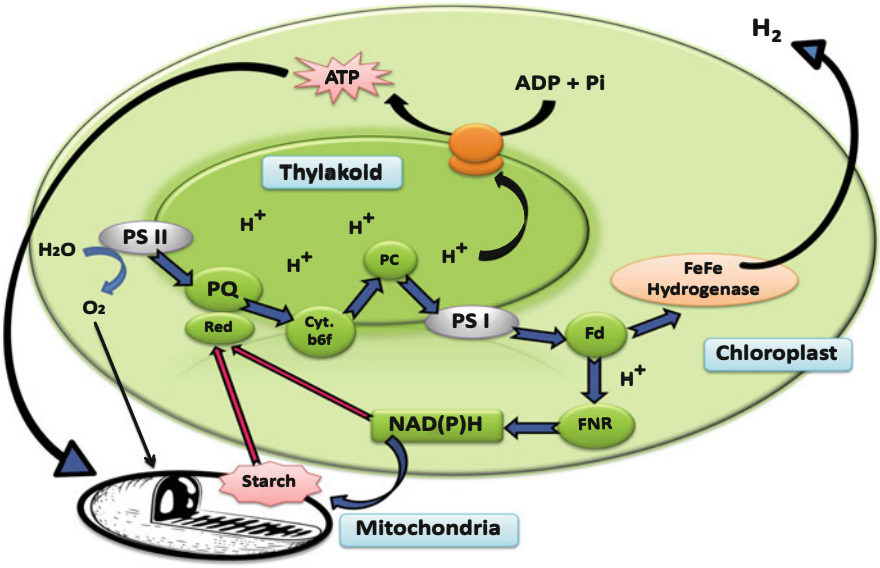


Fig. 13.5 Mechanism of hydrogen production in green algae. The blue arrows indicate electron transport from photosystem II to the [FeFe]-hydrogenase. The red arrows show oxidation of reducing equivalents and the NAD(P)H-plastoquinone oxidoreductase required for the respiration activity. *PS* photosystem, *PQ* plastoquinone, *Cyt. b6f* cytochrome b6f complex, *PC* plastocyanin, *Fd* ferredoxin, H^+ protons

large antenna pigments interfere with light levels due to fluorescence shadow and heat evolved and contributes to low generation of hydrogen. To increase the hydrogen potential, the electrons pass through the thylakoid membrane to push electrons towards photosystem II, where the water split reaction occurs and releases the remaining electrons to cytochrome b6f complex by plastoquinone (PC). The newly formed complex enters photosystem I, where this complex gets reduced by ferredoxin, following the production of NADPH by reducing NAD+. During this electron transfer, an electrochemical gradient has created that releases the protons out of transmembrane using ATPase to generate ATP. The Calvin cycle then uses the produced NADPH to fix carbon dioxide to glucose, which is used in the growth and other biological activities (Fig. 13.5).

13.4 Secondary Products Released During Metabolic Pathways

The energy crisis in the 1970s changed the people's outlook towards algal cultivation as a natural source of valuable products and not just as food and feed. In the midst of industrialization, the exploration of value-added products gained

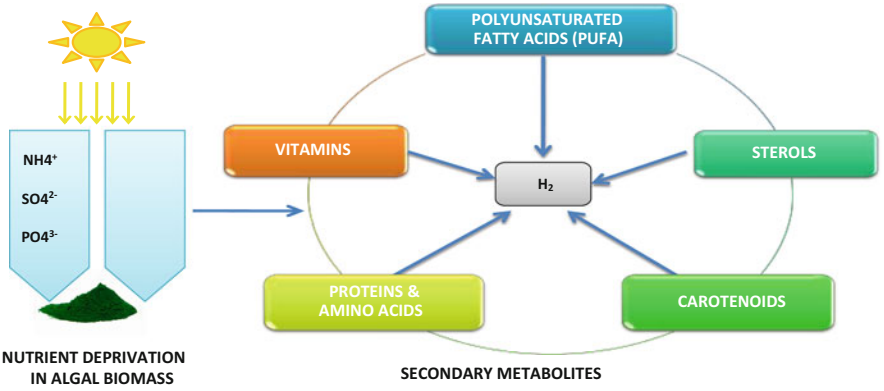


Fig. 13.6 Some secondary metabolites isolated from green algae during hydrogen production

momentum in various countries like Japan, Europe, and others, where the cultivation of algae is practised since ancient times as a food supplement. Every year the processed dried algal biomass generates bio-products revenue worth of US $1.25 × 109, which is a huge potential of the industrial market in the field of pharmaceuticals, nutraceuticals, cosmetics, and functional foods. A number of secondary compounds (primarily photosynthetic pigments) like carotenoids (astaxanthin, β-carotene, lycopene, etc.), polyunsaturated fatty acids (PUFA), sterols, vitamins, proteins, and amino acids. All these products are commercially used in medicinal activities as they have therapeutic effects on various diseases. The most common secondary metabolites isolated from microalgae during hydrogen production are graphically listed in Fig. 13.6.

13.4.1 Polyunsaturated Fatty Acids (PUFA)

The polyunsaturated fatty acids are the essential fatty acids present in many algae, necessary for various biological activities. They are generally produced during the nutrient deprivation of the growth stage, where the lipid content increases per cell in the algal culture based on certain stressed conditions like temperature, light intensity, and salinity. The oxidative end products like alcohol and aldehydes play an important regulatory role in enzymatic reactions (Enamala et al. 2018). The food supplements rich in PUFA are beneficial to humans as antibiotics, anti-inflammatory, and other chronic diseases (Riediger et al. 2009).

13.4.2 Carotenoids

The carotenoids are one of the major pigments having commercial applications as a dietary supplement and feed additives, pharmaceutical and cosmetic industries. The primary carotenoid lutein is produced at optimum salinity and high temperature and mainly used as a colourant (E161b) in foods and cosmetics (Jin et al. 2003). β-carotene has a crucial role in vision and the immune system, due to its relation to vitamin A by acting as a precursor of visual pigment. Astaxanthin, a secondary carotenoid is used as a food dye (E161g), giving colour to egg yolks and chicken skin; dietary supplements to poultry have been shown to be associated with increased vitamin E contents of the liver (Surai et al. 2003). β-carotene and astaxanthin also have strong effects on the enzymatic antioxidant defence system by preventing oxidative stress through scavenging of free radicals. Therefore, used as an encapsulated antioxidant product in nutraceuticals (Bishop and Zubeck 2012).

13.4.3 Sterols and Glycerol

Many algae, especially the diatoms, are rich in sterols where they are synthesized in large numbers during unfavourable conditions. They are useful in aquaculture nutrition as they act in signal transduction. Glycerol is another organic metabolite useful in osmoregulatory functions of enzymes. Its production in the algal cell is highly influenced by the light intensity and external water activity. Glycerol is widely used in cosmetics, pharmaceuticals, paint, food (E422), industries of pulp and paper, leather, and textiles, and as a feedstock for the production of various chemicals (Wang et al. 2001).

13.4.4 Vitamins

Many vitamins like β-carotene and tocopherols, nicotinic acid thiamine, riboflavin and biotin are readily produced by various algae. The studies showed that vitamin production is increased in stressed nutrient-deprived conditions (Durmaz 2007). They are widely used in the food industry as food preservatives, stabilizers, and as nutraceutical (E306), applicable in treating various memory-related diseases like Alzheimer's and Parkinson's (Skjanes et al. 2013).

13.4.5 Proteins and Amino Acids

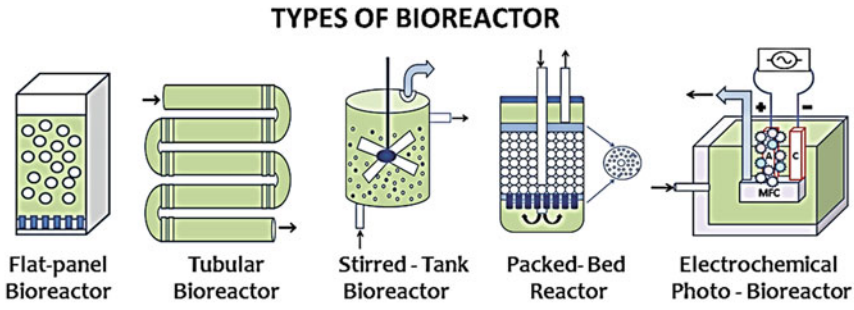
Proteins are the integral component of the light-harvesting complex, engaging various enzymatic actions in the photosynthetic process in the algal cells. Several species of green and blue-green algae are rich in protein content, which are commercially applicable in producing food supplements. However, the low digestibility of protein in humans is still debatable, e.g., *Chlorella* sps. and *Spirulina* sps. Besides these proteins, there are antifreeze proteins extracted from algae and other microorganisms that are able to bind to ice crystals, prevent re-crystallization, and protect other proteins from damage. These are used for various biomedical, agricultural, and industrial applications. In case of Chlorella nivalis, there are certain mycosporine-like amino acids bound to cellular chromatophores, which help in protection from ultraviolet radiation and sudden cold stress (Karsten et al. 2007).

13.5 Types of Bioreactors Used for Producing Biohydrogen

The design of bioreactor is significant in the production of biohydrogen besides fulfilling all the above nutrient requirements. In the past tradition, usual open raceway ponds are oval-shaped, made of concrete and the mechanical mixing and circulation of the liquid medium is done by paddle wheels. However, this system is unsuitable for hydrogen production and its collection due to lack of anaerobic environment. Also, the large and open area for its setup results in great water loss because of evaporation (Dasgupta et al. 2010). For the optimum algal biomass yield, a bioreactor must possess a gas and light radiation, liquid nutrient medium, low contamination rate, uniform mixing of biomass, simple to operate, and cost-effective in space and time. Though in past several photobioreactors designs are tried and tested in the algal biohydrogen production, only a few designs sustained in terms of cost-efficiency. The photochemical efficiency, light regime of light and dark cycles; trapping and withdrawal of hydrogen in the system, high-density algal cells are required for scale-up for maximum hydrogen production (Gutierrez-Wing et al. 2014). The modular designs of different photobioreactors based on successful operational costs and maintenance, resources required, and energy consumption are given in Fig. 13.7.

13.5.1 Flat-Panel Bioreactor (FBR)

The design of a bioreactor is a small rectangular box made up of glass or transparent plastic and joined by a perforated tube at the base. The hydrogen gas introduced from the tube gets aerated by photosynthetic algae. A separate degasser is installed to remove residual gases from the flat-panel reactor. The panels are illuminated by solar



FBR capture light efficiently, high gas transfer coefficient and temperature Control	*TBR capture light well and excellent temperature control*	*STBR perform uniform mixing and excellent temperature control*	*PBR is attached with support carriers (algal biofilm) – less turbulent mixing*	*EPBR is improved version of algal biofilm reactor mainly focused on higher hydrogen production efficiency*
Difficult to Scalable	*Reasonable to scalable*	*Difficult to Scalable*	*Economical viable feasible to upscale*	*Economical viable to scale up*
Low shear stress; oxygen build up at panel and low hydrogen yield	*Fouling smell and algal cells stick to walls*	*Hydrodynamic stress on algae*	*pH gradient sensitive; require recirculation*	*Continuous supply of electric current and carbon feedstock*

Fig. 13.7 Types of bioreactor and their advantage and disadvantage in hydrogen production

radiation at one end, where the diffusion of light can be controlled by tilting the orientation of bioreactor. It is successful at small-scale only with uniform algal strain, low power consumption, and high biomass yield. The main drawback of this design is the inefficient mixing of gases and controlled temperature (Kumar et al. 2020).

13.5.2 Tubular Bioreactor (TBR)

Closed tubular reactor made up of glass or plastic have with many loops. The long transparent tubes are designed diversely along different planes with several U-bends that increase the photochemical efficiency due to shorter light/dark cycles. The performance of the photobioreactor is dependent on the length and diameter of the tube as well as the mixing of biomass concentration by air-lift technology. The main advantage of this design is uniform nutrient supply and exposure to light. This led to a homogeneous gaseous gradient inside the long tubes (Dasgupta et al. 2010). The main drawback is the requirement of a large area for these tubular reactors, low CO_2 and high O_2 in the extended tubes during mixing of culture and high energy consumption.

13.5.3 Stirred-Tank Bioreactor (STBR)

In one of the most conventional bioreactors, a continuous stirring of the tank takes place by a stirrer placed either at the top or bottom of the tank. It needs continuous air supply for the thorough mixing of suspended algal cells in the liquid medium. The ratio of liquid height to tank diameter is crucial for optimizing the fermentation of algal biomass. The main drawback of this technique is that the high agitation of immobilized cells of substrate and product is required to reduce mass transfer and can be achieved by the use of alginate/glass beads (Akkerman 2002; Moreira et al. 2006).

13.5.4 Packed-Bed Bioreactor (PBR)

A simple tubular tank where the suspended microalgae cells are packed with immobilized enzymes or other biocatalysts. The microbial activity is limited within heterogeneous biomass and the hydraulic mixing is less as compared to the stirred-tank bioreactors. The main drawback of this bioreactor design is the uneven pH gradient, which is not circulated properly in the vertical column and recirculation flow is needed to achieve high hydrogen yield (Fang and Liu 2002).

13.5.5 Electrochemical Photobioreactor (EPBR)

An advanced bioreactor using electrogenic microorganisms consuming and releasing electrons, protons, and carbon dioxide. The hydrogen is produced from the microbial fuel cell platform placed in the bioreactor. The light intensity and high voltage power supply release hydrogen ions, which unite to form hydrogen gas. The main drawback of this photobioreactor is that it requires a continuous power supply and carbon-based feedstock to work efficiently, resulting in high operating costs (Hasnaoui et al. 2020).

A productive photobioreactor must have an optimum exposure of solar radiation and a large surface-to-volume ratio, in a closed controlled environment without contamination for the maximum efficiency of biohydrogen production. Recent improvements in the bioreactor designs such as, multi-stage type and hybrid type work on gas-tight systems that have routine nutrient supply to artificially cultured algae.

13.6 Challenges and Opportunities in the Algal Bioenergy Market

The green technology sector is keeping algal balls rolling to offset the emission of greenhouse gases, which are affecting the global climate change every year. The global market eyeing at the algal products is gradually gaining pace to develop commercialized algal bioreactor facilities. It is predicted that the global demand for biohydrogen is likely to increase by 4–5% during the next 5 years. Coming to the Asian countries a higher growth percentage rate of biohydrogen consumption is estimated, which is going to increase by 2030 as twice compared to the consumption rate of 15 years back (Saifuddin and Priatharsini 2016). The global algae market estimate is valued at US $717.14 million in 2018 and expected to achieve an ambitious goal of about US $1365.8 million by the year 2027 (Hydrogen Generation Market 2015).

The world needs biohydrogen as future fuel stock to meet various energy demands. The cost of production of biohydrogen from microalgae can be brought down by metabolic and genetic engineering. Presently, China has the highest biohydrogen market and will increase by the year 2050, which is followed by Canada, USA, Japan, and India. When compared with the global scenario, India stands in 5th position for primary energy consumer and the 4th largest consumer of petroleum-based products and India is expanding its algal resources to grow at 6–8% per year to combat this energy crisis (Hemaiswarya et al. 2012).

The high demand for these renewable energy sources obtained from algae could scale up to produce hydrogen fuel in the coming years at a competitive price and made us less dependent on fossil fuels. Thus, we can expect a healthier life as algal business models will produce potential value-added products. Also, there is a huge inflow of venture capital investments are going on in the four economies to generate a niche for this emerging energy sector (bioeconomy) by the year 2050.

13.7 Conclusion

The surging interest of biohydrogen production worldwide from algal resources might be a tough journey ahead as the recent findings are building up the process design. Advanced photobioreactors seem promising; further, intensify algal research on biophotolysis and anaerobic fermentation to ensure commercially scalable hydrogen to solve the global energy crisis. The next generation biohydrogen from algal systems (aerobic and anaerobic phase) remarkably reduces carbon emissions. The genetic breakthrough on certain algae such as *Chlamydomonas reinhardtii*, C. *moewusii*, *Ethanoligenens harbinense*, *Synechocystis*, etc. along with other microbes in the lab controlled environment shows promising results in terms of higher biohydrogen production. However, still, much improvement is needed in bioreactor process design and economic viability. At the molecular level, the

bottleneck is to characterize model microalgae from extreme environments in nutrient-deprived conditions and produce biohydrogen and other value-added products simultaneously. Genetic engineering of strains could be a possible approach to be considered to improve the hydrogen production using algae, while the possibilities are not that far.

Conflict of Interest There is no conflict of interest between authors.

Financial and Ethical Disclosure No financial and ethical issues involved.

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Chapter 14
Biopolymer: Production from Biomass

Rishi Gurjar and Manaswini Behera

Abstract Implementing biomaterials instead of synthetic materials and non-renewable resources that lead to pollution is becoming popular. These biomaterials are naturally derived polymers from plants and microbes and are termed as biopolymers. Their production can be carried out by utilizing food processing industries, lignocellulosic and organic fraction of municipal wastes, thereby developing a clean waste management system. This chapter mainly discusses the types of biopolymers such as polyhydroxyalkanoates, xylooligosaccharides, lipid wax, exopolysaccharides, and succinic acid, to be obtained from eco-friendly bio-chemical processes carried out in biorefineries. However, strict and enriched conditions are required for producing biopolymers. Therefore, a detailed discussion on the effect of various operating parameters and their range that can be used to maximize biopolymer yield is also included. Biopolymers have been used in numerous fields' especially biomedics, pharmaceutical, and food packaging industries. The chapter gives an overview of biopolymer production and challenges faced in developing large scale production unit that will enable biopolymer as a product of circular economy.

Keywords Biorefineries · Polyhydroxyalkanoates (PHA) · Xylooligosaccharides (XOG) · Lipid wax · Extracellular polymeric substances · Circular bioeconomy

Abbreviations

ADF	Aerobic dynamic feeding
EPS	Extracellular polymeric substance
PE	Polyethylene
PHA	Polyhydroxyalkanoates
XOS	Xylooligosaccharides

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14.1 Introduction

Petroleum-based polymers' applications have been on the rise since its inception. This growing demand has been a cause of pollution as petro plastics are produced from non-renewable sources and are non-biodegradable. The former increases its carbon footprints, whereas the latter ensures presence in the ecosystem for several decades, thus interfering in the functioning of biological entities. Short service life and improper plastic waste management lead to clogging of water and wastewater conduits, polluting water sources (e.g., rivers, beaches, and lakes) and landscapes, thereby affecting tourism revenues and additional expenditure to clean the environment. The various petro based plastics are polyethylene (PE), polyester, polypropylene, nylon, epoxy, and polytetrafluoroethylene. However, with technological advancements, materials having thermal and mechanical properties similar to petro plastics have been developed and are termed as *"biopolymers."* The production mechanism and substrate render biopolymers as organic and biodegradable. The major biopolymers being used are polyhydroxyalkanoates (PHA), xylooligosaccharides (XOS), extracellular polymeric substance (EPS), biosurfactant, lipids, waxes, etc. The classification of biopolymers is shown in Fig. 14.1.

The PHA, intracellular energy storage material, belongs to hydroxyalkanoates group that supplements the carbon unit to microbes under stress conditions. The PHA's thermal and mechanical property has allowed application in pharmaceutical industries, surgical and single-use components, while its biocompatibility has empowered use in biomedical engineering. Conversely, EPS and biosurfactants are extracellular products of microbes to prevent dehydration, predation, and assist in carbon consumption. The EPS also provides carbon substrate and defense under extreme conditions, while biosurfactants aid in consuming insoluble carbon sources. Also, it can be used as washing powder, fabric softener, etc. Another energy reserves

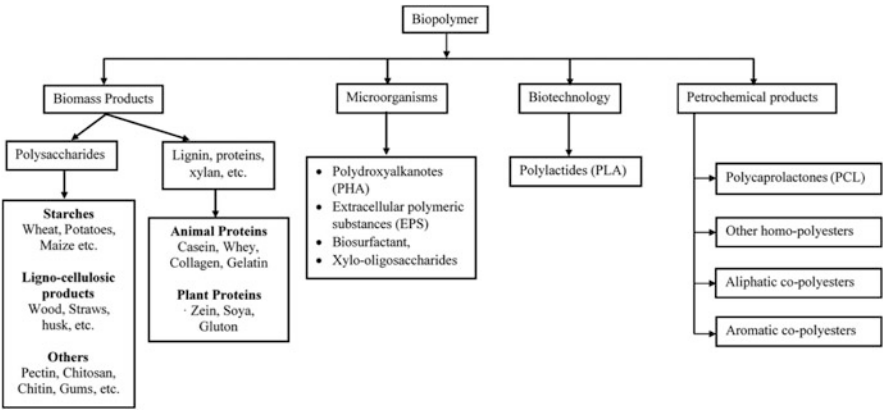

Fig. 14.1 Classification of biopolymers

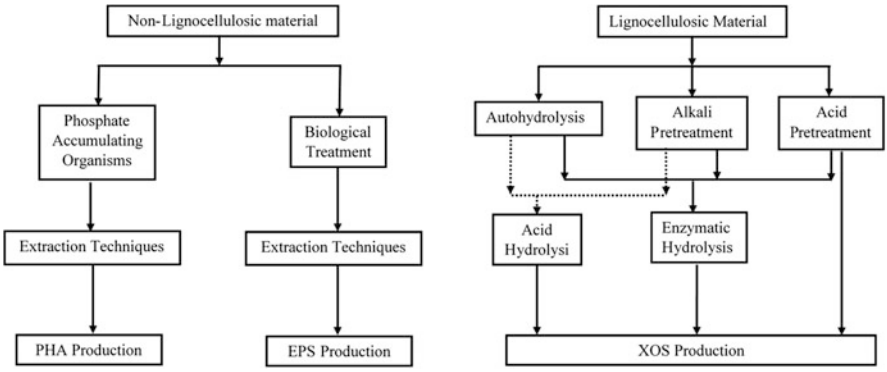

Fig. 14.2 The process flow diagram for the production of various biopolymers

in the form of lipids exist naturally in animals and plants as a component of the cell wall. The lipid includes various compounds comprising of fats, waxes, vitamins, and others. Finally, XOS is a non-digestible, non-carcinogenic, white powder obtained from xylan present in hemicellulose. The XOS as a low-calorie sweetener is being used in dietary supplements for diabetic individuals and a prebiotic for humans and animals. In animals, it is used as growth promoters instead of antibiotics that enter the food chain through the meat. It has been found to curtail the risk of having dental caries, cardiovascular disease, and colon cancer. The flow diagram for the production of biopolymers is shown in Fig. 14.2.

The biopolymer yield is governed by various factors, viz. substrate, specific microorganisms, process outline, metabolic pathway, polymer characterization, process modeling, reactor operation, and control strategies. Further, the biopolymer cost is hinged on the substrate; therefore, an economical, abundant, and readily available substrate is the need of the hour. The substrate includes biomass such as plant oil, lignocellulosic materials, and organic waste stream obtained from industrial and residential complexes. Further, the various components of the substrate are responsible for the generation of certain biopolymers that are produced by a disintegrating substrate, e.g., carbon, fatty acids, xylan, and lipids. These provisions will reduce reliance on conventional resources while simultaneously ensuring integrated waste management.

These virtues allow biopolymer applications in diverse sectors such as disposable biomedical accessories, food packaging, fishing nets, etc. by replacing conventional plastics. Their wide variety has made biopolymer applications in multiple fields. The use of a techno-economic solution to preserve raw materials while utilizing renewable biologically degradable commodity helps in developing sustainable infrastructure. These efforts allow a constructive implementation of biopolymers that generates economic advantages, thereby contributing to the circular bioeconomy. The chapter discusses biopolymers property, production mechanism, and parameters affecting their yield and substrate requirement.

14.2 Source of Biopolymer

14.2.1 Starchy Material

Starch is a white, tasteless, organic, renewable source abundantly produced by plants. It is a polysaccharide formed by combining excess glucose in α 1,4 linkages having a general chemical formula of $(C_6H_{10}O_5)n$. The starch is stored in granular form as seeds of wheat, rice, corn, and potato tuber. The stored glucose can be utilized by disintegrating starch with the help of certain enzymes (α-amylase) by a two-stage mechanism, liquefaction, and saccharification (Akaraonye et al. 2010; Pagliano et al. 2017). However, sometimes commercially available enzymes are implemented. This upturns the unit cost of end-products. The commercial starch, as a nutritional supplement, is mainly obtained after crushing, grinding, and removing impurities from corn, wheat, potato, etc. Also, the starch is used in the textile industry, as a thickener for baked goods, to increase the strength and surface sizing of paper and to produce packaging materials, e.g., paperboard, paper bags, boxed, tape, etc. A starchy wastewater stream is formed from food industries (processing potato chips and candy) and spent wash from distillery industries (using starch-rich jowar and rice grain) along with sources, as mentioned earlier (Khardenavis et al. 2007).

14.2.2 Industrial Wastewater

14.2.2.1 Whey Wastewater

The wastewater is generated from the dairy industry by refining and manufacturing of milk products such as butter, ice cream, cheese, yogurt, and desserts. The various processes that give rise to this wastewater are separation, clarification, centrifugation, pasteurization, coagulation, evaporation, drying, and chilling (Rivas et al. 2010). The dairy effluent characteristics are mainly governed by the final product of the manufacturing plant and contain chemical compounds (acidic and alkaline) from cleaning tanks, equipment, and bottles. High organic strength wastewater, whey wastewater, is generated as the main by-product of the manufacturing of cheese and casein. It is found that a total of 50% of whey is disposed of as waste, which consists of approximately 4.5% lactose, 0.8% protein, 1% salts, and 0.1–0.8% lactic acid (w/v). These components make the wastewater exert huge biological oxygen demand (40 g L^{-1}) (Pagliano et al. 2017). The effluent has a pH (3.3–9.0), suspended solids (0.1–22), TKN (0.01–1.7), and total phosphorus (0.006–0.5) (Carvalho et al. 2013).

14.2.2.2 Molasses

Molasses is a black, cheap, viscous by-product obtained after crystallization of sugars from sugarcane and beet. Repeated centrifugation results in varying degrees of molasses characteristics at subsequent stages of crystallization. During the first extraction, the molasses is light in color with higher sugar content. The ensuing extractions make the color darker with complete sugar removal rendering molasses downright dark, viscous, low grade, and a cheap waste product. The final product is used as animal feed or to generate citric acid, vinegar, etc.

Nonetheless, the molasses composition is determined by the source, either from sugarcane or beet. The molasses obtained from sugarcane contains 55% sucrose to 50% from beet (Akaraonye et al. 2010). Conversely, molasses also provides a compelling amount of minerals and vitamins with iron, calcium, potassium, and magnesium considered as foreign matter in sugar. The molasses is classified as sulfured and unsulfered molasses on the basis of use of preservative (sulfur dioxide) for unripe sources.

14.2.2.3 Food Processing Industries

The fast-food restaurants, potato processing plants, and snack food factories are capable of producing different types of waste vegetable oil, viz. olive, groundnut, corn, palm, soybean oil, etc. The worldwide production of waste cooking oil is more than 16.73 million tons/year, mainly from industrial deep fryer and restaurants (Gui et al. 2008). This waste oil can be an inexpensive source of carbon and lipids.

14.2.3 Sludge

The focus towards making the environment clean by preventing untreated wastewater from being discharged uncontrolled into the water sources is essential. The treatment of wastewater can be achieved by establishing wastewater treatment plants in residential and industrial areas with strict norms for wastewater disposal. However, a tremendous sludge quantity is generated by treatment plants, which is difficult to dispose of. The low dewatering ability makes this bulky waste challenging to transport. The excess sludge management includes 40–60% of entire operational and management costs of the treatment plant (Liu 2003). Nevertheless, the sludge waste has been found to carry organic material except for sludge from chemical precipitation tank.

The sludge waste on fermentation can yield VFAs (preferably short-chain fatty acids) that can be utilized for multi-purpose by-product recovery or biological nutrient removal techniques. This potential utilization of sludge will reduce handling costs and stress on landfills while developing a sustainable wastewater treatment

facility. Moreover, there are considerable challenges in adapting sludge as a carbon source. Therefore, significant research is required to optimize critical parameters, i.e., pH, hydraulic retention time, solids retention time, temperature.

14.2.4 Solid Waste

The increase in waste generation has made municipal solid waste a promising substrate. It consists of a biodegradable fraction constituted by kitchen and yard waste (grass, leaves, flowers, and branches). The kitchen waste has complex compounds, viz. carbohydrates, proteins, lipids, salts, and trace elements (Ma et al. 2018), whereas yard waste falls in the category of lignocellulosic material having cellulose, hemicellulose, lignin, and fibrous compound. These compounds must be disintegrated into simpler ones, before being used as a carbon source. Another abundant source of these compounds is agricultural/agro-industrial waste such as industrial fruit and vegetable waste, soybean husk, rice paddy straw, rice bran, wheat bran, wood straw, husk, etc.

14.3 Types of Biopolymer

14.3.1 Polyhydroxyalkanoates (PHA)

Polyhydroxyalkanoates (PHAs) are polymers that belong to the family of hydroxyalkanoates. A specific set of microorganisms produces PHAs, as an intracellular energy storage material. It acts as a carbon source when microbes succumb to famine conditions (Macrae and Wilkinson 1958). PHAs form in microbes by consuming carbon source with or without nutrients. The popularity of PHAs is increasing because of biodegradable nature and the ability to tailor thermal and mechanical properties by blending (Tokiwa et al. 2009). These virtues encourage its use for vivo- and vitro- studies in different sectors. The PHAs with an array of saturated, unsaturated, straight, branched, and aromatic structures have been able to form 150 different monomers (Steinbüchel and Valentin 1995; Witholt and Kessler 1999; Steinbüchel 2001). Their molecular weight varies between 2×10^5 and 3×10^6 Da, while the length of the side chain and functional group depends on the microorganism and operating conditions. These molecular changes influence polymers properties such as crystallinity, melting point, and glass transition temperature that determines its end-use.

The two primary classifications of PHAs based on the size of monomer are short-chain length PHA (less than 5 carbon atoms) and medium chain length PHA (6–14 carbon atoms). The most common copolymers of PHAs are PHB (poly (3-hydroxybutyrate)) and PHV (poly(3-hydroxyvalarate)). The variety of PHAs synthesized depends on substrate composition with the help of several microbes

such as *Cupriavidus necator, Alcaligenes latus, Pseudomonas putida,* and *Pseudomonas mendocina* (Akaraonye et al. 2010). However, several efforts are being made to make PHAs production economical by developing high yielding bacterial strains, efficient biological and recovery processes, and low-cost substrates. There are some microbes capable of producing 90% PHA (w/w) of dry cells (Madison and Huisman 1999). The cost of PHAs is heavily dependent on substrates as it almost takes up 30–50% of total production cost (Kim 2000; Salehizadeh and Van Loosdrecht 2004). The use of substrate such as glucose, sucrose, corn, etc. results in a higher cost of PHAs, making it unattractive for large scale production. Nowadays, the focus has shifted to renewable sources, i.e., waste organic by-products from food industries and municipal waste. The implementation of these sources can reduce the cost of PHA production by half (Serafim et al. 2004).

14.3.1.1 Biological Process for PHA Production

The recovery of PHAs is an expensive process. In order to make it economical and efficient, a high yield of PHAs accumulation is desirable. The high yield of PHAs depends on the type of bacteria and operating process, i.e., aerobic dynamic feeding (ADF) or feast and famine (Serafim et al. 2004). The bacteria used for PHA synthesis are classified based on culture conditions. One category of bacteria requires limitations of nutrients (N, R Mg, K, O, or S) with an excess carbon source (e.g., *Alcaligenes eutrophus*, *Protomonas extorquens* and *Protomonas oleovorans*, *Cupriavidus necator*, *Rhodopseudomonas palustris,* and *Methylobacterium organophilum*); whereas, other without nutrition limitations undergrowth phase (e.g., Al. latus, recombinant *E. coli*). Besides, the process of feast and famine improves PHA yield. During the feast phase (carbon excess), the bacteria uptake the substrate quickly and store it in the form of PHAs to be utilized later in the famine phase (carbon limited). In the famine stage, the PHA is used only for cell maintenance, while in a feast stage the cell growth and PHA storage are linear (Serafim et al. 2004).

This concept was studied by Mengmeng et al. (2009), which introduced fermentative substrates (2437.55 mgTOC/L of VFAs, 1835 mL) at regular intervals instead of at once (lowering the OLR), to improve PHA yield. The study was carried in batch mode under aerobic dynamic feeding with municipal wastewater biomass. The study improved PHA yield from 3.5% to 56.5% of the dry cell at the end of 3 h study. The substrate was introduced when an increase in DO concentration was detected due to substrate exhaustion. On further addition of the substrate, no significant change in the PHA yield was observed, which shows the exhaustion of PHA storage by microbes. Also, the PHA concentration improved due to the effect of ammonium consumption by the bacteria. As nitrogen is essential for microbial growth, the ammonium consumed in the early stages with carbon source results in growth and PHA accretion. However, in the absence of nitrogen, only PHA accumulation occurred without cell growth. This notion of cell growth limitation due to the lack of a nitrogen component is supported by lower volatile suspended solid in biomass.

This method allowed a higher PHA storage rate than a high substrate dosage. The specific PHA storage rate of 0.38 HAs/mg VSS h was higher than that obtained from pure cultures like *Ralstonia eutropha* (0.019 mg HAs/mg VSS h), Alcaligenes latus (0.031 mg HAs/mg VSS h) except for recombinant *Escherichia coli* (0.042 mg HAs/mg VSS h) (Kim et al. 1994; Lee et al. 1994; Yamane et al. 1996).

The primary precursor in the substrate that drives PHA accumulation is volatile fatty acids (VFAs). The VFAs mainly comprise acetic, butyric, propionic, and valeric acid. This variety of VFAs component not only improves PHA yield but also governs the composition of biopolymer produced, i.e., PHB and PHV. The higher amount of PHB is due to the presence of a high amount of acetic and butyric acids as compared to propionic and valeric acid. In contrast, propionic and valeric acid govern PHV production (Lemos et al. 2006; Bengtsson et al. 2010). Cheah et al. (2019) proposed that substrate must be well distributed with VFA constituents to obtain PHA and insisted on using a parameter having a ratio of even (C_2 + C_4) and odd carbon components (C_3 + C_5). The constituents of VFAs present or produced depend on the form of substrate, i.e., complex (carbohydrates, protein, and lipids) or pure (glucose and amino acid) on the operating condition. This working condition includes pretreatment and respiration microenvironment, i.e., aerobic and anoxic. The anoxic condition encourages the degradation of complex compounds to simpler ones while simultaneously consuming simple compounds to accumulate PHAs.

Conversely, the aerobic condition only helps in accumulating PHAs. The PHA yield under such conditions can be stepped up by employing a pretreated substrate. Generally, fermentation produces readily consumable products, VFAs. On these premises, Reddy and Mohan (2012) produced higher PHA (39.6%) under anaerobic conditions than under aerobic microenvironment (35.6%) for fermented food waste and unfermented food waste (35.6%). Further, reading about the pathway involved can be referred to in Bengtsson (2009).

14.3.1.2 PHA Application

The PHA has replaced silicone to be used as non-woven materials, pharmaceutical products, surgical tools, and single-use components employed in transplantology, pharmacology, and tissue engineering (Zinn et al. 2001). The PHAs before using in the human body are relished to be biocompatible (i.e., support cell growth and adhesion while allowing passage of nutrients and waste products) and are biodegradable without producing harmful by-products. These characteristics make PHA unique and are replacing petro plastic-based polymers in medical, coating materials, skincare products, and food packaging. A properties comparison of petro plastic and PHAs with its copolymer is compared in Akaraonye et al. (2010). The application of PHAs can be fostered by altering desirable properties based on end-users requirements through blending.

14.3.2 Xylooligosaccharides (XOS)

Xylooligosaccharides (XOS) are non-digestible, non-carcinogenic, white powder produced by hydrolyzing xylan. It contains xylose molecules linked with different β 1–4 bonds (Mäkeläinen et al. 2010) to obtain xylobiose (2 monomers), xylotriose (3 monomers), xylotetrose (4 monomers), xylopentose (5 monomers), xylohexose (6 monomers), etc. (Kumar and Satyanarayana 2011). It is a low-calorie sweetener as a dietary supplement for diabetic individuals and a prebiotic for humans and animals (Vazquez et al. 2000; Senani et al. 2009). Popularly, the XOS instead of fructo- and mannan-oligosaccharides is replacing antibiotic growth promoters for animals. These antibiotics cause the deposition of antibiotics in meat and enter into the food chain. The XOS is characterized as non-digestible by humans because they lack the required enzymes in the gastrointestinal tract for hydrolysis. The XOS having less than four monomers is beneficial for human. They promote growth of bifidobacteria (present in intestine and stomach), which improve bowel movement, lipid metabolism, calcium absorption by inhibiting putrefactive bacteria and pathogens (Aachary and Prapulla 2009). Studies suggest the use of 4 g XOS/days for 3 weeks can improve intestinal microbiota among adults (Chung et al. 2007). They develop changes in not having dental caries, cardiovascular disease, and colon cancer because of fatty acids formation (Gullón et al. 2008). The proportion of substitute, viz. acetyl, phenolic acids, and uronic depends on the constituents of the substrate utilized. The essential substrate to produce XOS is plant biomass or lignocellulosic material having a high content of xylan. Xylan is a main constituent of hemicellulose. The hemicellulose also produces other compounds such as ethanol, furfural, xylitol, lactic acids, and erythritol. But it is the heterogeneous biomolecule xylan that is broken down to XOS by the help of autohydrolysis, acid/alkali treatment, and enzymatic action. The xylan as an individual component has shown medicinal prospect as immune-stimulatory, ulcer protective, and antitumor agent (Nabarlatz et al. 2007a).

14.3.2.1 Treatment Techniques

The XOS extraction from lignocellulosic material comprises chemical (acid and alkaline hydrolysis), thermal pretreatment (autohydrolysis/hot water or steam), and enzymatic reaction. However, before pretreatment, the raw material is subjected to preliminary treatment to remove impurities with the help of ethanol or ethyl acetate (Vázquez et al. 2005). All the methods used have some merits and demerits. The acid and alkaline treatment are cost-intensive techniques, as it implements chemicals that are responsible for corrosion of containers. Additionally, these processes face problems in discharging chemical-laden waste. Conversely, the autohydrolysis do not require chemicals but need specialized containers to perform pretreatment at high temperatures. Further, the acid and thermal treatment may lead to undesirable components' production such as xylose and furfural. These unwanted substances

are easily restricted in the enzymatic reaction. However, the process requires a longer period for hydrolysis, strict operating conditions, and commercial enzymes to improve yield. Moreover, all the processes at every step demand efficient separation of components with high yield and least cross-contamination. The study of operational strategies and process economics is requisite prior to its scaling up to industrial scale.

Autohydrolysis

Autohydrolysis (also known as hot water or hydrothermal pretreatment with pressure) is an eco-friendly method that solubilizes hemicellulose and carries out deacetylation of xylan to produce acetic acid. It uses water as a reaction medium without any catalyst at a temperature range of 160 °C–240 °C for a period extending from few minutes to several hours, depending on substrate components (Mosier et al. 2005). At high-temperature hydronium ions [H_3O^+] are generated due to dissociation of water. The ion acts as a catalyst in an acidic medium because of acetic and other acid presence, to hydrolyze hemicellulose and a small quantity of acid solubilizes lignin to monomers. A desirable pH within 4 and 7 must be maintained during the whole treatment process to keep hemicellulose in the oligomeric form with simultaneous inhibiting monosaccharides formation and sugar degradation (Carvalho et al. 2013). The drop in pH below 3 occurs due to the production of acetyl and uronic acid. These acidic contents give the XOS high solubility in water. The temperature and reaction time are important factors that determine the characteristics of XOS, such as acetyl content and molar mass distribution (Shimizu et al. 1998; Nabarlatz et al. 2007a) along with substrate type. During hydrolysis, some unwanted materials are generated, viz. lignin furfural, monosaccharides, etc. and demands purification (Zhu et al. 2006) while cellulose and lignin remain in the biomass. Consequently, the autohydrolysis method is preferred over dilute acid hydrolysis as it may lead to the darkening of pulps, thereby requiring the additional cost of bleaching. Also, it is cumbersome to control the duration and temperature of the process at elevated temperature and pressure. The non-solubilize solid matter contains cellulose and lignin, which can be used for producing cellulose pulp and fuels. In order to separate lignin-derived compounds and XOS, the separation methods include centrifugation, ion exchange, spray drying into powder, membrane filtration, freeze-drying, and decoloration (Nabarlatz et al. 2007b). The operating principle of these processes is the molecular weight cut-offs of compounds.

Alkali and Acid Pretreatment

In this chemical pretreatment, alkaline and acidic compounds under varying concentration, temperature, and incubation conditions are performed. A higher temperature helps in softening lignin protective layer present in biomass. Yang et al. (2005) conducted a combination of acid and alkali treatment to alter the lignin–xylan

complex to improve xylan yield. The yield is improved by limiting the production of xylose and furfural using mild/dilute thermochemical treatment. To overcome this limitation, Brienzo et al. (2009) used hydrogen peroxide during alkali treatment of sugarcane bagasse to preserve acetyl groups, thereby producing higher xylan yield. The corncob pretreatment for acid (0.01 M H_2SO_4 at 60 °C in 12 h), alkaline (2% NaOH solution), and steam (135 °C/30 min) pretreatments reported XOS yields and concentrations were, respectively, 40.8, 39.2, and 40.0% (Aachary and Prapulla 2009). This study shows alkaline treatment being a slightly better method than other methods. However, a mild/dilute acid and longer time result in advanced degradation of xylan to xylose along with reducing equipment corrosion (Otieno and Ahring 2012). In another study, alkali treatment of S. nervosum grass with different concentrations of sodium and potassium hydroxide with an overnight incubation at room temperature (16 h, 25 °C) and autoclaving (45 min, 121 °C, 15 lbs) was performed. A staggering 97% of xylan was retrieved from S. nervosum grass with 12% NaOH and steam application. Moreover, the extraction of xylan by alkali is advantageous over other processes as the former is simple to perform and cost-effective (Aachary and Prapulla 2011).

Enzymatic

The xylan from the lignocellulosic biomass pretreatment is subjected to enzymatic hydrolysis for XOS production. The production of XOS with the help of enzymes is preferred from hemicellulose, unlike other chemical treatment process due to no harmful by-products generation. The enzymes that are responsible for XOS production are xylanases, which consist of exo-xylanases and endo-xylanase. Higher activity of the latter is desired for XOS production than the former, which produces undesirable xylose (Vazquez et al. 2002; Akpinar et al. 2010). These xylan enzymes are provided by various organisms *Phanerochaete, Aspergillus, Trichoderma, Streptomycetes, Chytridiomycetes,* and *Thermoascus* (Carvalho et al. 2013). A variety of these species can be obtained from various agro-industrial waste (Abdel-Sater and El-Said 2001). However, fungi secrete cellulases and xylanases that result in undesirable end-products (Balakrishnan et al. 1992). The yield and type of XOS production depend on the kind of enzymes produced, substrate, interaction between enzymes, and activity of xylanases at a different temperature, enzyme dose, pH, and incubation time. Despite the enzymatic process being a simple process with no harmful product generation. It is inherited with a slow hydrolysis rate and involves cumbersome pathways. Therefore, a prior pretreatment of the substrate is essential. Further, the focus has been shifted to a new form of enzymatic action by using solid-state fermentation of substrates.

14.3.3 Extracellular Polymeric Substances

The EPS are extracellular products of microbes that shield cells from dehydration, counteract phagocytosis, phage attack (Wilkinson 1958), UV radiation, extreme pH conditions, and antimicrobial antibiotics (Palomba et al. 2012) and act as an ATP sink (Neijssel and Tempest 1976). It acts as an adhesive agent for nutrients and carbon to be utilized by the microbes (Johnson et al. 1977; Decho and Lopez 1993). It is the main compound generated from microbes, which causes fouling in membrane techniques. The EPS comprise polysaccharides, proteins, glycolipids, etc. These compounds boost the development of a surface matrix to house microorganisms on inert surfaces. The components of EPS play a vital role in influencing properties of the matrix, viz. charge, porosity, water content, density, sorption, mechanical stability, and hydrophobicity of biofilms (Neu and Lawrence 2010). One of the prominent properties of EPS is jellification. The feature allows EPS use in food industries (for frozen foods, baked goods, and brewery production) and pharmaceutical sectors by replacing alternatives such as starch, caseinate, carrageen, gelatin. The EPS is produced in conjunction with other intracellular material; therefore, factors that boost the yield of one component will result in a decrease of the other and vice versa.

14.3.3.1 Biological Treatment

Several studies have used various microbial strains to produce both compounds (intracellular and extracellular) such as *Azotobacter* (Sutherland 1986), *Azotobacter beijerinckii* (Pal et al. 1999), *Klebsiella aerogenes* (Norval and Sutherland 1969), *Azotobacter chroococcum* (Quagliano and Miyazaki 1999), etc. The EPS are characterized by composition, structure, and viscosity, which depend on factors such as microbial strain, substrate, trace elements, mineral salts, and operating conditions (e.g., pH, temperature, culture volume, and nutrients). The EPS more than often is also consumed as a carbon source. The driving factors are selected to maximize cell growth, which will return a high yield. Danilova et al. (1992) found that a temperature of 35 °C was optimum for EPS yield by *A. beijerinckii*. Similarly, a higher yield was achieved when temperature conditions were increased from 20 °C to 37 °C, which ultimately increased cell growth (Norval and Sutherland 1969).

Another factor playing an important role in the yield of a biopolymer is the culture volume added to the reaction medium. It controls the biomass amount to be added initially to the substrate medium to maximize yield. This condition also controls the amount of oxygen to be present during the process, i.e., culture volume is proportional to oxygen limiting. On these premises, Pal et al. (1999) took different culture volumes of 10, 20, and 40 for glucose, fructose, and mannitol. A relatively high yield of EPS at 1.9 g/L (approx.) was obtained for 40% cultural volume in glucose. The study optimized yields of polymers by using different substrates concentration (glucose, fructose, mannitol in 1, 2, 3, 4% (w/v)), pH (6.0–9.0), temperature

(25, 30, 35, and 40 °C), nutrient limitation, and culture volume (10, 20, and 40%). The study reported a lower yield of EPS when different N-sources (beef extract, yeast extract, casamino acid, tryptose, tryptone, etc.), except for bacto peptone (comprising readily available nitrogen). The study observed mannitol to produce EPS higher than PHB at all concentrations and was highest for 3% (w/v) at 1.5 g/L among all the substrates at varying concentrations. Quagliano and Miyazaki (1999) reported that bacterium under various carbon sources individually (glucose, fructose, sucrose, molasses) and addition of 0.1 g/L of ammonium sulfate (except molasses) showed EPS production to reduce with the addition of ammonium sulfate except for sucrose and fructose. For molasses, the EPS production was highest at 1.1 g/L (72 h) than PHB at 0.2 g/L (72 h) decreased from 0.7 g/L (48 h). Therefore, a medium with a high C/N ratio is essential for EPS production (Quagliano and Miyazaki 1999). Conversely, Wang and Yu (2007) observed EPS production was closely related to cell growth, which is possible with sufficient nitrogen presence by *Rhizobium eutropha*.

Although the main factor governing EPS production is carbon source with or without nitrogen, but other factors bacterial strain and elements present in the medium are also equally crucial. The importance is evident from one study having two different strains of *Rhizobium meliloti.* One strain (Su47) produced fewer EPS than another strain (M5N1) for fructose, but the yield reversed when subjected to a medium having K_2HPO_4 and $MgSO_4$. Also, the EPS production improved for glucose for Su47 when a medium having yeast extract, K_2HPO_4, and $MgSO_4$ were used, thus, citing a strong correlation between EPS production in the presence of elements (Tavernier et al. 1997). Likewise, a study complementing the positive influence of phosphate and potassium on EPS and PHB production reported KH_2PO_4 (0.2% w/v) improved the production of polymer.

The change in substrate components plays a role in polymer yield by regulating pH. The transformation occurs when easily degradable compounds and acid-sensitive bacteria are employed. The fall in pH values is inevitable and only bacteria with acid tolerating capacity can produce EPS. In this regard, Pal et al. (1999) showed higher EPS yield when pH was increased from 6 to 7.7, thereby buffering the system and preventing a fall of pH, as supported by many studies. This increase in pH encourages biomass production and polymer formation. However, an increase in pH beyond neutral values had an inhibitory effect on growth. The yield of various by-products (PHA, XOS, and EPS) for various substrates is shown in Table 14.1.

14.4 Challenges Faced

The biopolymer production involves several aspects, which are required to be taken care of to maximize yield. The macrofactors driving their production are substrate, pretreatment, treatment techniques, and scalability. In the past few decades, the established costly substrates have been replaced with suitable, economical, abundantly available, and renewable resources. However, the full potential of these

Table 14.1 The yield of various by-products (PHA, XOS, and EPS) for various substrates and microbes

S. No	By-product	Substrate	Microorganisms	Yield %	References
1	PHB	Deproteinized jowar grain-based distillery spent wash	Activated sludge	43.2[a]	Khardenavis et al. (2007)
		Filtered rice grain-based distillery spent wash		40.0[a]	
		Raw rice grain-based spent wash di-ammonium hydrogen phosphate (DAHP)		67[a]	
2	3-hydroxybutyrate	Fermented molasses	Glycogen accumulating organisms	70.0 (Mol %)	Bengtsson (2009)
3	PHB	Glucose	*Alcaligenes eutrophus*	76.0[a]	Kim et al. (1994)
4	PHB	Starch	*Azotobacter chroococcum*	46.0[a]	Kim (2000)
		Whey	Recombinant *Escherichia coli*	80.0[a]	
5	PHA	Fermented sludge	Sludge treating municipal wastewater	56.5[a]	Mengmeng et al. (2009)
6	PHA	Unfermented food waste	Activated sludge	35.6[a]	Reddy and Mohan (2012)
		Fermented food waste		39.6	
7	XOS	Eucalyptus wood	*Hemicellulase* 90	67.0	Vazquez et al. (2000)
8		Corncob	*Aspergillus oryzae*	81.0	Aachary and Prapulla (2009)
9		Rice husk	Commercial xylanases	82.8	Gullón et al. (2008)
10		Tobacco stalk	Commercial xylanases	13.8	Akpinar et al. (2010)
			Acid hydrolysis	12.9	
11		Lignocellulosic materials	*Aspergillus fumigatus*	40.0	Carvalho et al. (2013)
12		Sehima nervosum	*Trichoderma viride*	11.0	
13	EPS	Glucose	*Azotobacter beijerinckii* WDN-01	1.5 (g/L)	Pal et al. (1999)
	PHB			2.73 (g/L)	

(continued)

Table 14.1 (continued)

S. No	By-product	Substrate	Microorganisms	Yield %	References
14	EPS	Fructose	*Azotobacter chroococcum*	1.1 (g/L)	Quagliano and Miyazaki (1999)
		Glucose		2.7 (g/L)	
		Molasses		1.3 (g/L)	
	PHB	Glucose		0.6 (g/L)[b]	
		Molasses		0.6 (g/L)[b]	

[a]Product weight/dry cell weight (w/w)
[b]Approximate values

sources is yet to be explored fully and is the key to obtain by-products on a large scale. The second and crucial aspect playing a significant role in biopolymer production is the treatment processes. There are various stakeholders in treatment processes governing the production of biopolymers, which are primitive to the method employed. The thermochemical process is energy and chemical-intensive and requires special equipment to perform the task. The factors mainly responsible are temperature, pressure, reaction time, chemical type, and dosage. The optimization of these parameters is essential as slightly higher or lower values will result in the production of undesirable, harmful by-products, high resource consumption, and corrosion of the vessels used, thereby rendering the process inefficient. These types of methods require sophisticated equipment, advanced technology, and skilled workforce to carry out an efficient production.

In contrast, the use of the biological process will reduce the consumption of chemicals and corrosion of equipment used. Nonetheless, it is time-consuming and depends on several operating parameters such as microbial strain, substrate, trace elements, mineral salts, and operating conditions (e.g., pH, temperature, culture volume, and nutrients). The incorporation of biological entities allows no extra initial cost, but needs to employ strict environmental condition to obtain a high yield of desired by-products. A specific biopolymer can be produced by pure culture or mixed culture. The pure culture can be retrieved either from isolation or commercially. This commercial strains of microbes are costly. In another way, a diverse culture will have different microbial species depending on the source of the inoculum.

Further, strict optimized conditions are required to be maintained to encourage the growth of a specific microbe to produce biopolymer at higher rates. These biological strains are capable of producing different biopolymers depending on the components of the substrate available and demand different factors to boosts yield of one component while depreciating others and vice versa. All the treatment techniques produce certain compounds as a by-product that are necessary to be removed by the purification process, thus increasing the per-unit cost of biopolymer.

Furthermore, the biopolymer must be added with some blends, plasticizers to improve its mechanical and thermal properties to deliver a commercial product.

14.5 Biopolymers and Circular Economy

The present global scenario of population explosion, increased industrial activities, high waste generation, limited resources, and endless demand for energy and goods has given rise to adopt a sustainable solution termed as "circular bioeconomy." It is a parallel economy that incorporates the development of value-added resources with the help of techno-economic biotechnologies without utilizing non-renewable sources, thereby enhancing the carrying capacity of the ecosystem.

The production of various types of biopolymers from biomass (agricultural, industrial, and residential waste) can help in producing valuable by-products. These biopolymers can replace synthetic polymer obtained from petroleum products and help reducing industrial carbon footprints. The numerous type of biopolymer exists with an array of thermal and mechanical properties and has expanded its applications in various sectors such as biomedical engineering, food industries, pharmaceutical, human and dietary requirement. The circular bioeconomy for biopolymer production can be achieved by process integration with zero liquid and solid discharge. This enhances the effective utilization of resources through the reduced generation of undesirable and harmful by-products generated during processing potential waste and production of biopolymer. Also, it will lead to the development of a better ecosystem for future generations, along with tempting job opportunities in biorefinery industries. However, the approach of circular bioeconomy lacks significant research gaps for the efficient recovery of biopolymers. This can be achieved by the integration of processes and optimizing the operating parameters with the help of mathematical tools.

14.6 Conclusion and Future Perspectives

The rising synthetic plastic waste generation and improper waste management have led the scientific community to develop an alternate solution in the form of biopolymer from agricultural, industrial, and residential waste. Although the waste provides an attractive proxy for non-renewable sources, it requires preliminary treatment and proper characterization of components prior to its use. The production of a biopolymer involves thermal, chemical, and biological processes that are dependent on several operating parameters. All thermochemical treatment suffers from being chemical and energy-intensive processes while generating objectionable compounds, whereas a biological process can provide a techno-economical solution. The effectiveness of biological processes for biopolymer generation banks on substrate composition and specific microbial strains employed. However, the

process is associated with a sluggish biopolymer generation rate and involves several parameters. The management of multiple parameters along with complex pathways of biopolymer production is cumbersome, thereby rendering the process ineffective. Moreover, all the treatment processes suffer from their inapplicability at large scale. Its overcoming these limitations with the help of mathematical tools such as density functional theory, factorial design methods, principal component analysis and response surface methodology must be carried out. This will evaluate the effect of various parameters on biopolymer yield and optimize the same. Finally, it will enable a biopolymer to be used as a product of circular bioeconomy.

Competing Interests All the authors declare that they have no competing interests.

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Chapter 15
Recent Advances in Biochar-Based Mitigation of Dyes, Agrochemicals, and Pharmaceutical Pollutants

Venkatesh Chaturvedi

Abstract Millions of tons of dyes, agrochemicals, and pharmaceuticals are produced globally per year and a substantial part is released in the environment. Several chemicals persist and accumulate in the environment and exhibit detrimental effects on organisms. They also lead to the development of carcinogenicity, endocrine disruption, antimicrobial resistance in pathogenic microorganisms, causing a great threat to health of humans. Chemicals present in the environment are removed by various biological and chemical processes. Among them, adsorption is better suited owing to its effectiveness, low cost, ease of operation. A number of adsorbates such as natural clay, charcoal, carbon nanotubes, and biochar have been utilized. Biochar is a carbonaceous material formed from plant biomass, when it is heated in limited supply of oxygen. It has porous structure, charged surface, and functional groups, which help in adsorption of vast array of pollutants. In this chapter, we have reviewed recent trends in biochar-based removal of dyes, agrochemicals, and pharmaceuticals. We have also discussed the mechanisms behind the adsorption and future prospects of this approach in abatement of these pollutants.

Keywords Biochar · Dyes · Agrochemicals · Pharmaceutical · Pollution · Adsorption

Abbreviations

AMR	Antimicrobial resistance
AOPs	Advanced oxidation processes
CEC	Contaminants of emerging concern
CIP	Ciprofloxacin
CTC	Chlortetracycline
DS	Diclofenac sodium
EDCs	Endocrine disrupting compounds
HC	Hydrochar

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MSW-BC	Municipal solid waste derived biochar
NMR	Nuclear magnetic resonance
NSAIDs	Non-steroidal anti-inflammatory drugs
OTC	Oxytetracycline
SDZ	Sulfadiazine
SMX	Sulfamethoxazole
SPY	Sulfapyridine
TC	Tetracycline

15.1 Introduction

Owing to increasing population, development of industries, and health care services around the world, the use of dyes, agrochemicals, and pharmaceutical compounds have increased tremendously. Dyes owing to their color, affects the rate of photosynthesis by aquatic photo autotrophs and when ingested by animals including humans leads to a number of health related issues. Agrochemicals such as pesticides are employed to enhance productivity of crops, but they enter in the body of the organisms and show their detrimental effects such as acute toxicity, mutagenicity, carcinogenicity, etc. Pharmaceutical compounds belong to a group Contaminants of Emerging Concern (CEC) (Patel et al. 2019). CEC consists of endocrine disruptors, antibiotics, non-steroidal anti-inflammatory drugs (NSAIDs), personal care products, plasticizers, etc. These chemicals after use are disposed in the environment in huge amounts, where they exhibit adverse effects on the environment (Tushara Chaminda et al. 2018).

The current technologies used in treatment of dyes, agrochemicals, and pharmaceutical present in the environment include biological processes using plants and microbes, electrochemical oxidation, advanced oxidation processes (AOPs), and adsorption (Watkinson et al. 2007; Cheng et al. 2019). Biological processes involve degradation/accumulation of pollutants by plants and microorganisms such as bacteria, fungi, micro-algae. These processes are economical, efficient, and do not pose any damage to the environment (Tiwari et al. 2017). Only drawback is that these processes are usually inefficient under in situ conditions, where apart from pollutant, other competing microorganisms, or toxic chemicals/metal ions are present, which affects the growth and survival of degrader microorganisms. Also, these processes do not work if the contaminant/pollutants are present in very low concentration or are insoluble in water. AOPs are chemical treatment processes, which depend on creation of extremely reactive hydroxyl radicals, which oxidize pollutants found in water or wastewater treatment plants (Deng 2009). The radicals are formed mainly from hydrogen peroxide or ozone. These processes are quick and effective for removal of pharmaceuticals, only disadvantage is that they consume enormous amount of energy and chemicals employed in oxidation process, significantly increase the operating costs (Deng and Zhao 2015). Besides, chemicals utilized in

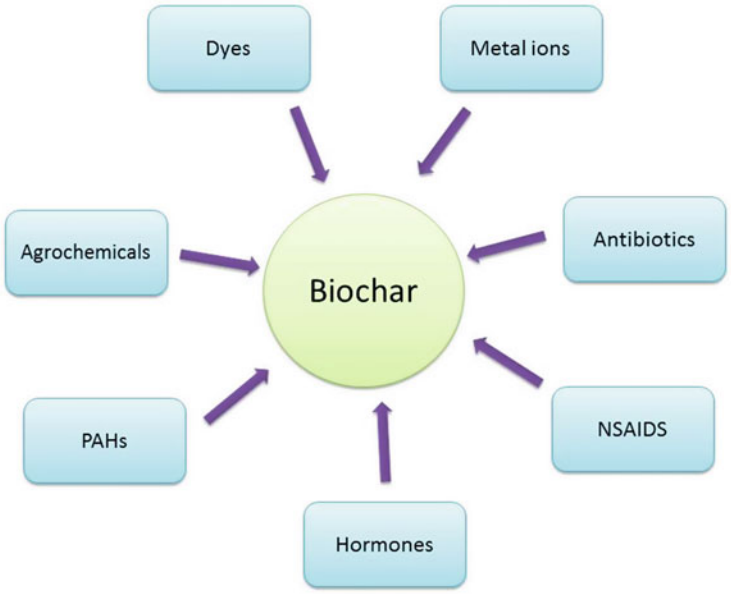

Fig. 15.1 Adsorptive removal of a wide array of pollutants by biochar

AOPs additionally lead to auxiliary contamination. Adsorption because of its ease, viability, simplicity of activity is presently considered as a promising innovation for evacuation of pharmaceutical contaminations. Various adsorbents have been tried for expulsion of pharmaceuticals from water system. Among them, biochar has gained a lot of importance, inferable from its minimal effort, ease in accessibility, and productivity in removing an immense range of contaminants (Zhang et al. 2019). Biochar is a carbonaceous substance acquired by heating plant biomass, for example, wood, fertilizer, horticultural waste, industrial waste, sewage waste, and so forth at high temperature (300–600 °C) without oxygen (Ronsse et al. 2013; Reddy 2015; Kameyama et al. 2016). Under such conditions, the biomass experiences thermal transformation to form biochar. Biochar has various applications in the territories of agriculture, environment (Inyang and Dickenson 2015), and energy (Liu et al. 2019). In the field of agriculture, biochar is utilized as soil amendment, to upgrade soil quality by diminishing acidity of soil and by expanding the accessibility of soil nutrients, which prompts improved crop productivity (Rondon et al. 2007; Elmer and Pignatello 2011; Zhang et al. 2017). It is an effective adsorbent for disposal of various toxins/contaminants from watery frameworks (Fig. 15.1), which incorporate dyes (Fan et al. 2016), metal ions (Cui et al. 2016), pharmaceuticals, for example, anti-infection agents, NSAIDs, hormones, agrochemicals, for example, pesticides (Mudhoo et al. 2019), insecticides (Mandal et al. 2017), and so on. In this chapter, we have examined the techniques for preparation of biochar, its properties, adsorption mechanism, and its utilization in disposal of dyes, agrochemicals, and pharmaceutical pollutants.

15.2 Methods of Preparation of Biochar

There are several methods through which biochar is prepared from biomass. The most commonly used methods are as follows;

15.2.1 Pyrolysis

It is thermal breakdown of biomass in absence/limited supply of oxygen at 200–1200 °C. Pyrolysis of biomass yields biochar, bio-oil, and gases such as methane, carbon dioxide, carbon monooxide, and hydrogen (Biswas et al. 2017). At low temperature (<450 °C), biochar is produced, at intermediate temperature (450–800 °C), bio-oil is produced, and at high temperature (>800 °C), gases are produced. Pyrolysis is divided into three types, i.e. slow and fast pyrolysis.

15.2.1.1 Slow Pyrolysis

Here, the biomass is heated at 400–600 °C, for a more drawn out timeframe (few hours to few days). The yield of biochar is typically 20–40% by weight (Lee et al. 2013).

15.2.1.2 Fast Pyrolysis

It includes heating the biomass at >800 °C for a brief timeframe (Wang et al. 2013). The productivity of biochar is typically 15–25% by weight. This technique is normally performed for development of bio-oil, utilizing fluidized bed reactors (Tripathi et al. 2016).

Slow and fast pyrolysis leads to biochar with different physiochemical properties. Bruun et al. (2012) made a comparative study on wheat straw biochar production by slow and fast pyrolysis. The results of their study showed that in slow pyrolysis, wheat straw was completely pyrolyzed, whereas in fast pyrolysis, residual carbohydrate was present. The two biochar had different pH, particle size, and BET surface area. Paethanom and Yoshikawa (2012) reported that slow pyrolysis of husk rice formed pores, which had higher BET surface area, however, the porosity and surface area was reduced considerably, with increasing the temperatures of over 600 °C.

15.2.2 Gasification

Gasification involves heating of biomass at a temperature >500 °C in limited supply of air to produce syngas (mixture of methane, carbon dioxide, carbon monooxide, hydrogen, and water), biochar, tar, and ash (You et al. 2017). Gasification of biomass occurs in four main steps, i.e. drying of biomass, pyrolysis, partial oxidation, and reduction (Hansen et al. 2015). The yield of biochar in this process is very low, 5–10% by weight. Brewer et al. (2009) did a comparison of the attributes of biochar produced by gasification and pyrolysis. Biochar produced by gasification was highly porous, with high particle density. However, due to high temperature involved in gasification, the aromaticity of biochar was a bit low as compared to slow and fast pyrolysis processes.

15.2.3 Hydrothermal Carbonization

It is also called wet torrefaction. In hydrothermal carbonization, the biomass is heated at 200–300 °C in closed container in presence of water, at a pressure of 2–10 MPa, leading to formation of hydrochar (HC) similar to biochar (Fang et al. 2018). Zhang et al. (2020) prepared spent coffee grounds hydrochar by this process. The yield was 64.5–80.3% at different temperature range. It was found that HC had lower level of carbonization, large number of amorphous domains, high polarity, as compared to spent coffee grounds biochar. The properties of HC rely mainly on the type of biomass. HC harbors numerous functional groups that contain oxygen and is hydrophobic (Sun et al. 2014). Hydrochar is used for soil improvement, carbon capture, and wastewater treatment (Taskin et al. 2019). A comparison between various methods of biochar production is provided in Table 15.1.

Table 15.1 Summary of the methods of production of biochar

Method of preparation	Temperature	Incubation time	Yield (w/w)	Reference
Slow pyrolysis	300–600 °C	Some hours to few days	20–40%	Lee et al. (2013)
Fast pyrolysis	(>800 °C)	Minutes to hours	15–25%	Wang et al. (2013)
Gasification	>500 °C	Seconds to minutes	5–10%	You et al. (2017)
Hydrothermal carbonization	200–300 °C	Few hours	60–80%	Fang et al. (2018)

15.3 Characteristic Properties of Biochar

The structure of biochar is amorphous and completely different from coal and coke, which exhibits graphite like layer structure (Suliman et al. 2016). Biochar possesses numerous pores, which have either honeycomb or slit shaped arrangement (Ahmed et al. 2016). The pore size ranges from few nm to several μm in diameter. A major part of biochar consists of micropores, which are <2 nm and are present inside. While mesopores range from 2 to 50 nm and they connect micropores to its surface. Due to its characteristic structure, biochar contains numerous adsorption sites, which are heterogeneously distributed (Sun et al. 2016). Biochar also contains surface charge, owing to functional groups on its surface. It is an important characteristic, which decides adsorption of charged species through columbic attraction (Bogusz et al. 2015). Surface charge comes from ionizable groups like hydroxyl and carboxyl on the surface. Surface charge is measured with the help of ion exchange capacity or zeta potential measurements (Cui et al. 2016).

Another important characteristic of biochar is aromaticity and polarity. Aromaticity is defined as the content of total aromatic carbon content as compared to total carbon present in biochar (Fryda and Visser 2015). Aromaticity depends upon type of biomass and pyrolysis conditions. For example, biochar derived from woody biomass has high aromaticity due to presence of high amounts of lignin, whereas biomass having lower lignin content exhibits lower aromaticity (Suliman et al. 2016). Pyrolysis conditions also determine aromaticity. It is observed that high temperature during pyrolysis increases aromaticity of biochar. Aromaticity of biochar is determined by hydrogen/carbon (H/C) index. A low H/C index represents high carbon content and aromaticity (Sun et al. 2014). Another important method to determine aromaticity is Nuclear Magnetic Resonance (NMR). Polarity of biochar is due to its aliphatic content. The amount of polar functional groups in biochar can be presented by ((O + N)/C) ratio. Greater amount of oxygen will lead to high polarity of biochar (Chen et al. 2016). High polarity of biochar causes high rate of adsorption of polar/ionic compounds, and a low affinity for hydrophobic compounds.

Biochar also contains some amount of ash. The content of ash in biochar varies from 1 to 80%, depending upon type of biomass and pyrolysis temperature. The amount of ash is high when waste material or grass is used as substrate. Ash content had a dominant role in pore size and polarity of biochar.

15.4 Adsorption Mechanism in Biochar

There are a number of mechanisms through which adsorption of compounds occurs on the surface of biochar (Fig. 15.2).

Some important adsorption mechanisms are as follows:

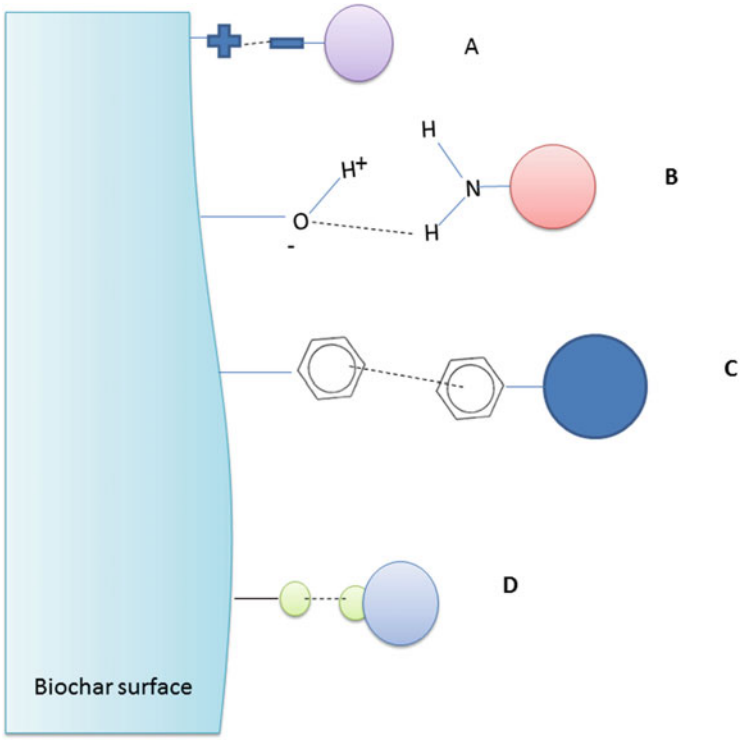

Fig. 15.2 Different mechanisms of adsorption taking place between biochar and pollutants. (**a**) Columbic interactions, (**b**) Hydrogen bonding, (**c**) π–π interactions, (**d**) Hydrophobic interactions

15.4.1 Columbic/Electrostatic Interactions

Columbic interactions take place between two charged species having opposite charge. In this case, the adsorbate and adsorbent have opposite charges. The adsorption of organic compounds to biochar depends upon pH and ionic strength, because these factors play an important role in dissociation of ionic groups in aqueous systems. For example, adsorption of methyl violet dye on plant based biochar occurs due to electrostatic interactions. At pH >9, methyl violet carries a net positive charge and it is adsorbed due to presence of (−)ve charge on the surface of biochar (Xu et al. 2011). Similarly, removal of lead (II) ions through waste derived biochar was studied by Xu et al. (2014). It was found that adsorption by organic fraction of biochar was by electrostatic interactions between carboxyl group of biochar and pb(II) ions.

15.4.2 Hydrogen Bonding

These are dipole interaction taking place between a H donor and acceptor. The H donor is normally bound to H acceptor atoms, i.e. O, N, Cl, F, or it may be present in organic functional groups and electron-rich π-systems. These hydrogen donor/acceptor functional groups are present on either biochar or organic molecules. For example, in spent coffee bean hydrochar, it was observed that hydrogen bonds had a critical role in adsorption of sulfonamide antibiotics on hydrochar. The results of FTIR spectra revealed that the disappearance of a band at 1163 cm^{-1} related to carboxyl group after the adsorption of hydrochar with sulfonamide antibiotics (Zhang et al. 2020).

15.4.3 π–π Interactions

These are dipole interactions, which are weaker than H bonding. These are defined as the attractions taking place between uncharged organic molecules and electron containing π-systems. It consists of π-bonds, which is formed by the overlapping of electronic orbitals, for example, C=C bonds and aromatic structure that can attract other charged molecules or other π-systems. It was observed that the aromatic nature of biochar predominates with increasing temperature of pyrolysis, owing to destruction of aliphatic groups. As shown by Zhang et al. (2020), coffee grounds biochar was used for removal of adsorption of antibiotics sulfadiazine (SDZ) and sulfamethoxazole (SMX). The results indicated that biochar had graphite like structure with less number of polar domains. Further, antibiotics acted as π-acceptors owing to the presence of amino groups and aromatic ring structure. Therefore, the graphite like structure acted as π-donor for SDZ and SMX by π–π interactions.

15.4.4 Hydrophobic Interactions

These are non-specific interactions which play a role due to entropy. They exist due to the tendency of non-polar groups to come together in aqueous system in order to avoid interactions with water. Hydrophobic interactions have been observed in certain antibiotics, such as sulfamethoxazole, where uncharged antibiotics molecules adsorb to aromatic groups present on surface of biochar, with the help of hydrophobic interactions (Huang et al. 2020).

15.5 Biochar Based Removal of Dyes

With the increasing human population, there is a tremendous increase in the number of textile, plastic, and printing industries around the world. These industries release a huge amount of waste water contaminated with mostly dyes, chemicals, and other toxic substances (Gupta 2009). In many countries, this waste water is directly released in the environment, without any prior treatment leading to pollution. The dye residues impart color to the water, leading to a drastic decrease in rate of photosynthesis by aquatic photo autotrophs. The dye containing water, when ingested by animals including humans leads to a number of health related issues owing to the acute toxicity of dye molecules (Aljeboree et al. 2017). Therefore, it was realized that removal of dye residues from the environment especially water systems is of prime importance. There are enormous amount of studies on adsorptive removal of dyes by biochar obtained from different plant biomasses. Sewu et al. (2017b) reported the removal of crystal violet by Korean cabbage biochar prepared by pyrolysis at 500 °C for 1 h. This biochar has highest adsorption power of 1304 mg/g for crystal violet. The kinetics' data showed best fit with pseudo-second order kinetics and isotherm data showed compliance with Langmuir model. The mode of adsorption was through electrostatic interactions. In a report by Yang et al. (2016), vermicompost biochar was employed for elimination of methylene blue and Congo red. The biochar was prepared by pyrolysis for 2 h at temperature ranging from 300 to 700 °C. Maximum adsorption power was different for both the dyes and biochar formed at different temperatures. For Congo red, maximum adsorption power was 17 mg/g for biochar formed at 700 °C. For methylene blue, maximum adsorption power was 70 mg/g for biochar formed at 300 °C. The data showed that for both the dyes, Langmuir and Temkin model was a perfect fit. The kinetics data revealed that the mode of adsorption was pseudo-second order. It was anticipated that H bonding and π–π interactions were responsible for the adsorption process. In a study by Sewu et al. (2017a), crystal violet removal was studied with the help of a mixture consisting of spent mushroom waste biochar and sea kelp biochar (9:1 ratio). Both the biomasses were mixed before pyrolysis. It was performed for 1 h at 500 °C. The results demonstrated that the mixture of biochar gave better results as compared to both the biochar used separately. The highest adsorption power was 610.1 mg/g and it complied with pseudo-second order kinetics and data of isotherm revealed that it complied better with Langmuir model. It was found that adsorption was through pore filling and film diffusion, respectively. Zhu et al. (2018) studied the elimination of methylene blue by cattle manure biochar, produced by pyrolysis at 200 °C for 30 min. The maximum adsorption power of the biochar was 241.9 mg/g and it followed pseudo-second order kinetics and Langmuir isotherm. The adsorption took place by means of H bonding and electrostatic interactions between biochar and dye molecules. Yu et al. (2018) reported the adsorption of methyl orange on chicken manure biochar, which was synthesized by pyrolysis for 2 h at 600 °C. The highest adsorption power was 39.5 mg/g and adsorption exhibited pseudo-second order kinetics. The isotherm data revealed close compliance with Langmuir isotherm

model. The mode of adsorption was through pore filling and columbic interactions. Chen et al. (2018) studied removal of Congo red, crystal violet, and malachite green with the aid of macroalgae residues biochar, prepared from pigment extracted algal biomass pyrolyzed at 800 °C for a period of 2 h. The highest adsorption power of the biochar towards these dyes was 5306.2 mg/g (malachite green), 1222.5 mg/g (crystal violet), 345.2 mg/g (Congo red), respectively. The data of kinetic studies suggested pseudo-second order for elimination of these dyes. Isotherm data exhibited close similarity with Freundlich model. The mode of adsorption was by π–π interactions. In a report by Abd-Elhamid et al. (2020), rice straw biochar was employed for the elimination of methylene blue and crystal violet. The biochar was formed by pyrolysis at 500 °C for a period of 2 h. The highest adsorption power of the biochar for both the dyes was found to be 90.9 mg/g (methylene blue) and 44.6 mg/g (crystal violet) respectively. With both the dyes the rate of adsorption showed compliance with pseudo-second order kinetics and isotherm data revealed that adsorption followed Langmuir isotherm. The mode of adsorption of dyes on the surface of biochar was through ionic interactions. A summary of adsorptive removal of dyes by biochar is presented in Table 15.2.

15.6 Biochar Based Removal of Pesticides

Pesticides are a broad group of chemicals used in agriculture for control of a variety of pests of the crop plants such as weeds, insects, and pathogenic microorganisms. Pesticides are employed to enhance productivity of crops, but after their use and during their application, they enter in various compartments of our environment such as soil, water bodies, etc. and cause pollution. From environment, they enter in the body of the organisms and show their detrimental effects such as acute toxicity, carcinogenicity, etc. They also accumulate in the body of organisms present in different trophic levels and exhibit biomagnification. Owing to various ill effects of pesticides on organisms, a large number of studies have been performed regarding removal of pesticide residues from the environment. Recently, biochar has emerged as an efficient, economical adsorbate for removal of pesticides present in nature. Ponnam et al. (2020) reported adsorptive removal of bentazone, a commonly used insecticides used to control of insect such as moths, by neem waste biochar. Bentazone is highly toxic to humans and its ingestion leads to a number of ailments (Ania and Beguin 2007). In their study, biochar was prepared from neem tree bark by pyrolysis at 300 °C for 2 h. The adsorption of bentazone to neem biochar was studied, the process was reliant on pH, and the rate of adsorption was high at low pH. It was anticipated that at low pH bentazone was negatively charged and it was adsorbed to the surface of biochar by means of H bonding. The highest adsorption power was 79.4 mg/g. The rate of removal showed pseudo-second order kinetics and Freundlich isotherm. In a different study, Zhao et al. (2018) studied the elimination of insecticide imidacloprid by utilizing peanut shell biochar. Imidacloprid is a widely used neonicotinoid class of insecticide, which is used to control insects such as

Table 15.2 Summary of biochar-based adsorption of dyes

Adsorbate	Biomass	Maximum adsorption capacity	Method/temp	Mechanism	Kinetics	Isotherm model	References
Methylene blue	Vermicompost	70 mg/g	Pyrolysis at 300 °C	H bonding π-π interactions	Pseudo-second order	Langmuir and Temkin	Yang et al. (2016)
	Cattle manure	241.9 mg/g	Pyrolysis at 200 °C	H bonding, electrostatic interactions	Pseudo-second order	Langmuir	Zhu et al. (2018)
	Rice straw	90.9 mg/g	Pyrolysis at 500 °C	Electrostatic interactions	Pseudo-second order	Langmuir	Abd-Elhamid et al. (2020)
Malachite green	Macro algae	5306.2 mg/g	Pyrolysis at 800 °C	π-π interactions	Pseudo-second order	Freundlich	Chen et al. (2018)
Congo red	Vermicompost	17 mg/g	Pyrolysis at 700 °C	H bonding π-π interactions	Pseudo-second order	Langmuir and Temkin	(Yang et al. 2016)
	Macro algae	345.2 mg/g	Pyrolysis at 800 °C	π-π interactions	Pseudo-second order	Freundlich	Chen et al. (2018)
Crystal violet	Mushroom waste and sea kelp	610.1 mg/g	Pyrolysis at 500 °C	Pore filling and film diffusion	Pseudo-second order	Langmuir	Sewu et al. (2017a)
	Rice straw	44.6 mg/g	Pyrolysis at 500 °C	Electrostatic interactions	Pseudo-second order	Langmuir	Abd-Elhamid et al. (2020)
	Macro algae	1222.5 mg/g	Pyrolysis at 800 °C	π-π interactions	Pseudo-second order	Freundlich	Chen et al. (2018)
	Korean cabbage	1304 mg/g	Pyrolysis at 500 °C	Electrostatic interactions	Pseudo-second order	Langmuir	Sewu et al. (2017b)
Methyl orange	Chicken manure	39.5 mg/g	Pyrolysis at 600 °C	Pore filling and columbic interactions	Pseudo-second order	Langmuir	Yu et al. (2018)

aphids, termites, beetles, etc. (Held and Parker 2011). Biochar was prepared by pyrolysis at varying temperatures (300–700 °C) for a period of 4 h. The results demonstrated that pyrolysis temperature had an impact on adsorption process as biochar at 700 °C showed maximum removal of imidacloprid. The maximum adsorption power was 8.68 mg/g. The kinetics data suggested that it followed pseudo-second order and the isotherm of imidacloprid adsorption followed both Langmuir and Freundlich models. In a different study, Baharum et al. (2020), reported the adsorption of pesticide diazinon by means of coconut shell biochar. Diazinon belongs to organophosphorus group of pesticides. This pesticide is highly toxic to animals, its affects the nervous system by inhibiting the enzyme acetylcholinesterase (Esfandian et al. 2016). The biochar was formed by pyrolysis for 2 h at 700 °C, it was further modified by acid and alkali treatment. The process of adsorption was pH dependent; the adsorption was highest at pH −3 by acid modified biochar. However, alkali modified biochar showed highest removal efficiency at pH −7. The highest adsorption capacity was shown by acid modified biochar, which was 5.69 mg/g. The isotherm data revealed that for alkali modified biochar, the adsorption complied with Langmuir model and for acid modified biochar, the adsorption followed Freundlich model. The results of this study demonstrated that electrostatic interactions played a major part in the process of adsorption. Wang et al. (2015) reported adsorptive elimination of chlorpyrifos with the aid of wheat straw biochar produced by pyrolysis at 750 °C for 2 h. The highest adsorption power was 16 mg/g. The rate of elimination complied with pseudo-second order kinetics and isotherm data complied with Freundlich isotherm. The mode of adsorption was through π–π interactions. In a similar study, Jacob et al. (2020) studied removal of chlorpyrifos, which is an organophosphate group of pesticide, by utilizing sugar cane bagasse biochar. The biochar was formed by pyrolysis at 450 °C, biochar was prepared in three sets, normal biochar, bagasse treated with acetone prior to pyrolysis, and bagasse treated with benzene prior to pyrolysis. The results indicated that the process of adsorption was dependent on the pH. Maximum adsorption was observed at pH −2. The highest adsorption power was found to be 3.2 mg/g after 1 h of incubation. The rate of reaction complied with pseudo-second order kinetics and isotherm data showed similarity with Freundlich model. The mode of adsorption of chlorpyrifos to the biochar was physisorption. In a report by Suo et al. (2019), elimination of triazine pesticides was studied with the help of corn straw biochar. Triazines are a broad group of pesticides. They are normally employed as weedicides for the control of common weeds. These pesticides are toxic to animals owing to their endocrine disrupting activity, carcinogenicity, and other detrimental effects (Hu and Chen 2013). Corn straw biochar was formed by pyrolysis at 300 °C for a period of 2 h. It was seen that with corn straw biochar, the highest adsorption power was 79.6 mg/g. The adsorption exhibited pseudo-second order kinetics and isotherm data complied with Freundlich isotherm. The mode of adsorption was through involvement of H bonding, columbic interactions, and Van Der Waals forces. A summary of adsorptive removal of pesticides by biochar is presented in Table 15.3.

Table 15.3 Summary of biochar-based adsorption of pesticides

Adsorbate	Biomass	Maximum adsorption capacity	Method/temp	Mechanism	Kinetics	Isotherm model	References
Atrazine	Wheat straw Peanut Shell	2.0 mg/g 1.5 mg/g	Pyrolysis at 350–550 °C	H bonding	Pseudo-second order	Freundlich	Zhou et al. (2016)
Bentazone	Neem bark	79.4 mg/g	Pyrolysis at 300 °C	H bonding,	Pseudo-second order	Freundlich	Ponnam et al. (2020)
Imidacloprid	Peanut shell	8.68 mg/g	Pyrolysis at 700 °C	Electrostatic interactions	Pseudo-second order	Freundlich Langmuir	Zhao et al. (2018)
Diazinon	Coconut shell	5.69 mg/g	Pyrolysis at 700 °C	H bonding,	Pseudo nth order	Langmuir	Baharum et al. (2020)
Chlorpyrifos	Wheat straw	16 mg/g	Pyrolysis at 750 °C	π-π interactions	Pseudo-second order	Freundlich	Wang et al. (2015)
	Sugar cane bagasse	3.2 mg/g	Pyrolysis at 450 °C	Physisorption	Pseudo-second order	Freundlich	Mariam Jacob et al. (2020)
Triazine	Corn straw	79.6 mg/g	Pyrolysis at 300 °C	H bonding, columbic interactions and Van der Waals forces	Pseudo-second order	Langmuir	Suo et al. (2019)

15.7 Biochar Based Removal of Pharmaceutical Pollutants

Over millions of tonnes of pharmaceutical are manufactured globally per annum. During the process of production, use, and disposal, these drugs and their degradation products enter into the surroundings. A large number of pharmaceuticals such as antibiotics and NSAIDs are used for human and veterinary consumption (Maia et al. 2019). Around 30 and 90% of the drug administered is excreted in urine. A huge amount of pharmaceuticals have been detected in aquatic systems, sewage sludge, and soils (Teodosiu et al. 2018). Many of them are persistent causing a number of eco-toxicological effects in animals such as impairment of the reproduction in fishes, reduction in vulture populations owing to poisoning with diclofenac (Ziylan and Ince 2011). The presence of antibiotics leads to the development of antimicrobial resistance (AMR), which is an emerging hazard to the health of humans. AMR can lead to morbidity, death, and healthcare expenses (Do Minh et al. 2020). Therefore, removal of pharmaceutical pollutants from the environment is very essential. In this section, we have dealt with biochar based removal of pharmaceutical pollutants such as antibiotics, NSAIDs, and hormones.

15.7.1 Antibiotics

15.7.1.1 Tetracycline Antibiotics

Tetracyclines are a broad group of antibiotics, which consists of tetracycline (TC), oxytetracycline (OTC), and chlortetracycline (CTC). They are low cost broad spectrum antibiotics employed in swine industry (Kim et al. 2014). Like any other antibiotics, TCs are not completely absorbed by animals, and 70–90% are excreted out. Therefore, a huge amount of TCs have been reported in swine wastewater (Cheng et al. 2019). TCs present in waste water show detrimental effects on aquatic animals and humans. Extended exposure of these antibiotics in the environment may cause the development of antimicrobial resistance (AMR) (Martínez 2008). Fan et al. (2018) studied removal of TC by rice straw biochar produced by pyrolysis at different temperatures. Highest adsorption power was shown by biochar produced at 700 °C, which was 50.72 mg/g at 35 °C. The results indicated that EDA π–π interactions and electrostatic interactions played a major role in adsorption. Jang and Kan (2019) studied removal of TC by alpha-alpha biochar formed by pyrolysis at 500 °C for 30 min. Highest adsorption power was 372 mg/g. It was by hydrogen bonding, electrostatic interactions between biochar and TC. In a study by Cheng et al. (2020), biochar derived from pomelo peel was used for adsorption of TC. Biochar was formed by pyrolysis for 2 h at a temperature of 400 and 600 °C, respectively. The biochar yield was 35.93% at 400 °C and 32.78% at 600 °C. Biochar at 400 °C exhibited low adsorption of TCs as compared to biochar produced at 600 °C. Under similar conditions, biochar modified by KOH exhibited high rate of

adsorption for TC, OTC, and CTC, respectively. The adsorption kinetics suggested that pore filling and chemisorption were the prominent modes of adsorption, whereas the adsorption by BC-KOH was due to π-π EDA mechanism. In all of the above studies, the kinetics based data showed similarity with pseudo-second order model and data of isotherm revealed close proximity with Langmuir model.

15.7.1.2 Sulfonamides

Sulfonamides are one of the widely used antibiotics, due to their low cost and efficiency against a number of bacterial infections. It is also supplemented to animal food (Ahmed et al. 2017). Sulfonamides have been reported from environments. They exhibit a number of detrimental effects. They can act as carcinogens (Shao et al. 2005) and show accumulation in marine animals. Yao et al. (2018) used raw and anaerobically digested bagasse biochar for adsorption of sulfamethoxazole (SMX) and sulfapyridine (SPY). The biochar from digested bagasse was formed at 600 °C, it exhibited excellent adsorption properties for SMX and SPY with highest adsorption power of 54.38 and 8.60 mg/g, correspondingly. SMX isotherm complemented with the Freundlich and Redlich–Peterson models and SPY with Redlich–Peterson model. The kinetics data indicated pseudo nth order model. The results also demonstrated that π–π interactions were the cause of adsorption. Huang et al. (2020) reported the adsorption of sulfamethoxazole (SMX) by ball milled hickory chips biochar produced at 450 °C. The results showed that hickory chips biochar showed 83.35% adsorption of SMX in comparison to the biochar formed at different temperature. FTIR studies indicated that electrostatic interactions, along with hydrophobic and π–π interactions were the dominant forces involved in adsorption, as the process was pH dependent. The data of kinetic studies was in compliance with the Langmuir model and highest adsorption power was 100.3 mg/g and 25.7 mg/g in wastewater correspondingly. Zhang et al. (2020) reported the elimination of sulfadiazine (SDZ) and sulfamethoxazole (SMX) by spent coffee grounds hydrochar (HC) and biochar (BC) produced by hydrothermal carbonization and pyrolysis, respectively. The highest adsorption power of biochar was 121.5 μg/g (SDZ) and 130.1 μg/g (SMX) at 25 °C, whereas the highest adsorption power of hydrochar were 82.2 μg/g (SDZ) and 85.7 μg/g (SMX), respectively. The results showed that for BC, adsorption occurred by π–π interactions and for HC, it was through hydrogen bonds. Further, the data of adsorption was in compliance with Langmuir model and adsorption kinetics was related to the pseudo-second order model.

15.7.1.3 Ciprofloxacin (CIP)

It is a fluoroquinolone based, broad spectrum second-generation antibiotic active against Gram-negative and Gram-positive bacteria. It acts by inhibiting the enzymes DNA gyrase and topoisomerase involved in bacterial DNA replication and

transcription, respectively (El-Shafey et al. 2012). It is commonly prescribed in urinary, respiratory, and gastrointestinal tract infections. A very high concentration of CIP has been reported in natural and engineered water environments. In water environment, a very low concentration of CIP can lead to mortality and genotoxicity in freshwater cyanobacteria and plants (Peng et al. 2016). Many reports have demonstrated the removal of ciprofloxacin by biochar. Li et al. (2018a) reported removal of CIP by potato stem and leaves biochar formed by pyrolysis at 500 °C. The biochar was modified by KOH treatment. It was observed that KOH treated biochar showed greater removal of CIP as compared to untreated biochar. The highest adsorption power was 23.36 mg/g at 308.15 K. The data of isotherm was in compliance with Langmuir model of adsorption isotherm. FTIR studies revealed that H bonding, π–π interactions played a vital part in adsorption. Li et al. (2018b) reported used tea leaves biochar for adsorption of CIP. BC was prepared by pyrolysis. The biochar formed at 450 °C exhibited highest adsorption power 238.10 mg/g at pH 6 and 40 °C. The isotherm data followed the Langmuir model. The kinetics data was in agreement with pseudo-second order model. It was observed that π–π interactions, H bonding and columbic interactions played dominant roles in removal of CIP. Ashiq et al. (2019) studied removal of CIP on composite material consisting of municipal solid waste derived biochar (MSW-BC) and bentonite clay. The biochar was formed by pyrolysis at 450 °C for 30 min. The highest CPX elimination was observed at pH 6, and the highest adsorption power of 190 mg/g. The data fitted into Hill isotherm model along with pseudo-second order and Elovich kinetic models showed the best fit. The results also indicated columbic interactions between CPX and composite, as the mechanism of interaction.

15.7.2 NSAIDs

15.7.2.1 Diclofenac

Diclofenac sodium (DS), a non-steroidal anti-inflammatory and analgesic drug (Barczak et al. 2018), it is commonly found in waste waters and soil. Owing to its low biodegradability, it is not readily degraded in water system and wastewater treatment plants (Beyki et al. 2017). A concentration of 250 ng/L in wastewater treatment plants has been reported. Zhang et al. (2019) reported removal of DS by waste sludge/leaf biochar. In their study, sludge to leaf ratio was 1:3, pyrolysis was performed at 200 °C for 1 h. A highest adsorption power of 877 mg/g was observed at 25 °C, initial DS concentration of 10 mg/L, and biochar dose of 0.005 g. The kinetics data showed compliance with pseudo-second order and Temkin model complied with isotherm data. Santos et al. (2020) reported adsorption of DS by MgAl layered double hydroxide sustained on *Syagrus coronata* biochar. This biochar was highly porous, had greater surface area and excellent ion exchange capacity. For MgAl/LDH and the composite, removal efficiency was close to 60%

for 30 mg/L of DS initial concentration at pH values from 5 to 12, adsorption was inhibited at pH 2. It was observed that electrostatic interactions were the cause of adsorption. Kinetic studies revealed that at 50 mg/L, the equilibrium was attained in 1 h of contact, removing 78% of the pollutant. Nevertheless, at a concentration of 200 mg/L, equilibrium reached only after 6 h of contact, achieving up to 82% of removal. Probably, in the initial hours the adsorption occurred by physisorption. For higher concentrations, besides the physisorption, chemical interactions also occurred inside the pores. For both the adsorbents, at lower concentrations, and contact time, the data showed compliance with the pseudo-first order model, for the highest concentration (200 mg/L), the pseudo-second order model fitted better. Bagheri et al. (2020), studied removal of DS by Moringa seed powder biochar, treated with phosphoric acid. It was found that highest adsorption power of 95.85 mg/g, corresponding to 82.8% removal was achieved at pH ~5. The removal was low at basic pH. It was anticipated that π–π caused the adsorption. The kinetics data showed compliance with pseudo-second order model and Sips model defined the isotherms data better. In another study by Correa-Navarro et al. (2020), fique bagasse biochar was formed by pyrolysis at 850 °C for 3 h. It showed highest adsorption capacity of 5.40 mg/g for DS. The experimental data fitted well into Redlich–Peterson isotherm model and kinetics data fitted well with pseudo-second order kinetics.

15.7.2.2 Ibuprofen

Ibuprofen [2-(4-isobutylphenyl) propanoic acid] is a commonly prescribed NSAID, as painkiller in a number of body aches (Chakraborty et al. 2018a). A high concentration of Ibuprofen has been observed in aquatic environment (Mondal et al. 2016). Therefore, it was realized that removal of this drug is important for maintaining health of animals. Mondal et al. (2016) studied adsorptive removal of ibuprofen by chemically treated congress grass biochar. Biochar was formed by pyrolysis at 300 °C for 1 h. After pyrolysis, it was treated with 2 N NaOH of 2 h. Highest adsorption power was 3.89 mg/g by using 2 g biochar at 100 mg/L of ibuprofen. The isotherm data showed compliance with Langmuir model. Data of kinetics was in compliance with pseudo-second order model. Chakraborty et al. (2018b) reported adsorption of ibuprofen by steam activated sugarcane bagasse biochar (SPAB) and chemically activated sugarcane bagasse biochar (SCAB). Biochar was formed by pyrolysis at 400 °C for 1 h. The maximum adsorption power was 13.51 mg/g (SCAB) and 11.90 mg/g (SPAB), respectively. The adsorption followed pseudo-second order kinetics. Both the biochars followed Langmuir and Freundlich isotherms. The mechanism of adsorption was H bonding and π–π interactions. Chakraborty et al. (2020) studied removal of ibuprofen by steam activated (DSPB) and chemically activated (DSCC) date seed biochar, it was observed that removal of ibuprofen was 87.01% for DSCC at 21 h and 96.24% at 18 h for DSPB and highest adsorption of ibuprofen was found to be 13.87 mg/g for DSCC and 10.51 mg/g for

DSPB, respectively. The experimental data fitted well with Langmuir isotherm. The adsorption kinetics showed compliance with pseudo-second order kinetics.

15.7.3 Hormones

Natural and synthetic hormones such as estrogens present in the environment act as Endocrine Disrupting Compounds (EDCs) and interrupt the endocrine systems of animals and humans through imitating the action of natural hormones, they also inhibit other hormones or inhibit normal functioning of endocrine or immune systems (Li et al. 2012). Therefore, they cause a number of disorders/ailments in organisms exposed to these hormones. Estrogens are divided into two types; natural such as Estrone (E1), 17 β-estradiol (E2), and estriol (E3), and synthetic such as 17α-ethynylestradiol (EE2) respectively. Both E2 and EE2 are potent estrogens and when present in the environment, they act as EDCs (Al-Khateeb et al. 2014). Wang et al. 2017 reported removal of E2 by rice straw biochar prepared at different temperatures. It was found that biochar at 600 °C showed highest adsorption power of 26.91 mg/g. The data of kinetics showed compliance with pseudo-second order model and it was postulated that film diffusion was the cause of adsorption. The data of isotherm was in agreement with the Freundlich model. Liu et al. (2019) studied adsorption of E2 by lotus seed pod biochar. Highest adsorption power was 100 mg/g by posttreating the biochar by KOH followed by heating at 650 °C. The results of kinetics data showed compliance with pseudo-second order model. The results of adsorption data indicated Langmuir model of isotherm. The results also indicated that chemisorption, π–π interactions, monolayer adsorption were the main adsorption mechanisms. A summary of adsorptive removal of pharmaceutical pollutants by biochar is presented in Table 15.4.

15.8 Conclusion

Biochar is an inexpensive, easy to produce carbon rich material, which is obtained by pyrolysis of biomass. The biomass used to produce biochar is often waste materials, which are either dumped in the environment or incinerated, which leads to increased carbon emissions and subsequently, greenhouse effect. Therefore, biochar production leads to carbon sequestration. Studies have shown that biochar, owing to a number of functional groups on its surface, is an excellent adsorbent for a wide range of pollutants such as dyes, metal ions, and pharmaceuticals. The adsorption properties of biochar also rely on type of biomass and pyrolysis conditions. Further, these properties can be enhanced by physical and chemical modifications. This is an important area of research, which requires utmost attention. Since, many

Table 15.4 Summary of biochar-based adsorption of pharmaceuticals

Adsorbate	Biomass	Maximum adsorption capacity	Method/ temp	Mechanism	Kinetics	Isotherm model	References
Tetracycline	Rice straw	50.72 mg/ g	Pyrolysis at 700 °C	EDA π–π interactions and electrostatic interactions	Pseudo-second order	Langmuir	Fan et al. (2018)
	Alpha-alpha	372 mg/g	Pyrolysis at 500 °C	Hydrogen bonding, electrostatic interactions	Pseudo-second order	Langmuir	Jang and Kan (2019)
	Pomelo peel	124.95, 124.91 and 124.99 mg/g for TC, OTC and CTC	Pyrolysis at 600 °C	Pore filling and chemisorption	Pseudo-second order	Langmuir	Cheng et al. (2020)
Sulfonamide	Raw and anaerobically digested bagasse	54.38 for SMX and 8.60 mg/g for SPY	Pyrolysis at 600 °C	π-π interactions	Pseudo nth order	Freundlich and Redlich–Peterson models	Yao et al. (2018)
	Hickory chips	100.3 mg/g	Pyrolysis at 450 °C	Electrostatic interactions, hydrophobic and π–π interactions	Pseudo-second order	Langmuir	Huang et al. (2020)
	Coffee grounds	121.5 μg/g (SDX) and 130.1 μg/g(SMX)	Pyrolysis at 500 °C	π–π interactions	Pseudo-second order	Langmuir	Zhang et al. (2020)
Ciprofloxacin	Potato stems and leaves	23.36 mg/g	Pyrolysis at 500 °C	π–π interactions, hydrogen bonding and electrostatic attraction	Pseudo-second order	Langmuir	Li et al. (2018a)
	Used tea leaves	238.10 mg/g	Pyrolysis at 450 °C	π–π interactions, hydrogen bonding and electrostatic attraction	Pseudo-second order	Langmuir	Li et al. (2018b)
	Municipal solid waste	190 mg/g	Pyrolysis at 450 °C	Electrostatic interactions	Pseudo-second order	Elovich	Ashiq et al. (2019)

(continued)

Table 15.4 (continued)

Adsorbate	Biomass	Maximum adsorption capacity	Method/ temp	Mechanism	Kinetics	Isotherm model	References
Diclofenac	Waste sludge/leaf	877 mg/g	Pyrolysis at 200 °C	π–π interactions, hydrogen bonding and electrostatic attraction	Pseudo-second order	Temkin	Zhang et al. (2019)
	Syagrus coronata	164 mg/g	Pyrolysis at 400 °C	Physisorption, chemical interactions	Pseudo-second order	Sips	Santos et al. (2020)
	Moringa seed powder	95.85 mg/g	Pyrolysis at 450 °C	π–π interactions	Pseudo-second order	Sips	Bagheri et al. (2020)
Ibuprofen	*Parthenium hysterophorus*	3.89 mg/g	Pyrolysis at 300 °C	Monolayer adsorption	Pseudo-second order	Langmuir	Mondal et al. (2016)
	Sugar cane	13.51 mg/g for SCAB and 11.90 mg/g for SPAB	Pyrolysis at 400 °C	π–π interactions, hydrogen bonding	Pseudo-second order	Langmuir and Freundlich	Chakraborty et al. (2018b)
	Date seed	13.87 mg/g for DSCC and 10.51 mg/g for DSPB	Pyrolysis at 800 °C	Monolayer adsorption	Pseudo-second order	Langmuir	Chakraborty et al. (2020)
17β-estradiol	Rice straw	26.91 mg/g	Pyrolysis at 600 °C	Film diffusion	Pseudo-second order	Freundlich	Wang et al. (2017)
	Lotus seed pod	100 mg/g	Pyrolysis at 500 °C	Chemisorption, π–π interactions, monolayer adsorption	Pseudo-second order	Langmuir	Liu et al. (2019)

workers have reported that these modifications cause a substantial increase in adsorptive power of biochar. Therefore, it can be concluded that although biochar from different biomasses has shown potential to remove a wide variety of pollutants but still a lot of work in this area is required to harness its utmost potential.

Competing Interests The authors do not have any competing interests to declare.

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Chapter 16
Electro-Fermentation of Biomass for High-Value Organic Acids

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Abstract Microbial fermentations are well recognized process for large-scale bio-conversion of organic waste biomass into high-value organic acids. It requires processes optimization, i.e., that should reach at maximum productivity and no feedback inhibition, to reduce the cost of up- and down-stream processing for commercialization. To achieve this, triggered metabolic activities are often needed that maximize the conversion of organic carbon into organic acids under non-sterile conditions. By regulating the redox balance in-situ, the specific organic acid production could be tailored in fermentation systems under mixed/mono-culture conditions. In recent years, bio-electro-fermentations (BEF) has developed as a promising approach for organic waste conversion into value products due to its

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sustainable nature but yet required better understand for further development. In BEF, the fermentative metabolic pathways are enhanced with poising electrodes that facilitate effective electron transfers towards end-product recovery. It is expected to maintain the required redox conditions and buffer the system by regulating reducing equivalents e.g. $NADH^+$ during fermentation. Moreover, microorganisms extract energy required to build biomass (anabolic process) from redox reactions (catabolism) through syntrophic interactions in BEF, while feedback inhibition of process could be overcome. In this chapter, we will elaborate the BEF process for organic acid production (mainly succinic, acetic, and muconic acids) and techno-economics of the process for commercialization.

Keywords Bio-electro-fermentations · High-value organic acids · Microbial fermentations · Redox reaction

Abbreviations

AA	Acetic acid
AD	Anaerobic digestion
BEF	Bio-electro-fermentations
CA	Citric acid
CAPEX	Capital expenditure
ccMA	*cis*,*cis*-muconic acid
ctMA	*cis*,*trans*-muconic acid
EET	Extracellular electron transfer
EF	Electro-fermentation
frd	Fumarate reductase
fum	Fumarase
LA	Lactic acid
LCA	Life cycle assessment
MA	Muconic acid
mdh	Malate dehydrogenase
MMC	Mixed microbial cultures
OW	Organic waste
ppc	PEP carboxylase
pck	PEP carboxykinase
pyc	Pyruvate carboxylase
SA	Succinic acid
TEA	Techno-economic analysis
ttMA	*trans*,*trans*-muconic acid

16.1 Introduction

Increasing population growth posing pressure on environment and exploit natural resources, while it generates huge volume of organic wastes for disposal or treatment (Mehariya et al. 2018; Jiang et al. 2019; Rago et al. 2019). Electro-fermentation (EF) are well recognized approach for bioconversion of organic waste (OW) into valuable bio-products such as biofuels, chemicals, and organic acids (Lovley 2011; Kumar et al. 2014a, b; Awate et al. 2017). EF requires processes that should reach at maximum productivity to reduce the cost of up and downstream processing for commercialization of technology. EF have the potential to tune the fermentation pathway for selective production of selective organic acids (Lovley 2011; Moscoviz et al. 2016; Jiang et al. 2019). Also, EF have substantial role on the selection of specific microbial species during the fermentation process in mixed microbial cultures (MMC) (Moscoviz et al. 2016). Therefore, EF maximize the conversion of biomass towards the accumulation of the selective organic acids with high titer under non-sterile conditions (Schievano et al. 2016; Moscoviz et al. 2018; Jiang et al. 2018, 2020). Moreover, during the mixed (open) fermentations there should be equilibrium of redox potential, which could be achieved by reusing the reducing power to assist the reactions for formation of alcohols, acids, and gases (Lovley 2011; Kiely et al. 2011; Shanthi Sravan et al. 2018). However, in the classical fermentation approach the alcohols and acids accumulates that lead to hamper the microbial growth and metabolic activity due to feedback inhibition and imbalances of pH that reduces the overall economics of organic acid productions (Schmidt 2005). In recent years, BEF considered to balance of redox potential and buffer the system by regulating $NADH^+$ during fermentation (Schievano et al. 2016). In the BEF system, electrodes could be used as electron acceptors or donors to overwhelm the balanced fermentation process (Shanthi Sravan et al. 2018). Moreover, micro-organisms extract energy required to build biomass (anabolic process) from redox reactions (catabolism) through syntrophic interactions due to electron donor/acceptor conditions (Khosravanipour Mostafazadeh et al. 2017). Also the applied potential could be avoid the over accumulation of feedback inhibition during fermentation and boost the metabolic activities and without syntrophic partners (Xafenias et al. 2017; Sasaki et al. 2018). The key advantages of BEF to recover the alcohols and organic acids from complex organic wastes and facilitate the recovery of selective products under open fermentation process. Moreover, the potential of BEF process discussed in subsection for recovery high-value organic acids and flow diagram of BEF showed in Fig. 16.1.

16.1.1 High-Value Organic Acids: Supply and Demand

Biomass can be converted into various organic acids such as lactic acid (LA), succinic acid (SA), citric acid (CA), acetic acid (AA), and Muconic acid (MA).

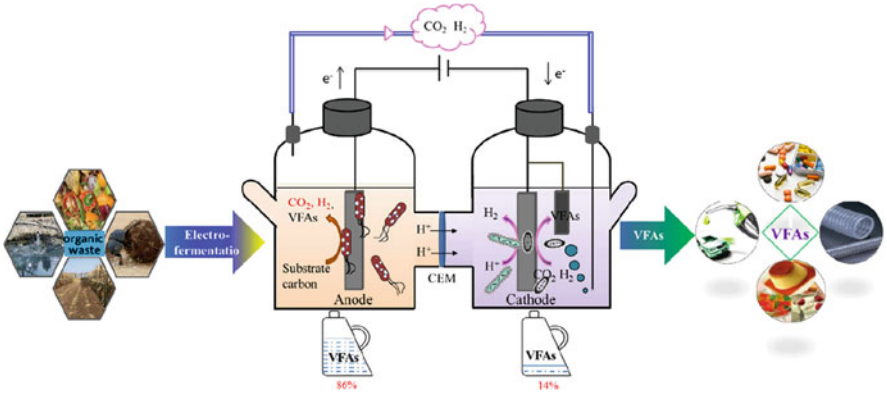

Fig. 16.1 Process flow diagram BEF for recovery of organic acids from biomass

LA is a platform chemicals, which have potential application in different industrial sectors such as leather, pharmaceutical, and textile sector. While due to availability of carboxylic and hydroxyl groups, it can be further converted into others biochemicals (Uçkun Kiran et al. 2014). Microbial fermentation cover approximately, 90% production of total LA for commercial uses globally, which mostly produced through the batch fermentation (Alves de Oliveira et al. 2018). While the productivity of LA is 90–95% w/w based on the initial sugar content (Ghaffar et al. 2014). Recently, lactic acids produced using the various types of biomass, while the productivity can be enhanced via BEF (Ghaffar et al. 2014; Shanthi Sravan et al. 2018; Teigiserova et al. 2019; Chu et al. 2020). Moreover, commercial production of LA via biological routes are in practices in China, USA and Europe (Alexandri and Venus 2017). Moreover, in 2016 the market demand of LA was around 1.2 mt (Alves de Oliveira et al. 2018). While the market demand of LA presents an annual growth of 16.2%, which will lead to estimated demand around 1.96 mt in 2025 (Alexandri et al. 2018; Alves de Oliveira et al. 2018).

SA is recognized as significant platform chemicals with high market demand and its production from biomass is inexpensive as compared to petrochemical based production, with a market value over 108 million USD (2013) and 100 million USD, respectively (Alexandri and Venus 2017). While, the commercial producer of SA are Myriant (corn glucose), Succinity and Reverdia (starch-based sugars) to meet the market demand (Mika et al. 2018). In 2015, global market demand of SA was expected around 90 kilotons (Teigiserova et al. 2019). Also, CA have numerous applications in various industrial sectors for manufacture of chemicals and materials. Due to various application of CA, the global CA production rate is increasing with 3.7% compound annual growth rate (Cavallo et al. 2017). One more high-value organic acid is levulinic acid, which has various applications in food and pharmaceutical sector, with high market price (1000–3000 USD/ton) (Mika et al. 2018). AA have various application pharmaceutical sectors for manufacturing different products as well as have application food, textiles, automobile industries. AA is

considered as potential organic acid in the chemical industry for synthesis of various products. The growing global demand of AA was 12.1 million tons in 2014, with forecasted to arrive 16.8 million tons by 2022 with compound annual growth rate (CAGR) of 6.8% (Caxiano et al. 2020). MA is a high value-added dicarboxylic acid, which connected by double bonds, with three isomeric forms, i.e., *cis*,*cis*-MA, *cis*, *trans*-MA, and *trans*,*trans*-MA (Khalil et al. 2020). Therefore, market demand of MA increasing year by year due to various industrial applications. MA is recognized as "bioprivileged" compound that can be converted into to multiple bioproducts (Bentley et al. 2020). Microbial fermentation considered for efficient conversion of biomass/organic waste into high-value organic acids. However, the tuning of fermentation parameters can alter the metabolic pathways of microorganisms for selectivity for organic acid production from biomass. Moreover, the strain selection and operating parameters have significant role on productivity of organic acids (Chu et al. 2020).

16.1.2 Current Production Platform and Costs

Biorefineries represent an opportunity for processing biomass into various high-value organic acids via microbial fermentation routes (Teigiserova et al. 2019). However, the production cost is high for production of individual acids, therefore synergic production for simultaneous production of various acids offer a cost-effective approach (Chavas and Kim 2010). The integration of various biomass conversion technologies allows the efficient valorization into numerous products could allow to meet the markets demand, which could lead to surge the revenues. Moreover, the integration various approach can increase the productivities of different organic acids in a single biorefinery. The integration of biorefinery can be coupled with BEF, which can minimize the demand of biomass as well as decrease the energy requirement for biomass treatment, which reduce the production cost (Chavas and Kim 2010; Xafenias et al. 2017; Shanthi Sravan et al. 2018; Chu et al. 2020). However, the production and recovery yields of organic acids have significant role in the CAPEX (Capital expenditure) of process. Therefore, the integration of process and development of new technologies to reduce the CAPEX cost is need for an hour (De Corato et al. 2018; Teigiserova et al. 2019).

16.2 Bio-Electro-Fermentation

In order to minimize the biomass (or organic waste) disposal and utilize the organic fraction of waste, different technologies utilizing microorganisms have taken huge leap with simultaneous production of commodities that have high value. Anaerobic digestion (AD) process is one such established process to recover a spectrum of biobased products from diverse organic biomass (Zhou et al. 2017). AD has been

conventionally hired to decrease the size and heaviness of waste, sludge stabilization, eradicate the pathogenic microbes, and utilize the bio-energy present in the sludge by producing bio-methane gas at the last stage of its process (Appels et al. 2008; Karthikeyan and Visvanathan 2013). AD process is usually well-known for treating sewage sludge in a more controlled and sustainable method when compared to other landfill or composting disposal ways. Nevertheless, the degree and competence of AD is low for organic biomass (other than sludge), particularly when inoculators are not supplemented which can be overcome by integrating with other potential technologies (Liu et al. 2005). Moreover, the limitations on biobased product recovery from organic biomass are leading to search for alternate, integrated strategies for producing electrical energy from these sources in a controlled manner and consumed on demand.

The rate kinetics and process performance of AD could be improved by the introduction of electrodes in the microenvironment. In order to optimize energy conversion efficiency and operational stability in the traditional AD, various strategies are being studied among which bio-electro-fermentation (BEF) would be an effective strategy (Schievano et al. 2016; Venkata Mohan et al. 2016; Rabaey and Rozendal 2010). Further, if the electrodes are connected externally while applying voltage/current across them, the process may be further enhanced (Villano et al. 2017; Stamenkovic et al. 2016). Electrochemical reduction of organic substrate to diverse biobased products can become an economic and commercially viable process with influence on the rate kinetics of a bioprocess (Choi and Sang 2016; Rabaey et al. 2011). The electrons utilized during the EF process certainly accounted for higher amounts when subjected to electrical energy supplementation. The microbial production of multiple biobased products from biomass with electrical energy as an influencing factor is economically feasible and sustainable process (Venkata Mohan et al. 2016; Moscoviz et al. 2016).

16.2.1 Biochemistry of the Fermentation and Organic Acid Productions

Apart from bioelectricity generation and waste remediation, the BEF technology process can be comprehensively used for generation of commodity and other products that have high value such as viable chemicals and fuel alternatives such as H_2, CH_4, organic acids, alcohols, etc., in the cathode chambers by applying supplementary voltage. The terminal reduction reaction is mainly dependent on the electron acceptance and donation at the cathode terminal. In a BEF system, the poised potential at the cathode provides the reducing power (electrons) required by the reaction. Microbes take up the redox species either in the form of e^- or H^+. Redox potential plays a significant role in reducing the substrate to specific product (Rabaey and Rozendal 2010). The in situ potential generated in the system along with externally supplied voltage can aid in product formation by the enriched

microbial community. These can be produced from variety of substrates, viz., inorganic carbon (CO_2), volatile fatty acids, glucose, different wastewaters, and organic biomass. The process of generating value-added chemicals, viz., ethanol, butanol, long chain fatty acids, etc. along with their recovery is referred to as microbial electrosynthesis (Choi et al. 2017). In BEF, the electrons obtained from working electrode (cathode) are used for reducing CO_2 into broad spectrum of commercial compounds which are organic in nature and that act as precursors for anticipated value addition (Rabaey et al. 2011). The thermochemical and electrochemical technologies require high potential input to carry out the reactions and produce diverse product range. The BEF is characterized as a promising way for efficient carbon consuming and sustainable development technologies. The CO_2 capture and utilization for value-added products generation will decrease the carbon footprints with complimentary biofuel and bio-energy synthesized products. (Mohanakrishna et al. 2015; Srikanth et al. 2016). Unlike other photo-electrocatalysis processes, BEF requires less energy input for crossing the energy barrier and form bio-products by biocatalyst. The BEF technology is trending across the world as the studies successfully reported the generation of valuable products by utilizing both organic and inorganic carbon material as substrates. The possibility to control the electron delivery to the cathode is improved in BEF. Contemporary studies have evidenced the microbial production of biobased products to increase in a significant manner which is also an economic feasible way forward towards sustainability. The microbial biocatalysts may be robust alternatives but when coupled with electricity driven strategies, the conversion efficiencies of organic substrates to biobased products are more effective than traditional biomass-based strategies (Moscoviz et al. 2016; Kumar et al. 2017).

16.2.2 Electrodes for BEF and Optimization of BEF

The electrode–microbe interaction plays a significant role in BEF technology that governs the reducing equivalents exchange between them. Hence, appropriate electrode materials are crucial for an effective biological generation of organic molecules or chemicals. The electrodes require the characteristics of cost-effective, high stability and long lasting, good surface to volume ratio, biocompatibility, etc. The reactor configuration involving EF pertaining to anode or cathode electrodes can occur in single chamber reactor or double chamber reactors divided by exchange membranes (cation/anion). The product conversion efficiencies were noticed to be higher in single chambered reactors. But, if an adverse consequence on the purity of the product, a double chambered reactor with separator is utilized for product synthesis. The counter electrode and its compatibility quotient with the working electrode need to be considered for operation of a single or dual chambered reactor. Cost-effective and viable electrodes also need to be optimized for efficient process performance. These critical factors pose a real challenge during upscaling of the process to industrial scale. Anode/cathode reactions involving variations in

oxidation and reduction potentials influence the reaction kinetics and product synthesis. The input electricity/potential determines the specific product synthesis during the BEF operation. The oxidation of electron donor (anode) (oxidation of acetate to HCO^{3-} vs. SHE (E^0) = −0.28 V) and reduction of electron acceptor (cathode) (reduction of O_2 to H_2O vs. $E^0 = 0.82$ V) with higher cathode electrode potential result in an overall positive potential of 1.10 V while producing power as product. But, the application of higher anode potential (acetate to HCO^{3-} vs. SHE (E^0) = −0.28 V) than cathode reduction potential (H^+/H_2 vs. SHE (E^0) = −0.41 V) results in an overall negative cell potential of −0.13 V, hence, an input of potential is essential. The presence of H_2O as electron donor (H_2O/O_2; $E^0 = 0.82$ V vs. SHE) requires higher energy inputs when compared to other forms of reactions. Redox shuttlers such as methyl viologen, neutral red, riboflavin, thionin, etc., can also enhance the electron transfer rates during the microbe–electrode interactions (Kotloski and Gralnick 2013). LaBelle and May (2017) reported maximum volumetric acetate generation of 19 g/L/day in a BEF from inorganic carbon using reticulated vitreous carbon foam cathode (unmodified).

Carboxylate platform is also a value addition in BEF, including short chain (C_2–C_5; volatile fatty acids) and medium chain (C_6–C_8) fatty acids. Volatile fatty acids produced from CO_2 as substrate with electricity/potential application on working electrode produced higher yields for acetic acid and propionic acid at −0.290 V vs. SHE (Lee et al. 2014; Marshall et al. 2012) and formic acid at −0.430 V vs. SHE (Srikanth et al. 2014). Medium chain fatty acids (MCFAs) such as butyrate, caproate, and minor portions of caprylate were formed in BES (Eq. (16.1)) on application of cathode potential of −0.9 V from acetate with in situ produced H_2 (Van Eerten-Jansen et al. 2013; Marshall et al. 2012; Steinbusch et al. 2011; Kucek et al. 2016; Grootscholten et al. 2013).

$$ {}_{n}CO_2 + (6_n + 2)\left(e^{-1} + H+\right) \rightarrow C_nH_{2n} + 2 + 2_nH_2O \tag{16.1} $$

These studies have shown the important application during the external stimulation in regulating the microbial metabolism for driving a bioprocess towards enhancing a definite product.

16.3 Microbiology of BEF Process and Organic Acid Production

The performance of BEF to form a specific product is highly influenced by the biocatalyst and the electron transfer mechanisms which need to be well understood. The anode respiring bacteria (ARB) or electrochemically active bacteria (EAB) present in the BEF can transfer electrons either by direct or indirect methods. *Shewanella* species can transfer electrons through conductive filaments or by mediated way of electron transfer, while, *Pseudomonas* sps. generate extracellular redox

shuttles (phenazines) and *G.sulfurreducens* requires nanowires (conductive pili) and c-type cytochrome OmcZ to encourage e^- transfer onto the terminal electron acceptor (electrode) (Summers et al. 2010). Early findings of methane generation from abiotic anode and biotic cathode adapted with pure strain *Methanobacterium palustre* by Cheng et al. (2009) laid the concept of microbial electrosynthesis. In another study, the electrons at cathode are utilized to convert inorganic carbon to acetate by the biofilms of *Sporomusa ovate* depicting electron consumption of 85% for product formation (Nevin et al. 2010). And with using mixed culture as source of inoculum obtained from brewery wastewater and CO_2 as carbon source, CH_4, acetate and H_2 were produced at working electrode (biocathode) poised with −590 mV (vs SHE) (Marshall et al. 2012). One of the value-added products generated from BEF is acetate.

Despite many research reports, little is known about the effect of electrodes on the structure and functions of the microbial community. The mechanism for the enhancement of exoelectrogenic microorganisms in the microbial communities with electrodes placement needs to be specifically focused to improvise the process efficiencies which can directly improvise productivity at industrial scale (Reguera et al. 2006; Gorby et al. 2006). To improve and enhance BEF technology, a systematic and thorough understanding of microbe–electrode interaction and potential extracellular electron transfer (EET) mechanisms in a bidirectional (away from and towards the electrode) way are crucial. Furthermost, the central research is focused more on anodic electron transfer and very less on cathodic reactions. The main hypothesized mechanisms are (1) direct EET through direct cells interaction that form a biofilms on the surface of electrodes that facilitate direct electron transfers (through mediated shuttle molecules or through nanowires) and (2) indirect EET in which the soluble redox molecules (or through artificial shuttle molecules) and extracellular polysaccharides that mediate reducing equivalents between microbe and electrodes. Some of the predicted redox proteins thought to be mediating the electron transfers are Cytochrome C (*Geobacter* sp., and *Shewanella* sp.), Ferredoxin (*Clostridium* spp., *Methanobacter* spp., etc.), Rubredoxin (e.g. *Desulfovibrio* sp.), and Hydrogenase and Formate dehydrogenase (*Desulfovibrio* sp., methanogens and methanotrophs). The understanding of mechanism aspects and microbial community structure on electrode influence helps in enhancing the specific products output during BEF, while mainly the redox potential and proteins involved in electron transports need to be well understood.

16.3.1 Mono-Culture vs. Mixed Culture Operations

Both the mixed and pure culture BEF operations have their own advantages and disadvantages based on the objective of the study. The current practices followed in research or in industries are gradually leaning towards microbiota driven fermentations for specific product and has better control on process control, prediction and microbial environment along with good growth/biomass for maximal product

Table 16.1 List of various microbes and substrates for the generation of value-added products

Product	Microorganism	Substrate	Concentration (g/L)/yield (%)	Reference
Succinate	*Actinobacillus succinogenes*	Corncob hydrolysate	3.84/26	Zhao et al. (2016)
	Actinobacillus succinogenes	Glucose	7.88/53	Zhao et al. (2016)
	Actinobacillus succinogenes	Xylose	5.24/35	Zhao et al. (2016)
	Actinobacillus succinogenes	Arabinose	4.7/31	Zhao et al. (2016)
Acetate	Mixed culture	CO_2	95 mg/d	Jiang et al. (2013a)
	Enriched electroactive culture biofilm (mixed)	CO_2	2.35 mM/d	Su et al. (2013)
	C. ljungdahlii DSM13528	CO_2	~105 μM	Nevin et al. (2011)
	Moorella thermoacetica	CO_2	~90 μM	Nevin et al. (2011)
Muconate	*C. glutamicum*	Catechol	85/100	Becker et al. (2018)
	E. coli sp.	Glucose	59/30	Frost et al. (2013)
	Pseudomonas DCB-71	Toluene	45/96	Chua and Hsieh (1990)
	Arthrobacter sp. *T8626*	Benzoate	44/96	Mizuno et al. (1988)

formation. The redox balance can be achieved by recovering all the electron equivalents in a single product via perfectly balanced reaction. Based on different factors, viz., carbon source, environmental conditions like pH, temperature, pressure, etc., a single microorganism can follow various metabolic pathways to generate diverse end products (Table 16.1). Such methods would assist for further enhancement and selectivity of target molecule/product and also aids in redirecting the pathway towards higher product formation. Hence, near-maximal yields and productivities are obtained thereby enhancing the economics and reducing the separation costs.

Mixed culture or co-culture may offer numerous other benefits like improving the overall process and its economics, symbiotic association for producing growth factors, provision of substrate, remove toxic substances or inhibitors present in the media, regulate the conditions required by other microorganisms for enhanced product formation. Some complex cascade metabolic pathways may not be happening in a single microorganism therefore requiring at least two or more different microorganisms for final molecule or product formation. The generation of new 1 deg or 2 deg intermediates can be activated in co-cultivation environments thereby presenting higher chances for generating new products. Due to their diverse nature, they can adapt easily, handle disparity in substrate composition, economic

procedures to preserve axenic environment, and evade contamination. Microorganisms have good ability to utilize mixed or unrefined or complex substrates. Apart from waste biomass, complex substrates present in various industrial wastes are being used by industrial biotechnology for generation of fuels or commodity chemicals. Hence, mixed culture consisting of group of microbes have broader collection of enzymes that targets a superior variety of substrates than a single strain operation. However, the practice of electrochemical technology can be an effective method in controlling the operation stability, broad the product spectrum, regulating metabolism of microorganisms for selective product formation, regulating target product generation, advancing diverse metabolic pathways, mainly when the substrate is not pure or sterile.

The product generation rates and efficiency are extremely dependent on the type of microbes, bioreactor size and design, electrode material type and size, type of operation, applied poised potential, etc. One approach to make BEF more economically viable is aiming product selection and divergence towards fuels or commodity chemicals with good worth. Few reports suggested the production of longer chain carboxylate synthesis in BEF by pure strains. But, in the case of mixed microbiota community, the interspecies communication such as redox species transfer between diverse species, dual usage of redox shuttles, etc. along with microbe–electrode interaction plays a significant role in enhancing the efficiency of BEF process. On the other hand, mixed culture operations have some specific disadvantages, e.g. maintaining optimum conditions to balance the growth of all active organisms to develop synergistic interactions, competition for similar nutrients and organic carbon, and any metabolic toxicity. Through facilitated electron transfer, i.e., adding artificial electron transfer or shuttle molecules (e.g. iron), the specific reaction rate could be improved during the BEF process and recover the desired end products.

16.3.2 Organic Acid Pathways and Requirements

In the process of BEF, the organic molecules or substrates act as e^- acceptor or donor based on the reaction specificity. Due to anaerobic conditions prevailing in the electrofermentor, ATP is maintained by the process of glycolysis. The precursor molecule, pyruvate, is metabolized into various value chemicals such as carboxylic acids, alcohols, organic acids, polyhydroxyalkanoates (PHAs), polyols, etc. by the mechanism of microbiota. Irrespective of pathway that takes care of carbon metabolism, the poised potential also affects the upsurge or diminution of intracellular redox factors such as $NADH^+$ or $NADPH^+$. The $NADH^+$ from glycolysis process cannot return to NAD^+ by releasing e^-. Hence, to regenerate the NAD^+ from the $NADH^+$, the electrons are dropped with an organic compound which allows glycolysis process to keep running by confirming a stable source of NAD^+. Various organic acids, viz., acetic acid, muconic acid, butyric acid, itaconic acid, pyruvic acid, etc. can be generated by the process of electro-fermentation when a small amount of potential is poised.

16.3.2.1 Acetic Acid

In BEF, acetate is one of the mostly generated and utilized compounds from substrates as intermediate molecule by the process of hydrolysis/substrate utilization and for generation of a wide range of compounds, respectively. Electro-fermentation of acetate takes place from glucose and CO_2. The glycolysis pathway aids in generation of pyruvate which in turn produces acetate. Acetate is produced by a group of bacteria called as acetogens, which are facultative autotrophs that utilize hydrogen (H_2) or carbon monoxide (CO) and reduce CO_2. Other than CO_2, other substrates such as alcohols, pentoses, methyl compounds, hexoses, formic acid, etc. are utilized for acetate production. The genera sporomusa and acetobacterium contain only acetogens while clostridium genera contain both acetogenic and non-acetogenic species. The mostly studied acetogens are *Clostridium ljungdahlii, Acetobacterium woodii,* and *Moorella thermoacetica*. These bacteria are chosen because of their metabolic flexibility for biocathode driven CO_2 fixation in BEF. Another approach of fixing CO_2 in multicarbon compounds such as chemicals and other liquid fuels by electricity driven reduction reactions has gained popularity. The generation of acetate by using CO_2 has showed considerable interest as the acetate is considered as the intermediary compound (Nevin et al. 2015). The process of electroacetogenesis was carried out by using *Sporomusa ovate* (pure culture) at a potential of -0.4 V on working electrode. Similar results were reported by using mixed culture when the poised potential of <-0.59 V vs NHE was applied on the cathode (Nevin et al. 2010). The recovery of electrons in the generated products was $>85\%$ of the e^- transferred at the working electrode. Consequent reports illustrated the process of CO_2 reduction using BES process by a broader range of microbes (Nevin et al. 2011). Similarly, Jiang et al. (2013b) reported maximum amount of CH_4 and acetate generation using inorganic carbon and mixed culture at biocathode. Usually, the acetogenic bacteria reduce the inorganic carbon by consuming H_2 as an electron donor. Irrespective of this, it was prominent that certain acetogenic bacterial groups such as *C. ljungdahlii, S. sphaeroides, C. aceticum, and M. thermoacetica,* etc. makes use of poised potential to generate organic acids like formate, acetate, etc. (Nevin et al. 2011). Similarly, acetate production was demonstrated by using mixed culture and cathode potential of -0.6 V in a continuous process from CO_2 (Batlle-Vilanova et al. 2016). The lower potential requirement for conversion of inorganic carbon to acetate is observed in microbial electrosynthesis (MES) but, thermodynamically, the potential for transformation requires -280 mV vs SHE. The probable reasons for requirement of lower potential in BEF are lower resistances (mass and charge transfer), involvement of redox mediators for efficient and effective electron transfer, and other design parameters that influence the process performance (Desloover et al. 2012). Unlike other photo-electrocatalysis processes, MES requires less energy input for crossing the energy barrier and form bio-products by biocatalyst. The technology of MES is still in its infancy, as only few studies successfully reported the generation of valuable products by utilizing CO_2 as a substrate. Nevin et al. (2010) first demonstrated the electron uptaking capability of biocatalyst and

generate product from CO_2, leading to new applications of MES. Mohanakrishna et al. (2015) showed production of acetate (4 g/l) by using MES from bicarbonate as substrate and as extension to this study, acetate (~1.5 g/l) was produced form direct CO_2 (Patil et al. 2015).

16.3.2.2 Succinic Acid

Apart from being end product of anaerobic fermentation, Succinate is also termed as essential intermediate molecule for cellular metabolism in the biological systems. Succinate is also being listed as one of the organic molecules that are generated from biomass among the top 12 value-added molecules listed by the US Department of Energy. The generation of succinate by biological process by integrating with electro-fermentation has gained much attraction. The biological routes for succinate production by the process of BEF have been documented in Cao et al. (2013). Park and Zeikus (1999) reported the production of succinate by the flow of e^- from cathode in a mediated fermentation of *Actinobacillus succinogenes*. The increase in ATP synthesis by enhanced e^- transfer and induced H^+ translocation via ATPase complex illustrated the cathodic EET for reduction reactions intracellularly. Instantaneously, the driving factor for ATP synthesis is the H^+ consumption leading to H^+ gradient. The glucose is transformed into phosphoenolpyruvate (PEP) and lastly to pyruvate by glycolysis process in both aerobic and anaerobic environments. In anaerobic conditions, the PEP acts as a substrate for the carboxylase-catalyzed anaplerotic reaction and gets converted to oxaloacetate by the help of enzymes PEP carboxylase (*ppc*) or PEP carboxykinase (*pck*). The end product of glycolysis step, pyruvate, can also be converted into oxaloacetate or malate by incorporating with CO_2 and pyruvate carboxylase (*pyc*) or malic (*mae*) enzymes, respectively. The oxaloacetate formed from PEP or pyruvate, with the help of malate dehydrogenase (*mdh*) is converted to malate. The malate formed can be transformed to fumarate and further to succinate by the help of fumarase (*fum*) and by fumarate reductase (*frd*), respectively. Figure 16.2 provides an outline of the succinate biosynthetic pathway rom glucose under anaerobic fermentation conditions in BEF.

16.3.2.3 Muconic Acid

Muconic acid also termed as 2,4-hexadienedioic acid is a high value-added product chiefly used as a dominant and central precursor molecule for the manufacturing of polyesters and polyamides. The muconic acid is used as starting material for synthesizing various compounds that have high value, bulk material for polymer reactions of muconic homo and copolymers, and production of its derivatives like terephthalic acid, caprolactam, adipic acid, etc. Muconic acid is present in three isomeric states, i.e., *cis*,*cis*-muconic acid (ccMA), *cis*,*trans*-muconic acid (ctMA), and *trans*,*trans*-muconic acid (ttMA) which vary by its physical and chemical properties along with their applications. Biotechnological routes of producing

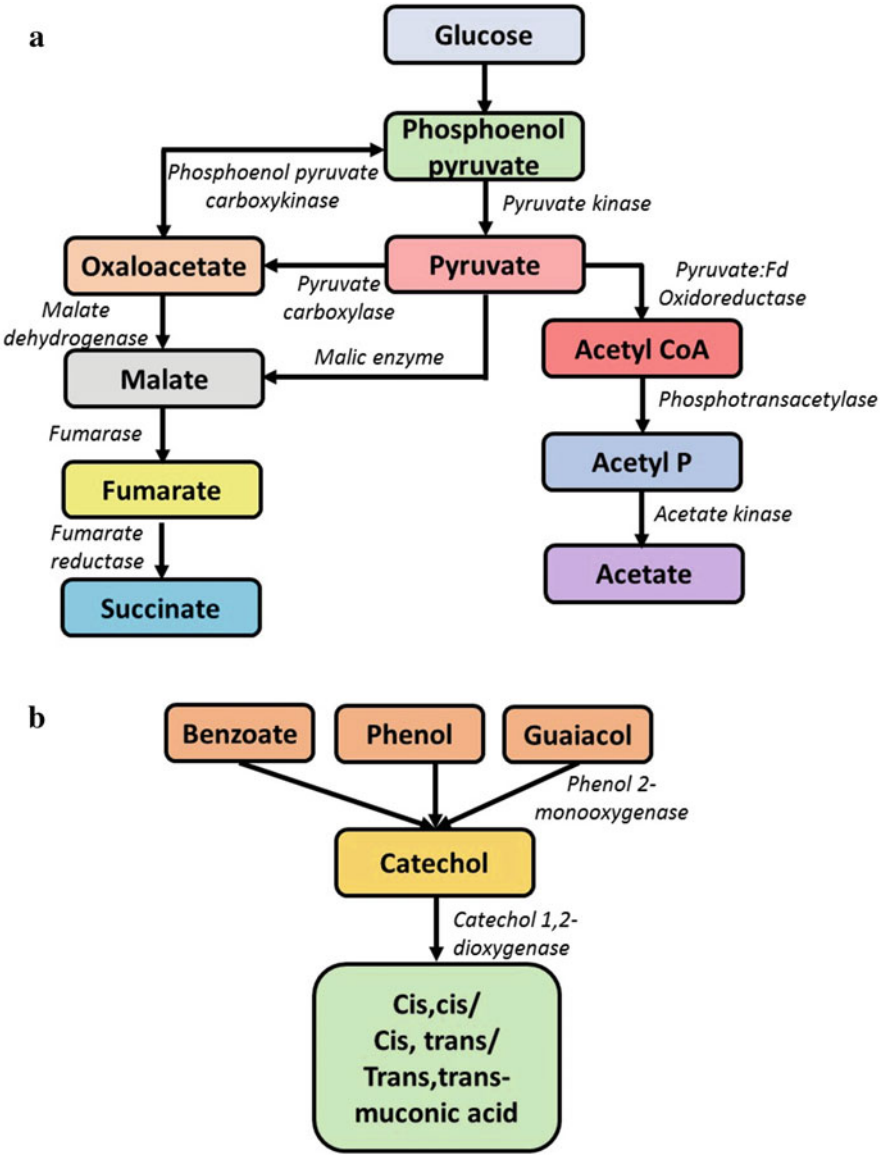

Fig. 16.2 (**a**) Metabolic core pathways involved for production of acetate and succinate (**b**) metabolic pathway for production of muconic acid from different aromatic compounds

muconic acid are developed by catechol ortho-cleavage. In this, the aromatic compounds of petroleum base, viz., phenol, toluene, guaiacol, benzene, benzoate, etc. were oxidized by specific microorganism to produce muconic acid. The biomass feedstocks will obtain catechol, phenol, and other aromatic compounds by the conversion technologies. The phenol 2-monooxygenase will assist in cleavage of

petroleum based aromatic compounds to catechol, which is an intermediary molecule for its conversion to muconic acid. The catechol 1,2-dioxygenase will aid in ortho-cleavage of catechol for producing muconic acid. The maximum muconic acid production was obtained by using microorganisms under fed-batch operation. The productivity of muconic acid is enhanced during the fed-batch mode of operation as the fed-batch regulates the substrates concentration less than the inhibitory levels thereby reducing the substrate toxicity on the microorganism during the operation process. The metabolically engineered the soil bacterium *Corynebacterium glutamicum* to produce lysine, aminovalerate, ectoine, and diaminopentane using diverse substrates. Remarkably, *C. glutamicum* degrades a wide range of aromatic compounds through the catechol division of the β-ketoadipate pathway, concerning muconic acid as a transitional molecule in the pathway. The β-ketoadipate pathway is the foremost pathway for aromatic compounds that are derived from lignin thereby illustrating the significance of engineered strains (*C. glutamicum)* to form muconic acid. Becker and Wittmann (2012) reported the ability of engineered *C. glutamicum* strain for the production of muconic acid by using catechol and glucose. The study yielded 85 g/L *cis*,*cis* muconate which is highest reported titer till date. The biotechnological process showed higher efficiency and selectivity of muconic acid production (100%) when compared to chemical process (35%).

16.4 Techno-Economics of BEF

The techno-economic calculation of the process mainly includes capital cost (or CAPEX) and operational cost (OPEX). The CAPEX are one-time investments with additional minimum investments over the longer operations in modifying the process flow or repairing the existing facilities. In specific, the CAPEX costs include the land cost of establishing the operation facility that is calculated based on the amount of biomass being treated, establishing basic infrastructures like buildings, energy supply, fermentation systems, electrodes and membranes, acid recovery units, and larger storage facilities, etc. On the other hand the OPEX are the costs that mainly includes the cost of day-to-day operation of the facility, manpower costs, substrate and pre-treatment costs, energy costs, and any additional chemical costs that fluctuates based on the location, size, and operational designs. A typical process flow of converting organic biomass into organic acid is presented in Fig. 16.3. The classical fermentation will also have the same process flow, while the CAPEX cost comparatively lower (~300$/t) than BES system was reported (Christodoulou and Velasquez-Orta 2016). This is mainly due to the additional costs for electrodes and membranes. The existing electrodes and their costs are very expensive, while they are less durable. However, the production costs or the final product costs are lower for BES compared to conventional fermentation due to high product titer in given time.

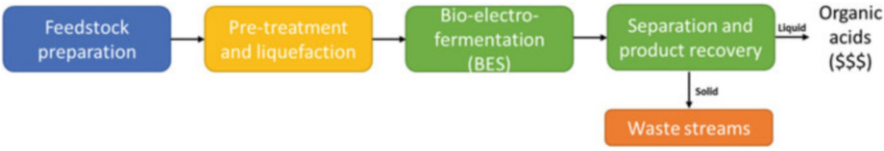

Fig. 16.3 Process flow of converting biomass into organic acid

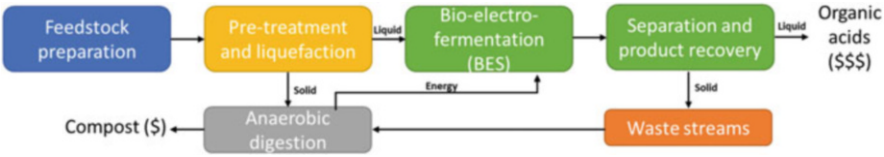

Fig. 16.4 Integrated process development for organic acid production from biomass

16.5 Integrated Process Development

BES system has a drawback associated with its CAPEX costs and operation difficulties in treating large volume of biomass. Therefore establishing the integrated approach (Fig. 16.4) of pre-processing of complex organic biomass is proposed. In this integrated process, the organic biomass is fraction separated and utilize only the soluble fractions (of organic carbons) for BES fermentation, while the solid residues are anaerobically digested to produce energy. The bio-energy from anaerobic digestion process could be used for BES operation and organic acid recovery. The CAPEX cost will be increased with the integrated process development, i.e., due to additional process flow, however, it helps to reduce the OPEX through additional revenue generated. However, the process integration required prior research and proper Life Cycle Assessment (LCA) and Techno-economic analysis (TEA) analysis. Also, it should be considered the type of biomass used for the process, optimized pre-treatment methods, and identified low-cost end-product recovery technologies.

16.6 Knowledge Gaps and Future Research Directions

Based on the detailed review and understanding of the process flow in treating biomass to produce organic acids through BES systems, the following key points need to be studied and well understood to make it competitively viable for commercialization.

a. Cheap electrodes: the cost of electrodes is high and that need to be reduced. Low cost electrodes that are highly conductive of electrons and support biofilm growth are need to be developed and it required extensive research.

b. Integrated process: establish the best integration approach for treating various types of organic biomass and optimize the conditions to reduce the OPEX and final product costs.
c. Microbes: Selection of microbes and the genetic modification to improve its capacity for high product titer is another key area for process improvements. In addition, better understanding of electron transport/shuttle molecules are required more attention.
d. Product recovery and purification: Recovery and purification of organic acids from the fermentation broth is another key area that required better process development to reduce the CAPEX and OPEX costs.

Competing Interests All the authors declare that they have no competing interests.

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Chapter 17
Bioenergy-Byproducts Based Electrodes for Flexible Supercapacitors

Neelam Bora, Rumi Narzari, Nilutpal Bhuyan, and Rupam Kataki

Abstract Among the various electrochemical energy storage devices, supercapacitor is a significant one due to its high power density, excellent charge/discharge mechanism, and longer lifespan. Supercapacitors have the application in various fields including digital communication devices, electric vehicles, and portable devices. In the recent times, flexible supercapacitors have acquired greater attention due to the higher demands for flexible, wearable, and portable electronic devices. Continuous investigations have been carried out in the past several years on the electrode materials of flexible supercapacitors to achieve excellent electrochemical performance. Among the different electrode materials which have been studied till now, biomass-derived carbon showed potentiality owing to its porous structure, large specific surface area, good chemical stability and electrical properties, low cost, and easy processing. In this chapter, various biomass and biowastes from which carbon materials can be derived were discussed along with the energy storage principle and electrochemical performances of flexible supercapacitor electrodes prepared from these biomass-derived carbons of different precursors.

Keywords Supercapacitors · Biomass · Carbon · Flexible · EDLC · Activated carbon · Pseudocapacitor

Abbreviations

EDLC	Electric double layer capacitor
PC	Pseudocapacitor
CNT	Carbon nanotube
ESR	Equivalent series resistance
ACs	Activated carbons
EIS	Electrochemical impedance spectroscopy
CV	Cyclic voltammetry

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GCD	Galvanostatic charge-discharge
MTBC	Micro-tube bundle carbon
PVA	Polyvinyl alcohol
PVDF	Polyvinylidene fluoride
PVDF-HFP	Polyvinylidene fluoride-co-hexafluoropropylene
SPE	Solid polymer electrolyte
GPE	Gel polymer electrolyte
PAA	Poly polyacrylate
PEO	Polyethylene oxide
PAN	Polyacrylonitrile

17.1 Introduction

Supercapacitor is an energy storage device which possesses benefits of excellent power density, good charging mechanism as well as longer lifespan. Supercapacitors are also known as electrochemical capacitor or ultracapacitor, which has acquired greater attention in the recent times as a supplier of energy in digital communication devices (such as computers, mobile phones, etc.), memory backup systems, electric vehicles, and various portable devices (Zhang et al. 2009; Zhang et al. 2009). Apart from the efficient charge/discharge mechanism, properties, viz. power density and lifespan are another reason for the growing popularity of supercapacitors over traditional energy storage devices. Based on the charge storing characteristic, supercapacitors are divided into (1) Electric Double Layer Capacitor (EDLC) and (2) Pseudocapacitor (PC) (Khanna 2019). In EDLC, the obtained capacitance is completely dependent on the pure electrostatic charge gathered at electrode/electrolyte interface. The capability of electrolyte ions to enter the electrode materials is the major influencing factor in this process (Palchoudhury et al. 2019). The electrode surface area signifies the total area of the porous reactive surface, which takes part in the adsorption-desorption of electrolyte ions. Double layer effect of EDLC comes under non-Faradaic process, where the processes such as electrolyte ions movement, reorientation of dipoles of electrolyte, and ionic sorption take place at electrode/electrolyte interface. Electric double layer formation process is depicted in Fig. 17.1. Pseudocapacitor is another type of supercapacitor, in which electro-active species are available, that initiates the fast and reversible Faradic reactions. In Faradaic process, movement of charge takes place at electrode/electrolyte interface because of oxidation/reduction reaction (Zhang et al. 2009). High capacitance of a supercapacitor can be obtained by inducing both the effects of double layer and pseudocapacitance. It can be achieved by using metal oxide, conducting polymer or metal-doped carbon materials, etc. in one of the porous carbon electrodes of EDLC. This mechanism results in two simultaneous processes: (1) Double layer formation at the carbon electrode, (2) Faradaic and surface reactions at the metal oxide electrode (Khanna 2019).

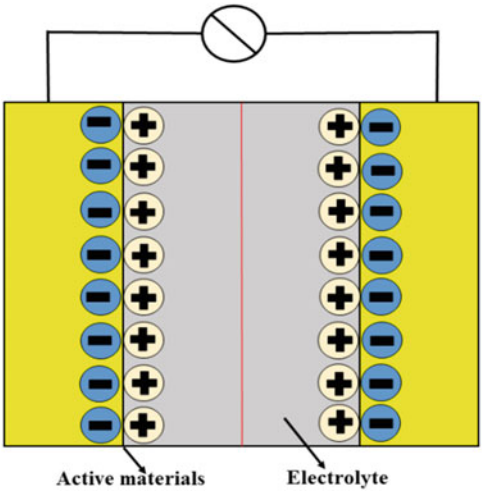

Fig. 17.1 Electric double layer formation in EDLC

17.1.1 Significance of Flexible Supercapacitor

In a race to produce electronics which are cheap, portable, environmental friendly, thin, light and offer high performance, a global diversion of research to develop such device can be observed. These would be helpful in medical purposes, military, and civilian purposes such as health tracking tools, mobile phones, computers, televisions, etc. (Kaempgen et al. 2009). Usually the conventional supercapacitors comprise components, viz. outer case, current collectors which are basically metal based, two electrodes, electrolyte, and separator which works on ion movement. But in case of the flexible supercapacitor, the structural architecture change takes place to make it a light weight and portable device. The two electrodes and current collector in flexible supercapacitor are replaced by flexible carbon network having high conductivity. Another important difference between the conventional and flexible supercapacitors is that the components comprising the flexible one such as electrodes and packing shell, etc. are flexible in nature (Shi et al. 2013; Liu et al. 2017). The utilization of metallic current collectors results in the increased mass of conventional supercapacitors and also makes the devices bulky, restricting the use in small space along with low weight. Due to these, conventional supercapacitors are unable to fulfil the demand of flexible devices with simplified designs. Figure 17.2 depicts the structure of a flexible supercapacitor. Similar to conventional supercapacitors flexible supercapacitors can also be categorized into two types: (1) EDLCs and (2) PCs (Niu et al. 2013).

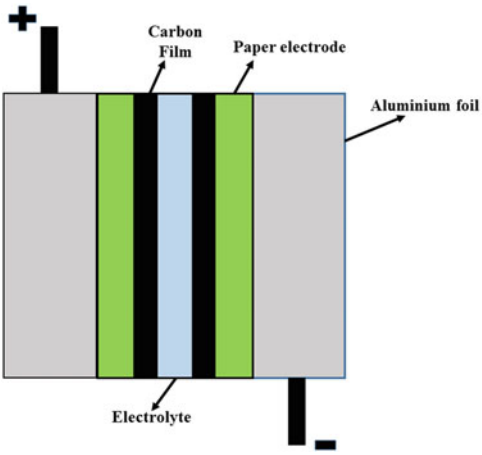

Fig. 17.2 Structure of flexible supercapacitor

17.1.2 Types of Electrode Materials

Electrode materials highly influence the performance and cost of supercapacitors and the other energy storage devices. The electrodes of supercapacitors are fabricated using mainly two kinds of materials. The first one is the pseudocapacitor material, viz. metal oxides and conducting polymers. Low electrical conductivity as well as poor capability rate are major drawbacks of the mentioned pseudocapacitive materials. The second type is the carbon-rich material such as activated carbons, carbon nanofibres, graphene sheets, etc. (Ma et al. 2017). Carbon materials employ the electrochemical double layer formation at electrode/electrolyte interface, for storing energy. Therefore, reactive surface area and other functionalities, pore distribution, electrochemical stability, conductivity, etc. highly influence the capacitance of carbon (Ganesan et al. 2014).

17.1.2.1 Carbon Nanotube (CNT)

Carbon nanotubes have acquired greater significance as an electrode material of supercapacitor because of distinctive pore distribution, excellent thermal as well as mechanical stability and excellent electrical characteristics (Cheng et al. 2011). CNTs can be prepared by carrying out catalytic decomposition of few hydrocarbons under controlled conditions, those results in nanostructures of different conformations of controlled crystalline structure. CNTs comprise inter-connected mesopores, which results in continuous charge distribution in every part of reactive surface area. Because of the diffusion of electrolyte ions into mesoporous network, CNTs possess lower Equivalent Series Resistance (ESR) in comparison to activated carbon (Tamilarasan et al. 2011). Carbon nanotubes are chiefly of two categories, viz. single-walled and multi-walled. The drawback of CNTs in comparison to activated carbons is that CNTs possess small specific surface area which results in low energy

density. In case of high power electrode materials, CNTs are the most suitable as they possess high electrical conductance couple with high surface area. In addition, higher mechanical resilience as well as open tubular network of CNTs offers better support to active materials. Specific capacitance of CNTs can be enhanced by chemically activating it with potassium hydroxide, thereby increasing the surface area and maintaining its nanotubular morphology at the same time (Halper and Ellenbogen 2006; Pandolfo and Hollenkamp 2006).

17.1.2.2 Graphene

Besides carbon nanotubes, graphene is of another kind of electrode material for supercapacitor, which is independent of pore distribution at solid state. In a study, among various carbon products used for electrodes, graphene possesses the higher surface area of 2630 m^2/g and capacitance of about 550 F/g (Liu et al. 2010; Moon et al. 2010). Graphene sheet is advantageous because both the major surfaces are outward, which can easily connect with the electrolyte. Various types of methods available for graphene preparation from graphite are chemical vapour deposition, micromechanical exfoliation, arc-discharge method, etc. (Ramachandran et al. 2013; Kuilla et al. 2010). The single layered graphene oxide sheet prepared using gas-based hydrazine when used in supercapacitor electrode showed capacitance of 205 F/g. This obtained capacitance was higher than the carbon-based supercapacitors electrode (Pope et al. 2013). To find the proper preparation method of graphene to be applied in supercapacitor, various methods were investigated. Graphene prepared from graphitic oxide using thermal exfoliation resulted in capacitance of 117 F/g when used for supercapacitor electrode (Kannappan et al. 2018). Chemical exfoliation is another method of preparation of graphene performed at a high temperature. When it was performed for graphene at low temperature, graphene possesses better electrochemical performance in comparison to the high temperature exfoliation (Lv et al. 2009). Highly corrugated graphene sheets can be prepared at high temperature by applying thermal reduction process in graphite oxide. The highly corrugated graphene sheet was used in supercapacitor electrode and achieved capacitance of 349 F/g (Yan et al. 2012).

17.1.2.3 Activated Carbon

Among the mentioned source of carbon electrode materials, activated carbons (ACs) acquire greater attention because of high porosity, greater surface area, good chemical stability as well as good electrical properties (Syarif et al. 2012). ACs are usually fabricated using fossil fuel-based materials, viz. coal, petroleum coke, asphalt, etc. by applying activation methods. In physical activation, carbon precursors are treated with oxidizing gases, viz. steam, carbon dioxide, etc. or using their proper combination between 700 and 1200 °C, whereas the other one, i.e. chemical activation is performed at temperature in the range 400–700 °C applying chemical activating

agents, viz. boric acid, phosphoric acid, potassium hydroxide, zinc chloride, etc. (Pandolfo and Hollenkamp 2006). Activated carbons show dissimilar pore distribution which comprises micropores (<2 nm), mesopores (2–50 nm), and macropores (>50 nm) (Iro et al. 2016; Tseng 2006). Along with the surface area, other aspects such as proportion of activating agent, electrolytes also influence the electrochemical performance of carbon materials. During chemical activation, optimum amount of chemical activating agent should be taken; otherwise excessive activation may cause large pore volume and in turn reduction in conductivity will lower energy and power density. Different electrolytes have altered effect on the capacitance of activated carbon, show greater capacitance in aqueous electrolytes in comparison to organic electrolytes (Iro et al. 2016).

17.1.3 Importance of Biomass-Derived Flexible Supercapacitor

The increase in demands for energy has caused rapid decline in fossil-based energy resources and the consequent exponential increase in fossil fuel consumption has led to global environmental damages. Due to these reasons, the world community is moving towards alternative energy source which are renewable, efficient, and cost-effective with lesser emissions. Out of the various abundant renewable sources of energy, biomass is one of them (Van Aardenne et al. 2001; Searle and Malins 2015). Considering the mentioned challenge along with the other aspects, viz. material cost, and various energy and environmental concerns, the application of biomass and biowaste for the preparation of activated carbons with good pore distribution, high electrical conductivity and pore density is receiving wide attention in recent years. Low-cost biomass, and agro-industrial waste, could easily overcome various problems associated with the fossil-based energy resources (Wei-Jiang et al. 2011). Biomass-derived carbon is a kind of human-made carbon material which is different from the fossil-based carbons like charcoal, graphite, petroleum coke, etc. Biomass-derived carbons can be prepared using various available biomasses, viz. microorganisms, plants, biowaste, and animal defecate into products through the application of methods like thermal carbonization followed by activation. In thermal carbonization, biomass is heated at high temperature in an inert atmosphere, where the elements which contain in biomasses escape leaving the porous carbon. After the activation, the carbon atoms produce conductive interconnected layered structures having large surface area, high pore density, which make them excellent electrode materials in energy storing devices. Similar to other electrode materials, the activated carbon produced from biomass is also capable of storing charges following the principle of electrical double layer (Subramanian et al. 2007; Liu et al. 2018).

This chapter describes the potential of biomass-derived carbon for electrode of flexible supercapacitor which follows the principle of electrical double layer. Various biomass feedstock which were already been utilized in production of carbon for

electrodes of flexible supercapacitor are discussed in this chapter. Further various electrochemical methods like electrochemical impedance spectroscopy (EIS), cyclic voltammetry (CV), and galvanostatic charge-discharge (GCD) techniques are discussed which have been mostly used for the evaluation of the performance of flexible supercapacitor. Electrolytes used in flexible supercapacitor also highly influence the capacitance of the supercapacitor. Hence, various electrolytes used in flexible supercapacitor are also explained in this chapter.

17.2 Biomass-Derived Carbon-Based Flexible Supercapacitor Electrode

17.2.1 Principles of the Precursor Selection for Biomass-Based Electrode Material

Biomass is a highly abundant renewable energy sources in the world. Any organic matter that originates from various sources like plants and animals is considered as biomass. Carbon materials can be prepared from various biomass precursors using carbonization and a subsequent activation process (Jahirul et al. 2012). The porous carbon structure can be formed by applying different thermal conversion methods to biomass precursors. Moreover, subsequent activation of the obtained carbon results in highly interconnected three-dimensional structures having good conductivity as well as higher porosity. This makes the biomass-based carbon suitable for many energy storage applications, especially in supercapacitors, but requires more research to investigate the optimum carbon structure and its surface properties. Among all the electrode materials available, biomass-derived carbon acquires attention because of lesser ash content, cheaper price, easy preparation methods, stability, mechanical properties, etc. (Liu et al. 2019). Biomass-derived carbons follow the mechanism of electrical double layer for storing charge in flexible supercapacitor which is based on the principle of EDLCs. The overall process of preparing flexible supercapacitor from biomass carbon is depicted in Fig. 17.3. In order to fabricate a perfect supercapacitor electrode, biomass-derived carbon should possess (1) large surface area and suitable pore division that results into large specific capacitance, which ensures the high energy and power densities, (2) small ESR for minimal voltage drop, and (3) higher stability for long lifespan of the supercapacitor. The electrochemical performance of various biomass-based carbons is dependent on capacitance, morphology and preparation method of the carbon material. Therefore, the selection of a suitable biomass for the production of carbon has greater significance. The selection is mainly dependent on the cost, intrinsic microstructure, and elemental composition of biomass feedstock (Madhu et al. 2014). In the preparation of biomass-derived carbons with properties such as good conductivity and high porosity, etc. along with a higher yield of carbon for flexible supercapacitors, below mentioned conditions should be considered:

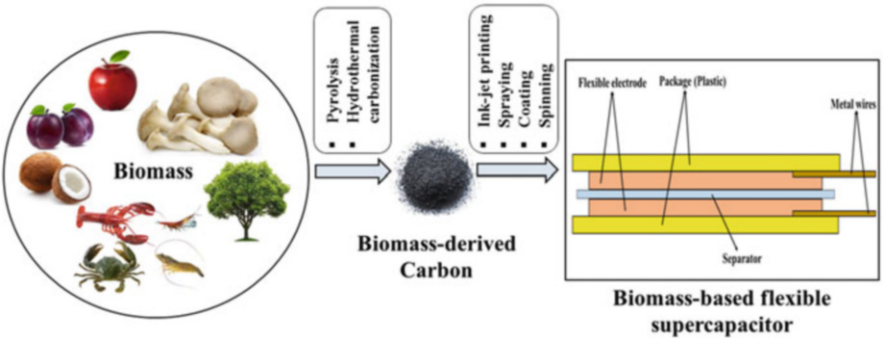

Fig. 17.3 Graphical representation of flexible supercapacitor fabrication from biomass

1. Biomass with higher thermally stable biomacromolecules having highly cross-linked structure and high molecular weight (e.g. lignin, chitin, and keratin) is favourable in maximum carbon production as well as formation of aromatic carbon.
2. Biomass with lower amount of non-cross-linked structure having small molecular weight, and aliphatic compounds
3. Biomass should have low oxygen and high nitrogen contents to produce fewer defects, high crystallinity, and formation of aromatic carbon and in situ creation of N-doped carbon having high conductivity (Liu et al. 2018).

17.2.2 Precursors for Biomass-Derived Carbon

In the present scenario, researchers are showing importance towards the application of activated carbon derived from biomass and biowaste, in the production of electrodes of high capacity flexible supercapacitors. The higher demand of such biomass-derived carbon is because of large surface area, good electrical conductivity, high availability of raw materials, non-toxicity, high chemical stability, etc. These properties of the biomass-derived activated carbons effectively promote higher charge accumulation at electrode/electrolyte interface, which promotes higher device capacitance (Nataliya et al. 2013). Various biomass and biowaste materials such as poplar catkin, lignin, corncob residue, sugarcane bagasse, rice husk, bacterial cellulose, rice straw, egg white, banana fibres, orange peels, tea waste, chitin, mushrooms, human hair, etc. were the feedstocks utilized for the production of biomass-based carbon (Liu et al. 2018) for electrode in various energy storage devices. These biomasses and biowaste materials can be classified into four major classes, viz. microorganism, fruit, plant, and animal-based biomaterials. Different types of electrodes are being utilized as energy storage devices already. Out of these, most commonly manufactured device is the electrical double layer capacitor where

carbon materials are used (Liu et al. 2018; Ma et al. 2017; Thambidurai et al. 2014; Khu et al. 2016).

Attention has been paid for searching unique biomass feedstock, which can deliver higher carbon contents consisting of nanoporous structure. The nanoporous structure and heteroatom compositions in the biomass materials are exceptionally advantageous for the development of interconnected micro and mesoporous structure, and nitrogen and oxygen comprising functional groups in thermal conversion and activation process. These factors greatly influence the properties of the materials to be used in the electrodes in flexible supercapacitor that based on EDLC-supercapacitor. Biomass and biowaste materials of different types which were used for the derivation of activated carbons for supercapacitor application are discussed in details (Li et al. 2012). Performance of carbon as supercapacitor material derived from various types of biomass is shown in Table 17.3.

17.2.2.1 Plant-Based Biomass

The yield of carbon from different plant biomass during thermal carbonization process is highly influenced by their chemical compositions, viz. lignin, cellulose, and hemicellulose contents; even though carbon fractions are relatively high (40–60%) in the plant biomass. Out of these components, lignin has the maximum thermal stability and has the highest contribution in the yield of the chars and activated carbon. The cellulose and hemicellulose contents have less influence in the yield of chars as well as carbon from biomass, in comparison to lignin. Based on these, plants with high lignin, low cellulose content, low oxygen and high nitrogen should be selected for higher yield of carbon having properties such as graphitic structure, less defects as well as excellent conductivity, as required by supercapacitive materials (Rutherford et al. 2005; Cagnon et al. 2009).

The elemental compositions, i.e. the amount of oxygen, and nitrogen of the plant biomass also highly influence the yield, pore distribution, conductivity as well as capacitance of biomass-derived carbon materials. Higher oxygen(O) containing plant biomass results in low yield of carbon along with more defects and less crystallinity, whereas the plants with high nitrogen (N) content can develop naturally N-doped carbon having excellent electrochemical characteristics (McDonald-Wharry et al. 2015; Hao et al. 2018). Table 17.1 depicts the percentage of elements contain by various plant biomass.

Zequine et al. (2017) manufactured a flexible supercapacitor from carbon material derived from jute using hydrothermal method. Electrochemical performance of the obtained char was improved after chemical activation. In GCD measurement, capacitance of 185 F/g was obtained at 500 mA/g, whereas in cyclic voltammetry test performed at 1 mV/s, 408 F/g of specific capacitance was obtained. The carbon material showed an outstanding cyclic stability over 5000 cycles. Activated carbon obtained from bamboo fibres was utilized in flexible supercapacitor and electrochemical properties were studied by Zequine et al. (2016). In GCD measurement, a capacitance of 510 F/g (at 0.4 A/g) was obtained. The carbon material showed an

Table 17.1 Various plant-based biomass and their elemental compositions (dry wt. % basis)

Biomass	C (%)	H (%)	O (%)	N (%)	S (%)	References
Bamboo fibres	47.66	6.18	46.01	0.15	–	Kalyani and Anitha (2013)
Tea waste	67.92	3.17	–	6.79	0.37	Arsene et al. (2013)
Argan nut shell	51.33	6.32	42.34	0.005	–	Miskam et al. (2009)
Banana steam	36.83	5.19	43.62	0.93	–	Rahib et al. (2019)
Corncob	65.30	4.30	29.35	1.06	–	Yang and Zhang (2018)
Rice husk	38.19	5.30	56.23	0.28	–	Kalyani and Anitha (2013)
Sugarcane bagasse	48.60	6.30	45.10	–	–	Rahib et al. (2019)
Sawdust	42.38	5.27	42.41	0.14	–	Hassan et al. (2014)
Coconut coir	46.22	5.44	40.47	0.36	–	Rahib et al. (2019)
Rubber seed shell	48.80	5.90	43.70	1.50	0.10	Latshaw and Miller (1924)
Saline corn stem	44.51	5.90	43.90	0.84	0.16	Abou Raya et al. (2014)

excellent cyclic stability and retaining its charge storage capacity over 5000 cycles. Elmouwahidi et al. (2012) prepared carbon material using argan seed shells which was further chemically activated using KOH. The prepared carbon material possessed surface area of 2100 m^2/g. In this study, the nitrogen (N) rich activated carbon offered capacitance of 355 F/g and retained 93% capacitance at 1 A/g because of the improved porosity as well as pseudocapacitance effects of *N* functionalities of the activated carbon.

In a study, corncob derived activated carbon exhibited surface area of 1471 m^2/g. The carbon materials displayed good electrochemical performance with capacitance of 293 F/g (Yang and Zhang 2018). The nanoporous carbon from waste rice husk which was chemically activated using KOH, possessed surface area, and capacitance of 2523.4 m^2/g and 250 F/g, respectively, was obtained for waste rice husk-derived carbon. The fabricated carbon electrode displayed excellent cycle life with stable capacitance for 10,000 cycles (Xu et al. 2014). Micro-tube bundle carbon (MTBC) was derived from paulownia sawdust and was chemically activated using NaOH. The prepared carbon showed surface area of 1900 m^2/g comprising abundant micropores and mesopores. In cyclic voltammetry, MTBC electrode displayed a higher capacitance of 227F/g at 2 mV/s (Liu et al. 2013). Senthilkumar and Selvan (2015) manufactured a flexible fibre supercapacitor from carbon prepared using *Ficus religiosa* leaves as electrode material, whereas polyvinyl alcohol-H_3PO_4 was used as gel polymer electrolyte. The fabricated flexible supercapacitor provided gravimetric capacitance of 3.4 F/g. Even after 4000 cycles this flexible supercapacitor retained 88% of its original capacitance. Yu et al. (2017) fabricated a wrinkled carbon membrane electrode material from cherry blossom flower petal for flexible supercapacitors application. In the preparation of the carbon material, pyrolysis followed by chemical activation methods was used. The carbon membrane showed capacitance of 332.7 F/g as well as retained 92.3% capacitance over 10,000 cycles. Using that carbon membrane in the electrode, a flexible supercapacitor was

fabricated, which displayed capacitance of 154 F/g and exceptional bending stability.

Supercapacitor was manufactured from all-biomaterial originated film for the first time using three-dimensional bacterial cellulose-based electrode. The bacterial cellulose electrode depicted maximum capacitance of 241.8 F/g. The supercapacitor prepared using the electrode depicted an outstanding capacitance of 289 mF/cm^2 and retained 66.7% over 100 cycles (Wang et al. 2016).

17.2.2.2 Fruit-Based Biomass

Fruits are composed of various chemical components such as carbohydrates, crude proteins, crude fibres, ash, lipids, etc. In comparison to plant biomass, fruit biomass has higher content of crude protein, moisture, and lipid. Though these components are high in fruits, but they have less influence in the carbon yield during thermal carbonization. This happens due to the degradation of both the lipid and crude protein contents at temperature below 300 °C, which releases volatile components like carbon dioxide, water vapour, ammonia gases, olefins, and methyl esters. The crude fibres in fruit-based biomass comprised of lignin, hemicellulose, and cellulose, which highly influence the carbon productivity. As the crude fibres of fruit-based biomass contains more cellulosic components than lignin, the fruit-based biomass is less suitable than the plants for preparation of carbon materials of graphitic structures with high yield (Liu et al. 2018; Orozco et al. 2014). The lignin, cellulose, hemicellulose contents of different fruit-based biomass along with carbon yield are shown in Table 17.2.

Activated carbon was prepared from banana fibres by applying $ZnCl_2$ for the enhancement of surface area as well as electrochemical properties as electrode material in EDLC. The prepared carbon depicted capacitance of 74 F/g in galvanostatic charge-discharge measurement. The $ZnCl_2$ treated sample showed surface area of 1097 m^2/g as well as outstanding cyclic stability of 88% coulombic

Table 17.2 The chemical compositions and percentage yield of carbon of different fruit-based biomass

Biomass	Lignin (%)	Cellulose (%)	Hemicellulose (%)	Experimental carbon yield (%)	References
Apple pulp	58.0	18.8	23.1	25.7	Cagnon et al. (2009)
Plum pulp	79.0	5.6	15.4	25.9	Cagnon et al. (2009)
Olive stones	75.3	10.7	14.1	30.9	Cagnon et al. (2009)
Plum stones	70.8	14.1	15.1	24.6	Cagnon et al. (2009)
Coconut shell	66.9	8.7	24.4	25.6	Cagnon et al. (2009)

efficiency for 500 cycles (Subramanian et al. 2007). Orange peel was used for the preparation of interconnected carbon structure using pyrolysis process and KOH activation. The electrode prepared offered a capacitance of 407 F/g and retained 100% capacitance for 5000 cycles (Ranaweera et al. 2017).

Analysis was performed on activated nanoporous carbon prepared using orange peel biowaste to check its feasibility to use in electrode of flexible supercapacitor. The prepared activated three-dimensional nanoporous carbon showed reactive area of 2160 m^2/g. Flexible supercapacitor fabricated using the carbon and aqueous electrolyte offered maximum capacitance of 460 F/g as well as maintained 98% capacitance for 10,000 cycles (Subramani et al. 2017). Highly porous foam-like carbon material was prepared from waste fig-fruit applying thermal conversion process followed by chemical activation. An electrode fabricated using the synthesized carbon material exhibiting specific surface area of 2000 m^2/g showed excellent capacitances of 340 and 217 F/g (Ba et al. 2018). In a study, nitrogen-doped porous carbon foam was prepared from banana peel to use in binder-free electrode in supercapacitors. The prepared carbon material exhibited a surface area of 1357.6 m^2/g and maximum capacitance of 210.6 F/g (Liu et al. 2016) (Table 17.3).

17.2.2.3 Microorganism

In addition to the above-mentioned biomass, various microorganisms, viz. fungi and bacteria cellulose are also considered as promising source of carbon. The highly abundant fungus such as mushrooms and yeasts is suitable biomass feedstock for carbon production. Similar to other biomass, microorganisms are composed of the major components such as carbohydrates, crude proteins, ash, etc., but the elements consisting of these components are significantly dissimilar to plants and fruits. The carbohydrates in the microorganisms are composed of chitins. These chitins are bio-cross-linked with glucan and contribute to carbon yield in thermal carbonization. In contrast, the carbohydrates in plants and fruits are comprised of less thermally stable as well as non-cross-linked sucrose and starch (Arroyo et al. 2016). Cellulose is the major constituent of crude fibres in microorganisms. Because of the presence of cellulose, microorganisms show similar carbonization characteristics like the plant and fruit-based biomasses, and also influence in the yield of carbon (Kalac 2009).

Out of the various microorganisms, the mushrooms are considered as the major precursors of the carbons derived from biomass. In comparison to the plants and fruits, the mushrooms acquire more attention as the carbon precursors because of the presence of high nitrogen content from 3–10% (in dry wt. % basis) and up to 17% in some other species. In the mushrooms, commonly fruiting body is used in carbon preparation (Liu et al. 2018; Pineda-Insuasti et al. 2014). Table 17.4 shows the chemical compositions of various microorganisms.

In a study, Kombucha, a microorganism-based biomass was used for porous carbon preparation by directly treating with KOH followed by in situ activation. The prepared carbon depicted surface area of 917 m^2/g and offered capacitance of 326 F/g, and also retained 91.3% capacitance over 5000 cycles (Dai et al. 2017).

Table 17.3 Performance of various types of biomass-derived carbon as supercapacitor electrode material

Feedstock	Activation method	Surface area (m^2/g)	Capacitance (F/g)	Scan rate	References
Jute	KOH	1769	408	1 mV/s	Zequine et al. (2017)
			185	500 mA/g	
Bamboo fibres	KOH	1120	510	0.4 A/g	Zequine et al. (2016)
Argan seed shell	KOH	2100	355	125 mA/g	Elmouwahidi et al. (2012)
Banana fibre	$ZnCl_2$	1097	74	500 mA/g	Subramanian et al. (2007)
Corncob	KOH	1471.4	293	1 A/g	Yang and Zhang (2018)
Rice husk	KOH	2523.4	250	1 A/g	Xu et al. (2014)
Sawdust	NaOH	1900	227	2 mV/s	Liu et al. (2013)
Cherry blossom flower petal	Air	509	332.7	10 mV/s	Xu et al. (2014)
Ficus religiosa leaves	–	157	3.4	1 mA	Senthilkumar and Selvan (2015)
Bacterial cellulose	KOH	491	241.8	0.1 A/g	Wang et al. (2016)
Orange peel	KOH	2521	407	0.5 A/g	Ranaweera et al. (2017)
Orange peel	KOH	2160	460	1 A/g	Subramani et al. (2017)
Fig-fruit	KOH	2000	340	0.5 A/g	Ba et al. (2018)
			217	20 A/g	
Banana peel	N-doped	1357.6	210.6	0.5 A/g	Liu et al. (2016)
Kombucha	KOH	917	326	1 A/g	Dai et al. (2017)
Shiitake Mushroom	H_3PO_4 and KOH	2988	306	1 A/g	Cheng et al. (2015)
Crude auricularia	–	80.08	196	5 mV/s	Zhu et al. (2011)
Human hair fibres	KOH	1306	340	1 A/g	Qian et al. (2014)
Chicken eggshell membrane	KOH	221	297	0.2 A/g	Li et al. (2012)

Shiitake mushroom was used as biomass feedstock for porous carbon preparation applying two steps activation process which includes H_3PO_4 activation followed by KOH activation and exhibited surface area of 2988 m^2/g, capacitance of 306 F/g, and retained 95.7% capacitance over 15,000 cycles (Cheng et al. 2015). Activated

Table 17.4 The compositions of different microorganisms (in dry wt. % basis)

Biomass	Carbohydrates (%)	Crude fibre (%)	Crude protein (%)	Crude fat (%)	Ash (%)	References
Pleurotus ostreatus	57.05	8.25	26.05	2.79	5.86	Latshaw and Miller (1924)
Agaricus bisporus	42.56	13.21	33.85	2.41	7.97	Latshaw and Miller (1924)
Tricholoma terreum	31.1	30.1	20.1	6.6	12.1	Yin and Zhou (2008)
Tricholoma portentosum	34.6	30.1	19.6	5.8	9.9	Yin and Zhou (2008)
L. crocipodium	12.8	37.9	29.3	1.0	5.8	McDonald-Wharry et al. (2015)
B. edulis	30.6	15.3	28.7	4.1	9.2	McDonald-Wharry et al. (2015)
Lactarius volemus	15.0	40.0	17.6	6.7	13.3	Zhou and Yin (2008)
R. virescens	13.4	32.8	28.3	1.5	11.9	Zhou and Yin (2008)

carbon was produced using *crude auricularia*, a type of fungi and the electrochemical performance were analysed. The prepared activated carbon depicted surface area of 80.08 m^2/g, capacitance of 196 F/g, and retained 99% capacitance after 1000 cycles (Zhu et al. 2011).

17.2.2.4 Animal-Based Precursors

Biomass-derived carbon can also be prepared from various animals including crustaceans, insects, mollusks, etc. The chitin which can be extracted from these animals can be considered as the promising source for biomass-derived carbon production because of high nitrogen, chemical stability, and natural abundance. Chitin is different from cellulose found in other biomass, which has the capability to form strong intermolecular hydrogen bonding as well as crosslinking structures with catecholamine and glucan complexes. Due to these reasons, chitin possesses significantly higher thermal stability and results in higher carbon yields. The cuticles, sloughs, pupae, and pupa of various animal-based precursors such as crustaceans, insects, mollusks, etc. are composed of relatively good chitin content. Other than the cuticles and sloughs of animals, biomass-derived carbon can also be prepared from the "keratin", a fibrous protein found in claws, hooves, hairs, horns, etc. (Liu et al. 2018; Arbia et al. 2013; Percot et al. 2003). Like the chitin, keratin also possesses better chemical and thermal stability due to its intensive intermolecular bonding. As because the molecules in keratin form strong covalent

Table 17.5 The chitin contents of different animal-based biomass, viz. crustaceans, insects, and mollusks

Crustaceans		
Crustaceans	Chitin (%)	References
Cancer (crab)	72.1	Liu et al. (2018)
Alaska shrimp	28.0	Liu et al. (2018)
Nephro (lobster)	69.8	Liu et al. (2018)
Lepas (goose barnacle)	58.3	Liu et al. (2018)
Carcinus (crab)	64.2	Liu et al. (2018)
Insects		
Insects	Chitin (%)	Reference
Blatella (cockroach)	18.4	Liu et al. (2018)
Coleoptera (ladybird)	27-35	Liu et al. (2018)
Pieris pupae (butterfly)	64.0	Liu et al. (2018)
Bombyx (silk worm)	44.2	Liu et al. (2018)
Cicada sloughs	36.6	Liu et al. (2018)
Diptera pupae	54.8	Liu et al. (2018)
Mollusks		
Mollusks	Chitin (%)	Reference
Clam	6.1	Liu et al. (2018)
Shell oysters	3.6	Liu et al. (2018)
Squid pen	41.0	Liu et al. (2018)
Krill, deproteinized shells	40.2	Liu et al. (2018)

and non-covalent bonds, therefore, display better stability in thermal carbonization, resulting in higher yield of good quality biomass-derived carbon (McKittrick et al. 2012; Qian et al. 2014). The chitin contents of various animal-based biomass such as crustaceans, insects, and mollusks are shown in Table 17.5.

In a study, human hair fibres were used in heteroatom doped porous carbon flakes preparation, which was further used in electrode of supercapacitor. Carbonization at a temperature of 800 °C was used to prepare these carbons, and electrochemical performance was evaluated. The electrode fabricated using the carbon material showed specific capacitance of 340 F/g as well as outstanding cyclic stability over 20,000 cycles in aqueous electrolyte (Qian et al. 2014). Three-dimensional carbon films from chicken eggshell membranes were prepared by carbonization process, the performance of which was further investigated for the application in electrode of supercapacitor. Though the carbon material displayed surface area of 221 m^2/g, capacitance of 297 F/g was achieved with an excellent capacitance of 97% over 10,000 cycles in basic electrolyte (Li et al. 2012).

17.3 Characterization Methods of Flexible Supercapacitor Electrode

The performance of flexible supercapacitors can be analysed by considering the factors, viz. specific capacitance, operating voltage, equivalent series resistance (ESR), cyclic stability, energy, and power densities. Capacitance refers to the capacity of the electrode materials to store charge within certain voltage range. The electrochemical techniques, viz. cyclic voltammetry (CV), galvanostatic charge-discharge (GCD), and electrochemical impedance spectroscopy (EIS) are used to study their performance. Each of these techniques can be performed using a two or three-electrode set-up. In the previously reported literatures, it was mentioned that specific capacitance obtained from the three-electrode set-up is twice that of the two-electrode system. The three electrodes used in the system are the reference, working, and counter electrodes. (Frackowiak et al. 2013).

17.3.1 Cyclic Voltammetry

Cyclic voltammetry is applied in investigation of electrolysis mechanisms in an electrochemical cell. In this technique, voltages are applied to the electrode in a fixed range and the corresponding flow of current is observed. Cyclic voltammetry is performed in a two or three electrode system. Applying the system, a current–potential polarization curve can be recorded using a potentiostat, which controls the voltage between WE and RE, and measures the current flow between WE and CE. Area of the closed curve in CV graph determines the capacitance of supercapacitor (Mabbott 1983). A sampled CV graph is shown in Fig. 17.4.

The specific capacitance of the working electrode obtained from the CV curve, tested in a three electrode set-up can be calculated by following the Eq. (17.1),

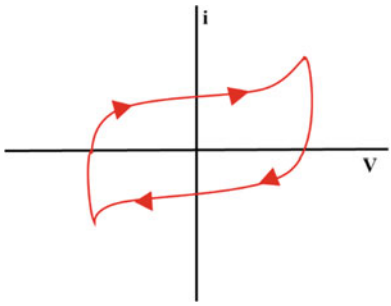

Fig. 17.4 Cyclic voltammogram of EDLCs

$$C = \frac{\int_{v_1}^{v_2} \mathrm{IdV}}{\mathrm{mv}(V_2 - V_1)} \tag{17.1}$$

17.3.2 Galvanostatic Charge-Discharge (GCD)

GCD can be used for the determination of capacitance, power densities, and cyclic stability of the electrode. In GCD, current is applied to the working electrode and the potential obtained is measured against a reference electrode as a function of time.

The discharging time from maximum to minimum voltage is used in determination of capacitance by the Eq. (17.2).

$$C = \frac{I}{\mathrm{dV}/\mathrm{dt}} \tag{17.2}$$

$$C = \frac{I\Delta t}{\Delta V} \tag{17.3}$$

where I denote discharge current or set current, ΔV refers to potential window, and Δt denote discharging time in GCD curve.

Equations (17.4) and (17.5) can be used to calculate Energy (E) and the power densities (P) of supercapacitors,

$$E = \frac{\mathrm{CV}^2}{2} \tag{17.4}$$

$$P = \frac{V^2}{4R} \tag{17.5}$$

where V is the applied voltage, C is the capacitance, and R is the equivalent series resistance (Qu et al. 2015; Halper and Ellenbogen 2006).

17.3.3 Electrochemical Impedance Spectroscopy (EIS)

Electrochemical impedance spectroscopy is an electrochemical graphical method used to determine the capacitance of an electrochemical cell by evaluating ESR within the cell. In supercapacitor, all the information regarding electrode/electrolyte interface are possible to acquire from the analysis of the impedance data. This method is based on the alternating current electrode processes. EIS is performed by applying a small amplitude alternating signal (5–10 mV) within a wide frequency range in order to get a linear response and to study behaviour of the electrode when comes to rest (Negroiu et al. 2017).

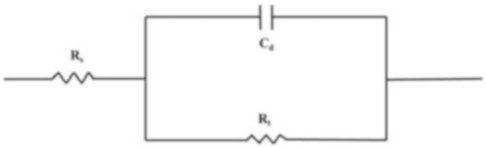

Fig. 17.5 Equivalent electric circuit of EDLCs. R_t charge transfer resistance, R_s resistance of the electrolyte solution, C_d electric double layer capacitance, Equivalent series resistance (ESR) = $R_t + R_s$

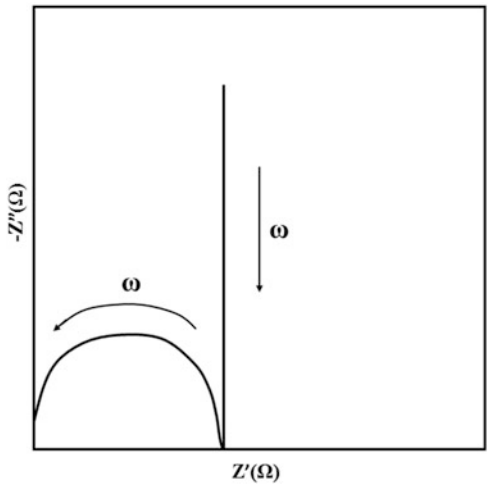

Fig. 17.6 Nyquist plot of EDLCs

EIS displays direct connection between real system and idealized equivalent circuit, that consists of discrete electrical elements such resistance and capacitance, etc. in series and parallel combination which is shown in Fig. 17.5.

In EIS, the impedance is plotted as function of frequency $Z(f)$ by changing the frequency over a wide limit. The function of frequency $Z(f)$ consists of real and imaginary part in this method. The capacitance of a supercapacitor can be determined from the bode plot between log|Z| and log f. Another graph, viz. Nyquist plot can be plotted between the imaginary part of the impedance $Z(f)''$ against the real part of impedance $Z(f)'$. The ideal Nyquist plot between the imaginary and real parts of the impedance is shown in Fig. 17.6. The capacitance can be determined by the following Eq. (17.6),

$$C = \frac{1}{2\pi f \mid Z \mid} \tag{17.6}$$

(Zhao et al. 2012; Bi et al. 2019).

17.4 Types of Electrolyte for Flexible Supercapacitors

The major advantages of supercapacitors over conventional energy storage systems are high power output, longer lifespan, as well as good charge/discharge processes within seconds (Lu et al. 2014). However, drawbacks such as high cost of packaging material and technique coupled with manufacturing difficulties of small and flexible supercapacitor devices using liquid electrolytes are also prevalent. Thus, flexible solid-state supercapacitors have acquired considerable attention in recent year because of its added advantages like light weight, wide range for operation temperature, small size, ease of handling, and excellent reliability (Zhong et al. 2015). The performances of supercapacitors strongly depend on the capacitance power of electrode materials, potential window of electrolytes (electrolyte salt + solvent), and the structure of the integrated systems (Shi et al. 2013).

The combination of both salt and solvent is known as electrolyte. It helps in charge separation on both the electrode in the cell through ionic conductivity. Electrolyte is the key component for the development of electrical double layer (EDL) in EDLC and in pseudocapacitors it helps in reversible redox process for storing charge. The cycle life, power and energy density and EDL capacitance and pseudocapacitance are governed by the following factors of an electrolyte: (1) type and size of ion; (2) solvent and ion concentration; (3) ion/solvent interaction; (4) electrolyte/electrode interaction; and (5) potential window (Shi et al. 2013). The electrolytes applied for flexible supercapacitors are as follows: liquid electrolytes and solid electrolytes or quasi-solid state electrolyte (Jiménez-Cordero et al. 2014).

17.4.1 Liquid Electrolytes

17.4.1.1 Aqueous Electrolytes

The low energy density of aqueous electrolyte discourages its commercial usage. However, its ease of handling and low cost are the prime reason for its extensive use in laboratory for research and development. Based on the size of both hydrated and unhydrated cations and anions, ion movements, and the corrosive degree, the electrolytes are selected. The aqueous electrolyte can be further subdivided into three groups:

Acid Electrolytes (H_2SO_4 or H_3PO_4 Aqueous Solution)

H_2SO_4 is most widely used acid electrolyte because of excellent ionic conductivity, which highly depends on the electrolyte concentration. Different studies suggest that specific capacitances of electrical double layer capacitors (EDLCs), obtained from

strong acid electrolyte, are greater in comparison to neutral electrolytes which may be attributed to high ionic conductance and low equivalent series resistance (Torchała et al. 2012; Wu et al. 2013; Zhang et al. 2012; Conway 1999). Apart from electrostatic EDL capacitance, H_2SO_4 electrolyte also exhibits some pseudocapacitance, which could be helpful for redox reactions occurring on certain surface functionalities (Lang et al. 2012). The introduction of hetero atoms, viz. oxygen, nitrogen, phosphorous, etc. can further enhance the pseudocapacitance or functional groups of carbon surfaces (Jiménez-Cordero et al. 2014).

Alkaline Electrolytes (KOH Aqueous Solution, LiOH, or NaOH)

Owing to high ionic conductivity, KOH is usually preferred alkaline electrolyte. However, various scientific articles suggest that aqueous KOH and H_2SO_4 electrolyte are very much similar to each other in terms of EDLC capacitances and energy densities. A tremendous amount of research is being carried out in order to increase the capacitance or widen the voltage window which will eventually increase the energy density of supercapacitors using base electrolytes. The capacitance is dependent on the nature of electrolyte used as it was observed in some studies that the pseudocapacitance of carbon electrode doped with nitrogen was enhanced due to usage of KOH electrolyte (Slesinski et al. 2018). Further attempt for the improvement of performance of supercapacitors, Wang et al. (2013) doped carbons with phosphorus and nitrogen. From this experiment it was observed that specific capacitance, potential window, and stability were greatly improvised over phosphorus-free counterparts. Apart from this, it was observed that negative polarization can help in storage of hydrogen when potential smaller than thermodynamic value for water reduction is being applied (Kang et al. 2012). Alkaline electrolyte was found to be more favourable to achieve the increased pseudocapacitance.

Neutral Electrolytes

High working potential windows, higher safety, and low corrosion are the driving force which encourages the usage of neutral electrolyte. The most extensively used conducting salts in this type of electrolytes are Lithium (Li) (e.g. Lithium Chloride (LiCl), Lithium Sulphate (Li_2SO_4), and Lithium Perchlorate ($LiClO_4$)), Sodium (Na) (such as Sodium Chloride (NaCl), Sodium Sulphate (Na_2SO_4), and Sodium Nitrate ($NaNO_3$)), Potassium (K) (such as Potassium Nitrate (KNO_3), Potassium Sulphate (K_2SO_4), and Potassium Chloride (KCl)), Calcium (Ca) (e.g. Calcium Nitrate ($Ca(NO_3)_2$)), and Magnesium (Mg) (e.g. Magnesium Sulphate ($MgSO_4$)). Out of these, Na_2SO_4 is the highly applied neutral electrolyte in pseudocapacitors as well as hybrid supercapacitors (Jiménez-Cordero et al. 2014).

17.4.1.2 Organic Electrolytes

The combination of salt and organic solvent is known as organic electrolytes. 1 M Lithium Hexafluorophosphate ($LiPF_6$) in Ethylene carbonate/Diethyl carbonate (EC/DEC = 1:1) or Ethylene carbonate/Propylene carbonate /Dimethyl carbonate (EC/PC/DMC = 1:1:1) and 1 M Tetraethyl ammonium tetrafluoroborate (Et_4NBF_4)/ Propylene carbonate (PC) solutions are mostly utilized as organic electrolytes (Yu et al. 2009; Sekhon 2003). These electrolyte-based SCs are of higher interest in market in the present scenario, because of its high potential window (2.5–2.8 V) which significantly improves the energy and power densities. Apart from this, low-cost materials can be used as current collectors as well as outer parts in case of organic electrolytes. However, high cost, small capacitance value, low conductivity, and various safety issues are some of its major limitation. In addition to this, difficulties arise during purification and assembling process and are performed in strict controlled atmosphere to eliminate any unwanted matters discourage its usage for research work (Lang et al. 2012).

17.4.2 Solid-State Electrolytes

Ease to handle, superior reliability as well as broader range of operation temperature make this form of electrolyte an excellent candidate in comparison to liquid electrolytes. In addition to that solid-state electrolytes are leak proof thereby reduces cost involved in packaging. Gel polymers are the most extensively used solid-state electrolytes in supercapacitors. The important properties to qualify as an excellent solid-state electrolyte are cheaper cost, non-toxicity, greater ionic conductivity, exceptional stability as well as wide potential range (Łatoszynska et al. 2015). A sol–gel method is used to prepare solid-state electrolyte using gel agent, solute, and solvent. The gel agents are Poly(vinyl alcohol) (PVA), Poly(vinylidene fluoride) (PVDF), or Poly(vinylidene fluoride-co-hexafluoropropylene) (P(VDF-HFP)) (Jiménez-Cordero et al. 2014). The polymer-based solid electrolytes are classified as: solid polymer electrolyte (SPE), gel polymer electrolyte (GPE), and polyelectrolyte. Quasi-solid state electrolyte is another name for GPEs because of the existence of liquid phase (Zhang et al. 2017).

17.4.2.1 Gel Polymer Electrolyte (GPE)

This electrolyte consists three components: (1) host (polymeric framework), (2) plasticizer (an organic/aqueous solvent), and (3) electrolytic salt. Poly (polyacrylate) (PAA), Poly(vinyl alcohol) (PVA), Poly(ethylene oxide) (PEO), Polyacrylonitrile (PAN) are some of the most commonly used host polymers. Usually two or more organic solvents are mixed together to produce plasticizer, for obtaining high ionic

conductivity, low viscosity as well as wide potential window (Zhong et al. 2015). Hydrogel polymer electrolyte consists of water as a plasticizer and 3D polymeric networks that helps in water retention in the matrices owing to tension. To facilitate mobile ions, the electrolytic salt must have large anions and low dissociation energy. Gel polymer electrolytes are classified based on electrolytic salt as: (1) lithium ion gel polymer electrolytes, (2) proton conducting gel polymer electrolytes, (3) alkaline gel polymer electrolytes, (4) other ion gel polymer electrolytes (Shi et al. 2013).

17.5 Future Perspective

Biomass-derived carbon proved as a cheap product as well as environment friendly having a high potential for application in various areas. Available literatures might have paved the path for upcoming investigation on the advancement and utilization of various biomass and biowaste-based carbon material instead of conventional fossil fuel-based carbon for the production of electrode materials for supercapacitor without compromising their quality. Unfortunately, supercapacitors derived from biomass have some disadvantages such as it suffers from low energy density and also rate capability is not adequate. Although the presence of higher oxygen content in the carbon matrix is important for enhancing the hydrophilicity of carbon materials as well as for assisting the electrolyte infiltration, the electrical conductivity was reduced by these groups. Therefore, a combine research on theoretical and experimental sciences should be carried out in near future on the development of an efficient approach for maintaining the equilibrium between hydrophilicity and electric conductivity of the carbon framework.

Feedstock having different compositions, different biochar carbon preparation criteria and activation methods as well as activation parameters (i.e. activating agent and time of activation) may have shown considerable influence on the properties of resulting activated biochar carbon including porosity, surface area, and surface functionalities, with which electrochemical performances of the electrodes for supercapacitor application are dependent on. The control and tuning of these properties are necessary to optimize the energy and power densities for compensating future energy demands. The advancement in research on these properties for supercapacitor application is still in budding stage and hence, in future, the researchers need to develop new methods which will help in fabricating biomass-derived supercapacitors with maximum performances. Furthermore, as the electrolyte systems highly influence the overall electrochemical performance of supercapacitors, therefore development of a proper electrolyte system is considerably essential for future generation of supercapacitors. Future thrusts should be also dedicated to explain the detailed mechanisms of activated biochar carbon's functionalities in the supercapacitor applications through the various viable characterization methods, which may help to develop more appropriate and novel activation methods and also the improvement of prevailing methods. Undesired pollutants such as alkali/acid (KOH, NaOH, H_2SO_4, HNO_3, etc.) and undesired gases (SO_2, NO_2,

etc.) may be also released into the environment during activation as well as application. Thus, significant measures must be taken in evaluation and minimizing the potential environmental contamination during a process.

Lastly, adequate information for large-scale production of biomass-based supercapacitor is limited. Only the cross-cutting collaboration between the scientists/researchers of different areas, i.e. materials science, environmental science, chemistry, physics, economics, etc. can make the large-scale production of the bio-based supercapacitor feasible by their new, novel and cost-effective production methods by using easily available environmental friendly materials.

17.6 Conclusion

Over the past decade renewable energy demand has gained tremendous research attention worldwide. A modern society considers the availability of economically viable and reliable energy source as one of its prime characteristics. However, it is quite unfortunate that a majority of such societies are substantially dependent on non-renewable energy sources and in particular, the fossil sources. Considering alternative materials for various applications of renewable sources, the feasibility in utilization of biomass as a source for energy generation and storage offers significant commercial and scientific opportunities. So far, various porous carbon products prepared from non-renewable resources have been utilized for fabricating flexible supercapacitors. Unfortunately, the greenness unavailability in the method, use of fossil resources, and the high cost of the products excavate the extraordinary prospects for developing high-performance electrode materials for flexible supercapacitors in a more sustainable way by using carbon derived from naturally abundant biomass resources (especially from waste biomass). In this chapter, the importance and potential of biomass-derived carbon as an electrode material in flexible supercapacitor are discussed with various reported biomass feedstock. A summary of the different electrochemical techniques used for the assessment of flexible supercapacitor's performance was also discussed. Various electrolytes used in flexible supercapacitor are explained in this chapter as electrolyte is the significant factor for specific capacitance of supercapacitors. Thus, it can be concluded that utilization of biowaste for the production of supercapacitor electrode serves multiple purpose such as sustainable waste management, its utilization and economic benefits. Therefore, needless to say that utilization of biomass for development of carbon material for flexible supercapacitors is a right choice.

Competing Interests All the authors declare that they have no competing interests.

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Chapter 18
A Sustainable Bio-Jet Fuel: An Alternative Energy Source for Aviation Sector

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Abstract In the modern world, the aviation sector plays a vital role in human life in terms of public transport and freighting of goods. Depletion of fossil resources and concerns of their effect to global warming is encouraging researchers to search for alternative options. The current work emphasizes the updated thermochemical process technologies over different heterogeneous catalysts with different feedstocks. The main parameters are availability of feedstock, challenges in the use of feedstock and process details. Besides environmental influence, also development status of bio-jet fuels will be presented.

Keywords Bio-jet fuel · Thermochemical conversion technique · Feedstock · Process developments

Abbreviations

ASTM	American Society for Testing and Materials
DTO	Distilled tall oil
GHGs	Greenhouse gases
HDC	Hydrodecarboxylation
HDO	Hydrodeoxygenation
HEFA	Hydro-processed esters and fatty acids
HVO	Hydrotreatment of vegetable oils

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HTL Hydrothermal liquefaction
MAH Monocyclic aromatic hydrocarbons

18.1 General Overview

The modern world is facing one of the major setbacks—pollution. This can be classified into various types and, among that, the major part of pollution is caused due to combustion of fossil fuels. Fuels are produced predominantly from non-renewable resources principally petroleum, coal and natural gas. Due to high demand of fuels, particularly in transportation, fuel consumption and the level of greenhouse gases (GHGs) are constantly increasing (US-EPA 2016). Fossil fuel-based energy systems lead to alarming levels of pollution. Due to increasing energy demands of the population and the depleting resources of fossil fuels, the world is facing an urgent task-search for sustainable alternative resources to ensure our energy supply. It is highly important to use natural resources to attain sustainable future.

In terms of the transportation sector, aviation has a significant role in world's economy, and it is a major way of transporting people and freights among various countries beyond the boundaries. Developing of this sector leads to increasing concentration of anthropogenic CO_2 emissions whereupon the fuel requirement is expected to double in the upcoming 20 years (Gutiérrez-Antonio et al. 2017). The transportation sector occupies the eight largest places throughout the globe to generate GHGs. If summarized, people produced 448 megatons (Mt) of CO_2 during the year of 2010. If no preventive measures are taken, the CO_2 emissions increase ranging from 682 Mt to 755 Mt by 2020 and as high as 2700 Mt by 2050 (Weber et al. 2010). This can be reduced yearly by 1.5% via engine modifications, optimized navigation systems and airport modifications, despite the fact that a long-term reduction of emissions requires more renewable and sustainable fuels. However, terms of sustainable and alternative propulsion technologies such as electric or solar-powered aircraft and cryogenic hydrogen-powered engines are still under development stage and will not be commercially available until 2050 (van Dyk et al. 2017). Thus, according to the international energy agency evaluations, biofuels could replace diesel and jet fuels by 2050 with a credit of a 27% of all fuels in the transportation sector (Eisentraut et al. 2011; EIA 2012) and can be used directly without any jet-engine modifications. Therefore, it is of major relevance to develop an efficient and sustainable process for the production of green jet fuels.

A bio-jet fuel can be determined as a fuel produced from biogenic materials such as vegetable oils, sugars, animal derived fats, waste cooking oils, lignocellulosic material, forest waste, etc. through sustainable and cost effective process (biochemical and thermochemical) technologies and resulting in C_8–C_{16} carbon chain length, which can be used in a straight line of existing aviation engine (turbine engine) without any alterations. According to the American Society for Testing and Materials (ASTM), up to 50% bio-jet fuel can be blended with fossil fuels, depending

upon the processing technologies and feedstock. The current chapter emphasizes the updated thermochemical process technologies over different heterogeneous catalysts and different feedstocks. The main parameters are availability of feedstock, challenges in the use of feedstock and process details. Besides environmental influence, also development status of bio-jet fuels is presented.

18.2 Types of Bio-Jet Fuels

Bio-jet fuels are produced from the range of various feedstocks including starchy crops, sugary crops, lignocellulose crops, oily crops, waste fats, oil and greases, agriculture residues, forest residues, microalgae, municipal solid waste, industrial waste gases and carbon dioxide (CO_2). Among the various feedstocks, only five products of bio-based aviation fuels have been accepted for blending with fossil-derived jet fuel. Not all above-mentioned feedstock products are able to reduce greenhouse gases in a larger extent and the carbon savings might be minimal. From these feedstocks, sugar and starch only result in minor decrease of GHGs, whereas those made from edible and non-edible oil derived fuels exhibit higher carbon intensity than the conventional aviation fuels (E4Tech 2014; Gill 2016a; IATA 2016a, b). Finally, lignocellulose derived aviation fuels will provide also extensive emission declines related to other feedstocks. According to the use of feedstock corresponding to the conversion techniques, the bio-jet fuels can be classified mainly into conventional and advanced bio-jet fuels.

18.2.1 Conventional Bio-Jet Fuels

The utilization of biomass plays a significant role in production of bio-jet fuels. The main drawback of the first-generation bio-jet fuel produced from, e.g. edible crops—wheat, corn, edible oils, etc., is the utilization of land, which is competing with food-based crops (Healey et al. 2015). These challenges lead towards second-generation biofuels, especially demanding oleochemicals. The term conventional bio-jet fuels is primarily aimed to exploitation of non-edible vegetable oils such as camelina, jatropha, palm, used cooking oils and waste animal fats. These contain high amount of fatty acids and can be utilized in the bio-jet fuel production (Azad et al. 2014).

18.2.1.1 Oleochemical Conversion Process

The hydro-treated vegetable oil conversion process, utilizing triglycerides, saturated or unsaturated fatty acids, tallow and used cooking oil as a feedstock is already a matured technique compared to other conversion process technologies for production of jet fuel (Rye et al. 2010). Oleochemical source derived fuels are called

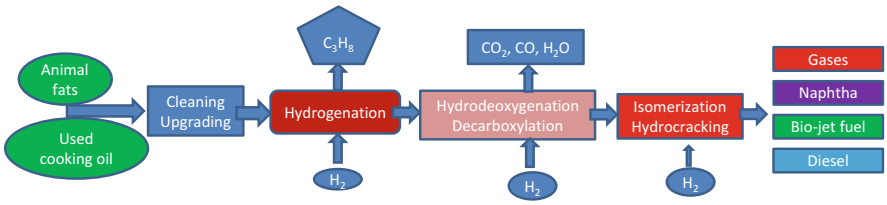

Fig. 18.1 Schematic diagram of hydrotreatment of oils [Redrawn from (Wei et al. 2019)]

hydro-processed esters and fatty acids (HEFA bio-jet), certified by ASTM in 2011 and the technology is in commercial stage (van Dyk et al. 2017).

Hydrotreatment of vegetable oils (HVO) is principally composed of hydrogenation, hydrodeoxygenation, decarboxylation, isomerization and hydrocracking reactions. The schematic process diagram can be seen in Fig. 18.1. The process is divided into two phases. In the first phase, unsaturated fatty acids and triglycerides are converted into saturated fatty acids via catalytic hydrogenation. Herein β-hydrogen elimination occurred resulting in formation of saturated fatty acids that are further transformed into straight chain alkanes (C_{15}–C_{18}) through hydrodeoxygenation and decarboxylation reactions (Morgan et al. 2012; Yang et al. 2013). Carboxyl group is removed via decarboxylation, while decarbonylation also occurred. In addition to formation of alkanes, H_2O and propane were also formed as by-products during the first phase. In the second phase, deoxygenated straight chain alkanes are additionally isomerized and cracked under hydrogen to yield up branched alkanes in a mixed liquid fuel. Further separation is based on boiling points of various fractions.

18.2.1.2 Catalysis Over Heterogeneous Catalysts

In the first phase, particularly noble metals supported with zeolites or oxides or active carbon have been used for hydrogenation of vegetable oils and used cooking oils. However, due to deactivation of the catalyst, by poisoning and assembly of cracking species, selection of other transition metals such as Ni, Mo, Co or their supported bimetallic composites is preferred in the current mode of operations (Murzin et al. 2009; Markkanen et al. 2010; Wang et al. 2013).

The catalytic materials used for hydrodeoxygenation and hydrodecarboxylation reactions of vegetable oils, used cooking oils or animal fats are mainly industrial noble metals such as Pd, Ru and Pt, see Table 18.1. Simakova et al. (2008) worked on the hydrogenation of vegetable oils over Pd on nano composite carbon catalysts (Pd/Sibunit). The reaction was conducted in an autoclave at 98 °C under 1–10 bar hydrogen pressure for 2–3 h. The fast hydrogenation rates were achieved due to mesoporous structure of the carbon support and edible oil hydrogenation resulted in monogenic products with a high ratio of *cis/trans*. Pd/Sibunit has the ability to keep low rate for trans-isomerization at the range of temperatures from 170 to 200 °C,

Table 18.1 Hydrotreatment of oleochemicals over noble metal supported catalysts

S. No.	Feedstock	Catalyst	Reaction conditions	Yield	References
1	Sunflower oil	1% Pd/Sibunit	170 °C, 60 bar H_2, 3 h, 1100 rpm	[a]25 wt.%	Simakova et al. (2008)
2	Oleic acid	5 wt.% Pd/C	300 °C, 15 bar, 6 h, 1100 rpm	[b]84%	Snåre et al. (2008)
3	Oleic acid	5 wt.% Pd/Zeolite	375 °C, 15 bar H_2, 3 h	[c]60.81 wt.%	Susanto et al. (2014)
4	Jatropha oil, oleic acid	3 wt.% Pd/SBA-16	325 °C, 10 bar H_2, 5 h, *n*-decane	[b]97.4 mol %, [b]99.8 mol %	Grosso-Giordano et al. (2016)
5	Glyceryl trioctanoate	5 wt.% $Pd/NbOPO_4$	180 °C, 30 bar H_2, 48 h, cyclohexane	[c]96.4 wt.%	Xia et al. (2016)
6	Waste cooking oil	1 wt.% Ru/Al_{13}-montmorillonite	350 °C, 20 bar H_2, 15 h	[c]89.8 wt.%	Liu et al. (2012b)
7	Stearic acid, Jatropha oil	1 wt.% Ru/La $(OH)_3$	200 °C, 40 bar H_2, 8 h	[c]98 wt.%, [c]80.7 wt.%	Guo et al. (2015)
8	Jatropha oil	1 wt.% Pt-20 wt.% Re /H-ZSM-5	270 °C, 60 bar H_2, 12 h	[c]67 wt.%	Murata et al. (2010)
9	Jatropha oil	2 wt.% Pt-10 wt.% Pd/Al_2O_3	350 °C, 30 bar H_2, 2 h	[c]82 wt.%	Gong et al. (2012)
10	Jatropha oil	0.01 wt.% Pt-20 wt.% USY/√-Al_2O_3	390 °C, 15 bar H_2, 35 h	[c]75 wt.%	Garcia-Dávila et al. (2018)
11	Palm oil	1 wt.% Pt/N-AC	300 °C, 30 bar H_2, 2 h	[c]91 wt.%	Jin et al. (2020)

[a]Saturated fatty acids
[b]Conversion
[c]Sotal liquid hydrocarbons (C_{11}–C_{20})

resulting in the absence of dienoic acids in the reaction mixture. *Cis/trans* ratio was conversion independent and this means that the activation energy for hydrogenation of di-unsaturated acids should give similar results (Simakova et al. 2008). Pd/Sibunit catalyst was tested under industrial conditions such as both three-phase slurry and up-flow fixed bed reactors being active and stable with respective to the hydrogenation of edible oil selectivity and hydrogenation of total non-edible oils (Simakova et al. 2008). Catalyst deactivation and the influence of gas atmosphere on the catalyst were also investigated at 300 °C in a continuous reactor. The results indicate that the catalyst deactivated quickly under Ar gas flow (Madsen et al. 2013). Deoxygenation reaction mechanism of unsaturated fatty acids over Pd/C was investigated and 87% conversion was obtained. The results indicated that at 300 °C and under 15 bar hydrogen, the reaction proceeds initially via parallel double bond hydrogenation and deoxygenation reactions, resulting in saturated fatty acids. The hydrogenation and deoxygenation reactions were enhanced under high hydrogen pressure and, in addition, isomerization also occurred (Snåre et al. 2008). Vegetable oil model compound such as oleic acid was hydro-deoxygenated over Pd/zeolite catalyst,

which was synthesized through microwave polyol method (Susanto et al. 2014). The reaction was performed at 375 °C and 400 °C and under 15 bar H_2 initial pressure; sample and catalyst ratio of 100:1 was applied. The obtained liquid products contain required density and viscosity. The deoxygenation process of oleic acid occurred through decarboxylation reaction (Susanto et al. 2014). Grosso-Giordano et al. (2016) investigated various modified and unmodified silane supports such as organosilane-modified silica or amino propylsilane modified or phenylsilane modified or unmodified silica supported palladium catalyst for deoxygenation of stearic acid under inert atmosphere at 300 °C. Capping silanols with phenylsilane is ineffective, whereas co-deposition of phenylsilane and aminopropyl silane before Pd incipient-wetness impregnation method enhanced the heptadecane yield more than 85% (Grosso-Giordano et al. 2016). Enhanced catalytic deoxygenation of fatty acids and vegetable oils over cubic mesoporous structure with a spacer group *Im3m* SBA-16 supported Pd was studied (Raut et al. 2016). The results showed that above 320 °C, 5 h reaction time, 3% palladium content on silica surface enables the complete conversion of feedstock through hydrodeoxygenation and decarboxylation due to higher surface hydrophobicity and metal dispersion (Grosso-Giordano et al. 2016). The Lewis acidity of catalyst enhances C–O bond breaking in hydrogenation or hydrodeoxygenation of fatty acids and waste cooking oils (Liu et al. 2018). A bi-functional Ir-ReO_x (Re/Ir = 3) supported SiO_2 Lewis acid catalyst was used for hydrotreatment of vegetable oils and waste cooking oil under 20 bar H_2 at 180 °C for 3 h (Liu et al. 2018). The obtained results indicated that yields up to 85% of diesel range alkanes under mild conditions with combined effect of Ir and ReO_x with SiO_2 could be achieved (Liu et al. 2018).

Hydrodeoxygenation of stearic acid and jatropha over Ru-based bi-functional catalysts with different supports: HZSM-5, ZSM-5, SiO_2-Al_2O_3, SiO_2, ZrO_3, Mg $(OH)_3$, La $(OH)_3$ and La_2O_3 was tested. Out of all these supports, La $(OH)_3$ showed remarkable performance at 200 °C, 40 bar of initial hydrogen pressure for 8 h and 98 wt.% and 80.7 wt.% of liquid hydrocarbons were obtained, respectively (Guo et al. 2015). Reaction path hydrogenation-decarbonylation: Murata et al. were hydrotreating jatropha oil over Pt based catalysts with different supports, H-H-ZSM-5, H-MOR, γ-Al_2O_3, USY, Beta, and SiO_2 with a ratio of jatropha/catalyst of 1:1 (Murata et al. 2010). Among these, Pt/H-ZSM-5 was very active at 270 °C and 60 bar of hydrogen pressure in transforming of jatropha oil. To enhance the catalytic activity of Pt/H-ZSM-5, it was modified with rhenium as Pt-Re/Al_2O_3 (Murata et al. 2010). The Pt-Re based catalysts are used in naphtha reforming and other industrialized processes. Under similar conditions, Pd-Re/Al_2O_3 was also active, but Pd based catalysts were active over hydrodeoxygenation via decarboxylation-decarbonylation reactions and exhibit poor C–O bond breaking capacity (Murata et al. 2010). The triglyceride conversion into hydrocarbons over catalysts decreased as Pt-Re/H-ZSM-5>Pd-Re/ H-ZSM-5>Re/ H-ZSM-5 >Ru-Re/ H-ZSM-5 > Co-Re/H-ZSM-5 > Ni-Re/ H-ZSM-5 > Pt/ H-ZSM-5 (Murata et al. 2010). The bimetallic function of Pt and Re plays an important role in hydrotreating reactions (Murata et al. 2010). Hydrodeoxygenation of triglycerides over novel catalytic property of $NbOPO_4$ supported Pd was applied to produce diesel range alkanes

under mild conditions with high selectivity, without catalytic any notable catalyst deactivation (Xia et al. 2016). The unique structure of $NbOPO_4$ with strong Lewis acidity combined with palladium enhanced the mass yield of fuel range alkanes (Xia et al. 2016).

The hydrotreatment of oleochemicals over noble metals is an expensive way due to cost of metal and deactivation of the support by poisoning, assembly of cracking species, leading towards the selection of other transition metals or transition metal composites, mainly Ni, Mo or Co with supported zeolites and sulphided catalysts (Table 18.2). The hydrogenation catalyst Raney nickel was used as a hydrotreating catalyst in palm crude oil transformation into hydrocarbon under 90 bar hydrogen initial pressure at 360 °C for 5 h and resulted in the formation of hydrocarbons ranging from 54% to 60% (m/m) of C_{15}–C_{17} due to pyrophoric nature of Raney nickel (Table 18.2, entry 3) (Studentschnig et al. 2013). The Mo based sulphided catalysts were very active at an optimum temperature of 200 °C and the catalytic performance was drastically decreased at 400 °C (Valencia et al. 2019). Wang et al. (2018) investigated the hydrotreatment of vegetable oil and FAME over Mo_2C/AC and the activity performance was compared with different catalytic materials such as MoO/Al_2O_3, MoS_2/Al_2O_3, Mo/Al_2O_3, NiP/Al_2O_3, Ni/Al_2O_3 or MoO/AC. Among all, Mo_2C/AC showed high catalytic activity giving complete conversion and 21.01% cracking ratio under 30 bar H_2 at 370 °C for 3 h (Wang et al. 2018). The characterization results of the spent catalysts showed that oxidization of Mo_2C occurred leading to the transformation of MoO_2 to MoO_3, which resulted in catalyst deactivation through formation of coke on the surface of the catalyst (Wang et al. 2018).

Bi-functional NiMo catalyst was used in hydrotreatment of distilled tall oil (DTO) into hydrocarbons in a continuous flow reactor at 325–450 °C (Anthonykutty et al. 2013). The fraction of hydrocarbons in treated DTO is about 50 wt.% paraffins (nC_{17}+C_{18}) obtained at 400 °C through hydrodeoxygenation, hydrodecarboxylation/decarbonylation and hydrodecarbonylation reactions, and the catalyst was stable up to 30 h (Anthonykutty et al. 2013). However, non-selective deoxygenation and cracking occurred at high temperatures (Anthonykutty et al. 2013). The catalytic activity of NiMo was enhanced by supporting on zeolites. Strong acid sites in H-Y, H-ZSM-5 are active for formation of gasoline ranged (C_5–C_{10}) hydrocarbons (Liu et al. 2011). The hydrotreatment of vegetable oil was studied over $NiMo/SiO_2$ or $NiMo/SiO_2$–Al_2O_3 under 40 bar hydrogen at 350 °C. Although $NiMo/SiO_2$ forms long-chain hydrocarbons such as n-C_{18}-C_{38}, n-C_{17}-H_{36}, n-C_{16}-H_{34} with a high pour point of 20 °C, $NiMo/SiO_2$-Al_2O_3 used for the hydrodeoxygenation of vegetable oil, on the other hand, resulted in the formation of hydrocarbons with a low pour point −10 °C via transforming some C_{15}-C_{18} n-paraffins to *iso*-paraffins and light paraffins due to appropriate acidic strength (Liu et al. 2011). Traditional catalysts such as $CoMo/Al_2O_3$ and $NiMo/Al_2O_3$ prepared through wet-impregnation method and used in hydrotreatment of jatropha oil under optimum conditions 30 bar H_2 at 380 °C for WHSV (weight hourly space velocity) 2 resulted in a conversions of 97% and 88.6% and hydrocarbon yields were 62.6% and 63%, respectively (Patil and Vaidya 2018). However, when compared with commercial catalysts, these

Table 18.2 Hydrotreatment of oleochemicals over transition metal supported catalysts or transition metal supported bimetallic composites

S. No.	Feedstock	Catalyst	Reaction conditions	Yield	References
1	Jatropha oil	Ni-Mo/Al_2O_3	350 °C, 50 bar H_2, LHSV 2 h^{-1}	[a]97.9 wt.%	Kumar et al. (2010)
2	Methyl Hexadecanoate	7 wt.% Ni-HZSM-5	220 °C, 40 bar H_2, 3 h	[a]90 wt.%	Shi et al. 2012)
3	Palm oil	Raney Nickel	360 °C, 90 bar H_2, 5 h	[a]60 wt.%	Studentschnig et al. (2013)
4	Fatty acids and triglycerides	Ni-Al (Layered Double Hydroxide)	350 °C, 10% H_2/N_2, 6 h	[a]42 wt.%, [a]90 wt.%	Santillan-Jimenez et al. (2014)
5	Stearic acid	5 wt.% Ni/H-Y-80	300 °C, 30 bar H_2, 6 h	[a]94 wt.%	Hachemi et al. (2017)
6	Stearic acid	NiCu/SiAl	300 °C, 40 bar H_2, 3 h, decane	_	Rafiani et al. (2020)
7	Jatropha, palm oil, canola oil	Ni-Mo/SiO_2-Al_2O_3	350 °C, 40 bar H_2, LHSV 7.6 h^{-1}	[a]83.5 wt.%, [a]81.4 wt.%, [a]82.1 wt.%	Liu et al. (2011)
8	Sunflower oil	20 wt.% Mo_2C/CNT	260 °C, 20 bar H_2, 3 h, hexane	[b]90%	Han et al. (2011)
9	Jatropha oil	NiMoLa/Al_2O_3	370 °C, 35 bar H_2, LHSV 0.9 h^{-1}	[a]78 wt.%	Liu et al. (2012a)
10	Palm oil	$NiMoS_2$/γ-Al_2O_3	300 °C, 50 bar H_2, LHSV: 1–2 h^{-1}	[a]95.5 wt.%	Srifa et al. (2014)
11	Canola oil	NiMoS	375 °C, 90 bar H_2, 8 h	[a]96.7%	Zhang et al. (2014)
12	Tall oil	NiMo/Al_2O_3	325 °C, 50 bar H_2, WHSV 1 h^{-1}	[a]90%	Anthonykutty et al. (2015)
13	Oleic acid	Cu–WO_x/Al_2O_3	300 °C, 30 bar H_2, 1 h	[a]93%	Janampelli et al. (2020)
14	Palm oil	Fe/natural zeolite	375 °C, 12 bar H_2, 2 h	[a]86%	Putra et al. (2018)

LHSV liquid hourly space velocity, *WHSV* weight hourly space velocity
[a]Total liquid hydrocarbons (C_{11}–C_{20})
[b]Lipid conversion

catalysts were less active (Patil and Vaidya 2018). The reaction temperature was reduced by applying a supercritical fluid medium, CO_2, propane or *n*-hexane over CoMo/γ-Al_2O_3. Out of these gases, propane actively reduced reaction temperature up to 57%, and suppressed the formation of coke and water. As a result, the catalyst lifetime also increased (Kim et al. 2014). The deoxygenation of oil proceeds more via decarbonylation and decarboxylation with a lower H_2 consumption than upon the

hydrodeoxygenation pathway (Kim et al. 2014). In terms of environmental aspects, human health point view, sulphurization of catalyst during hydrotreatment process raises issues, which can be excluded by applying lanthanide or actinide metals combined with transition metals such as Ni, Mo or Co supported on alumina. The next generation investigation was performed by Liu et al. (2012a). Hydrotreatment of jatropha oil over non-sulphided NiMoLa5.0/Al_2O_3 catalyst, at an ideal temperature of 370 °C under 35 bar H_2 initial pressure for WHSV (weight hourly space velocity) 0.9 gave rise to 94% selectivity, 83% conversion and 78% yield of C_{15}–C_{18} hydrocarbons Dividing to the gas and liquid phase distributions showed that the hydrotreatment process followed two paths. Whereas hydrodeoxygenation (HDO) resulted in the formation of a combination of C_{16}, C_{18} hydrocarbons and water (Liu et al. 2012a), another path was hydrodecarboxylation (HDC) which comprises decarbonylation and decarboxylation, resulting in the formation of odd number of hydrocarbons C_{15}, C_{17} with CO and CO_2 (Liu et al. 2012a). Thus, these results revealed that the increasing La-content was proportional to the ratio of (C_{15}+C_{17})/(C_{16}+C_{18}) hydrocarbons, indicating that HDC path was preferred when increasing La-content compared with HDO path (Liu et al. 2012a).

In the second phase, the focus was mainly on the conversion of normal alkanes to branched alkanes such as iso-alkanes formed via hydroisomerization. The straight chain normal alkanes enhance the viscosity and thermal stability of the fuel (Liu et al. 2018). In spite of this, they have some negative consequences on other fuel properties such as pour point and freezing point. Therefore, the fuel needs to be enriched via hydroisomerization to obtain branched-chain alkanes, which have decreased pour point and increased viscosity index, resulting in improved cold-flow properties, whereas other fuel properties remained the same (Liu et al. 2018). The fuel specification of cold-flow property generally depends on the geographical region where the fuel is used; the temperatures might change from tropical region −3 °C to cold region −35 °C (Mäki-Arvela et al. 2018). On the other hand, hydroisomerization occurs all the time collectively with hydrocracking as a consequential reaction, decreasing the yield of isomerized products (Jaroszewska et al. 2019). The products of hydroisomerization include light hydrocarbons (C_1–C_5), gasoline (C_5–C_8), jet fuel (C_9–C_{14}) and diesel (C_{15}–C_{18}) (Mäki-Arvela et al. 2018). Owing to these properties in the refinery industry for generating high quality bio-jet fuel being continued the search of more active, stable and selective catalysts.

Bi-functional catalytic materials have been used since more than five decades for hydroisomerization of long-chain paraffins (Batalha et al. 2013; Gomes et al. 2017; Mäki-Arvela et al. 2018). In bi-functional catalyst, the role of metal is to deliver hydrogenation or dehydrogenation reactions, whereas acid sites of support are accountable for skeletal isomerization. The hydroisomerization mechanism of *n*-alkanes starts via dehydrogenation of *n*-alkane on a metal site developing an alkene, subsequently a carbenium ion is formed, which is isomerized on an acidic site to an iso-olefin via formation of an iso-carbocation, and as the result, bi- or multi-branched alkanes are formed (Mäki-Arvela et al. 2018; Jaroszewska et al. 2019). The produced alkylcarbenium ions can also undertake β-scission reaction forming cracking products (Hengsawad et al. 2018; Mäki-Arvela et al. 2018) The important challenge is to

prepare bi-functional catalysts by means of outstanding catalytic implementation for complex synergistic effects between metal and Brønsted acid sites (Wang et al. 2019). This can be overwhelmed by the frequently used approaches, for example, the development of metal-acid balance and the synthesis of hierarchical acid supports (Lee and Ihm 2013; Batalha et al. 2015; Hengsawad et al. 2018; Mäki-Arvela et al. 2018; Jaroszewska et al. 2019; Wang et al. 2019). The preferable selection of catalyst for hydroisomerization of *n*-paraffins (C_9–C_{14}) is that the support has lower acidity when compared with catalyst applied for hydroisomerization of short chain *n*-paraffins (C_5–C_8). It is preferable to obtain long-chain alkanes while decreasing the isomerization selectivity. Most preferred hydrogenation metals for hydroisomerization are Pt or Ni supported on $SiO_2-Al_2O_3$, WO_3-ZrO_2, ZSM-22, SAPO-11, H-BETA or mesoporous ZSM-48, all of which have been applied as acid supports (Busto et al. 2013; Bauer et al. 2014; Regali et al. 2014; Miller et al. 2016; Thaker et al. 2016; Zhang et al. 2016). SAPO-11 is a moderate acidic zeolite which is widely used as a support in hydroisomerization of *n*-alkanes. A sulphided hollow tubular NiMo supported on SAPO-11 (0.4 Si/Al molar ratio) catalyst was applied to obtain the HDO product of castor oil hydroisomerization of *n*-alkanes (C_{15}–C_{18}) at 350 °C under 30 bar hydrogen pressure and H_2/oil ratio 800 with 1 h^{-1} liquid hourly space velocity (LHSV). This gave rise to 97.2% conversion and 81.6% selectivity towards jet fuel range hydrocarbons (Xing et al. 2019). The results indicated that when increasing the temperature proportionally, the hydroisomerization and hydrocracking were enhanced (Xing et al. 2019). The length of carbon chain is decreasing with increasing the temperature, no matter conversion, hydrocracking degree or isomerization tendency (Xing et al. 2019). Environmental issues encourage to abandon sulphided catalyst and non-sulphided catalysts should be applied. A single step hydrotreatment process of soybean oil was applied combining deoxygenation and isomerization reactions over a bi-functional Pt/zeolite catalyst. The catalyst synthesized with SAPO-11 and ZSM-22 zeolites as supports facilitated direct production of high quality diesel with a low pour point at 357 °C, under 40 bar hydrogen and 1 h^{-1} (LHSV), giving complete soybean oil conversion with 80% organic yield, with 100% alkane yield and 63% *i*-alkane selectivity (Wang et al. 2012). When using less strong Lewis acid sites of bi-functional catalysts, the reaction proceeds through decarboxylation and decarbonylation pathways (Wang et al. 2012). If the Pt/zeolite catalyst support with a low acidity is applied, the temperature needs to be high to attain high conversion. Brønsted acidic sites on the catalysts are responsible for the isomerization selectivity, whereas Lewis acid sites play a significant role in hydrodeoxygenation of soybean oil (Wang et al. 2012). Hydroisomerization of *n*-hexadecane was investigated over shaped Pt supported on AISBA-15 catalyst under continuous flow of 50 bar hydrogen at 320–360 °C with 3.5 h^{-1} (LHSV) (Jaroszewska et al. 2017). AISBA-15 (Aluminium incorporated SBA-15) was prepared by using aluminium isopropoxide, and shaped with binder, the Pt/AISBA-15 showed good isomerization selectivity. However, alongside it delivers high cracking products (Jaroszewska et al. 2017). To minimize the amount of cracking products and enhance the selectivity to isomerization, a bimodal composite material containing Pt/AISBA-15, a zeolite was used, where AISBA-15 with

20 wt.% of BEA zeolite synthesized through a gel method (Jaroszewska et al. 2019) and AISBA-15 with 25 wt.% Pt/ZSM-22 as a *co*-catalyst (Jaroszewska et al. 2020). The results indicated that Pt/AISBA + zeolite showed a high yield of mono-branched alkanes and a low yield of cracking products was obtained (Jaroszewska et al. 2019). Nevertheless, Pt/AISBA-15 with a *co*-catalyst resulted with 67% mono-branched isomers under 300–370 °C and 50 bar H_2, 3.5 h^{-1} (WHSV)) (Jaroszewska et al. 2020). Liu et al. investigated hydrotreatment of castor oil over Ni based bi-functional catalyst supported on (3-aminopropyl)-triethoxysilane (APTES) modified MCM-41/USY composite to produce aviation fuel range alkanes (Liu et al. 2015a). The reaction was conducted in a continuous flow fixed bed micro-reactor under 30 bar H_2 at 300 °C and 2 h^{-1} WHSV, with a 160 mL min^{-1} H_2 flow rate and obtained 91.6 wt.% of aviation fuel range alkanes with a high isomer/*n*-alkane ratio of 4.4 (Liu et al. 2015a).

An interesting research was conducted by Sun et al. via applying a Keggin type, W-based heteropolyacid on mesoporous MCM-41 supported Pt catalyst for the selective cracking and isomerization of *n*-alkanes. The reaction was performed in a tube with two piles of quartz sand and a flow rate of 1 h^{-1} (WHSV) whereupon about 20 wt.% of jet fuel over Pt/40 wt.% HPMo/MCM-41 was obtained. The obtained results showed that a high number of Brønsted acid sites with few strong acid sites promotes selective cracking and isomerization. The stability of the catalysts lasted for about 100 h (Sun et al. 2020).

18.2.1.3 Feedstock Availability

According to the bio-based updated news (2018–2019), the world-wide production of oleochemical feedstocks is about 204 Mt, which consist of 188 Mt of vegetable oil, 19.7 Mt of animal fat, micro algae and tallow oil (BBN 2018). These oils have been used in the production of special chemicals—polymers, polyamides, polyurethanes, polyethers, polyolefins, epoxy resins, surfactants, synthetic lubricants, plasticizers and other speciality chemicals—drilling fluid, hardener, fragrance, cosmetics, general purpose chemicals, fine chemicals and medicines (Jeon et al. 2019). Out of these products, conventional biofuels produced world-wide ca. 5 billion liters through the conversion of oleochemicals such as vegetable oils, used cooking oils, animal fats and other lipids to fully saturated products via hydrotreatment pathways (van Dyk et al. 2019). Despite this, procurement of large quantities of sustainable oleochemical based feedstocks is not economically feasible (van Dyk et al. 2019). The production of bio-jet fuel through hydrotreatment of vegetable oil mainly produces renewable diesel (van Dyk et al. 2019). Bio-jet fuels produced through oleochemicals hydrotreatment in a straight line compete with renewable diesel; where there is more demand to develop supply chains (van Dyk et al. 2017). As discussed previously, some vegetable oil based feedstocks have a price per ton that is higher than the fuels and is also competing with supply chains (van Dyk et al. 2019). To overcome these price and sustainability issues connected with oleochemical conversion pathways, several industries have accepted to use waste oleochemical

based feedstocks such as cheap used cooking oil and animal fats. However, the availability of these feedstocks is limited (van Dyk et al. 2019). Alternatively, lipid feedstocks such as jatropha, camelina, carinata, etc., which can be commercially grown on marginal lands to reduce the land usage matters are a viable option. Despite many efforts, generation of alternative feedstock supply chains has turned out to be a challenge and feedstocks are only partially accessible (van Dyk et al. 2019).

18.2.2 Advanced Bio-Jet Fuels

High amounts of bio-jet fuels are produced from oleochemical based feedstocks. Nevertheless, due to the availability and sustainability issues of the feedstock and competition between bio-diesel and bio-jet demands, one is unable to satisfy the demand requirements. The oleochemical based bio-jet fuel will not bring into being all future demands, but the core technology can be applied to set up the preliminary supply chain. Long-standing targets can only be achieved by utilizing advanced technologies such as thermochemical technologies (gasification-FT synthesis, pyrolysis-FT synthesis and liquefaction); however, it will likely take about 5–10 years span to reach industrial maturity. The utilization of these technologies for lignocellulosic based feedstocks, which are generated from the forest, agriculture residues, municipal, industrial solid waste streams and algae, is termed as advanced bio-jet fuels.

18.2.2.1 Thermochemical Conversion Process

In the search of sustainable feedstocks for the production of advanced bio-jet fuel, a second-generation lignocellulosic biomass will be an acceptable choice. The lignocellulosic material is a combination of cellulose, hemicellulose and lignin. These can be classified as follows: cellulose is a long-chain polymer of D-glucose and hemicellulose containing branched heterogeneous five and six-membered carbon sugars. Lignin is a group of branched polymeric phenols such as sinapyl (S), coumaryl (H) and coniferyl (G) alcohols (Albersheim et al. 2010). Lignocellulosic feedstock is very abundant with a low cost and it is not competing with the food chain. The conversion of lignocellulosic biomass into bio-jet fuel is performed *via* biochemical or thermochemical processes. The advanced bio-jet fuel production pathways follow thermochemical conversion technologies, as an alternative to biochemical pathways. The reason is that middle distillates derived through biochemical pathways are more expensive for consumers outside the aviation field (van Dyk et al. 2017).

The thermochemical pathways are described in Fig. 18.2. The lignocellulose conversion mainly follows three different pathways, such as gasification via FT-synthesis, pyrolysis followed by catalytic upgrading of bio-oil and direct liquefaction followed by catalytic upgrading of bio-oil.

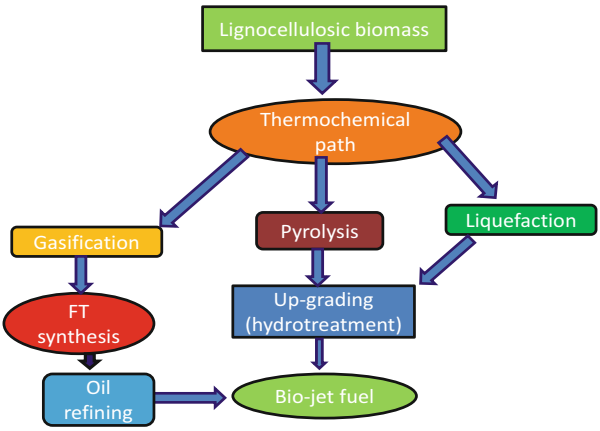

Fig. 18.2 Schematic diagram of bio-jet fuel production through thermochemical conversion techniques

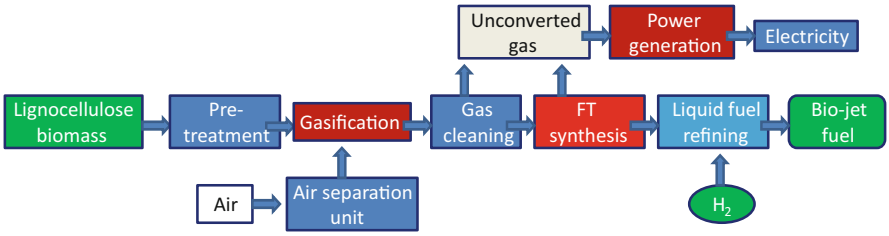

Fig. 18.3 Schematic diagram of FT-synthesis process to produce bio-jet (redrawn from (Wei et al. 2019))

Gasification-FT-Synthesis Followed by Liquid Fuel Refining

The schematic diagram of the Gasification Fischer–Tropsch synthesis followed by liquid fuel refining and can be seen in Fig. 18.3. In the first phase feedstock to gasification, feedstock must be dried and grinded to minimize the size of the wood particles. Thereafter, the pre-treated biomass is transferred into a gasification chamber. Various parameters, e.g. temperature, biomass species, particle size, heating rate, gasifying agent, operating pressure, equivalence ratio, reactor arrangement, catalyst addition, will affect the product and configuration of syngas (Samiran et al. 2014).

Gasification is the process to decompose long-chain hydrocarbons into small molecular weight gases such as CO, H_2, CO_2, H_2O and CH_4, formed via reforming and partial oxidation. Process requires heat and oxygen or steam as feed and, upon addition of heat, straight partial oxidation of fuel can occur (Li et al. 2004). Before the feedstock is fed into the gasifier, it needs to be dried so that moisture content would be ranging from 5% to 35% on wet basis. When increasing the temperature, small molecular weight molecules start volatilizing until about 200 °C (Basu 2010).

Pyrolysis reactions start ranging from 200–600 °C, whereupon exothermic dehydration of biomass starts in the range of 100–300 °C, resulting in the formation of water, CO and CO_2 by-products. Above 300 °C, cracking of volatiles into char and non-condensable gases occurs. The main product obtained during the pyrolysis stage is tar with high viscosity and acidity. Tar formation via condensation of high molecular weight molecules is frequently produced from aromatic hydrocarbons and creates major issues during gasifying and in reactor maintenance. Above pyrolysis temperatures, the gasification process starts at around 1300 °C. Gasification includes thermal breaking of large hydrocarbons into small condensable and non-condensable molecules (Di Blasi 2008; Basu 2010; Kwiatkowski et al. 2014). The available thermogravimetric analysis data gives the decomposition temperatures of cellulose at 275–350 °C, hemicellulose at 150–350 °C and lignin at 250–500 °C (Basu 2010; Kwiatkowski et al. 2014). The resulting equation below explains an average net pyrolysis reaction and main pyrolysis products (Di Blasi 2008).

$$\underset{\text{Biomass}}{CH_{1.46}O_{0.67}} \rightarrow 0.71\,\underset{\text{Bio-oil}}{CH_{1.98}O_{0.76}} + 0.21\,\underset{\text{Char}}{CH_{0.1}O_{0.15}} + 0.08\,\underset{\text{Gas}}{CH_{0.44}O_{1.23}}$$

The reactor system for the gasification of wood biomass generally occurs in a fluidized bed or moving bed (or fixed bed) or in a continuous flow system. The available reactor systems for gasification can be found in open literature (Li et al. 2004). Removal of ash and tar can be performed with direct-quench of syngas cooling system next to the gasifier (Williams et al. 2009). Then after the reaction (Fig. 18.3), the product syngas enters into an acid gas scrubber system, which also removes CO_2, H_2S and sulphides. The elimination of CO_2 and H_2S enhances the process kinetics and catalyst lifetime. Thereafter the clean gas enters a conditioning system to maintain the ratio between H_2 and CO with the help of water-gas-shift reaction. In FT synthesis (Fig. 18.3), the ratio of CO and H_2 is important. The main products are $C_nH_{2n}O_2$, $C_nH_{2n}O$, $C_nH_{2n+2}O$, C_nH_{2n+2} and C_nH_{2n} obtained upon FT synthesis and unconverted syngas might be reprocessed in the FT reactor after upgrading. The obtained liquid products need to be refined in order to obtain the required fuel quality. Electricity will be produced with the unprocessed gas from FT-synthesis.

Pyrolysis Followed by Catalytic Upgrading of Bio-Oil

The process of wood biomass decomposition into bio-oil in the absence of oxygen, at high temperatures and in a short span of time is termed as pyrolysis. The resulted product is a mixture of vapours, liquids and solids (coke and ash). The obtained "bio-oil" can be used for the production of fuels and used as a source of different platform molecules. By changing the process conditions, various properties of bio-oils can be obtained (Collard and Blin 2014; Kan et al. 2016; Dhyani and Bhaskar 2018).

In this section, specific interest is given to pyrolysis classification and especially to fast pyrolysis. Desirable technologies to enhance the bio-oil yield are pyrolysis and fast pyrolysis prior to production of aviation fuels. The process of pyrolysis can

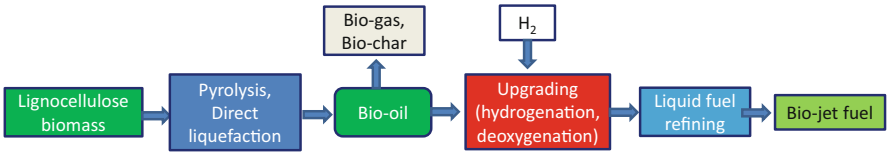

Fig. 18.4 Schematic diagram of pyrolysis or direct liquefaction process to produce bio-jet (redrawn from (Wei et al. 2019))

be divided into slow, fast and flash pyrolysis based upon the heating rate and residence time. The process of slow pyrolysis conducts at a slower heating rate, with moderately long solid and vapour residence times varying from 0.1 °C to 1 °C and 5–30 min. The long reaction time in the reactor leads to the formation of secondary reactions and results in greater percentage of char. The formation of char during pyrolysis affects the quantity of bio-oil fraction extensively. The slow pyrolysis is conventionally used for the production of charcoal (Mohan et al. 2006; Jahirul et al. 2012). Hence, if the target product is bio-oil, slow pyrolysis is not the desired method for the conversion of biomass into bio-oil.

The elevated technique of pyrolysis is termed as fast and flash pyrolysis. Upon fast pyrolysis, rapid heating rates and high temperatures (10–200 °C/s and 500–900 °C) together with shorter residence time (0.5–5 s) are applied. Pyrolysis is used for the conversion of biomass into bio-oil and process factors are cautiously coordinated to obtain high oil yield. The motive of fast pyrolysis is to avoid advanced cracking of pyrolysis products and it can be more challenging to increase the bio-oil yield *via* changing process parameters directly upon fast volatilization of the solid biomass (Bridgwater 2012). Flash pyrolysis can be achieved at high temperatures (800–1000 °C), using very fast heating rate and short residence time (1000 °C/s, < 0.5 s), and feedstock must be composed of much smaller particles ($<$ 0.2 mm). Flash pyrolysis is challenging to be used in industrial scale and to design the reactor system is difficult since the input biomass can reside for a very short time under extremely high heating rate. The major drawback of the obtained bio-oil is its instability and corrosiveness. These properties are harmful for the reactor system, and moreover deeply enhance the char/ash development. The presence of char in bio-oil can increase the viscosity of bio-oil by enhancing formation of polymerized products in bio-oil (Scott et al. 1985; Horne and Williams 1996; Radlein and Quignard 2013). Consequently, fast pyrolysis is suitable method to produce adequate amounts of bio-crude for the production of green aviation fuels. A schematic flow sheet for production of bio-jet fuel is depicted in Fig. 18.4.

Direct Liquefaction of Wood Biomass Followed by Catalytic Upgrading

Direct liquefaction or hydrothermal liquefaction (HTL) of wood biomass into bio-oil is an another interesting thermochemical approach. In earlier times, the wood biomass was directly liquefied in water used as a solvent at supercritical point, under H_2 atmosphere and using 1–3 h residence time. At supercritical point water

acts as a hydrogen donor, however, the critical point of water is about 374 °C with 220 bar internal pressure which renders the process difficult to operate. Thus, an alternative solvent with a low critical temperature and pressure with hydrogen donating property, at a critical point, is needed. Methanol, ethanol, 2-propanol and tetraline have been identified as suitable solvents for liquefaction of wood biomass into bio-oil (Akhtar and Amin 2011). In the pyrolysis process whole lignocellulosic biomass has been utilized, whereas in direct liquefaction process, hemicellulose, and lignin have been underexploited and residual cellulose from the wood has been consumed as an initial material in pulping and packing industry. There is no need to pre-dry to the feedstock; wet-biomass can be applied directly in the reactor in the presence of solvent. Before the start of the reaction, reactor needs to be flushed with an inert gas to remove residual oxygen. Thereafter the reactor is filled with hydrogen gas and heated up to the required temperature whereupon wood fractionation starts. After fractionation the reactor is rapidly cooled down to the room temperature and liquid samples are collected for further processing (Jogi et al. 2019a). The main reactions occurring during the liquefaction were depolymerization and decarboxylation of wood biomass. The production of green aviation fuel follows direct liquefaction of wood biomass into crude bio-oil and further hydrotreatment applied to bio-oil followed by liquid fuel refining (Fig. 18.4). The direct liquefaction process is equivalent to the fossil fuel formation under the earth-crust. The first pilot scale liquefaction process has been demonstrated in late 1970s at the U.S. Bureau of Mines Pittsburgh Energy Research Centre (PERC) (Lindemuth 1978). In that process dried wood biomass (sieved to about 300μm) was used and mixed with plant-recycled oil, processed in the presence of aqueous Na_2CO_3 as a catalyst at 350 °C under CO gas atmosphere and using 25 min runtime, bio-oil and flue gases were obtained in this process as the main products (Lindemuth 1978).

18.2.2.2 Catalysis over Heterogeneous Catalysts

High quality bio-oil and further green aviation fuel via catalytic thermochemical conversion techniques over heterogeneous materials such as gasification-FT-synthesis, fast pyrolysis and direct liquefaction have been studied in recent years as discussed below.

Catalytic Gasification and FT-Synthesis

In biomass gasification, specific catalyst properties are required to enhance the conversion of biomass and cracking of tars. In-house prepared catalysts can be used simply in the fluidized bed gasifier to enhance conversion, reaction rates, to diminish tar content, and regulate the final gas streams. In addition, blast furnace slag can also increase the gasification of char, tar cracking, and participate in the reforming of hydrocarbons in a moving-bed biomass gasifier (El-Rub et al. 2008; De Lasa et al. 2011; Luo et al. 2012). The operating conditions and process parameters depend on the catalyst properties (Lindemuth 1978; Jogi et al. 2019a).

The selection of catalyst depends on its methane reforming capacity, efficiency to crack tars, support to obtain the required syngas ratio, catalyst stability-regeneration capacity and price (Sutton et al. 2001).

Two different types of efficient catalysts have been applied for gasification of the biomass, namely natural minerals (dolomite, olivine, clay minerals and ferrous oxides) and synthetic catalysts (alkali metal carbonates and transition metal-based catalysts). The catalytic gasification of biomass over natural minerals such as limestone, calcined dolomite and olivine in a fluidized bed reactor occurred at 800–1000 °C with 100 °C increments. The obtained results indicated that relative to limestone and olivine, 49.1% of H_2 (mole-fraction) was achieved at 1000 °C with 1.0 steam/biomass ratio using dolomite catalyst. In addition, high efficiency towards gas formation and tar cracking was observed (Tian et al. 2018). Yu et al. (2018) studied catalytic gasification of biomass components such as cellulose, hemicellulose and lignin, straw and pine over Na_2CO_3 and dolomite on a small-scale flow gasifier. Hemicellulose gasification was extensively enhanced and tar production also reduced over Na_2CO_3 and dolomite (Yu et al. 2018). However, gasification of hemicellulose occurred over Na_2CO_3, whereas this catalyst was not efficient for gasification of cellulose, lignin, straw and pine biomass. Dolomite was an active catalyst for gasification of cellulose, hemicellulose, lignin, straw and pine. Results indicated that the selection of catalyst is crucial and depends upon the biomass type used (Yu et al. 2018).

Different bi-functional and multi-functional heterogeneous catalysts have been applied upon gasification of biomass to produce syngas. The catalytic gasification of algal biomass was performed over alkali and alkaline earth metal-based catalysts including NaOH, $KHCO_3$, Na_3PO_4 and MgO (Ebadi et al. 2019). Among those catalysts, NaOH was a very efficient catalyst upon production of hydrogen gas above 900 °C. Tar cracking was enhanced at the same time with an enhancement of syngas calorific values (Ebadi et al. 2019). In addition, the catalyst particle size (0.5–0.25 mm) did not have any influence on the process (Ebadi et al. 2019). Highly acidic catalyst SAPO-34 was used to gasify biomass in order to achieve high amounts of lower hydrocarbons, where Ni promoted SAPO-34 was less active (Doan et al. 2020). Among various transition metals, Ni enhanced the water-gas-shift reaction and from that point of view various bi-functional and multi-functional catalysts have been prepared and applied such as Ni-La_2O_3/Al_2O_3 (Mazumder and de Lasa 2016), La-Ni/SBA-15 (Oemar et al. 2015), Ni/SLC (Wang et al. 2015), Ni-CeO_2/Al_2O_3 (Peng et al. 2017), CeO_2-NiO (Granados-Fernández et al. 2020) and Ni-Fe-Mg-Al (Watanabe et al. 2015).

In FT synthesis, several metals, e.g. Fe, Co, Ni and Ru are used as catalysts (Vannice and Garten 1980; Soled et al. 2003). Among these, Ru based catalysts showed efficient performance and good selectivities were obtained. Nevertheless, the high price of Ru has limited its general application (Steynberg et al. 2004). In industrial scale, mostly Fe and Co are used. Fe-based catalysts have a long space time, but, their life time is short. In case of Co-based catalysts, efficient carbon chain growth power was achieved and the obtained products have less oxygen compounds, and less carbon deposition occurred (Luque et al. 2012). To regulate the catalyst properties promoters such as alkali metals, alkaline earth metals, together with Cu

and other transition metals were used (Xu et al. 2005; Zhang et al. 2006; Li et al. 2016).

Catalytic Fast Pyrolysis

Upon fast pyrolysis, different zeolites have been applied to produce bio-oil with extreme superiority, despite the fact that zeolites with different pore size and Si/Al ratio can promote the formation of aromatics (Carlson et al. 2010; Stefanidis et al. 2011; Ben and Ragauskas 2013) and especially catalyst acidity promotes the deoxygenation reactions of oxygenates. The catalytic efficiency of zeolites has been increased via applying, *e. g.* Pd, Ni, Zn, Fe, Mo, Ga, and Co (Zhang et al. 2013; Li et al. 2014; Liang et al. 2017), to obtain high product selectivity. One of the major drawbacks was catalyst deactivation. Ni, Mo and Ga supported on H-ZSM-5 catalysts showed high selectivity (Vichaphund et al. 2014), whereas Fe supported on ZSM-5 showed better catalytic performance towards deoxygenation process and limiting repolymerization of monocyclic aromatic hydrocarbons (MAH) (Sun et al. 2016). High selectivity to benzene, toluene and xylene was obtained when using fresh active sites (Zhang et al. 2018). The enhancement of monocyclic aromatic selectivity with better energy value was obtained by applying modulated zeolites with the addition of *co*-catalyst such as ZnO or MgO, though Brønsted acid sites were reduced in the parent catalyst by the creation of Lewis acid sites (Rezaei et al. 2014; Fermoso et al. 2016; Widayatno et al. 2016). The selection of zeolite based catalysts is economically more viable than other heterogeneous catalysts. Microporous zeolites are less active due to fast deactivation, whereas hierarchal mesoporous zeolites are highly attractive for catalytic pyrolysis and large pore-zeolites are active for production of polyaromatics (Rahman et al. 2018). More information about catalytic fast pyrolysis of biomass can be obtained in references (Liu et al. 2014; Venderbosch 2015; Nishu et al. 2020).

Catalytic Liquefaction

The main purpose in catalytic liquefaction of wood biomass is to obtain the enriched bio-oil which is a blend of phenolic compounds and sugars. In order to get the fuel components and fine chemicals through the refining of bio-oil, hydrotreating is applied. The process of direct liquefaction of wood biomass has been performed under various heterogeneous catalysts such as Pd (den Bosch et al. 2015), Pt (Ouyang et al. 2019), Ru (den Bosch et al. 2015), Ni (den Bosch et al. 2017), Co (Rautiainen et al. 2019), CoMo (den Bosch et al. 2017) and Fe (Xu and Etcheverry 2008; Jogi et al. 2019b) supported on active carbon (den Bosch et al. 2015), alumina (den Bosch et al. 2017) and zeolites (Xu and Etcheverry 2008; Beauchet et al. 2011; den Bosch et al. 2015; Huang et al. 2016; den Bosch et al. 2017; Jogi et al. 2019b). The applied catalysts have been classified into non-noble metals (Ni (Li et al. 2012; den Bosch et al. 2017), Co (Rautiainen et al. 2019), Fe (Xu and Etcheverry 2008; Jogi et al. 2019b) and noble metals (Pd, Ru (den Bosch et al. 2015), and Pt (Ouyang

et al. 2019)). Upon catalytic liquefaction of biomass, metal plays a vital part to fractionation of biomass to phenolic compounds and metal selection regulates what kinds of products are formed (Table 18.3).

Catalytic liquefaction of biomass over non-noble metal catalysts has been extensively studied (Araya et al. 1986; Xu and Etcheverry 2008; Beauchet et al. 2011; Li et al. 2012; Ferrini and Rinaldi 2014; den Bosch et al. 2015; Huang et al. 2016; Renders et al. 2016; den Bosch et al. 2017; Jogi et al. 2019b; Ouyang et al. 2019; Rautiainen et al. 2019). Liquefaction of birch over Lewis acidic Ni/Al_2O_3 was

Table 18.3 Direct liquefaction of wood biomass over heterogeneous catalysts

S. No	Feedstock	Catalyst	Solvent	Reaction conditions	Yield (%)	References
1	Birch	Ni-W_2C/AC	Water	235 °C, 60 bar H_2, 4 h, 1000 rpm	[a]46.5	Beauchet et al. (2011)
2	Birch	Ni/Al_2O_3	Methanol	200 °C, 30 bar H_2, 3 h, 750 rpm	[a]44, [b]87	den Bosch et al. (2017)
3	Straw: wood: grass mixture	Raney Ni	Tetraline	330 °C, 16 bar H_2,15 min, 1000 rpm	[c]18	Beauchet et al. (2011)
4	Poplar	Raney Ni	2-proponal: water (7:3 v/v)	220 °C, no external H_2,3 h	[b]87, [c]26	Ferrini and Rinaldi (2014)
5	Birch	Co-Phenanthroline/C, HCOOH (co-solvent)	Ethanol: water (5:5 v/v)	200 °C, no external H_2, 4 h	[a]34	Xu and Etcheverry (2008)
6	Pine	Co-Mo/γ-Al_2O_3	Tetraline	400 °C, 100 bar H_2, 2 h	[c]36	Araya et al. (1986)
7	Jack pine	$FeSO_4$	Ethanol	350 °C, 50 bar H_2, 40 min	[c]63, [d]88	Rautiainen et al. (2019)
8	Birch	Fe/H-Beta-150	Ethanol	243 °C, 5 bar H_2, 1 h, 300 rpm	[c]25, [e]68	Jogi et al. (2019b)
9	Birch	Ru/C or Pd/C	Methanol	250 °C, 30 bar H_2, 3 h, 700 rpm	[a]48, [a]49 [b]90, [b]90	den Bosch et al. (2015)
10	Birch	Pt/γ-Al_2O_3	Methanol: water (1:2 mol/mol)	230 °C, 30 bar N_2, 3 h, 500 rpm	[a]46	Rautiainen et al. (2019)

[a]Phenolic monomer yield
[b]Pelignification yield
[c]Crude bio-oil yield
[d]Biomass transformation
[e]Total phenolic yield

performed in methanol (den Bosch et al. 2017). When liquefaction of wood was also performed in the absence of any catalyst, results showed that ether bond can cleave both in the presence and absence of catalyst, in the meantime the product lignin oil remains unaltered. However, monomer yields are enhanced over Ni/Al_2O_3 (den Bosch et al. 2017). The unsaturated intermediates were stabilized and additionally control the repolymerization of intermediates were suppressed (den Bosch et al. 2017). However, catalyst deactivation was taking place but it might be regenerated under thermal H_2 treatment (den Bosch et al. 2017). In another work (Beauchet et al. 2011), the liquefaction of green waste (straw: wood: grass mixture) was performed over Raney nickel catalyst at 320 °C in tetraline. This approach increased the gas yield due to dehydration of the solvent resulting in the formation of an enhanced bio-oil quality through hydrogenation. Furthermore, the catalytic activity was also confirmed upon liquefaction of wood in 2-propanol:water (7:3 v/v) solvent medium (Ferrini and Rinaldi 2014). Corresponding to these results (Beauchet et al. 2011; Liu et al. 2015b), the catalyst was responsible to transform lignin fragments into monomers through depolymerization and hydrogen transfer reactions during liquefaction of wood biomass. Likewise, wood fractionation was performed over $Ni\text{-}W_2C/AC$ and Co-phenanthroline/C (Rautiainen et al. 2019). The results by Rautiainen et al. (2019) indicated that among other catalysts Co catalyst showed high activity (Table 18.3). Upon one-pot hydrocracking of wood biomass over bimetallic $Ni\text{-}W_2C/AC$ catalyst, W_2C enabled the conversion of cellulose and hemicellulose along with lignin in water [142]. Both cellulose and hemicellulose were converted into valuable ethylene glycol and other diols with a sum of 75.6% yield although phenolic monomers were obtained especially through lignin conversion, giving about 46.5% yield [142]. Upon catalytic liquefaction of birch over Co-phenanthroline/carbon catalyst at 200 °C (Rautiainen et al. 2019), Co enables formation of reactive intermediates from organosolv treating via hydrogenolysis and hydrogenation reactions (Rautiainen et al. 2019). In addition, β-O-4 bonds were breaking via hydrogenolysis (Rautiainen et al. 2019). Co-Mo or Pt metal supported on $\gamma\text{-}Al_2O_3$ have also been applied as catalysts at 400 °C in tetraline and methanol: water (1:2 mol/mol) medium (Araya et al. 1986; Ouyang et al. 2019). Co-Mo and Pt metals boosted the hydrotreatment which leads to the formation of lighter liquid and gaseous products. In Ouyang et al. (2019) different catalysts, i.e. Pd/C, $Cu/ZnO/Al_2O_3$, Cu/CeO_2 or Mo_2C/AC were tested upon birch fractionation and these catalysts gave enhanced hydrogenation yields (Rautiainen et al. 2019). The catalytic liquefaction of wood has been investigated over both homogeneous and heterogeneous iron based catalysts such as $FeSO_4$ and Fe-H-Beta-150 (Beauchet et al. 2011; Huang et al. 2016; Jogi et al. 2019b). Liquefaction of wood over homogeneous $FeSO_4$ catalyst was performed in ethanol medium (sub and supercritical points) under 50 bar initial hydrogen pressure giving 88% of wood conversion (Xu and Etcheverry 2008). The obtained results revealed that Fe-based catalyst promoted bio-oil yields with a relative low gas yield (Xu and Etcheverry 2008). Moreover water and char were also formed. At high temperatures, hydrogen gas catalysed condensation reactions through the development of free radicals (Xu and Etcheverry 2008). In a liquefaction process, supercritical primary and secondary alcohols function as a hydrogen donor (Xu and Etcheverry 2008). In case of heterogeneous

catalyst, Fe-H-Beta-150 (150 denoted as SiO_2/Al_2O_3 ratio), liquefaction was performed under 5 bar hydrogen initial pressure in supercritical ethanol (Jogi et al. 2019b). The outcomes of the liquefaction have shown that low hydrogen pressure enhances the degradation of hemicellulose and lignin due to the presence of strong Brønsted sites of the Fe-H-Beta-150 catalyst (Jogi et al. 2019b).

Birch fractionation was also investigated over noble metal catalysts (den Bosch et al. 2015; Ouyang et al. 2019). Birch fractionation over Ru/C or Pd/C catalysts in a methanol medium gave about 50% yield including the sum of phenolic monomers, di- and oligomers [133]. Pd/C catalyst promotes the phenolic monomer yield (den Bosch et al. 2015). The sub-components of lignin were composed of $–CH_2OH$ replaced with ethylene bonds (den Bosch et al. 2015). The cleavage of C–H bonds is reliant on metal in a liquefaction process and it means that –OH comprising yield is higher over Pd/C when compared with Ru/C (den Bosch et al. 2015). *n*-propyl end-chain products form over Ru based catalysts, whereas the dimers are covering the *n*-propyl end-chains with structural identity over Pd/C (den Bosch et al. 2015). In addition, the higher hemicellulose degradation efficiency was achieved in Pd/C related to Ru/C (den Bosch et al. 2015). It was reported that a Pt/γ-Al_2O_3 based catalyst was responsible for the depolymerization of lignin and the development of hydrogen through methanol reforming (Ouyang et al. 2019), Pt/γ-Al_2O_3 is also powerful in terms of cleavage of α-O-4, β-O-4 bonds along with formation of unsaturated conferyl/sinapyl alcohol and oxidizing syringyl units in an inert atmosphere (N_2) (Ouyang et al. 2019). Birch liquefaction was also performed over a bimetallic Pd-W_2C/AC catalyst and the results showed that the catalyst stabilized the phenolic monomers of lignin via hydrogenation into −OH products. However, other noble metal-promoted catalysts promoted dehydroxylation reactions (Li et al. 2012). Upon wood liquefaction, a proper support selection is also a foremost task (Ouyang et al. 2019). According to the updated literature, γ-Al_2O_3 support was more unstable at high temperatures in aqueous medium when compared with carbon supports (Ouyang et al. 2019). Upon comparison of Pt/C with Pt/γ-Al_2O_3 under equal liquefaction conditions, the obtained phenolic monomer yields were 45% and 46%, respectively (Ouyang et al. 2019). Apparently, under equal wood liquefaction conditions, γ-Al_2O_3 was more unstable than carbon support (Ouyang et al. 2019). The enhancement of the delignification efficiency by the addition of a *co*-catalyst or a *co*-solvent along with heterogeneous catalysts is a more appropriate strategy. Different research groups have been working in that theme and the results can be seen in Table 18.4. Notably, high delignification efficiency of poplar was obtained over Pd/C with H_3PO_4 (Renders et al. 2016).

Feedstock Availability

In all-purpose, the possible amount of feedstock for aviation fuel will be constrained by the competing users from the other sectors to the same resources. Biochemical, power generation (electricity and heat) and other than aviation transportation sectors offer the best ecological or economic performance. However, when one tries to couple various methods, they have benefits and drawbacks as well (Pavlenko et al.

Table 18.4 Direct liquefaction of wood biomass over heterogeneous catalysts besides with different *co*-catalyst or *co*-solvent media

S. no	Feedstock	Solvent	Catalytic	Reaction conditions	Yield (wt. %)	Ref.
1	Poplar	Methanol	Pd/C, H_3PO_4 or NaOH (co-solvent)	200 °C, 20 bar H_2, 3h, 750 rpm	[a]96 or [a]85	Li et al. (2012)
2	Birch	Ethanol: water (5:5 v/v)	Co-Phenanthroline/C, HCOOH (co-solvent)	200 °C, no external H_2, 4 h	[b]34	Rautiainen et al. (2019)
3	Pine	Aqueous Phosphoric acid	$LiTaMoO_6$ layered over Ru/C	230 °C, 60 bar H_2, 24 h, 450 rpm	[b]28, [c]63	Ferrini and Rinaldi (2014)
4	Birch	Methanol	Pd/C combined Yb $(OTf)_3$	200 °C, 20 bar H_2, 700 rpm, 2 h	[b]55	Renders et al. (2016)

[a]Delignification efficiency of biomass
[b]Phenolic monomer yield
[c]Alkanes yield

2016). The supply of lignocellulosic material is ultimately limited by the world-wide land area. It has been reported in (Searle and Malins 2015) as the plausible edge that biomass availability by the year of 2050 is 60–120 EJ/year comprising energy crops, forestry, crop residues and wastes. This is the ultimate amount of biomass in a drastic scenario that allows for the utilization of unused grassland and savannah. Due to the competing users of biomass, its availability to biofuel production has been estimated as 10–20 EJ/year in 2050. The production of aviation fuels needs additional costs related to the road transport of biofuels. The process economics of aviation fuel depends on various variables, such as the composition of feedstock, cost of feedstock, product yield, process design and maturity of the technology (Milbrandt et al. 2013; Wang and Tao 2016). Edible, non-edible oils and animal fats require less processing compared to other feedstock because they contain triglycerides and fatty acids. Sugar and starch materials can be fermented to intermediate yields, and lignocellulose feedstock needs additional steps because they must be hydrolysed to simpler sugars, or turned into intermediate syngas or bio-oil. Waste and residues (municipal solid waste and waste gas) require the most complex processing conditions because of the nature of the feedstocks and the complexity of processing involved. The cost of feedstocks follows a different trend: waste and residues are the cheapest, whereas vegetable oils and fats are the most expensive (Diederichs et al. 2016). The expected production cost of lignocellulosic aviation fuel varies from 1000 to 8000 $/ton, but these definite price ranges are sometimes greater than the assessed values. Nevertheless, renewable aviation fuel has greater revenue range than conventional jet fuel, which is the reason behind to establishment of plants for the production of aviation fuel (Gill 2016b).

18.3 Environmental Challenges and Future Outlook

The increasing demand for alternative bio-jet fuel similarly needs to overwhelm more tasks to substitute bio-jet fuel instead of conventional jet fuel, *e.g.* feedstock availability and sustainability.

For the industrial scale range of bio-jet fuel production, the main challenge is the accessibility of sustainable feedstock. The sustainable feedstock should follow some of the important specifications, which include large yield with a long delivery window, easy to transport, should not be responsible for deforestation and not compete with food production. Along with feedstock, industrial scale production should be cheap and feasible (IREES 2010). Upon large scale production of bio-jet fuels, the feedstock accessibility is related to policies and, thus, research and development should be promoted. The main key-aspect in the production of bio-jet fuel is to increase feedstock production by providing minimum requirements such as land, quality of water and supplements (ICAO 2013).

Upon industrial scale production, a wide range of environmental, societal and economic outcomes set the boundaries. For instance, large scale biomass production may highly influence on the use of land, water in addition to biodiversity, soil degradation and significant variations in countryside society and native publics (ICAO 2013). In order to utilize bio-jet fuels in a sustainable way, some polices need to be developed, together with sustainable goals and carry out with devoted actions, particularly in terms of land use and food security in order to produce bio-jet fuels in a large scale (Kandaramath Hari et al. 2015). It might be so that only genetically modified energy crops can produce sufficient amount of feedstock in order to develop industrial scale production of bio-jet fuels and can also minimize the environmental concerns. These crops need to be grown very fast with a high portion of required biocomponents, such as cellulose, hemicellulose and lignin.

18.4 Conclusions

The development of greenhouse gas (GHG) emissions from the aviation sector can be minimized by applying the bio-jet fuels. These fuels can be produced from various biomass sources such as vegetable oils, animal fats, waste cooking oils, lignocellulose biomass, algae, etc. Regardless the route of bio-jet fuel production, the biomass transformation technologies play a vital role. Among these, we have here summarized conventional and advanced technologies, which include hydrotreatment of oleochemicals (HVO) and thermochemical conversion techniques.

The production of bio-jet fuel through hydrotreatment of vegetable oils (HVO) is a promising approach; however, it will suffer from the rivalry with the modern bio-diesel industry. In that case, it is important to develop bio-jet fuel from other than

oleochemical based feedstocks, such as lignocellulosic biomass, municipal waste and algae via advanced thermochemical conversion ways.

The production of aviation fuels through FT-synthesis approach is the first option to attain the required bio-jet fuels. The combined process of gasification and FT-synthesis is already a well-established process technique in coal gasification towards FT-synthesis. Therefore, there is no need of capital cost investment on this technique to develop it further. The produced jet fuel has some interesting properties; it is sulphur free, exhibiting high specific energy and high thermal stability while it emits lower levels of anthropogenic carbon. Nevertheless, there are also some negative impacts, such as lower energy density, lower aromatic content which results in bio-based jet fuel to be less competitive and to have a higher production price.

The production of bio-jet fuels through pyrolysis in industrial scale is challenging due to fact that bio-oil through fast pyrolysis contains about 40% of oxygen and, thus, there is a further need to hydrotreat the bio-oil with high amounts of hydrogen. It adds to the equipment and production costs. A distinct advantage of pyrolysis is that it can be performed inside the premises of a conventional oil refinery, thus minimizing the capital cost of the reactor system.

Building our quest for a sustainable process to produce green aviation fuel and in relation to other thermochemical conversion techniques, direct liquefaction of wood biomass might be a better approach than pyrolysis. In this process, one obtains bio-oil with less than 10% moisture content and the intermediate is easy to further process to retain bio-jet. The obtained residual cellulose from liquefaction can be used for the production of ethanol through biochemical methods or green hydrogen production through aqueous phase reforming.

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Chapter 19
Biogas Biorefinery

Sumitha Banu Jamaldheen and Arun Goyal

Abstract Biogas is a gaseous fuel comprising methane and carbon dioxide as major constituents. It is produced by microbial consortium of acidogenic, acetogenic and methanogenic bacteria in the absence of oxygen from the organic substrates through anaerobic digestion. Main advantage of the process is a variety of organic wastes like wastewater, municipal solid waste and agrowaste can be used as the feed. Anaerobic digestion can be integrated with other processes like fermentation or microalgae cultivation for liquid fuel production. Disposal of produced carbon dioxide and ammonia is the main challenge. Conversion of CO_2 to valuable product like methane and post-treatment of digestate from anaerobic digestion can make waste disposal safer for environment leading to a better circular economy. This chapter gives an overview of biogas production, integration of anaerobic digestion process with other processes to produce biofuels and chemicals. It also briefs the significance of post-treatment of digestate after anaerobic digestion for an improved biorefinery.

Keywords Anaerobic digestion · Biogas · Post treatment · Digestate · Biorefinery

Abbreviations

AD	Anaerobic digestion
CSTR	Continuous Stirred-Tank Reactor
MSW	Municipal Solid Waste
VFA	Volatile Fatty Acids

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ANR	Advanced/Autotrophic Nitrogen Removal
PN	Partial Nitritation
Anammox	Anaerobic Ammonium Oxidation
HRAP	High Rate Algal Ponds
TDS	Total Dissolved Solids
MEM	Mountain Effective Micro-organisms
PS	Polysulphone
PES	Polyether Sulphone
PVDF	Polyvinylidene Fluoride

19.1 Introduction

Anaerobic digestion (AD) is a highly explored biological process, where the organic substrates are converted into biogas under anoxic condition. The steps involved in biogas production process are hydrolysis followed by acidogenesis, acetogenesis and methanogenesis (Sarker et al. 2019). Hydrolysis of the complex organic matter is carried out by hydrolytic enzyme secreting microorganisms. The hydrolysed products are then utilized by acidogenic bacteria from *Lactobacillus*, *Streptococcus* and *Clostridium* sp. to produce volatile fatty acids (VFA). Acetogenic bacteria convert the VFAs into acetate, CO_2 and H_2, which are then utilized by the methogenic bacteria to produce CH_4 and CO_2 (Moraes et al. 2015). The major constituents of biogas are methane (50–80%), CO_2 (20–50%) and H_2S (1%) (Chen et al. 2015). Various kinds of organic substrates originating from municipal solid wastes, agricultural wastes, industrial wastewater sludge, etc. can be fed for anaerobic digestion (Sarker et al. 2019).

Several municipal solid waste (MSW) based AD plants are being operated worldwide (Angelonidi and Smith 2015). Operation of AD plants at farm-scale level using second-generation energy feedstocks and animal husbandry wastes have been explored in the recent years (Holliger et al. 2017). This flexibility of AD process with the substrates makes it a sustainable option to integrate with various processes like ethanol fermentation or microalgae cultivation. This helps in producing multiple sustainable products such as ethanol, biodiesel, biogas, etc. from a series of processes (Bauer et al. 2009; Bose et al. 2020).

The two major challenges related to biogas biorefinery are CO_2 and H_2S removal from the biogas and production of a dischargeable quality effluent (digestate). Overcoming these challenges through biogas upgradation and post-treatment of the digestate can pave way for achieving more value-added products (Vyrides et al. 2010; Stangeland et al. 2017). The products include CH_4 from CO_2 methanation, lipid and protein, nitrogen, phosphorus and ammonium-based fertilizers, depending on the post-treatment, besides the eco-friendly water discharge (Vaneeckhaute et al. 2013). This chapter gives a detailed overview of the integrated

biogas biorefinery system and methods involved in biogas upgradation and post-treatment of anaerobic digestate.

19.2 Substrates for Anaerobic Digestion

Besides municipal solid and liquid wastes, agricultural wastes such as straw and bagasse, animal husbandry waste like piggery manure and microalgal biomass serve as the substrates for anaerobic digestion (Ekman et al. 2013; Molinuevo-Salces et al. 2016; Duan et al. 2019). For example, Microalgal biomass produced from a farm-scale piggery wastewater treatment has been used as a substrate for biogas production (molinuevo-salces et al. 2016). Chlorella and Dunaliella are the widely investigated microalgal species for their utilization as substrate for anaerobic digestion (Sialve et al. 2009; Collet et al. 2011). A comparative study involving microalgal strains, viz. *Arthrospira platensis*, *Chlamydomonas reinhardtii*, *Chlorella kessleri*, *Dunaliella salina* and *Scenedesmus obliquus* for their potential as a substrate for biogas production was carried out by Mussgnug et al. 2010. The results showed that the biogas production potential from microalgae is not specific to the classification of the organism but to the strain. For example, both *C. reinhardtii* and *D. salina* are from class Chlorophyceae. *C.reinhardtii* was the best substrate for the biogas production among the compared strains, whereas *D. salina* gave the lowest biogas yield (Mussgnug et al. 2010). Besides microalgae, the macroalgae such as *Macrocystis* sp., *Laminaria* sp., *Gracilaria* sp. and *Ulva* sp. also have been investigated for their potential as substrate for biogas production. *Laminaria* sp., *Macrocystis* sp. and *Gracilaria* sp. were able to produce methane from 300–500 dm^3 CH_4/kg organic dry matter (Dębowski et al. 2013). Black water from toilet can also be treated by anaerobic digestion to produce biogas (Gao et al. 2012). Depending on the total solid content of the feed, AD is classified as dry and wet AD. The dry solid content used in the dry AD is up to 40%, whereas the wet AD feed may contain up to 15% dry solid matter (Angelonidi and Smith 2015). The type of product from the biogas biorefinery system varies depending on the substrate used. For example, a farm-scale biorefinery utilizing second-generation energy feed stocks may focus mainly on producing electricity from biogas along with organic fertilizer from the digestate (Oleskowicz-popiel et al. 2012).

19.3 Role of Anaerobic Digestion in Biorefinery

19.3.1 Integration with Ethanol Production Unit

By-products such as Bagasse and liquid hydrolysate produced from sugarcane crop based first and second-generation bioethanol industry are anaerobically digested to produce biogas (Rabelo et al. 2011). Hydrothermal treatment like steam explosion of

bagasse helps in increasing the biogas yield and production rate in the above AD process. Sequential integration of ethanol and biogas production in this manner can lead to higher energy independence (De Paoli et al. 2011). Similarly, sequential ethanol and biogas production from a mixed substrate containing corn stover and swine manure can result in obtaining positive energy balance up to 5.5 MJ/Kg dry raw feed (MacLellan et al. 2013).

Wheat and other straw based biorefineries also have the potential of producing more than one fuel by integrating biogas unit with other processes (Ekman et al. 2013). Cellulose rich solid portion from the hydrothermally pretreated wheat straw serves as the substrate for ethanol production. The hydrolysate from the above pretreatment can be used for biohydrogen production and the waste coming from both the ethanol and biohydrogen production processes can be used as feed for anaerobic digestion to produce biogas. The overall energy output from the multiple processes is higher than the ethanol production process alone (Kaparaju et al. 2009). Ethanol fermentation of oat straw acts as an additional pretreatment for anaerobic digestion. The use of residual biomass after fermentation increases the biogas production rate and decreases the retention time (Dererie et al. 2011). In the biorefinery systems using second-generation ethanol feedstocks, mesophilic condition is favourable for anaerobic digestion to operate at higher substrate loading rate. It is also notable that the suspended solid concentration of the influent is directly proportional to the retention time of the anaerobic digestion. Hence, removal of suspended solids from the effluent will aid in reducing the retention time for biogas production (Uellendahl and Ahring 2010).

The ethanol industry also produces a huge volume of wastewater, called vinasse during the distillation process right after fermentation. Vinasse contains non-fermentable organic matter that is responsible for the higher Chemical Oxygen Demand (COD). It also contains higher total potassium and other components like total calcium, magnesium, phosphorus, sulphate and nitrogen due to the addition of various chemicals during the ethanol production process. The application of vinasse on soil directly without treatment causes emission of harmful gases such as CH_4 and N_2O, metal and sulphate leaching from the soil and bad odour (Moraes et al. 2014). Recent studies focused on anaerobic digestion of vinasse for biogas production, where the integration of the ethanol distillation unit to AD can result in two biofuels, viz. ethanol and biogas in place of only ethanol and safe disposal of the waste stream (Moraes et al. 2014; Moraes et al. 2015). Table 19.1 shows the list of earlier studies that focused on integrating anaerobic digestion with various processes and their products.

19.3.2 Integration with Microalgae Cultivation Unit

A series of processes to treat fertilizer industry wastewater was reported by Chavan and mutnuri 2020. The processes include struvite production from the wastewater, microalgae cultivation from the residual effluent and biogas production from the

Table 19.1 Integration of anaerobic digestion with other processes and their products

Substrate	Main product	Other products from integrated system	Reference
Wheat straw	Ethanol	Biohydrogen and biogas	Kaparaju et al. (2009)
Citrus waste	Limonene	Pectin, ethanol and biogas	Pourbafrani et al. (2010)
Oat straw	Ethanol	Biogas	Dererie et al. (2011)
Sugarcane crop	Ethanol	Biogas	De Paoli et al. (2011); Safari et al. (2017)
Whey + rye grain	Ethanol	Biogas	Oleskowicz-popiel et al. (2012)
Corn Stover + swine manure	Ethanol	Biogas	MacLellan et al. (2013)
Microalgal biomass	Amino acid/lipid	Biogas	Ramos-Suárez and Carreras (2014)
Pine wood	Ethanol	Biogas	Safari et al. (2017)
Wheat straw	Ethanol	Biogas and fungal based animal feed	Nair et al. (2018)
Fertilizer industry wastewater	Struvite	Biogas	Chavan and mutnuri (2020)

microalgal biomass. Most of the TKN, NH_4-N (nitrogen in the form of ammonium) and PO_4-P (phosphorus in the form of orthophosphate) were removed during struvite production. Microalgae cultivation aided in COD removal and then anaerobic digestion resulted in the conversion of algal biomass to biogas (Chavan and mutnuri 2020). Extraction of either amino acids or lipids from the microalgal biomass before anaerobic digestion results in higher methane yield than digestion of the raw biomass (Ramos-Suárez and Carreras 2014). As the C:N ratio of microalgal biomass is lesser, co-digestion of the microalgal cells with other carbon rich organic substrates such as maize silage, corn straw, swine manure, paper waste, etc. is required for higher methane yield. Addition of microalgal biomass to the agrowaste improves the biogas production compared with the respective agrowaste substrate alone (Dębowski et al. 2013).

19.3.3 Integration with Chemical Extraction Unit

Biogas production unit can be integrated in a such way that multiple valuable products other than biofuels can also be produced from a favourable substrate. For example, citrus waste can be used to extract commercially valuable chemical like limonene, pectin, ethanol and biogas. After recovering limonene from the acid treated citrus waste, the hydrolysate can be used for pectin precipitation and ethanol production. The solid residue from the overall process can be used for biogas production (Pourbafrani et al. 2010). Process simulation and economic analysis of the above biorefinery process show that citrus waste can serve as a sustainable feed

for the production of the above mentioned products. The ethanol cost may change based on the transportation cost of the feed, i.e. citrus waste (Lohrasbi et al. 2010).

19.3.4 Integration with Farm-Scale Biorefinery

Farm-scale biorefinery uses different substrates available from the farm associated activities such as agricultural and dairy wastes. A single substrate will not be available in plenty as in industrial operations. Therefore, mostly multiple substrates are co-digested to produce valuable products. Anaerobic digestion being a flexible process, it can be integrated with processes consuming any kind of organic substrate. For example, a farm-scale level ethanol producing unit consuming dairy waste can be integrated with a biogas unit by adding a co-substrate like clover grass silage. Apart from ethanol being produced as a commercially valuable product, integrated biogas unit helps in attaining self-sufficiency at the farm-scale level through the generation of electricity and organic fertilizer (oleskowicz-popiel et al. 2012).

19.4 Challenges

The presence of CO_2 and H_2S gases in the biogas compromises the biofuel quality and results in the emission of these harmful gases into the environment during combustion. These gases need to be removed to increase the purity of methane (Stangeland et al. 2017). The digestate coming from anaerobic digestion is a mixture of solid and liquid matters. The digestate is directly used as a soil fertilizer. Transportation of the digestate from the biogas plant to agricultural field increases the fertilizer cost. For this purpose, the digestate needs to be dried to get rid of the water and stored before transportation. This requires a large storage space, which is a challenge for the biogas plants (Camilleri-rumbau et al. 2014). Over supply of digestate towards its direct use as bio-fertilizer in the areas having several biogas plants is another challenge. For example, China is the largest producer of pig and pig manure. Anaerobic digestion units using pig manure as the feed has increased in the recent years which in-turn increased the supply of digestate as bio-fertilizer in China (Duan et al. 2020). Anaerobic digestate contains a large amount of organic matters and other nutrients like inorganic phosphorous, ammonia, etc. Direct usage of digestate as soil fertilizer leads to dumping nutrients more than the soil's requirement and to the emission of volatile matters from the digestate in the field. Post-treatment of the digestate is employed to recover the excessive nutrients in the form of valuable products and meet the regulations for its further utilization as fertilizer (Muñoz et al. 2019).

19.5 Biogas Upgradation

Biogas can be upgraded by removing the CO_2 and H_2S gases, thereby increasing the purity of CH_4. Biological way of upgrading biogas is through microalgae cultivation to remove CO_2. Oxygen released by the microalgae is simultaneously used by the sulphur oxidizing bacteria to convert H_2S to SO_4^{2-}. Controlling the gas to liquid ratio in the high rate algal pond (HRAP) in the above method helps in higher CO_2 and H_2S removal to achieve a better quality CH_4 (Rodero et al. 2020). Kao et al. 2012 reported the positive effect of intermittent gas switching with desulphurized biogas/air on a mutant Chlorella strain growth and subsequent CO_2 capture. H_2S can be chemically converted to sulphate by adjusting the pH above 8.0, which can be utilized by the microalgae (*Chlorella* sp.) as nutrient in the absence of other sulphate macronutrient in the medium. The negative impact of this method is that CO_2 in the form of bicarbonate at alkaline pH inhibits the growth of microalgae due to the exertion of osmotic pressure on the cells (González-Sánchez and Posten 2017).

19.5.1 CO_2 Methanation

Conversion of CO_2 to methane through Sabatier reaction is known as CO_2 methanation. The reaction involves CO_2 and H_2 to produce methane in the presence of a catalyst (Stangeland et al. 2017). The Sabatier reaction for CO_2 methanation is an exothermic reaction. It takes place between CO_2 present in the biogas and H_2 produced from electrolysis to produce methane and water in the presence of a catalyst as follows (Turks et al. 2017):

$$CO_2 + 4H_2 \leftrightarrow CH_4 + 2H_2O \; \Delta H_R = -165.1 \text{ KJ/mol} \tag{19.1}$$

Temperature and pressure are the significant factors in the CO_2 methanation process (Gao et al. 2019; Abate et al. 2016). At temperatures above 400 °C, reversed water gas shift (RWGS) reaction takes place where CO_2 and H_2 combines to form CO and H_2O and then CO reacts with H_2 to produce methane as follows (Turks et al. 2017):

$$CO_2 + H_2 \leftrightarrow CO + H_2O \; \Delta H_R = +41.2\text{KJ/mol} \tag{19.2}$$

$$CO + 3H_2 \leftrightarrow CH_4 + H_2O \; \Delta H_R = -206.3\text{KJ/mol} \tag{19.3}$$

Ru, Ni, Rh, Fe, Ir, Co, Pt, Mo, Au, Os, Pd and Ag are the widely studied metal catalysts for their activity and selectivity during the CO_2 methanation process. Ru followed by Rh and Ni provide better CO_2 conversion, whereas Pd followed by Pt, Ir and Ni provide better selectivity for CH_4 (Younas et al. 2016). Higher cost of Ru makes it economically a non-viable catalyst for CO_2 methanation. Ni is cheaper and commercially available too. Therefore, Ni catalyst is widely used for CO_2

methanation process (Turks et al. 2017). The catalyst is typically loaded on an oxide support such as Al_2O_3, TiO_2, CeO_2, SiO_2 and ZrO_2. Ni supported on alumina (Al_2O_3) is the highly explored and commercially available catalyst (Stangeland et al. 2018). Ni/Al_2O_3 catalyst has been acclaimed to be stable at full scale operation for several hours (Dannesboe et al. 2020). When Ni is promoted with a small amount of Ru, the activity and stability of the catalyst increases (Stangeland et al. 2018). The challenges linked with the catalysts are chemical and physical deactivations. The chemical deactivation of the catalysts occurs due to the interaction of impurities such as ammonia, chlorine, or sulphur compounds present in the feed gas. Physical deactivation rendered by the reaction conditions such as higher temperature or pressure on the catalysts results in sintering or degradation of the catalysts (Younas et al. 2016).

19.5.2 Bio-methanation

An alternative method for chemical CO_2 methanation process is bio-methanation. It is a biological way of converting CO_2 to methane using hydrogenotrophic methanogens (Lee et al. 2012). The different types of reactors used for bio-methanation are fixed-bed reactor, three phase system, trickle bed reactor, hollow fibre membrane bio-film reactor and continuous stirred-tank reactor (CSTR) (Ju et al. 2008; Alitalo et al. 2015; Burkhardt et al. 2015; Rachbauer et al. 2016). Plug flow reactor with bio-film and increased gas residence time eliminates the cost of agitation linked with the CSTR for bio-methanation (Savvas et al. 2017). Simulations show that the removal of water from the reactor can help in achieving higher methane yield (Faria et al. 2018).

19.6 Post-Treatment of Anaerobic Digestate

19.6.1 Ammonia Recovery

Advanced/autotrophic nitrogen removal (ANR) is a combined process of Partial Nitritation (PN) and Anaerobic ammonium oxidation (Anammox) to recover ammonium from the anaerobic digestate (Abma et al. 2007). PN and Anammox can be carried out in a single stage or double stage reactors (Magri et al. 2013). Partial nitritation is carried out by the aerobic ammonium oxidizing bacteria related to *Nitrosomonadales* sp. and *Nitrosomonas* sp. (Almeida et al. 2018). They convert part of the ammonium present in the digestate into nitrite through aerobic oxidation. The remaining ammonium is anaerobically converted into nitrogen gas and nitrate by anammox bacteria by utilizing the nitrite from the partial nitritation process (Vlaeminck et al. 2012). The major microorganisms involved in the anammox process are *Candidatus kuenenia*, *Candidatus anammoxoglobus* and *Candidatus*

brocadia (Gu et al. 2018). The main factors affecting the ANR process are the type of sludge and the wastewater compositions (Magri et al. 2013). However, ANR is the most economically feasible method of post-treatment for anaerobic digestate containing high concentration of ammonium (Khiewwijit et al. 2018).

Anaerobic digestion of black water (toilet water) containing hormones and pharmacuiticals such as paracetamol, metoprolol, ibuprofen, diclofenac, carbamazepine and cetirizine removes most of the paracetamol only. Partial nitritation followed by anammox is able to remove metoprolol and ibuprofen up to 67% and 77%, respectively (De Graaff et al. 2011). The micropollutants such as diclofenac, carbamazepine and cetirizine are hard to be removed by ANR post-treatment (De Graaff et al. 2011). Anammox treatment is also not effective on the effluents containing tetracycline hydrochloride and chloramphenicol. It is because the antibiotics inhibit the anammox organisms and decrease the nitrogen removal efficiency (Fernández et al. 2009). The pharmaceutical micropollutants can be removed by physical or chemical treatments like membrane filtration, activated carbon adsorption or ozonation (Joss et al. 2006; Hollender et al. 2009). Alternative method for ammonia recovery from the anaerobic digestate is ammonia stripping absorption. This method helps in the separation of ammonia as ammonium sulphate and gets rid of water, which makes the transportation of the digestate easier (Törnwall et al. 2017). The recovered ammonium sulphate has its commercial value as a mineral fertilizer (Menkveld and Broeders 2017).

19.6.2 Microalgae Cultivation

Anaerobic digestion of sewage or any other wastewater followed by post-treatment by using microalgae consortium in high rate algal ponds (HRAP) or photobioreactor is one of the biological ways of treating the digestate (Xu et al. 2015). The microalgal post-treatment helps in the removal of remaining COD and NH_4-N from the digestate (Vassalle et al. 2020). Microalgae cultivation in the anaerobic digestate helps in decreasing the electrical conductivity and total dissolved solids (TDS) as compared with the initial digestate (Muñoz et al. 2019). As microalgae can effortlessly assimilate organic nitrogen from the digestate, the biomass will be protein rich. Hence, protein can be extracted from the microalgal cells as a valuable product and then the residual biomass can be used as soil fertilizer (Vaneeckhaute et al. 2016) or it can be recirculated along with the sewage as a substrate for anaerobic digestion to increase the biogas yield (Vassalle et al. 2020). Flocculation of the turbid organic matter before microalgae cultivation decreases the COD of the digestate and increases the growth rate of microalgae (Zhou et al. 2019). Figures 19.1 and 19.2 show the schematic presentation of a simple and complex biogas biorefinery systems, respectively, with post-treatments and their products. The simple (Fig. 19.1) scheme shows the integration of only the effluent from the ethanol process with AD process with post-treatment to produce concentrated fertilizer. The complex biorefinery system (Fig. 19.2) depicts the utilization of each energy resource

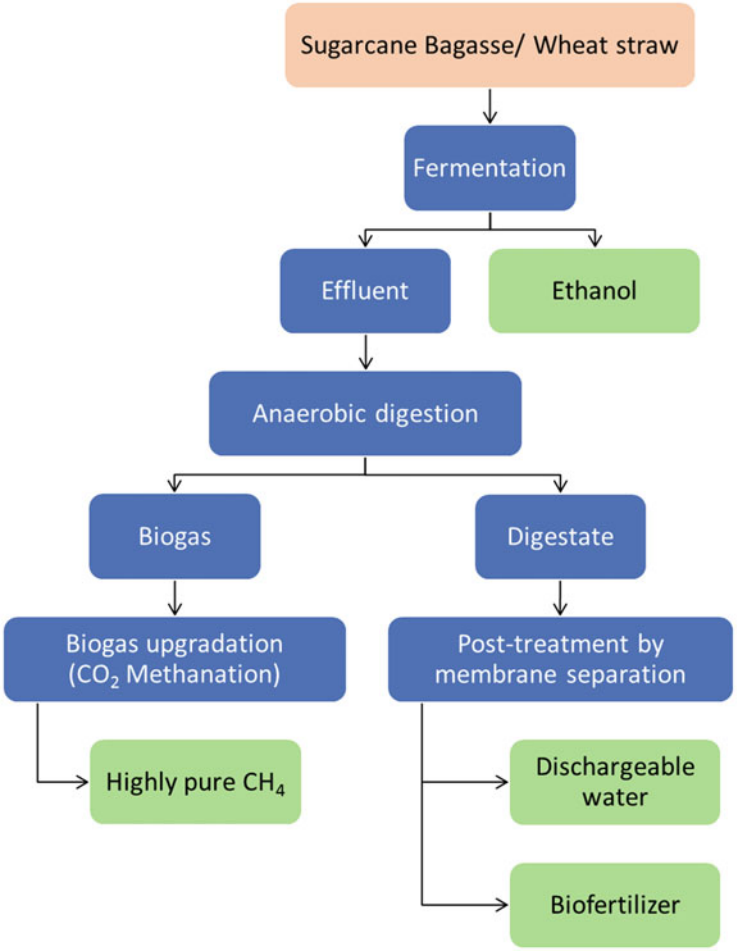

Fig. 19.1 Schematic layout of a simple biogas biorefinery system

(waste) coming out from the ethanol production process including pretreated hydrolysate, effluent from fermentation and vinasse to feed AD. The algal post-treatment (Fig. 19.2) can help in either recirculation of the algal biomass into AD as substrate or extraction of lipid and protein from the biomass and then the final disposal of the residue as bio-fertilizer.

19.6.3 Fermentation

The effluent from the anaerobic digester can contain 1×10^3 *E. coli*/100 ml for its use as fertilizer as per the WHO regulations. Fermentation of the anaerobic digestate

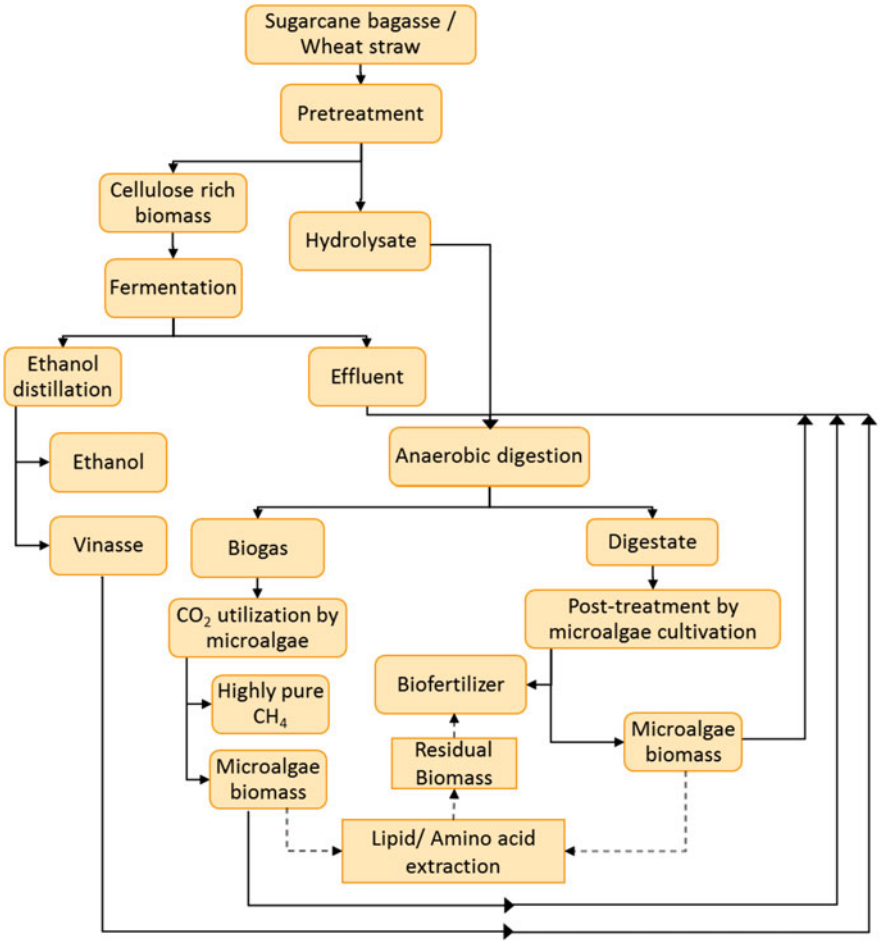

Fig. 19.2 Schematic layout of a complex biogas biorefinery system with better resource management

using mountain effective micro-organisms (MEM) as a post-treatment has been reported to reduce the number of coliforms in the digestate (Barzallo-Bravo et al. 2019). MEM is a mixture of forest mulch, molasses and water, consisting of natural consortium of bacteria and fungi.

19.6.4 Membrane Separation

Reverse osmosis, micro and ultra-filtration are used for concentration of the liquid fraction obtained from the anaerobic digestate. Polysulphone (PS), polyether

sulphone (PES) and Polyvinylidene fluoride (PVDF) membranes are commonly used for liquid digestate filtration purpose (Zarebska et al. 2014; Vaneeckhaute et al. 2016). The type of membrane material influences the fouling mechanism and specific product recovery. For example, Polysulphone (PS) membrane helps in separating phosphorous from the anaerobic digestate liquid fraction than PES and PVDF membranes (Camilleri-rumbau et al. 2014). Reverse osmosis is capable of recovering most of the NH_4-N and total Potassium from the digestate (Vaneeckhaute et al. 2013). Multi-stage conditioning of digestate involving decantation, ultra-filtration and reverse osmosis can deliver dischargeable quality water, solid fertilizer rich in organic nitrogen and phosphorus and liquid fertilizer rich in nitrogen and potassium. The major bottleneck in the membrane separation process is the high membrane cost and pumping energy required for filtration. Pumping energy can be reduced by involving enzymes to break down the organic matter and in turn, reducing the viscosity of the digestate (Gienau et al. 2018).

19.7 Conclusion

Integration of anaerobic digestion unit with other valuable product yielding processes like ethanol fermentation and microalgae cultivation can help in producing multiple biofuels, electricity and chemicals. CO_2 methanation process can be made more economically viable if hydrogen from electrolysis can be replaced by biohydrogen. PNR is the cost-effective post-treatment method for ammonia rich digestate. Recirculation of microalgal biomass from post-treatment to anaerobic digestion helps in increasing biogas yield, thereby increasing the energy output. Membrane process is best suitable for micropollutant removal from the digestate. Membrane process is costlier than the biological post-treatment processes, but it reduces the time for nutrient recovery. Exploring the routes to reduce the cost of membrane process can make the concentrated fertilizer production from digestate economically reliable.

Competing Interests All the authors declare that they have no competing interests.

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Chapter 20
Multiproduct Algal Biorefineries: Challenges and Opportunities

Ayesha Shahid, Sana Malik, Aqib Zafar Khan, Chen-Guang Liu, and Muhammad Aamer Mehmood

Abstract Establishing cost-effective algal biorefineries is a cornerstone research to enhance the process value by exploiting the full potential of microalgae and utilizing each component (protein, carbohydrates, lipids, and pigments) for biofuel and myriad industrial applications. Additionally, algal biorefineries offer environmental sustainability by reducing atmospheric CO_2, greenhouse gases, heavy metals, toxic compounds, and nutrients by utilizing flue gas as carbon source and wastewater as low-cost cultivation media. However, industrial applications of such biorefineries are still at infancy due to various challenges encountered during cultivation, harvesting, downstream processing, incomplete biomass utilization, uncertain nature of adopted technologies, and reduced economic viability. This chapter highlights the key challenges and opportunities to configure optimal algal biorefineries. A combination of integrated-biorefinery and cascading approaches is evaluated to systematically address the challenges. It emphasizes to consider the environmental, technical, and economic aspects to enhance the sustainability of algal biorefinery. Analysis and assessment of current frameworks in terms of raw biomass (especially indigenous algae), cultivation conditions (seasonal influx and wastewater sources), harvesting approaches (chemical and biological), processing technologies, and product-based biorefinery schemes are discussed to design a self-sustainable multiproduct algal biorefinery to achieve commercial robustness.

Keywords Algal biorefinery · Sustainable production · Efficient harvesting · Combined algal processing · Biomass valorization

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Abbreviations

CFB Cytophaga/flavobacterium/bacteroidetes
DHA Docosahexaenoic acid
EPA Eicosapentaenoic acid
HMF 5-hydroxymethyl-2-furaldehyde
ILs Ionic liquids
PHA Polyhydroxyalkanoates
PHB Polyhydroxybutyrate
PPLC Pigment, protein, lipid, and carbohydrate
SCP Single-cell protein

20.1 Introduction

Fossil fuels have been the main sources to meet the global energy needs for several decades. While, with the improving industrial demands after the industrial revolution, more and more fossil fuels have been extracted and consumed, which has caused air pollution and diminution of the resources. Carbon dioxide (CO_2) emission has been an uprising, and 1.9% of emission was increased in 2018 as compared to 2016–2017, making it a total of 37.9 gigatons (Crippa et al. 2019). This CO_2-rise has caused a gradual increment of the atmospheric temperature and is leading to global climate change. To overcome these challenges, in the 1990s, the concept of biorefinery was introduced which is often defined as "*a concept that represents a broad class of processes that refine forms of biomass into one or many products or services*". Environment friendly, sustainable, novel pathways, and products are common words which are also used for biorefinery (Björn Sandén 2014). Strategically, a biorefinery (can also be termed as bio-industry or green industry) is meant to devise a process for the cost-effective and eco-friendly transformation of biomass to various products that can substitute the petroleum-based fuels and chemicals (Chandra et al. 2019).

For biorefinery, first-generation feedstock (sugarcane, corn, etc.) and second-generation feedstock (lignocellulosic) were used in the beginning but due to issues related to food security with the former and complex molecular structure of the latter, it became difficult to utilize these feedstocks for biorefinery. Though nature has evolved biological processes for the photosynthetic fixation of the atmospheric CO_2 but its higher emission in the environment is still causing problems. After identifying the CO_2 fixation potential of microalgae and cyanobacteria, these have been considered as third-generation feedstock (Fig. 20.1) to produce fuel and chemicals (Sydney et al. 2019). There are some other characteristics which make algal biomass suitable for biorefinery such as fast growth rate and photosynthetic efficiency, growth on fresh and even seawater (which means that there is no competition with arable land

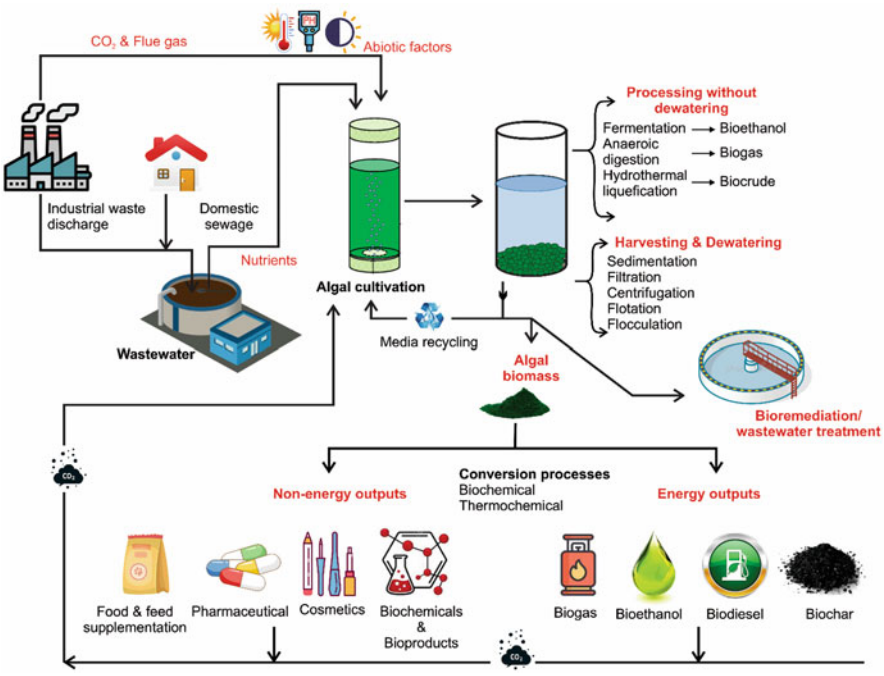

Fig. 20.1 Overview of the multiproduct algal biorefinery from cultivation to product formation

as in first-generation feedstock), high CO_2 sequestering capacity, high lipid content, and ability to grow in varied climatic zones (Fan et al. 2020).

For decades, algal studies have been focused on the hyperaccumulation of the single target molecule (lipid, for instance) in the cell by providing and identifying right nutrient supply and culture conditions (Mizuno et al. 2013; Sforza et al. 2014) which resulted in the accrual of three valuable components (proteins, lipids, and carbohydrates) inside cell especially when nitrogen content was modified in the media (Adnan et al. 2017; Bonnefond et al. 2017; Chu 2017; Gifuni et al. 2018). Algal proteins are attractive food, feed, and health products because of their nutritional characteristics (Francavilla et al. 2013). While the presence of lipids such as triglycerides, phospholipids, free fatty acids, and glycolipids has shown potential replacement of fossil fuels by producing biodiesel from algal biomass via extraction and transesterification (Lee et al. 2010). Algal carbohydrates (cellulose, starch, glucose, and polysaccharides) are precursors for many commercial chemical compounds such as ethanol, acetone, butanol, and terpenoids (Castro et al. 2015).

Although these three types of biochemicals are potential for algal biorefinery and produce minimum waste. However, targeting a single chemical compound is not feasible for algal biorefinery due to low growth (<3 g/L) depending upon the nature and size (carbon chain) of the target molecule. Synthesis of long-chain molecules is often inversely proportional to algal cell growth (reduced cell density) which is often difficult to harvest and takes more energy. Utilization of single biochemical is not

profitable as there are more than 40% down processing cost (Davis et al. 2016). Therefore, it is necessary to produce more than one chemical from the algal biorefinery to cover the cost incurred in cultivation, harvesting, and bioprocessing of the biomass. But optimization of their extraction from algal cells is still a challenge (Klok et al. 2013). Harvesting and fractioning are other factors for algal biorefinery which required optimization to make it commercially compactable. Previous decades have witnessed an intensive development of various biorefinery technologies for the recovery and utilization of algal biomass and its constituents. Among these technologies, thermochemical processes have been widely explored because of their high efficiency and economic viability (Chen et al. 2015a). Process integration biorefinery concepts for algal conversion are required for optimizing the exploitation of algal-derived value-added products.

Bottlenecks in microalgal biorefineries are present during all operational units. At laboratory-scale research, strain selection, strain engineering, and nutrient supply strategies are currently optimized for the hyperaccumulation of a single product. In the culture step, industrial photobioreactors have high costs and reach low biomass concentrations (~3 g/L, while at least a ten-fold higher concentration is necessary for efficient bioreactor exploitation and downstream operations); accumulation of different products often requires different or opposite cultivation strategies. Finally, downstream processes are designed for a single main product, and the remainder is frequently "waste" that must find a destination, with additional cost implications (Gifuni et al. 2019).

20.2 Designing a Sustainable Multiproduct Algal Biorefinery

A biorefinery based on algal biomass offers many advantages over other conventional biomass-based biorefineries, including higher growth, higher annual oil productivity, cultivation using brackish waters, recycling of waste nutrients, use of non-arable land, recycling flue gas of power plants, and production of many valuable products from the same biomass. The multiproduct paradigm of algal biorefinery makes this concept more attractive for the commercialization of algae-based products. However, the concept of the multiproduct algal biorefinery is not as much straightforward as it seems. In developing the process, there are different challenges associated with algal biomass cultivation, harvesting of biomass, and processing of biomass in an algal biorefinery (Gifuni et al. 2019). To develop a successful multiproduct algal biorefinery, sustainability, technological know-how, and efficient cost-analyses are required. An integrated-biorefinery approach can provide power, biofuel, and value-added bioproducts from single biomass, which can minimize the use of non-renewable energy and can lower down the carbon and water footprint.

20.2.1 Low-Cost Cultivation Approach

Cultivation of microalgae at a commercial scale incurs more cost than cultivating traditional crops because microalgal cultivation requires a huge amount of water and nutrients like carbon, nitrogen, and phosphorus. The high cultivation cost due to the consumption of plentiful water and nutrients is one of the major causes of expensive algal-based fuels. In an algal biorefinery, the first and most important task is to reduce the cultivation cost. Using wastewater for the growth of microalgae seems to be a promising strategy to reduce the need for freshwater resources and chemical fertilizers, additionally, microalgae can recycle the waste nutrients and help in the primary treatment of wastewater.

For the cultivation of microalgae open-ponds and photobioreactor systems are being used. The photobioreactors are of different types (airlift, flat plate, tubular, and bubble column) which provide several facilities for mass cultivation including maximum solar energy capture, efficient CO_2 sequestration, controlled conditions, and non-contaminated environment. Despite all these advantages, photobioreactors are not considered as a preferable choice when it comes to commercial-scale cultivation because of their high operational cost. The open-pond cultivation system is used for microalgal cultivation at commercial scale because of low capital and operational cost. But the open-pond cultivation system presents its challenges like poorly controlled conditions, higher risk of contamination, low biomass concentration, evaporation losses from the surface, inadequate mixing, poor control of temperature and pH, and less penetration of sunlight (Mathimani et al. 2019). These drawbacks limit the use of an open-pond system when microalgae are cultivated for pharmaceuticals, food, and feed industries. So, the attempts are underway to develop a sustainable closed culture system with low operational and capital costs for the mass cultivation of microalgae.

Microalgae have ten times greater potential for atmospheric CO_2 fixation through photosynthesis as compared to terrestrial plants. Each microalgal cell comprises carbon 36–56% of dry mass. Each kilogram of algal dry biomass fixes 1.3–2.4 kg of CO_2. In a biorefinery, flue gases (a mixture of CO_2, SO_x, and NO_x) from various industries can be used directly or indirectly for cost-effective and energy-efficient microalgal cultivation (Singh et al. 2015). For direct utilization of flue gases, the microalgal strains must have tolerance against the mixture of gases. Advanced scientific technologies must be introduced for the effective utilization of flue gases in microalgal cultivation to combat the environmental issues and to gain carbon credits.

To increase the production volume, scaling-up is critical in an algal biorefinery, shifting the algal culturing facilities from a lab-scale to an industrial-scale unit is challenging. The change in the volume of culture media affects different factors like reaction kinetics, light penetration, temperature, air bubbling, and contamination (da Silva and Reis 2015). The need is to assess all these technical and biological problems for the successful scale-up operation of algal cultivation.

Among other challenges, selection of a suitable potential algal strain having fast growth rate, maximum stress-tolerance ability, high growth and lipid productivity, and capable of dominating the contamination is crucial. Among their diverse group of organisms, very few microalgae have shown potential to be grown efficiently at lab-scale as well as pilot-scale using open-pond and photobioreactor systems to produce biodiesel and a variety of valuable bioproducts (Muhammad et al. 2020). For an algal biorefinery, green algae have shown the ability for mass cultivation owing to their higher growth rates and lipid content. There are algal repositories present globally, having potential algal strains to meet the biorefinery concept but locally isolated strains will have the native adaptability to the environment that would be beneficial for their mass cultivation. These strains can be isolated from freshwater or brackish water reservoirs, and then these are evaluated for their potential based on their growth characteristics, photosynthetic efficiency, carbon fixation rate, biomass and lipid productivity, nutrients recovery, and lipid profiling (Borovkov et al. 2020).

Contamination is another issue during mass cultivation of microalgae using an open-pond system and these contaminants (bacteria, fungi, etc.) must be controlled to obtain the monocultures for a biorefinery. To cope with the contaminants, the use of antibiotics, odd pH, ultraviolet radiation, and chemicals is not considered a good option, because they are non-eco-friendly, incur the high operational cost, and may interfere with the nature of end products (Holdmann et al. 2019). Therefore, the important factor which needs to be considered for mass cultivation is the selection of bio-preservative strain with the stress-tolerance ability and adaptability to environmental factors. To develop efficient and economic contamination control strategies, the specific interaction between environmental factors and microalgal species must be deciphered.

The selection of a suitable cultivation method is of great importance to achieve higher biomass and lipid productivity in a cost-efficient manner as it influences the algal growth pattern. The three main cultivation methods, photoautotrophic, mixotrophic, and heterotrophic, have been used for the mass cultivation of microalgae. The photoautotrophic cultivation is an energy-efficient method as it utilizes the sunlight as an energy source but due to poor light penetration, it results in lower biomass concentration and productivity. In heterotrophic cultivation, organic compounds serve as an energy source as well as the carbon source in a bioreactor independent of light. But the use of organic compounds and bioreactor increases the operational cost which makes this cultivation mode very expensive. Among these, the mixotrophic cultivation method seems a more effective mode of algal cultivation which allows the microalgae to use both organic and inorganic carbon sources which give higher biomass productivity (Wang et al. 2014). But we need to further explore the status and prospects of this model.

The seasonal changes also influence the growth characteristics and lipid profile in microalgae in the outdoor cultivation system for mass cultivation. The light, temperature, and moisture content vary during different seasons which have a great impact on lipid accumulation and content in microalgae (El-Sheekh et al. 2019).

These seasonal variations must be considered to meet a sustainable biorefinery concept that involves mass-scale outdoor cultivation.

The efforts are underway to commercialize the microalgal biofuels and bioproducts, but the high operational cost and energy demands associated with the mass cultivation of microalgae are major roadblocks that need to be overcome to achieve commercial feasibility. The multiproduct algal biorefinery is an attractive concept which has a promising future and can answer to all the problems. Using this approach the algal biomass can be cultivated in an open-pond system by employing stress-tolerant and bio-preservative strain, using wastewater as a growth medium and flue gases as CO_2 source to minimize the environmental concerns, to reduce the carbon and water footprint, and to save the cost and energy. The biorefinery concept has become imperative for the sustainable production of algal biomass.

20.2.2 Low-Cost Harvesting Approach

The algal biomass harvesting presents a significant challenge in the successful commercialization of algae-based products during mass cultivation in many industries (Shastri 2017). To satisfy the capital investment and to balance the economics of an algal biorefinery, the harvesting of algal biomass should be made efficient and cheap because it contributes to 20–30% of total production cost. Selecting a suitable technique to separate the algal biomass from culturing media mainly depends upon the characteristics of that strain (size and density), stability state, and the value of desired bioproducts. In the harvesting process of algae, they are first introduced on to a screen by using a micro strainer or vibrating screens having different aperture sizes. Then the microalgal cells are concentrated to form a slurry or cake, this slurry is then dewatered using different physical, chemical, or biological methods. At the end of the process, the algal biomass is dried out for further processing. There are different technologies to harvest microalgal biomass including electrical, mechanical, chemical, and biological flocculation (Table 20.1). Each one of these technologies presents its own advantages and challenges.

The electrical methods to harvest algal biomass involve electrophoresis of algal cells, in which the negatively charged algal cells are concentrated by applying the electric field. In mechanical flocculation of algal biomass different techniques are used, namely flotation, gravity sedimentation, centrifugation, filtration, attached algal biofilms, and flocculation (Roselet et al. 2019). The electro and flotation techniques are not very energy efficient as compared to the sedimentation techniques. Centrifugation and filtration methods can effectively dewater the algal biomass up to 20% (w/w) (Show et al. 2013). These techniques are highly efficient but are energy-intensive and require maintenance, thus make the overall harvesting costly. In chemical flocculation, various types of organic and inorganic flocculants like salts, chitosan, and multivalent cations, synthetic polymers, and electrolytes are used to flocculate the algal biomass. But the use of chemicals is not an eco-friendly

Table 20.1 Comparison of different harvesting techniques for microalgal biomass. Adapted from (Uduman et al. 2010; Christenson and Sims 2011; Ummalyma et al. 2017)

Harvesting technique	Biomass concentration (%)	Water removal concentration factor	Biomass recovery (%)	Advantages	Limitations
Gravity sedimentation	0.5–3	Lamella separator, 16	10–90	Low-cost and pilot-scale operability	Less reliable and slow process
Tangential filtration	5–27	5–40	70–90	Reliable and efficient	Energy-intensive and requires periodic filter replacement
Centrifugation	12–22	Disk stack centrifuge, 120	>90	Reliable and very efficient	Requires high energy input and costly
Flotation	3–6	N/A	50–90	Reliable and pilot-scale operability	Usually requires flocculants
Electro-flotation	3–5	300–600	N/A	Reliable	Energy-intensive and requires electrodes replacement
Flocculation	N/A	200–800, efficiencies of ≥80%	95	Cost-effective, contamination-free, and sustainable	Slow process and scale-up issues
Electrolytic flocculation	N/A	N/A	95	Reliable and efficient	Energy-intensive, metal contamination, and requires electrodes replacement
Flocculation-flotation	N/A	N/A	90	Reliable	Energy-intensive and requires electrodes replacement
Electrocoagulation	N/A	N/A	95	Reliable and efficient	Energy-intensive and requires electrodes replacement
Chemical flocculants	N/A	N/A	90	Reliable and sustainable	Non-ecofriendly and may contaminate the final products

approach and these chemicals may interfere with the downstream processing and nature of the final products.

Biological flocculation includes the use of other flocculating organisms (algae, plants, bacteria, and fungi) to harvest algal biomass. These self-flocculating organisms release extra polymeric substances from their surface which helps the microalgal cells to make aggregates together and settle down in the media. However, the use of bacteria or fungi may contaminate the final product and make the process inefficient. Among all harvesting technologies the most popular, reliable, efficient, cheaper, and eco-friendly approach is the use of self-flocculating microalgae. Self-flocculation is a very interesting phenomenon in which algal cells secrete extra polymeric substances from their outer surface, which help the other cells to make aggregates or flocs spontaneously. These flocs of microalgal cells then settle down in the growth media and make the harvesting cost-effective and easy. This amazing property of microalgae is also known as auto-flocculation. So far, very few microalgae species have been discovered which have the natural flocculating ability, namely *Ettlia texensis, Ankistrodesmus falcatus*, *S. obliquus* AS-6-1, *Tetraselmis suecica* (Zhao and Bai 2009), *Chlorococcum sp.* (Lv et al. 2018), *C. vulgaris* JSC-7 (Alam et al. 2014), and *Skeletonema marinoi* (Taylor et al. 2012). Such microalgae species are used in the co-cultivation system to flocculate the other non-flocculating microalgae (Schenk et al. 2008). The changes in different abiotic factors like temperature, pH, dissolved oxygen, and nitrogen levels are found to induce self-flocculation in some microalgae, but these changes can alter the cell composition leading to uncontrolled flocculation of microalgae. So, it is very important to find out those conditions which favor the higher flocculation rate without causing any undesirable change in the composition of the cell. The major cost and energy concerns related to the bulk production and harvesting of microalgal biomass must be tackled for the realistic commercial applications of algae-based products. Research efforts should be intensified to develop a cost-effective and energy-efficient harvesting technology to achieve the economic sustainability for a multiproduct algal biorefinery paradigm.

20.3 Processing of Algal Biomass: The Multiproduct Scenario

Downstream processing of biomass is an important step that refers to the extraction of high-value metabolites and the conversion of these metabolites into final products (Fig. 20.2).

This step typically accounts for 20–40% of the total process cost that can even go up to 50–60% of the total cost mainly due to (1) diluted nature of biomass, (2) lack of multiproduct harvesting methods, (3) incomplete valorization of biomass, (4) focus on one product while remaining to consider as waste, and (5) neglecting many valuable products (Gifuni et al. 2019). To make algal biorefineries economically

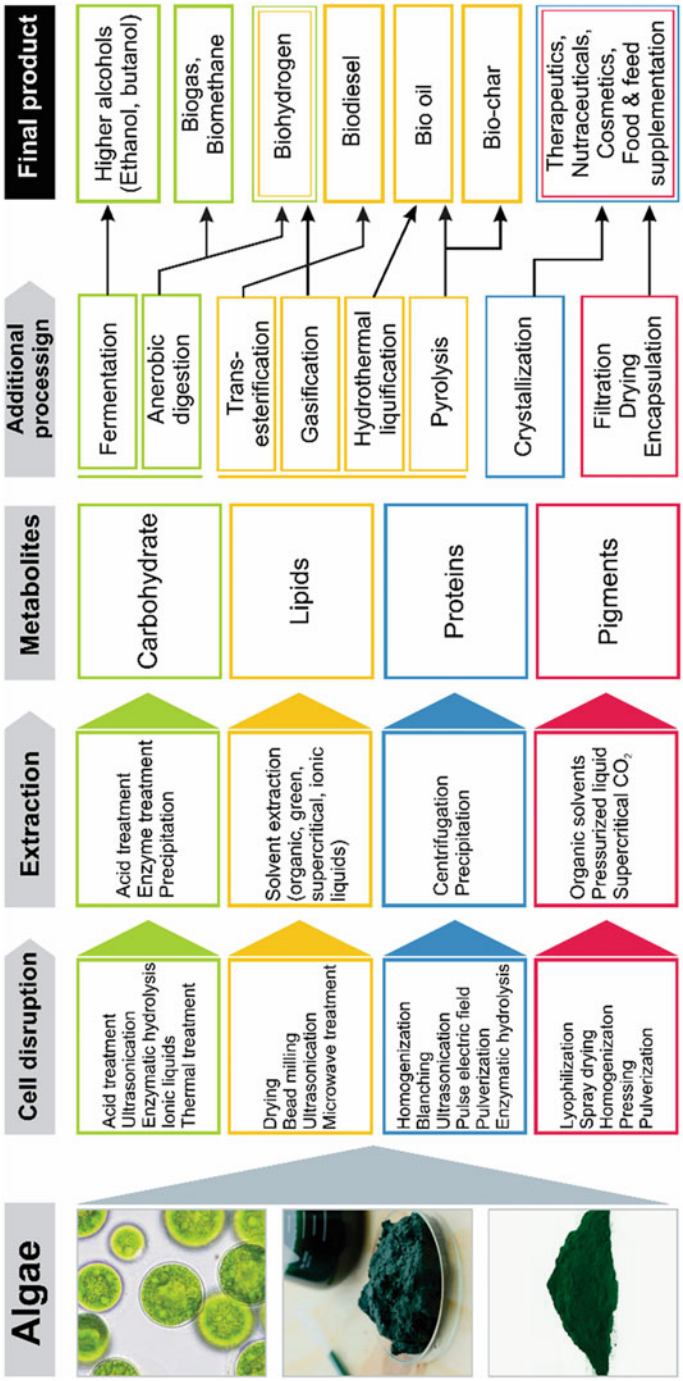

Fig. 20.2 Overview of the algal downstream processing technologies, from biomass to final product(s)

feasible, it is necessary to reduce the processing cost which is possible by enhancing biomass production (challenges and opportunities are discussed in biomass production section) and valuable metabolite accumulation by employing cost-effective, sustainable strategies. Other problems should be addressed by employing more efficient harvesting methods or by using combined algal processing methods and cascading approaches through which more than one product could be obtained from a single process. Moreover, residual biomass can be subjected to direct combustion or thermochemical conversion to develop a true circular bioeconomy based multiproduct algal biorefinery. The co-production of high-value commodities along with low-value commodities or specialized chemicals and product could enhance the overall profitability of the process. Recently, the attempts are being made for efficient and cost-effective downstream processing. Optimization of previously employed methods and development of new methods and strategies is a recent area of interest particularly to obtain multiple products (Vermuë et al. 2018).

20.3.1 Cell Disruption and Metabolite Extraction

The major barrier in the development of efficient downstream processing strategy is the low recovery of metabolites which is due to the rigid nature of algal cell walls that act as barriers to contain intracellular metabolites (Shahid et al. 2020a). Multiple layers of thick resistant cell walls hinder the release of cellular content. The use of an appropriate cell disruption method is a crucial step to recover maximum metabolites from the algal cell. However, the selection of appropriate disruption methods is challenging as it must completely disintegrate the cell wall without disrupting the metabolite's structure to ensure the efficiency of the extraction process (Phong et al. 2018).

The structural and chemical nature of the cell wall, nature of metabolite of interest, and quality and quantity of biochemical constituent are deciding factors affecting the disruption method's efficiency but in general, cell disruption must be done under stabilizing conditions to ensure the intactness of the metabolites of interest. Common cell disruption/extraction methods include physical (ultrasound, high-pulsed electricity), mechanical (bead beating, high-pressure homogenization), and chemical (enzymatic treatment, thermal treatment, alkali treatment) disruption method. Bead beating and high-pressure homogenization are widely used and effective methods, but high energy consumption, difficult cell debris removal, and non-specific metabolite extraction make them less favorable for algal biorefinery. Ultrasonic treatment is not suitable for proteins, enzymes, and pigments due to heat production. Though temperature control could be achieved by using solvents, but it greatly reduces the efficiency of the process. Comparatively, microwave-assisted extraction offers high efficiency with fewer solvents but is only suitable for polar compounds. Pulse electric field method could be easily scaled-up but process efficiency is affected by the media's conductivity and release of intracellular metabolites. Enzymatic treatment is highly specific and easy to scale-up but is

time-consuming, costly, depends on the enzyme of choice, and has low production as chemical or mechanical methods. Chemical methods are highly efficient, but product quality is affected by chemical contamination or by oxidation (Günerken et al. 2015).

Another problem associated with cell disruption is higher energy consumption. Many researchers have suggested the use of selective plasma membrane breakdown to resolve this issue, but it requires a complete understanding of the chemical nature and physiology of plasma membrane and it may vary depending upon the selected biomass or algal strain under study (Bhattacharya and Goswami 2020). For the development of an efficient biorefinery scheme, there is a need to develop more robust and sensitive methods that could be applied to a wide variety of biomass. In the last decades, research has been conducted to identify algicidal bacteria that are capable of cell lysis. These bacteria mainly belong to cytophaga/flavobacterium/bacteroidetes (CFB) group (50%), γ-proteobacteria (45%), and Gram-positive bacteria (5%) like *Bacillus*, *Micrococcus,* and *Planomicrobium*. Though their exact mode of action is not fully understood it is estimated that 70% of these bacteria release the algicidal compounds that breakdown the cell, while 30% of them have a direct-action mechanism for cell lysis. This unconventional method is low-cost, sustainable, non-toxic, and easy to scale-up but has the drawbacks of slow processing and difficult bacterial culture and end-product maintenance (Wang et al. 2020).

In a sustainable biorefinery both primary (protein, carbohydrates, lipids) and secondary (carotenoids, phycobilins, sterols, terpenoids) metabolites must be extracted from the harvested biomass (algae) (Shahid et al. 2020b). Solvent (hexane, acetone, methanol, chloroform, ethanol, organic acids, alkali, acids) or supercritical fluids (CO_2, N_2O, ethylene, butane, propane, dimethyl ether) extraction-based methods are used to obtain basic/primary and bioactive compounds, respectively. Supercritical fluids are preferable over traditional solvents due to their non-toxic, inflammable, eco-friendly nature, and being inert solvents, no residual solvents are left after extraction which reduces the cost and energy required for product purification. The use of CO_2 as supercritical fluid is most favored due to its low-cost, non-inflammability, non-toxicity, and critical temperature (31 °C) and pressure (7.28 MPa) conditions. The low-temperature requirement makes CO_2 suitable for heat-sensitive intracellular compounds. It is most suitable for neutral lipids and shows less affinity towards polar lipids, polysaccharides, proteins, and salts. The use of co-solvents is recommended to enhance the affinity towards other compounds which may increase the cost, making it unsuitable for industrial processes (Khoo et al. 2020).

Ionic liquids (IL) are molten synthetic salts having a melting temperature below 100 °C. They are preferred green solvents for industrial processing due to their vast adaptability, non-volatile nature, low viscosity, low vapor pressure, high chemical and thermal stability, high conductivity, high specificity, and high solubilization abilities. A combination of IL with traditional metabolite extraction techniques has been adopted to enhance the extraction efficiency. Though the use of IL is suitable for the extraction of lipids, proteins, carbohydrates, pigments, and bioactive

compounds their application at commercial scale is hindered by its non-biodegradability and toxic nature which may pose a dangerous threat to the environment if discharged untreated. Moreover, it is difficult to synthesize IL due to their complex nature and specific structure which enhances the production cost. However, the use of IL is a relatively newer approach; hence, further studies are required to cut back the production cost and to improve the efficiency (Tan et al. 2020).

Traditionally, metabolite extraction is performed on the dried biomass which enhances the energy requirements and process cost due to tedious and expensive drying steps. A substitution to address these shortcomings is the use of concentrated wet algae which eliminates the requirement of dewatering. The use of wet biomass for lipid extraction is well developed and has 80% of extraction efficiency, but more studies are required to study the impact of wet biomass-based lipid extraction on the biochemical constituent of the under-consideration cell. Moreover, the treatment of wet biomass with non-polar solvents results in the formation of a stable emulsified structure which could only be de-emulsified by energy-intensive processes like centrifugation (Amorim et al. 2020; Ansari et al. 2017a). This method is still at its infancy and requires further modifications (Howlader and French 2020) so it can be applied to recover all (or at least major) metabolites and to address the challenge of low metabolite recovery.

20.4 Routes of Metabolite Conversion to Products

Extracted metabolites are further processed through biochemical and thermochemical conversion methods to obtain the purified final product (Moncada and Aristizábal 2016) for which several routes have been suggested. The selection of feasible extraction and processing route is a key consideration to design an efficient algal biorefinery. The feasibility of algal biorefinery depends on the employed method that should be efficient, cost-effective, and provide a purified final product. Sustainable multiproduct biorefinery should be based on the methods that can be integrated in a sequential manner where residual biomass from one process is used as a feedstock in the next process to allow the complete valorization of single biomass in a variety of valuable products (Ferreira et al. 2019). However, the main challenge is the cost-effective conversion of targeted metabolites without producing toxic by-products so that the residual biomass can be utilized to obtain other valuable products.

20.4.1 Production of Biofuels and Utilization of Residual Biomass (Conventional Routes)

The commonly used methods are designed to target the biofuel (liquid, gaseous) production, where algal biomass/metabolite is fractionated through transesterification, biochemical conversion (fermentation and anaerobic digestion), and thermochemical conversion (gasification, hydrothermal liquefaction, pyrolysis) methods. Each one of them is specialized in the conversion of a specific product and has its own set of opportunities and challenges (Table 20.2).

Biochar (carbon-rich product of biomass) is produced as a result of anaerobic thermal decomposition. Production yield and composition (chemical and physical) vary depending on the biomass, conversion process, and process parameters. The proportional increase in the yield of biochar is associated with the carbohydrate content of algae. A maximum biochar yield of 65% has been obtained corresponding to 37% of carbohydrate content (Yu et al. 2017). High cation-exchange capacity, low surface area, high carbon and nutrient content, and higher pH make biochar beneficial for growth promotion and soil amendment (especially to neutralize acidic soils). Additionally, they have high bio-absorbent properties for contaminants, making them useful for wastewater treatment. Production optimization, cost, and energy effectiveness are some major challenges which lie in the commercial production of algal biochar. Focusing on the biofuel associated biochar biorefinery could help in addressing these challenges (Xie et al. 2015; Yu et al. 2017).

20.4.2 Production of High-Value Commodities (Non-Conventional Routes)

Though most of the discussed techniques focus on biofuel production, few also target the production of industrially important chemicals. Mostly these chemicals are produced as a by-product during the main process. However, the formation of both bioproducts and by-products in biorefinery depends on the algal composition which may vary according to the selected strain and the cultivation conditions. Thus, it is worth exploring the combination of the outstanding algal portfolio in terms of non-conventional algal products like cosmetics, food source, feed supplementation, pharmaceuticals, biopolymers, nanoparticles, and biofertilizers to meet the market demands for natural production (Sengupta et al. 2020; Trivedi and Atray 2020).

Solvent extracted crude pigments from the algae are a mixture of valuable fat-soluble chlorophylls (a and b) and carotenoids (astaxanthin, β-carotene, xanthophylls), while cyanobacteria can also produce water-soluble phycobilisomes (phycocyanin, allophycocyanin, phycoerythrin) (Fabris et al. 2020) which are purified through filtration and processed by encapsulation and drying before commercial use. Being natural colorant, these pigments are employed in cosmetic, textile, and food industries. Apart from these, they have applications in pharmaceutical and

Table 20.2 Summary of the commonly employed downstream technologies for biofuel production

Metabolite conversion processes	Basic principle	Challenges	Opportunities	Major product	Yield	References
Transesterification	Direct conversion of free fatty acids to the valuable product by catalyst under acidic conditions	Process affected by different factors like water content, catalyst type, reaction temperature, free fatty acid content, requires powerful machinery	In situ transesterification is less time consumption, lipid extraction and conversion can be done in a single step	Biodiesel, biochemicals like levulinic acid	80–91%	(Skorupskaite et al. 2016)
Fermentation	Conversion of complex sugars into alcohol by the action of microbes and enzymes	Requires hydrolysis of polysaccharides, cost and processing efficiency may vary depending upon hydrolysis strategy	An established method does not require pretreatment, high production	Bioethanol, bio-butanol	40–90%	(Ho et al. 2013)
Dark fermentation	Conversion of organic substances into gaseous fuels in the absence of light	Limited biodegradability due to chemical barriers of biomass, difficult harvesting of end products	Potential to treat wise variety of biomass, produce volatile/organic acids and alcohols as by-products	Biohydrogen, biohythane, biomethane	5–25%	(Ghimire et al. 2017)
Anaerobic digestion	Fermentation of organic material in the absence of oxygen	Sensitive process affected by temperature, pH, substrate, operational parameters, inhibition due to ammonia toxicity	Can be applied on dry or wet biomass, use of wet biomass reduces energy consumption	Biogas, biohythane	75–95%	(Ghimire et al. 2017)
Hydrothermal liquefaction (HTL)	Conversion of wet biomass in liquid oils in hot-compressed liquid system with or without catalyst	Requires highly sophisticated equipment, high cost, high energy requirement, contamination of bio-oil by nitrogen, low biofuel yield	Directly applicable to raw biomass, suitable for high moisture containing biomass, high biofuel quality	Bio-oil, biochar	–	(Gu et al. 2020)

(continued)

Table 20.2 (continued)

Metabolite conversion processes	Basic principle	Challenges	Opportunities	Major product	Yield	References
Pyrolysis	Biomass fractionization by combustion at extreme temperature (400–600 °C) in anaerobic condition	Requires dry mass, high energy requirement, requires further treatment, poor biofuel quality	Low capital cost, high bio-fuel yield	Bio-crude oil, hydrocarbons, biochar	–	(Chen et al. 2015b)
Gasification	Conversion of carbonaceous polymers of biomass into gaseous products in the presence of gasification agent at high temperature (800–1000 °C)	Product quality influenced by operational conditions, gasification agent, and temperature	High conversion, eco-friendly due to less CO_2 production	Biohydrogen	73–97%	(Adnan et al. 2017; Fan et al. 2020)

nutraceutical industries and in molecular and clinical research due to its useful therapeutic properties including anti-inflammatory, anti-oxidative, anti-cancerous, anti-neurogenic, anti-angiogenic, and hepatoprotection (Syrpas and Venskutonis 2020). Algal-based pigments are highly marketable but have high production costs which could be reduced by combining wastewater treatment and biorefinery approach with pigment production.

Numerous chemicals can also be produced during anaerobic digestion of biomass which is divided into four major steps. During the first step, i.e., hydrolysis, the interaction of fermentative and hydrolytic microbes with monomers and polymers of biomass produces hydrogen, acetate, and volatile fatty acids such as butyrate and propionate. The second step (acidogenesis) produces short-chain (C_1–C_5) organic molecules like acetic acid, butyric acid, and propionic acid which are then converted to alcohols, hydrogen, and CO_2. Hydrogen and acetic acid are produced as a by-product during the third stage (hydrogenesis), while methane is the major product that is obtained at the end of the fourth stage (dehydrogenation or methanation). Third and fourth stage consumes the products of previous stages. Extraction of by-products in moderation at each stage could enhance the process efficiency (Sengupta et al. 2020). Liquefaction of the carbohydrate content of algae results in the production of acetic acid, levulinic acid, formic acid, lactic acid, and 5-hydroxymethyl-2-furaldehyde (HMF), while pyrolysis of carbohydrates produces furans, ketones, aldehydes, acids, and pyrans (Fan et al. 2020).

Cellulosic and the starch portion of algal carbohydrate are used as precursors in combination with polymer/additive for the conversion of cellulose acetate, polylactic acids, and starch into cellulosic plastics, bio-ethylene, thermoplastic, and hybrid plastics. In addition to primary metabolites, algae can store valuable compounds like polyhydroxyalkanoates (PHA) and polyhydroxybutyrate (PHB) which are known for their thermostability, biodegradability, and biocompatibility, making them ideal to produce bioplastics, tissue engineering scaffolds, and dissolvable sutures. The aqueous phase which is obtained after algal hydrolysis and fermentation is a rich source of valuable extracellular metabolites and can be utilized to obtain PHB (Rahman and Miller 2017). Algae-based bioplastics have superior mechanical properties as compared to their synthetic or petroleum-derived counterparts. Though these bioplastics have the potential to replace the traditional plastics to address the environmental problems caused by synthetic plastics, algal-based bioplastic production faces some challenges. Product quality depends on the extraction and processing technique employed. These bioplastics may have unpleasant odors (due to the presence of lipids) which restrict its application. Reduced production, time consumption, and cost are major constraints in industrial-scale bioplastic production which occur due to agglomeration of carbohydrates in biomass. Protein-rich biomass could produce high-quality bioplastics as the incorporation of algal protein strengthens the product. A combination of biodiesel and bioethanol production with bioplastics production enhances process efficiency, economics, and sustainability (Beckstrom et al. 2020). However, algal-based plastics and biopolymers are still at infancy and require further research for process improvement.

Nanoparticles are another interesting product that can be synthesized by algae. Algae are foreseen as a model alternative for green synthesis of metallic nanoparticles. Reduction of the aqueous solution of salts by algae (a bioactive compound containing extract or whole-cell) can produce extracellular or intracellular nanoparticles depending on their production site (Khanna et al. 2019; Kumar and Kundu 2020). Metallic nanoparticles especially gold and silver nanoparticles have therapeutic applications and are generally employed for anticancer, antimicrobial, antifungal activity, drug delivery system, wound healing, biosensors, and as enzyme co-factors (Barwal et al. 2011). These nanoparticles have the advantage of low toxicity, energy efficiency, eco-friendly nature, high productivity, and low-temperature activity over the synthetic nanoparticles. These particles can be produced by (1) algal biomolecules, (2) whole cells, (3) cell-free supernatant, and (4) live-cell culture. In case of biomolecule-based synthesis, reduction of metal salts to individual particle is done by the algal protein. Although this is an efficient method, yet it requires extensive, time-consuming, and costly processing which may increase the process cost. While in case of (2) and (3) method, concentrated algal cells and cell-free supernatant are utilized, respectively, for the reduction of metal salts into nanoparticles. The second method is easy, efficient, and reproducible, while the third method is light-dependent. In the last method, the aqueous solution of metallic salts is provided in the media for algal cultivation. This method provides the added benefit of continuous production along with easy harvesting, easy scalability, and economic feasibility (Kumar and Kundu 2020). Change in reaction parameters (temperature, pH, reaction mixture ratio, and exposure time), nature of metal salt, and algal species could modify the size and shape of nanoparticles. Continuous nanoparticle production and coupling of processes with other bioprocesses could enhance the economics and sustainability of the process (Khanna et al. 2019).

20.4.3 Whole-Cell Utilization as a Feed Supplement or Biofertilizer

In most cases, algal metabolites are extracted and processed to obtain valuable products. However, the exceptional algal composition makes it a suitable candidate for direct utilization and application as biofertilizers and food/feed supplementation. Microalgae contain high amounts of macronutrients, micronutrients, and plant promoting substances (cytokinin, auxins, polyamines, amino acids, and vitamins) that are suitable to promote the growth and development of crops, nutrient uptake, cellular metabolism, antioxidant content, and resistance to abiotic stresses. Moreover, they also improve the nutrient availability and water holding capacity of soil for its improvement. Direct application of algae as biofertilizers and bio-stimulants is an attractive interest. These biofertilizers are a cost-effective and eco-friendly alternative to chemical fertilizers. The characteristics of these fertilizers depend on algal

species and processing techniques (Ronga et al. 2019). Exhaustive research in terms of suitable strain selection, cultivation conditions, selection of carrier material, prototype development, and scale-up trials and storage conditions are required to improve the production and processing (Mahapatra et al. 2018).

High crude protein content (60–70% of total biomass) of algae makes them a suitable candidate for single-cell protein (SCP) production. Algal SCP production is favored due to the co-production of carotenoids (astaxanthin) and omega-3-fatty acids like docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) (Jones et al. 2020). High protein content, high substrate to product conversion efficiency, small production footprint, climate, and soil characteristics independence are few advantages of algal SCP (Hülsen et al. 2018). Toxic nature, difficult cell disruption, expensive scale-up, and high production cost are the major challenges in algal SCP production and utilization. The cost of carbon substrate is major cost input which could be reduced by using wastewater and industrial/agricultural residues for the SCP production. It also enhances the bio-valorization, bio-potential, and process economy but faces the challenges of limited control on the regulatory processes (Hülsen et al. 2018; Jones et al. 2020). A combination of algae-based wastewater treatment with SCP production can improve the input/output ratio in terms of capital and production cost.

20.5 Cascading the Algal Biomass Processing

Optimization of algal conversion for integrated biorefinery is required for the exploitation of multiproduct algal biorefinery. Loss of resources, high energy, and high input cost are major barriers in the complete valorization of algae and thus restrict in attaining the full potential of multiproduct algal biorefinery. Combined processing focusing on the cascading algal biorefinery system can enhance resource utilization, energy recovery, process profitability, and biorefinery sustainability (Fan et al. 2020). Combined processing includes the combination of lipid extraction with residual biomass utilization, combined lipid and carbohydrate processing, an amalgamation of pigment extraction, and carbohydrate processing (Fig. 20.3).

20.5.1 Starting with the Lipid Content (Lipid-Rich Microalgae)

Liquid biofuels are the most common commodity produced by algae. Therefore, utilization of lipid-extracted biomass as feedstock for bioethanol production through fermentation (Ghimire et al. 2017) or biomethane (97% methane obtained by removing CO_2 from produced biogas) and biohydrogen production through anaerobic digestion (Bose et al. 2020) can enhance the process efficiency, sustainability,

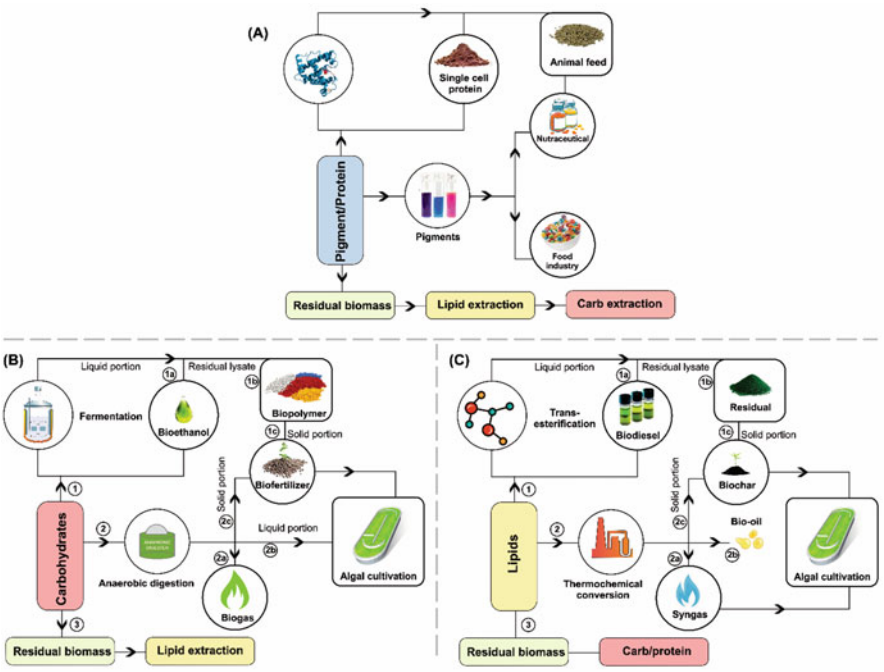

Fig. 20.3 Cascading biomass conversion schemes of (**a**) protein-rich, (**b**) carbohydrate-rich, and (**c**) lipid-rich algae for multiproduct algal biorefinery

and economics due to absence of lignin and high amount of available simpler sugars (Ansari et al. 2017a). Biomethane and biohydrogen can be used as an energy source for combustion, produce electricity, or for algal processing. As a result of liquid biofuel production, 60–70% of nutrient-rich algae remain as residual biomass which contains high amounts of valuable compounds like proteins, bioactive compounds, minerals, and pigments. The residual biomass is of great economic importance and could be either utilized for algal production (in combination with the remaining proportion of biogas) or used directly as biofertilizer or as a food additive for animal and aquaculture feed (Bose et al. 2020; Ghimire et al. 2017).

20.5.2 Starting with the Carbohydrate Content (Carbohydrate-Rich Microalgae)

In the case of carbohydrate-rich algal biomass, a cascading approach targeting carbohydrate and lipid portion is suggested, where, in first step algal biomass is pretreated (acidic, alkaline, or thermal) for carbohydrate extraction which is then subjected to ethanolic fermentation, while, in second step lipids are extracted by solvents (hexane, methanol, chloroform) and upgraded to renewable biodiesel blend

stock through hydrocracking and hydrotreating. This process has shown to achieve 87% of the fatty acid recovery and 74% of carbohydrate conversion, leading to 88% of total energy yield from the algal biomass (Dong et al. 2016). Besides, algal bioprocessing in combination with consolidated bioprocessing has also been suggested as an efficient system to obtain pigments and bioethanol through a single process. A 10–31% enhanced ethanol productivity was obtained when pigment-extracted algae were fermented with enzyme-displaying yeast. Life cycle assessment of the process indicated enhanced resource utilization (2.34 kg ethanol and 5 g lutein production from 1 kg algae), increased economic output (60.87 $), and lower environmental impact (2.7–10.7 fold) (Huang et al. 2020).

20.5.3 Starting with the Protein Content (Protein-Rich Microalgae)

Several algal strains have shown to be protein-rich, and this protein content is believed to hinder the lipid extraction process (especially from wet algal biomass) due to stabilized emulsions formation which reduces the process efficiency. Protein extraction followed by lipid extraction has been suggested as an efficient strategy for wet algae processing. Implementation of this strategy reduces the pretreatment steps and enhances the metabolite recovery for sustainable biorefinery development. Though this process results in the formation of less stable emulsion and is efficient for protein and solvent recovery but has decreased lipid yield (Amorim et al. 2020). The thermochemical conversion of residual biomass (lipid and protein-free) for value maximization is progressive for multiproduct algal biorefinery. As a result of such a cascading approach, 10% proteins, 14% lipids, and 33.2% of bio-oil yields have been obtained (Muñoz et al. 2015). However, further optimization is required for effective resource recovery and implementation at large scale. Moreover, improvement of protein extraction efficiency is required to enhance the bio-oil quality as protein in remnant biomass could contaminate the bio-oil (Fan et al. 2020).

Sequential extraction of protein, lipid, and carbohydrate (PLC) from algal biomass has been suggested as a feasible sequence to maximize the metabolite recovery. By following the PLC extraction scheme, 500 g of metabolites were extracted from 1 kg of dried algal biomass; out of which 240 g were protein, 180 g were lipids, and 80 g was carbohydrate. Net value gain (66.5%) of this process compensates for the algal production cost (Ansari et al. 2017b). It is hereby suggested that before protein extraction biomass should be subjected to pigment extraction, making the extraction sequence as pigment, protein, lipid, and carbohydrate (PPLC), while residual biomass can be analyzed and then subjected to thermochemical conversion or direct utilization, making the whole process more economical leaving almost no-waste.

20.6 Conclusion

In bio-based bioeconomy, multiproduct biorefinery is a promising possibility that curtails harmful environmental impacts, values waste, and focuses on the restoration and regeneration of products. Algal biomass is one of the best feedstocks for the multiproduct biorefinery due to its immense advantages and potential to produce diverse products. The commercial-scale implementation of these exceptional biorefineries is hindered by costly cultivation, difficult harvesting, and incomplete valorization. Wastewater-based cultivation, bioprospecting of novel indigenous strains, bio-flocculation of algal biomass for easy harvesting, and subsequent extraction of multiple metabolites in a single process offer the opportunities to make the process economical and environmentally friendly. Advances in processing techniques for biomass production, metabolite accumulation, and multiple product extraction will facilitate the transition from small-scale production towards large-scale production of bulk commodities by overcoming the associated challenge related to sustainability and profitability.

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Chapter 21
Genetic Engineering for Enhancement of Biofuel Production in Microalgae

Venkatesh Chaturvedi, Rahul Kumar Goswami, and Pradeep Verma

Abstract In recent decades, there has been a tremendous increase in fuel requirement all around the world. This has caused severe exploitation of conventional fuel resources leading to subsequent increase in the level of pollution owing to their consumption. There is a growing concern regarding exploration of alternate, safe sources of energy. Since the time immemorial, algae are known for producing a vast array of useful products, which has benefited the mankind. Algae also harbor high amount of biomolecules such as lipids and carbohydrates, which can be converted into biofuels. Therefore, algal biofuels have gained tremendous attention in past few years and several technologies related to algal biofuels have been developed. The major drawback in this area is the limited potential of algae to synthesize biomolecules to be converted into biofuels. Since, the demand of biofuels is very high and production is very low, therefore, genetic engineering technologies were explored to increase the yield of biofuels. In this chapter, we have discussed different types of biofuels produced from microalgae and recent genetic engineering-based studies employed for sustainable biofuel production.

Keywords Microalgae · Biofuel · Genetic engineering

Abbreviations

ACCase	Acetyl-CoA carboxylase
ACP	Acyl carrier protein
BOD	Biological oxygen demand
COD	Chemical oxygen demand
DAG	Diacylglycerol

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DGAT	Diacylglycerol acyltransferase
DHAP	Dihydroxy acetone phosphate
FAs	Fatty acids
G3P	Glyceraldehyde-3-phosphate
GEMs	Genome-scale metabolic models
GPAT	Glycerol-3-phosphate acyltransferase
LPA	Lysophosphatidic acid
LPAAT	Lysophosphatidic acid acyltransferase
PA	Phosphatidic acid
PAP	Phosphatidic acid phosphohydrolase
PDK	Pyruvate carboxylase kinase
RuBisCO	Ribulose biphosphate carboxylase
SHF	Separate hydrolysis and fermentation
SSF	Synchronous saccharification and fermentation
TAGs	Triacylglycerols
TAGs	Triglycerides

21.1 Introduction

Algae are photosynthetic autotrophs, which regularly flourish in different kinds of aquatic systems; for example, lakes, waterways, and oceans (Richmond 2004). They are able to withstand a number of biotic and abiotic conditions. They synthesize oxygen by means of photosynthesis, which converts water and carbon dioxide (CO_2) into sugars utilizing sunlight (Fehling et al. 2007). Algae are a broad group, ranging from unicellular prokaryotes to multicellular eukaryotes, exhibiting remarkable properties (Falkowski et al. 2004). Minute single celled algae are generally referred to as microalgae. Microalgae can be prokaryotic or eukaryotic photosynthetic microorganism, which fix CO_2 from the atmosphere as essential carbon source (Raja et al. 2008). Microalgae are believed to be one of the pioneer microorganisms on earth (Richmond 2004).

Microalgae contain high amounts of carbohydrates (polysaccharides), proteins, lipids, and other metabolites, which can be used for creation of various value-added products (Milledge 2011) (Fig. 21.1).

Along these lines, they are favored choice for microbial biorefinery. Microalgae are likewise a wellspring of biofuels because of high amount of oil. Microalgae due to their bestowed properties are considered as excellent source of biofuels (Skjånes et al. 2013). A few properties of microalgae, which make them favored organisms for biofuel creation are;

- Microalgae can synthesize oil (lipid) in every season.
- Microalgae synthesize high amount of oil as compared to oil crops.
- The amount of oil in microalgae is 20–50%, which is greater than plants.
- Biodiesel from algae is highly biodegradable and non-toxic.

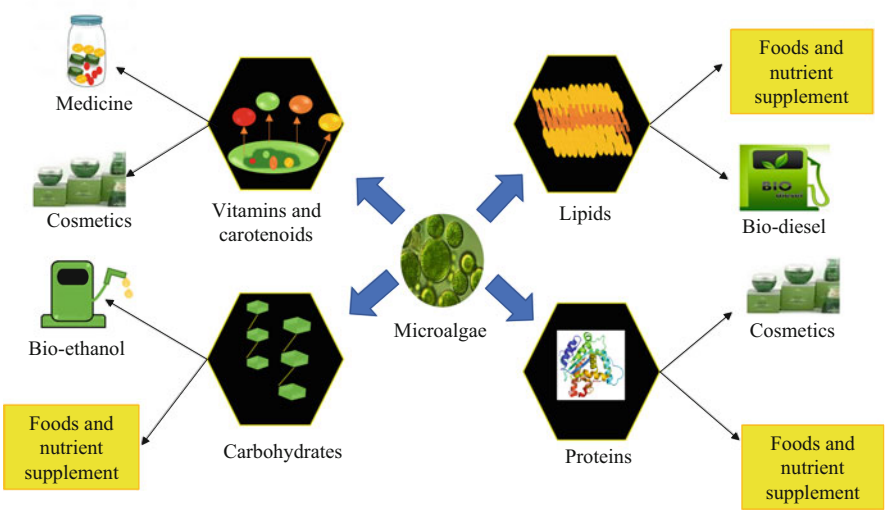

Fig. 21.1 Different value-added biomolecules produced from microalgae biomass

- Microalgae exhibit biomass yield as compared to terrestrial plants.
- Microalgae have short life cycle as compared to plants.
- They can grow in seawater, wastewater, or non-arable fields (Mutanda et al. 2011).

In this way, cultivation of microalgae for biofuels can efficiently diminish fuel versus food struggle. Some other properties of microalgae, which makes them a suitable choice for biofuels are that they require less amount of water during growth, so, they can be cultivated in water deficit regions, they can be grown in wastewater, where they can utilize chemical oxygen demand (COD), biological oxygen demand (BOD), phosphate, and nitrate, present in the form of pollutants. In this way, they can act in bioremediation of wastewater and increase their biomass. Besides, biodiesel produced from microalgae releases less amount of toxic gases (Mata et al. 2010), as compared to sustainable power sources, for example, sunlight, geothermal, wind, and so on. Microalgal growth is continuous all year long in comparison with plants, which is season specific. Therefore, microalgae culture can generate biofuel without using potable water or productive land (Borowitzka 2013).

21.2 Different Generations of Biofuels

Energy requirements are expanding worldwide because of urbanization and industrialization, prompting the misuse of accessible natural resources (Kuila and Sharma 2017). The creation of biofuels has achieved tremendous impetus because of the accessibility of biomass, the restricted accessibility of fossil fuels, and increasing air

pollution owing to burning of fossil fuels leading to greenhouse effect (Kumar et al. 2017). Therefore, there is an ever-increasing thirst for production of clean renewable energy sources to cater future energy requirements of the society (Abdullah et al. 2019). Also, biofuels formed from diverse biomasses should have cost-viability, natural supportability, and minimization of wastage during the activity and provide bioenergy sustainability to society (Vasudevan and Fu 2010). Biofuels will assume significant role by focusing on the huge fuel market and keeping up with worldwide energy safety (Shuba and Kifle 2018). At present, the commitment of non-renewable energy sources is almost 70% of the aggregate, overall energy requirement, contrasted with the overall power request, which contributes just 30% (Abdullah et al. 2019). Biofuels are very important for the society yet a large number of technologies are centered around the production of electricity from renewable sources of energy (Medipally et al. 2015). Different types of biomass have been used for the creation of biofuels to decrease the reliance on customary non-renewable energy sources (Shuba and Kifle 2018). Biofuels are the fuels, which are derived from biomass (plants, animals, and microorganisms). Based on biomass, biofuels are arranged into following types;

21.2.1 First-Generation Biofuels

They are derived from food producing crop plants, in which the oil from seeds is converted to biodiesel or sugars are converted to ethanol by fermentation (Naik et al. 2010). For creation of biofuels, food crops like wheat and sugarcane are employed for bioethanol production, similarly, oil from oil seeds is employed for production of biodiesel. These biofuels have a variety of problems (Hanaki and Portugal-Pereira 2018). There are reports that combustion of these biofuels leads to emission of large amount of greenhouse gases and in no way, they help in reduction of air pollution. They also release more CO_2 than the amount they capture during photosynthesis. Another issue is use of food crops in production of biofuels leads to an increase in prices of these food crops and they also cause food shortage in the world (Chaturvedi and Verma 2013).

21.2.2 Second-Generation Biofuels

These biofuels were devised to reduce the deleterious effects of first-generation biofuels (Naik et al. 2010). They are synthesized from plant biomass and agricultural leftover biomass, which is considered as waste and normally incinerated (Chaturvedi and Verma 2013).

21.2.3 Third-Generation Biofuels

They are centered on formation of biomass by microorganisms such as algae, which are mass cultivated on barren land and wastewater to produce biomass, which is then transformed into biofuel (Bahera et al. 2015). Growth of algae on wastewater and pollutants helps in remediation of the environment. Another benefit of algae is that a number of fuels like diesel, ethanol, and hydrogen (H_2) can be created from different algal biomolecules (Oncel 2013).

21.2.4 Fourth-Generation Biofuels

These are focused on creating reasonable energy as well as a method of sequestering CO_2. They utilize genetically engineered algae to capture CO_2. Algal biomass is then transformed into fuel utilizing identical procedures of second-generation biofuels. The biofuels are C neutral; that is, they capture more carbon than they release (Abdullah et al. 2019). Different generations of biofuels are represented in Fig. 21.2.

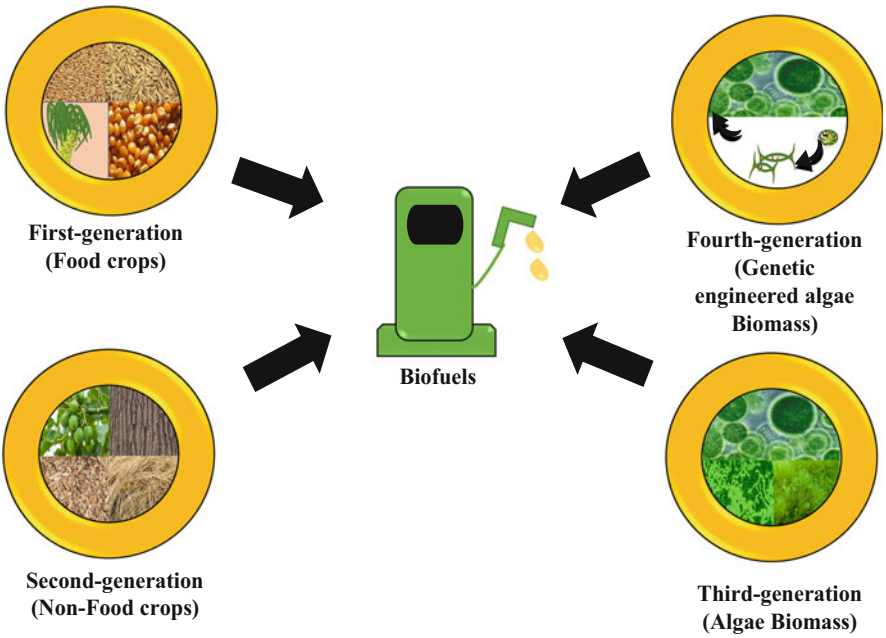

Fig. 21.2 Different generations of Biofuels

21.3 Biofuels from Microalgae

A number of biofuels can be synthesized from algae and cyanobacteria; for example, biodiesel and H_2 gas (Hossain et al. 2008), bioethanol (De Bhowmick et al. 2019). Furthermore, whole biomass can be used for formation of syngas, H_2 formation by dark fermentation, ethanol via fermentation, etc. (Shuba and Kifle 2018) (Fig. 21.3). Thus, microalgae can be efficiently employed for creation of different biofuels.

The significant biofuels delivered by microalgae are as per the following.

21.3.1 Biodiesel

Oil producing microalgae contain large amount of lipids, majority of which are triglycerides (TAGs) and remaining mono and diglycerides, with small amount of free unsaturated fats. Stearic, oleic, and palmitic acid are the main fatty acids found in algae (Kumar and Thakur 2018). The oil content of some microalgae is depicted in Table 21.1. Biodiesel is formed by trans-esterification. The procedure of trans-esterification of lipids occurs in presence of either acid or base or enzyme such as lipase in presence of an alcohol, leading to formation of diesel and glycerol

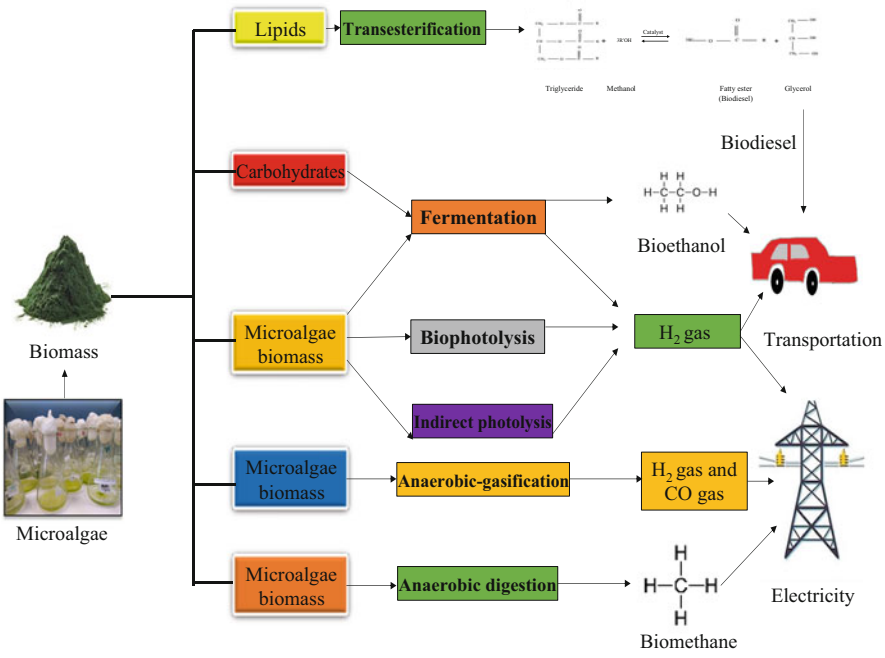

Fig. 21.3 Different types of biofuels produced from microalgae and their bioenergy application

Table 21.1 Oil content of selected microalgae

Name of microalgae	Oil content (%)	Reference
Chlorella vulgaris	40	Liu et al. (2008)
Nitzschia sp.	45–47	Christi (2007)
Chlorella vulgaris	56.6	Liu et al. (2008)
Parietochloris incisa	62	Solovchenko et al. (2009)
Chlorella emersonii	63	Mata et al. (2010)
Nitzschia laevis	29–65	Zhou et al. (2015)
Nannochloropsis sp.	30–68	Mata et al. (2010)
Dunaliella tertiolecta	16–71	Zhou et al. (2015)
Schizochytrium sp.	50–77	Zhou et al. (2015)

(Kumar et al. 2017). Different alcohols are utilized as co-solvent in the trans-esterification procedure, yet as a rule, methanol and ethanol are ideal because of physical and chemical properties and their low cost. In light of steps associated with the creation of biodiesel, trans-esterification is divided into two categories: extractive and in situ trans-esterification. Extractive trans-esterification includes following stages, i.e. cell drying, breakdown of cells, lipid extraction, trans-esterification, lastly downstream procedures and biodiesel cleansing (Saifuddin et al. 2015). This procedure is long and tedious because of quality of water in the biomass; this procedure gets non-commercial for a huge scope. In situ trans-esterification, the separation of lipids and trans-esterification are done in a single step, bringing about a decreased handling time and reduced usage of solvents (Tang et al. 2016). Alcohols are both co-solvent and extraction solvent in this process due to the fact that it penetrates algal cells and encourages the removal of lipids during the reaction. The decreased creation of waste, handling time, and energy utilization alongside better return of biodiesel make this procedure progressively practical at the modern scale.

21.3.2 Bioethanol

It is a carbon neutral fuel, and synthesized mainly from plant biomass. Similarly, biomass from algae can also be used for production of bioethanol via fermentation by using microorganisms (Chaudhary et al. 2014). Presently, *Saccharomyces cerevisiae* and *Zymomonas mobilis* are viewed as main microorganisms for the creation of ethanol through microbial fermentation. Numerous algal species contain different carbon sources, for example, mannitol, agar, glucose and galactose, alginate (Özçimen et al. 2015), which can be transformed into ethanol through fermentation. The amount of sugar in microalgae is roughly 40–50% of their biomass. For the synthesis of ethanol, the algal biomass first undergoes pretreatment and followed by saccharification (Scholz et al. 2013). Pretreatments of plant biomass by acid and base are broadly satisfactory in light of the fact that they are economical, productive

procedures for elimination of undesirable substances, with the appearance of sugar (Vincent et al. 2014). Saccharification is done with the help of microbes, which produce the enzyme amylase, which breaks down polysaccharides into fermentable sugars, which in later stage is transformed to ethanol by the process of fermentation (Jankowska et al. 2017). It is of two types; separate hydrolysis and aging/fermentation (SHF) and synchronous saccharification and aging/fermentation (SSF) (Özçimen et al. 2015). SHF is carried out in two separate reactors, saccharification in one and fermentation in another (Cesaro and Belgiorno 2015). The removal of algal biomass is the main challenge in SHF as it restricts the productivity of ethanol (Offei et al. 2018). Therefore, the retrieval of ethanol is done in situ, which drastically increases the productivity. In SSF, saccharification and fermentation are performed simultaneously, thereby reducing the problem of substrate hindrance as sugars formed during saccharification are converted to ethanol. The efficiency of SSF is very high as compared to SHF (Nguyen et al. 2017).

21.3.3 Biohydrogen

Various algal species are found to create H_2 under specific conditions. Biohydrogen creation in algae happens by three unique processes. The primary strategy is direct photolysis, in which with the help of chlorophyll a and photo system II, where water is broken down to form oxygen and H_2 gas, respectively (Das and Veziroglu 2008). The second process is indirect photolysis, in which during the process of photosynthesis, in presence of enzyme hydrogenase, H_2 gas is produced (Prince and Kheshgi 2005). Third process is dark fermentation in which, in absence of sunlight, microorganisms ferment algal biomass to form H_2 and a number of other metabolites such as acids (Vardar-Schara et al. 2008).

21.3.4 Biomethane

The creation of biomethane (CH_4) by means of anaerobic processing of algal biomass is notable because it leads to formation of gases having high proportion of methane followed by CO_2. The final productivity of biomethane from algal depends upon a number of factors, for example, temperature, rate of biomass stacking and its volume, time, type of bacteria, and cell wall properties of algae (Jankowska et al. 2017). It is observed that Carbon/Nitrogen ratio also plays an important role in biomethane formation. In the event that the proportion is low, at that point yield is additionally low. The process of biomethane formation is completed in four main steps; hydrolysis of biomass, acidogenesis followed by acetogenesis, and finally methanogenesis (Ayala-Parra et al. 2017), and where

methanogenesis is the rate determining step. The economic feasibility of biomethane formation depends mainly on the cost incurred during large-scale culturing on biomass, the technology used for treatment of biomass and biomethane production. (Wu et al. 2019). The final biomethane productivity depends upon the nature of biomass and its constituents.

21.3.5 Biosyngas

It is produced by gasification of biomass in presence of air. The end products of this reaction are gases such as methane, H_2, carbon monoxide (CO), different hydrocarbons, and ash. In gasification process, the biomass (not more than 20% water content) is heated at 800–1200 °C in presence of air. If the process takes place in boilers and turbines, then electricity along with other industrially important compounds can be produced. The yield of different biofuels from microalgae is shown in Table 21.2.

Table 21.2 The yield of selected biofuels from microalgae

Biofuel	Name of microalgae	Yield	Reference
Ethanol	*Chlorella vulgaris*	40%	Lee et al. (2011)
	Chlorococum sp.	38%	Harun et al. (2010)
	Chlamydomonas reinhardtii	29%	Nguyen et al. (2009)
	Chlorococcum infusionum	26%	Harun et al. (2010)
Biodiesel	*Chlorella* sp. BDUG 91771	60% methyl ester	Mathimani et al. (2015)
	Chlorella pyrenoidosa	92.5%	Cao et al. (2013)
	Nannochloropsis oculata	97.5%	Umdu et al. (2009)
	Chlorella sp.	0.12 g/L	Li et al. (2011)
Methane	*Chlorella sorokiniana*	0.22–0.28 L/gVS	Ayala-Parra et al. (2017)
	Spirulina maxima	0.25–0.34 L/gVS	Samson and Leduyt (1986)
	Chlorella vulgaris	0.24 L/gVS	Ras et al. (2011)
	Scenedesmus obliquus	0.18 L/gVS	Mussgnug et al. (2010)
Hydrogen	*Anabaena* sp. PCC 7120	14.9 mL H_2 L-1 h-1	Lindblad et al. (2002)
	Anabaena variabilis	4.1 mL H_2 g dcw-1 h-1	Yoon et al. (2006)
	C. reinhardtii (CC124)	1.3 mL H_2 L-1 h-1	Oncel and Kose (2014)
Syngas	*Chlorella* sp.	50–90%	Liu et al. (2018)
	Scenedesmus sp.	50–90%	Liu et al. (2018)
	Chlorella bulgari	<89%	Hossain and Mahlia (2019)

21.4 Genetic Engineering Strategies to Increase Biofuel Production

Different classes of biofuels are being produced from microalgae, but owing to limited capacity of microalgae to produce biomolecules such as lipids and carbohydrates, biofuel production cost is very high. Their yields are also very low and thus, algal biofuels are not competitive as compared to fossil fuels in terms of their cost (Zhou et al. 2015). In order to increase the yield of biofuels, a number of techniques such as optimization of growth medium, varying environmental conditions were tested but optimum yields were not obtained (De Bhowmick et al. 2015). In order to increase biofuel production, genetic engineering techniques such as heterologous expression of foreign genes, gene silencing by RNA interference (RNAi), gene knockout were performed in a number of microalgae and in many cases fruitful results were obtained. In this section, we have discussed the use of genetic engineering for increasing biofuel production.

21.4.1 Genetic Engineering Strategies to Increase Biodiesel Production

Biodiesel is formed from lipids/fats by a process called trans-esterification (Saifuddin et al. 2015). Numerous microalgae have been shown to produce and accumulate high amounts of lipids, i.e. Triacylglycerols (TAGs) in their biomass. Biosynthesis of TAGs occurs in two compartments; chloroplast and endoplasmic reticulum (Radakovits et al. 2010). During photosynthesis, CO_2 and water combine in presence of light to form sugars, which later form starch. During respiration, starch is metabolized to form glucose, which is then utilized as the fuel to the cell. During metabolism of glucose, it is first converted to pyruvate via glycolysis (Hu et al. 2008). The pyruvate is then converted to Acetyl CoA, to be used in TCA cycle. However, some amount of Acetyl CoA enters into fatty acids (FAs) synthesis pathway. The transformation of Acetyl CoA to Malonyl CoA is the initiating step of fatty acid synthesis (Deng et al. 2013). This step is catalyzed by the enzyme acetyl-CoA carboxylase (ACCase), followed by binding of malonyl CoA with acyl carrier protein to form malonyl-ACP in presence of enzyme malonyl-CoA: acyl carrier protein (ACP) transacylase (MCAT). It then enters in FA synthesis pathway, which is catalyzed by a group of enzymes called ketoacyl-ACP synthases (Radakovits et al. 2010). This group of enzymes is collectively called the FAs complex. After formation of fatty acids, they enter in TAG synthesis pathways, which occur in chloroplast and ER (Coleman and Lee 2004). Some fatty acids are released in the cytoplasm, from there; they are transported to ER for synthesis of TAGs. Formation of TAGs occurs through Kennedy pathway, which starts by acylation of glyceraldehyde-3-phosphate (G3P) to form lysophosphatidic acid (LPA) in presence of enzyme glycerol-3-phosphate acyltransferase (GPAT). In the

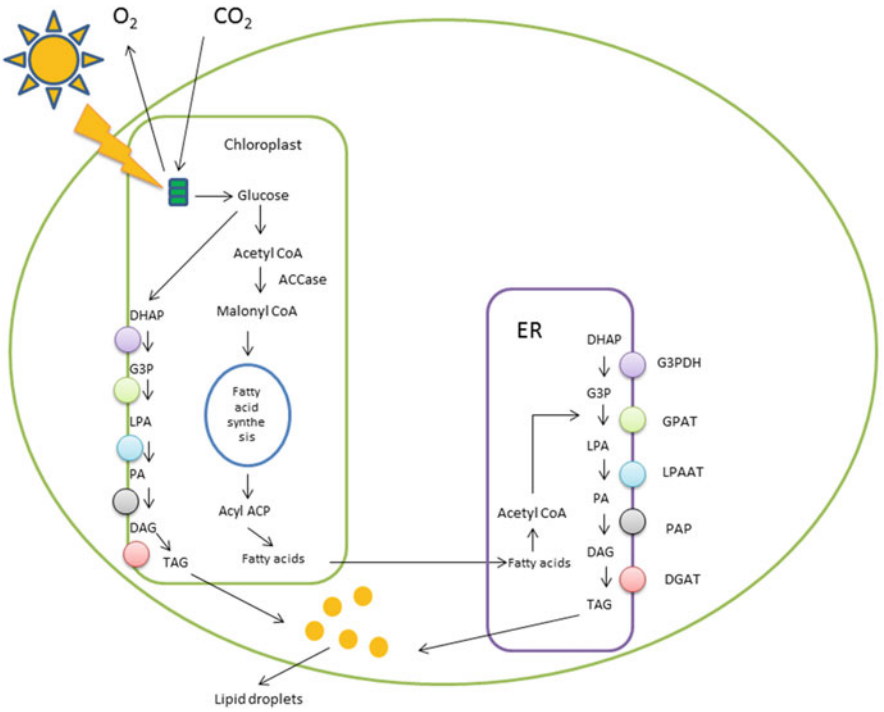

Fig. 21.4 Pathway of TAG biosynthesis in microalgae. Substrate abbreviations: Dihydroxy acetone phosphate (DHAP), glyceraldehyde-3-phosphate (G3P), lysophosphatidic acid (LPA), phosphatidic acid (PA), diacylglycerol (DAG), triacylglycerol's (TAGs). Enzyme abbreviations: acetyl-CoA carboxylase (ACCase), glycerol-3-phosphate acyltransferase (GPAT), lysophosphatidic acid acyltransferase (LPAAT), phosphatidic acid phosphohydrolase (PAP), acyl-CoA: diacylglycerol acyltransferase (DGAT)

second step, LPA is converted to phosphatidic acid (PA), catalyzed by lysophosphatidic acid acyltransferase (LPAAT), PA in then converted to diacylglycerol (DAG), in presence of enzyme phosphatidic acid phosphohydrolase (PAP). In the last step, acyl-CoA: diacylglycerol acyltransferase (DGAT) converts DAG to TAG (Lung and Weselake 2006). The pathway of TAG synthesis is presented in Fig. 21.4.

In order to increase biodiesel production from microalgae, the lipid content has to be increased significantly. A number of genetic engineering-based techniques have been used in microalgae, so as to escalate its lipid content. In most of these studies, one or more genes coding for enzymes playing important role in biosynthesis of lipids were up-regulated or over expressed, in order to increase the rate of lipid production (De Bhowmick et al. 2015). In another strategy, the process of lipid oxidation was significantly reduced by either inhibiting or knocking out key enzymes involved in the process (Liang and Jiang 2013). In *Chlamydomonas reinhardtii*, overexpression of lipid biosynthesis genes such as LPAAT (Yamaoka

et al. 2016), GPAT (Iskandarov et al. 2016), DGAT2 (Iwai et al. 2014) led to a significant increment in TAG production. Similarly, in *Nannochloropsis oceanica*, overexpression of MAT (Chen et al. 2017), DGAT1 (Wei et al. 2017), DGAT2 (Zienkiewicz et al. 2017) also increased TAG yield. TAG yield depends mainly upon the concentration of acetyl CoA, which is the substrate in this pathway. If the concentration of acetyl CoA is increased, then rate of production also increases. The enzyme mitochondrial pyruvate dehydrogenase complex, which converts pyruvate to acetyl CoA, is the main enzyme, which produces acetyl CoA. It is inhibited by pyruvate carboxylase kinase (PDK). It was observed that inhibiting the expression of PDK resulted in substantial increase in lipid biosynthesis (Ma et al. 2014). Similarly, malic enzyme also forms pyruvate from malate, which is subsequently converted into acetyl CoA. It was observed that by increasing the expression of malic enzyme, lipid production was significantly increased (Xue et al. 2015).

Another strategy is suppression of competing pathways, such as starch biosynthesis pathway and lipolysis pathway, which decreases the production of lipids. It was observed that by inhibiting starch biosynthesis pathway enzymes such as ADP-glucose pyrophosphorylase (Li et al. 2010), phosphoenolpyruvate carboxylase I and II (Deng et al. 2014), UDP-glucose pyrophosphorylase (Zhu et al. 2016), lipid yield was increased. Similarly, by silencing lipid catabolizing enzymes such as lipase, phospholipase, lipid production rate was shown to be increased (Trentacoste et al. 2013).

21.4.2 Genetic Engineering Strategies to Increase Bioethanol Production

Polysaccharides exist in the cell and cell wall in the forms of starch and cellulose. Carbohydrates metabolism starts with photosynthesis process. It is by light dependent and light independent pathways. In light dependent pathways, light and water are used for the production of ATP and $NADPH_2$. In light independent pathways, ATP and $NADPH_2$ are utilized for fixation of CO_2 from the atmospheres (Calvin Benson Cycle). The Calvin cycle comprises carboxylation (first stages), where ribulose biphosphate carboxylase (RuBisCO) helps in binding of CO2 with pentose sugar and which forms two moles of phosphoglycerates. In reduction process (second stage) ATP and $NADPH_2$ are required for conversion of phosphoglycerate into diphosphoglycerates and ADP, reduction of diphosphoglycerate into phosphoglyceraldehyde and $NADPH_2$. Last stages are regeneration stages, where ribulose bisphosphate is regenerated with the help of different enzymes (Richmond 2004; Ho et al. 2011; Zhao and Su 2014). After Calvin cycle glucose molecules are formed, which polymerize and synthesize a wide variety of polysaccharides (Chen et al. 2013; Ferro 2019). However, only (10–50%) polysaccharides are formed, which can be used for the production of bioethanol via fermentation by using different suitable microorganisms (Markou and Nerantzis 2013; Choo et al. 2020). The microalgae

biomass is pretreated (saccharification) firstly by using mechanical, chemical, and biological systems. After that, polysaccharides are converted into simple fermentable sugars. The microalgae-based bioethanol production depends on the amount of polysaccharides in biomass, saccharification and conversion process. The low carbohydrate composition and undeveloped refinery system are the bottleneck of bioethanol production. However, this problem can be resolved by enhancing the carbohydrate synthesis in microalga cells by using genetic engineering and developing an integrated co-production biorefinery process for production of bioethanol and biodiesel (Hamed 2016; Kim et al. 2020). Overexpression of starch synthesis, knockout of starch inhibiting genes, knock in of starch synthesis gene or enzymes enhances the starch productivity inside cells (Work et al. 2012; Jagadevan et al. 2018). ADP-glucose pyrophosphorylase (starch synthesis enzyme) overexpression enhances the synthesis of carbohydrates in microalgae cells. Furthermore, knock out of (starch inhibiting genes) glucan-water dikinases and amylases gene, which inhibits the starch degradation and increases the accumulation of starch inside cells (Chang et al. 2016; Jagadevan et al. 2018).

21.4.3 Genetic Engineering Strategies to Increase Biohydrogen Production

There is an urgent requirement for development of new strategies for biofuel production in order to avoid the complications associated with consumption of fossil fuels. Among different types of biofuels available today, biohydrogen is considered as a very important fuel due to a number of characteristics such as high energy density, ease of storage and transport, and it does not emit CO_2 or pollutants after combustion. In microalgae and cyanobacteria, H_2 is produced during photosynthesis by a process called biophotolysis (Eroglu and Melis 2011). The H^+ ions, which are substrate in hydrogen production, come from breaking down of water by photosystem I (PSI). During photosynthesis, PSII produces reduced ferredoxin, which donates electrons to the enzyme hydrogenase, the key enzyme in H_2 production. By using H^+ ions and electrons, hydrogenase produces biohydrogen (Eroglu and Melis 2016). Hydrogenases are of two main classes; Iron containing [Fe-Fe] and nickel containing [Ni-Fe], respectively. In microalgae, there are some cell membrane bound uptake hydrogenases, which consume hydrogen. For higher hydrogen production, these enzymes should be silenced (Ghirardi 2015). In some nitrogen fixing cyanobacteria, the enzyme nitrogenase, key enzyme in nitrogen fixation, also produces some amount of H_2. In this reaction, ferredoxin is also produced during photosynthesis and acts as electron donor (Fig. 21.5). If uptake of nitrogen in inhibited, H_2 formation can be substantially increased (Gutthann et al. 2007). In some green algae, it is observed that under sulfur scarcity and anaerobic conditions, when algal culture is illuminated, hydrogen is produced. This phenomenon occurs due to the presence of damaged D1 protein (Torzillo et al. 2009).

In past few decades, a number of studies have shown upregulation of H_2 production using several genetic engineering techniques. The rationale behind

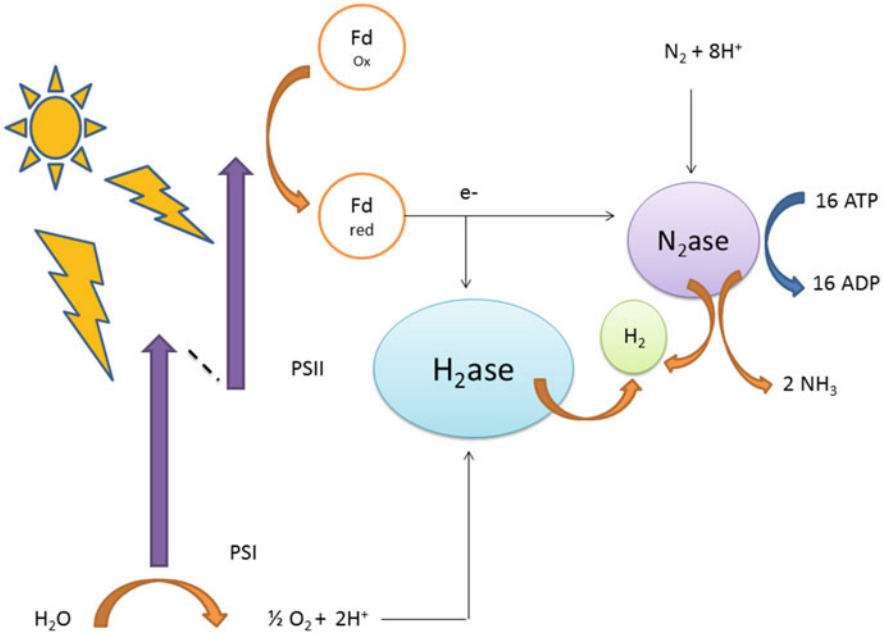

Fig. 21.5 Mechanism of H_2 production by direct photolysis

these techniques is up/downregulation of key enzymes/proteins involved directly/indirectly in H_2 production in these microorganisms. As the enzyme hydrogenase is sensitive to oxygen, it was realized that developing an oxygen resistant hydrogenase would upregulate H_2 production (Chang et al. 2007).

Oxygen resistance was increased by overexpression of hydrogenase gene (Asada et al. 2000). In another study using antisense technology, sulfur uptake genes were silenced, which led to increased oxygen resistance (Melis and Chen 2005). In a similar study, inhibition of D1 protein by site-directed mutagenesis (Torzillo et al. 2009) and inhibition of subunit O of PSII by antisense technology led to overproduction of H_2 (Lin et al. 2013).

Another strategy is inhibition of uptake hydrogenase, which utilizes hydrogen. It was observed that in several knockout strains of microalgae in which these enzymes were silenced, production of H_2 was significantly high in comparison to normal strains (Lindberg et al. 2012; Khetkorn et al. 2012; Raleiras et al. 2016). H_2 production is also dependent upon concentration of electron donors like ferredoxin and NAD(P)H_2 in the cell. These electron donors are involved in other competing reactions as well. Therefore, by channeling these donors towards hydrogenase, H_2 production can be increased. It was observed that by inactivating complex I of respiratory system, H_2 production was increased (Cournac et al. 2004). It was found that nitrate assimilation pathway is also a competitor of H_2 production; therefore, by inhibiting this pathway, H_2 production was increased (Baebprasert et al. 2011) (Table 21.3).

Table 21.3 Different genetic engineering strategies for enhancing of lipid, carbohydrates, and hydrogen substrates for production of different bioenergy products

Biomolecules	Bioenergy product	Strategy of genetic engineering	References
Lipids	Biodiesel	Overexpression of lipid biosynthesis genes LPAAT	Yamaoka et al. (2016)
		Overexpression of lipid biosynthesis genes GPAT	Iskandarov et al. (2016)
		Overexpression of lipid biosynthesis genes DGAT2	Iwai et al. (2014)
		Overexpression of lipid biosynthesis genes MAT	Chen et al. (2017)
		Overexpression of lipid biosynthesis genes DGAT1	Wei et al. (2017)
		Overexpression of lipid biosynthesis genes DGAT2	Zienkiewicz et al. (2017)
		Upregulation of DGAT2	Niu et al. (2013)
		Inhibition of pyruvate carboxylase kinase (PDK)	Ma et al. (2014)
		Increasing the expression of malic enzyme	Xue et al. (2015)
		Inhibiting starch biosynthesis pathway enzymes such as ADP-glucose pyrophosphorylase	Li et al. (2010)
		Phosphoenolpyruvate carboxylase I and II	Deng et al. (2014)
		UDP-glucose pyrophosphorylase	Zhu et al. (2016)
		Silencing lipid catabolizing enzymes lipase, phospholipase	Trentacoste et al. (2013)
Carbohydrates	Bioethanol	Overexpression of ADP-glucose pyrophosphorylase starch synthesis enzyme	Work et al. (2012)
		Knockout of glucan-water dikinases and amylases genes	Jagadevan et al. (2018)
Complete biomass	Biohydrogen	Inhibiting the uptake of dinitrogen	Gutthann et al. (2007)
		Sulfur scarcity and anaerobic conditions (damaged of D1 protein)	Torzillo et al. (2009), Batyrova and Hallenbeck (2017)
		Design an oxygen resistant hydrogenase gene	Chang et al. (2007)
		Overexpression of hydrogenase gene	Asada et al. (2000)
		Silencing of Sulfur uptake gene	Melis and Chen (2005)
		Inhibition of D1 protein via site-directed mutagenesis	Torzillo et al. (2009)
		Inhibition of subunit O of PSII by antisense technology	Lin et al. (2013)

21.5 Conclusion

Microalgae are well suited microorganisms for biofuel production owing to a number of useful characteristics. A number of microalgae have been exploited for biofuel production. But still, algal biofuel is not preferred over fossil fuels due to a number of reasons. First and foremost reason is the high cost of these biofuels due to high amount of expenditure in separation of algal biomass, removal of water from biomass, and costly techniques involved in biofuel production. Another important reason is low biomass yield as compared to plant biomass and very low amount of lipids and carbohydrates, which makes the overall process very much energy intensive. This hinders mass production of algal biofuels. A number of genetic engineering techniques were employed to uplift biomass yield and amount of biomolecules from microalgae. Some of these approaches have shown prominent results, where significant increase in the yield was obtained. However, it is felt that still a large amount of research is required in this area to harness the full potential of these microorganisms. With the advent of gene editing techniques such as CRISPR/Cas9 system and in silico approaches such as genome-scale metabolic models (GEMs), it is believed that real breakthroughs in biofuel production will be achieved.

Competing Interests All the authors declare that they have no competing interests.

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Chapter 22
Decentralised Anaerobic Digestion Systems as Basis for Future Biorefinery Platforms

Ehiaze Ehimen and Seán O'Connor

Abstract The production of chemicals and industrial raw materials via the application of the anaerobic digestion (AD) process on agricultural and bio-industry derived wastes and by-products has been increasingly discussed. While the implementation of such schemes for raw material production is foreseen to potentially increase the valorisation capacity and the overall circularity of AD systems (i.e. by availing the production of other economic outputs other than the energy carrier methane, CH_4), widespread implementation of such approaches is still lacking. This chapter provides a comprehensive review of the current state of the art related to AD-based biorefinery approaches and highlights some operational and economical limitations preventing the uptake of such systems. The chapter then further draws attention to some potential candidate processes and products which could be produced from decentralised AD (with a focus on small-scale AD) systems to meet intended biorefinery objectives.

Keywords Small scale · Anaerobic digestion · Biogas · Decentralised systems · Value-added products

Abbreviations

AD	Anaerobic digestion
CHP	Combined heat and power
EU	European Union
FIT	Feed-in tariffs
GHG	Greenhouse gases
IEA	International Energy Agency
RHI	Renewable heat incentives
SSAD	Small-scale anaerobic digestion
VFAs	Volatile fatty acids

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22.1 Introduction

The rapid rise of the world population over recent decades has contributed to considerable increases in energy demand, material consumption and waste generation (Surendra et al. 2015). The excessive use of fossil fuels to meet these demands has had severe implications for the environment resulting in the average global temperatures increasing by 1.1 °C since pre-industrial levels due to human activity (IPCC 2013, 2014, 2018). If current trends continue, the temperature will likely surpass 1.5 °C between 2030 and 2052, with potentially disastrous environmental consequences, including extreme weather phenomena, depleting natural resources and desertification of fertile areas (IPCC 2018). Therefore, the need to pursue and develop alternative, environmentally friendly sources of energy is apparent (Farrell et al. 2006; Semelsberger et al. 2006).

To address these environmental challenges, renewable energy technologies have been developed and promoted (i.e. wind, solar, hydro, geothermal and biomass) as potential alternatives to petroleum-based fuels. Consequently, the global power generation derived from renewables has grown tremendously increasing from 1347 GW in 2010 to 2537 GW in 2019 (d'Ortigue et al. 2015; IRENA 2020). Of the available technologies, anaerobic digestion (AD) has emerged as a promising GHG mitigator with the capacity to mitigating greenhouse gas emissions while generating renewable energy. The process can efficiently convert a diverse range of biodegradable feedstocks to produce a methane (CH_4) rich gaseous output commonly referred to as biogas. The AD process is usually characterised by having the organic matter feedstock broken down by natural microbial communities over four consecutive steps (hydrolysis, acidogenesis, acetogenesis and methanogenesis) in an oxygen-deficient environment. The biogas generated can be consumed directly by heating applications, applied to a combined heat and power (CHP) unit to produce electricity and heat, or upgraded to produce biomethane (with higher methane and thus energy content than the original gas mixture). The production of bioenergy from AD, therefore, affords a promising technology route for the achievement of climate change reduction goals while providing a useful waste treatment and management route for the reduction of potentially polluting waste streams. AD systems aimed primarily at heat and energy production provide several advantages, with the main advantage being the potential replacement of fossil fuels with the CH_4 gas produced. Furthermore, the organic residues remaining post-digestion can be applied as a mineral-rich fertiliser. The technology is especially promising considering that over 90 million tonnes of organic waste are generated yearly from the agricultural and agri-food industries within the EU-28 (Stenmarck 2016).

Despite these advantages which have encouraged an increased use of AD systems (Fig. 22.1) globally, AD use in smaller-scale scenarios have however been observed to still face some limitations with regards its uptake. This is mainly since the economies of scale favour improved profitabilities obtained via the establishment and operation of larger AD systems aimed for biogas production (with higher biogas volumes produced mitigating the high levels of process and equipment

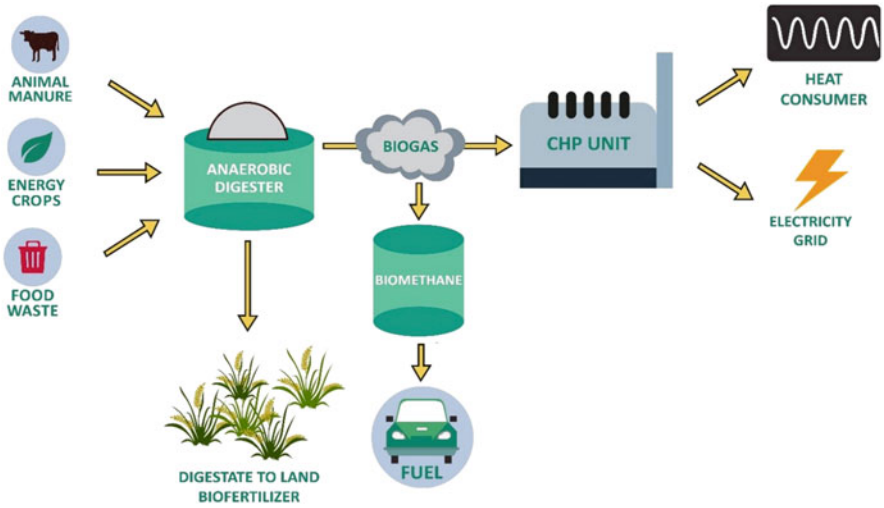

Fig. 22.1 Schematic of farm-scale anaerobic digestion plant

investments). However, AD systems principally focused on biogas as the sole valuable outputs usually suffer "carbon wastage", since a large proportion of the total carbon available in the biomass is not converted to biogas. Previous studies have reported that only about 40% of the initial carbon in the biomass was converted to biogas in AD processes (Dinh et al. 2019). Even though the waste treatment uses of AD systems are desirable, these limitation issues are especially problematic for the future expansion and implementation of AD in farm and small scales since the operation of such systems will be highly dependent on its economic viability. Schemes which could improve the value-added outputs emanating from the AD process which in turn maximises the overall valorisation of the feedstock biomass composition into a larger variety of products would therefore be beneficial. The application and integration of biorefinery concepts to AD systems could potentially be used to address such identified limitations facing the viability of small-scale AD installations.

The International Energy Agency (IEA) defines biorefineries as systems which support the "sustainable processing of biomass into a spectrum of marketable products (food, feed, materials and chemicals) and energy (fuels, power, heat)" (van Ree and van Zeeland 2014). The concept of biorefining and biorefineries has seen increased use in recent time especially due to an increased drive by businesses to be more resource-efficient, increased government-driven policies promoting the sustainable use of biomass to meet fuel and products production and to meet overall fossil fuels demands reduction and more consumer interest and request for "greener" products and fuels.

The purpose of this chapter is to present an overview of the reported use of AD-based biorefinery systems for the production of chemicals and industrial raw materials. This review focuses on the integration of the biorefinery to small-scale

anaerobic digestion (SSAD) applications by examining the technological, operational and economic considerations of various approaches. In addition, the chapter investigates different configuration and production scenarios to improve the use of AD systems to meet this goal. The work highlights progress in the field, problems and difficulties faced and further outlook.

22.2 Role of Decentralised Systems as Biorefineries

22.2.1 Small-Scale AD Systems

Over the past 20 years, the biogas industry within the European Union (EU) has grown tremendously with biogas electricity production peaking at 10,532 MW by the end of 2017 across 17,783 plants (European Biogas Association 2019). The growth in the industry has been primarily driven by the EU commitment to reduction of greenhouse gas (GHG) emissions through the promotion of climate action policies such as the 2020 climate & energy package and the EU's 2030 Policy Framework for Climate and Energy (EC Communication 2008) (EC Communication 2014). These European directives have encouraged national governments to introduce climate action incentives, such as feed-in tariffs (FIT) and the renewable heat incentives (RHI) resulting in increased public and private investment as the financial viability of potential projects became more attractive to investors.

The biogas plants deployed have typically consisted of large-scale centralised facilities using vast quantities of biomass feedstock from several surrounding sources (Raboni and Urbini 2014). However, facilities outside the catchment area or stand-alone agricultural environments cannot typically implement the technology as the feedstock quantities available do not justify the investment (Štambaský 2016). Small-scale anaerobic digestion (SSAD) (plant CHP capacity between 15 and 100 kW_e) hold promise to potentially overcome this barrier as its operation provides greater portability and flexibly with lower capital costs. The application of SSAD technology in agricultural environments which would have been previously considered financially unsustainable (on basis of centralised feedstock supplies and availability) could therefore enable the valorisation of waste biomass in such environments. The adoption of SSAD plants within the EU-27 has increased sharply in recent years. In particular, Germany has seen rapid growth in the adoption of SSAD plants mainly contributed to the introduction of the Renewable Energy Source Act in 2012, which included a special feed-in tariff for biogas plants with an installed electrical capacity up to 75 kW. In 2016, approximately 560 manure-based biogas plants were in operation across the country, producing 271.3 GWh_e annually (Daniel-Gromke et al. 2018). Similarly, the Flemish region in Belgium has also seen growth spurred by the Flemish Climate Fund for the development of over 80 SSAD plants operating in the region (De Dobbelaere et al. 2015).

A wide variety of agricultural by-products and dedicated energy crops are available for the production of biogas at SSAD plants. A diverse range of digesters

designs can be implemented with the selection primarily based upon the feedstock available. Although all digester types conduct the same basic operation, they can vary widely depending on operational performance, regional environmental factors. These systems are generally classified by three main categories: passive systems (i.e. covered lagoon), low-rate systems (i.e. garage-type, plug-flow, complete mix) and high-rate systems (i.e. fixed film and suspended media). Furthermore, other variables can have a substantial impact on plant performance, such as substrates characteristics, operating temperature, pH level and retention time (Weiland 2010).

Some farm-scale anaerobic digestion (AD) plants have implemented pre-treatment technologies because of their potential to enhance biogas production and increase biomass digestibility (Zhang et al. 2014). These pre-treatment schemes are increasingly integrated with AD process operations in small-scale scenarios due to the growing incorporation of lignocellulosic biomass (i.e. grass and other plant based substrates) in the digestion process along with the traditional manure feedstocks. These feedstocks are often difficult to digest because of their highly recalcitrant structures. Therefore, the incorporation of pre-treatments is necessary to improve the overall digestibility of the biomass feedstocks and is normally concentrated on enhancing the disruption of the lignocellulosic biomass structure into simpler to digest C5 and C6 sugars. Pre-treatments are generally categorised into three main classifications, being physical, chemical and biological methods (Jain et al. 2015). Physical pre-treatment, through mechanical or thermal disruption, can be applied to positively change the biomass feedstock at the molecular level (Hartmann et al. 2000). Although thermal pre-treatments have many advantages in terms of being a relatively low-cost solution to increase the digestibility of the organic feedstock and to remove pathogens, the methane yield is not necessarily higher than that obtained from untreated wastes (Carrere et al. 2016). Similarly, mechanical pre-treatments are often necessary to introduce lignocellulosic biomass into the digester but can require significant capital costs (Elliott and Mahmood 2012; Ren et al. 2018). Alternatively, chemical pre-treatments, primarily in the form of acids and bases, have proven to be effective (Croce et al. 2016). In addition, biological pre-treatments have become a popular research topic in recent years, mainly for their capacity to increase the AD rate and promote hydrolysis (Ren et al. 2018).

A growing trend by SSAD manufacturers is to use a modular approach in the design of their digesters. The main advantage of this approach is the flexibility in treatment capacity it provides, as individual modules can be easily added or removed, depending on the required feedstock stream (Kougias and Angelidaki 2018; Biolectric 2019). Other benefits include increased ease and speed of installation, lower capital costs and greater mobility, as the modules are not fixed to a single location. Several businesses are in the testing phase or have fully commercialised various modular digesters for sale in the European market place (Alchemy Utilities Holdings Ltd 2018; Bio Ferm Energy Systems 2018; Demetra Ltd 2018; Earthlee Pty Ltd 2018; QUBE Renewables Ltd 2018; SEaB Energy 2018).

22.2.2 AD-Based Biorefinery Platforms

While the co-digestion of lignocellulosic biomass can be seen as a useful route to improve the digestion outputs of SSADs (O'Connor et al. 2020), the application of pre-treatment for SSAD aimed at mainly biogas production appears to not only be costly but will also lead to the production of an appreciable quantity of side wastes streams that require additional treatment before final disposal. The inhibitive high pre-treatment cost for SSAD operators and the loss of potentially valuable biomass components in the side waste streams are thus important challenges which could affect the overall viability, and the achievement of the economic and environmental sustainability of SSADs aimed primarily at energy production.

As highlighted in Sect. 22.1, the incorporation and implementation of a biorefinery concept for traditional and lignocellulosic AD substrates in SSAD systems could be a potentially useful mechanism for realising the economic viability and environmental goals of the use of such systems. This is with the goal of utilising the "biorefinery" as a platform that builds on the primary AD processes, by integrating additional conversion processes to afford the production of value adding fuels and chemicals as co-products from the system (Fig. 22.2). The AD-based biorefinery concept can be presented as analogous to conventional fossil fuel and petroleum-based refineries which are used for the production of several fuels, chemical products and power from a single input feedstock (crude oil) and using a plant in a single location. The production of several outputs from the AD-based conversion system could, therefore, provide greater market opportunities which in turn improve its economic viability and sustainability.

In addition, the biorefinery concept application could provide an opportunity to utilise diverse feedstocks which would otherwise be seen as a challenge for conventional AD plants focused on just biogas production. This is since the substrate composition is usually closely monitored to prevent variation in the consistency, digestibility characteristics and eventual outputs of such systems. With the biorefinery approach, however, such variation could be regarded and seen as an opportunity and be exploited for the production of more diverse products.

22.3 Technologies to Enhance Decentralised and Small-Scale AD Plants Use as Biorefineries

22.3.1 Nutrient Recovery from Digestate

The last decades have seen a growing emphasis on the preservation of natural resources and the improvemnet of sustainability practices in agriculture (Drosg et al. 2015). Encouraged by this trend, many countries in the EU have introduced regulations for their respective agriculture sectors focused on nutrient and manure management (Drosg et al. 2015). To date, digestate is typically used as a

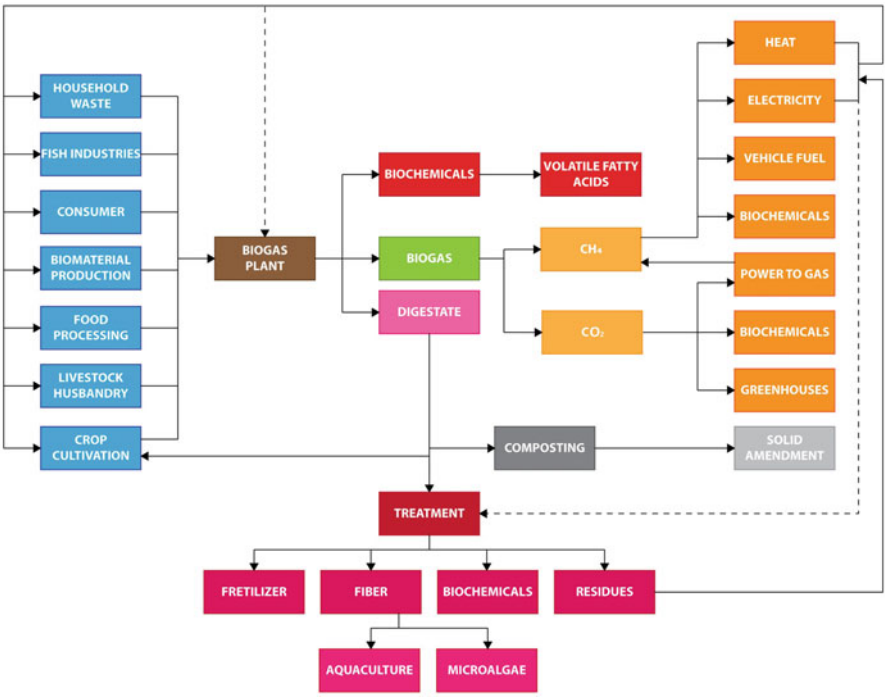

Fig. 22.2 AD-based biorefinery scenarios (arrows indicate biomass and/or energy flows, dashed arrows indicate in process energy flows)

bio-fertiliser without any further treatment, minimising the need for industrially produced mineral fertilisers (Drosg et al. 2015; Romero Güiza et al. 2016). However, the increasing production and availability of AD digestate could creates problems related to nitrate leaching, nutrient overdose and ammonia emissions (Monlau et al. 2015; Romero Güiza et al. 2016). At the EU level, legislation such as the European Nitrate Directive 91/676/EEC has been introduced to tackle this, which restricts the amount of nitrates that can be applied annually to agricultural land within EU member states. Both for environmental and legislation reasons the processing of digestate is increasingly shifting from nutrient removal and disposal towards integrated nutrient recovery and recycling (Drosg et al. 2015; Monlau et al. 2015). These nutrient recovery technologies include those that are capable of separating nutrients from digestate in mineral form or can produce an end-product with a higher concentration of plant nutrients as presented in Fig. 22.3 (Drosg et al. 2015). The integration of nutrient recovery technologies in AD plants in some EU regions has been further neccessitated due to specific regulations restricting the direct use and disposal of digestate on farm lands due to the presence of manure in the substrate (Kayser et al. 2015). The integration of nutrient recovery technologies holds promise by potentially adding value to the digestate by-product and improving

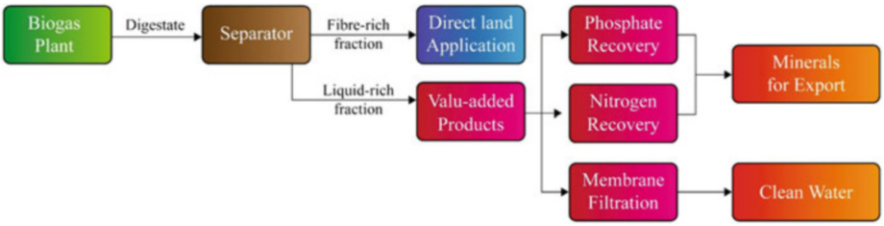

Fig. 22.3 Nutrient recovery technologies integrated into AD-biorefinery

profitability of AD processes (Drosg et al. 2015). Several technologies are in development or on the market at different degrees of technical maturity (Drosg et al. 2015). Technologies successfully applied to agriculture digestate include drying, evaporation, stripping and membrane filtration (Arbor project n.d.; Bernet and Béline 2009; Fuchs and Drosg 2013; Drosg et al. 2015; Sheets et al. 2015; Monfet et al. 2018).

22.3.1.1 Phosphorus Recovery

The recovery of phosphorus through struvite precipitation from agricultural digestate has been widely studied (Zeng and Li 2006; Zhang and Lau 2007; Fernandes et al. 2012; Huchzermeier and Tao 2012). The process works by adding magnesium salts to form a complex compound called struvite (magnesium, ammonium phosphate or MAP), which precipitates and is insoluble (Doyle and Parsons 2002; El Diwani et al. 2007; Uysal et al. 2010; Gerardo et al. 2013). Struvite can then be separated and recovered for use as a slow-release fertiliser supplying nitrogen, phosphorus and magnesium for plant growth in the horticulture and agriculture sectors (Le Corre et al. 2009; Capodaglio et al. 2015; Lin et al. 2015). The process can be heavily influenced by the pH level where it is often necessary to artificially increase to achieve an optimal range for precipitation of approximate pH 10 (Pell Frischmann Consultants Ltd 2012). Other influencing factors include reaction time, chemicals added, temperature and the presence of other ions in the solution (Lin et al. 2015).

Researchers have experimented using precipitate struvite for small-scale, decentralised applications. Ganrot et al. (2009) designed a micro-scale phosphorus recovery unit which combined zeolite adsorption and struvite precipitation (Ganrot et al. 2009). The method separated urine from faeces with an "Aquatron" system, and the phosphorus was precipitated to produce struvite. Another study applied struvite precipitation digestate produced from cattle manure to provide a cost-effective solution (Castro et al. 2018). The highest recovery rates of PO_4^{3-} and NH_4^+ achieved through this study were 58 ± 7.72% and 55 ± 4.94% at a 450 rpm stirring speed, 1.5 molar ratio and a 50 min reaction time.

The feasibly of integrating such technology with farm-based anaerobic digestion is not easily generalised, with the support schemes available in the different countries and regions often being a decisive factor (Drosg et al. 2015). Moreover, the

sources used for the magnesium and alkali chemical agents can have a dramatic effect on the cost of the process as they can contribute up to 75% of total production expenses (Dockhorn 2009; Campos et al. 2019).

22.3.1.2 Nitrogen Recovery

Ammonia stripping (or air or steam stripping) is another nutrient recovery technology which could be used in the removal of ammonia from digestates (Liao et al. 1995). The by-product of this process consists of a high-quality fertiliser rich in macronutrients, nitrogen and sulphur (Vaneeckhaute et al. 2013, 2014, 2017). The process works by introducing air or steam into a stripping tower with the use of compressors, with liquid digestate flowing in a converse direction (Zeng et al. 2006; Zarebska et al. 2015). After contact, the ammonium ions (NH4+) in the liquid digestate convert to ammonia gas (NH_3) and are carried away by the flow of air or steam (Shi et al. 2018). The gaseous NH_3 can be then converted back to the liquid phase with the use of a liquid acid solution (Jamaludin et al. 2018). The transfer rate of ammonia from the liquid to the gas phase is dependent on the processes temperature, pH and mass transfer area (Limoli et al. 2016).

Recent studies on ammonia stripping have now moved from lab to full-scale systems with a range of approaches being trialled, including thermal evaporation, or the use of air, biogas, steam and nitrogen as stripping agents, and with and without solid–liquid separation (Bonmatí and Flotats 2003; Zeng et al. 2006; Walker et al. 2011; Jiang et al. 2013; Ledda et al. 2013; Nie et al. 2015; Li et al. 2016). Researchers have focused on the testing of operating conditions for optimum stripping efficiency with the agriculture residues used including piggery wastewater (Liao et al. 1995; Bonmatí and Flotats 2003; Lei et al. 2007; Gangagni Rao et al. 2008; Zhang et al. 2012) and livestock manure digestate (Zeng et al. 2006; Jiang et al. 2014; Tao and Ukwuani 2015; Bousek et al. 2016; Huang et al. 2016). The main limitations associated with integrating the technology with AD have included the high electrical requirement, use of chemical reagents and the high investments costs required for appropriate machinery (Drosg et al. 2015). It is envisioned with the continued advancement of the technology and an increasing legislative emphasis on the environment such systems will increasingly become more financially sustainable.

22.3.2 Extraction of Volatile Fatty Acids

Various difficulties can be experienced in the management of agriculture waste streams such as a high moisture content and rapid decomposition under atmospheric conditions (Yin et al. 2014; Surendra et al. 2015). A potential method to overcome these difficulties is the bioconversion of organic feedstocks through the AD process into value-added chemicals such as volatile fatty acids (VFAs). VFAs are short-

chain aliphatic monocarboxylate compounds, which are formed during the methanogenesis stage of the AD process. These acids include mixed or purified chemicals that have been commonly used in the pharmaceutical food, leather, plastic and textile industries (Wainaina et al. 2019; Lukitawesa et al. 2020). The VFAs produced and extracted from the AD process could further be considered for used as a principal feedstock and carbon source in the production of biodiesel, the production of electricity in microbial fuel cells, and as a carbon source in biological denitrification processes (Lim et al. 2006; Fei et al. 2011; Chen et al. 2013; González-García et al. 2019).

As previously discussed in Sect. 22.1, AD comprises of a complex biochemical process which involves four stages including hydrolysis, acidogenesis, acetogenesis and methanogenesis. Within the biorefinery approach, VFAs production occurs in the first two stages, where complex organic polymers in the feedstock are broken down into simpler organic monomers in the hydrolysis phase. The acidogenic stage follows which sees the conversion of the hydrolytic produced monomers to mainly VFAs, including acetic, propionic, isobutyric, butyric, isovaleric, valeric and caproic acids (Lukitawesa et al. 2020). The quantity of VFAs produced is highly dependent on the bioreactors operating conditions with optimal parameters including a short hydraulic retention time (1–7 days), a marginally acidic pH (6.0–7.0), a thermophilic operating temperature, appropriate use of chemical methane inhibitions and a high organic loading rate (Lim et al. 2008; Atasoy et al. 2018; Wainaina et al. 2019; Battista et al. 2020). VFAs have been successfully extracted from various agriculture feedstocks such as starch-rich potato-processing wastewater, food waste and olive mill wastewater (Elefsiniotis and Wareham 2007; Scoma et al. 2013; Trevisan et al. 2014).

Studies have reported the integration of VFA extraction technologies in agriculture AD plants is both environmentally friendly and cost-effective (Alkaya and Demirer 2011; Trevisan et al. 2014). The extraction process selected is key to these plants financial viability as it typically encompasses the highest costs (excluding pre-treatments). The processes available include solid–liquid extraction, ultrasound, supercritical fluid extraction, biochemical, etc. (Fermoso et al. 2018). Furthermore, the plant's economics is heavily reliant on the value of the extracted product, which can fluctuate depending on the market price. A recent study demonstrated the potential of the technology by analysing the productiveness of a pilot plant comprising of a two-stage AD process with VFAs and hydrogen produced in the first stage and biogas generated in the second stage (Righetti et al. 2020). The yield of acids produced was 0.13 gCODVFA/gTVS, which was comparable to similar previous studies, i.e. a yield of 0.15 gCOD/gTVS from manure and maize silage (Cavinato et al. 2017).

22.3.3 Ethanol Production

Bioethanol has gained significant recent attention in the research community as a potential contender to replace liquid fuels produced from oil (Cesaro and Belgiorno 2015). It can be produced from sugar and starch-rich biomass similar to those employed by the biogas industry, this is through a process involving hydrolysis, fermentation, distillation and dehydration as seen in Fig. 22.4 (Scott et al. 2013). Despite recent progress, the technology still experiences significant technological issues primarily in the generation of large fractions of wastes from lignocellulose-based biomass (also known as stillage) when it is used as feedstock for ethanol production. The stillage produced is commonly used as an animal feed due to its macromolecular and mineral-rich composition, i.e. containing amino acids, free sugars and proteins (Wilkie et al. 2000; Kim et al. 2008; Moestedt et al. 2014). However, the ethanol production processes related to the pretreatment of the biomasss can be energy intensive accounting for up to 30–45% of the total energy consumption (Ziganshin et al. 2011; España-Gamboa et al. 2012). This provides an oppourtunity where such biomass can be degraded anaerobically through the process of AD to both treat the organic material and simultaneously produce biogas for energy recovery (Eskicioglu et al. 2011; Martin and Eklund 2011; Westerholm et al. 2011). The digestate residue produced from the AD process can further be returned back into the ethanol-production plant (Surendra et al. 2015) to add to the overall ethol yields obtainable from the biomass. Other benefits include stillage having a high chemical oxygen demand (COD) value of over 50% ideal for the conversion to biogas (Cesaro and Belgiorno 2015). In addition, the energy necessary to convert the stillage by-product is dramatically reduced as the residue has already been partially degraded through the fermentation process (Cesaro et al. 2014; Cesaro and Belgiorno 2015).

The combination of biogas and bioethanol production achieves economic advantages by improving the process competitiveness and stability in comparison to individual fermentative plants, as they are heavily reliant on the market value of its main product (Cesaro and Belgiorno 2015; Manzetti and Andersen 2015), furthermore, improving the energy and mass balance of the plant. In 2009, one of

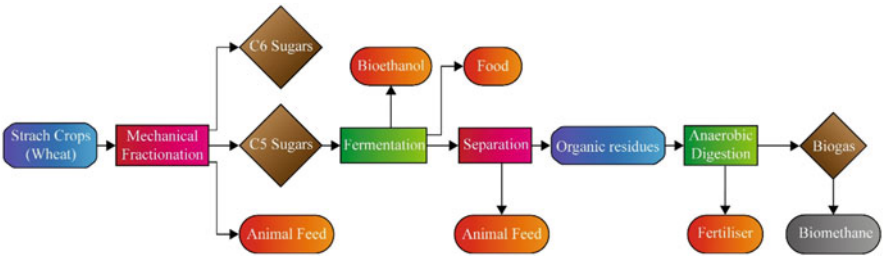

Fig. 22.4 Biorefinery schematic for the production of biomethane, fertiliser, animal feed, food and bioethanol from starch crops

the first plants of this kind was constructed and opened at Kalundborg, in Denmark (Cesaro and Belgiorno 2015; Chemical Technology 2020). This plant was designed to treat 30,000 t/year of biomass, with the aim of producing 5.4 million litres of ethanol, 11,100 t of C5 molasses and 13,000 t of lignin pellets which was separated from the initial substrate (Chemical Technology 2020). The average ethanol yields produced were between 200 and 280 L/ton (Cesaro and Belgiorno 2015).

22.3.4 Composting

Composting is a natural process commonly used for the decomposition of organic matter, normally waste products. This process is dependent on several factors including temperature, pH, porosity, nutrient balance and moisture content (Wainaina et al. 2020). These factors combined to control the rate of organic matter degradation and microbial growth, which are key considerations for process optimisation (Das and Keener 1996; Agnew and Leonard 2003; Wainaina et al. 2020). Composting and AD technologies often compete against each other for their potential implementation on waste disposal projects with final selection primarily based on economics, project-specific requirements, treatment requirements and technological capability. The characteristics of the feedstock available to be processed can play a decisive factor in these deliberations with biomass comprising of a high biomethane potential (BMP) content often necessary for the plant to be economically viable.

In recent years, a new approach has been trailed which combines both composting and AD technologies using synergies to enhance plant performance. The process includes both an AD digester and composter where digestate from the AD plant becomes the feedstock inputs for the composting process and vice versa (Kraemer and Gamble 2014). Through this process many advantages can be achieved including the removal of the effluent treatment process depending on the feedstock, unsuitable biomass can bypass the AD process and greater economies of scale can be achieved. Table 22.1 presents an economic comparison between composting only and compostion combined with AD.

22.4 Discussions and Conclusion

One of the main advantages for the implementation of AD in small-scale scenarios is the ability to afford bioenergy production via the biogas outputs from the system. The produced energy can in turn be used to meet various on-site applications, as well as to potentially meet the power and heating energy needs of surrounding demands (where energy excesses are available from the AD). For power production, the use of gas generators or more ideally, CHP will be applied.

Table 22.1 Net present value comparison of composting only, versus composting and AD. Reprinted with permission from Kraemer T, and Gamble S (2014) 'Composting Digestion Synergy', Biocycle, pp. 32–36

20-year project life (costs)	Scenario 1 40,000 tons/year (tpy) Composting ($)	Scenario 2 30,000 tpy composting 10,000 tpy AD ($)	Scenario 3 30,000 tpy composting 20,000 tpy AD ($)
Total initial capital spending	$12,818,848	$18,064,370	$23,168,419
Total equipment replacement	$5,210,000	$5,320,000	$5,470,000
Total operating and maintenance	$22,041,857	$27,263,214	$33,273,080
Total grants and revenues	$47,307,323	$57,049,597	$84,055,659
Net present values	$1,895,081	$1,012,888	$11,068,631

The high capital costs of the biogas installations as well as the low energy-conversion efficiencies and high capital and maintenance/operating costs of such CHP systems are major shortcomings of current AD systems focused solely on biogas production. This is especially more pronunced in SSAD applications where the ability to access capital is limited, and the volumes of biogas produced are not substantial enough to ensure sustained higher revenues. In this context, the utilisation of the SSAD system for the generation of additional or alternative value-added products and processes and other higher-value outputs could result in a higher return on investment. In this chapter, the additional stripping of nutrients, the extraction of volatile fatty acids (VFAs), the integration with ethanol production and the implementation of composting processes to the AD process have been highlighted as potential side processes which could be integrated with existing SSAD systems.

While these value-added products could potentially improve the sustainability and overall economic viability of SSAD, the extent of how the added value products could economically contribute to current SSAD operations were not covered in this chapter.

With little demonstrations of such integrated systems in practice, more work (modelling and pilot) is therefore still required to ascertain the financial and operational advantages accruable with the incorporation of such side processes and products to existing biogas systems especially as applicable to small-scale concepts.

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Competing Interests All the authors declare that they have no competing interests.

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Chapter 23
Techno-Economic Assessment of Biomass-Based Integrated Biorefinery for Energy and Value-Added Product

Bikash Kumar and Pradeep Verma

Abstract Biological feedstocks and waste biomass represent a suitable alternative for the generation of biofuels and value-added materials replacing fossil fuels. However, the application of biobased feedstocks resulting in full-scale generation of biofuel and chemicals is limited and some of the technologies are in their early developmental stages. The literature on the techno-economic aspects of the different steps and technologies involved in biomass-based biorefinery is lacking. The present chapter will attempt to suggest the viability of integrated biorefinery over single feedstock-based biorefinery. The techno-economic viability and limitations that need attention while considering the development associated technologies with the major steps of the integrated biorefineries such as cultivation, harvesting, collection, logistics, pretreatment, fermentation, separation have been discussed in detail. This chapter also assesses the policy implementation in different developed and developing countries for promoting biomass-based biorefinery.

Keywords Techno-economic · Feedstocks · Biofuels · Biochemicals · Integrated biorefinery

Abbreviations

AEDP	Alternative energy development plan
CDM	Clean development mechanism
GHG	Greenhouse gas
IREDA	Indian Renewable Energy Development Agency
LCA	Life cycle assessment
LPMO's	Lytic polysaccharide monooxygenases
MoA	Ministry of Agriculture
MoEF	Ministry of Environment and Forests

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SFA	Swiss Federal Administration
SHF	Separate hydrolysis and fermentation
SmF	Submerged fermentation
SSCF	Simultaneous saccharification and co-fermentation
SSF	Simultaneous saccharification and fermentation
SSF	Solid-state fermentation

23.1 Introduction

The energy and chemical needs of modern-day civilization are usually met by the traditional petrochemical refinery (non-renewable) (Frumkin et al. 2009). The human population is increasing at a very high rate that resulted in rapid increase in demand for fuels/chemicals. Also negative environmental impacts such as Greenhouse Gas (GHG) emission, global warming, and pollution associated with these fossil fuel utilization (Frumkin et al. 2009; Clews 2016a; Clews 2016b; Heo et al. 2019). To protect the environment, different governing bodies have enforced stringent environmental laws. Thus, a shift in focus toward the greener and renewable sources has been observed in recent times (Owusu and Asumadu-Sarkodie 2016; Dechezleprêtre and Sato 2017). Some of the renewable sources are wind, hydel power, wind, tidal, and geothermal sources (Owusu and Asumadu-Sarkodie 2016). These renewable resources are available in huge quantities yet its major limitation is lack of the ability to generate chemicals that were earlier fulfilled by the petroleum or fossil fuels-based refinery. The biological feedstocks are available in abundant quantities as a huge rise in the demand for food, feed for fulfilling the need of humans has resulted in the generation of huge amounts of waste biological biomass. Therefore, an alternative concept utilizing this biomass in refinery is developed and termed as "Biorefinery" that can sustainably meet the energy and chemical needs (Tan et al. 2019).

The word biorefinery was first used by Kerckow et al. (1997). From year 1997, the biorefinery has come a long way and different classification of biorefinery has been available based on the type of substrate used such as lignocellulosic feedstock biorefinery, whole crop biorefinery, forest-based biorefinery, oleochemical biorefinery (Koutinas et al. 2006; Cherubini et al. 2009b; Cheng and Wang 2013; Devappa et al. 2015; Günerken et al. 2015; Schneider et al. 2016; Cesário et al. 2018; Dahiya et al. 2018). Several biomasses can be potentially used for the generation of a wide variety of energetic and non-energetic products with a series of advanced technologies. However, substrate-specific biorefinery has several limitations such as difficulty to maintain year-round availability of the biomass, cost efficiency, infrastructure requirement. The concept of integrated biorefinery was introduced for utilization of various waste feedstock as substrates for generation of different biofuels and biochemicals at a single industrial unit (Bozell and Petersen 2010; Wang et al. 2018; Özüdoğru et al. 2019). The generation of value-added compounds

along with biofuel can be a sustainable approach. The understanding of the techno-economic viability of the different steps of the integrated biorefinery is necessary to plan for its future development from merely a concept to reality. The evaluation of the techno-economic viability of integrated biorefineries has been performed (Vlysidis et al., 2011; Wan et al., 2016; Zang et al., 2020). This chapter will give a basic idea of available feedstock for integrated biorefinery, different energetic and non-energetic products that can be produced. The techno-economic evaluation of different steps of biorefinery with special focus on the essential factors have been highlighted. The life cycle assessment, environmental sustainability, and socio-economic sustainability in terms of integrated biobased refineries have been discussed. At last, policies improvisation in different developing and developed countries for promotion of integrated biorefineries have been elaborated.

23.2 Feedstocks Available for Biomass-Based Biorefineries

Several bioorganic constituents derived from living organisms can act as a potential substrate for biorefinery based products such as biofuel and value-added biochemicals (Ur-Rehman et al. 2015). These biomasses can be further grouped based on their origin as the microbial, plant (agricultural, forest), and animal-derived biowastes. Apart from this, the treatment of huge amounts of solid waste and wastewater is generated from modern urban anthropogenic activities (i.e., household, municipal, and industrial application) and has been a headache to mankind. These solid waste and wastewater either go to the treatment plant or are dumped causing pollution. As these wastes are rich in nutrients, they can be potentially used for deriving energy and chemicals. Different available bioorganic wastes are discussed below in Table 23.1.

23.3 Various Products from Biomass-Based Biorefinery

The biomass as discussed above is subjected through a series of steps resulting in a wide range of biorefinery products that can be classified as energetic and non-energetic products. The energetic products are usually the fuels (Fig. 23.1) and non-energetic products are biochemicals, respectively.

23.3.1 Energetic Products

The energetic products are those components that can be used as fuel source replacing the conventional firewood, petrol, diesel, etc. These energetic products are further classified as solid, liquid, and gaseous and are discussed below.

Table 23.1 Different available bioorganic wastes used as biorefinery

Feedstocks	Types	Example	Resulting generation of biofuel	References
Agricultural residues	Food crops	Edible crops such as sugar-rich beet, malt, and cane or starch-rich corn, barley, maize, and tuber	1st	Balaman (2019), Ramesh et al. (2019)
	Primary residues	Straw, stalks, or leaves	2nd	Balaman (2019), Ramesh et al. (2019)
	Secondary residues	Husk, cob	2nd	Hattori and Morita (2010)
	Non-food crops	Switchgrass, Napier grass, *Miscanthus,* reed canary grass, *Erianthus*	2nd	Mehmood et al. (2017)
Forest residues	Primary residues	Waste generated during season falls and harvesting of timber such as short twigs, leaves, and tops	2nd	Balaman (2019), Woo et al. (2019)
	Secondary residues	Waste generated during the processing of timber such as bark, sawdust, and scrap wood, mill waste, etc.	2nd	Balaman (2019), Woo et al. (2019)
Microbial biomass	Primary products	Algal proteins, lipids, i.e., can be converted to biofuel also the metabolites (anthocyanins, astaxanthin, β- carotene, phycoerythrins, phycobilins, and lutein), etc. as value-added chemicals	3rd generation	Dong et al. (2016), D'Este (2017), Timmis et al. (2010), Barsanti and Gualtieri (2014), Linares et al. (2017), Molino et al. (2020)
	Secondary products	Algae polysaccharide-rich components which can be converted to biofuel	3rd	Gajda et al. (2015), Ndayisenga et al. (2018)
Animal wastes	Primary	Waste generated during cultivation as animal excreta bedding materials, feathers, renderings, waste feed, etc.	2nd	Thyagarajan et al. (2013); Chen and Jiang (2014)
	Secondary	Waste generated during processing and finishing such as eggshells, feathers hair, scales, waste skin, etc.	2nd	Jayathilakan et al. (2012), Thyagarajan et al. (2013), Muduli et al. (2019)

(continued)

Table 23.1 (continued)

Feedstocks	Types	Example	Resulting generation of biofuel	References
Food wastes	Pre-consumer	Edible or non-edible food partly or completely damaged/ rotten during supply chain to industry for processing such as grains, fish, meat, and also waste generated during harvesting of food items, i.e., farm wastes	1st	Gustavsson and Sonesson (2011), Carmona-Cabello et al. (2018)
	Manufacturing wastes	During the food processing at industries such as contaminated food, expired and inedible components, processing industry discharge wastewater	1st and 2nd	Gustavsson and Sonesson (2011), Carmona-Cabello et al. (2018)
	Post-consumer wastes	Waste generated at household or food services cooking wastes, e.g., fruits and vegetable peels, bones, and non-consumed and discarded food portions	1st and 2nd	Gustavsson and Sonesson (2011), Carmona-Cabello et al. (2018)
Municipal and industrial waste	Solid	Household, residential complexes, and offices: food wastes, papers, plastics, metals, waste furniture and clothing, industrial setup: By-products, hazardous waste from hospital, etc. as well	–	Ravindran and Jaiswal (2016), Esteban and Ladero 2018)
	Liquid	Municipal (residential and official setups) sewage sludges and industrial wastewater (pharmaceuticals, chemical, paper, pulp, textile, and tanner industries)	–	Rasul et al. 2006, Singh (2015), Ranade and Bhandari 2014, Singh (2015)

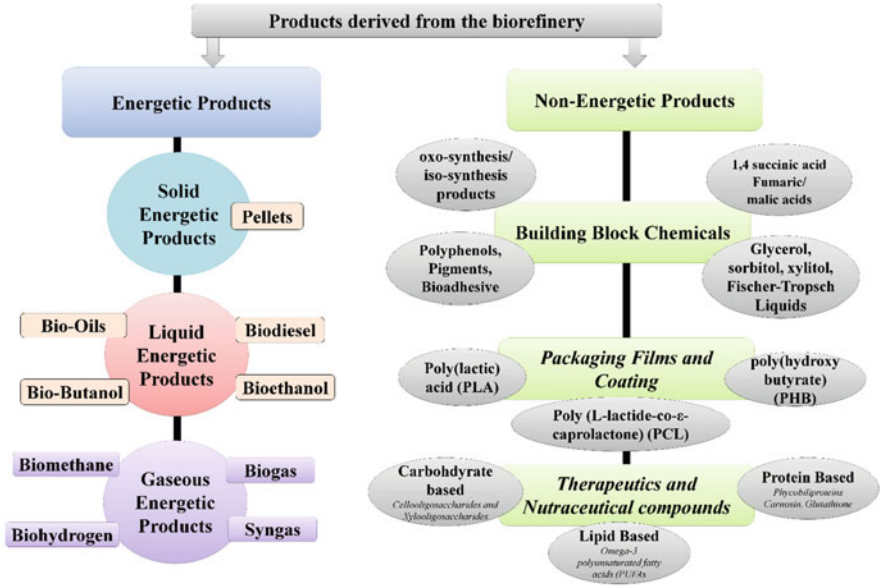

Fig. 23.1 Schematic representation of products from biomass-based biorefinery

23.3.1.1 Solid Energetic Products

The energy density of the natural biomass such as agricultural, energy crop, cattle waste (manure), and forest residues is low. Thus this biomass can be compressed and densified through a series of steps such as drying, milling, palletization to improve the energy/heat content (Nunes et al. 2014; Tauro et al. 2018). Pellet preparation processes often involve several physical (torrefaction, hydrothermal carbonization), chemical, physicochemical (steam explosion), and biological methods (Brodeur et al. 2011; Wang et al. 2011; Lam et al. 2015). Apart from being energy efficient the palletizations also help in the reduction of storage carbon release (30–40%) during combustion as compared to the normal biomass (Hamzah et al. 2018). The storage and logistic cost for the pellets also decrease (Ranade and Bhandari 2014; Singh 2015).

23.3.1.2 Liquid Energetic Products

The production of biomass-based liquid fuels such as bioethanol, biodiesel, and biobutanol, etc. has gone up due to the fast depletion of the fossil-based petrol and diesel. Bioethanol is generated from different biomass via a series of steps, namely pretreatment, hydrolysis, fermentation, and distillation (Gupta and Verma 2015; Gavahian et al. 2019; Rosales-Calderon and Arantes 2019; Kumar et al. 2020).

Vegetable oils, lipids, and lipid rich waste can be subjected to biological (in presence of oleaginous microbes) or chemical-based transesterification for generation of biodiesel (Kulkarni and Dalai 2006; Hossain et al. 2008; Vicente et al. 2009; Zahan and Kano 2018). The major steps involved in generation of biodiesel from lipid-rich substrates are pretreatment, transesterification, and separation (Knothe et al. 2005; Abbaszaadeh et al. 2012; Beltrán-Ramírez et al. 2019). Biodiesel can also be produced from the Algal biomass *(Chlorella vulgaris*) and filamentous fungi (*Mucor circinelloides*) (Vicente et al. 2009; Levine et al. 2010).

Bio-oils are also one of the potential liquid fuels that can be used as liquid fuels and can be derived from direct pyrolysis and liquefaction of different waste biomass (Xu et al. 2011; Graça et al. 2013). The low cost of pyrolysis as compared to liquefaction makes it more preferable process for bio-oil production (Xu et al. 2011; No 2014). The bio-oils need to be pre-processing (cracking and hydrotreating) before being used as fuel (Xu et al. 2011). Different agro-residues (neem oil cakes, palm kernel), specialized oil-based crops (mustard, sesame, jatropha), lipid-rich algal biomass, and waste plastic can be subjected to pyrolysis for bio-oils production (Alper et al. 2015).

The combustion properties of butanol are in close similarity with the gasoline that has driven its blending with gasoline. Although the biological process for the production of biobutanol has been known since 1915 rising petrol prices led to gaining interest in this process in the early 1980s (Calam 1980; Häggström and Molin 1980; Maddox 1980; Rosenberg 1980; Wiegel 1980; Zeikus 1980; Birgen et al. 2019). Blending of biobutanol to gasoline has mutual positive effects such as gasoline help in improvement in the volatility of butanol, whereas the heat of combustion of gasoline is improved by mixing of butanol (Hönig et al. 2014). Biological assisted butanol production can be performed by anaerobic fermentation of different biomass by anaerobic bacterial strains from *Clostridia* genus, e.g., *C. aurantibutylicum*, *C. beijerinckii*, *Clostridium acetobutylicum,* and *C. tetanomorphum* (George et al. 1983; George and Chen 1983; Gottwald et al. 1984; Kolesinska et al. 2019). For example, rice bran when subjected to anaerobic fermentation using *C. saccharoperbutylacetonicum strains,* high biobutanol yield was observed (Al-Shorgani et al. 2012). Similarly, several groups have reported utilization of agricultural and forest residues such as coffee silver skin, oil palm empty fruit bunch, wheat bran, wheat straw for the generation of biobutanol (Qureshi et al. 2008; Al-Shorgani et al. 2012; Ibrahim et al. 2012; Hijosa-Valsero et al. 2018; Salleh et al. 2019).

Aerobic fermentation of carbohydrate-rich waste feedstock using *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas putida*, and *Saccharomyces cerevisiae* can also result in biobutanol production (Rathour et al. 2018). The difference in some of the chemical and physical properties of biobased liquid fuel often requires modification in existing engines. Thus, high percentage blending of biodiesel, biobutanol, and bioethanol to fossil-based diesel, gasoline, and petrol, respectively, has been suggested to be used and even tested for utilizing the already existing engines without the requirement of modifying or redesigning it (Maiti et al. 2016).

23.3.1.3 Gaseous Energetic Products

Biogas is generated via the decomposition of the waste biomass and its major constituents are methane, carbon dioxide, traces of hydrogen, hydrogen sulfide, ammonia, and carbon monoxide (Deublein and Steinhauser 2011; Wellinger et al. 2013; Solarte-Toro et al. 2018). The anaerobic digestion of several waste substrates (manure, lignocellulosic waste, etc.) in the presence of anaerobic microorganisms or microbial consortium results in generation of biogas (Rabii et al. 2019). The generation of biogas at the industrial scale involves a multistep process involving hydrolysis, acidification, acetate production, followed by the generation of methane (Sarkar et al. 2018). Organic matters are decomposed by several diverse metabolic pathways (Kougias and Angelidaki 2018). Co-digestion of different biomass together helps in improving the biogas yield, e.g., food waste in combination with wood chips (Oh et al., 2018), animal manure with corn stalk (Lv et al., 2018) etc. Regattieri et al. (2018) demonstrated biogas production by utilization of human organic waste and suggested anaerobic digestion as an efficient mode of rural and urban waste management and meeting energy needs simultaneously.

Syngas and biohydrogen are other two gaseous products that can be produced via the utilization of different waste biomass. Syngas is a mixture of hydrogen, carbon monoxide with minute quantity of carbon dioxide (Lieuwen et al. 2009). On pyrolysis of nine different hybrid and non-hybrid perennial grass-based composts, it resulted in syngas production with a high heating value (HHV) of up to 17.43 MJ/Kg (for tall fescue grasses) (Hlavsová et al., 2014). Except for pyrolysis some of the other strategies for syngas production are subjecting the biomass to dark fermentation, gasification, and reforming (Ayodele et al. 2019). Syngas is also generated via oxygen-steam assisted gasification and Fischer–Tropsch reaction of solid, gaseous, and liquid fossil fuels (coal, natural gas, and liquid hydrocarbon) (Nakyai and Saebea 2019; Mehariya et al. 2020). The off-gases generated from steel mills and petrochemical industries are also rich in syngas (Spath and Dayton 2003; Capodaglio and Bolognesi 2019) (Fig. 23.1).

The combustion of biohydrogen results in the generation of very high specific value of energy (~143 GJ/ton) and only by-product, i.e., water, thus considered as one of the cleanest sources of energy (Boyles 1984; Forsberg 2007). Thermochemical gasification, fractional oxidation, and reforming of fossil-based solid or liquid fuel have been demonstrated to have huge biohydrogen generating potential (Das and Veziroğlu 2001; Levin et al. 2004). The fast depletion of fossil fuels and the costly nature of conversion processes result in shifting in focus towards waste biomass assisted biohydrogen production (Claassen et al. 1999; Das and Veziroğlu 2001; Levin et al. 2004; Show et al. 2019). The microbial assisted method for biohydrogen production can be classified as light-dependent processes (direct and indirect photolysis, photo-fermentation) (Melis 2002; Zhang et al. 2002; Hankamer et al. 2007; Sağır et al. 2018) and light-independent process (dark fermentation) (Ntaikou et al., 2010; Sağır et al. 2018). Agro residues, food wastes, and wastewaters have been utilized for microorganism assisted biohydrogen production (Schröder

et al. 1994; Ueno et al. 1995; Okamoto et al. 2000; Liang et al. 2001; Kanai et al. 2005).

23.3.2 Biochemicals

The decomposition of the waste biomass can result in the generation of several values added chemicals which acts as major building blocks compounds (Werpy and Petersen 2004; Kumar et al. 2017) for the generation of polyphenols, pigments, (carotenes, terpenes, sterols, and tocopherols) and bioadhesives (Ayala-Zavala et al. 2010; Ignat et al. 2011; Joshi et al. 2012; Rodrigues et al. 2014; Kantifedaki et al. 2018; Mishra et al. 2019). Based on different compositional elements of biomasses, different types of bioadhesives are lignin, tannin, starch, protein-based bioadhesives (Onusseit 1992; El Mansouri et al. 2007; Li et al. 2009; Navarrete et al. 2010; Mansouri et al. 2011; Tan et al. 2011). The waste biomass residues can also be utilized in synthesis of bio-composite (Beltrán-Ramírez et al. 2019; Zuccarello and Scaffaro, 2017), packaging films or coatings materials (De Azeredo et al. 2014; Vartiainen et al. 2014; Šuput et al. 2015), therapeutics and nutraceuticals compounds (Attanzio et al. 2018; Krishna et al. 2019; Ramesh et al. 2019) (Fig. 23.1).

23.4 Technical Evaluation of the Biomass-Based Biorefinery

The development of biomass-based biorefinery is targeted for the generation of different biofuels and value-added products. Various steps are involved in the development of integrated biomass-based biorefinery. The processes start right from the cultivation of biomass source, its harvesting (agro-residues), collection, and supply from the field to the biorefinery sites. The biomass as reached to the biorefinery is subjected to sorting, storage, pretreatment, hydrolysis, fermentation, and separations. Therefore, the development of a sustainable system requires better understanding of the different technical aspects of the biomass-based biorefinery. The technical development of different aspects of biomass-based biorefinery has been discussed below.

23.4.1 Techno-Economic Evaluation of Biomass Collection and Supply to the Biorefinery Plants

In the petroleum-based biorefineries, crude fuel emerges from a single place, i.e. mining fields and oil fields. Due to the single point of the production, crude

fuels can be collected and transported to the processing or application site through road, rail, or pipelines easily. Whereas the source of waste biomass is not single, it is often generated at different locations and quantity also varies seasonally. Thus, therefore understanding the cultivation, harvesting, sorting, and supply is very much needed (Xie et al. 2014; USDA 2017). The major contribution to biomass-based biorefinery comes from substrates from agricultural residues, food crops (corn, millet, rice, wheat, dedicated energy crops), and microbial systems (algae). Therefore, the seasonal and regional variability, the time required from sowing to harvesting, harvesting frequency, harvesting method applied are important parameters to be discussed apart from the pre-processing and supply or mode of transport used (Sokhansanj et al. 2009; Yue et al. 2014; Yue and You 2016; Zandi Atashbar et al. 2018). Several genetic modifications in the plants have been performed to get higher biomass yield, overcome seasonal and regional variability, improve harvesting frequency. The wasteland or underutilized land has been suggested to be used for growing energy crops (Jones et al. 2015; Mathan et al. 2016). The main contributor to the third-generation biofuel, i.e., microalgae cultivation requires specialized bioreactors or often done at open pond-based bioreactors. The regulation of growth parameters such as the composition of the media, O_2/CO_2 supply, pH, temperature, etc., (Becker 1994; Grobbelaar 2000) is required for improved efficiency. These parameters are easy to control in specialized illuminated photobioreactors but in the pond system regulation is a bit tiresome. Whereas considering the economics of the process the photobioreactors system is costly and very difficult to scale up (Thein 1993; Pulz and Scheibenbogen 1998; Borowitzka 1999; Tredici 2004; Werner et al. 2004; Ehimen 2016).

The forest-based wastes and wood/timber are available throughout the year, however, the conservation of traditional forests and improving the commercial foresting need to be promoted without disturbing the already existing ecosystem balance. In general for utilizing the lignocellulosic waste (agricultural, forest, food crops) several parameters as discussed above must be taken care of for scheduling the collection and transport maintaining a year-round supply of the biomass at the biorefinery site (Sokhansanj et al. 2009; Yue et al. 2014). Several types of harvesting have been suggested (a) Single-pass harvesting, (b) Multipass, and (c) Whole crop harvesting (Sambra et al. 2008; Zandi Atashbar et al. 2018). Followed by harvesting collection of the generated waste is done using different techniques such as baling, loafing, and stacking. These biomasses are even chopped, dried, collected, and stored at the pre-processing units. This collection method helps in reducing the space required during storage and transportation (Sambra et al. 2008; Yue and You 2016; Zandi Atashbar et al. 2018). The other major contributors to the biomass-based biorefinery are food and animal-based waste, municipal and industrial sludges, and wastewater. The cultivation and harvesting modes are not a matter of concern in the supply chain management of food, animal, industrial, and municipal wastes (Holm-Nielsen 2016; Toka et al. 2016; Whittaker and Shield 2016; Liu et al. 2017; Zandi Atashbar et al. 2018). However, pre-processing, packaging, storage, supply, and transport mode need to be considered while analyzing the technology and cost involved (Holm-Nielsen 2016). These wastes are collected manually or by

machines stored in tanks/silo and then moved to the storage points or biorefineries (Kaltschmitt et al. 2002; Toka et al. 2016). The exponential growth of the human population resulted in increase in agricultural, animals, and other municipal/industrial wastes which ends up at dumping sites or often burnt causing environmental pollution. Therefore, there is a need for collective measures for the minimizing pollution and developing empirical models for waste biomass collection, and supply to the biorefinery plants for enabling a year around biomass supply system analogous to the petroleum biorefineries.

One such approach for maintaining a year-round supply of biomass is establishing decentralized biomass pre-processing depots as suggested in the work of Lamers et al. (2015). The techno-economic comparison of the suitability of the decentralized depots for both conventional (pelleting) as well as sophisticated technologies (AFEX) is performed. It is suggested that stability, density, and flowability of the feedstock may be maintained by *Standard depots* which are further supported with *Quality depots* (Lamers et al. 2015). Quality depots often involve leaching or chemical-based pretreatment. Thus, the economics of the overall process is dependent on energy consumption. The evaluation was performed using the Biomass Logistics Model consisting of SQL database engine and Powerism calculation engine which indicated that depot processing cost could be possibly between US$ 30–63 per dry metric tonne of biomass (Lamers et al. 2015). This overall cost often depends on the specific technologies and equipment (dryers, grinders) used at depots and their associated energy consumption. Thus, the integration of depots with the supply chain of waste biomass will outweigh the overall management costs of depot and thus can be aggressively followed (Lamers et al. 2015).

23.5 Technical Economic Evaluation of Different Technologies Associated with the Biomass-Based Biorefinery

23.5.1 Pretreatment of the Waste Biomass

Pretreatment being an important and rate-limiting step in the biomass-based biorefinery. Pretreatment is required to overcome the natural recalcitrance of the biomass making the biomass accessible to enzymes and microbes in the subsequent steps. The pretreatment method can be broadly classified as physical, chemical, physicochemical, and biological. Different pretreatment methods are often combined and show better efficiency. Table 23.2 shows different available pretreatment methods for their stage of development and techno-economic aspects that need to be considered for future biorefinery.

Table 23.2 Different available pretreatment technologies and their techno-economic economic aspects that need to be taken care for future application

Name of technology			Stage of development	Techno-economic aspects that need to be taken care for future biorefinery application
Physical pretreatment	Mechanical	Extrusion	Commercial	• Low/restricted flowability.
		Mechanical grinding	Commercial	• Large scale feasibility is questionable due to the very high energy requirement
	Irradiation	Microwave	Commercial	• Sophisticated microwave systems are required • High inhibitory compounds formation • High energy and cost-intensive
		Ultrasound	Laboratory	• Sophisticated ultrasound systems are required • High energy and cost-intensive
		Gamma-ray	Laboratory	• A sophisticated irradiation chamber with advanced safety measures enhances the cost on a large scale
		Electron beam	Laboratory	• Sophisticated electron beam accelerator • limits the large scale application
		Pulsed electric energy	Laboratory	• In its prime stage, require more experimental proofs at laboratory scale to suggest the feasibility of the process
	Direct thermal hydrolysis	Pyrolysis	Commercial	• High-temperature requirement up to—1000 °C or even more thus energy-intensive
		Liquid hot water	Commercial	• Formation of fermentation inhibitory products due to high pressure and temperature
Chemical pretreatment		Acid pretreatment	Commercial	• Requirement of non-corrosive reactors • Requirement of intensive neutralization of acid after pretreatment • Results in high yield of fermentation inhibitory compounds
		Alkali	Commercial	• Longer residence time • Intensive post-pretreatment processing • Not suitable for high lignin waste

(continued)

Table 23.2 (continued)

Name of technology		Stage of development	Techno-economic aspects that need to be taken care for future biorefinery application
	Oxidative	Pilot-scale	• The oxidative chemical as often costly and bear low stability • High cost in the regulation of oxidative reaction due to the requirement of sophisticated reactors • Intensive safety measures would be required on industrial application
	Organosolv	Laboratory	• The high volatility of organosol makes it difficult to recover the solvents • Often highly reactive and safety concerns
	Deep eutectic solvents (DES)	Laboratory	• A very early stage of development
	Ionic liquid (IL)	Laboratory	• High cost of ILs and recycling is energy-intensive • Often toxic to fermenting microorganisms
	Hydrotropic	Laboratory	• An early stage of development requires adaptation at industrial scale
	Salts	Laboratory	• Early stage of development requires adaption at industrial scale
Physicochemical pretreatment	Steam explosion	Commercial	• Selection of suitable temperature-pressure • High cost of post-processing
	Ammonia fiber expansion (AFEX)	Commercial	• Cost of ammonia • Sophisticated equipment requirement • High capital cost
Biological pretreatment	Direct microbe and enzyme-based pretreatment	Laboratory	• Time intensive process due to slow growth rate • Specific temperature and growth requirement for maintaining microbes result in the high operational cost • Biomass is used by microbes for their own growth thus overall yield may be low

23.5.2 Techno-Economic Evaluation of Advancement in Enzyme-Based Technologies in Biorefinery

The research communities are working in developing several enzyme-based systems to be used in different steps of the biorefinery (Kumar et al. 2020; Kumar and Verma 2020). The industrial application of the enzymes is limited due to low availability (lower yield of enzyme production by microorganism) and stability concern at varying pH and temperature. The high yield and stability concerns are attempted to be solved by the application of several optimization processes or recombinant DNA technology (Bhardwaj et al. 2017; Kumar et al. 2018b; Agrawal et al. 2019; Bhardwaj et al. 2019, 2020b). The other important parameters that need to be considered for the re-utilization of enzymes are its recyclability which is usually attained by immobilization (Ai et al. 2005; Mohamad et al. 2015; Jamie et al. 2016; Milessi et al. 2016; Lin et al. 2018).

Different enzymes important for biorefinery are the cellulase, hemicellulose, ligninolytic, amylase, lipases enzymes acting on cellulose, hemicellulose, lignin, starch, and lipids, respectively, at different steps of the biorefinery (Taherzadeh and Karimi 2007; Binod et al. 2019). Recently discovered lytic polysaccharide monooxygenases (LPMOs) are also used in combination with the other hydrolyzing enzymes under a controlled environment for enzyme assisted conversion of biomass to biofuel and biorefinery. In recent years several groups are working on the one-pot culture (Li et al. 2012) or consolidated system (Xin et al. 2019; Liu et al. 2020; Wen et al. 2020). In the consolidated systems, key enzymes used usually in different steps are merged in a single step via different approaches. Some of them are integrating different enzymes into a single microbe by utilizing different modern-day advanced biotechnological tools such as cell surface engineering (Gao et al. 2017; Saeui et al. 2015; Tanaka and Kondo 2015), metabolic and protein engineering (Selim et al. 2018), synthetic & system biology (Mukhopadhyay et al. 2008; Jullesson et al. 2015; Vickers et al. 2017; Joseph et al. 2018) approaches for the development of microbial cell factories (Gustavsson and Lee 2016), and multienzyme cascades (Pröschel et al. 2015). In some cases, different mutually compatible microbes are used in a single step consolidated process for the generation of biofuel and biochemicals utilizing biomass. Also, genomic-based advanced next-generation sequencing tools are used for making the overall enzyme-based system industrially compatible (Kries et al. 2013; Steinkellner et al. 2014; Huang et al. 2016; Jeffries et al. 2016).

Enzymes have a key role to play in the growth in the waste biomass-based biofuel and biochemicals production. Thus, the production of industrially compatible enzymes has undergone a major shift, e.g., the USA alone produces 14.7 billion gallons of cellulosic ethanol annually which will rise in the next few years. In these biorefineries, cellulase enzymes have a key role to play. These enzymes have also replaced several other chemicals based on environmentally hazardous methods in paper, pulp, textile, and food processing industries. Therefore, a huge rise in hydrolytic enzyme production will be observed such as the annual cellulase enzyme market is expected to rise by 5.5% and reach 2.3×10^3 million USD by 2025

(Markets-and-Markets 2014). The major key players are Novozyme and DuPont. One other approach which is gaining interest is in-house enzyme production using a part of the biomass and using it for biofuel production resulting in complete valorization of biomass (Kumar and Verma 2020).

23.5.3 *Techno-Economic Evaluation of the Fermentation Steps in Biorefinery*

The hydrolyzed biomass is subjected to microbial fermentation for the production of biofuels and different value-added compounds (Balat and Balat 2009). Several bacteria and fungi play crucial role in the fermentation, e.g., *Pichia pastoris, Saccharomyces cerevisiae, Zymomonas mobilis,* and modified or recombinant *E. coli* strains. Several types of fermentations are classified based on medium condition, oxygen presence/absence, substrate concentration, and sometimes the number of steps involved during hydrolysis and fermentation. Different types of fermentation based on different parameters are shown in Fig. 23.2.

Different techno-economics parameters of different fermentation type that need to take *care for future biorefineries are mentioned below*

- *Solid-State Fermentation (SSF)*—Operation at a large scale is very tiresome due to poor mixing and agitation. Require longer incubation time.
- *Submerged Fermentation (SmF)*—Contamination prone and metabolite accumulation may affect process efficiency negatively.
- *Anaerobic*—Maintaining anaerobic condition makes the overall process difficult and costly.
- *Aerobic*—As exposed to open environment air chances of contamination are high.
- *Very high gravity fermentation (VHG)*—High concentration of substrate results in high osmotic pressure often causing loss of cell viability and thus accompanied with very low ethanol yield.
- *Separate hydrolysis and fermentation process (SHF)*—High overall cost due to more number of steps, longer duration, feedback inhibition, high risk of contamination.

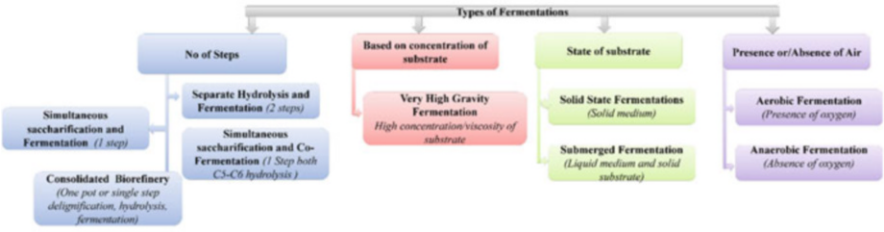

Fig. 23.2 Different type of fermentation technology available

- *Simultaneous Saccharification and Fermentation (SSF)*—Finding microbes capable of surviving and active at the varying temperature of saccharification (50 °C) and fermentation (usually 32–37 °C).
- *Simultaneous Saccharification and Co-Fermentation (SSCF)*—Maintaining varying temperature and high enzyme load requirement.

Although consolidated biorefinery is in the preliminary stage of development but has shown great potential minimizing the need for multistep process overcoming cost and time limitations. But the ways to conceive the concept at large scale is a big dream to achieve.

23.5.4 Techno-Economic Evaluation of the Separation Technologies Steps in Biorefinery

The separation of the derived products during different stages of biorefinery (for separating the products or by-products) is crucial and several technologies have been implemented and suggested for future biorefineries (Hestekin et al. 2002; Ramaswamy et al. 2013; Datta et al. 2014). The separation techniques required to separate the fermentable pulp from the pretreatment hydrolysate, recovery of bioethanol or other biofuels, purification of impurities, and recovery of the catalysts or chemicals used during different stages of biorefinery for their reuse (Huang et al. 2008; Ramaswamy et al. 2013; Datta et al. 2014). Each step of biorefinery adds up to the cost of the overall process therefore the cost of technologies involved in the separation, purification, distillation affects the final cost of the yield (Aditiya et al. 2016).

Different techniques were used during different stages of the biorefinery for the separation of raw material for the next step and by-products. Some of the technologies are discussed below

- A series of steps are involved after pretreatment for separation of different component generated by the breakdown of biomass (cellulose, hemicellulose, lignin, inhibitors) and the chemical used for, e.g., washing, drying, acidification, centrifugation, evaporation (vacuum based), precipitation (N'Diaye et al. 1996). The chemical if recovered is recycled and reused and the precursor molecules are subjected to the processing for generation of value-added chemicals.
- The fermentable pulp is separated and free from chemicals and inhibitory compounds are subjected to hydrolysis and fermentation. Distillation is performed followed by fermentation to recover the bioethanol or similar products from mixture.
- After hydrolysis, the pulp is subjected to fermentation and the broth is subjected to distillation to separate different products of the fermentation mixture. Several distillation technologies are available such as simple distillation, azeotropic distillation (Chianese and Zinnamosca 1990; Luyben 2006; Kumar et al. 2010), extractive distillation (Ravagnani et al. 2010; Gryta 2012; Lei and Chen 2013),

adsorption distillation (Hu and Xie 2001; Carmo et al. 2004), chemical dehydration (Ladisch and Tsao 1982; Ligero and Ravagnani 2003), diffusion distillation (Qariouh et al. 1999), membrane distillation (Huang et al. 2008; He et al. 2012; Izquierdo-Gil 2013; Wang and Chung 2015), and a most advanced hybrid membrane pervaporation-bioreactor (HMPB) and vacuum membrane distillation (VMD) (Gostoli and Sarti 1989; Qariouh et al. 1999; Huang et al. 2008; Wang et al. 2013)

The cost involved during downstream processing/separation depends greatly on products recovered and the followed technology. For example, modern membrane-based technologies are efficient but often the high cost of specialized membrane technology restricts its use. Therefore, the choice of separation system is crucial in biorefinery, and often the purity of substance regulates the final price of the product.

23.6 Need of the Integrated Biorefinery

As discussed in the earlier part of the chapter that the generation of biofuel from biomass-based biorefinery is a multistep process. At the industrial scale, the more the number of steps more is the cost involved thus there was a need to reduce the overall cost and make the biorefinery process sustainable as similar to petroleum biorefinery (Aro 2016). Combining the multiple steps through consolidated biorefinery as suggested in the above section is one such approach where pretreatment/delignification, hydrolysis, fermentation are merged into a single step or two-step process (Bhardwaj et al. 2020a). However, the consolidated biorefinery is snowed under several technical limitations for the growth and stability of microbes and enzymes used in the process (Wiltschi et al. 2020).

The concept of the integrated biorefinery is a broader term which can target several biomass simultaneously. This can be understood as biomass is made of complex polymeric structure and it can be a source to several chemicals similar to petroleum refinery (Bozell and Petersen 2010; Wang et al. 2018; Özüdoğru et al. 2019). Thus, it has been suggested that value-added chemicals can also be produced along with the biofuel to make the overall process economical and sustainable. Hughes et al. (2014) demonstrated the coffee waste biomass utilization for the generation of bioethanol along with amino acids/peptides and biofertilizers using strains of *K. marxianus*, mutant *Y. lipolytica,* and recombinant *Saccharomyces cerevisiae*. Also, integrating a wide range of biomass-based biorefinery such as lignocellulosic biomass biorefinery, food waste biorefinery, algae-based biorefinery onto one site is also suggested (Awasthi et al. 2020). Integrating different feedstock based biorefinery can help to overcome the year-round supply of biomass (Junginger et al. 2019). Also, earlier enzymes that are used during hydrolysis steps are usually sourced from commercial producers that resulted in high cost. The production of enzymes (in-house) using the waste biomass as a substrate and enzyme producing microorganism is one such approach to reduce the overall cost (Lau et al. 2012; Johnson 2016). This concept of integrating all the steps of biorefinery and the

different feedstock based biorefinery can be mutually beneficial and helps in overcoming the cost barrier associated with different biorefinery based approaches. Thus, integrated biorefinery can result in the generation of biofuels and value-added chemicals at one site from different varieties of feedstocks.

In an integrated biorefinery, product generated at one of the feedstock based biorefinery can be an energy source for the next steps or even act as starting material for different chemicals, thus making the overall process self-sustainable (Bušić et al. 2018). One such example is the H-M-Z-S model of integrated biorefinery where the municipal waste valorization is performed. The first stage for the dark fermentation of municipal waste results in biohydrogen production. The product of the first step is used in the fuel cells that meet the energy demand for the other steps of the H-M-Z-S biorefinery. The strain *T. reesei* MCG80 utilizes the organic waste for cellulase production which is used during the enzymatic hydrolysis step of biorefinery and finally the organic waste is subjected to anaerobic digestion for generation of biofuel (Escamilla-Alvarado et al. 2013; Escamilla-Alvarado et al. 2014, 2015). Several earlier reports suggest that biomass-based integrated biorefinery utilizing a wide range of waste feedstock can be evolved as a future alternative to the petroleum-based refinery. Also, an interesting point is suggested by Jungmeier et al. (2014) and Ketabchi et al. (2019), for the operation of petroleum refinery infrastructure and technologies (chemical catalyzed reaction can be mimicked) for the conversion of waste biomass into long-chain hydrocarbons (as fuels) and value-added chemicals during integrated biorefinery.

23.7 Life Cycle Assessment of Integrated Bio-Based Biorefineries

To analyze the overall impact of any industrial process and technology on the environment, economy and social factors are assessed by the mean of life cycle assessment (LCA) approach (McKone et al. 2011). During life cycle assessment of the integrated biorefineries, important factors that need to be considered are the raw materials (feedstocks), generated products, land, and resource utilization. Roes and Patel (2007) suggested that toxicity, accident, work-related illness, and technological disorders also important parameters for LCA during evaluating the risk management factor. The assessment in general is just the comparison of materialistic (food and feed) and energetic potential of the biomass. Several previous LCA studies for the generation of biofuels and value-added chemicals from different biobased biorefinery suggested biofuels and biochemical's production from biobased biorefinery. Zah et al. (2007) suggested that the global warming potential of the biomass depends upon the species/origin of biomass and type of biomass produced based on the survey by the Swiss Federal Administration (SFA). Manik and Halog (2013) suggested that the growing population has increased energy and food utilization thus causing changes in the land use pattern. Thus, suggesting that more

biomass and energy production will cause a greater release in the GHG and jeopardize all GHG savings gained by the biomass-based biorefineries. There is need of larger amount of agricultural crops to cater to the food needs of the growing population. To increase the productivity, the use of harsh chemical fertilizers is promoted that results in high food production cost and negatively affect the soil quality, compromise human health and ecotoxicity (Bai et al. 2010; Cherubini and Jungmeier 2010; Cherubini and Strømman 2011). Also, the use of nitrogen-rich fertilizers causes an increase in GHG emission (Zah et al. 2007).

Based on life cycle assessments of different biomass as a potential substrate for the biomass-based biorefinery (Cherubini et al. 2009a; Stoeglehner and Narodoslawsky 2009; Cherubini and Jungmeier 2010), it is found that second-generation substrates such as lignocellulosic biomass are preferred substrates for biorefinery. Patel et al. (2006) and Hermann et al. (2007) evaluated the biotechnological routes for the production of 21 and ten bulk chemicals from different feedstocks. Different parameters such as global warming potential, non-renewable energy usage, land usage for growth/cultivation of biomass are compared to fossil fuels/petrochemical industries. It was suggested that 25–35% of GHG emission reduction and non-renewable energy usage can be attained using biological feedstocks. Alvarenga et al. (2013) also suggested that better understanding of LCA can lead to development of efficient technologies used in integrated biorefineries and will further impact on the overall process.

23.8 Environmental and Social Sustainability Assessment of the Integrated Bio-Based Biorefineries

The environmental and social sustainability of any developed industrial process is needed to be studied. Therefore, sustainability assessment of the biomass-based biorefineries is required to understand how these approaches can help in environmental pollution mitigation (environmental) and their positive social impacts (social sustainability) (Awasthi et al. 2020). This sustainability analysis helps in improving overall efficiency and the cost-effectiveness (Mateos-Espejel et al. 2011; Gassner and Marechal 2013). Environmental and social sustainability assessment has been discussed below.

23.8.1 Environmental Assessment of the Integrated Bio-Based Biorefineries

The development of the biomass-based integrated biorefinery will have its long-term implication on the environment similar to any anthropogenic activity on earth. Different environmental parameters that interact with the integrated biorefineries

are complex (Näyhä and Horn 2012). The integrated biorefineries are newer techniques as compared with the fossil fuel-based refinery technologies thus to assess its environmental impact of the newly developed process could be compared with the old one which is in use (Schebek and Mrani 2014; Moncada et al. 2017). The major parameters considered for the biomass-based integrated biorefineries are pattern and intensity of land use, soil quality, pollution, negative impact on the ecosystem and biodiversity, GHG emissions, change in carbon pool, and pattern of biomass or fossil fuels resources (Hasenheit et al. 2016). The impact of the integrated biomass-based biorefinery could be summarized as earlier the biomass left untouched to decompose or burnt contributing to the GHG emission. Also, the combustion of the fossil-based fuel worsened the situation by contributing to the pollution caused by waste biomass dumping or burning (Perera 2018). Thus, the recent advancement in the field of integrated biomass biorefinery which utilizes a wide range of waste feedstocks to be converted to fuels or the chemicals can not only compensate for the chemical and energy need that was fulfilled by the fossil fuels. But at the same time also helps in overcoming the environmental hazards earlier caused by the dumping and burning of biowaste and fossil fuels (Cherubini 2010; Näyhä and Horn 2012; Weiss et al. 2012; Purohit and Chaturvedi 2018; Takkellapati et al. 2018; Ferronato and Torretta 2019). Therefore, advanced technologies need to be developed for advanced integrated biorefinery for utilizing the biological waste (waste management) replacing the fast depleting pollution-causing fossil fuels (Romero-García et al. 2018). Similarly, utilization of wastewater streams from different industries as a nutrient-rich substrate for the growth of microalgae and bioelectricity generation can also have positive environmental impacts (Usher et al. 2014; Kumar et al. 2018a) Thus, while evaluating the environmental impact of biobased biorefinery the impact of technologies used at different stages of biorefinery must be evaluated. Thus, while developing any process in biorefinery evaluation of its environmental impact is must.

23.8.2 Socio-Economic Assessments of the Integrated Bio-Based Biorefineries

The economic and social sustainability of any industrial process must be evaluated before its implementation on a larger scale. The major points while considering the socio-economic assessment must be that

- Low investment cost in terms of energy and infrastructure (Lange 2007; Alles and Jenkins 2010).
- Production of high-end chemicals combined with the in-house production of energy such as biofuel from integrated biorefineries makes the overall process sustainable and energy efficient (Werpy and Petersen 2004). (Luo et al., 2010).
- The choice of a location near to the availability of substrate makes the overall process cheaper (Yu and Tao 2009; Giarola et al. 2011; Akgul et al. 2012).

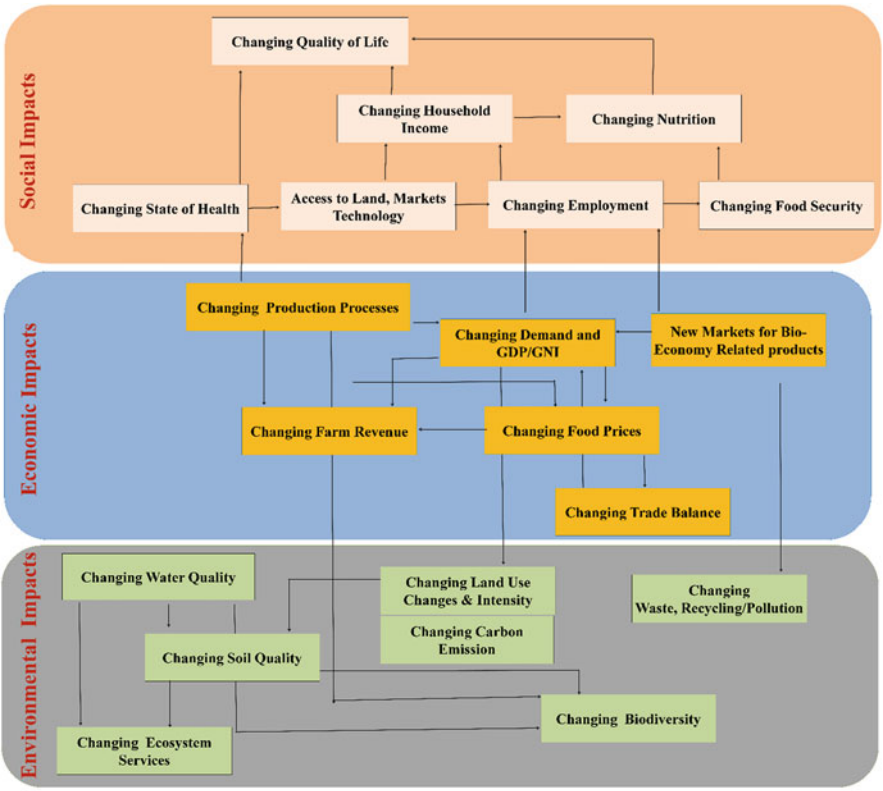

Fig. 23.3 Key factors of social, economic, and environmental sustainability analysis in biorefineries (Adapted from Hasenheit et al. 2016)

- Key parameters such as net energy balance, the market for generated products, farming pattern, and associated revenues are key to the socio-economic analysis (Schebek and Mrani 2014; Hasenheit et al. 2016; Rakotovao et al. 2018).
- Social factors such as employment opportunity, social habit, food security, land access, quality of life, and health are also very key factors for the socio-economic analysis (Schebek and Mrani 2014; Hasenheit et al. 2016; Rakotovao et al. 2018).

Overall life cycle assessment, environmental and socio-economic impacts are closely related aspects that need to be considered while developing the integrated biobased biorefinery. This correlation between the social, economic, and environmental impact of the biorefinery development has been shown in Fig. 23.3.

23.9 Designing of Policies for Integrated Biobased Biorefinery

The regions which are rich in petroleum-based resources are present in the most politically unstable regions. The fast depletion rate of fossil fuels and associated environmental concerns has obligated the policy-makers to find a way to endorse biomass-based integrated biorefineries. The government of developed and developing countries such as the USA, Argentina, Canada, Germany, India, Brazil has introduced several acts and rules to promote the growth of the biorefineries. The US government in the 2005 Energy policy Act had set up the target to produce 4 billion gallons of biofuels by 2006 which was further modified to 36 billion gallons by 2022. Similarly, in 2007, the USA introduced Energy Independence and Security Act, that set up a target to achieve the contribution of renewable energy up to 18% (Yacobucci and Bracmort 2009; Sorda et al. 2010). The Biomass Program, 2008 by the US government focussed on promoting the production of corn-based bioethanol and subsequently decreasing the consumption of gasoline by 30% till 2030 (Sorda et al. 2010; Saravanan et al. 2018). Similarly, the European Union introduced policies and designed road map to develop a low carbon emission economy and meet domestic energy requirement (up to 20%) and transportation fuel (up to 10%) via the renewable mode by 2020 (Smyth et al. 2010; Czyrnek-Delêtre et al. 2017). Germany, through the Biofuel Quota Act, 2007, and National Renewable Energy Action Plan set up a target of diesel blending by 4.4% with biodiesel and meet overall energy consumption through renewable energy by 2020 (Su et al. 2015; Saravanan et al. 2018).

Canadian Environmental Protection Act, 1999 (BillC-33), "Argentinean Regulation and Promotion of the Production and Sustainable Use of Biofuels regime, 2006, Thailand Alternative Energy Development Plan (AEDP) are several programs and acts introduced for improving the participation of renewable energy to the overall energy consumption (Sorda et al. 2010; Kumar et al. 2013). Several incentives have been provided for the promotion of the application of renewable energy production and utilization. The Ministry of Renewable Energy and Indian Renewable Energy Development Agency Ltd. (IREDA) have been providing several incentives to the biofuel producers such as capital subsidies, depreciation benefits, tax holiday, custom and excise duty concessional, sales tax exemption, and carbon credit through Clean Development Mechanism (CDM) (IEA 2020; EAI 2012). Several programs such as the "Ethanol Blending Programme" and "National Biodiesel Mission" are introduced by the Indian government for promoting bioethanol and biodiesel production (EAI 2012; Saravanan et al. 2018). Currently in India, different government agencies are working in close coordination such as MNRE, Ministry of Petroleum and Natural Gas, Ministry of Agriculture (MoA), Rural Development, Panchayati Raj, Tribal Affairs, Science and Technology, Ministry of Environment and Forests (MoEF), and Finance for attaining the objectives laid by national bioenergy policy (Saravanan et al. 2018). The government of India is also promoting carbon emission reduction by lowering the use of fossil fuel and promoting renewable energy sources (IEA 2020).

23.10 Conclusion

The availability of a wide array of biological waste feedstocks and the opportunity of its conversion to a wide array of energetic (liquid, solid, or gaseous fuels) as well as non-energetic (biochemicals) with application in manufacturing, packaging, pharmaceuticals, etc has been elaborated. The conversion of these available feedstocks to biofuels and chemicals involves multiple steps right from production/cultivation, harvesting, collection, pretreatment, hydrolysis, fermentation, to separation/recovery, etc. Based on several studies it suggested that to overcome the major limitation of biorefinery, i.e., year-round supply of the biomass and sustainability integrated biorefinery could be an ideal approach for future replacement of fossil-based refinery by the biomass-based biorefinery. The different techno-economic aspects of the different stages of biomass to biofuel/biochemicals conversion have been discussed suggesting the parameters that need to be considered for economic and environmentally friendly approaches for integrated biorefinery. It is also suggested to utilize the existing petroleum refinery infrastructure to the biobased refinery. The development of greener yet compatible technologies/processes can help in utilization of existing petroleum refinery infrastructure to the full extent. Therefore, overcoming the economic burden that is required during the early phase of commercial exploitation of integrated biorefinery.

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Index

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