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Bhaskar Singh *Editors*

Novel Feedstocks for Biofuels Production

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 serves global readership on this theme.

Abhishek Guldhe • Bhaskar Singh
Editors

Novel Feedstocks for Biofuels Production

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Preface

Biofuel sector is rapidly evolving to cater the renewable energy demands. Novel feedstocks are being investigated for techno-economic feasibility to produce biofuels. Environmental concerns, food vs fuel debate, energy security, economic feasibility and availability are the major drivers for exploring different feedstocks for biofuel production. Researchers and national polices worldwide have led to exploration and application of novel feedstocks for biofuels production. In recent times, several novel feedstocks have shown promising results in terms of production efficiency as well as economic viability. This book *Novel Feedstocks for Biofuels Production* aims to critically evaluate recently investigated feedstocks for different types of biofuels production.

Chapters in this book give a complete outline of the novel feedstocks, their characteristics, potential biofuels produced and techno-economic aspects of the overall production process. Initial chapters in the book give an overlook of current biofuel scenario, different types of biofuels, challenges of conventional feedstocks and potential novel feedstocks for biofuel production. Subsequent chapters discuss novel feedstocks such as non-edible oils, potential microorganisms, algae, aquatic weed and animal fats for the production of biodiesel, bioethanol, biomethane and bio-oil. Different waste materials such as wastewater, solid wastes, agricultural lignocellulosic waste and food waste are also discussed for their application as feedstock for biofuels production. A chapter also deals with application of microorganisms in microbial fuel cells for electricity generation. Exploration of novel feedstocks will lead to catering the global renewable energy demand through sustainable biofuels production.

Mumbai, Maharashtra, India — Abhishek Guldhe
Ranchi, Jharkhand, India — Bhaskar Singh

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Abhishek Guldhe

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Bhaskar Singh

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Chapter 1
Biofuel Production from Conventional Feedstocks: Challenges and Alternatives

Indu Kumari and Abhilash T. Nair

Abstract The growing need for alternative renewable and sustainable fuels has incited the accelerated production of biofuels. Biofuel has received significant attention as an economical and environmentally friendly fuel. Favourable regulatory frameworks have supported increased production and consumption of biofuel in many countries. The present chapter discusses about the conventional feedstocks used for biofuel production and associated environmental, social and technological challenges in biofuel feedstock cultivation. Extensive biofuel feedstock cultivation has led to indirect land-use change, excessive water consumption and deforestation. In addition, biofuel feedstock cultivation has escalated the food and land prices. Lastly, the chapter discusses various waste materials used as an alternative biofuel feedstock, which reduces the cost of biofuel generation and solves the issues related to waste disposal.

Keywords Biofuel · Feedstock · Biodiesel · Bioethanol · Waste

1.1 Introduction

Due to the Industrial Revolution, increased energy consumption has directly impacted the environment and natural resources (Cheng et al. 2021). The depleting fossil fuel resources and rising greenhouse gas (GHG) emissions are two significant concerns for the energy sector. The scientific community is working on alternative renewable energy sources such as biofuels (Adeniyi et al. 2018). Biofuels are considered as superior alternative fuels to fossil fuels such as natural gas, coal and oil as they are eco-friendly, renewable, biodegradable and sustainable (Shahid et al.

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A. Guldhe, B. Singh (eds.), *Novel Feedstocks for Biofuels Production*, Clean Energy Production Technologies, https://doi.org/10.1007/978-981-19-3582-4_1

2021). Biofuel feedstocks include solid wastes, plant biomass containing oils and carbohydrates, lignocellulosic biomass, animal fats and algae. Innovative ideas and research towards the development of cleaner and sustainable energy resources are growing due to fossil fuel depletion, escalating fossil fuel prices and environmental-related issues (Peters et al. 2020).

1.2 Types of Biofuel Feedstock

1.2.1 First Generation

The first-generation or traditional biofuels are produced from starch and sugar-containing biomass like wheat, potato, barley, sugarcane, sugar beet, etc. Starch, sugar or vegetable oil extracted from edible crops through fermentation are converted into biodiesel, bioethanol or biogas (Bothast and Schlicher 2005; Mohr and Raman 2013; Lee and Lavoie 2013). Biogas is obtained from manure or other biomass resources through fermentation. Production of biofuel from edible biomass depends on various factors such as the chemical composition of biomass, cultivation and contribution to biodiversity, cost of storage and transport of biomass, etc. Bioethanol fuel is generated from corn, wheat and other sugar-containing crops through fermentation.

Compared to wheat, maize and other edible crops, sugarcane is largely used in bioethanol production. This is because the water required for sugarcane is less in comparison to other biomass. High bioethanol production and mitigation of GHG benefits are two critical factors that can be considered for selecting crop plants. Crop plants are classified into two groups: (a) crops that contain starch, viz. rice, wheat, corn, barley, etc., and (b) crops that contain sugar, viz. sugarcane, beetroot, fruit, etc. Starch contains a long chain of glucose molecules, so it cannot be converted into ethanol directly through conventional fermentation. Instead, starch-containing crops are converted to mash which contains 15–20% starch. Different processes like grinding, mixing with water, cooking and using enzymes such as amylase, pullulanase and glucoamylase are involved. Yeasts are used for the fermentation process.

Biodiesel fuel is obtained from edible or non-edible oil crops such as coconut, sunflower, olive, castor, linseed, etc. Bioethers or fuel ethers are produced by using bioethanol with iso-olefins. Wheat and sugar beet are primary natural sources for the production of bioethers.

1.2.2 Second Generation

Second-generation biofuel feedstocks include non-edible biomass, especially lignocellulosic biomass and waste plant biomass, including grasses, willow, jatropha,

eucalyptus, other seed crops, oil crops and wood (Naik et al. 2010). The cell wall of a plant contains 75% polysaccharides which are used for biofuel production. Agricultural wastes alone cannot fulfil the demand for biofuels. Lignocellulosic substances derived from cellulose, hemicellulose and lignin are converted into liquid fuels through physical and chemical treatments suitable for transportation from one place to another. Hydrolysis, fermentation, product separation and distillation of lignocellulosic biomass produces bioethanol. Different processes such as gasification, fermentation or catalysed reaction can be used for bioethanol production (Rekhate and Prajapati 2019). Different types of yeast (*Saccharomyces* species), bacteria or mould are used for fermentation. Biodiesel fuel is obtained from common energy crops such as *Jatropha*, salmon oil, *Madhuca longifolia* and jojoba oil after processing it via transesterification. In addition, non-edible oil crops such as castor, linseed, animal fats and beef tallow can be used for biodiesel fuel production (Naik et al. 2010). Due to higher octane number, non-corrosiveness, lesser waste generation and sustainability, second-generation feedstocks are better than feedstocks of the first generation. The main drawback of biodiesel is its lower efficiency at low temperature. Biobutanol is also gaining popularity as a biofuel. Biobutanol contains 21.6% oxygen, so when used in an internal combustion engine, it releases only carbon dioxide and water, which is eco-friendly. Iso-butanol is less toxic than n-butanol, so proper distillation is required to produce iso-butanol from wine using yeast.

1.2.3 Third Generation

Third-generation biofuel feedstock includes different types of microorganisms, especially microalgae. Based on size and morphology, there are two types of algae—macroalgae and microalgae. Microalgae can grow in nature as well as in artificial conditions. It requires lesser space, and through photosynthesis, it produces special chemicals and nutritional products (Nair et al. 2019). The amount of oil varies in different microalgae. Some contain more than 80% oil, while others contain 15–40%, so microalgae are the best feedstock for bioethanol and biodiesel production compared to other oil crops. Biofuel derived from microalgae produces less carbon monoxide and sulphur dioxide than fossil fuels (Jalilian et al. 2020). The main drawback of microalgae-based bio-oil is high cost, being less stable, highly unsaturated and more volatile at high temperature. Bioethanol production using algae is simpler than lignocellulosic biomass due to the absence of enzymatic steps. Algae bioreactors are used for scaling up biofuel production to commercial levels.

Biodiesel fuel can be obtained from yeast, oleaginous algae and bacteria with high lipid content. The development of microalgae with high lipid content depends on factors such as nitrogen and phosphate concentration, temperature, and cultivation condition. Recombinant DNA technology also plays a vital role in improving biodiesel production (Brar et al. 2021).

1.2.4 Fourth Generation

Fourth-generation biofuel feedstocks include genetically modified organisms. Modified cyanobacteria, yeast, microalgae, fungi, etc. are the main sources for the production of biofuels (Brar et al. 2021). *Synechocystis* is a freshwater, non-filamentous, non-nitrogen-fixing organism that is both photoautotrophic and heterotrophic and could be genetically modified. Gene transformation into nucleus, mitochondria and chloroplast produces genetically modified microalgae. *Chlamydomonas* can be modified by genetic transformation. It produces a large amount of recombinant proteins, including monoclonal antibodies and mammalian therapeutic enzymes. *Chlorella* sp. is unicellular green algae that can be modified by gene transformation. Genetically modified organisms can be used for commercial production due to their low complexity of structure, high oil content, high growth rate and modified genes. Different techniques, such as pyrolysis, gasification, upgrading, etc. are used in biofuel production (Brar et al. 2021).

1.3 Types of Biofuel

1.3.1 Bioethanol

Microbes ferment sugar, starche or cellulose present in the feedstock to produce various types of alcohol including ethanol, propanol and butanol. The pure form of ethanol E100 cannot be used as fuel for vehicles as it is not good for cold starting due to lower evaporative pressure than gasoline. Hence, it is mostly employed as an additive to improve the octane of gasoline. On the other hand, biobutanol or biogasoline may be directly used as motor fuel.

Bioethanol is widely used in Brazil as a transport fuel. Sugar obtained from various sources like molasses, beet, sugarcane, corn, starch and wheat are fermented, distilled and dried to obtain bioethanol (Xu et al. 2016). As the energy density of ethanol is lower than gasoline, a larger quantity of fuel is required to produce the same energy. However, ethanol has higher octane than gasoline, which increases the engine compression ratio and thermal efficiency (Iodice et al. 2018). Hence, ethanol is blended with gasoline for use in petrol engines.

1.3.2 Biodiesel

Biodiesel is generated from oils or fats by the process of transesterification. It is made of fatty acid methyl or ethyl esters. Pure form of biodiesel is B100 which is used as fuel for vehicles. Pure biodiesel (B100) is also known as “neat” biodiesel. This is because it reduces carbon monoxide and hydrocarbon emissions from diesel-

Table 1.1 Advantages and disadvantages of biofuel

Advantages	Disadvantages
Less polluting due to reduced disposable carbon, sulphur and particulate emissions	High initial investment and production cost
Decrease engine wear and tear and enhanced combustion efficiency	Environmental and social impacts due to uncontrolled cultivation of feedstock
Renewable source of energy	Complicated certification standards
Reduce reliance on crude oil, thus improving energy security	Reduce land for foodgrain cultivation, thus inflating the food and land price

powered vehicles. Vegetable oils, mustard, soybean, sunflower, waste animal fats, jatropha, mahua, flax, hemp and algae are the commonly used feedstocks for biodiesel production (Agarwal 2007; Raud et al. 2019).

Biodiesel blended with mineral diesel can be used in any diesel engine. The pure form of biodiesel (B100) used in diesel engines has some maintenance and performance issues in low temperatures. The viscosity of biodiesel increases with decrease in temperature. Biodiesel has a high flash point (164 °C) than petroleum diesel (52 °C) (Agarwal 2007; Chhetri et al. 2008). Biodiesel usage can reduce the emission of particulate matter, carbon monoxide and unburned hydrocarbons from conventional engine (Agarwal 2007). Advantages and disadvantages of biofuels over fossil fuel have been summarised in Table 1.1.

1.4 Challenges with Conventional Biofuel Feedstocks

1.4.1 Environmental Impacts

Biofuel production systems have often been considered sustainable because of biomass renewability. However, sustainability cannot be established solely on biomass renewability or on the conservational element. Biofuel has a severe effect on the ecosystem from its generation till its end-use. The scale of the impact varies from local, to regional, to even global levels. The environmental effects vary depending on topographical setting and spatial arrangement of biofuel feedstock production (Banse et al. 2011). The land classification and land area required for producing biofuels is at the core of the discussion on biofuel feedstock production. Converting the grassland into fields for biofuel feedstock production can lead to loss of habitation and growth of invasive species. Also, biofuel feedstock production can lead to undue stress on wildlife habitats and bird species (Hoekman and Broch 2018). The conversion of forestland or grassland into agricultural land releases the carbon sequestered by soil and changes the carbon storage capacity of the soil, which is termed as indirect land-use change (ILUC) emissions. ILUC leads to an instant and one-time huge surge in GHG releases (Rajagopal 2016).

Accelerated consumption of fertilisers and pesticides releases GHGs into the atmosphere. A substantial portion of the nutrients in applied fertilisers is lost because of mechanisms like tile drainage, sediment transportation, surface run-off and infiltration (Hoekman et al. 2018). Nutrient pollution can lead to eutrophication of surface water bodies (Lankoski and Ollikainen 2011). Higher concentrations of suspended solids, nitrogen and phosphorus have been reported in watersheds near intensive biofuel feedstock production sites, causing severe threats to aquatic life and recreational events, for example, swimming and fishing. Nitrate contamination in drinking water can lead to methaemoglobinaemia in infants, reproductive effects and cancer (Hoekman et al. 2018). High nitrogen availability leads to N_2O emission by the bacteria present in the soil through nitrification, denitrification and degradation of crop residues (Carter et al. 2011). N_2O has 298 times higher global warming capability than CO_2 in a 100-year time horizon (Carter et al. 2011). The N_2O released from fertiliser applied to corn and rapeseed crops for biofuel production has higher global warming potential than abated by fossil fuel savings (Crutzen et al. 2016).

Similarly, increased production of fertiliser and pesticides can lead to increased CO_2 emissions from the manufacturing plants (Lankoski and Ollikainen 2011). For example, 39–54 kg of CO_2-eq is released per hectare of biofuel feedstock production from pesticide production, transportation and application (Lankoski and Ollikainen 2011). Similarly, various cultivation practices like sowing, ploughing, harvesting and transportation of feedstock release an average of 196 kg of CO_2-eq per hectare, while biofuel processing activities emit 0.22–0.52 kg CO_2-eq emissions per kg production of biofuel feedstock (Lankoski and Ollikainen 2011).

1.4.2 Socio-economic Issues

The social aspect of sustainability is not considered often in biofuel production as the tools and methodologies used to evaluate social aspects are scarce and are not common in engineering sciences. Also, environmental and monetary sustainability aspects are measured quantitatively, whereas, social aspects are commonly measured in qualitative terms (Palmeros Parada et al. 2017). The social issues can differ typically based on political environment, market, location and climate. Intensive biofuel feedstock cultivation can reduce the agricultural area presently utilised for food crops and animal feed, challenging food security (Islam 2012). In developing countries, mainly the larger farmers have benefitted from the demand for more biofuel feedstock production. A large land area is required to increase feedstock production, often taken away from socially and economically weak people (Amigun et al. 2011). ILUC for more biofuel feedstock production is responsible for increasing the price of land. Carbon-rich, non-cultivation land is rapidly converted to carbon-deficient cultivation land (Rajagopal 2016). The food prices will also rise due to the growing feedstock demand for biofuel production (Ravindranath et al. 2011). Increased food prices can lead to increased malnutrition in developing

nations. According to International Food Policy Research Institute (IFPRI), the increasing demand for biofuel production between 2000 and 2007 was responsible for a 30% rise in weighted average grain rates (Tirado et al. 2010).

Also, increased biofuel feedstock production will perhaps lead to higher water consumption. This problem is more severe in many tropical countries due to water scarcity and falling groundwater levels (Ravindranath et al. 2011). For instance, several first-generation biofuel crops, e.g. palm, sugarcane and maize, have comparatively greater water requirements. Widespread feedstock cultivation for commercial applications might cause competition for freshwater between biofuel and food production (Ravindranath et al. 2011; Islam 2012). Land and water scarcity can lead to increased food prices. Underdeveloped nations with a low level of industrialisation should emphasise small-scale bioenergy systems incorporating prevailing crops and livestock.

1.4.3 Technological Issues

The high cost of feedstock production is one of the most critical problems in biofuel production. Typically, feedstock production has 70–95% overall biodiesel production cost (Chhetri et al. 2008). Hence, several cheaper alternatives like waste cooking oil (WCO), algal oil and animal fats have been used to reduce the cost of biofuel production (Barnwal and Sharma 2005; Zhu and Ketola 2012; Sodhi et al. 2017). In addition, these feedstocks do not challenge food security as they do not compete with edible food crops. However, these alternatives require additional pretreatment and refinements due to high moisture content and free fatty acid (FFA), increasing the cost of biofuel production (Sodhi et al. 2017).

1.4.4 Certification Issues

A sustainable biofuel production system safeguards economic viability, environmental conservation and social well-being. Sustainability in biofuel production is still a topic of debate as several advantages and risks are involved in every stage of biofuel production. Many enterprises are developing biofuel sustainability standards and certification schemes. These certification standards were prepared to resolve social, local environmental, global environmental and commercial management problems related to biofuel production (Pols 2015). These certification schemes are based on reporting criteria such as air quality, land rights, biodiversity protection, carbon saving, soil preservation, sustainable water consumption and workers' rights (Scarlat and Dallemand 2011). The biofuel suppliers can use the Carbon Calculator tool to estimate the carbon emission savings of every set of biofuels. However, the practical scope of most certifications is inadequate (de Man and German 2017). Although certification results in sustainable biofuel production, its practice comes

with a few risks. Certification is weakest on the most severe issues like food security, competition for land and problems associated with rights that are tough to tackle through market-based governance only (de Man and German 2017). Companies with more organisational funds can spend on certification processes and have more power in the international biofuel trade than small farmers (de Man and German 2017).

1.5 Application of Waste Materials as Feedstock for Biofuel Production

1.5.1 Waste Oil

Second-generation biofuel production focuses on biomass production from waste deployment. Using WCO discarded as waste after deep frying is a sustainable biodiesel production option. Deep frying causes oxygen to dissolve in the oil and initiates various chemical reactions, increasing the polar materials and reducing the unsaturated fatty acids (Sodhi et al. 2017). The low unsaturated fatty acids make it unsuitable for reuse in cooking and human consumption (Sodhi et al. 2017). Further, it is economical and readily available from various sources like food processing industries, households and restaurants. Hence, recycling of WCO provides economical feedstock and environmental protection and safeguards public health. WCO is usually converted to biodiesel through the transesterification process. An alkaline catalyst such as KOH, NaOH, or CaO increases reaction efficiency (Bharti et al. 2020). However, alkaline catalysts can only be used for WCO with low FFA content. The reaction of alkaline catalysts with high FFA content leads to saponification, resulting in low production of fatty acid methyl esters (FAME), i.e. biodiesel (Sodhi et al. 2017; Goh et al. 2020). In such a scenario, a two-step biodiesel production is adopted. The first step involves the pretreatment esterification process using an acid catalyst like H_2SO_4 or HCl. The second step involves the conventional transesterification process with an alkaline catalyst (Sodhi et al. 2017; Goh et al. 2020). However, the energy content of vegetable oil is 10% lesser than petroleum diesel due to its high viscosity and the presence of a significant quantity of oxygen in it. Also, the specific gravity of biodiesel is around 0.88, which is higher than petroleum diesel, whose specific gravity is around 0.85. Hence, per unit volume, the total energy content is nearly 5% lesser than petroleum diesel (Agarwal 2007). Also, the low volatility of vegetable oil leads to a high amount of carbon deposition, thickening of lubrication oil, sticky oil ring and oil degradation, which ultimately results in weak cold starting, misfire and delayed ignition (Meher et al. 2006; Agarwal 2007). Biofuel produced from vegetable oil deteriorates during long storage due to hydrolytic and oxidative reactions (Meher et al. 2006). Hence, more research on preservatives is required to improve the storage time of biofuel from waste oil.

1.5.2 Fishery Waste

Approximately 60% of the total fish processed is disposed as waste which includes the outer skin, head, stomach and fatty layer (Moraes et al. 2020). The fish waste contains saturated, monounsaturated and polyunsaturated fatty acids required in appropriate concentrations for high-quality biodiesel (Moraes et al. 2020). This waste can be used as feedstock for biodiesel production after the transesterification process. The fishery industry also produces omega-3-rich oils for human consumption. Almost 45% of the entire fish captured is released as waste after omega-3-rich oil production. These wastes are the viscera skin, which comprises 1.4–40.1% (w/w) of oil, which can be processed for bio-oil/biofuels (Jayasinghe and Hawboldt 2013). The properties of biodiesel produced from fish waste meet the standards established by regulatory agencies and are reliable for storage and transportation (Kudre et al. 2017). The main challenge with fishery waste is storage time. The enzymes and microbes present in the waste degrade the lipids in the waste (Jayasinghe and Hawboldt 2013). Hence, establishing biofuel production unit near the fishery industry can reduce the cost of biofuel production and reduce the impact of fishery waste disposal.

1.5.3 Animal Fats

Waste animal fats (WAFs), by-products from meat processing industries, include tallow, pork lard and chicken fat and grease (Banković-Ilić et al. 2014). In addition, poultry waste such as chicken blood, feathers and carcass lesions are the source of chicken fat, containing significant quantities of fatty acids (Foroutan et al. 2021). The two-step reaction method is the most commonly used method for transforming animal fat into biodiesel. The first step involves esterification of FFAs using an acid catalyst, and in the second step, transesterification of lipids is performed using an alkaline catalyst (di Bitonto and Pastore 2019). WAFs contain a large quantity of saturated fatty acids (SFA) comprising of myristic, palmitic and stearic acids and free fatty acid (FFA) (Banković-Ilić et al. 2014). Hence, biodiesel produced from WAFs possesses a higher cetane number than most of vegetable oils. Likewise, high SFA content in WAFs provides improved oxidative stability for biodiesel (Adewale et al. 2015). Thus, WAFs used for biodiesel production provide food security, and environmental and economic advantages over using food crops as feedstock.

1.5.4 Agricultural Waste

The wastes from apple, barley, date, grape, maize, orange, potato, rice, sugar beet, sweet lemon, sugarcane and wheat are utilised for biofuel production (Mohammad

and Khademalrasoul 2018). The cellulose and hemicellulose components of agricultural residue are popular feedstocks for bioethanol production. Microbes break down the hexose and pentose present in these residues through fermentation process to produce bioethanol. Sugarcane and sugar beet are significant sources for sugar production. However, the waste after juice extraction, i.e. molasses, is fermented to produce bioethanol. Therefore, many sugar factories commonly adopt integrated production of sugar and bioethanol. Sucrose ($C_{12}H_{22}O_{11}$) in the feedstock is hydrolysed by yeast *Saccharomyces cerevisiae* to form hexose ($C_6H_{12}O_6$), which is further converted to ethanol (C_2H_5OH) and CO_2 (Jung et al. 2021). To reduce the complexity of the total bioethanol production methods, genetically engineered enzymes have been developed for synchronised hydrolysis of starch and bioethanol fermentation. Moreover, various kinds of glucoamylases have been developed to directly convert the starch into monosaccharides in mild operational settings and improved ethanol productivity (Xu et al. 2016).

1.5.5 Food Waste

Farming fields, food processing industries, marketplaces, eateries and kitchens are the prominent sources of food waste. Around 33% of the municipal wastes is food waste. Almost 130 million tons of food wastes are produced worldwide every year (Theppitak et al. 2020). Carbohydrates (35–69%), proteins (3.9–21.9%) and fats (10–40%) present in food waste are easily degradable compared to other organic waste (Li and Yang 2016). Fats in the food waste can be used to produce biodiesel, while carbohydrates can be fermented to produce biogas (Karmee 2016; Li and Yang 2016). The disintegration of glycoside linkages during carbohydrate hydrolysis in food waste results in polysaccharides being released as oligosaccharides and monosaccharides, which are more fermentable (Li and Yang 2016). Moreover, anaerobic digestion of food waste can produce biomethane, a high calorific value biofuel (Kavitha et al. 2017). Segregation of carbohydrate, fats and other carbon components increases the cost of biofuel production. Hence, more research for process optimisation and catalyst is required to produce biofuel without separation of components from the food waste.

1.5.6 Microalgae Biofuel Feedstock

Presently, biofuel production from microalgae has gained significant consideration as it can grow in diverse environmental conditions and have high photosynthetic efficiency, high lipid content and high growth rate (Zhu and Ketola 2012; Mehrabadi et al. 2016). Algae are chlorophyllous, unicellular or multicellular organisms of the size range in micrometres. The microalgal species with high lipid and carbohydrate content are appropriate feedstock for biodiesel and bio-alcohol, respectively (Jalilian

et al. 2020). Microalgae also can uptake nutrients from wastewater and CO_2 from the atmosphere to produce biomass and lipids, helping to purify water and air (Thomas et al. 2016; Nair et al. 2019; Mohan Singh et al. 2020). Microalgae can be cultivated in wastewater or wasteland, thus no competition for agricultural land, safeguarding the environmental biodiversity and food security (Mehrabadi et al. 2016). In addition, microalgae can consume CO_2 from the atmosphere, flue gas and soluble carbonates for their growth (Zhu and Ketola 2012; Nair et al. 2019). 1 kg of microalgal biomass can consume approximately 1.8 kg of CO_2 for its own metabolic activities (Thomas et al. 2016). Various algal species have been studied for biofuel production, including *Scenedesmus*, *Schizochytrium limacinum*, *Botryococcus braunii*, *Isochrysis galbana*, *Nannochloropsis*, *Chaetoceros calcitrans* and *Chlorella* species (Sandesh and Ujwal 2021). However, the main challenge with microalgal biofuel is the high fuel price. Microalgal biofuel production requires a high capital cost in the initial phase. A high amount of chemicals and energy is required for harvesting and dewatering the microalgal biomass. Also, the techniques used to extract oil from microalgae are expensive for large-scale production (Zhu and Ketola 2012). Hence, co-production of other value-added products like dyes, pigments, enzymes and biochar can recover the operational cost of biofuel production.

1.6 Summary and Future Research

Biofuel production induces significant environmental impacts. Hence, a land and water utility plan must be prepared before establishing a biofuel production industry. Land-use optimisation to reduce the adverse environmental impact is a complex process. However, the negative environmental impacts can be reduced by selecting suitable energy crops and proper distribution in the watershed areas. Hence, further studies using simulation models can help us choose the best option for land management with minimal impact on hydrology and water quality.

Similarly, crops with low nitrogen demand can help reduce nitrogen pollution in water bodies. Low nitrogen demand biofuel feedstock also leads to lesser N_2O emission. The use of life cycle assessment can help estimate the land, water, nutrient and energy demand and its resultant global warming potential in the various stages of biofuel production.

The economic viability of biofuel production can be achieved through resource optimisation, improvement in production efficiency, market research and long-run cost-effectiveness. For example, changing the feedstock from edible crops to high oil content crops and waste products can reduce the feedstock production cost. This also reduces the negative environmental and social impacts of inefficient waste management. Also, applying advanced technologies such as genetic engineering to develop high-yielding strains can further address the biofuel production issues.

Many certification schemes are available in the market with different criteria, making it difficult for the biofuel producers to choose the suitable one. Also, the

large administrative resource requirements make it difficult for small-scale producers to participate in the certification schemes. These prevent them from international trade and funding opportunities. Hence, harmonisation between the certification schemes is essential to bring more consistency and transparency. Additional studies are required to synchronise the government policies, framers and public participation to tackle the problem.

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Chapter 2
Novel Feedstocks for Biofuels: Current Scenario and Recent Advancements

Zaira Khalid, Shahrukh Nawaj Alam, Abhishek Guldhe, and Bhaskar Singh

Abstract New policy initiatives combined with rise in demand for transport fuel have stimulated an increase in biofuel production throughout the world. Since the beginning of bioenergy era, biofuel industries have been mostly dependent on feedstocks with agricultural importance especially for production of bioethanol and biodiesel. The main problem of conventional feedstocks such as edible crops or oilseeds lies with the availability, demand and the cultivation of raw material which may impact food production. Moreover, they require large arable land masses and irrigation facilities giving rise to secondary problems such as high water requirement leading to increase in production cost. Therefore, the current situation demands such raw material for biofuel production that can overcome food versus fuel scenario and water dependency. Various novel feedstocks like lignocellulosic waste, municipal wastes, waste oils, sewage waste, non-edible oil seeds, forest residues, microalgae, aquatic weeds and others which can be used to overcome aforesaid issues and reduce the production cost have been mentioned in this chapter.

Keywords Bioenergy · Biofuel · Conventional feedstocks · Novel feedstocks

2.1 Introduction

The potential of biofuel as an alternative to fossil fuel is immense which has led to commercial production of biofuel for reduction in carbon emission (Paul et al. 2019). New policy initiatives combined with rise in demand for transport fuel have stimulated increase in biofuel production throughout the world. Adoption of mandates by countries has increased regarding the consumption of biofuels produced domestically for energy security and improvement of air quality (IEA 2018). Predominantly,

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biofuels are obtained from renewable photosynthetic matters such as biomass, micro- and macroalgae and various vascular plants. The primary products of biofuels can be in solid, liquid or gaseous forms like burning pellets, or other forms of liquid and gaseous biofuels but can be converted to other forms using various physical, chemical and thermal techniques (Rodionova et al. 2017). However, the main problem with conventional feedstocks lies with the availability and demand. The cultivation of raw material requires large arable land masses and irrigation facilities giving rise to secondary problems like food shortage and high water requirement. Therefore, the current scenario demands such raw material for biofuel production that can overcome 'food versus fuel' and water dependency. As a result, researchers, energy sector and policy makers are showing great interest in searching novel feedstock that can overcome aforesaid problems. Considerable research is currently being held in the field of identifying raw materials that can be supplied continuously without competing with food crops, optimizing and advancing conversion techniques to gain more output and reducing the overall cost of production keeping in view the environmental aspects. Emphasis is being given on waste materials such as lignocellulosic waste, municipal wastes, waste oils, sewage waste, non-edible oil seeds, forest residues, microalgae, aquatic weeds and other biomass which are showing great potential for the production of biofuels (Alam et al. 2021; Vasić et al. 2021). This chapter discusses current scenario of the biofuel production from novel feedstocks.

2.2 Biofuels

Biofuels may be described as liquid fuels derived from biomass used for transportation as an alternate to fossil fuel, including bioethanol derived from sugar, starch and lignocellulosic feedstocks, and biodiesel derived from oils and fats. According to the EASAC (2012) report, biofuels can be classified as first-, second- and third-generation biofuel that is primarily based on the origin or the raw materials from which they are extracted from such as biomass, waste materials or cultivated algae, whereas the concept of fourth-generation and fifth-generation biofuel is still at the elementary level of research. Biofuels of any generation are mainly derived from cellulose, hemicellulose, sugar, starch vegetable and animal fats. However, the general structure of biofuel doesn't change with the change in biofuel generation.

2.2.1 First-Generation Biofuels

First-generation biofuels consist of edible feedstocks or food crops such as corn, sugarcane, wheat, soya bean, rapeseed, coconut, palm, mustard, olive and others. The uses of food crops were quite popular for the production of biofuel in the beginning. High cost, competitiveness with food supplies and requirement of

extensive growth conditions created problems at the beginning of biodiesel era. Availability of crops and comparatively easy conversion procedure are the main benefits of the first-generation feedstocks. The risk of competing with arable land and food supply, high cost of production and requirement of extensive growth conditions were the main disadvantages in the use of these feedstocks that increase the cost of food products creating ethical and sustainability issues (Gerbens-Leenes 2017). These drawbacks pushed researchers and policy makers to shift onto the different alternate sources for biofuel production (Tariq et al. 2012).

2.2.2 Second-Generation Biofuels

Drawbacks associated with first-generation biofuel feedstocks attracted researchers to work on non-edible feedstocks such as forest or waste-derived lignocellulosic biomass (LCB). The main advantages of these feedstocks apart from their no food value are minimal environmental impact and not requiring surplus amounts of fertilizer or water. The most prominent second-generation feedstocks include forest-derived lignocelluloses like switchgrass, miscanthus, Indian grass seed crops like jatropha, camelina, palm and rapeseed, waste cooking oil and municipal solid waste (Shi et al. 2009; Pandey et al. 2012; Ho et al. 2014; Bharti et al. 2020).

The main disadvantages of second-generation fuels are that the yield of many important non-edible plants like jatropha, jojoba and Karanja are not of the required value to compete with fossil fuels. However, these plants can be cultivated in nonarable and degraded lands. This being the main reason directly influences the economy of society without hampering the food production. The second-generation biofuel feedstock's carbon footprint is much lower than fossil fuels (Naik et al. 2010); however, requirement of alcohol in large quantity during the production process is one of the main drawbacks of second-generation biodiesel (Tariq et al. 2012).

2.2.3 Third-Generation Biofuels

First- and second-generation biofuels due to their various limitations demanded exploration of alternative raw materials for the production of biofuels superior to their predecessors. This led to the explorations of algal biomass for the third generation of biofuel. Both microalgae and macroalgae have been greatly explored owing to their high lipid content producing larger quantity of biofuel or indirectly as feedstock biogas production through fermentation in shorter period of time. They can convert light and carbon dioxide into various chemical compounds through cellular activities like carbohydrate, lipid, protein, vitamin, etc. that can be utilized in health, food supplement, energy and pharmaceutical industry (Costa and De Morais 2011). The advantages of third-generation biofuel feedstock include high growth

rate and productivity much higher than terrestrial plants that can be harvested in just 5–6 days after cultivation, high carbon sequestering potential, higher amount of oil percentage and lesser influence on food supply. The main disadvantages of third-generation biofuels are requirement of large investment, surplus amount of sunlight and difficulties in oil production (Liew et al. 2014; Lamichhane et al. 2021).

2.2.4 Fourth-Generation Biofuels

Fourth-generation biofuels are derived by genetically modifying microorganisms to enhance quality and productivity. These microorganisms are modified to increase intake of carbon dioxide for photosynthesis, creating an enhanced carbon sink to enhance the overall growth. Some of the examples include *Phaeodactylum tricornutum* sp., *Chlamydomonas reinhardtii* sp., *Chlorella vulgaris*, *Thalassiosira pseudonana* sp., etc. which have been modified genetically to enhance the adaptability and growth rate to increase the production and hence biofuel (Illman et al. 2000; Rizwan et al. 2017; Abdullah et al. 2019).

The genetically modified microorganisms and their environmental advantages may include higher carbon dioxide sequestration and assimilation, the reduction of GHGs and higher nutrient accumulation as well as nutrient tolerance making them suitable for wastewater treatment (Zhu et al. 2017; Leong et al. 2019). Some microorganisms and their modifications which have been reported in a few studies are shown in Table 2.1.

Table 2.1 Modifications in microorganisms

Microorganism specie	Modification result	Reference
Chlamydomonas reinhardtii	Two-fold increase in starch content and 2.4-fold higher accumulation of TAG	Rengel et al. (2018)
Chlamydomonas reinhardtii	Increased productivity and 28.5% increase in lipid content	Kao and Ng (2017)
Chlamydomonas reinhardtii	56% increase in total lipid	Tan and Lee (2017)
Chlorella sorokiniana and *Chlorella vulgaris*	2.2-fold increase in lipid accumulation	Lin et al. (2018)
Chlorella vulgaris	Increased productivity and 67% increase in lipid content	Sarayloo et al. (2018)
Phaeodactylum tricornutum	2.4-fold increase in lipid content	Xue et al. (2017)
Nannochloropsis salina	Biomass productivity increased 2.4 fold	Vikramathithan et al. (2020)
Thalassiosira pseudonana	Three-fold increase in lipid content	Trentacoste et al. (2013)
Synechococcus elongatus	41% increase in carbon uptake	Chen et al. (2012)
Chlamydomonas reinhardtii	50% increase in photosynthetic efficiency	Beckmann et al. (2009)

2.3 Types of Biofuels

With the reference to the source and feedstock, biofuels may be categorized into two types: primary and secondary biofuels. Primary biofuels are obtained from the raw material which can be applied in the biofuel production process in their natural/raw form without needing any types of pretreatment or processing and are used to produce heat and electricity. Some examples of primary biofuels include firewood, animal waste, crop waste, etc. Secondary biofuels are generated from processed waste or biomass and are converted into desired product by using various physical, chemical and biological means. The first generation of biofuels is the production of ethanol from starch. Biofuel can be further classified based on the state, nature and raw material into bioethanol, biodiesel and biogas.

2.3.1 Bioethanol

Bioethanol are alcohols produced by fermentation of simple sugar, carbohydrate or starch from crops such as maize, sugarcane, sorghum, soya bean, corn, etc. (Kumar et al. 2018). Bioethanol are largest produced liquid biofuel used in transportation industry as eco-friendly alternative to fossil fuel. Ethanol in its purest form possess relatively low energy density and poor storage characteristics and are therefore mostly used as additives in the blend of gasoline to enhance the energy density and octane number and reduce vehicle emission (Goldemberg and Teixeira Coelho 2004; Radakovits et al. 2010). Cellulose-based biomass can be utilized as effective feedstock to produce bioethanol, and several additions in the field of pretreatment and microorganism-assisted fermentation have been adopted to enhance the production process (Fatma et al. 2021).

2.3.2 Biodiesel

Biodiesel are produced from fats and oils from plant and animal origin through the process of esterification and transesterification. Biodiesel is the second largest liquid fuel utilized and produced after bioethanol used by transportation sector as a blend with fossil fuel in any kind of biodiesel engine. Biodiesel are mostly used as blends as the pure biodiesel burning may add up to NOx emissions and also cause problems during winter due to low viscosity leading to performance and maintenance issues (Ferreira et al. 2009). However, in blend it minimizes the emission of hydrocarbon and particles (Fisher et al. 1995).

2.3.3 Biogas

Biogas are obtained by fermenting organic feedstock with the help of anaerobic microorganisms. Biogas is regarded as one of the cleanest burning biofuel from a wide range of raw material. The most prominent advantage of biogas includes possibility of liquefaction, hence enhancing the storage capacity and transportation and can be supplied by same pipelines used to supply natural gas (Urban 2013). It is also easy to make without any complications and therefore can be produced even by farmers by using available raw materials like cow dung. The by-product after the extraction of biogas can be used again as fertilizer.

2.4 Biofuel Production from Various Novel Feedstocks

The search for novel feedstock that is environmentally and economically better than its predecessors has been a major research area since the first attempts at biofuel production. Currently, the major focus is on the biofuel feedstocks that are readily available, do not impact the global environment and are preferable if they assist in carbon reduction, can achieve multiple outputs or otherwise are not a nuisance to society; thus, biofuel production provides a mode of management. In current time, biofuel production from lignocellulose-based feedstock such as non-edible feedstocks, waste materials, algae, weeds both terrestrial and aquatic, etc. is in momentum and shows great potential for reducing fossil fuel dependency in the future.

2.4.1 Biofuel Production Using Biomass and Lignocellulose-Based Feedstocks

Lignocellulosic biomass is one of the most attractive feedstocks for biofuel production mainly due to its high energy content and renewable and inexpensive nature. Lignocellulose-based feedstocks are predominant in cellulose (33–55 wt%), hemicelluloses (20–40 wt%) and lignin (10–25 wt%) along with several kinds of extractives such as flavonoids, terpenoids, steroids, fats, carbohydrates and lipids which can be converted to various types of biofuels (Nanda et al. 2013). Most researches related to the utilization of lignocellulosic feedstocks for biofuel production focus on the biomasses which are considered waste in some regard or residues from other mainstream human activities such as agriculture, forestry, industrial domestic, etc. The main advantage of these kinds of feedstock includes elimination of food versus fuel competition faced by biofuel production system mainly concerning dilemma of fuel over food from land utilization. Most of the lignocellulosic wastes, in the present time, either end up in landfills, burnt or get discarded in waterbodies. Therefore, the effective utilization of these waste materials for the production of biofuel can lead to

several environmental impacts such as decreasing waste pollution and decreasing GHG emission, thus called the next-generation biofuel feedstock. Some of the lignocellulosic feedstocks currently being utilized for the production of biofuel are mentioned below.

2.4.1.1 Non-edible Forest Products

Non-edible forest products and forestry residues represent a massive source of readily available biomass not needing additional land and other resources for biofuel production. These can be obtained from the by-products of raw material which are planted, processed and consumed. It is estimated that throughout the world around 501 million dry tonnes of forestry residues are generated every year (IEA 2010). These include non-consumable forest residues which are the second-largest lignocellulosic biomass source after agricultural residues. Forest products generally refer to non-edible or sometimes toxic fruits and seeds, parts of trees and low wood value species which can be important sources of LCB (lignocellulosic biomass) and utilized in the production of bioenergy. Forestry residues are mainly generated during and after logging and pruning operations and during the processing of woods in industries. Forestry residues can be found in a considerable amount for the production of bioenergy in the regions with large forest covers and high industrial use of wood. These types of forest residues may include woodchips, barks, hardwoods and sawdust which are utilized to produce burning pellets, pyrolysis oil, liquid biofuels, etc. Ren et al. (2012) studied the microwave pyrolysis of Douglas fir sawdust pellet and showed the highest bio-oil conversion of 57.8%. Similarly, Heo et al. (2010) studied furniture sawdust bio-oil production using a fluidized bed pyrolysis reactor and found the highest bio-oil conversion of 65%, whereas ethanol production from sawdust was studied by Tulashie et al. (2021) where they studied different acid hydrolysis for the conversion of substrate to bioethanol and found the production to be as high as 80%. Wood chips and pruning residues like barks also possess great biofuel potential which have been studied by researchers like Chukwuneke et al. (2019) where they analysed mahogany wood pyrolysis to produce bio-oil and found the maximum bio-oil yield to be 69.5 wt%.

Non-edible forest products include non-edible seed oils. These seeds may contain some harmful compounds and therefore may be unfit for human consumption; however, they can be successfully applied for the production of biofuels overcoming the economic, environmental and food versus fuel problems. The oil extracted from these non-edible seeds is mostly applied to produce biodiesel due to its liquid nature, higher combustion efficiency, lower sulphur content, easy availability and appropriate aromatic content (Shikha and Chauhan 2012). Also, it can help the competitiveness of biodiesel in price when compared to the biodiesel production from edible vegetable oils. A detailed description of the non-edible seed oil is discussed later in the chapter.

2.4.1.2 Aquatic Weeds

Aquatic weeds are nuisance causing plants that grow in water interfering with the intended use of water harming the environment and human welfare (Dhadse et al. 2021). Some aquatic biomasses such as *Eichhornia crassipes* (water hyacinth), *Pistia stratiotes* (water lettuce), *Salvinia molesta* (water fern) and *Lemna minor* (duckweed) have very high reproductive and doubling rate and invaded freshwater ecosystem completely taking over the waterbody causing considerable socio-economic problems (Alam et al. 2021). These aquatic weeds greatly affect the water quality and biodiversity throughout the world but owing to their unique physicochemical characteristics can be effectively used to produce several types of biofuel. Aquatic weeds also possess the ability to surpass other kinds of biofuels owing to their high reproductive rate. Other than that, aquatic weeds have a notable amount of cellulose, hemicellulose, lignin, carbohydrate, sugar, etc. which are essentially converted to several kinds of biofuels. Sugar undergoes direct fermentation to produce bioethanol; lignin parts are utilized to produce bio-oil, heat energy and combustible gases through thermochemical conversion. Aquatic weed also possesses lipids which are essentially made up of modified fatty acids which are converted into biodiesel through the process of transesterification (Naik et al. 2010). This biomass can also be utilized to produce liquid biofuel like biomethanol, biobutanol and gaseous biofuels like biohydrogen using biological conversion method and biomethane using anaerobic digestion (Bhattacharya and Kumar 2010; Nong et al. 2020). Different biofuels produced from various aquatic weeds are mentioned in Table 2.2.

Table 2.2 Different types of bioethanol extracted from aquatic weed species

Aquatic weed specie	Biofuel produced	Yield	Reference
Pistia stratiotes (water cabbage)	Bioethanol	15.385 g/L	Whangchai et al. (2021)
Pistia stratiotes (water cabbage)	Biomethane	72.5%	Güngören Madenoğlu et al. (2019)
Eichhornia crassipes (water hyacinth)	Bioethanol	0.23 g/g	Figueroa-Torres et al. (2020)
Eichhornia crassipes (water hyacinth)	Biomethane	53–58%	Rathod et al. (2018)
Eichhornia crassipes (water hyacinth)	Biohydrogen	65 mmol/L	Carreño Sayago and Rodríguez (2018)
Victoria amazonica (giant water lily)	Bioethanol	4.82 g/L	Junluthin et al. (2021)
Pichia stipites (kariba weed)	Bioethanol	13.7 g/L	Chupaza et al. (2021)
Wolffia globosa (Asian water meal)	Bioethanol	170 g/kg	Soda et al. (2015)
Ceratophyllum demersum L. (coontail)	Bioethanol	2.92 g/L	Kusolsongtawee et al. (2018)
Lemna gibba L. (duckweed)	Bioethanol	20%	Dhruba et al. (2010)

2.4.1.3 Microalgae

Biofuel derived from algae has become a promising alternative fuel which ensures sustainable and stable transport fuel supply. Moreover, the use of algal diesel blend in gas turbine systems, compression ignition engines as well as aviation fuel has proven to be viable (Chiong et al. 2018). The required setup for harvesting, pretreatment and production questions the feasibility of microalgal biofuel generation. The extractives having nutraceuticals, therapeutics and cosmetic value derived from algal biomass, before as well as after oil extraction, have been reported in various studies. It has been reported that β-carotene, an algal chemical in its cis form, can create a profit of about USD600 million/kg. Additionally, leading market analyst companies have estimated that the value of omega fatty acids will stand at USD18.95 billion by 2020, carotenoid at USD1.53 billion by 2021, astaxanthin at USD814.1 million by 2022 and lutein at USD357.7 million by 2024 (Kumar and Bharadvaja 2020). *Botryococcus*, *Chlorella* sp., *C. reinhardtii*, *Dunaliella*, *Isochrysis galbana*, *Monodus subterraneus*, *Nannochloropsis*, *Phaeodactylum tricornutum*, *Scenedesmus*, *Spirulina* and *Tetraselmis* are biodiesel-rich microalgae genera with higher biomass productivity of about 20–200 mg/L/day. A study conducted using *S. dimorphus* and *S. obliquus* used chelate promoter, Ni(II)-Schiff base and Ni/H_2 catalyst, to carry out higher yield of algal oil. The microalgal biodiesels were found to have higher cetane number and oxidation stability (Vadivel et al. 2020). It was found that through solvent extraction method, maximum ester yield of 82.33% was derived from *Botryococcus braunii* at 55 °C. It was also noted that the rate of conversion increases with increasing temperature (Prasad et al. 2015). Through Soxhlet extraction method, it was observed that in *Spirogyra* the lipid yield (55–80%) was higher in 100% dried sample and lowest in 50% dried sample (Konga et al. 2017), while *Cladophora* in similar growth conditions showed higher yield (90–95%) (Verma et al. 2016). Moreover, the concept of genome editing has revolutionized the biotechnological sector with its unique ability to identify, manipulate as well as isolate nucleic acid sequences changing the landscape of microorganism and crop-based biofuels (Shokravi et al. 2021). Few of the recent advancement in genome editing are described in Table 2.3.

2.4.2 Biofuel Production Using Non-edible Oilseeds

Urban expansion and agriculture have led to increase in deforestation leading to the decline in biodiversity and destruction of ecosystem. The competition towards the same resource in food and biofuel sector raises the debate over food versus fuel. Due to food scarcity in developing countries, conversion of food crop to biofuel could create a food shortage problem. Non-edible oil-based biodiesel production provides fuel security without compromising food supply (Islam et al. 2018). Furthermore, it can be grown in unproductive and waste land assisting in land reclamation (Francis

Table 2.3 Recent account of genome editing in microorganisms

Species	Technique used	Targeted gene	Impact	Reference
Chlamydomonas reinhardtii	CRISPR-Cas9	APT	Enhanced editing efficiency	Guzmán-Zapata et al. (2019)
Chlorella vulgaris UTEX395	CRISPR/Cas9	NR, APT	Enhanced editing efficiency	Kim et al. (2021)
Tetraselmis sp.	CRISPR-Cas9 RNP	AGP	Enhanced lipid production	Chang et al. (2020)
Chlamydomonas reinhardtii	CRISPRi	PEPC1	Enhanced biomass concentration and lipid accumulation rate	Kao and Ng (2017)

et al. 2005). Non-edible oil crops such as *Jatropha curcas*, *Pongamia pinnata*, *Calophyllum inophyllum*, *Madhuca indica*, *Ricinus communis*, *Hevea brasiliensis* and *Azadirachta indica* have proven to be promising alternatives as a biodiesel feedstocks (Azam et al. 2005). *Carica papaya* is a tropical fruit that weighs from 200 g to 3000 g. The seed content is 15–20% of wet weight of papaya fruit that is generally discarded (Daryono 2017). The oil content of these seeds is 30–34% with properties very similar to that of olive oil. Wong and Othman (2014), through enzymatic transesterification, extracted biodiesel from papaya seed using lipase at a molar ratio of 6:1 of methanol/oil. Daryono (2017) produced biodiesel from papaya seed using alkaline catalyst, and sodium hydroxide for the process of transesterification. The papaya seed oil can also be transesterified using KOH as a catalyst through single-stage method with 10:1 molar ratio of methanol/oil (Anwar et al. 2018). It has been observed that the physicochemical properties of biodiesel derived from papaya seed oil are very similar to that of diesel (Anwar et al. 2019). The typical yield of seed pods annually for *Ceiba pentandra*, a drought-resistant plant habitable in both subhumid and humid tropical regions, is estimated to be in the range of 300–1000 (Kachrimanidou et al. 2016). These pods contain cotton-like lustrous fibre embedded with about 120–175 seeds with the oil yield of 28% w/w. Under suitable conditions, the yield of seeds from *Ceiba pentandra* may be 30 kg annually. The pods are typically 10–25-cm-long ellipsoidal capsule with a diameter of 3–6 cm. According to Anwar et al. (2014), the iodine number for *Ceiba pentandra* seed lies at 80–100 which indicates nondrying on exposure to air and also has high free fatty acid content. The presence of cyclopropenoid fatty acids such as sterculic and malvalic acids causes physiological reaction in animals which makes *C. pentandra* a non-edible feedstock (Arumugam et al. 2020). *Citrus aurantium* is a fruit grown in Iran that has a lot of seeds that are regarded as waste. The oil content in seeds is about 38%. The maximum yield obtained from the novel feedstock via transesterification process at the temperature of 60 °C with catalyst concentration of 1 wt% was 97%, consistent with the ASTM standards (Almasi et al. 2021). Different non-edible oilseed and their oil content are mentioned in Table 2.4.

Table 2.4 Oil content in non-edible seeds

Species	Oil content (%)	Reference
Ricinus communis (castor)	49.2	Román-Figueroa et al. (2020)
Azadirachta indica (neem)	30–60	Karmakar et al. (2012)
Pongamia pinnata (karanja)	40	Calica (2017)
Moringa oleifera (drumstick)	30–40	Mohammed et al. (2003)
Hevea brasiliensis (rubber)	40–50	Ramadhas et al. (2005)
Jatropha curcas (jatropha)	40	Abdelrahman et al. (2020)
Sapindus mukorossi (soapnut)	51	Uzoh et al. (2014)

2.4.3 Biofuel Production Using Waste Products

Globally, every year millions of tonnes of waste are generated from household, industrial activities and agriculture that can create critical environmental and health issue if not disposed or managed properly. Through processes like gasification, pyrolysis, combustion and biological treatments, waste products/biomass may be converted to useful forms (Bhatt et al. 2018). Lignocellulosic waste as a feedstock has become popular for biofuel production (Kumari and Singh 2018). In recent years, focus on sustainability assessment of biofuel production has become vital as the emphasis on food versus fuel debate and the need for reduction in greenhouse gas emission has increased. Keeping these issues in mind, industrial waste residue, lignocellulosic waste and municipal solid waste are deemed as promising potential feedstocks (Cortez et al. 2018).

2.4.3.1 Municipal Solid Waste

Municipal solid waste (MSW) is commonly referred to as trash or garbage, discarded after use. MSW includes myriad of materials such as plastics, metals, medical wastes and hazardous materials. They generally have higher sulphur. This makes the selection of operating conditions and appropriate process paramount (Mukherjee et al. 2020). MSW can be categorized as recyclable consisting of non-lignocellulosic (glass, plastic, rubber, metals and others) and non-recyclable consisting of lignocellulosic (paper, wood waste, textile waste, yard waste, food/kitchen waste) components. In the lignocellulosic component, the main constituents are cellulose (15.30–65.80%), lignin (11.40–43.80%) and lastly hemicelluloses (7.20–16.50%) (Abdulyekeen et al. 2021). The average specific heat of combustion of MSW is 5–10 MJ/kg, while the elemental analysis depicts H_2, O_2, C, H_2O and ash to be 1.5–3.4, 8–23, 17–30, 24–34 and 18–43% (Fabry et al. 2013). There are 765 MSW-based waste to energy (WTS) plants globally. They are relatively scarce due to lack of support from the government and high capital cost (Wilson and Velis 2015). It is estimated that that per tonne MSW, the yield achieved can be 5.7 kg acetone, 12.2 kg butanol, 1.5 kg ethanol and 0.9 kg hydrogen (Meng et al. 2019). The fraction of MSW composed of kitchen waste, food waste and remnants from

Table 2.5 Bioenergy potential in various MSW

Component	Fixed carbon (%)	Moisture content (%)	Ash content (%)	Calorific value (MJ kg^{-1})	References
Kitchen food waste	7.19–16.60	9.60–79.00	0.80–20.93	15.34–18.10	Liu et al. (2014), Samad et al. (2018), and Huang et al. (2019)
Wood waste	17.29–20.16	5.21–66.00	5.29–7.31	17.73–19.46	Zhou et al. (2014), Samad et al. (2017), and Rago et al. (2020)
Paper	9.60–12.11	4.43–13.15	0.23–12.20	14.00–18.24	Zhou et al. (2014) and Rago et al. (2020)
Plastic	0.56	0.02–1.1	0.04–0.50	21.90–46.69	Zhao et al. (2016) and Rago et al. (2018)
Textile	0.71–13.75	5.25–13.75	0.41–3.56	16.51–20.16	Zhao et al. (2016) and Rago et al. (2020)

restaurants, residents, cafeterias, factory lunch rooms and gardens are called the organic fractions of municipal solid waste (OFMSW) (Campuzano and González-Martínez 2016). Since the availability of OFMSW is high and free of cost, its use in energy production could be an economical and technically viable alternative (Romero-Cedillo et al. 2017; Tyagi et al. 2018). It was observed that in source-segregated OFMSW, the biogas yield per tonne was slightly higher (111.1 m^3/tonne) in comparison with mechanically sorted OFMSW (105.3 m^3/tonne) (Seruga et al. 2020). Various component of municipal solid wastes and their bioenergy potential are mentioned in Table 2.5.

2.4.3.2 Waste Oils

Cooking and waste lubricating oil, degraded or contaminated after use, is generally referred to as waste oils. Waste oils derived from transmission oil, engine oil, cutting oil and hydraulic constitutes waste lubricating oil. Waste cooking oil is derived from coconut, soya bean, palm tree, sunflower, rapeseed, olive and cotton seed. Due to the presence of undesired substances and degraded additives, they are known to be hazardous substances which could bring about negative impacts to human health (e.g. reproductive, mutagenic and carcinogenic effects) and environment (e.g. fragile ecosystem, soil and water pollution and climate changes) (Lam et al. 2015). The open frying process alters the structure of cooking oil by free radical mechanism resulted by oxidation reaction. Through this primary oxidation process, hyperperoxide is produced which may oxidize further into 4-hydroxy-2-alkenals, a very reactive and toxic compound (Choe and Min 2007). Approximately, 50% of lubricating oil is produced as waste after operations resulted due to inefficiency of machinery. This has led to the generation of 20 million tonnes of waste oil.

There are new developments in waste oil-derived biofuel. Mićić et al. (2019) suggested a novel drying method which used silica as an absorbent instead of using carrier gas or distillation for water removal. It was noted that at 220 °C highest conversion can be obtained and FFA was reduced from the initial 8.6–1.6% at optimal conditions. Lam et al. (2019) mixed empty fruit batch from palm oil industry with waste oil for the production of high-quality solid fuel product with a higher heating value of 28 MJ/kg. Altalhi et al. (2021) performed catalytic pyrolysis of WCO through synthesis of heterogeneous acidic catalyst derived by sulphonation of modified alumina. Through the engine test investigation, the blend of biofuel-diesel indicated the suitability of B30 blend. Jahromi et al. (2021) studied the reaction between WCO and cyclic oxygenated hydrocarbons for novel biolubricant production through the process of hydrolysis, ketonization and Friedel-Crafts alkylation followed by hydrotreatment.

2.4.3.3 Sewage Wastes

The quantity of sewage sludge has increased with rapid growth in population globally. High content of organic matter, nutrient, salt, microelements, pathogens and heavy metals poses serious threat to health and well-being of human and ecosystem making its appropriate disposal mandatory (Kijo-Kleczkowska et al. 2016). Sewage sludge accounts for 1–2% of wastewater treated generated by wastewater treatment plants (Wzorek 2021). The relationship between generation of sewage sludge and the efficiency of treatment systems is proportional; the greater the sophistication of treatment plant, the higher waste generation occurs (Wzorek 2021). It has been estimated that the total electrical energy output utilized by these facilities is about 1–3% of a country (Capodaglio and Olsson 2020). In comparison with industrial sewage sludge, municipal sewage sludge contains higher amount of organic matter; this makes the municipal sewage sludge more suitable in regard to energy generation (Djandja et al. 2020). Wood processing and industrial pulping results in cellulose and lignin content in sewage sludge. Cellulose content ranges from 8.0 to 15.0, 8.0 to 15.0 and 7.0 to 9.7 wt% in untreated, digested and secondary sludge, respectively (Kacprzak et al. 2017). For higher heating value, lignin and volatile content in sewage sludge generally accounts for 11–26 MJ/kg, 23–29% and 30–88 wt%, respectively (Kacprzak et al. 2017).

Thermal processes including gasification, combustion and pyrolysis are applied for reducing both volume and mass of sewage sludge (Oladejo et al. 2019). Gasification and pyrolysis along with mass reduction can also generate gaseous and liquid fuel (Capodaglio et al. 2016). The pyrolysis product of sewage sludge includes CO_2, CO, H_2, CH_4, condensable compounds, hydrocarbons, bio-oil and biochar (Gao et al. 2016). It was observed that fast pyrolysis of sewage sludge at the temperature of 450–550 °C in fluidized bed reactors provides the oil yield of 30–70 wt% (Arazo et al. 2017), while for conventional pyrolysis, the yield of bio-oil extracted was around 51–80 wt% (Alvarez et al. 2015). Through fast pyrolysis, it was observed that depending on the material weight input, the yield of oil, gas and char was

between 60 and 70 wt%, 10 and 20% and 15 and 25%, respectively (Djandja et al. 2020). For the production of solid biofuel, hydrothermal carbonization on sewage sludge was performed at different temperature and residence time. It was observed that hydrochar with highest HHV was produced at 150 °C for 30 min, while the maximum yield of hydrochar was found at 150 °C for 60 min (Silva et al. 2020). Wang et al. (2020) studied hydrothermal carbonization of sewage sludge mixed with phenolic wastewater and found that the hydrochar yield and higher HHV increased substantially by 1.83–31.11% and 1.01–10.01%, respectively, while ash content decreased by 1.39–25.68% (Wang et al. 2020). Ghodke et al. (2021) carried out pyrolysis of sewage sludge and obtained maximum yield of bio-oil, gas and biochar (22.4%, 18.9% and 58.7%, respectively) at 500 °C.

2.5 Challenges of Using Novel Feedstocks

There is an immense need for novel feedstocks for overcoming the demand for viable, feasible as well as sustainable biofuels. The biggest challenge of using novel feedstock is lack of available literature regarding the same. In regard to non-edible forest products, the main challenges are collection, harvest, seasonal availability and improper marketing channels (Shaah et al. 2021). Aquatic weeds have relatively lower lipid content in comparison with other biodiesel feedstock which results in lower biodiesel yield. High water content (≈90%) in tissue of aquatic weed may affect biofuel conversion process. The high content of sulphur in water hyacinth may result in production of corrosive substance that can reduce fuel efficiency (Nawaj Alam et al. 2021). Moreover, irregular supply, complex structural makeup and high pretreatment cost of aquatic weed pose a challenge (Alam et al. 2021). The high cost of production of biofuels from microalgae at industrial scale and concerns regarding the impacts of genetically engineered microalgae on environment are major challenges (Guldhe et al. 2017; Varela Villarreal et al. 2020). While the biowaste biorefinery has gained attention for its utilization of biowaste and converting it into high-value bioproducts, the basic problem is high pretreatment cost. With conventional approach, significant amount of chemicals is used generating large volume of hazardous sludge that requires safe disposal. There is a need for further research to look for alternatives and technology to overcome these issues.

2.6 Future Prospects and Conclusion

Biofuels as a renewable energy source have notable advantages. In comparison with fossil fuel, they significantly reduce carbon emission, particulate matter and micropollutants. They can be available on demand, are transportable and are easily storable energy source. For biofuel and bioenergy production, copious volume of feedstock is required. This has resulted in the development of novel feedstocks and

novel techniques for existing feedstocks. To overcome the debate of food versus fuel, the potential of unconventional feedstocks such as non-edible oilseeds and forest products, aquatic weed, macro-/microalgae as well as waste products (waste oil, municipal solid waste and sewage waste) is being investigated. Though these novel feedstocks prove to be a promising source, there lays certain challenges in their implementation like irregular supply, high harvesting and pretreatment cost and improper marketing channel. Moreover, the genetic manipulation of feedstock causes a debate of its safety towards the environment. The future of these biofuels is based on developing cost-effective approaches for the most operationally efficient technologies and development of policies encouraging sustainable energy production through the recognition of various environmental benefits. Moreover, the study into circular economy as well as life cycle assessment is imperative to analyse the pros and cons of these novel feedstocks for biofuels.

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Chapter 3
Non-edible Oil Plants for Biodiesel Production

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Abstract About 80% of the energy consumed in the world comes from the use of oil and its derivatives, while biofuels represent less than 1% in the global scenario. Biodiesel production has a history of overcoming difficulties, as technologies, despite high conversions, are still incipient and can be improved. At industrial level, homogeneous catalysts are used, which cannot be reused, and methanol used as a reagent in the transesterification process is from fossil origin. In addition, the use of raw materials that compete with the food chain is a matter of systematic debate due to the demand for the use of agricultural land to plant production for energy and/or foods. Based on this, among some of the main challenges in the biodiesel production still prevail: the search for more industrial applications of glycerol and the improvement of the technological process to reduce production costs and increase the efficiency and diversification of alternative sources of inedible oils. In this context, this chapter aims to discuss the potential of different sources of inedible raw materials for the production of biodiesel through information reported in the literature about its oil content, physicochemical properties, and quality of biodiesel produced by different routes as well as the challenges that must be faced to make this process more competitive and attractive from a technological and environmental point of view.

Keywords Non-edible feedstocks · Physico-chemical properties · Biodiesel production · Emissions from unconventional biodiesel

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3.1 Introduction

Global warming caused by the increase in greenhouse gases from the constant use of fossil fuels and industrial activity is a concern constantly discussed by the international scientific community and government around the world. In 2016, global greenhouse gas emissions were recorded at 52 Gt CO_2-equivalent with a forecast to reach 58 Gt CO_2-equivalent per year by 2030. These annual emissions need to be reduced by up to 30 Gt CO_2-equivalent to limit the average global warming temperature to 1.5 °C (Rogelj et al. 2018). However, still, about 80% of the energy consumed in the world comes from the use of oil and its derivatives (REN21 2020), and despite efforts, the use of renewable energy (biomass, solar, and geothermal, among others) is not enough at 20% and biofuels remain between 0.8 and 1% in the world scenario (Fig. 3.1).

Several countries have investigated, have developed, or are considering the introduction of biofuels in their national energy programs (Perez et al. 2014), thus producing biofuels such as bioethanol (de Andrade et al. 2021; Dussán et al. 2019), biodiesel (Pinheiro et al. 2019; Silveira Junior et al. 2019a, b, 2020), HVO (Dimitriadis et al. 2020; Li et al. 2019), and biogas (Liaw et al. 2020) in order to minimize the environmental impact caused by the use of fossil fuels.

Unlike biotechnological ethanol production, whose technology is well established, the biodiesel process is still in its infancy, despite the innovations over the years. In general, the methods used in this process are well described in the literature, and chemical transesterification with methanol and basic homogeneous

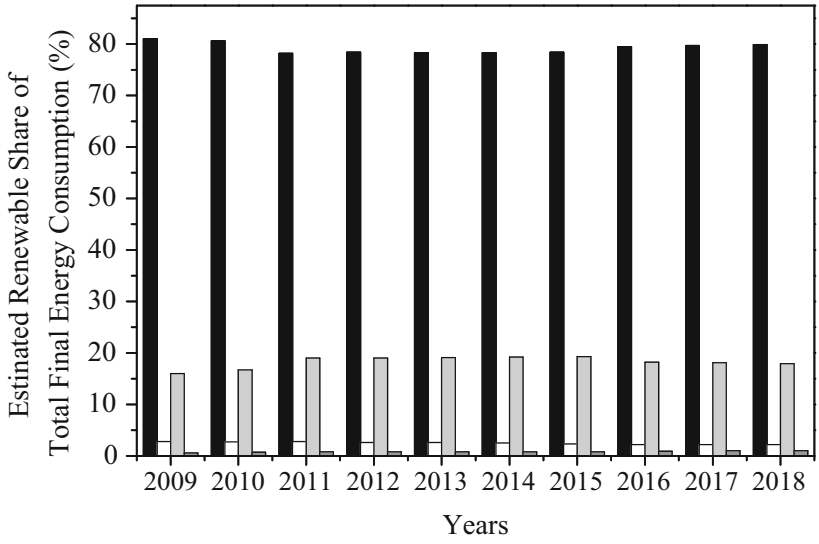

Fig. 3.1 Estimated share of renewable sources in total final energy consumption. Symbols: ■ fossil fuels; □ nuclear power; ■ all renewables; ■ biofuels. (REN21 2020)

catalysts such as NaOH and/or KOH in the form of alkoxides has been the technology adopted as a conventional route for its production on an industrial scale. However, depending on the presence of free fatty acids for acid value above 3%, esterification may be required, using mainly H_2SO_4 and HCl as catalysts. Many discussions about the technological disadvantages of this process, which generates effluents that require treatment, as well as the limitations of catalyst's reuse, which increase production costs, have been documented in literature (Perez et al. 2014). Furthermore, when the raw materials used constitute food sources for human and/or animal consumption, the controversial competition between energy vs. food, sustainability and limited land use and deforestation reappears (Elbehri et al. 2013).

In this context, the main challenges in biodiesel production are focused on the following aspects: (a) diversification and use of non-edible oils as raw material, despite the conventionally adopted sources, (b) continue exploring new applications for glycerol, and (c) technological improvement aimed at reducing production costs, including the development of more environmentally friendly processes, including the use of low-cost heterogeneous catalysts, among others (Silveira Junior et al. 2019b).

The chapter aims to address aspects related to the production of biodiesel in the world, reinforcing the potential of using oils from non-edible raw materials, established technological routes and alternative processes, as well as unconventional methods of characterization of both raw materials and their respective biodiesels, and finally, the challenges that must be addressed to make these processes more competitive and attractive in terms of prevailing industrial and environmental concerns.

3.2 Global Scenario of the Biodiesel Production

The global production of biodiesel increased by 13% in 2019, reaching more than 47 billion liters (Fig. 3.2). In this scenario, Indonesia, the USA, Brazil, Germany, France, and Argentina together account for 57% of global biodiesel production (REN21 2020). In 2019, the mandatory biodiesel program implemented by the Indonesian government managed to increase the market demand of crude palm oil products, of which biodiesel was the main product (APROBI 2021). Then, in the year 2020, Indonesia become the largest global producer of biodiesel, since its production reached 7.9 billion liters using palm oil as its main raw material.

In Brazil, there was an 11% increase in biodiesel production achieving record 5.9 billion liters in 2020. The determining factors for that were the increase in the biodiesel/diesel binary mixture from 10 to 11% and the need to meet the higher demand expected of biodiesel with the introduction of the RenovaBio program implemented by the ANP (ANP 2020).

The USA has been the most prominent producer during last several years; however, its biodiesel production decreased by 7% that could be attributed to the factories closing as consequence of the withdrawal of national biodiesel blend credit.

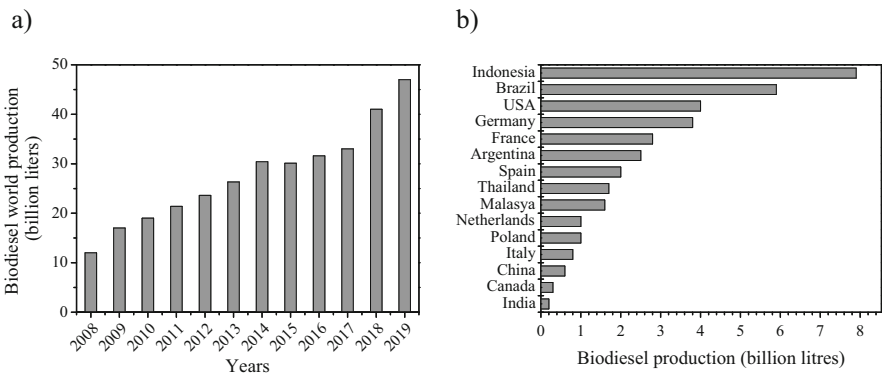

Fig. 3.2 Global scenario of biodiesel production: (**a**) biodiesel world production in the last decade and (**b**) global ranking of biodiesel production in 2019 (REN21 2020)

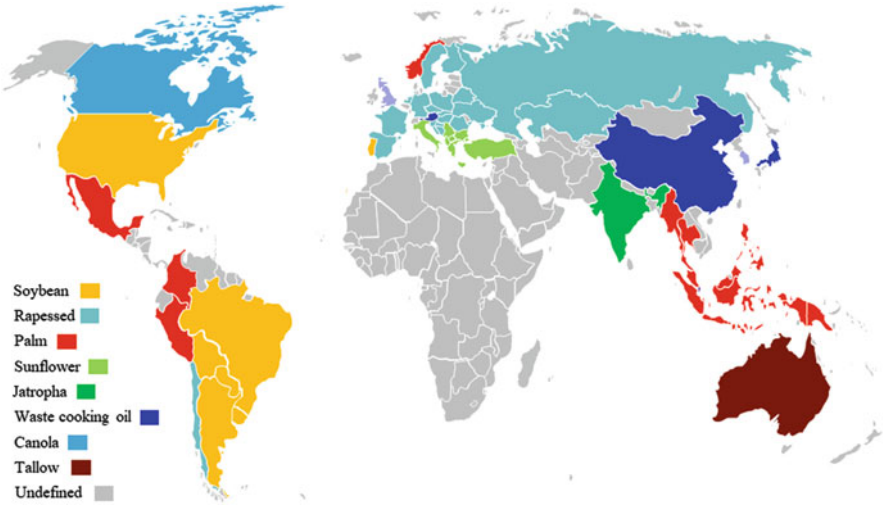

Fig. 3.3 Global geographic distribution of the main edible and non-edible oil plants by countries

In addition, the weakening of the American market and ongoing US taxes on biodiesel imports have also affected the production of biodiesel in Argentina, reducing Argentine exports of biodiesel from 1.6 billion liters in 2018 to 1.2 billion liters in 2019.

Figure 3.3 shows the global geographic distribution of the main edible and inedible oil plants by countries used for the production of biodiesel. Soybean is widely cultivated in the world; in 2019 the global production reached 122 million hectares, the largest producers being Brazil, the USA, Argentina, India, and China, resulting in 106 million hectares in the world for this oilseed cultivation, representing more than 85% of all sown area in the world (LATIFUNDIST 2020).

Thus, soybean is the predominant raw material on the American continent. Brazil is the largest producer of soybean biodiesel, using in its energy matrix around 71% soy oil for biodiesel production, followed by beef tallow which has a participation of 13% in current scenario (USDA 2020a). The USA in 2020 consumed 744 million kilograms of soy oil to produce biodiesel (EIA 2020).

Rapeseed oil is the predominant feedstock in European Union (EU) countries, accounting for 43% of total biodiesel production in 2019. However, its use has been decreasing as the demand and use of residual cooking oil increased in last years (USDA 2020b). Its biggest producers are Germany, France, and Spain, which together add up to a production of 8.6 billion liters of biodiesel. In Germany, rapeseed is the main raw material used for the production of biodiesel according to the data reported by Verband der Deutschen Biokraftstoffindustrie (VDB) in 2017; its participation in this scenario was 58%, with a cultivation area of 713,000.00 hectares. Other sources such as waste frying oil (27%), palm oil (2%), and soybean oil (5%) were also used (VDB 2018). France is currently the fifth largest producer of biodiesel in the world; however, consumption of hydrogenation-derived renewable diesel (HDRD) is expected to increase at the expense of conventional biodiesel. Spain also stands out on the world stage, however, depends heavily on imported raw materials since their oilseed production is essentially limited to olive and sunflower oils, destined almost exclusively for food purpose. Then, for the production of biodiesel, the internal supply is limited to animal fats and waste frying oil amounting to around 14% (USDA 2020c). In 2020, with the slowdown in the activity of hotels, restaurants, and institutions, these raw materials have become more difficult to obtain, and this has increased imports of Argentine soybean biodiesel, reaching 28% in 2019. However, the largest import is in palm oil for the production of biodiesel, coming from Indonesia, representing 48% in the year 2018/2019.

Other highlights can also be commented, as the Austria case where the Münzer Bioindustrie GmbH is the largest biodiesel production plant, with an annual production of 206,000.00 tons of biodiesel produced, using residual cooking oil as a raw material (Münzer Bioindustrie GmbH 2021). In Norway, 126 million liters of biodiesel from palm were produced in 2019. However, there is a negative environmental impact in this country due to deforestation that has been taking place because of the increase in the cultivation of palm. Rapeseed is believed to be a potential option for use in biodiesel production in the near future (Rainforest Foundation Norway 2020).

The UK has adopted used cooking oil as its main raw material; the largest biodiesel production center in Liverpool, the Olleco biorefinery, has been using this feedstock (Olleco 2021). Sewage grease (fatbergs) also enters in the productive matrix, however to a lesser extent. According to the Renewable Energy Association (REA 2019), significant investments have been made in the UK to encourage the production of biodiesel from all types of waste, from used cooking oils to sewage grease (fatbergs) and other waste that often ends up in "waste landfills." With this incentive, 900,000 tons of biodiesel from waste oils are produced, which is equivalent to more than 99% of all biodiesel used in the UK.

The use of *Jatropha* was encouraged in India through a program called the National Biodiesel Mission that aimed to meet 20% of the country's diesel needs (Goswami and Hazarika 2016). However, the implementation of this program has seen challenges due to various issues such as absence of institutional support, technology, and financial support (Kumar Biswas and Pohit 2013; Syafiuddin et al. 2020). The tendency is for the country to adopt the use of residual frying oil for biodiesel production in the coming years. On the other hand, China is becoming a major producer of biodiesel with residual frying oil. In the year 2018, China produced 834 million liters of biodiesel (USDA 2019).

As seen in Fig. 3.3, palm has a large participation in the global scenario of biodiesel production; Indonesia is the largest producer of palm oil, followed by Malaysia (Abdul Kapor et al. 2017). It is noteworthy that from the processing of palm oil, it is possible to obtain palm fatty acid distillate (PFAD), a residue that becomes a potential raw material to produce biodiesel because it is cheap and abundant, e.g., from 1 ton of palm oil, 3.25% of PFAD are produced. According to the Malaysian Palm Oil Board (MPOB 2018), in 2018, around 782,048 tons of PFDA were produced.

3.3 Agricultural Aspects About Non-edible Plants

Over the years, it is predicted that some non-edible oil plants will gain space in the biodiesel production chain. The expansion of their use as feedstocks will become more significant as technological advances in agriculture are achieved, especially for those oilseeds with higher energy density than soybean (Table 3.1), i.e., the higher oil content of the seeds.

Geographical conditions also contribute to the advancement of these oilseeds in this scenario, as regions where there is a vast territory for cultivation, in addition to water resources, regular rainfall, high biodiversity, and well-developed agricultural

Table 3.1 Several non-edible plants with potential para-biodiesel production

Non-edible feedstocks	Yield of seeds (kg/ha)	Oil content (%)	Oil yield (kg/ha)	Reference(s)
Babassu	1480	60–68	120	Freitas et al. (2009)
Cotton seed	1727	15	259	CONAB (2020)
Crambe	2650	38	1000	Carlsson (2009)
Castor	621	45–55	341	CONAB (2019)
Forage turnip	1150	42	486	Silveira Junior et al. (2019a)
Jatropha	6000	34	2000	PESAGRO (2008)
Macaw	24,000	20–25	6000	EMBRAPA (2015)
Rapeseed	345	40	138	Azcan and Danisman (2008) and Dušek et al. (2021)
Rubber seed	150	40–50	75	Ramadhas et al. (2005)

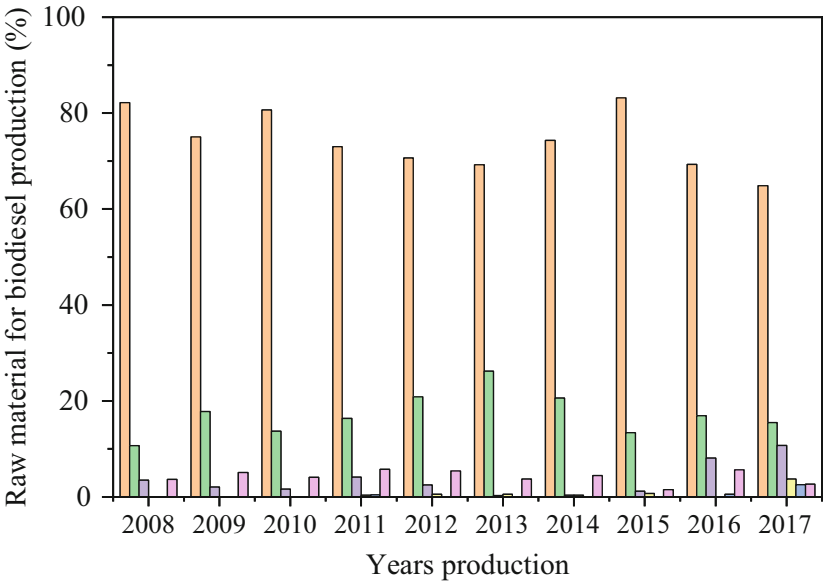

Fig. 3.4 Biodiesel production in Brazil from various feedstock: soybean oil; beef tallow; other fatty materials; pork fat; palm oil; other fonts (ANP 2021)

technologies, certainly provide potential for the production of biodiesel. Furthermore, with advances in research in genetic improvement, it was possible to increase the oil productivity of crops such as soybean and cotton by 64 and 14%, respectively. Both their oilseeds are technologically well developed, and have a large number of cultivars registered (Dias 2011). Soybean, for example, has more than 820 registered cultivars (EMBRAPA 2011). The explanation for this fact is due to investments in the production chain over decades, which have resulted in the development of new varieties of species, genetic improvement and pest control, and, consequently, a good productivity in relation to other oilseeds, resulting in a relatively lower soybean production cost (Perez et al. 2014). Brazil has a very favorable scenario for introducing new raw materials into the biodiesel production chain due to its territorial extension, agricultural development, and climate conditions. However, soybean oil has been the main raw material since the beginning of the biodiesel program, representing around 70 to 80% of all raw materials between 2008 and 2017, followed by tallow and some other raw materials in smaller quantities (see Fig. 3.4).

On the other hand, as discussed earlier, rapeseed is widely used in Europe to produce biodiesel; however, for human consumption, it has an unpleasant taste and a greenish color due to the presence of chlorophyll, in addition to the high concentration of erucic acid. Thus, in the 1970s, there was the first attempt to minimize the negative aspects of this oilseed, and with that, rapeseed emerged through the crossing of two rapeseed cultivars, *Brassica napus* and *Brassica rapa* (Pederson and Storgaard 2015), a cultivar with low erucic acid content. Subsequently, canola

was genetically modified aiming at tolerance to the use of herbicide, and then the Roundup Ready canola (www.roundupreadycanola.com.au) got developed, a cultivar tolerant to the herbicides through the use of genetic engineering, providing excellent weed control to canola growers and enabling greater yield potential. With advances since then, canola grown today is disease resistant and drought resistant as well as herbicide tolerant. Of the 31 million hectares of canola grown worldwide, 26% are considered genetically modified crops (Beckie et al. 2011; Johnson et al. 2008). Jatropha stands out for its high oil content and for being a perennial plant that can produce for up to 40 years (Dias 2011).

Among the non-edible oilseeds shown in Table 3.1, jatropha and macaw are the most attractive in relation to their oil yield per hectare. According to the Agricultural Research Institute of Rio de Janeiro (PESAGRO 2008), the production of *Jatropha curcas* starts in the first year of planting, and with a good agricultural management, it can produce 400 kg/ha in the first year of planting, reaching 1000 kg/ha in the second year, 3000.00 kg/ha in the third year, and reaching up to 6000.00 kg/ha from the fourth year onward, the period in which oil production reaches 2000 kg/ha. Some experimental works have evaluated different planting conditions, such as increasing spacing between planting lines and between plants in the row, which allows an increase from 1000 plants to 5000 plants/ha, and consequently, increasing fruit yield and oil. The main reasons for the initial interest in *Jatropha* sp. were based on the fact that it is an non-edible oil, has a good adaptability and is drought tolerant, is available in many tropical and subtropical regions, has fast growth capacity (commercial production after 5 years of planting), has the capacity to produce seeds for many years, and needs less nutrients when compared to other oilseeds in addition to its high oil production (Baral et al. 2020). However, there is still fear in cultivating *Jatropha curcas* on a large scale because research studies with this oilseed are still limited; moreover, its cultivation system is not yet consolidated, and the lack of studies aimed at genetic improvement of this plant are insufficient to recommend its commercial cultivation (Drumond et al. 2016). Finally, the harvest of *Jatropha curcas* is another important bottleneck, as there is still no mechanical harvester for this operation (PESAGRO 2008).

Another oilseed that deserves to be highlighted is macaw, the fruit with high oil content, reaching up to 6000 kg/ha (see Table 3.1). However, the insertion of this oilseed in the biodiesel production chain still depends on technological development in various stages of the production system (EMBRAPA 2015). Researchers from the São Paulo Agribusiness Technology Agency of Brazil (http://www.apta.sp.gov.br/) and the Fraunhofer Institute of Germany (https://www.fraunhofer.de/en.html) have dedicated efforts to assess the genetic variability existing in macaw species and thus select matrices for greater production of oil from the pulp and seed of the plant in the different environments where it can be found naturally and thus develop its production chain, as well as new products, in addition to adding value.

3.4 Physicochemical Properties of Non-edible Oil Feedstock

Other non-edible oil plants are gaining space as raw material sources to produce biodiesel. It is expected that over the years, there will be an increase in the use of these raw materials, as technological advances in agriculture are achieved, especially for those oilseeds with higher energy density than soybean (Table 3.2), i.e., the highest oil content of oil plants. An important aspect to be noted is the high acidity index of some oils (Carvalho et al. 2013; Roschat et al. 2017; Silveira Junior et al. 2019a) (Table 3.3). In this case, these oilseeds should be considered low-quality raw materials, as there is a direct relationship between lipid quality, measured as the inverse of the free fatty acid (FFA) content, and cost (Knothe et al. 2005).

3.5 Biodiesel Production from Non-edible Oils: Case Studies

Several studies have demonstrated the potential of some inedible oilseeds for the production of biodiesel, and in Table 3.4 some successful examples are presented in which the yields of biodiesel production reached above 90% and, in some cases, it reaches complete conversion. Obviously, the choice of suitable raw materials must fulfill the biodiesel quality standards (ASTM, EN); therefore, the physicochemical properties of both the oil and the biodiesel formed must be observed. Thus, depending on the chemical composition of the raw material, some properties of the biodiesel produced may be undesirable (Perez et al. 2014), e.g., the production of biodiesel from oils with a high iodine content can result in a product susceptible to oxidation. Likewise, raw materials with a high content of saturated fatty acids can result in biodiesel that tends to have solidification problems with temperature variations (Knothe et al. 2005).

As can be seen in Table 3.4, the work found in the literature have explored not only raw materials from different sources but also different reaction conditions and types of catalyst such as homogeneous (Moreira et al. 2013; Pinheiro et al. 2019; Shrivastava et al. 2020; Zullaikah et al. 2005; Pierezana et al. 2015), heterogeneous (da Costa and Lima 2021; Foroutan et al. 2021; Roschat et al. 2017; Silveira Junior et al. 2019a), and enzymatic catalysts (Carvalho et al. 2013; Da Rós et al. 2014), thus making it difficult to compare the results. Except for enzymes that operate under moderate conditions, it is observed that in almost all cases, there were high conversion (around 82–100%), but for higher temperature (da Costa and Lima 2021; Zullaikah et al. 2005) and long reaction time (Carvalho et al. 2013; da Costa and Lima 2021; Da Rós et al. 2014; Roschat et al. 2017; Shrivastava et al. 2020; Silveira Junior et al. 2019a; Zullaikah et al. 2005) which is still undesirable from an industrial point of view. Another relevant point is the oil's high acidity value (Carvalho et al. 2013; Roschat et al. 2017; Silveira Junior et al. 2019a), because when the acidity is high (above 3%), an esterification reaction with acid and then a transesterification

Table 3.2 Fatty acid composition of several non-edible oils used as feedstocks for biodiesel production

Non-edible oils from several sources	Fatty acid composition														Refs.
	C8	C10	C12	C14	C16	C16:1	C18	C18:1	C18:2	C18:3	C20	C20:1	C22	C22:1	
Andiroba oil	–	–	0.1	0.1	29.0	–	10.0	47.0	10.7	–	–	–	–	–	Carvalho et al. (2013)
Babassu oil	4.5	3.5	44.7	17.5	9.7	–	3.1	15.2	1.8	–	–	1.05	–	–	Da Rós et al. (2014)
Butia capitata oil	14.6	15.9	42.2	6.9	3.4	–	2.5	10.6	2.7	–	–	–	–	–	Pierezana et al. (2015)
Castor oil[a]	–	–	–	–	0.7	–	0.9	2.8	4.4	0.2	0.3	–	–	–	Conceição et al. (2007)
Cotton oil	–	–	–	–	22.92	–	2.22	15.39	57.64	–	–	–	–	–	Zhao et al. (2018)
Crambe oil					2.0			16.8	8.4	4.8	0.5	3.4	2.3	56.1	Costa et al. (2018)
Damascus seed oil	–	–	–	–	5.85	–	2.51	63.84	25.34	0.51	–	–	–	–	Anwar et al. (2019)
Forage turnip oil	–	–	–	–	–	–	–	35.04	22	17.97	–	5.28	–	12.81	Silveira Junior et al. (2019a)
Jatropha oil	–	–	–	0.1	12.9	–	5.6	39.8	40.0	–	–	–	–	–	Carvalho et al. (2013)
Karanja oil	–	–	–	–	11.65	–	7.50	51.59	16.46	2.65	–	–	–	–	Shrivastava et al. (2020)
Macaw oil	5.4	4.0	36.1	10.2	8.7	–	3.6	27.7	3.4	–	–	–	–	–	Carvalho et al. (2013)
Moringa oleifera oil	–	–	–	0.6	8.2	2.4	4.7	66.5	–	1.4		5.8	4.7	1.6	Foroutan et al. (2021)
Rapeseed oil	–	–	–	–	4.6	–	3.2	60.7	20.5	9.3	0.6	–	–	–	Raman et al. (2019)
Rubber seed oil	–	–	–	–	9.1	–	5.6	24.0	46.2	14.2	–	–	–	–	Roschat et al. (2017)
Scheelea phalerata oil	3.6	3.9	30.7	11.9	10.5	–	3.4	30.5	5.2	–	–	–	–	–	Pierezana et al. (2015)

Syagrus romanzoffiana oil	6.0	6.0	37.0	11.0	8.0	–	3.0	24.0	5.0	–	–	–	–	–	Moreira et al. (2013)
Terminalia catappa oil	–	–	–	–	–	33.5	4.5	37.5	22.6	–	–	–	–	–	Pierezana et al. (2015)

[a]Castor oil contains C19:1 1, 2-hydroxy-cis-9-octadecanoic fatty acid (90.2%) and C19 dihidroxyestearic fatty acid (0.5%)

Table 3.3 Physicochemical properties of non-edible oils for biodiesel production

Non-edible oils	Properties								Refs.
	Acidity value (mg KOH/g oil)	Density (kg/m^3)	Kinematic viscosity 40 °C (mm^2/s)	Saponification (mg KOH/g oil)	Water content (% w/w oil)	Iodine value (g I_2/ 110 g oil)	Peroxide value (meq/kg of oil)	Oil content (%)	
Andiroba oil	0.1	–	38.7	200	–	69	1.7	45	Cabral et al. (2013) and Carvalho et al. (2013)
Babassu oil	–	–	29.5	–	–	–	–	42	Da Rós et al. (2014) and Moreira et al. (2018)
Castor oil	6.08	–	35.94	174.36	–	38.61	0.17	45	Umeuzuegbu et al. (2021)
Cotton oil	0.24	–	29.22	187.94	–	68.91	80	2.71	Onukwuli et al. (2017) and Li et al. (2009)
Damascus seed oil	1.65	910	34.54	173	–	103	–	53.97	Anwar et al. (2019) and Mandal et al. (2007)
Forage turnip oil	8.79	926 (20 °C)	36.0	–	–	134	–	42	Silveira Junior et al. (2019a)
Jatropha oil	0.3	–	34.5	141	–	101	4.2	40–50	Atabani et al. (2012) and Carvalho et al. (2013)
Macaw oil	16.1	–	29.8	223	–	28	3.9	55.7	Carvalho et al. (2013) and Oliveira et al. (2021)

Moringa oleífera oil	2.7	916.2	–	192.5	–	–	–	35–45	Foroutan et al. (2021) and Ayerza (2012)
Rapeseed oil	2.92	916	34.9	184	–	100.4	–	40	Azcan and Danisman (2008)
Rubber seed oil	10.60	894 (15 °C)	7.54	–	0.013	130	–	24	Roschat et al. (2017)
Scheelea phalerata oil	1.2	–	–	–	–	–	–	34.2	Pierezana et al. (2015)
Syagrus romanzoffiana oil	1.80	–	30.12	–	699	–	–	52	Moreira et al. (2013)
Terminalia catappa oil	0.9	–	–	–	–	–	–	46.1	Pierezana et al. (2015)

Table 3.4 Case studies of biodiesel production using non-edible oils from several sources

Non-edible oils from several sources	Catalysts	Reaction conditions		Biodiesel yield (%)	Refs.
		Oil/alcohol molar ratio and catalyst weight (g or %)	Reaction parameters		
Andiroba (*Carapa guianensis*) oil	Immobilized *Burkholderia cepacia lipase on* SiO_2- PVA	Oil/ethanol (1: 9) Catalyst 20%	45 °C; 150 rpm; 1440 min	100	Carvalho et al. (2013)
Babassu oil	SiO_2- PVA	Oil/ethanol (1: 12) Catalyst 20%	50 °C; 150 rpm; 600 min	100	Da Rós et al. (2014)
Butia capitata oil	KOH	Oil/ethanol (1: 7) Catalyst 0.4 g	65 °C; 200 rpm; 60 min	85–88	Pierezana et al. (2015)
Cotton oil	KF/bentonite	Oil/ethanol (1: 13) Catalyst 10%	140 °C; 360 min	95	da Costa and Lima (2021)
Crambe oil	$CH_3CH_2O^-Na^+$	Oil/ethanol (1: 6) Catalyst 2%	65 °C; 200 rpm; 120 min	100	Pinheiro et al. (2019)
Forage turnip oil	K_2CO_3/sepiolite	Oil/ethanol (1: 12) Catalyst 2%	70 °C; 300 rpm; 240 min	99.9	Silveira Junior et al. (2019a)
Jatropha oil	Immobilized *Burkholderia cepacia lipase on* SiO2- PVA	Oil/ethanol (1: 9) Catalyst 20%	45 °C; 150 rpm; 1440 min	100	Carvalho et al. (2013)
Jatropha oil	Bi_2O_3- La_2O_3-	Oil/methanol (1:15) Catalyst 2%	150 °C; 240 min	93	Nizah et al. (2014)
Karanja oil	KOH	Oil/methanol (1:6) Catalyst 1%	64 °C; 1440 min	96.89	Shrivastava et al. (2020)
Macaw palm oil	Immobilized *Burkholderia cepacia lipase on* SiO_2- PVA	Oil/ethanol (1: 9) Catalyst 20%	45 °C; 150 rpm; 1440 min	100	Carvalho et al. (2013)
Moringa oleifera oil	MgO/K_2CO_3	Oil/methanol (1:2.9) Catalyst 4%	70 °C, 200 min	99	Foroutan et al. (2021)
Rapeseed oil	CH_3NaO	Oil/methanol (1:6) Catalyst 0.5%	60 °C; 90 min	92.3	Moser (2008)
Rice bran oil	H_2SO_4	Oil/methanol (1:5) Catalyst 0.54%	100 °C; 300 rpm; 480 min	99.8	Zullaikah et al. (2005)

(continued)

Table 3.4 (continued)

Non-edible oils from several sources	Catalysts	Reaction conditions		Biodiesel yield (%)	Refs.
		Oil/alcohol molar ratio and catalyst weight (g or %)	Reaction parameters		
Rubber seed oil	CaO	Oil/methanol (1:9) Catalyst 9%	65 °C; 200 rpm; 210 min	97.74	Roschat et al. (2017)
Scheelea phalerata oil	KOH	Oil/ethanol (1:7) Catalyst 0.4 g Oil/methanol (1:7) Catalyst 0.4 g	65 °C; 200 rpm; 60 min	73 90	Pierezana et al. (2015)
Syagrus romanzoffiana oil	NaOH	Oil/methanol (1:2) (v/v) Catalyst 0.54%	60 °C; 60 min	98.5	Moreira et al. (2013)
Terminalia catappa oil	KOH	Oil/ethanol (1:7) Catalyst 0.4 g Oil/methanol (1:7) Catalyst 0.4 g	65 °C; 200 rpm; 60 min	82 93	Pierezana et al. (2015)

step is performed using sodium ethoxide or potassium methoxide by homogeneous route (Fig. 3.5). However, when acidity is high, this method is costly, as it requires, in addition to the reaction steps, washing and purification steps for biodiesel and the resulting wastewater that needs to be neutralized. An attempt to eliminate these reaction steps could be using heterogeneous bifunctional catalysts which have shown promise in the synthesis of biodiesel from oils with a high acidity value (Nizah et al. 2014).

3.5.1 Physicochemical Proprieties and Biodiesel Quality

Some important aspects that must be observed regarding the properties and quality of biodiesel include stability problems and sediment formation during the storage stage. As can be seen in Table 3.5, biodiesel produced from inedible plants meets many of the quality monitoring parameters provided for ASTM and EN standards. However, there is still a need to explore important parameters of oxidative stability in order to minimize the above mentioned degradation problems. The use of ethanol in the production of biodiesel (Table 3.4) should also be seen as a positive approach due to the resulting environmental advantages when compared to the use of methanol (Perez et al. 2014; Sánchez et al. 2015; Baird and Cann 2012). Briefly, it should be noted that the use of methanol should be discouraged since it is basically

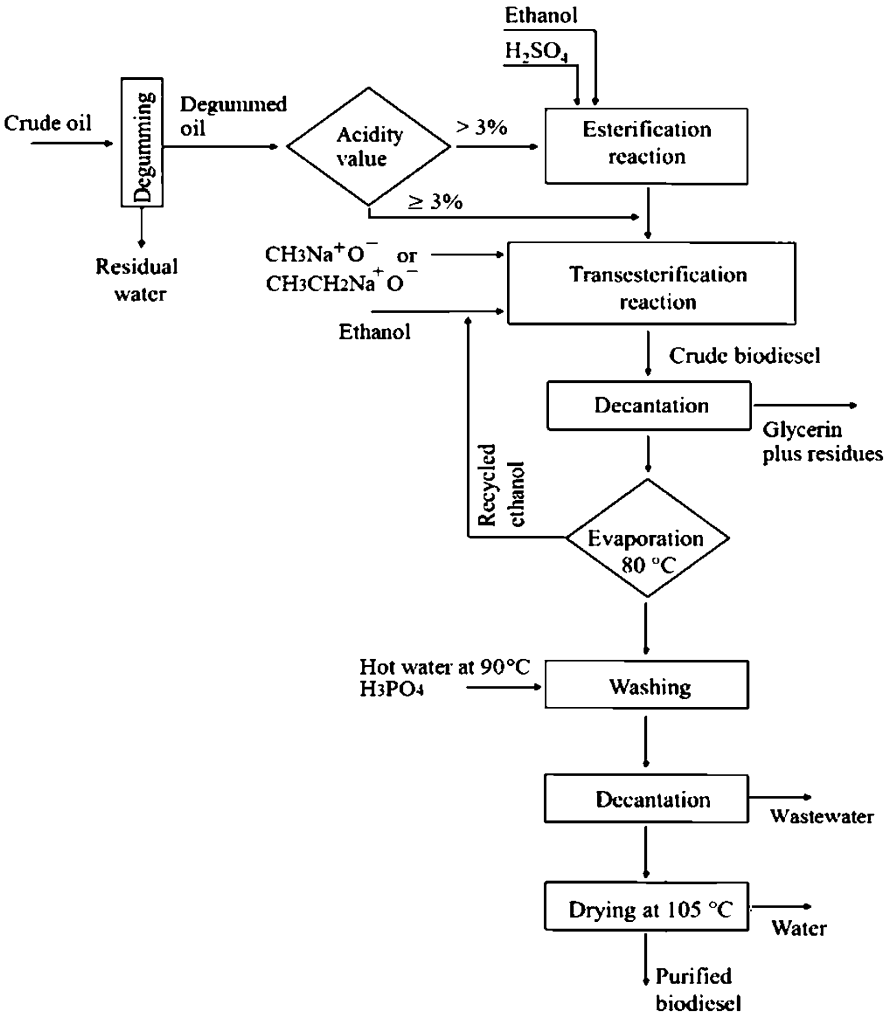

Fig. 3.5 Flowchart of biodiesel production from several feedstocks by chemical ethanolic route

produced from fossil sources, such as natural gas. In addition, biodiesel produced by the ethyl route has greater oxidation stability and lower cloud point and pour point, which improves engine starting at lower temperature, and the extra carbon atom provided by the ethanol molecule slightly increases the combustion heat and cetane number of the fuel (Pinheiro et al. 2019).

Challenges still remain as the question to be answered is whether these non-edible oil plants are available to provide biofuel in sufficient quantities to meet global demand. One strategy that has been adopted is the elaboration of blends between esters obtained from edible and non-edible oils, with the objective of reducing the proportion of use of edible oils (da Silva et al. 2020). The feasibility of preparing

Table 3.5 Physicochemical proprieties of produced biodiesel from non-edible feedstocks

Biodiesel from non-edible oilseeds	Cetane number	Acidity value (mg KOH/g oil)	Density (kg/m^3)	Kinematic viscosity (mm^2/s)	Water content (% w/w oil)	Iodine value (g I_2/ 110g of oil)	Ref.
Andiroba (*Carapa guianensis)*	–	–	888	6.0	–	–	Carvalho et al. (2013)
Babassu	63.7	–	870	4.2	–	–	Da Rós et al. (2014)
Butia capitata	–	0.187	–	3.86	–	5.77	Pierezana et al. (2015)
Castor bean	43.7	–	9461	15.40	0.15		Keera et al. (2018)
Crambe	–	0.48	874	5.5	–	87.8	Pinheiro et al. (2019)
Cotton	–	–	878	4.7	–	–	da Costa and Lima (2021)
Forage turnip	50.90	–	879.5	4.53	–	119	Silveira Junior et al. (2019a)
Jatropha	–	–	882	4.9	–	–	Carvalho et al. (2013)
Karanja	52	0.12	880–913	3.99–5.71	–	–	Shrivastava et al. (2020)
Macaw	–	–	880	4.6	–	–	Carvalho et al. (2013)
Moringa oleifera	57.2	0.12	876	4.4	–	–	Foroutan et al. (2021)
Rapeseed		0.22	882	4.02	–	–	Serqueira et al. (2014)
Rubber seed	–	0.35	880	4.84	0.023	–	Roschat et al. (2017)
Syagrus romanzoffiana		0.08		3.16	453		Moreira et al. (2013)

these improved biodiesel blends is already being reported in the literature and revealed that the optimization of fatty acid ester profiles by blending biodiesel from different sources can result in high-quality fuels (Adepoju et al. 2021; Albuquerque et al. 2009; Moser 2008; Ong et al. 2019; Sarin et al. 2009). For example, the blend of *Jatropha* biodiesel and palm biodiesel provided a biodiesel with substantial improvements in oxidative stability and thermal properties (Sarin et al. 2007). More recently, Adepoju et al. (2021) carried out a quaternary mixture of oils from *Carica papaya*, *Citrus sinensis*, *Hibiscus sabdariffa*, and waste oil in proportions of 25:25:25:25 and obtained a biodiesel with low viscosity and adequate volatility.

Binary blends of oil from three species from the Brazilian biome known as "Cerrado" such as *S. phalerata*, *T. catappa*, and *A. moluccanus* with soybean oil in proportions 10:90, 20:80, 30:70, 40:60, and 50:50 were investigated for biodiesel production (da Silva et al. 2020). Then, the formed biodiesel was subjected to thermal and oxidative analysis and attained results were compared with the biodiesel from each oilseed, and it was possible to conclude that biodiesel from blends was more suitable as biofuel, because their thermal and oxidative properties were improved. As can be seen in Table 3.4, the oil from *S. phalerata* has a predominance of saturated fatty acids, while the oil from *T. catappa* shows an equilibrium in the fatty acid profile, with 40.5% saturated and 57% unsaturated, whereas the oil of *A. moluccanus* presents a predominance of unsaturated fatty acids (90.0%). This varied chemical composition reflected in different degrees of thermal stability as follows: *A. moluccanus* > soybean > *T. catappa* > *S. phalerata*. A fast and efficient way to monitor thermal stability is through thermal analysis (TG-DTG), and in this case, the DTG analysis of these esters can be performed in an oxidative atmosphere of synthetic air, under a linear temperature ramp. Oxidative stability data provided by thermal analysis are also indicators of the quality of biodiesel, whose technique has been used to investigate oxidation stability and indicate low-temperature flow properties (Borugadda and Goud 2014; Nicolau et al. 2018).

3.5.2 Emissions of Biodiesel from Non-edible Oils

In general, biodiesel causes less adverse impacts on the environment in relation to fossil diesel due to the reduction of carbon dioxide (CO_2) and particulate emissions into the atmosphere. However, it is known that NOx levels increase as a function of the biodiesel content in diesel blends or even for pure biodiesel. In this context, the main question is whether biodiesel from non-edible sources pollutes more than other conventional sources, including edible ones, or of animal origin, such as beef tallow, pork fat or waste frying oil, or even algae oils or lipids from oleaginous microorganisms.

Previous studies suggest that biodiesel from raw materials with a higher composition of unsaturated fatty acids emit more NOx. Knothe et al. (2005) showed a

correlation of NOx emission with the increase in the "iodine number," which is a good indicator of unsaturation degree of the fatty acids.

In the current scenario, there are still no fully consistent studies on the reduction of GHG emissions from biodiesel produced, considering the diversity of sources of non-edible raw materials available. With biodiesel from non-edible feedstocks with higher iodine indices, it is likely that their percentage of emissions is similar to that of biodiesel synthesized from soybean.

In order to investigate the generation of greenhouse gases emitted by burning biodiesel produced by edible and non-edible oils and their mixture in diesel, several studies have been carried out to evaluate the emission levels of N_2O, NOx, NO, CO_2, and CO (Navaneeth et al. 2021; Pinheiro et al. 2019; Rocha et al. 2014). In this context, Pinheiro et al. (Pinheiro et al. 2019), in a comparative study, evaluated the effect of combustion of blends of diesel and ethanolic biodiesel produced from various raw materials, among which are soybean, crambe, macaw, sunflower, and residual cooking oil using an engine operated in low and high rotation where the emission levels of NOx, N_2O, NO, CO_2, and CO in the atmosphere were monitored. To assess the effect of combustion, biodiesel/diesel blends were prepared in the following proportions: 10% (B10), 15% (B15), 25% (B25), and 50% (B50). The findings showed the formation of NOx during the combustion of all prepared biodiesels, regardless of the engine's operating mode. However, as the biodiesel content in diesel increases, the NOx concentration also increases; this is expected because biodiesel emits more nitrogen oxides than diesel, and this issue is still a matter of great concern. Regarding N_2O, it is noteworthy that this is a gas that also raises concerns as it has great potential to retain around 300 times more heat in the atmosphere than CO_2 and deplete the ozone layer. Comparatively, the levels produced were similar for all blends, except for blends containing crambe and macaw biodiesel, whose emissions were reduced, and therefore, these raw materials were found to be the most environmentally sustainable for the production of biodiesel as they generated fewer emissions of greenhouse gases. These results are particularly attractive considering that in addition to being inedible sources, their resulting biodiesel can favorably impact the environment. On the other hand, this study pointed that blend containing biodiesel from waste cooking oil generated highest N_2O comparatively; thus, this source does not seem to be a good option for biodiesel production and thus, more studies must be carried out to verify which one is the techno-economically feasible and environmentally friendly option, waste oil disposal or its use for biodiesel.

3.6 Concluding Remarks

This chapter presents the potential of several non-edible feedstocks for biodiesel production. The expectation of using non-edible oil plants as feedstock for the production of biodiesel is a matter of great relevance in order to diversify alternative sources, although more research is needed to improve the current processes and

reduce production costs. In general, additional efforts must be made to develop pest-resistant varieties that adapt to the climate and soil conditions of different regions. Brazil, which is one of the major global producers of biodiesel, has a very favorable scenario to find a way to increase the use of non-edible raw materials in the biodiesel production chain, since soybean has been the main raw material throughout all these years. Challenges still remain, as the question to be answered is about the availability of these non-edible oil plants to provide a sufficient quantity of biofuel to meet global demand and logically within the quality standards required by each legislation. On the other hand, the debated issue about the conflicts between energy and food may be increasingly mitigated as new raw materials that do not compete with the food chain start to gain more attention in the production of biodiesel.

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Chapter 4
Role of Microorganisms in Production of Biofuels

Abha Kumari, Pankaj Kumar Kundu, Manju M. Gupta, Kumud Bala, Shivani Chandra, Rudrani Dutta, and Aushmita Das

Abstract Several types of microbes such as whole cells of algae, fungi, yeast, and bacteria are employed to produce biofuel which include several steps such as aerobic and anaerobic fermentation, transesterification, etc. for biofuel production. Present chapter aims to review the wide range of applications of microbes and enzymes used in the pretreatment of diversified lignocellulosic biomass, starchy biomass, and oily biomass having complex structure for the development of a sustainable and economically significant biofuel. Numerous microorganisms have been reported to be involved in biofuel productions such as bioethanol/biobutanol, biogas, biohydrogen, and bioelectricity production. A special focus has been laid on recent microbial resources identified for these purposes from saline and other environmental conditions. Specific applications of microorganisms in pretreatment of solid waste and wastewater are also discussed.

Saccharomyces sp., *Kluyveromyces* sp., *Clostridium* sp., and *Trichoderma* sp. have been extensively exploited to obtain a high yield of simpler sugars, lower concentration of inhibitory compounds, and high biofuel yield. Several steps have been taken in recent years to develop genetically engineered microorganisms to enhance saccharification of lignocellulosic biomass, decrease the production of inhibitory sugars, and increase the tolerance level of the fermenting microorganisms for desirable end products.

To overcome the challenges associated with municipal solid waste-derived and agricultural feedstocks for enzymatic hydrolysis, potential of diverse microorganisms of biotechnological interest have been identified for fermenting this complex feedstock. This chapter further covers the collective approaches of genetic engineering and metabolic engineering currently being researched to develop mutant and engineered strain of microorganisms for the production of various biofuels (e.g., alcohol, hydrogen, biodiesel, and biogas) from multifarious feedstock materials. The

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concept of a rational and designed whole-cell catalyst for the production of fourth-generation biofuel and the prospects of microorganisms developed by genetic and metabolic engineering and synthetic biology for second- and fourth-generation biofuel production are also discussed. The chapter concludes with a discussion of metabolic engineering techniques being highly efficient, rapid, precise, and rational when compared to the conventional strategies for development of strain, for instance, mutagenesis. Biosynthetic pathways need to be altered, and it is even possible to introduce and optimize an entirely new pathway in microbes to ensure that we get the final product of our interest from them. There is a need to integrate biofuel fermentation technology and metabolic engineering with an aim to improve metabolism and enhance heterogeneity in gene expression.

Keywords Bacteria · Algae · Yeast · Fungi · Biofuel · Bioethanol

4.1 Overview

Biofuels are the fuels produced by biological agents from biomass. The total cellular dry weight or organic material produced by an organism (usually from CO_2 and sunlight) is the biomass. Usually, biofuels are meant for use in transport as a substitute for the nonrenewable and rapidly declining fossil fuels extracted from petroleum. Economic and industrial developments in the developing countries have contributed to an increased use of fossil fuels, which, in any case, are nonrenewable and limited in supply and are declining rapidly. Therefore, humans are forced to seriously consider the option of biofuels as a replacement for fossil fuels. Biofuels are customarily distinguished into four categories based on types of biomasses employed for their production based on the feedstock used and the processing technology adopted (Abbasi et al. 2021; Lu et al. 2011; Naik et al. 2010):

(i) First-generation biofuels are directly related to food biomass that is altogether wholesome.
(ii) Second-generation biofuels are defined as fuels produced from a wide array of different organic feedstock, ranging from lignocellulosic feedstocks to municipal solid wastes.
(iii) Third-generation biofuels are derived from algal biomass and seaweed grown on different types of feedstocks.
(iv) Fourth-generation biofuel is derived from genetically modified plant biomass.

In the present chapter, the role of microbes such as bacteria, fungi, yeast, algae, seaweed, etc. in production of biofuel is reviewed. These microorganisms belong to different taxonomic groups and possess unique characteristics which make them useful in production of all generations of biofuels (Table 4.1). Fungi such as yeasts and molds, protists such as algae, bacteria such as *Escherichia coli*, and archaea such as methanogens are extensively employed in production of biofuels (Willey et al. 2020; Madigan et al. 2021). Members of domain Archaea possess distinctive rRNA sequences, but they lack peptidoglycan in their cell walls, and unique membrane

Table 4.1 A comparison of the characteristic features of bacteria, archaea, algae, and fungi (molds and yeasts)

Characteristic features	Bacteria	Archaea	Algae	Fungi (molds)	Fungi (yeasts)	References
Cell type	Prokaryotic (unicellular)	Prokaryotic (unicellular)	Eukaryotic (microscopic and unicellular to very large and multicellular)	Eukaryotic (multicellular)	Eukaryotic (unicellular)	Madigan et al. (2021)
Reproduction	Binary fission	Binary fission	Asexual and complex	Either asexual or sexual	Budding	Riedel et al. (2019)
Source of energy	Both organic and inorganic matter	Methanogenesis Phototrophs	Autotrophs	Secrete hydrolytic enzymes that degrade biopolymers into simpler substances that can be absorbed	Convert carbohydrates to alcohol and CO_2 anaerobically through fermentation	Anderson et al. (2016) and Ryan (2018)
Examples used in biofuel production	*Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi*, etc.	*Thermosphaera aggregans, Staphylothermus marinus*, *Sulfolobus tokodaii*	Seaweed and freshwater moss	*Alternaria, Aspergillus, Fusarium, Mucor, Penicillium*, etc.	*Saccharomyces cerevisiae*, *Cryptococcus neoformans*, etc.	Pommerville (2011)

lipids. Peculiar metabolic characteristics are possessed by some archaea, for instance, methanogens can produce methane gas. Many archaea inhabit or thrive in extreme environments, for instance, extreme halophiles can thrive in high concentrations of salt and thermophiles flourish in environments with high temperatures (Riedel et al. 2019). This makes them especially useful for production of biofuels. Fungi are a diverse group of microbes that range from unicellular forms such as yeasts to molds and mushrooms and are extensively employed in fermentation.

Microorganisms directly and indirectly contribute to production of diverse biofuels. Heterotrophic microorganisms are being used for commercial production of biofuels such as biogas and fuel alcohols from organic matter. Photosynthetic microorganisms convert inorganic carbon and water to potential fuels (e.g., fuel alcohols, biohydrogen) and fuel precursors (e.g., biomass, starch, lipids). Only a few natural microbial processes are used for commercial production of biofuels and enhanced production capabilities being achieved through microbial metabolic engineering approaches (Jang et al. 2012). Processes that previously required multiple steps of feedstock pretreatment and subsequent conversion to fuel are being consolidated into single-step microbial processes using metabolically engineered species. Microorganisms with the ability to produce fuels from feedstock that could not be used previously are now being engineered (Seungwoo et al. 2016). The ultimate goal of metabolic engineering is to be able to use these organisms to produce valuable substances on an industrial scale in a cost-effective manner.

This chapter discusses some of the genetic and metabolic engineering approaches being used to enhance the commercialization potential of microbial biofuels including fuel alcohols, biodiesel, and biohydrogen. At present, all biogas production relies on native populations of methanogens, and this does not seem likely to change in the near term. Potential fuels from microalgae, cyanobacteria, and other photosynthetic bacteria, whether native or engineered, have distant prospects of commercial use (Liao et al. 2016). Metabolically engineered yeasts' surface displaying various hydrolytic enzymes appears to hold the greatest potential for near-term commercial use in generating bioethanol from starch, pretreated lignocellulose, and other polysaccharides. The bacterium *Zymomonas mobilis* metabolically engineered to make bioethanol from pentose sugars is already being commercialized. Other similar examples are likely to emerge as more engineered microorganisms become available (Paul et al. 2021).

In Fig. 4.1, several steps in production of different types of biofuels from solid waste are summarized. The chapter systemically highlights the wild and engineered strains of potential microorganism involved in pretreatment of solid waste and wastewater pretreatment and in production of biofuels, namely, bioethanol, biodiesel, biogas, and biohydrogen, and electricity generation and conversion of complex lignocellulosic waste to biofuel and lower form of carbohydrates. Concept of microbial factory cell, and whole-cell catalyst along with prospects for improvement in metabolic engineering for new strain development using advanced technologies for second-, third-, and fourth-generation biofuel, is also highlighted.

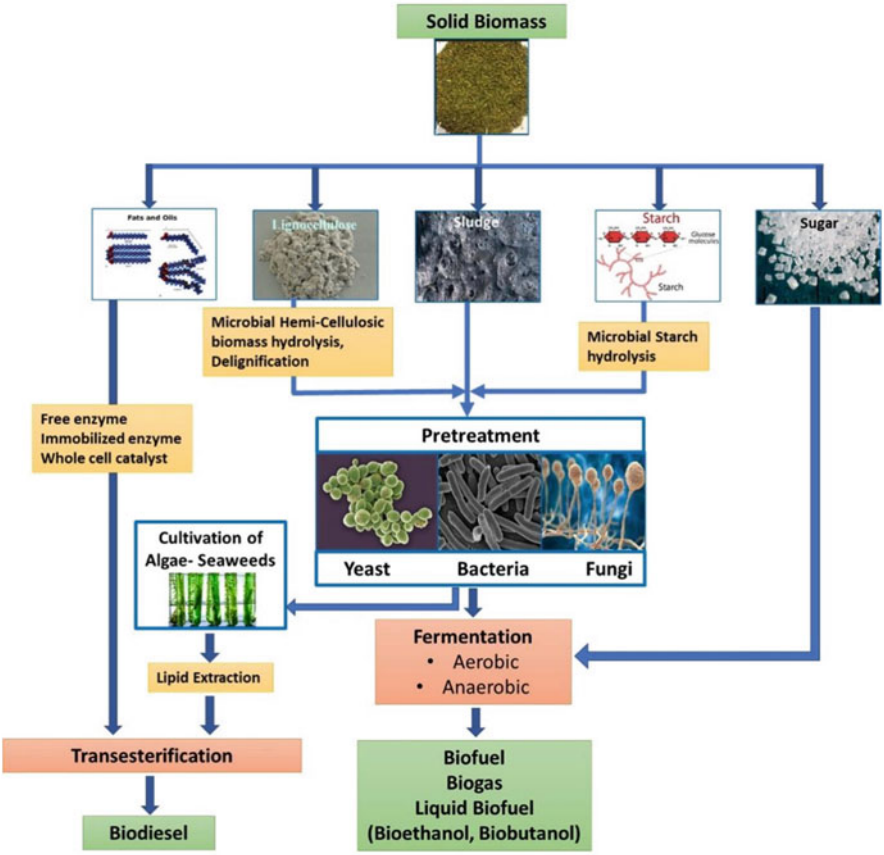

Fig. 4.1 Microorganisms used in several steps of biofuel production

4.2 Application of Microorganisms for Waste Treatment

Wastes of different types such as municipal solid waste, agricultural residues, wood and wood residues, etc., and the dedicated energy crops, which are a good source of starch, pectin, and lignocellulosic biomass have been tried to be converted to biofuels after biological degradation (Abbasi et al. 2021; Lu et al. 2011; Naik et al. 2010). The compounds present in these waste feedstocks have the potential of being converted to energy including pectin, starch, hemicellulose, cellulose, and lignin. In this section, structures of these energy compounds in waste material along with several hydrolytic enzymes obtained from different microbes used for their conversion are discussed. A special focus is laid on recent microbial resources identified for these purposes from saline and other environmental conditions. Specific applications of microorganisms in the pretreatment of solid waste and wastewater are also discussed.

4.2.1 Microorganisms as Source of Hydrolytic Enzymes

4.2.1.1 Starch and Saccharification Enzymes

Plants store starch as their reserved form of carbohydrate and thus is one of the most persistent biomass on Earth because it is synthesized by plants every year in large amounts. Starch undergoes the process of hydrolysis, and as a result, high-fructose corn syrup, glucose syrup, and glucose are produced which are of industrial importance. Bioethanol can be produced by fermenting the glucose which is obtained by the process of hydrolysis (Barancewicz and Gryta 2012). The principal enzymes of the starch industry comprise of glucose isomerase, alpha-amylase, beta-amylase, and glucoamylase (Chai et al. 2016). Examples of microorganisms applied in several studies for the effective production of these enzymes along with optimum conditions for their functioning are listed in Table 4.2.

Alpha-Amylase

The enzyme alpha-amylase is basically an endo-1,4-alpha-D-glucan glucanohydrolase which cleaves alpha-1,4 linkages between adjacent glucose units of starch, and as a result, glucose, maltose, and maltotriose are produced to form linear chains of amylose (Sharma and Chapadgaonkar 2021). Alpha-amylase enzyme is reported in numerous groups of halophilic microbes, for instance, marine bacteria, archaea, actinobacteria, fungi, and bacteria. Molecular weight of these alpha-amylase enzymes ranges from 30 to 140 kDa. Most of them can work efficiently when there is high salt concentration, but a few of them are active in a wide range of temperature and pH values. The alpha-amylase produced from *Nesterenkonia* sp. strain F has been extensively studied for this purpose and has been reported to produce ethanol and butanol directly from glucose (Sarikhan et al. 2011). This substantiates the fact that with a few modifications, it is possible to produce ethanol and butanol from starch only by making use of this strain. Selected examples of non-halophilic microorganisms producing alpha-amylase are used in industry for hydrolysis of starch (Table 4.2).

Beta-Amylase

Beta-amylase is basically an exoenzyme which removes maltose from the nonreducing end of the starch by hydrolyzing the starch. Beta-amylases are not so common. Many species of the genus *Bacillus*, for instance, *Bacillus megaterium*, *Bacillus cereus*, and *Bacillus polymyxa*, secrete the enzyme beta-amylase (Barchiesi et al. 2018). At present, there are only two halophilic bacteria known in which the presence of beta-amylase enzyme has been reported. Two moderately halophilic bacteria, namely, *Halobacillus* sp. strain LY9 and *Salimicrobium halophilum* strain

Table 4.2 Selected microorganisms used in industry for hydrolysis of starch

Enzyme	Microorganism	Optimum temperature (in °C)	Optimum pH range	Enzyme activity	Hydrolysis rate	Reference
α-Amylase	*Haloferax mediterranei*	60	7–8	–	–	Pérez-Pomares et al. (2003)
	Haloarcula sp.	50	7	–	–	Fukushima et al. (2005)
	Natronococcus sp.	55	8.5	0.12 U/ml	–	Kobayashi et al. (1992)
	Salimicrobium halophilum	70	10	573.5 units mg^{-1}	–	Li and Yu (2012)
	Micrococcus halobius	50–55	6–7	81.6 U/ml	35%	Onishi and Sonoda (1979)
	Aspergillus penicillioides	80	9	118.42 Umg^{-1}	–	Ali et al. (2015)
	Acinetobacter sp.	50–55	7	–		Onishi and Hidaka (1978)
	Nesterenkonia sp.	45	7.5	181 units/mg	38%	Shafiei et al. (2012)
β-Amylase	*Halobacillus* sp.	60	8	78.0 U/ml	–	Li and Yu (2011)
	Streptomyces sp.	40	6.7	–	–	Shinke et al. (1974)
	Bacillus cereus	40	7	–	–	Shinke et al. (1974)
	Salimicrobium halophilum	70	10	–	–	Li and Yu (2012)
Glucoamylase	*Halolactibacillus* sp.	70	8	–	–	Yu and Li (2014)
	Halorubrum sp.	50	7–7.5	–	–	Siroosi et al. (2014)
	Alkalilimnicola sp.	55	9.5	240 U/mg	–	Mesbah and Wiegel (2018)
	Aspergillus niger	30	5	–	137 U/ml	Wang et al. (2008)

'–': Not Reported

LY20, have been reported to have the presence of beta-amylase enzyme (Sittipol et al. 2019). Activity was shown by these enzymes under high temperatures and pH values which suggested that these microbial beta-amylases will prove to be promising in industrial processes. Selected examples of non-halophilic microorganisms producing beta-amylase are used in industry for hydrolysis of starch (Table 4.2).

Glucoamylase

Glucoamylase catalyzes the sequential cleavage of alpha-(1, 4) and alpha-(1,6) glycosidic bonds from the ends of starch which are not reduced, and related oligosaccharides and glucose are produced as the only end products (Zhang et al. 2019). Filamentous fungi, for instance, members of the genera *Rhizopus* and *Aspergillus*, are mainly responsible for the production of glucoamylases for industrial purposes. However, certain constraints like acidic pH requirement, moderate thermostability, and increased process costs because of slow catalytic activity often hinder industrial uses of fungal glucoamylases (Van den Burg 2003). Selected examples of non-halophilic microorganisms producing glucoamylase are used in industry for hydrolysis of starch (Table 4.2).

4.2.1.2 Pectins and Pectinolytic Enzymes

Pectin is one of the essential structural components of plant cell walls, and it has been observed that pectin acts as a shield between cellulose and hemicellulose, thereby blocking their exposure to hydrolytic enzymes. Homogalacturonic acid is the backbone of pectin and it is comprised of the side chains of xylose, arabinose, l-rhamnose, and galactose. Pectin polymer is degraded by some of the pectin degrading enzymes such as exopolygalacturonase, endopolygalacturonase, polymethylgalacturonase, exopolygalacturanosidase, etc. Rhamnogalacturonan is hydrolyzed by α-l-rhamnosidase and l-arabinose side chains are hydrolyzed by α-l-arabinofuranosidase, and arabinan side chains are hydrolyzed by endoarabinase and galacturonic acid polymer is hydrolyzed by pectate lyase, pectate disaccharide lyase, and pectin lyase.

4.2.1.3 Hemicellulose and Hemicellulolytic Enzymes

The plant species exhibit varied composition of hemicellulose in a backbone structure, branching, and modifications. Hemicelluloses comprise 20–40% of lignocellulose and are polysaccharides with heterogeneous and linear chains (Tu and Hallett 2019). Hemicellulose is covalently attached to sheaths of lignin in a lignocellulosic complex and connects with cellulose via hydrogen bonds (Xu et al. 2005; Eleonora et al. 1998). Thus, hemicelluloses are classified based on composition of the polymer of carbohydrate including xylan (D-xylose), xyloglucan (D-xylose and D-glucose),

glucomannan (D-glucose and D-mannose), galactoglucomannan (D-galactose, D-glucose and D-mannose), and arabinogalactan (D-galactose and L-arabinose). Hemicelluloses are polysaccharides having high molecular masses and firm structures. Therefore, numerous hemicellulolytic enzymes called as hemicellulases are required for the conversion of those large polymers into smaller oligosaccharides, disaccharides, and monosaccharides. Esterases are required for the conversion of hemicelluloses to remove acetyl and ferulic acid modifying groups and glycoside hydrolases to disintegrate the sugar backbone and branched sugar residues. Thus, numerous enzymes with discrete specificity and function are needed to be produced by microbes involved in hemicellulose degradation (Gao et al. 2011).

Microorganisms have potential to produce hemicellulolytic enzyme for hydrolyzing hemicellulose. Studies dealing with application for several hemicellulose hydrolyzing enzymes such as endo-β-1,4-xylanase, exo-β-1,4-xylanase, endo-β-1,4-mannanase, β-mannosidase, acetyl xylan esterase, α-glucuronidase, α-arabinofuranosidase, and α-galactosidase along with optimum temperature and pH are listed in Table 4.3. Hemicellulose hydrolyzing enzymes vary among mesophilic bacteria and fungi, and intriguingly, involvement of extremophilic bacteria have also been reported (Gao et al. 2011; Black et al. 1996; Hemansi et al. 2019). Recently, a few new halophilic archaea and bacteria exhibiting hemicellulose degrading activity have been described, and a few halotolerant and halophilic hemicellulases have been purified.

Xylan is the most frequently found hemicellulose in terrestrial plants among all hemicelluloses (Hsieh and Harris 2019). Xylan is a heterogeneous polysaccharide and its backbone consists of D-xylose linked by β-1,4 glycosidic bonds. Glucuronosyl, acetyl, and arabinosyl groups are frequently linked to the units of xylopyranoside. This is the reason why large spectra are exhibited by the composition of xylan among species of plants. Degradation of xylan requires two kinds of enzymes—(1) the chain is fragmented by endo-β-xylanases, and (2) xylooligomers are converted to monomers by β-xylosidases (Bokhari et al. 2008; Jorge et al. 2005). Other than that, auxiliary enzymes, for instance, ferulic and p-coumaric acid esterases, acetyl xylan esterase, and α-glucuronidase, are required for the removal of residues of side group (Motamedi et al. 2021). In the recent past, numerous halotolerant and halophilic microbial species possessing the ability to degrade xylan have been characterized. However, at present, there are no reports of these microbes being used for production of biofuel.

Halophilic microbes have been used to characterize, isolate, and purify various enzymes like xylanases and xylosidase. These enzymes have been isolated from distinct microbial types, for instance, bacteria belonging to the genera *Marinimicrobium*, *Halomonas*, *Gracilibacillus*, *Bacillus*, *Flammeovirga*, and *Chromohalobacter* and also *Halorhabdus* belonging to the Archaea domain (Carol 2011). Halophiles were used to characterize numerous xylosidases. The presence of xylosidases was also confirmed from the composition of hydrolysate. Xylosidase aids in the full degradation of xylan to D-xylose. The enzyme xylosidase was found to be present in *Halorhabdus utahensis* and *Gracilibacillus* sp. TSCPVG (Kumar et al. 2014; Sanghvi et al. 2014).

Table 4.3 Hemicellulose and hemicellulolytic enzymes

Enzyme	Microorganism	Optimum temperature (in °C)	Optimum pH range	Reference
Exo-β-1,4-xylanase	*Geobacillus thermoleovorans*	80	8.5	Verma and Satyanarayana (2012)
	Paenibacillus macerans	60	4.5	Dheeran et al. (2012)
	Thermoanaerobacterium saccharolyticum	63	6.4	Hung et al. (2011)
	Clostridium sp.	75	6	Lo et al. (2011)
	Actinomadura sp.	80	6	Sriyapai et al. (2011)
	Bacillus subtilis	60	8	Saleem et al. (2012)
	Nesterenkonia xinjiangensis	55	7	Kui et al. (2010)
	Bacillus stearothermophilus	75	6.5	Khasin et al. (1993)
	Bacillus flavothermus	70	7	Sunna et al. (1997)
	Bacillus thermoleovorans	70–80	7	Sunna et al. (1997)
	Bacillus thermantarcticus	80	5.6	Lama et al. (2004)
α-Glucuronidase	*Pichia stipitis*	60	4.4	Ryabova et al. (2009)
α-Arabinofuranosidase	*Acremonium zeae*			Bischoff et al. (2009)

4.2.1.4 Cellulose and Cellulolytic Enzymes

Cellulose is a significant component of plant tissues, and it is found among all other available biomass on Earth. Cellulose comprises of D-glucose molecules linked by beta-1,4 linkages. Cellulosic biomass availability is approximated to be near 30 gigatonnes every year through global terrestrial production, and this has led to the development of a varied, multifarious approach for biodegradation of cellulose in the natural world (Devi 2012). The degradation of cellulose is generally achieved by an inducible system of enzymes and proteins which are cellulolytic in nature, working collectively and consisting of a discrete cellulase system (Wong 1995). Beta-D-glucosidase, exoglucanase, and endoglucanase constitute the cellulose complex from cellulolytic microbes, and this cellulose complex has been recommended as an admissible strategy in the conversion of cellulose biotechnologically.

Beta-1,4-glucosidic bonds between the glucosyl residues in cellulose are hydrolyzed by the cellulase enzyme (Yang et al. 2014). Microorganisms have the ability to produce cellulolytic enzyme to convert cellulose into water-soluble sugar (Table 4.4). There is no doubt that there are numerous producers of cellulase in the microbial world with higher activities, but halophiles exhibiting cellulase activity will prove to be more useful in extremely salty conditions. There are many reports of halophilic microbes exhibiting cellulolytic activity; nowadays, focus of research devoted to bioenergy is the screening of such highly adaptable cellulose-producing microbes and utilizing them in the production of biofuel. Materials of cellulosic origin are hydrolyzed to sugars by the enzyme cellulase and other cellulytic enzymes, and afterward these sugars are fermented to generate bioethanol and other products of biological origin.

4.2.1.5 Lignin and Ligninolytic Enzymes

In numerous species of plants, lignin is the main component of cell wall structure, and it is the next most abundant raw material on Earth after cellulose. Lignin present in plants gives them strength, is responsible for their rigidness, and assists in transportation of water (Xiao et al. 2019). Most microbes are unable to degrade the rigid structures of lignin. In aerobic conditions, phenol oxidases and peroxidases are the principal enzymes in the degradation of lignin. Polyphenol oxidases and laccases are the two groups of phenol oxidases. Phenyl phosphate synthases and phenyl phosphate carboxylases are the principal enzymes responsible for the degradation of lignin under anaerobic conditions. Numerous lignin-containing plants thrive in or inhabit the salt marshes and coastal regions. It appears that halotolerant and halophilic microbes play a significant role in lignin metabolism, particularly degradation of lignin in environments like these and disclose the scope of production of biofuel from lignin.

Microorganisms have the ability to produce enzyme for delignification of lignocellulosic biomass (Table 4.5) even in environments such as sediments and salt

Table 4.4 Cellulose and cellulolytic enzymes

Enzyme	Microorganism	Optimum temperature (in °C)	Optimum pH range	Enzyme yield	Reference
Endo-β-1,4-glucanase	*Pleurotus florida*	45	4.4	480 UL^{-1}	Goyal and Soni (2011)
	P. ostreatus	–	–	420 UL^{-1}	Goyal and Soni (2011)
	P. sajar-caju	–	–	450 UL^{-1}	Goyal and Soni (2011)
	Thermobifida halotolerans	55	8	–	Zhang et al. (2011)
	Clostridium thermocellum	60	5.4	6.4 ± 0.4 U ml^{-1}	Peng et al. (2011)
	Thermoanaerobacter tengcongensis	75–80	6.0–6.5	294 U mg^{-1}	Liang et al. (2011)
	Acidothermus cellulolyticus	80	5.1	36 U mg^{-1}	Lindenmuth and McDonald (2011)
	Fervidobacterium nodosum	80–83	5.0–5.5	–	Wang et al. (2010)
	Paenibacillus campinasensis	60	7	–	Ko et al. (2010)
	Rhodothermus marinus	95	7	–	Hreggvidsson et al. (1996)
	Thermotoga neapolitana	106	6.0–6.6	–	Bok et al. (1998)
	Caldibacillus cellulovorans	80	6.5–7.0	–	Huang and Monk (2004)
Exo-β-1,4-glucanase	*Pleurotus florida*	45	4.4	102 UL^{-1}	Goyal and Soni (2011)
	P. ostreatus	–	–	82 UL^{-1}	Goyal and Soni (2011)
	P. sajar-caju	–	–	54 UL^{-1}	Goyal and Soni (2011)
β-Glucosidase	*Pleurotus florida*	45	4.4	980 UL^{-1}	Goyal and Soni (2011)
	P. ostreatus	–	–	880 UL^{-1}	Goyal and Soni (2011)
	P. sajar-caju	–	–	856 UL-1	Goyal and Soni (2011)

'–': Not Reported

Table 4.5 Delignification enzyme (pectinases, lignin peroxidases, xylanases, mannanases, manganese peroxidases, and feruloyl esterase)

Enzyme	Microorganism	Optimum temperature (in °C)	Optimum pH range	Hydrolysis rate	Reference
Xylanases	*Bacillus* spp.	40–60	5.0–9.0	71.4–81.3%	Thite and Nerurkar (2020)
	Phlebia sp.	50 °C	5.0	–	
	Cyathus stercoreus	–	–	57%	
	Aspergillus nidulans	–	–	–	
Pectinases	*Bacillus* spp.	40–70	6.0–11.0	71.4–81.3%	Thite and Nerurkar (2020)
	T. harzianum	–	–	–	
	Aspergillus niger	–	3.5–4.0	75–90%	Beldman et al. (1984)
Manganese peroxidase	*Phlebia* sp.	–	4.5	40.7%	
Lignin peroxidase	*Trametes hirsuta*	50	4.8	52.69%	Saritha et al. (2012)

'–': Not Reported

marshes, waterlogged wood, coastal seawater, and anaerobic sediments; most abundantly found prokaryotes play significant roles in lignin polymer degradation. A marine aerobic bacterium named *Sagittula stellata* was the first strain that exhibited halophilic nature and is capable of degrading lignin into smaller units. Laccase is the most intriguing enzyme in environmental applications among all the enzymes responsible for the degradation of lignin. There is no requirement of extra components like manganese or H_2O_2 by the laccase enzyme for its activity. In addition to this, lignin is simultaneously degraded or broken down and decolorized by the laccase enzyme in conditions of hypersalinity. A laccase (LccA) has been purified from a halophilic archaeon named *Haloferax volcanii* (Haque et al. 2020). This enzyme could tolerate high salt concentrations, and it was capable of oxidizing a wide spectrum of organic substrates, for instance, syringaldazine and bilirubin (Haque et al. 2020).

Chromohalobacter sp. was responsible for the purification of yet another laccase enzyme from it. $CuSO_4$ was the most effective inducer for production of laccase from *Chromohalobacter* sp., and a laccase enzyme was purified from a bacterium named *Bacillus* sp. strain WT which forms halotolerant endospores. An extremely stable laccase enzyme (extracellular in nature) was possessed by the halophilic bacterium named *Aquisalibacillus elongatus*. This laccase enzyme was exceedingly stable against organic solvents, temperature, and pH. Laccase activity was also noted in a halophilic archaea and bacteria named *Bacillus safensis* sp. strain S31. Samples of soil collected from a chromite mine located in Iran served as the source of endospores of this bacterium. A laccase enzyme of fungal origin was isolated from

Pestalotiopsis sp. SN-3. Lignin was efficiently metabolized by this halotolerant fungus, and toxic matter could also be potentially decomposed by this fungus from its surroundings. The laccase enzyme isolated from *Pestalotiopsis* sp. SN-3 exhibited high activity and could even withstand high concentrations of salt. Two isoforms of laccase (thermotolerant in nature) are obtained from *Pycnoporus sanguineus*, and even at high temperatures, these enzyme isoforms could retain their stability which prolonged their shelf life to a great extent (Vite-Vallejo et al. 2009). Numerous halophilic fungi have been reported to have potent and expansive abilities to metabolize lignin. Furthermore, a few marine species like *Rhizophila marina*, *Ascocratera manglicola*, *Cryptovalsa halosarceicola*, *Linocarpon bipolaris*, and *Astrosphaeriella striatispora* showed considerable amounts of solubilization of lignin (Kis-Papo et al. 2003; Oren and Gunde-Cimerman 2012).

4.2.1.6 Mannan

Mannan is present in cell walls of many algal species and a few seeds of plants. Mannan is a homopolymer polysaccharide and it has resulted from D-mannose monomers linked with β-1,4 glycosidic bonds. Glucomannan is most commonly found in the soft trees and it is a heteropolymer of D-mannose and D-glucose. Firstly, oligomers are produced from polymers by mannanases and afterward the monomeric sugars are obtained by hydrolysis. Hydrolyzing enzymes, for instance, β-glucosidase, β-1,4-mannanase, and β-1,4-mannosidase, required for the hydrolysis of mannan and glucomannan are generally extracellular in nature and are produced by a few species of bacteria. A few halophiles have been reported to have the ability to degrade mannan. The bacterium *Pantoea agglomerans* A021 was responsible for the isolation of the halo stable enzyme β-mannanase from it, and this new mannanase gene (man26P) has been cloned and expressed successfully (Chauhan et al. 2012). The bacterium *Marinimicrobium haloxylanilyticum* strain SX15 is fairly halophilic, and it has been reported to have the ability to metabolize mannan. This bacterium was isolated from the Great Salt Lake, and it is capable of degrading a few polysaccharides like galactomannan, starch, carboxymethyl cellulose, and xylan (Møller et al. 2010).

4.2.2 Microorganisms for Pretreatment of Solid Waste

Solid waste generated from various sources including agricultural, municipal, or forestry sources is a matter of grave concern (Abbasi et al. 2021; Lu et al. 2011; Naik et al. 2010). An unlimited amount of annual global waste is being generated, much of which is not managed in an environmentally safe manner. Hemicellulose, lignin, and cellulose are present in the firm plant parts and are also not easily biodegraded. To initiate the procedure for fermentation reactions, it is essential to expose this biomass to high temperature or extreme conditions of pH, a method which is called

Table 4.6 Microorganisms used in pretreatment of Solid waste

Class of microorganisms	Name	Biomass	Pretreatment conditions	Reference
Bacteria	*Micrococcus luteus* SR-1	Sawdust	20–55 °C Aerobic mode	Carlsson et al. (2012)
	Citrobacter freundii SR-3	Sawdust	20–55 °C Aerobic mode	Carlsson et al. (2012)
	Clostridium straminisolvens (CSK1)	Cotton stalk	20–55 °C Anaerobic mode	Carlsson et al. (2012)
	Brevibacillus sp. M1-5	Cotton stalk	20–55 °C Anaerobic mode	Carlsson et al. (2012)
	Bacillus subtilis	Rice straw	20–55 °C Aerobic mode	Carlsson et al. (2012)
Fungi	*Auricularia auricula-judae*	Chestnut leaves and hay	37 °C	Ali et al. (2017)
	Ceriporiopsis subvermispora	Chestnut leaves and hay	37 °C	Ali et al. (2017)

pretreatment (Archambault-Leger et al. 2012). Poplar wood, corn stover, switchgrass, and wheat straw have been treated with lime, a method which is known as pretreatment with alkali. During pretreatment process, it is possible to replace lime with salts which are alkaline in nature. As a result, concentration of salt and pH will render the immediate environment alkaline saline. Reduced sugars are produced by biomass which is hydrolyzed, and then microorganisms act on these reduced sugars and convert them to ethanol.

The term municipal solid waste (MSW) incorporates any non-modern waste originating from household and public or business organizations. As populace development, industrialization and urbanization increased MSW volumes are projected to rise impressively to 3.4 billion tons for every annum by 2050 (Sawatdeenarunat and Surendra, 2015). The natural part of MSW regularly comprises ~50% lignocellulose-rich material. Lignocellulosic materials are the best sources utilized for biofuel production. The lignocellulosic biomass pretreatment before anaerobic digestion is viewed as a huge advance to work on its biodegradability, and furthermore the biogas creation. The benefits of microbial (biological) pretreatment in contrast to nonbiological methods are a sustainable method of waste utilization, diminished involvement of inhibitory substances on the grounds, the diminished application synthetic substances, diminished energy input, and lower costs for squander store. By and large, the utilization of microorganisms is undeniably more financially savvy than the utilization of hydrolytic chemicals (Parawira 2012). The effective microorganisms for biological pretreatment are the types of white-rot fungi from the *Basidiomycetes* class, *Phanerochaete chrysosporium*, owing to their high development rate and lignin debasement abilities. Few examples of bacterial and fungal pretreatments are listed in Table 4.6.

4.2.2.1 Bacterial Pretreatment

In bacterial pretreatment, microorganisms can be utilized as inoculum-containing bacterial consortia, or in the mix with other organisms. The microbial consortium containing microorganisms with lignocellulolytic movement (the bacterial genera *Citrobacter*, *Klebsiella*, *Exiguobacterium*, *Lactococcus*, and *Micrococcus* and the *yeasts Sugiyamaella* and *Vanrija*) improved the hydrolysis and biomethanation of sawdust. Similarly, bacterial consortium containing a few strains of *Bacillus*, *Pseudomonas*, *Streptomyces*, and *Staphylococcus*, expanded the methane yield (Chandra et al. 2012). For upgrading anaerobic digestion, a few analysts utilized a bacterial consortium like the one that contained *Clostridium straminisolvens* (CSK1), *Clostridium* sp. *FG4b*, *Pseudoxanthomonas* sp. strain M1-3, *Brevibacillus* sp. M1-5, furthermore, *Bordetella* sp. M1-6. The critical bacterium for cellulose debasement is viewed as CSK1, an anaerobic cellulolytic bacterium (Ventorino et al. 2018).

4.2.2.2 Fungal Pretreatment

Fungal pretreatment works on the debasement of lignin and hemicellulose, which is significant for the anaerobic digestion cycle. These classes include the ascomycete (e.g., *Trichoderma reesei*) and basidiomycete species, which are gathered into white-, brown-, and delicate decay growths. Likewise, some anaerobic genera (e.g., *Orpinomyces* sp.) were found, having the ability to decay cellulose in the lots of ruminants. It was tracked down that the basidiomycetes' white-decay growths are the most proficient between all parasitic genera for delignification measure. The most utilized types of white-decay growths for biomass treatment contain *Phanerochaete chrysosporium*, *Pleurotus ostreatus*, *Trametes versicolor*, *Flammulina velutipes*, *Ceriporiopsis subvermispora*, *Streptomyces viridosporus*, and *Trichoderma viride*, *Pleurotus ostreatus*, and *Trichoderma reesei* were utilized for straw pretreatment all together to expand its decay and methane yield (Ali et al. 2017).

4.2.3 Microorganisms for Treatment of Wastewater

With the development of populace and the improvement of science and innovation, more water is burned through in day-to-day existence and creation, and more wastewater is produced. Lately, both according to the point of view of creation necessities and natural prerequisites, wastewater treatment has been a significant part that cannot be disregarded. The disclosure and use of microbial flocculants have a background marked by over 100 years. In 1876, Louis Pasteur found microbial flocculation while concentrating on yeast. The most punctual bacterial flocculant-creating microorganism was *Zoogloea*, which was separated from the enacted ooze screened by Butterfield in 1935 (Bao and Jiang 2012). Likewise, the bacterial

species, for example, *Paenibacillus* sp., *Alcaligenes latus*, *Pseudomonas aeruginosa*, *Bacillus* sp., *Rhodococcus* sp., and *Acinetobacter* sp., are harmless in nature to the biological system when in the utilization of a flocculant. Normal microorganisms that can acquire microbial flocculants include gram-positive microscopic organisms (*Rhodococcus erythropolis*, *Nocardia calcarea*, *Corynebacterium*, and so on), gram-negative microbes (*Alcaligenes latus* and so on), and other microorganisms (*Agrobacterium*, *Dematium*, *Acinetobacter*, soy sauce *Aspergillus*, *Paecilomyces*, and so on) (Guo et al. 2013). Among them, the flocculant delivered by *Paecilomyces* has a decent flocculation impact on food wastewater, coal slurry wastewater, and material wastewater, and the flocculant created by *Rhodococcus erythropolis* has a decent flocculation impact on fine coal wastewater, papermaking wastewater, and enacted muck (Zhang and Zhang 2013).

Phanerochaete chrysosporium was utilized as flocculant-creating microbes for coal slurry (Hou et al. 2013). Microbial flocculant, as a green flocculant, is additionally applied to the cycle of flocculation of substantial metal wastewater. *Bacillus* flocculant has a decent treatment impact on Cu^{2+} and Pb^{2+}. The expulsion pace of Cd^{2+} by *Pseudomonas* can reach 93.5%. *Merulius aureus*, an unidentified class, and *Fusarium sambucinum*, to shape a cementing parasitic complex, were utilized for exploratory examination on the treatment of kraft paper wastewater. Advancement of flocs framed essentially has a few stages: (a) flocculant particles are bit by bit scattered in the arrangement; (b) collisions with different particles in the molecule conveying flocculant adsorption; (c) adsorption of flocculant atoms on the outer layer of particles; and (d) small totals develop bigger and further through ceaseless impact and adsorption. Wastewater, food wastewater, and also, other wastewater are perplexing parts, which can give energy to microorganisms while decreasing the substance of natural matter and nitrogen in wastewater. Examples of microbial pretreatment of wastewater are given in Table 4.7.

4.3 Potential Microorganisms for Biofuel Production

The waste biomass is classified into three categories, starch, lignocellulosic, and oil/fat biomass. In this section, we discuss how and which microorganisms are employed for conversion of different forms of organic biomass to different forms of biofuels, that is, bioethanol, biodiesel, biogas, biohydrogen, butanol, acetone, and advanced biofuel. Starch biomass and lignocellulosic biomass are pretreated with microbe/enzyme to break matrix of biomass. Microbial/enzymatic hydrolysis results into formation of sugar and lower form of carbohydrate (Sect. 4.2). Water-soluble sugar is directly converted to biofuels by microbes, whereas oil and fat are transesterified to yield biodiesel. Microalgae are cultivated on carbohydrate-enriched liquid stream and wastewater, and lipid is extracted followed by transesterification to yield biodiesel. Figure 4.1 shows that microorganisms are used in several steps of biofuel production.

Table 4.7 Microorganisms used in treatment of wastewater

Microorganisms used in pretreatment of wastewater	Biomass	Pretreatment conditions	References
Bacteria			Bao and Jiang (2012)
Bacillus subtilis	Coal slurry wastewater	34 °C, aerobic	
Mycobacterium tuberculosis	Coal slurry wastewater	32 °C, aerobic	
Klebsiella	Municipal wastewater	32 °C, facultative anaerobe	
Pseudomonas	Heavy metal wastewater (cd^{+})	32 °C, aerobic	
Enterobacter sp.	Papermaking wastewater	32 °C, facultative anaerobe	
Fungi			Guo et al. (2013)
Fusarium sambucinum	Kraft paper wastewater	37 °C (pH 6, for 60–72 h)	
Rhizopus oryzae	Kraft paper wastewater	37 °C (pH 6, for 60–72 h)	

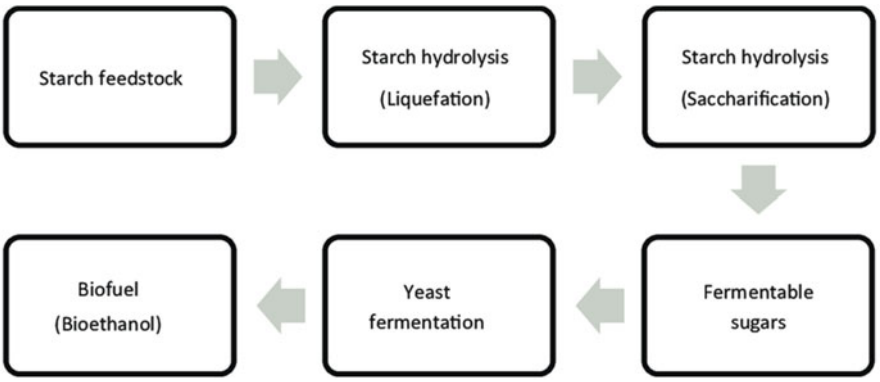

Fig. 4.2 Schematic diagram for ethanol production

4.3.1 Bioethanol

Ethanol produced by microorganisms from biomass is called bioethanol. During the present time, bioethanol (two carbon atoms) is not cost-competitive as compared to petrol (4–12 carbon atoms) but is being used for transport due to subsidies from the government due to its sustainable nature. Bioethanol can be produced from starch or sugar crops following enzymatic hydrolysis and chemical hydrolysis (Fig. 4.2). Bioethanol has drawn attention from scientists because it is identified as a potential replacement and/or additive to gasoline (Sharma et al. 2016). Environmentally friendly bioethanol production from various enzymes from various biomasses is

gaining more popularity when compared to other processes. Starch, hemicellulose, lignin, and cellulose constitute plant biomass or lignocellulosic biomass which exhibit the property of renewability. Pretreatments of biomass through enzymatic hydrolysis, fermentation, distillation, and sieving are the four main steps which are involved in bioethanol production from biomass (Zhu et al. 2020). Bioethanol is produced from several wastes using different microorganisms through different processes and obtained high bioethanol yield (Table 4.8).

Bioethanol with 100% purity is produced by fermentation of water-soluble glucose in anaerobic condition followed by distillation and sieving. It is produced from starch and cellulosic biomass through similar method after microbiological pretreatment. Pretreatment, cellulase synthesis, enzymatic hydrolysis, microbial fermentation, and product recovery are the five-unit processes that make up ethanol production from cellulosic biomass. Cellulase generation, enzymatic hydrolysis, and microbial fermentation are all biologically mediated processes in consolidated bioprocessing (CBP). CBP has the potential to be a game-changing technology for the biological conversion of cellulosic biomass to ethanol. Efficient and cost-effective process of continuous recovery of ethanol needs to be developed because such a process would keep ethanol concentration in the bioreactor broth to low levels and would avoid product inhibition of yeast cells. More efficient and cheaper methods of ethanol recovery need to be developed to reduce the cost of recovery. Some possible approaches may be reverse osmosis, selective adsorption using solid adsorbents, and use of supercritical CO_2 to selectively extract ethanol.

Yeast (*Saccharomyces cerevisiae*) is usually selected for maturation of sugars to bioethanol because of its capacity to endure high ethanol fixations and inhibitors delivered during the aging system. The solid-state fermentation (uses solid support) is the most favored maturation strategy for bioethanol production from lignocellulose. The cycles of enzymatic hydrolysis and aging are done inside the equivalent bioreactor which reduces the cost, giving ethanol yield, by diminishing the dangers of pollution and protein restraint by the finished results of hydrolysis (Althuri and Venkata Mohan 2020). Of the different microbes employed for fermentation, *Saccharomyces cerevisiae* is the microbe of choice for ethanol production from hexoses like glucose, and sucrose *Saccharomyces cerevisiae* can yield ethanol up to 18% of the fermentation broth. *Zymomonas mobilis* has up to 2.5 times specific ethanol productivity than *Saccharomyces* species, but it ferments only glucose, fructose, and sucrose. In either case, both these microbes are unable to ferment xylose which is the second most abundant sugar in the hydrolysate from lignocellulosic biomass. Several bacteria such as *Escherichia coli*, *Klebsiella oxytoca*, and *Clostridium thermocellum* can also utilize pentoses, but they are poor producers of ethanol (Bakhat et al. 2019). Some yeasts, such as *Pichia stipitis*, *Candida shebatae*, etc., can also use xylose but their ethanol yields are poor (Mohd Azhar et al. 2017).

Efforts have been made to genetically engineer *Saccharomyces cerevisiae* to enable it to ferment xylose. The focus has been on conversion of xylose to xylulose, overexpression of native xylulokinase, intracellular redox balance, xylose transport, and pentose phosphate pathway. Genes XYL1 (encoding xylose reductase) and XYL2 (encoding xylitol dehydrogenase) from *Pichia stipitis* were made to express

Table 4.8 Bioethanol production from several wastes using different microorganisms and processes

Substrate	Microorganisms	Pretreatment	Method/process	Ethanol yield (g/L)	Reference
Sugarcane	*Trichoderma reesei* (fungi)	Enzymatic	Consolidated bioprocessing (CBP)	9.7 ± 0.2	Huang et al. (2014)
Date palm sap	*Saccharomyces cerevisiae (wild)*	Acid hydrolysis	Batch and fed-batch (FB) fermentation	86.8 ± 0.35	Ben Atitallah (2021)
Wheat straw (treated by NMMO)	*Mucor indicus*	Boiling water	Ethanolic fermentation	9.9–10.6	Asachi and Karimi (2013)
Mix fruit waste	*Yeast Wickerhamomyces* sp. *UFFS-CE-3.1.2*	–	Alcoholic fermentation	21.63 (in 9 h)	Zanivan et al. (2022)
Brewer spent grains	*Escherichia Coli* (recombinant)	Acid pretreatment	Separate hydrolysis fermentation	16	Shen et al. (2021)
Wood and rice straw	*Pichia stipitis* NBRC1687 (UV-mutagenesis)	–	Two-phase fermentation	12.7	Watanabe et al. (2011)
Orange peel	*Saccharomyces cerevisiae*	–	Consolidated bioprocessing	7.53	Jeong et al. (2021)
Spent coffee ground	*Pichia stipitis* (wild)	Acid hydrolysis	Separate hydrolysis fermentation	19.9	Thomas et al. (2021)
Corn stover	*Aspergillus oryzae*	Untreated, dilute acid and dilute alkaline peroxide	Simultaneous saccharification and fermentation	0.39, 0.42, and 0.43, respectively	Hossain (2013)

Table 4.9 Engineered microorganisms used for bioethanol production

Engineered microorganism	Microorganism type	Characteristics	Ethanol yield (g/L)	Reference
Trichoderma reesei CICC 40360	Fungi	Improved ethanol yield by CBP	9.7 ± 0.2	Huang et al. (2014)
Pichia stipitis NBRC1687 (UV-mutagenesis)	Yeast	Adapted to increased hydrolysate concentration	12.7	Watanabe et al. (2011)
Thermoanaerobacter mathranii BG1L1	Bacterium	Deletion of lactate dehydrogenase for improved ethanol yield	Ethanol yield from 3% to 35%	Olson et al. (2015)
Zymomonas mobilis A1 strain	Bacterium	Converting alginate to ethanol, thus ethanol production improved	13	Takeda et al. (2011)
Pichia stipitis TJ2–3	Yeast	Ferment xylose, produce 1.5 times more ethanol than wild-type *Pichia stipitis*	11.9	Shi et al. (2014)
Thermoanaerobacterium saccharolyticum ALK1	Bacterium	Maximum ethanol titer increasing from 25 to 50 g/l	33	Olson et al. (2015)
Saccharomyces cerevisiae MF01-PHO4	Yeast	Improving ethanol yield and replacement of PHO4 gene	114.71 (max.)	Wu et al. (2020)
Trichoderma reesei PB-3	Fungi	Increase ethanol production by efficient biomass saccharification	54.2	Li et al. (2018)

in *Saccharomyces cerevisiae* (Moysés et al. 2016). Activities of these enzymes together convert xylose into xylulose, which is utilized by *Saccharomyces cerevisiae*. This was the first report demonstrating xylose fermentation by transgenic *Saccharomyces cerevisiae* (Chu and Lee 2007). The results from such genetic engineering efforts have been promising, but no such strain has been taken to commercial production yet. Examples of several engineered microorganisms used in the production of bioethanol are listed in Table 4.9.

Practical exploitation of the superior capabilities of *Zymomonas mobilis* is very well reported (Victor et al. 2019). This anaerobic bacterium is a better producer of ethanol than yeast as it has a relatively slower growth rate coupled with higher sugar conversion rate. Immobilized *Zymomonas mobilis* could be used to achieve high rates of ethanol production. *Zymomonas mobilis* production rates can be as high as 60 g l^{-1} h^{-1} as compared to only 30 g l^{-1} h^{-1} for yeast.

A few bacterial species with ethanol creation capacity have been recognized, alongside with this hereditarily changed bacterial species like *Escherichia coli* and *Bacillus subtilis* likewise can deliver high measures of subordinates of unsaturated

fats, isoprenoids, and bio-liquor. *Thermococcus*, *Thermotoga*, *Caldicellulosiruptor*, and *Pyrococcus* species can create hydrogen in higher sums, but these species produce ethanol in lesser amount. Ethanol could be productively created by co-culture of *Thermoanaerobacter* species alongside cellulolytic creatures. Bacteria such as *Clostridium thermocellum* and fungi such as *Neurospora crassa*, *Fusarium oxysporum*, *S. cerevisiae*, and *Paecilomyces* sp. come in handy for ethanol production using CBP approach (Schuster and Chinn 2013). *Saccharomyces cerevisiae* and *Zymomonas mobilis* are the best-known alcohol fermenting microbes with the ability to ferment hexose sugars and sucrose into ethanol but are inhibited by end products (Chandel et al. 2013).

Biofuels' creation from algal growth for the most part relies upon lipid content, with the utilization of algal biomass. Algae have a place with the gathering of photosynthetic living beings and can be named macroalgae (multicellular) and microalgae (unicellular), and in biofuel research, microalgae are vital. A few microalgal varieties like *Chlorella*, *Scenedesmus*, and *Chlamydomonas* have starch substance up to half of dry weight (Singh and Gu 2010). In the cell mass of microalgae, the normal parts are gelatin, cellulose, hemicelluloses, protein, and sugars. By corrosive or enzymatic hydrolysis, these parts can be changed over into monomers to deliver bioethanol. In one study, a marine yeast recognized as *Candida* sp. was discovered, categorized, and used for production of bioethanol utilizing *Kappaphycus alvarezii*, biomass from red algae.

There is evidence to substantiate the fact that halophilic microbes can be utilized as inedible feedstocks for production of bioethanol. For instance, it has been reported that *Arthrospira platensis*, which is a halophilic filamentous *cyanobacterium*, can be directly converted to ethanol without any processes such as enzymatic hydrolysis and pretreatment. It was pinpointed that *Arthrospira platensis* is a notable carbohydrate feedstock appearing as glycogen, which is a potential material for bioethanol production and many other chemicals of commercial importance (Klanchui et al. 2018). Until this finding, large quantities of ethanol were triumphantly obtained from cyanobacterial cells (non-pretreated) without the addition of any amylases, utilizing lysosome and a strain of *Saccharomyces cerevisiae* which expresses amylase. Overall yield of ethanol based on consumption of glycogen was 86% which is the highest yield of bioethanol from a microbe that is oxygenic photosynthetic (Klanchui et al. 2018). Halophilic microbes can play roles in the process of hydrolysis of other available feedstocks also where the products obtained therefore can be fermented to general biofuels, particularly bioethanol.

4.3.2 Biodiesel

Biodiesel is the diesel-like liquid extracted from materials of biological origin. Diesel usually has 9–23 carbon atom hydrocarbons. Biodiesel fuel refers to fatty acid alkyl esters which has attracted considerable attention as an environmentally friendly alternative fuel for diesel engines. Alkali catalysis is widely applied for the

commercial production of BDF. However, enzymatic transesterification offers considerable advantages, including reducing process operations in biodiesel fuel production and an easy separation of the glycerol by-product. The high cost of the lipase enzyme is the main obstacle for a commercially feasible enzymatic production of biodiesel fuels. To reduce enzyme-associated process costs, the immobilization of fungal mycelium within biomass support particles as well as expression of the lipase enzyme on the surface of yeast cells has been developed to generate whole-cell biocatalysts for industrial applications.

Bacteria, yeast, filamentous fungi, and microalgae can accumulate high content of lipids (more than 20% w/w on the basis of cell dry weight) in their cellular compartments and are considered as oleaginous feedstock for biofuel production. Oleaginous bacteria are a good source of triacylglycerols. However, their utilization for biodiesel production is limited compared to microalgae and yeast (Cho and Park 2018). Some important genera of oleaginous bacteria are *Rhodococcus* sp., *Gordonia* sp., *Acinetobacter* sp., and *Arthrobacter* species. Among them, *Rhodococcus* sp. has been the most extensively studied because of its ability to grow on versatile substrates (Wei et al. 2015; Sriwongchai et al. 2012). Within the biorefinery concept to produce biofuels, lignin is often left underutilized. Only certain fungi (mainly white-rot fungi) and prokaryotes have lignin-depolymerizing enzymes (Reiter et al., 2013). Recently, *Rhodococcus* sp. was studied for its potential to degrade lignin and finally assimilate lignin monomeric compounds into the lipid accumulation pathway (Kosa and Ragauskas 2012, 2013). In a study, *Rhodococcus opacus* attained a lipid content of 26.8% w/w when cultivated on aromatics obtained from organosolv pretreatment of loblolly pine along with lignocellulosic pretreatment effluents containing various sugars (Wells et al. 2015). This species was also applied to convert oxygen- pretreated kraft lignin into valuable lipids (Wei et al. 2015). Several microorganisms such as algae and seaweed are capable of secreting high content of lipid (Table 4.10). The schematic diagram for biodiesel production is given in Fig. 4.3.

Two-stage cultivation with glycerol and triethylamine enhanced the lipid productivity of *Dunaliella tertiolecta*, indicating that two-stage cultivation is an efficient strategy for biodiesel production from microalgae (Liang et al. 2019). Green microalgae produce a higher quantity of biofuel in comparison to blue-green algae. *Chlorella* sp., *Chlorococcum* sp., and *Neochloris oleoabundans* are identified to be potential biodiesel feedstocks. Ethyl acetate is a promising extraction solvent for biodiesel and biogas production. Solid nanoparticles (like alumina (Al_2O_3), CERIA, carbon nanotubes (CNT), Co_3O_4, ZrO_2, La_2O_3, CeO_2, SiO_2, Ni_2O, TiO_2, ZnO, Fe_2O_3, CuO, CexZr (1–x) O_2, nano-liquids, or nano-beads) with biofuel and nonrenewable energy source were demonstrated to further develop the fuel lubricity, cetane number, consuming rate, synthetic response, synergist execution, fire/streak point, warmth and mass exchange effectiveness, and water co-dissolvability just as lessening postpone period (Tajudeen et al. 2015). Three yeast strains of *Candida lipolytica*, *Candida tropicalis*, and *Rhodotorula mucilaginosa* have shown that the maximum lipid content could be achieved in the medium containing 8% molasses solution and 1.0 g/L $(NH_4)_2SO_4$ at pH 5 after 4 days of incubation time. Biodiesel

Table 4.10 Strains of microorganisms such as algae, yeast, and seaweeds for biodiesel production and the % of lipid content

Strain of microorganisms	Lipid content (%)	Substrate on which it grows	Reference
Oedogonium sp.	9.2	BBM and 3N-BBM	Abu-Khader (2006)
Chlamydomonas reinhardtii	21	ASM-1 medium	Almaraz-Delgado et al. (2013)
Dunaliella tertiolecta	19	Sterilized seawater	Shuba and Kifle (2018)
Chlorella zofingiensis	19.44	Agar slants using sterilized BBM medium	Ahmad et al. (2014)
Spirulina platensis	8	Sterile modified Zarrouk liquid medium	Prasertsit et al. (2013)
Nannochloropsis salina	92	Municipal wastewater	Chisti (2007)
Scenedesmus sp.	12	BG11	Campbell et al. (2011)
Botryococcus braunii	25–75	BBM and 3N-BBM	Chisti (2007)
Chaetoceros sp.	18	Conway medium	Campbell et al. (2011)
Chlorella emersonii	18.5	Agar slants using sterilized BBM medium	Ahmad et al. (2014)
Chlorella protothecoides	18	Agar slants using sterilized BBM medium	Ahmad et al. (2014)
Chlorella vulgaris	17	Agar slants using sterilized BBM medium	Ahmad et al. (2014)
Phaeodactylum tricornutum	20–30	Polycarbonate flasks (Walne medium)	Silva Benavides et al. (2013)
Porphyridium cruentum	11	Sterile Conway medium	Salim et al. (2011)
Bellerochea sp.	15	Sterile modified Zarrouk liquid medium	Prasertsit et al. (2013)
Rhodomonas sp.	15	Erlenmeyer flasks (f/2 medium)	Salim et al. (2011)
Spirogyra sp.	16	Anaerobically digested piggery wastewater	Borowitzka (1999)
Amphora sp. (Persian Gulf)	24	Liquid Moh202 culture media	Beetul et al. (2014)
Ankistrodesmus sp.	17.5	Bold basal medium (BBM)	Beetul et al. (2014)
Crypthecodinium cohnii	20	RSM-based medium composed of (per liter) RSM hydrolysate (7% vol/vol), molasses (6% vol/vol), and sea salts (25 g)	Greenwell et al. (2010)

(continued)

Table 4.10 (continued)

Strain of microorganisms	Lipid content (%)	Substrate on which it grows	Reference
Cylindrotheca sp.	16–37	f/2 medium	Aresta et al. (2005)
Dunaliella primolecta	23	Liquid Moh202 culture media	Ataya et al. (2008)
Isochrysis sp.	25–33	Methacrylate ponds	Aresta et al. (2005)
Monallanthus salina	>20	f/2 medium	Bhatia et al. (2021)
Neochloris oleoabundans	35–54	BOLD 3N medium with 2% agar	Brennan and Owende (2010)
Nitzschia sp.	45–47	f/2 medium	Chew et al. (2017)
Schizochytrium sp.	50–77	Waste glycerol	Brennan and Owende (2010)
Tetraselmis suecica	15–23	f/2 medium	Chew et al. (2017)
Lipomyces starkeyi	68	Sewage sludge	Angerbauer et al. (2008)
Candida lipolytica	59.9	Molasses	Wang et al. (2012)
Candida tropicalis	46.8	Molasses	Wang et al. (2012)
Rhodotorula mucilaginosa	69.5	Molasses	Patel et al. (2017)
Saccharomyces cerevisiae	12.8	Molasses	Patel et al. (2017)
Ulva lactuca	16	Saltwater tanks	Meher et al. (2006)
Padina boryana	46.7	Saltwater tanks	Cardone et al. (2003)
Ulva intestinalis	17	Saltwater tanks	Cardone et al. (2003)

was produced using the lipase immobilized on the magnetic nanoparticle. The reusable stability of the catalyst was performed, and it was found that the immobilized biocatalyst can be used for five cycles. This was first studied on the conversion of *Aspergillus* lipid into biodiesel. Palmitic acid (C16:0) showed the highest proportion in all studied seaweed species. Most of the nano-added substances from the exploratory examination were not all around described as far as molecule size, shape, and size dispersion just as grouping. Appropriate nano-additives are not available in large amount. Many nano-catalysts are quite expensive. An important limitation of large-scale algae cultures for protein or energy production

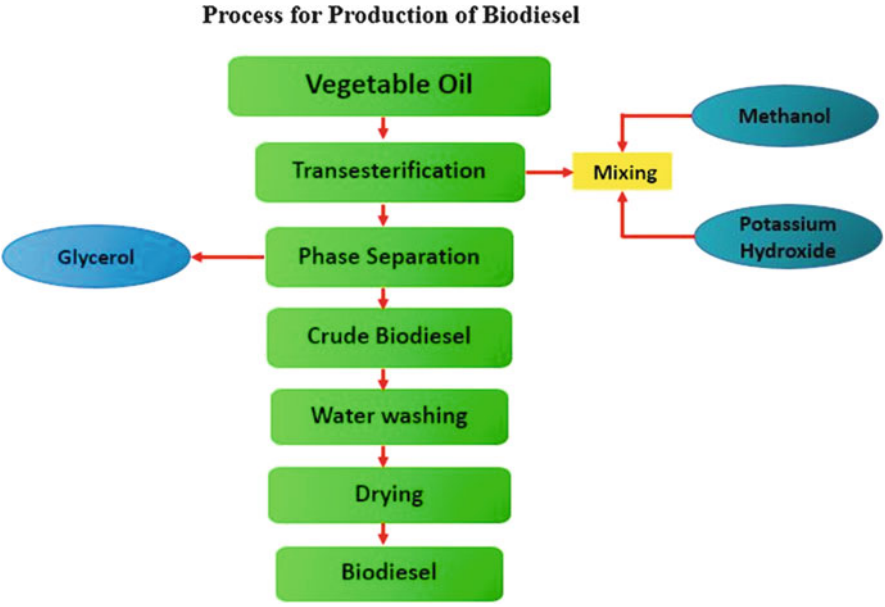

Fig. 4.3 Schematic diagram for biodiesel production

is competition for freshwater with traditional crops. To avoid or reduce the impact on freshwater resources, algae must be cultivated in brackish water or seawater. Algal biodiesel has additionally lower dependability during standard occasional temperature on the grounds that, during handling, microalgae contrast in polyunsaturated from which is one more type of biodiesel and polyunsaturated fats can hold their smoothness at a lower temperature during winter, yet it will have likewise lower steadiness during customary occasional temperature. Table 4.11 shows lipase enzyme isolated from microbes is used for transesterification of lipid obtained from algae, yeast, and seaweed.

High lipid quantities were produced by *Mortierella ramanniana*, *Mucor* sp., and mainly *Mortierella isabellina*, with glycerol being more adequate for *M. ramanniana* and glucose for *Mucor* sp. and *M. isabellina.*

The most used lipase in biodiesel production, the Novozyme 435 (*Candida antarctica*), is generally immobilized by adsorption on the surface of an acrylic resin. It is widely used industrially because of its nonspecific regioselectivity and its activity (10,000 U/g) (Lotti et al. 2015). Alcohol is the second important parameter to consider during the enzymatic transesterification. Alcohols used are MeOH, ethanol, propanol, 2-propanol, 1-butanol, 2-butanol, isobutanol, and 2-ethyl-1-hexanol. The lipase *Candida* sp. 99–125 (30% w enzyme/w oil) are tested during the biodiesel production from microalgae oil (*Chlorella protothecoides*) in the presence of MeOH (alcohol/oil molar ratio of 3:1), 10% (w/w) of water and hexane as cosolvent (pH = 7.0, temperature of 38 °C and a reaction time of 12 h). The fatty acid methyl ester (FAME) yield was 98% (w/w) (Xiong et al. 2008**).** Microalgae

Table 4.11 Transesterification of lipid obtained from algae, yeast, and seaweed strains at optimum temperature and suitable catalyst

Microorganisms	Temperature (°C)	Enzyme	Source of enzyme	Reference
Oedogonium sp.	60	*Novozyme 435*	*Candida antarctica*	Abu-Khader (2006)
Chlamydomonas reinhardtii	21	*Novozyme 435*	*C. antarctica*	Almaraz-Delgado et al. (2013)
Dunaliella tertiolecta	32	*Novozyme 435*	*C. antarctica*	Shuba and Kifle (2018)
Chlorella zofingiensis	35	*Lipase AY*	*Candida rugosa*	Ahmad et al. (2014)
Spirulina platensis	65	*Novozyme 435*	*C. antarctica*	Prasertsit et al. (2013)
Nannochloropsis salina	65	*Novozyme 435*	*C. antarctica*	Chisti (2007)
Scenedesmus sp.	65	*Novozyme 435*	*Candida antarctica*	Campbell et al. (2011)
Botryococcus braunii	25–27	*Lipozyme TL IM*	*Thermomyces lanuginosus*	Chisti (2007)
Chaetoceros sp.	50–60	*Novozyme 435*	*Candida antarctica*	Campbell et al. (2011)
Chlorella emersonii	15 to 26	*Novozyme 435*	*Candida antarctica*	Ahmad et al. (2014)
Chlorella protothecoides	15 to 26	*Lipase AY*	*Candida rugosa*	Ahmad et al. (2014)
Chlorella vulgaris	15 to 26	*Lipase AY*	*Candida rugosa*	Ahmad et al. (2014)
Phaeodactylum tricornutum	17 to 21	*Lipozyme TL IM*	*Thermomyces lanuginosus*	Silva Benavides et al. (2013)
Porphyridium cruentum	50–60	*Lipozyme TL IM*	*Thermomyces lanuginosus*	Salim et al. (2011)
Bellerochea sp.	65	*Lipase AY*	*Candida rugosa*	Prasertsit et al. (2013)
Rhodomonas sp.	25–30	*Lipase AY*	*Candida rugosa*	Salim et al. (2011)
Spirogyra sp.	35	*Novozyme 435*	*Candida antarctica*	Borowitzka (1999)
Amphora sp. *(Persian Gulf)*	20	*Novozyme 388 immobilized*	*Aspergillus oryzae*	Beetul et al. (2014)
Ankistrodesmus sp.	21 ± 1	*Lipozyme 62350*	*Candida* sp.	Beetul et al. (2014)
Crypthecodinium cohnii	22	*Lipozyme RMIM*	*Rhizomucor miehei*	Greenwell et al. (2010)
Cylindrotheca sp.	25 ± 1	*Novozyme 435*	*Candida antarctica*	Aresta et al. (2005)
Dunaliella primolecta	20	*Novozyme 388 immobilized*	*Aspergillus oryzae*	Ataya et al. (2008)

(continued)

Table 4.11 (continued)

Microorganisms	Temperature (°C)	Enzyme	Source of enzyme	Reference
Isochrysis sp.	62	*Lipozyme RMIM*	*Rhizomucor miehei*	Aresta et al. (2005)
Monallanthus salina	65	*Lipozyme 62350*	*Candida* sp.	Bhatia et al. (2021)
Neochloris oleoabundans	25	*Lipozyme RMIM*	*Rhizomucor miehei*	Brennan and Owende (2010)
Nitzschia sp.	25 ± 1	*Novozyme 435*	*Candida antarctica*	Chew et al. (2017)
Schizochytrium sp.	27	*Lipozyme 62350*	*Candida* sp.	Brennan and Owende (2010)
Tetraselmis suecica	25 ± 1	*Lipozyme RMIM*	*Rhizomucor miehei*	Chew et al. (2017)
Lipomyces starkeyi	28	*Novozyme 388 immobilized*	*Aspergillus oryzae*	Angerbauer et al. (2008)
Candida lipolytica	28	*Novozyme 388 immobilized*	*Aspergillus oryzae*	Wang et al. (2012)
Candida tropicalis	28	*Lipase AY*	*Candida rugosa*	Wang et al. (2012)
Rhodotorula mucilaginosa	28	*Lipozyme TL-100 L immobilized*	*Aspergillus oryzae*	Patel et al. (2017)
Saccharomyces cerevisiae	28	*Lipozyme TL-100 L immobilized*	*Aspergillus oryzae*	Patel et al. (2017)
Ulva lactuca	28	*Lipase AY*	*Candida rugosa*	Meher et al. (2006)
Padina boryana	28	*Lipozyme 62350*	*Candida* sp.	Cardone et al. (2003)
Ulva intestinalis	28	*Lipozyme TL-100 L immobilized*	*Aspergillus oryzae*	Cardone et al. (2003)

biodiesel contained a significant amount of shorter chain fatty acid methyl ester derived from myristic acid (C14:0, 10%). Yeast biodiesel is composed primarily of methyl oleate (C18:1, 60%) and is almost exclusively monounsaturated (60%), containing only 6% polyunsaturated fatty acid methyl ester (FAME). The enzymatic transesterification of lipid using lipase enzyme gives high biodiesel yield (Table 4.12).

The model strain for biofuel creation needs the capacity to use a high measure of substrate, transportation of sugar through quick and liberated pathways, capacity to endure inhibitory mixtures and finished results, and expanded metabolic transitions to deliver a further developed maturation item. The ideal strain can either be a characteristic cellulolytic biofuel-creating organism or a designed mechanical strain met with the gene(s) to deliver biofuel. In such manner, microbial creatures, for

Table 4.12 Biodiesel yield after enzymatic transesterification using lipase from various microorganisms

Microorganism	Biodiesel yield (%)	Reference
Bacillus subtilis	>90	Ying and Chen (2007)
Burkholderia cepacia	>80	Kaieda et al. (2001)
Candida antarctica	97	Royon et al. (2007)
Pseudomonas cepacia	85.4	Wu et al. (1999)
Candida rugosa	98	Shah and Gupta (2007)
Enterococcus aerogenes	94	Kumari et al. (2007)
Penicillium expansum	92.8	Li et al. (2009)

example, *Escherichia coli (E. coli)* and *Saccharomyces cerevisiae*, are investigated broadly for their capability to create biofuels. *E. coli* strains can normally use an assortment of carbon sources (counting sugars and sugar alcohols) under both vigorous and anaerobic conditions (Bond-Watts et al. 2013). Different life-forms, for example, *Corynebacterium glutamicum* and *Closteridium* species, are additionally effectively utilized in the creation of different biofuels relying upon the idea of the objective material and the kind of biofuel.

The diesel creation from algal growth is efficient and simple. Various species like *Tribonema*, *Ulothrix*, and *Euglena* have great potential for biodiesel creation. The screening of microalgae (*Chlorella vulgaris*, *Spirulina maxima*, *Nannochloropsis* sp., *Neochloris oleoabundans*, *Scenedesmus obliquus*, and *Dunaliella tertiolecta*) was performed to pick the best one(s), as far as amount and quality as oil hotspot for biofuel creation. *N. oleoabundans* and *Nannochloropsis* sp. (marine microalga) ended up being reasonable as crude materials for biofuel creation considering their high oil content, 35% and 28%, respectively (Gouveia and Oliveira 2009). *S. obliquus* presents the most satisfactory unsaturated fat profile, specifically as far as linolenic and other polyunsaturated unsaturated fats. *Oedogonium* and *Spirogyra* were contemplated to look at the measure of biodiesel creation. Algal oil and biodiesel (ester) creation was higher in *Oedogonium* than *Spirogyra* sp. (Chisti 2007). The three yeast strains of *Candida lipolytica*, *Candida tropicalis*, and *Rhodotorula mucilaginosa* showed that the most extreme lipid content could be accomplished in the medium containing 8% molasses arrangement and 1.0 g/L $(NH_4)_2SO_4$ at pH 5 following 4 days of hatching time. The most extreme lipid substance was estimated as 59.9% for *C. lipolytica*, 46.8% for *C. tropicalis*, and 69.5% for *R. mucilaginosa* (Chen et al. 2012).

Lipids created from filamentous fungal growths show incredible guarantee for biofuel creation; however, a significant restricting component is the high creation cost ascribed to feedstock. Eleven potential lipid-creating growths are *Aspergillus niger*, *Aspergillus terreus*, *Chaetomium globosum*, *Cunninghamella elegans*, *Mortierella isabellina*, *Mortierella vinacea*, *Mucor circinelloides*, *Neosartorya fischeri*, *Rhizopus oryzae*, *Mucor plumbeus*, and *Thermomyces lanuginosus*, which were refined in the medium with xylose as the sole carbon source (Andre et al. 2010).

Seaweed is considered as a suitable feedstock for biofuel. Between 85 and 90% of seaweed is water, which implies ocean growth is entirely reasonable for biofuel production techniques like anaerobic absorption to make biogas what's more, aging to make ethanol. Furthermore, numerous seaweed species, like sugar kelp, have high sugar and low lignin content that is ideal for making bioethanol. Seaweed is quite possibly the most efficient species, particularly in engrossing supplements like phosphorous, as well as nitrogen.

4.3.3 Biogas

Several hundred species of bacteria are known to be involved in the anaerobic digestion and biogas production. They can be classified into hydrolytic, acetogenic, and methanogenic bacteria. Flow sheet for biogas production is shown in Fig. 4.4.

4.3.3.1 Hydrolytic Bacteria

This group includes both obligate and facultative anaerobes and may occur up to 10^8–10^9 cells/ml of sewage sludge digesters (Pan et al. 2018). They remove the small amounts of O_2 present and create anaerobic conditions. These bacteria hydrolyze and ferment the organic materials, for instance, cellulose, starch, proteins, sugars, lipids, etc., and produce organic acids, CO_2, and H_2. Digestion of complex polysaccharides is rate-limiting, and lignin associated with cellulose often shields the

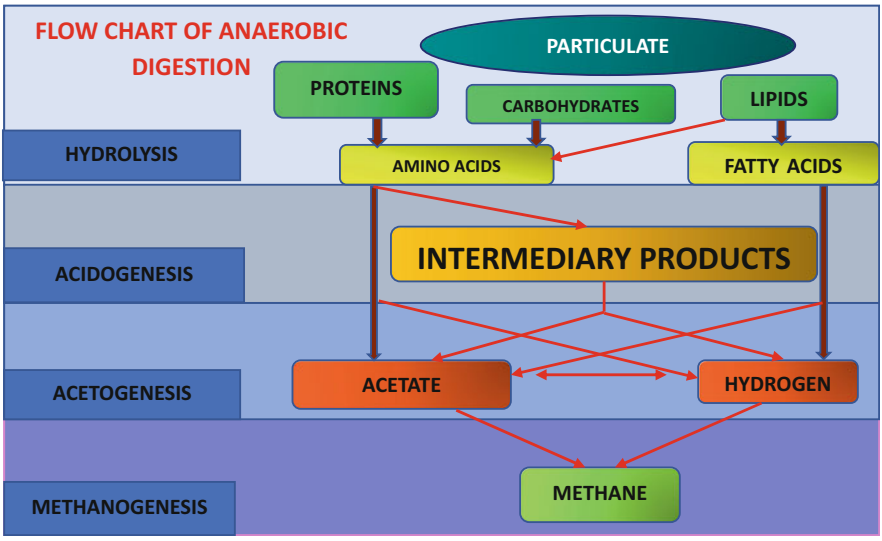

Fig. 4.4 Flow sheet for production of biogas

latter from enzymatic action. Thus, quite often, only 50% of the polysaccharide present in the waste may be digested (Menzel et al. 2020).

4.3.3.2 Acetogenic Bacteria

These bacteria oxidize hydrogen by reducing carbon dioxide to acetic acid which is then used up by methanogens to generate methane, CO_2, and hydrogen. Thus, acetogenic bacteria also remove hydrogen and enable the obligate hydrogen-producing bacteria to continue their function.

4.3.3.3 Methanogenic Bacteria

This group of bacteria converts acetate and CO_2 + H_2 into methane. Thus, methanogens remove the H_2 produced by obligate H_2-producing bacteria, thereby lowering the partial pressure of hydrogen and enabling the latter to continue producing hydrogen. Methanogenic bacteria are the strictest possible anaerobes known. They may occur up to 10^6–10^8 cells per ml of the slurry in digestors. These belong to the new kingdom called Archaebacteria and oxidize hydrogen by reducing carbon dioxide to obtain energy. Examples of methanogenic bacteria are *Methanosarcina barkeri*, *Methanobacterium omelianskii*, etc.

Affected by feedstock type and microbial inoculum, diverse microbial gatherings should exactly cooperate for top-notch biogas yields. A different and dynamic local area described by bacteria (82–88%) and a lot of Archaea (8–15%) introduced profiles specific to each phase of biogas creation. Eukaryotes (2.6–3.6%), for the most part, parasites, were a minor however stable part (Saini et al. 2015). Methane-delivering microorganisms distinguished during biogas creation were *Methanothrix soehngenii*, *Methanococcoides methylutens*, and *Methanoculleus bourgense*. The types of microbes separated from the substrate before assimilation were *Micrococcus* sp., *Klebsiella* sp., *Shigella* sp., *Escherichia coli*, *Pseudomonas* sp., *Staphylococcus aureus*, and *Citrobacter* sp., while parasitic species included *Fusarium* sp., *Aspergillus* sp., *Penicillium* sp., and *Mucor* sp.

In the second week stretch of processing, species, for example, *Salmonella* sp., *Serratia* sp., *Proteus vulgaris* and *Mucor* sp., along with those disconnected in the main week aside from *Klebsiella and Fusarium* sp., were segregated (Azizi et al. 2019). The third and fourth weeks (25–30 days) of absorption included *Staphylococcus aureus*, *Micrococcus* sp., *Pseudomonas aeruginosa*, *Bacillus* sp., *Escherichia coli*, *Citrobacter*, and *Mucor* sp. These living beings except for *Bacillus* sp., *Pseudomonas aeruginosa*, and *Mucor* sp. were solely prevailed by the previously mentioned methanogens recognized as *Methanothrix soehngenii*, *Methanococcoides methylutens*, and *Methanoculleus bourgense*. Anaerobic digestion includes various gatherings of microscopic organisms, for example, hydrolyzing, acidifying, acetogenic, and methanogenic microorganisms which in the last stage produce CO_2 and methane (Hendriks and Zeeman 2009).

4.3.4 *Biohydrogen*

When hydrogen is produced by biological agents such as bacteria and algae, it is called as biohydrogen. Biohydrogen is produced mainly by two routes, during anaerobic fermentation and by photolysis of water. In addition, an in vitro system created by coupling together the photosynthetic unit (for instance, from green plants or algae) and hydrogenase (from bacteria like *Clostridium* sp.) is a potent hydrogen generating system.

Hydrogen (H_2) has caught the attention of scientists because it can be easily converted to electricity. Schematic diagram of biohydrogen is shown in Fig. 4.5. Photosynthetic microbes which include cyanobacteria, photosynthetic bacteria, and green algae as well as bacteria which do not photosynthesize including anaerobic bacteria and nitrogen-fixing bacteria are the favored biological producers of hydrogen. Non-photosynthetic and halophilic photosynthetic bacteria can produce hydrogen, and a number of studies have reported this observation. In the case of photosynthetic bacteria, it has been reported that a community of halophilic bacteria which emerged from night soil treatment sludge vigorously produced hydrogen from raw starch in the presence of light and 3% sodium chloride (Patel and Kalia 2013). *Proteus vulgaris*, *Vibrio fluvialis*, and *Rhodobium marinum* were the successful strains of the community which produced hydrogen. The amount of hydrogen produced from starch by co-culturing *Rhodobium marinum* and *Vibrio fluvialis* was approximately equal to the community of bacteria, and this showed the significant role of these two halophilic bacteria on production of hydrogen from starch.

Few cases of microorganisms used in biohydrogen production are discussed below:

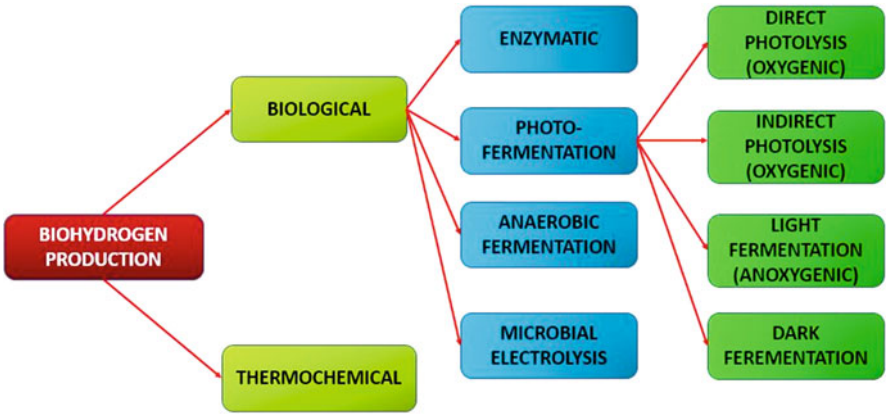

Fig. 4.5 Schematic diagram for production of biohydrogen

4.3.4.1 Syntrophic H_2-Producing Bacteria

This group is also called obligate H_2-producing or obligate proton-reducing bacteria since they oxidize NADH by reducing H^+ to H_2, thereby producing hydrogen. These bacteria break down organic acids having greater than two carbon atoms in their chain to produce acetate, CO_2, and H_2. However, they can grow freely and generate H_2 only under low H_2 partial pressure, which is maintained by methanogens. Sewage sludge digestors have about 4×10^6 cells/ml of this group. Examples of these bacteria are *Syntrophomonas wolfei* and *Syntrophobacter wolinii.*

4.3.4.2 Anaerobic Bacteria

Anaerobic bacteria oxidize the substrate by reducing NAD^+ to NADH. But for a continued substrate oxidation, it is essential to remove NADH from the cell environment and regenerate NAD^+. The electrons from NADH are transferred to H^+ ions to produce hydrogen gas, thereby regenerating NAD^+; the reaction is catalyzed by the enzyme hydrogenase. If hydrogen-producing bacteria are grown in the absence of hydrogen-utilizing species of bacteria, hydrogen accumulates and can be collected. The substrate for such a digestion can be any biodegradable organic material, including cellulose that has first been hydrolyzed (enzymatically or chemically).

4.3.4.3 Photosynthetic Algae

Some microscopic algae and cyanobacteria produce hydrogen when exposed to, particularly, low levels of sunlight. The photosynthetic apparatus splits water molecules into hydrogen and oxygen most likely as follows: the photosystem 1 produces reduced ferredoxin, the ferredoxin is then reoxidized, and protons (H^+) act as electron acceptors to produce hydrogen. The efficiency of production of hydrogen is reasonable at low light intensities (about 15% of the energy is stored as chemical energy in the form of H_2). But the efficiency is much lower at higher and more realistic light intensities. It is imperative that this problem is certainly resolved for making this route of hydrogen production of commercial interest. Isolation of suitable mutants and metabolic engineering may enhance hydrogen production to attractive levels.

4.3.4.4 In Vitro Photosynthetic-Hydrogenase System

The photosynthetic apparatus of higher plants, that is, chloroplast and the hydrogenase produced by hydrogen-producing bacteria, have been combined in an in vitro system to generate hydrogen from water using solar energy. The photosystem 1 generates H^+, e^-, and O_2 from H_2O, while hydrogenase combines e^- and H^+ to

yield H_2. This system functions and produces H_2 but is unstable due to the high sensitivity of hydrogenase to the O_2 produced by the photosystem 1 component of the arrangement. Further research is needed to resolve the instability problems; if this does happen, this H_2 production system may develop into an economically attractive process. The hydrolyzing, acidifying, acetogenic, and methanogenic microorganisms involved in biogas production are given in Table 4.13.

4.3.5 Microbial Fuel Cell

Microbial fuel cells (MFCs) are basically batteries powered by microorganisms. Electricity can be generated by directly capturing electrons from the microorganism's electron transport chain (ETC). Heterotrophic microbes can pass the electrons directly to an electrode when organic material is oxidized by them. A microbial fuel cell captures these electrons to produce electricity. To achieve this, a rich diet of organic substrates is continuously fed to the microorganisms in order to minimize de novo biosynthesis. In this way, during catabolism, much of the organic substrate is oxidized and electrons are donated to the ETC.

Power can be generated in MFCs by many different types of heterotrophic microorganisms. The sole requirement is that the organic matter must be anaerobically oxidized by them. In fact, communities of microbes can be recruited. The biggest restriction to obtaining maximum efficiency seems to be electrons' delivery to the electrode. This can be achieved in many ways. Often a chemical mediator is used to shuttle electrons from inside the cell, across the cellular membrane, to the anode. Some microorganisms, for instance, the γ-proteobacterium *Shewanella oneidensis*, give rise to "nanowires" to transmit electrons to the anode (in nature, these nanowires shuttle electrons to external electron acceptors such as Fe^{3+}).

Shewanella oneidensis is a promising fuel cell microbe that thrives in low-oxygen environments. It turns out that its efficiency is limited by the bacteria's membranes, through which electrons have a hard time escaping. So, the researchers tackled this problem by essentially implanting transmission wires inside the bacteria (Cao et al. 2021). The team grew *Shewanella* on electrodes made of graphene oxide with silver ions embedded in it. The bacteria reduce these ions into nanoparticles that are incorporated inside their cells, which helps more electrons escape to the outside of their membranes. It is being said that with these enhancements, the bacteria now shuttle 81% of the electrons they produce into the electrode. That generates 0.66 milliwatts of power per square centimeter, which the researchers claim is the highest power density for a microbial fuel cell by quite a margin. The breakthrough could help make microbial fuel cells more practical for real-world use (Cao et al. 2021).

The utilization of MFC as an elective hotspot for power age is considered as a dependable, perfect, effective cycle, which uses sustainable techniques and doesn't create any poisonous result. A MFC is a framework where microorganisms convert substance energy created by the oxidation of natural/inorganic mixtures into ATP by successive responses in which electrons are moved to a terminal electron acceptor to

Table 4.13 Hydrolyzing, acidifying, acetogenic, and methanogenic microorganisms for biogas production

Process	Microorganism used	Temperature and pH	Reference
Hydrolysis	*Escherichia coli*	27 °C–29 °C (pH, 6.9–7.4)	Saini et al. (2015)
	Micrococcus sp.	27 °C–29 °C (pH, 6.9–7.4)	
	Shigella sp.	27 °C–29 °C (pH, 6.9–7.4)	
	Klebsiella	27 °C–29 °C (pH, 6.9–7.4)	
	Pseudomonas sp.	27 °C–29 °C (pH, 6.9–7.4)	
	Staphylococcus aureus	27 °C–29 °C (pH, 6.9–7.4)	
	Citrobacter sp.	27 °C–29 °C (pH, 6.9–7.4)	
	Mucor sp.	27 °C–29 °C (pH, 6.9–7.4)	
Acidogenesis	*Salmonella* sp.	28 °C–39 °C (pH, 6.9–7.4)	Azizi et al. (2019)
	Serratia sp.	28 °C–39 °C (pH, 6.9–7.4)	
	Proteus vulgaris	28 °C–39 °C (pH, 6.9–7.4)	
Acetogenesis	*Escherichia coli*	28 °C–39 °C (pH, 6.1–7.2)	Hendriks and Zeeman (2009)
	Micrococcus sp.	28 °C–39 °C (pH, 6.1–7.2)	
	Bacillus sp.	28 °C–39 °C (pH, 6.1–7.2)	
	Pseudomonas sp.	28 °C–39 °C (pH, 6.1–7.2)	
	Staphylococcus aureus	28 °C–39 °C (pH, 6.1–7.2)	
	Mucor sp.	28 °C–39 °C (pH, 6.1–7.2)	
Methanogenesis	*Methanothrix soehngenii*	28 °C–39 °C (pH, 6.1–7.2)	Brown and Shi (2012)
	Methanococcoides methylutens	28 °C–39 °C (pH, 6.1–7.2)	
	Methanoculleus bourgense	28 °C–39 °C (pH, 6.1–7.2)	

produce an electrical flow. An ordinary MFC comprises of anode and cathode compartments, which are isolated by a cationic film. Microorganisms live in the anode compartment, where they use natural mixtures, for example, glucose, which

Table 4.14 Microorganisms used for generating higher voltage in microbial fuel cell

Microorganism used in MFC	Voltage (in volts)	Reference
Bacteria (gram −ve)		Bond et al. (2002)
1. *Bacillus cereus*	0.463	
2. *Escherichia coli*	0.534	
3. *Pseudomonas mendocina*	0.627	
4. *Proteus vulgaris*	0.35	
5. *Shewanella putrefaciens*	0.72	
6. *Geobacter metallireducens*	0.2	
Bacteria (gram +ve)		Bond et al. (2002)
7. *Clostridium beijerinckii*	0.759	
8. *Bacillus subtilis*	0.298	
9. *Paenibacillus lautus*	0.727	
Yeast		Chou and Whiteley (2014)
10. *Saccharomyces cerevisiae*	0.183	

go about as electron giver. *Saccharomyces cerevisiae* and microscopic organisms, for example, *Escherichia coli*, were first utilized for influence age in MFC (Behera et al. 2010). Microbial fuel cell generates high voltage due to microorganism (Table 4.14).

4.4 Microbial Factories for Biofuels

Microbial cell factory approach for biofuels considers microbial cells as a production facility in which the optimization process largely depends on metabolic engineering. They use lignocellulosic and other feedstocks including acetate, lactate, syngas, and glycerol or food crops for biofuel production. Additionally, photo-biorefineries which help in conversion of CO_2 and light energy into useful chemicals are also being developed (Lindberg et al. 2010; Lan and Liao 2013; Oliver et al. 2014). Microbes utilize organic substrates, and its subsequent metabolism leads to the generation of the most valuable biofuel. However, criteria such as selection of microorganisms and choice of substrates and the process for optimum production of biofuel are of crucial importance. For example, ethanol production by microorganisms from corn requires utilization of more energy from fossil fuel than to a process where sugarcane is used as the substrate (Goldemberg et al. 2008). Hence, a biofuel that has a better positive net energy balance might be considered as the best suited for commercialization.

Selection of an efficient substrate, for the microorganism to produce biofuels, is very important. Lignocellulose-containing substrates, namely, plant biomasses and agricultural wastes, can be considered as the most desirable alternatives in comparison to the feedstocks of other types. However, some microbes do not completely degrade lignocellulose into its fermentative constituents, e.g., *Saccharomyces*

cerevisiae (Chang et al. 2013). Lignocellulosic biomasses undergo deconstruction to form biofuel. This conversion starts with a pretreatment which is followed by an enzymatic hydrolysis step or by consolidating these two steps in one reactor (Mosier et al. 2005; Singh and Trivedi 2013). The process of cellulolytic hyphal penetration can be carried out either by physical or chemical or biological methods or by a combination of all three. The resultant biomass is then hydrolyzed by cellulolytic microbes or by a cocktail of cellulolytic enzymes (Lynd et al. 2002).

4.5 Whole-Cell Catalyst for Biofuel Production

Many studies have reported the utilization of microorganisms such as bacteria, yeast, and fungi as whole-cell biocatalysts in attempts to improve the cost-effectiveness of the bioconversion processes (Ban et al. 2001; Fujita et al. 2002; Narita et al. 2006). The cost of lipases considerably restricts their use for the mass production of chemicals and fuels. This led researchers to explore the potential use of microbes, for instance, filamentous fungi, bacteria, and yeast that might be employed as whole-cell biocatalysts because of their ability to immobilize and the display of functional proteins of interest on the surface of their cell. Moreover, simple techniques of immobilization and relative ease of process scale up of filamentous fungi make these particularly practical whole-cell biocatalysts with numerous commercial advantages (Nakashima et al. 1990). Table 4.15 lists the fungal and yeast whole-cell biocatalysts that so far have been used for production of biodiesel fuels.

4.6 Bioprospecting Microorganisms by Genetic and Metabolic Engineering and Synthetic Biology for Second- and Fourth-Generation Biofuel Production

Metabolic engineering is considered as an important tool in improving biofuel production either by refining the microbial fermentation for better utilization of broad range of biomass or engineering feedstock crops (Yuan et al. 2008). Advances in molecular biology and synthetic biology allow modification of the biosynthetic pathways in the host organism. Such modifications have successfully resulted in the production of value-added chemicals, for instance, alcohols, esters, and higher fatty acids that can be utilized as fuel source (Lee et al., 2008; Clomburg and Gonzalez 2010; Lü et al. 2011; Runguphan and Keasling 2014).

The bacteria *Escherichia coli* and *Bacillus subtilis* have been most widely used to construct ethanol-producing strain because the molecular biology of these microbes is well understood. *Escherichia coli* was probably the first microorganism to be successfully modified for ethanol production through metabolic engineering (Zhou et al. 2005). An important problem with mesophilic bacteria such as *Escherichia coli*

Table 4.15 Comparison of biodiesel fuel production methods that used different whole-cell biocatalysts

Whole-cell biocatalyst	Oil	Alcohol	Solvent	ME (%)	Time, hour(s)	Temperature	Reference
BSPs with *R. oryzae*	Soybean	Methanol	None	80% to 90%	72	32 °C	Ban et al. (2001)
BSPs with *R. oryzae*	Soybean	Methanol	None	90%	48	350 °C	Hama et al. (2007)
BSPs with *R. oryzae*	Soybean	Methanol	*t*-butanol	72%	NA	35 °C	Wei et al. (2007)
BSPs with *R. oryzae*	Jatropha	Methanol	None	89%	60	30 °C	Tamalampudi et al. (2008)
BSPs with *R. oryzae*	Rapeseed (refined)	Methanol	*t*-butanol	60%	24	35 °C	Li et al. (2007)
BSPs with *R. oryzae*	Rapeseed (crude)	Methanol	*t*-butanol	60%	24	35 °C	Li et al. (2007)
BSPs with *R. oryzae*	Rapeseed (acidified)	Methanol	*t*-butanol	70%	24	35 °C	Li et al. (2007)
Mycelium of *R. chinensis*	Soybean	Methanol	None	86%	72	NA	Qin et al. (2008)
S. cerevisiae (intracellular ROL)	Soybean	Methanol	None	71%	165	37 °C	Matsumoto et al. (2001)
S. cerevisiae (cell surface ROL)	Soybean	Methanol	None	78%	72	37 °C	Matsumoto et al. (2002)

All processes are batch reaction with a stepwise addition of 3 mol methanol

and *Bacillus subtilis* is their poor ability to hydrolyze carbohydrate polymers, poor tolerance to extreme pH values, and inability to withstand high salt concentrations (Jin et al. 2014). Thus, bioprocesses based on these microbes are easily contaminated with other unwanted species, making them poorly suited for use in large-scale production operations.

4.7 Challenges and Prospects

More efficient processes, especially continuous processes, must be developed to reduce the cost of production of biofuels. To achieve the above goal, organisms with higher product yields and greater product tolerance must be developed. Efficient processes to utilize low-cost substrates for biofuel production are urgently needed. The continuous processes of biofuel production cannot allow for continuous selection programs to maintain the desirable features of microorganisms. In any case, such selection programs add to the production cost. Thus, it is important to develop genetically stable, high-producing microorganisms. The sources of biomass or their derivatives to be used as substrates should be identified and their cheap and abundant supply should be ensured. For instance, genetic and agronomic improvements of energy crops are expected to reduce biofuel production costs. The substrate utilization ability of excellent ethanol producers like yeast and *Zymomonas mobilis* must be increased. Also, there is a need to enhance the alcohol tolerance of yeast. The ethanol production ability of bacteria capable of utilizing cellulose, hemicellulose, pentoses, etc. should be increased.

Practical exploitation of the superior capabilities of *Zymomonas mobilis* is needed. This anaerobic bacterium is a better producer of ethanol than yeast as it has a relatively slower growth rate coupled with higher sugar conversion rate. Immobilized *Zymomonas mobilis* could be used to achieve high rates of ethanol production. *Zymomonas mobilis* production rates can be as high as 60 g/l/h as compared to only 30 g/l/h for yeast.

Efficient and cost-effective process of continuous recovery of ethanol needs to be developed because such a process would keep ethanol concentration in the bioreactor broth to low levels and would avoid product inhibition of yeast cells. More efficient and cheaper methods of ethanol recovery need to be developed to reduce the cost of recovery. Some possible approaches may be reverse osmosis, selective adsorption using solid adsorbents, and use of supercritical carbon dioxide to selectively extract ethanol.

There isn't a single organism known yet that can produce components of biofuel on an industrial scale or is capable of effectively converting cellulose to ethanol. Thus, biofuel's future heavily relies on emerging technologies such as CRISPR (Cas-based genome editing tools) which will pave the way for the development of microbial biofuels of the future. However, to develop bioenergy crops that are sustainable, there is a need to understand the basic biology behind components of

cell wall, regulation of cell wall synthesis, fatty acid biosynthesis and regulation, and also, efficiency of the process of photosynthesis.

The considerable amount of biomass generated by plants can be converted to biofuel, but species of microalgae are the preferred feedstocks of choice for the production of components of biofuel. This is because highly efficient photosynthesis is carried out by microalgal species coupled with growth rates, and they are able to give rise to varied groups of metabolites. Recent metabolic engineering developments have already revealed the possibility to interfere with metabolic pathways by manipulating them for the excess production of metabolites in model organisms, for instance, *Saccharomyces cerevisiae* and *Escherichia coli*. Numerous worthful efforts have been made by scientists to engineer the metabolic pathways in microbes and plants to mold them into best platform for biofuel production. Rational design and plant biomass engineering with an aim to decrease content of lignin and increase content of carbohydrate to ensure that there is higher conversion to bioethanol will play a crucial role in developing desired plants for production of biofuels. Scientists will be able to analyze and evaluate the metabolic dynamics of varied biomass feedstocks with the easy access to tools for genetic manipulation, next-generation sequencing technologies, and techniques for analysis. Present and future research revolves around analyzing, solving, and making the best use of these metabolic pathways to enhance the yield of biofuels. The present objectives in this field are directed toward gaining a specific or exact control on the metabolic pathways and diverting the flux to get desired products without affecting the feedstocks' in-built physiological state. Techniques of metabolic engineering will also broaden over time to develop novel biomass feedstock by introducing the biochemical pathway not present naturally in the system.

Initially, butanol was produced by strictly anaerobic fermentation by *Clostridium acetobutylicum* utilizing molasses or cornmeal as substrate. However, at present, it is produced from propylene for industrial applications because propylene is cheaper than biobutanol. Furthermore, butanol obtained either from fermentation or from propylene is costlier than ethanol. Thus, efforts must be focused on reducing the cost of production of biobutanol to make it an attractive biofuel. Gene cloning experiments using *Clostridium acetobutylicum* are being carried out with a view to expand its substrate utilization range (for instance, by expressing cellulase encoding genes in this bacterium), to overexpress or modify the genes resulting in enhanced yield of butanol, and possibly to overcome inhibition of product (Antoni et al. 2007). There is a need to develop nonbiological approaches (for instance, developing efficient but cheap techniques for recovery of product) to decrease cost of production. If these developments are proven to be successful, then it might lead to the production of biobutanol for its use as a fuel.

In addition to low yields, production of butanol from *Clostridium acetobutylicum* fermentation presents problems of toxicity to the producer organism (higher than that of ethanol), and the complex genetics of the bacterium *Escherichia coli* has been engineered to express two enzymes of broad-substrate range, namely, a 2-keto acid decarboxylase and an alcohol dehydrogenase. This strain of *Escherichia coli* is able to produce a few potentially valuable alcohols which include isobutanol, 3-methyl-1-

butanol, 1-butanol, and 1-propanol. Metabolic engineering is being used to modify other microbes to ensure that they are able to produce low-cost biofuels, and there is a possibility that it might even venture to produce new replacements for existing biofuels (Nilsson et al. 2019).

There are many limitations of biogas as a fuel. The process of production of biogas is not economically very attractive as compared to other biofuels on a large industrial scale (Rodionova et al. 2017). Recombinant DNA technology and even techniques for improvement of strain cannot be used to enhance the efficiency of the process because the conditions of digestion are non-aseptic and exert their own selection pressure. Thus, improvement in the process can be brought about only by optimizing the environmental conditions of the anaerobic digestion. Also, biogas contains a few gases as impurities which are corrosive to the metal parts of internal combustion engines (Mittal et al. 2018).

The main limitation of hydrogen (H_2) as a fuel is the absence of a cost-competitive technology for production and utilization of hydrogen (H_2) (Aman 2018). At the present time, it can be considered as an objective of long-term research and development (Kumar and Sehgal 2018).

4.8 Conclusions

Microbes play a pivotal role in the manufacturing of biofuel. Nevertheless, the fact is that the yield of product by strains which are native is not economical, and therefore it is the need of the hour to apply the concepts of genetic engineering and metabolic engineering to develop and improve the strains of the microbes employed to produce biofuel. In the recent times, focus has been on applying the concepts of metabolic engineering to model development of strain to ensure that production is high and production cost is cheaper. In the future, database mining could be the reason for the emergence of peculiar metabolic pathways for production of biofuel. Therefore, the involvement of these pathways in industrial fermentation hosts might overcome any shortcomings associated with the use of lignocellulosic biomass as a renewable feedstock for fermentation. It is expected from metabolic engineers to exploit the "omics" technology and CRISPR-Cas 9 system to design and produce new strains of microorganisms with improved ability to generate biofuel from a wide array of substrates by insertion of appropriate genes into the genome or deletion of intervening ones.

Presently, metabolic engineering is making huge strides in the field of science, and metabolic engineering techniques have the potential to effectively produce biofuels by making use of diverse groups of microbes. Metabolic engineering techniques are reported to be highly efficient, rapid, precise, and rational when compared to the conventional strategies for development of strain, for instance, mutagenesis. Biosynthetic pathways can be altered, and it is even possible to introduce and optimize an entirely new pathway in microbes to ensure that we get the final product of our interest from them. It is difficult to optimize the process of

fermentation for production of biofuel in large bioreactors found in industrial setups because it becomes a costly affair. Thus, there is a need to integrate biofuel fermentation technology and metabolic engineering with an aim to improve metabolism and enhance heterogeneity in gene expression. Moreover, fermentation engineers thoroughly need to know about microscopic and macroscopic parameters to address the performance gap between the lab-scale studies and its industrial applications. But, for commercialization of the biofuels, it must be ensured that the strains used as microbial factories are better in their performance when compared to the wild-type strains. Advancing progress in the development of metabolic engineering techniques will enable the microbes to make use of substrates which they couldn't make use of earlier. This characteristic of the engineered microbes will help to improve the economic feasibility of utilizing biofuels as alternative fuels to fossil fuels. Genetic engineering and metabolic engineering along with synthetic biology and systems biology will enable the development of high-powered cell factories for the purpose of biofuel production.

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Chapter 5
Algal Biomass for Biodiesel and Bio-oil Production

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Abstract Microalgae serve as a potential feedstock for the production of third-generation biofuel to overcome economic and environmental challenges associated with petroleum-based fuels. Microalgae offer more advantages than traditional plant-based feedstocks used for biofuel generation. Besides biodiesel, microalgae can be utilized for bio-oil, ethanol, syngas, biohydrogen, etc. production. Industrial-scale production of biodiesel and bio-oil possesses hurdle because of expensive harvesting and downstream and conversion processes. Efficient and economical techniques need to be developed for sustainable production of biofuels. The present chapter provides an overview on different culturing, harvesting, and drying methods for microalgae to be used as feedstock for biodiesel and bio-oil production. Different techniques for extraction of lipids from wet and dry biomass and various catalysts used for conversion of lipids to biodiesel and bio-oil along with their advantages and limitation are explained.

Keywords Biodiesel · Bio-oil · Microalgae · Transesterification · Extraction

5.1 Introduction

Biofuels are receiving great attention as they are the source of renewable energy and are considered as safer, cost-effective, and environmentally friendly alternative to fossil fuels. With rising petroleum prices and increasing concern over the emission of greenhouse gases which contribute to global warming, researchers are paying attention toward developing new-generation biofuel, which is economic and sustainable. Biofuels can be produced from cheap renewable sources like crops, crop residues, food waste, animal waste, and algae biomass. Terrestrial crop plants including corn, sugarcane, soya, etc. require a huge amount of fertile land, water,

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nutrients, and a long time to grow and are highly dependent upon the climatic conditions, which becomes a hurdle in the economic production of biofuels. Algae, on the other hand, can be grown in ponds and tubular or vertical photobioreactors. They do not require soil or even freshwater to grow throughout the year, show higher tolerance to carbon dioxide content, need less consumption of water, and don't require pesticides or herbicides and wastewater containing nitrogen, and phosphorus nutrients can be utilized for algal cultivation. Their ability to grow rapidly under harsh conditions like seawater, saline water, and brackish water makes them noncompetent with the agricultural land (Behera et al. 2015). Algae can grow in harsh regions which makes it possible to use arid and non-fertile lands for their cultivation, and they give exponential biomass results when compared to terrestrial plants. They have the potential to produce a volume of biomass and biofuel many times greater than that of most crops and oilseeds. An acre of algal culture can produce 2000 to as many as 5000 gallons of biofuels per year.

Algae also efficiently remove atmospheric carbon. Like other plants, they use carbon dioxide and sunlight with water to perform photosynthesis to produce oxygen and convert atmospheric carbon dioxide to organic carbon. Algal biofuels can be an outstanding alternative for the growing crisis of fossil fuels. They are eco-friendly and cleaner and can be used in existing motor engines with slight or without any modifications. It is estimated that algae-based biodiesel (produce through fatty acid methyl transesterification) can reduce greenhouse gas emissions by more than 60% compared to petroleum diesel.

Microalgae (microscopic algae) are vast group of aquatic organisms with much lesser complex systems than plants and are capable of converting solar energy into chemical energy. They are found in diverse regions; exist in freshwater, sea, and saline environments; and are mostly photoautotrophic. Microalgae can be unicellular or multicellular that exist individually or in a group or chain. They can synthesize huge amounts of triglycerides (20–70% of dry cell weight) and polysaccharides which can be used as potential feedstock/raw materials for the production of biodiesel and bioethanol (Slade and Bauen 2013). Some microalgae like *Neochloris* sp., *Nannochloropsis* sp., and *Scenedesmus* sp. are proved to be suitable as raw materials for biofuel production (Gouveia and Oliveira 2009). Microalgae show two different modes of energy consumption which are photoautotrophic and heterotrophic. Photoautotrophic microalgae perform photosynthesis by using sunlight and carbon dioxide to produce energy for their growth. Heterotrophic microalgae can grow in dark by consuming organic compounds such as carbon as an energy source. Equally, both photoautotrophic and heterotrophic microalgae additionally require water and nutrients for growth.

Bio-compounds derived from microalgae have tremendous value in the market. Hence, microalgae are one of the assuring and sustainable sources of numerous industrially important products and biofuels (Tan et al. 2020). Algae have faster growth rates as compared to terrestrial plants (Randrianarison and Ashraf 2017). Therefore, microalgae are among the best candidate to be utilized as feedstock for biofuel production. The reason behind it is their high photosynthesis efficiency and capacity of accumulating high amounts of bio-compounds (Tan et al. 2020). They

can generate numerous chemical intermediates like hydrogen, hydrocarbons, carbohydrates, lipids, proteins, etc. which can be transformed into biofuels and products (Randrianarison and Ashraf 2017).

Several techniques are employed to generate abundant biomass of algae and exploit it to produce bio-oils and biodiesel. Research in this field discloses great potential of using algae as the feedstock to produce sustainable and biological-derived fuels for energy. Major challenges are to lower their price and make them available at good quality without compromising on their potential.

5.2 Microalgae Cultivation

Microalgae can be extensively exploited for the production of biomass which can be used as a renewable feedstock to produce biodiesel. They also show growth in adverse environments as they require very minimal nutritional requirements. Wastewater, brackish water, and even saline water can be used for algae cultivation. Agitation of the culture broth ensures uniform distribution of the nutrients and exposure to light. Microalgae can utilize CO_2 in the atmosphere as the carbon source. Phototrophic cultivation has an environmental advantage since it requires CO_2 as a source of inorganic carbon and sunlight as a source of energy to produce chemical energy by photosynthesis. However, this type of cultivation is limited by the photoperiod, intensity of light, and insufficient supply of CO_2. Moreover, irregular distribution of light also affects productivity. Heterotrophic cultivation includes microalgae that grow under the absence of light, in the dark, and utilize organic carbon as a source of energy, hence avoiding the problem with limited duration and intensity of light. It is necessary to add a source of organic carbon in case of heterotrophic cultivation which may lead to an increase in the cost of production of biofuel. In mixotrophic cultivation, microalgae grow in the presence of light and utilize CO_2 (inorganic carbon source) to perform photosynthesis so they can additionally acquire organic carbon provided as a source of energy. Mixotrophic cultivation is a combination of both phototrophic and heterotrophic cultivation which results in an increase in growth, resource utilization, and biomass production. It eliminates the disadvantages from both cultivations; hence, it is the most preferable cultivation system in the case of biofuel production from microalgae. Several species of microalgae can be cultivated in two broad ways, open raceway ponds and closed photobioreactors.

5.2.1 Open Raceway Ponds

Open raceway ponds are shallow ponds usually 0.25–0.4 m deep and open to the environment which can be built using concrete or can be simply dug from the ground and lined with plastic-like PVC or polyethylene. In some designs, paddle wheels are

used for constant mixing of the algal culture and maintain uniformity. Paddle wheels facilitate aeration and proper mixing of nutrients which increases their uptake by the algae. Based on the requirements, there are several designs of paddle wheels available. They require less space, are easy to cultivate, are highly scalable, and have low investment and low operating costs. But on the other hand, they have less yield of biomass when compared to other methods, a huge amount of water loss due to evaporation, and a high risk of contamination. The major drawback of this method is that the physiological and biological factors can't be controlled efficiently. However, open raceway ponds are the most preferable system for cultivation as they are less expensive in both operation and production costs (Chiaramonti et al. 2013).

Usually, the specific species of microalgae grow best under their respective optimal temperature, light, and climatic conditions. Also, the nutritional requirement varies from species to species. The optimal conditions are required for maximum yield. Carbon dioxide and minimal nutrients like phosphorus, nitrogen, hydrogen, and other elements are taken up for growth, and oxygen is released out. Though the marine photosynthetic algae can utilize the atmospheric CO_2, additional CO_2 is supplied to enhance their productivity. To ensure uniform mixing of nutrients and to avoid sedimentation, most of the open raceway ponds are supplied with rotating/agitating metal devices known as paddle wheels (Banerjee and Ramaswamy 2017).

These marine microalgae can be cultivated in freshwater, seawater, or even brackish water based on their purpose and the amount of yield required. Photoautotrophic algae are the best suitable source as they can utilize natural sunlight and fix more CO_2 and thus reduce the production cost. Their productive potential is dependent on the geographical location, species, availability of nutrients, and climate especially the factors like relative humidity, solar irradiance, wind velocity, etc. (Banerjee and Ramaswamy 2017). For assessment of microalgae growth in different climatic conditions and to determine their productivity potential in open raceway ponds, a bioreaction kinetics-based growth model was invented. The capital and operating costs for the cultivation were majorly determined based on mass and energy balances. Open raceway pond cultivation is suitable for high-volume and low-value products like biofuels.

5.2.2 Photobioreactors

Closed photobioreactors (PBRs) have enclosed medium in tubes or plates and the central reservoir circulates the microbial broth (Slade and Bauen 2013). They can intensify the culture biomass and have fewer chances of contamination as they are highly controlled systems. Different parameters required for the optimal growth of microalgae like the pH, temperature, nutrient level, CO_2, etc. can be regulated based on the requirements. There are two types of PBRs, tubular and flat/horizontal bioreactors used for different purposes.

Several algal species can grow and increase their cell mass using very low-quality resources like CO_2 from industries, wastewaters, etc. using this method. Algal species which exhibit a high growth rate, are capable of growing readily in any culture, have the ability to grow in a wide range of environmental conditions, and show high lipid production are chosen for cultivation (Dogaris et al. 2016). Recently, horizontal photobioreactors (HBR) are being developed for reducing operational costs and energy consumption and maximizing the yield with minimal nutrients (Dogaris et al. 2016). The prototype consists of an eight-blade paddle wheel which requires much lesser energy than the air compressor system for the culture mixing. Also, the results show that it has double the efficiency of airlift systems which is an added advantage. HBR is equipped with ceramic gas micro-diffuser which facilitates better CO_2 supply and circulation.

Flat panel photobioreactors are also extensively used for the cultivation of specific species in different geospatial locations. A heat and mass transfer model was developed to assess the microalgae productivity in flat panel bioreactors (Banerjee and Ramaswamy 2019). Many researchers usually carry out several indoor experiments using photobioreactors before actual cultivation to determine the optimal growing conditions, the economic reliability, and the production costs.

Another fascinating bioreactor called liquid foam-bed photobioreactor was invented as a novel approach for the reduction of operating and production costs (Janoska et al. 2018). The main principle of this reactor is that it contains algal suspension which can be circulated from the bottom to the top of the stabilized foam and then it drains back to the bottom through the foam. This ensures homogeneous distribution of algae and optimal nutrient mixing. For stabilizing the foam, a specific compound called Pluronic F68 was used in recent studies (Janoska et al. 2018).

5.3 Biomass Harvesting and Drying

Effective harvesting and drying of biomass after the cultivation step is crucial for efficient biofuel production (Chen et al. 2011). There are various techniques available for harvesting such as filtration, centrifugation, flotation, gravity sedimentation (Fig. 5.1). All these techniques aimed to remove as much culture media from microalgae biomass as possible so it can ease down the downstream proceedings (Tan et al. 2020). The presence of a high amount of water in the medium makes downstream processing such as extraction of metabolites and conversion to biofuel challenging. Some important points that need to consider while deciding on harvesting technique are choice of microalgae and the desired end product, the processing technique for further extraction and conversion, and the impact on these processing techniques.

Usually, microalgae harvesting is a two-step process, which includes bulk harvesting and thickening. Bulk harvesting aims to separate microalgal biomass from bulk suspension. It can accomplish with the techniques such as flocculation, flotation, gravity sedimentation, etc. Thickening aims to concentrate the slurry. It can

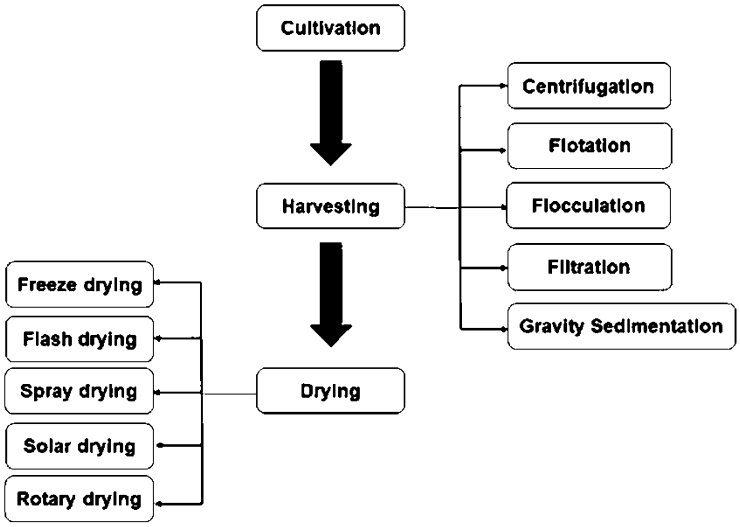

Fig. 5.1 General types of microalgae harvesting and drying techniques

be achieved with the techniques such as flotation, centrifugation, etc. (Brennan and Owende 2010).

5.3.1 Centrifugation

The centrifugation technique helps to separate a mixture containing molecules of various densities by applying centrifugal force. This force helps to create a differential pressure required for a particle to get separated from suspension (Al Hattab et al. 2015). The efficiency of this technique solely depends upon the algal size, settling properties of algal cells, retention rate and time of the slurry in the centrifuge, and density difference of the media components (Najjar and Abu-Shamleh 2020).

Disk stack centrifuge is one of the standard types of centrifuges commercially used in high-value algal products plants and in algal biofuel plants (Molina Grima et al. 2003). Separation of the material is done based on their densities. This technique is most suitable for separating the molecules of size ranging from 3 to 30 μm, and it has been reported that *Spirulina* sp. can be harvested using disk stack centrifuge (Al Hattab et al. 2015). This type of centrifuge is widely used to separate algal biomass for numerous applications including biodiesel plants (Najjar and Abu-Shamleh 2020). It requires high-energy input (Uduman et al. 2010); however, such high-energy input can be reduced by pre-concentrating the biomass using combination of separation techniques (Milledge and Heaven 2013). This centrifugation method can disrupt the cell and hence cause damage to the cell which

ultimately affect the total solid content and overall efficiency of centrifugation (Najjar and Abu-Shamleh 2020).

Basket centrifuges are beneficial for separating microalgae, mycelia, etc. They are most often used with a perforated basket overlaid with a filter bag of nylon, cotton, etc. Separation is done through a perforated wall which is based on the variation in the densities of solid and liquid phases. These centrifuges are usually operated at speeds of up to 4000 rpm for feed rates of 50–300 dm^3 min^{-1} and have a solid holding capacity of 30 to 500 dm^3.

Decanting centrifugation uses a specific settling tank in which the solids in suspension forced to settle down with the help of gravitational forces. It runs continuously by pumping the cultivated microalgae biomass inside the centrifuge bowl. Inside this bowl, the suspended particles in a liquid forced to settle down at the bottom of the bowl (hattab, 2015). This centrifuge is more suitable for high solid suspensions rather than those generated by microalgal growth ponds. It has suggested that decanter centrifuge can be of use to concentrate the microalgal slurries from the harvesting methods (Milledge and Heaven 2013). Total 28.5% harvesting efficiency at flow rate of 18 L/min has been observed through the use of continuous flow decanter centrifuge. It is advisable to first thicken the biomass by concentrating it up to 2–3% using dissolved air flotation method for better efficiency (hattab, 2015). Total microalgae of concentration of 22% (w/v) along with energy consumption of 8 kWh/m^3 using decanter centrifuge has been observed (Molina Grima et al. 2003).

Hydro-cyclones have a top cylindrical part connected to a conical bottom. Feed is introduced tangentially into an inlet opening near the top of the cylindrical part, and then the particles undergo the radial centrifugal force. If the radial centrifugal force is greater than the drag force, then the particles will be left from the fluid and move within the conical base. Unlike centrifuges, hydro-cyclones are comparatively inexpensive and possess no moving elements (Pahl et al. 2013). Hydro-cyclone can be used only for confined strains of microalgae and its effectiveness is reliant on solid concentrations. Even if hydro-cyclones are cost-effective, only limited strains of microalgae can be harvested, and its performance relied on a solid concentration (Milledge and Heaven 2013).

Centrifugation is an efficient process at both lab and large scales; it can be used for all microalgae. It has recovery rate of >90%. At larger scale, centrifugation process becomes time-consuming and highly expensive. Operational and maintenance cost is high for this method (Singh and Patidar 2018). Sometimes, fragile microalgal cells get disrupted during centrifugation due to gravitational or shear stress (Najjar and Abu-Shamleh 2020).

5.3.2 Filtration

Filtration techniques simply utilize a semipermeable membrane, through which when cultivated biomass moves, microalgae retain on the membrane and liquid

media is passed through it. Algal biomass retained on the membrane can be collected later. The semipermeable membrane used in this technique can have variable pore sizes depending upon the size of microalgae (Al Hattab et al. 2015).

Pressure difference that aids in filtration can be provided by vacuum, pressure, or gravity. Vacuum filtration method separates solids from the liquids by capturing solid particles on the membranes and allowing liquid to pass through it by using suction. It has been reported that vacuum or pressure filtration method is more suitable for separation of large size microalgae, but incompatible for smaller size microalgae (Molina Grima et al. 2003). *Spirulina platensis* species has been separated with the help of vacuum filtration equipped with membrane filter of pore size 0.45 μm (Stucki et al. 2009). Different types of membrane can be used in vacuum filtration, namely, vacuum drum filter, belt filter, starch precoated drum filter, and suction filter. Out of them, suction filter and starch precoated drum filter were found to be more compatible to filter out *Coelastrum* microalgae species. However, filter thickeners are not advisable because of high-energy consumption and less total solid recovery. Also, drum filters are not recommended because of high chances of clogging. However, filtration apparatus equipped with diatomaceous earth can avoid clogging of filters and helps in recovering microalgae species like *Chlamydomonas reinhardtii* and *Dunaliella* species (Brennan and Owende 2010; Gudin and Chaumont 1991; Molina Grima et al. 2003).

Pressure filtration technique can be used to separate solid particles of microalgae from a liquid suspension into their compact form with the help of pressure. In this filter, the major driving force for filtration is the liquid pressure generated by pumping or through the force of gas pressure in the feed vessel. Pressure filtration apparatus is equipped with plate and frame filter presses or pressure vessel for harvesting. Pressure filtration is more suitable for large size microalgae like *Coelastrum proboscideum* and *Spirulina platensis*; however, this method is not suitable for small size microalgae like *Dunaliella* and *Chlorella* species (Harun et al. 2010).

In cross-flow filtration, sample passes tangentially over a membrane. The microalgae having a large size than the membrane pores are retained and are known as the retentate. The microalgae having a smaller size than the membrane pores can pass through the membrane; this fluid extract which passes through the membrane is known as the permeate. Cross-flow filtration is more beneficial than other conventional harvesting methods like sedimentation, flocculation, etc. as this method completely removes debris and microalgae cells and maintains the shape and properties of recovered microalgae (Al Hattab et al. 2015). Cross-flow filtration and pressure filtration methods are energy-efficient methods (Harun et al. 2010).

The advantage of using filtration is that it is a cost-effective method and has an efficient recovery rate. There is no need of chemicals in filtration process. Low shear stress on the microalgae also helps in preventing the disruption of microalgae. Also, water that has been used in filtration process can be recycled. Natural and pressure filters consume less energy. However, vacuum pressures consume high energy. Limitations of this method include requirement of pressure and vacuum and high operational and maintenance cost. This method is not suitable for smaller algae.

Also, it is slower as compared to others. Sometimes, membrane used in filtration might get clogged and hence required maintenance or replacement. It increases operational and maintenance costs (Singh and Patidar 2018).

5.3.3 Flocculation

Flocculation involves the aggregation of unicellular microalgae to form aggregates known as floc, which allows sedimentation and easy extraction from the culture medium (Tan et al. 2020). Flocculating reaction occurred due to the addition of flocculating agent. They do so by shrinking ionic double layer, or by bridging between the particle with the help of high molecular weight polymer, or by neutralizing the surface charge present on the suspended particles. It includes changes in pH and the use of several compounds to modify the ionic compound (Branyikova et al. 2018). There are various ways to use flocculation for microalgae harvesting like chemical flocculation, bio-flocculation, and auto-flocculation.

In auto-flocculation, suspended microalgal cells impulsively aggregate to build large flocs. Then they induce the gravitational sedimentation on their own; such phenomenon is observed in some algal species. Such reaction is seen only under substandard conditions like cultural aging and change in pH. In some species, it was reported that alkaline or acidic condition decreases the intensities of the negative charge present on the microalgal cell and consequently promotes their self-aggregation (Ibrahim A. Matter et al. 2019).Carbonate salts can be used to precipitate algal cells in auto-flocculation (Chen et al. 2011).This technique is cost-effective and eco-friendly, no chemical flocculants are required, and the used medium can be reused after the harvesting process. However, this process is slow and species-specific (Ibrahim A. Matter et al. 2019).

Chemical flocculation works based on charge neutralization and electrostatic bridging between the suspended algae in the culture medium. There are two types of chemical flocculants, namely, organic and inorganic. Many organic polymers are widely used as flocculants in the microalgae harvesting process. Usually, cationic polymers are considered more suitable for microalgae harvesting, because they induce neutralization of the negative charge present on the surface of the algal cells, which eventually leads to the flocculation of the algal cells. Cationic polymers like chitosan (deacetylated chitin), cationic inulin, cationic starch, and poly-L-lysine can be used as an organic flocculant to harvest microalgae (Ibrahim A. Matter et al. 2019). It has been reported that chitosan can be used as a flocculant for harvesting microalgae *C. sorokiniana* (Xu et al. 2013). Inorganic flocculants work best under low pH environments for cationic hydrolysis products; under such conditions, these inorganic flocculants form polyhydroxy complexes (hattab, 2015). Salts of iron and aluminum form cationic hydroxides in an aqueous media and destabilize negatively charged algal cells, subsequently causing flocculation of microalgal cells (Ibrahim A Matter et al. 2019). The combined flocculation process operates in a multistep manner, requiring the usage of more than one type of flocculants. To flocculate

seawater, two methods have been discovered. The first method involves the use of polyelectrolytes with inorganic flocculants like alum or ferric chloride. The second method involves the use of ozone oxidation and then the addition of flocculants (Chen et al. 2011).

Electrochemical flocculation is performed by moving a direct electrical current with the help of an electrode into a culture medium. Sacrificial and non-sacrificial electrodes are two types of electrodes available to perform this technique. A sacrificial anode is made up of iron and aluminum or magnesium, copper, zinc, or brass. This anode releases positively charged metal ions. The surface of microalgae has a negative charge; hence, they get attracted toward the positively charged ions and eventually form flocs. A non-sacrificial electrode is made up of carbon. Under a direct current, negatively charged microalgal cells travel toward this anode. Once these cells reached the charged anodes, they get neutralized, meaning they lost the negative charge present on them, eventually resulting in flocculation (Matter et al. 2019).

Bio-flocculation involves the use of self-flocculating microorganism or their part in the culture medium to harvest targeted algae. This microorganism can be a fungus, yeast, bacteria, or algae itself (Fig. 5.2). Algal-fungal bio-flocculation involves the use of fungi as a bio-flocculant. Various filamentous fungi have been reported to possess self-pelletizing capacities. With the help of this capability, they can entrap and adsorb microalgal cells. Four fungal species (*Aspergillus lentulus*, *A. terreus*, *Polyporus* sp., and *Rhizopus oryzae*) have been reported to hold co-pelletizing capability (Matter et al. 2019). Algal-yeast bio-flocculation involves the co-cultivation of yeast with algae; together they stimulate the production of biomass and lipid. It also supports efficient flocculation harvesting. Synergistic increase in lipid content (60–70%) and biomass production (40–50%) by co-culturing yeast, *Rhodotorula glutinis*, and algae, *S. obliquus*, has been reported. This technique seems to be environmentally friendly (Matter et al. 2019).

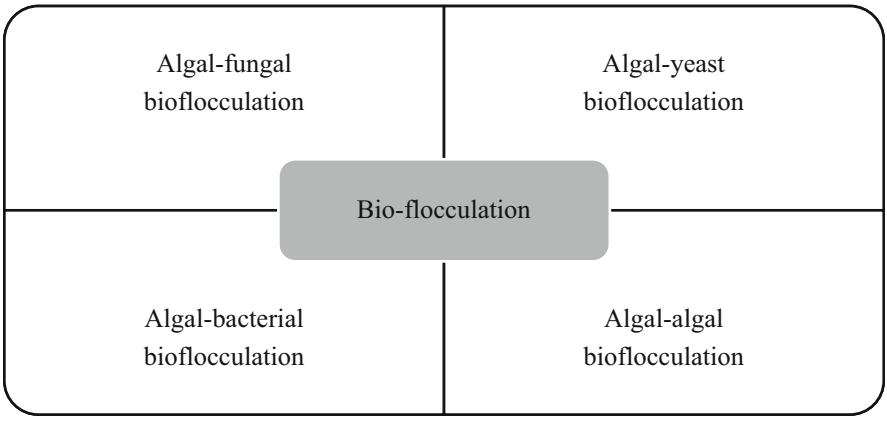

Fig. 5.2 Bio-flocculation of algae with other microorganisms

Some bacteria already endure during the algal cultivating process; their interaction with algae can either be inhibitory or beneficial. Various algae-associated bacteria like *Flavobacterium*, *Terrimonas*, and *Sphingobacterium* have been reported to stimulate the formation of larger flocs which aid in efficient harvesting (Lee et al. 2013). The faster growth rate of bacteria provides more benefits as compared to other bio-flocculants like fungus or yeast. The drawbacks of this method are that these bacteria are species-specific and might have some environmental safety issues (Matter et al. 2019). Algal-algal bio-flocculation involves the usage of self-aggregating algae or their extracellular polymeric substance (EPS) as a flocculating agent to harvest targeted non-flocculating algae. Such self-aggregating species of algae (*C. vulgaris JSC-7*, *Ankistrodesmus falcatus*, *S. obliquus AS-6-1*, *Scenedesmus* sp. *BH*, *E. texensis*, and *T. suecica*) have been reported (Matter et al. 2019).This method can be enhanced by optimizing environmental conditions like pH, e.g., pH 4.5 improves the harvesting efficiency by 90% for *C. zofingiensis* and *C. vulgaris*, utilizing three self-aggregating algae, specifically *C. nivale*, *C. ellipsoideum*, *and Scenedesmus sp.* (Liu et al. 2014) As this technique involves the use of algae only, it decreases the chances of microbial contamination and is also beneficial for post-purification processes (Matter et al. 2019).

Flocculation is a fast and easy technique with lesser chances of cell damage. It can be applied for various species. It can be utilized for larger scale. Flocculation-based biomass harvesting is potentially energy efficient. Auto-flocculation or bio-flocculation may be an inexpensive technique. Disadvantages of flocculation involve limited culture medium recycling, chances of mineral or microbial contamination, and difficulty in coagulant separation from harvested biomass. Additionally, it is a pH-dependent technique and chemical utilized in flocculation can be expensive. Also, efficiency varies as per the type of coagulant used (Ibrahim A. Matter et al. 2019).

5.3.4 Flotation

The flotation technique uses micro-air bubbles to trap microalgae in it so that they can float on the surface of the culture. As they float on the medium, they can be harvested with ease by skimming process (Tan et al. 2020). Many types of flotation systems are available that produce air bubbles to trap microalgae. Dissolved air flotation system produces air bubbles using compressed air. This air can saturate the culture and then releases culture at atmospheric pressure (Tan et al. 2020). Supply of air into the system can be regulated by managing the pressure of the saturator. Also, the size of the bubbles can be managed with the help of a saturator. Small bubbles of range 10–100 μm are advantageous.

Dispersed air flotation system generates air bubbles by means of a mechanical agitator or sparger. This system can generate air bubbles of size 700–1500 μm. Chemicals like SDS (sodium dodecyl sulfate), CTAB (cetyltrimethylammonium bromide), chitosan, saponin, etc. have been used to improve the flotation.

Electrolytic flotation technique uses hydrogen bubbles formed by the electrolysis of water. This hydrogen bubble traps microalgae in the culture medium. Cathode used in electrolytic flotation is made up of inactive metals like steel which generates hydrogen bubbles (Al Hattab et al. 2015). Advantage of this technique is that no chemicals are required, while high-power requirement and cathode fouling are a few of the disadvantages (Tan et al. 2020). In ozone dispersed flotation rather than using atmospheric air, ozone is used as a source to produce bubble. Ozone is a strong oxidizing agent; it oxidizes soluble organic matter and charged bubbles separate the microalgae. But it is considered as one of the costlier processes. Flotation is suitable for large-scale process; it is essentially a low-cost process, and it requires minimal space and less time for operation. Limitations of flotation are that they required surfactant which creates issue in further downstream processing. Technique like ozone-dispersed flotation is expensive (Singh and Patidar 2018).

5.3.5 *Gravity Sedimentation*

Usually, this method is used to separate microalgae from water (Chen et al. 2011). This method is solely based upon Stokes' law, i.e., radius and the density of cells will determine the sedimentation rate. Although its efficiency can be enhanced by using lamella separators and sedimentation tanks, this process is slow and energy demanding and might require high maintenance. This method is only suitable for microalgae having a size of >70 μm such as *Spirulina.* The harvesting efficiency of this method is >95% (Brennan and Owende 2010).

Gravity sedimentation can be applied to harvest algal species like *Micractinium*, *Scenedesmus*, and *Spirulina* with cluster diameter ~ 60 μm; however, smaller algal species like *Chlorella* and motile microalgal species like *Euglena* and *Chlorogonium* do not get settle down easily and hence cannot be harvested using gravity sedimentation (Nurdogan and Oswald 1996). Sedimentation process of microalgae depends upon the light intensity, amount of nutrients, age of microalgal cell, lipid content, temperature, sedimentation time, etc. (Nurdogan and Oswald 1996). It has been observed that dinoflagellates and many other species of microalgae propel upward approaching the light. Also, insufficient amount of nutrients decreases the sedimentation rate and increases in case of older microalgal cells and long sedimentation time (Milledge and Heaven 2013).

5.3.6 *Combination of Harvesting Methods*

It has been observed that use of combination of various harvesting methods instead of applying single harvesting method is the more economical choice. Microalgae first can be concentrated up to 2–7% total suspended solid using flocculation method, and then cells further can be concentrated using another method such as

filtration or electrophoresis. By integrating harvesting methods dissolved air flotation and chemical flocculation, increase in the recovery efficiency from 88 to 95% for *Chlorella vulgaris* was observed. In a method combination of electrolytic coagulation and electrolytic flotation is used for continuous harvesting of microalgae. With the help of electrodes, the current creates two phases. The first phase acts to destabilize the microalgae cells having a negative charge and eventually form flocs. Metal ions discharged from the electrode induce the formation of flocs. In the second phase, the metal ion production is halted, and the bubbles created from each electrode elevate the flocs to the top of the solution inducing them to float (Kim et al. 2012). Harvesting efficiency of 98.9% in 14 mins was reported for *Botryococcus braunii* when two harvesting methods, i.e., electrolytic flocculation and dispersed air flotation, are combined (Xu et al. 2010). Such various combinations of harvesting methods can be employed for efficient recovery as well as to reduce the cost.

Extensive study has been made on numerous harvesting techniques of microalgae. As the microalgae population has great variation, it is challenging to select one specific method as a preferred one. Selection of harvesting method will depend on the aspects of targeted microalgae (Uduman et al. 2010). Centrifugation is a potential microalgae harvesting technique; however, centrifugation by itself is not suitable for large-scale production of biofuel from microalgae, because of high cost and high-energy consumption. The most widely used and most efficient centrifuge device is a disk stack centrifuge. If the centrifuge is being utilized in microalgae harvesting at a larger scale, one can combine centrifuge with any other inexpensive method like flocculation to decrease the cost (Najjar and Abu-Shamleh 2020). Centrifugation is preferred over a flocculation method because of its high recovery rate (Molina Grima et al. 2003). Whenever microalgae in the process are fragile, centrifugation cannot be employed. In such cases, filtration is the preferred alternative (Brennan and Owende 2010).

5.3.7 Drying Techniques

Drying process removes moisture contained in the wet material. After harvesting process, biomass slurry is perishable and hence needs processing. Biomass viability can be extended by drying or dehydration process (Brennan and Owende 2010). Major algae drying methods involve rotary drying, spray drying, solar heat drying, flash drying (Fig. 5.1). In areas where energy supply is an issue, solar heat drying can be used to dry the microalgae biomass. It can be performed by direct solar radiation or by use of a solar heater. But algal drying by direct solar radiation can dehydrate the algal chlorophyll, thereby changing the texture and quality of the final algal product. The major limitation of this method is overheating and requirement of large space and operational unreliability. Also, there are possibilities of spillage and risk of fermentation (Show et al. 2015a, b). Total triglyceride recovery can get affected at higher temperature. Fatty acids present in this triglyceride get oxidized at higher

temperature. Solar drying at higher temperature is not advisable, as the biodiesel obtained from the microalgae consist of both saturated and unsaturated fatty acid ester compounds and they can get affected at such higher temperature (Mallick et al. 2016).

The rotary drying method involves the use of a rotary dryer having a slope. This dryer can be used to transfer algae being dried from one edge to the other by gravitation. This method has dual advantages: that it can be used to sterilize the sample as well as to break the cell wall of microalgae. Microalgae species *Scenedesmus* has been successfully dried using rotary drying method at 120 °C for 10 s (Show et al. 2015a, b). Limitations of rotary drying include the high processing time and temperature. High temperature used to heat up the drum can deteriorate the quality of microalgal biomass (de Farias Neves et al. 2020).

Flash drying method can be used to rapidly dry the harvested algal biomass to remove its moisture content by spraying or injecting a mixture of wet algal biomass into a hot gas stream. This hot gas stream has served as a carrier for mass transfer.

Spray drying is one of the common methods utilized to dry microalgal biomass (de Farias Neves et al. 2020). It involves liquid atomization, gas droplet mixing, and drying from liquid droplets. The water droplets which are atomized are sprayed downward within a vertical column into which heated gases pass downward. The dried product then can be removed from the vertical column. Utilizing this method, drying can be achieved within a few seconds (Show et al. 2015a, b). Factors that affect the spray drying process are air temperature, size of droplet, liquid flow, and biomass characteristics like surface tension, liquid density, composition, and viscosity (de Farias Neves et al. 2020). The limitations of this method are the high operational cost and low digestibility of dried algae for its food or feed application (Show et al. 2015a, b) and loss of volatile compounds, and heat-labile products. Also, high pressure can disrupt the cell membrane of microalgae during atomization; it can lead to oxidation and degradation of functionally important compounds (de Farias Neves et al. 2020). This can be avoided by mixing the microalgal biomass along with some encapsulating agents like gum Arabic, maltodextrin, etc., and by this strategy, microcapsules can be made using spray drying method, which retains the important functional groups in microalgae (de Jesús Bonilla-Ahumada et al. 2018).

Freeze-drying is one of the well-known drying methods. In this method, biomass is first frozen and then transferred to the vacuum chamber to sublimate the water. Freeze-drying method can be applied to the microalgal biomass which are sensitive to the high temperature and oxygen exposure. Limitations of this method are high installation and operational cost. Also, this method can cause oxidation of lipids and pigments due to low content of water; hence, vacuum packaging is advisable to store the biomass. It avoids the degradation of functionally important component of microalgae during storage (de Farias Neves et al. 2020).

Even though drying of microalgae helps in the handling of the dried microalgal biomass, drying methods are considered as inexpensive and energy inefficient (Show et al. 2015a, b). Usually, heat drying is used to dry wet algal biomass (Mallick et al. 2016). It is considered as a primary choice for drying algal biomass,

as it is a cheaper and pollution-free method. However, it requires large land area to dry large amount of wet algal biomass (Mallick et al. 2016).In terms of installation and operational cost, spray drying and freeze-drying methods are the only recommended for small-scale operations. If algal biomass is to be used for human, spray drying is the preferred method, because it is sufficient for the large-scale production. However, this method can disrupt the cell structure (de Farias Neves et al. 2020). Necessary temperature and time to dry the microalgae will depend on the microalgae species. One such study has been done on *Scenedesmus* species, which was dried at various temperatures. They found out that lipid recovery was more than >90% when the initial sample partially dried with 10% end moisture content, which results in less consumption of power at later stage of drying (Bagchi et al. 2014). Such studies can be done before applying any drying method at large scale, in order to save energy.

5.4 Biodiesel Production from Microalgae

Microalgal biodiesel is gaining interest because of its suitable fuel properties, high lipid accumulation in microalgae, and faster growth rates. The interest in biofuel has been growing because it successfully decreases the dependence on imported oil in the transport sector (Pinzi et al. 2014). Biodiesel production from microalgae includes several steps such as cultivation, harvesting, biomass drying, extraction of lipids, and conversion to biodiesel (Table 5.1). Biodiesel can be produced through two routes, i.e., a dry route where wet algae biomass is dried using several drying techniques before extraction, whereas in the wet route, direct wet algal biomass is used for extraction (Fig. 5.3). An investigation suggested that though these two routes produce similar quality biodiesel, there is a difference in overall energy consumption and energy-intensive steps.

5.4.1 Lipid Extraction from Microalgal Biomass

Microalgal biomass is comprised of carbohydrates, lipids, and proteins. Lipids are converted to biodiesel by using the transesterification process. Thus, it is important to extract lipids from harvested microalgal biomass. Various extraction techniques are studied for the recovery of lipids from microalgal biomass. These techniques involved solvent extraction methods coupled with cell disruption techniques to improve yields (Guldhe et al. 2016). Cell disruption techniques used for both dry and wet algal biomass are autoclaving, microwave, electroporation, bead beating, oxidative stress, and ultrasound (Ghasemi Naghdi et al. 2016). The efficiency of the lipid extraction process depends on various factors such as cell wall structure, quality of lipids, microalgal strain, etc.

Table 5.1 Various microalgae used for biodiesel production, extraction methods, lipid yield, catalyst used, and biodiesel conversion (%)

Sr. no.	Microalgae strain	Extraction method	Lipid yield (%)	Catalyst (temp/rpm/ time)	Biodiesel yield (Y) and conversion (C) (%)	Reference
1	*Scenedesmus obliquus* FR751179.1	Microwave-assisted solvent extraction	29%	Lipase (35 °C / 200 rpm/ 12 h)	C = 66.55%	Guldhe et al. (2015)
2	*Acutodesmus obliquus*	Microwave-assisted solvent extraction	54.04%	H_2SO_4 (60 °C/ 200 rpm/ 4 h)	C = 94.76%	Singh et al. (2016)
3	*Acutodesmus obliquus*	Microwave-assisted solvent extraction	33.9%	Lipase (50 °C/ 200 rpm/ 8 h)	C = 95.36%	Guldhe et al. (2019)
4	*Chlorella* sp. KM401849 and *Nannochloris* sp. KP119843	In situ supercritical methanol transesterification	12% & 21%	Catalyst free	Y = 45.62% and Y = 21.79%	Jazzar et al. (2015)
5	*Spirulina platensis*	Osmotic shock and solvent extraction	8.9%	H_2SO_4 (60 °C/ 200 rpm/ 3 h)	C = 79.5%	Sumprasit et al. (2017)
6	*Chlorella pyrenoidosa*	n-Hexane/ methanol solvent extraction	–	H_2SO_4 (120 °C/ 3 h)	C = 93.2%	Cao et al. (2013)

Organic solvents commonly used for the extraction of lipids from microalgae are benzene, hexane, acetone, chloroform, methanol, etc. (Harun et al. 2010). Two types of microalgal lipids are found such as neutral lipids (e.g., triglycerides) and polar lipids (e.g., phospholipids). Hexane is one of the most efficient nonpolar organic solvents which extract neutral lipids by penetrating the cell membrane and interacting with cytoplasmic neutral lipids, while polar solvents extract polar lipids (Guldhe et al. 2016). Many researchers advised that solvents like chloroform and methanol, i.e., the mixture of polar and nonpolar solvents, are efficient for lipid extraction, though both are highly toxic in nature.

Soxhlet extraction technique is one of the most widely used organic solvent extraction processes for oil extraction by oil industries from different dry microalgae and plants (Kirrolia et al. 2013). It is a temperature-dependent method, ranging from 30 to 60 °C, and temperature above this decreases lipid yield. The Soxhlet extraction method is simple, but it is time-consuming as well as lipid yields are very low. Sonication is a mechanical disrupting method where microalgal cells are disrupted because of sound waves which create cavitation in it. The energy or shockwave

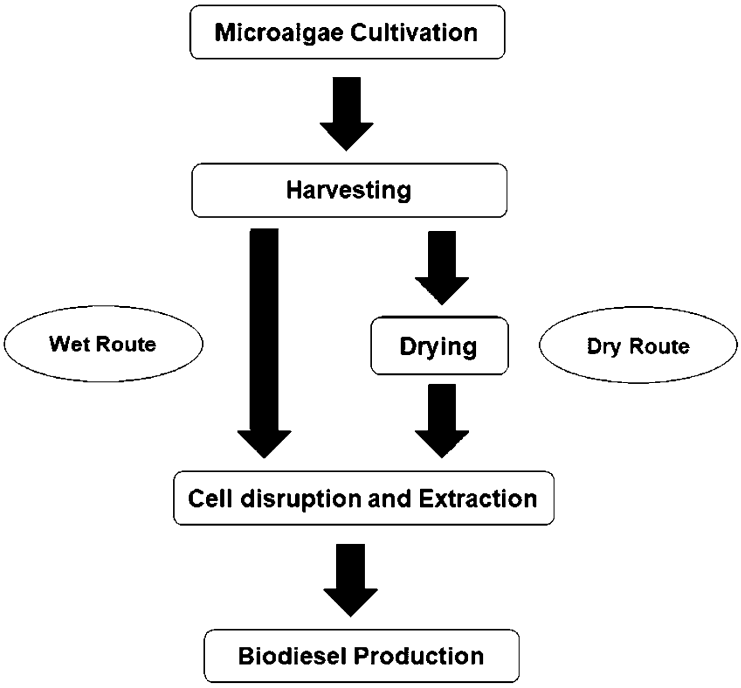

Fig. 5.3 Microalgae biodiesel production routes

generated from this technique by moving the bubbles in the media which collapse with each other helps to shatter the cell wall to release the intracellular content (Suslick and Flannigan 2008). This technique could be cost-intensive because it needs sophisticated instrumentation.

The microwave technique is one of the efficient cell disruption methods in lipid extraction, first established in the mid-1980s. Disruption of cells occurs due to energy generated by the rotation of the molecular dipole and by tremendous pressure which disrupts the hydrogen bonds. Heat produces water vapor within the cells which ruptures the cell membrane to release the intracellular contents. This technique can also be used for extraction from wet microalgal biomass. The higher temperature in the microwave technique results in higher oil extracting efficiency compared with the Soxhlet extraction method (Aarthy et al. 2018). Recently, the microwave method is evaluated to be a cost-effective technique for wet lipid extraction. However, at commercial scale, maintenance costs act as a limiting factor (Ghasemi Naghdi et al. 2016). Autoclaving technique is also an efficient method for microalgal biomass lipid extraction; the cell disruption occurs by the diffusion of heat from the surrounding of the cell. Autoclaving is an energy-intensive process, and the lipid yield is lower than the sonication and microwave method. In the bead beating technique, beads are continuously ground against the cells inside the vessels for the disruption of the algal cell wall which leads to cell wall rupture, resulting in the release of intracellular contents into the solvent medium (Aarthy et al. 2018).

Supercritical fluid extraction is a promising green technology as well as an effective thermochemical process. Specific extraction temperature and pressure are required here; this should be above the critical liquid temperature and pressure. The obtained lipid from this extraction technique does not need to go through the solvent recovery step because it is free from solvents (Guldhe et al. 2016). Supercritical CO_2 is the frequently used microalgal lipid extraction solvent, as it has critical pressure as well as moderate temperature. Supercritical lipid extraction technique has several advantages compared with the traditional extraction methods used for microalgal lipid extraction which includes shorter extraction time, has higher selectivity, and does not use conventional toxic organic solvents (Santana et al. 2012).

5.4.2 Conversion of Lipids to Biodiesel

Biodiesel is a mixture of fatty acid methyl ester which is obtained by transesterification of animal fats, plant oils, and microalgal lipids as raw materials (Abomohra 2016). For any catalytic reaction, raw material along with substitute material is needed that will help to develop into the product. In transesterification methanol or ethanol will interact with microalgal oil to generate the biodiesel, and chemical catalysts are homogeneous or heterogeneous or enzyme biocatalysts (Fig. 5.4). Transesterification is mainly a three-step reversible process where triglycerides first are converted to diglycerides, then diglycerides converted to monoglycerides, and then finally monoglycerides converted to glycerol and fatty acid alkyl esters (biodiesel). Stoichiometrically, for transforming one mole of triglyceride into an ester (biodiesel), three moles of alcohol are required.

5.4.2.1 Chemical Catalysis

Catalysts play an important role in transesterification; they can be either heterogeneous or homogeneous (Fig. 5.5). Chemical catalysts used are acidic or alkaline. Homogeneous catalysts have several advantages such as high selectivity, fast reaction rate, and easy availability. NaOH, KOH, CH_3ONa, and CH_3OK are some commonly used alkali catalysts for biodiesel production. In the presence of water and free fatty acid, alkaline catalysts produce soap which reduces the biodiesel yield and quality (Thangaraj et al. 2019). In such cases, a two-step process is followed which includes esterification of free fatty acids by acid catalyst and

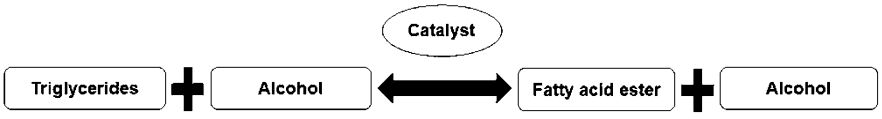

Fig. 5.4 Transesterification process

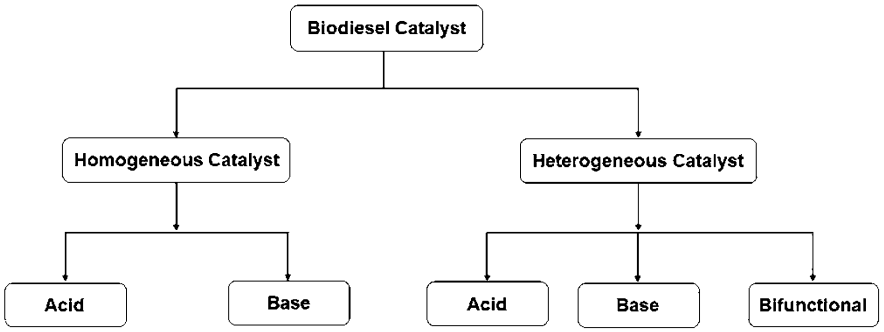

Fig. 5.5 Catalysts for biodiesel production

transesterification by an alkaline catalyst. Homogeneous catalysts require multiple washing steps for their removal from the product. To improve the transesterification process, heterogeneous catalysts are being used because they eliminate the extra processing cost which is involved in the removal of homogeneous catalysts. Heterogeneous catalysts (solid base, solid acid, acid-base) can resist harsh conditions like high temperature and pressure. The commonly used heterogeneous catalysts include CaO, MgO/KOH, SrO, K_2CO_3/Al_2O_3, KF/ZnO, sodium silicate, dolomite, CaO/Fe_3O_4, Mg-Al hydrotalcite, KNO_3/CaO, etc. (Faruque et al. 2020).

5.4.2.2 Biocatalysts

Biocatalytic conversion is a greener approach that can overcome the demerits of chemical catalysts. Biocatalyst employed for the transesterification of microalgal lipid biomass is mainly the enzyme lipases, such as free lipase and immobilized lipase. Enzymes are more efficient than homogeneous chemical catalysts because of their environmental acceptability, biocompatibility, and biodegradability (Thangaraj et al. 2019). They also have great catalytic activity as well as stability in a nonaqueous environment. Microbial lipases are drawn for esterification and transesterification from various microorganisms such as *Aspergillus niger*, *Bacillus thermoleovorans*, *Candida rugosa*, *Chromobacterium* sp., *Geotrichum candidum*, *Fusarium heterosporum*, *Humicola lanuginosa*, *Mucor miehei*, *Chromobacterium viscosum*, *Rhizopus thermosus*, *Rhizopus usamii*, *Rhodotorula rubra*, *Staphylococcus hyicus*, *Pseudomonas putida*, *Rhizopus arrhizus*, *Rhizopus japonicus* NR 400, etc. (Gunner and Alexander 1964). Intracellular lipase from these microorganisms can reduce the cost and processing steps as a biocatalyst. Whole microbial cell-based catalysts can be used in transesterification. Immobilized lipases are stable than free lipases because the turnover number of free lipases at 50 and 60 °C is 88.46 and 75.38%, but in the case of immobilized lipase, it is 95.86 and 81.26%, respectively. The product separation and purification process is easier in enzyme catalysis than the chemical catalysis.

5.5 Bio-oil Production from Microalgae

Microalgae can be converted to various fuel types through different conversion routes (Fig. 5.6). Bio-oil is a viscous liquid with a distinctive smoky odor. It is dark brown in color. Depending on feedstock type, operating conditions, thermochemical process, and physical properties, the chemical composition of bio-oil differs. Bio-oil is a complex mixture of different organic compounds, mainly containing alcohols, acids, aldehydes, ketones, esters, phenols, and guaiacols. Some of these mentioned compounds are also responsible to add undesirable properties of bio-oil. Therefore, bio-oil needs to be processed before use (Saber et al. 2016). For the production of bio-oil, microalgae are considered as an option. Many factors contribute to this choice such as high photosynthetic efficiency, ease of cultivation, and fast growth rate, they don't occupy the arable land area, and they have the potential to be converted into bio-oil as well as other biofuels (Hao et al. 2021). Compared with biofuel from the plant, biofuel from microalgae has a low viscosity, a low density, and a high caloric value. These properties make microalgae a suitable option to produce biofuel (bio-oil) than lignocellulosic materials (Kiran Kumar et al. 2018). For producing bio-oil, mainly the techniques used are organic solvent extraction, mechanical disruption, hydrothermal liquefaction (HLT), ultrasound, microwave radiation, and supercritical fluid extraction (Hao et al. 2021). The techniques that are used to produce energy from microalgae are mainly classified into biochemical and thermochemical. The development of bio-oil production techniques is carried out by several researchers (Chaiwong et al. 2013). The synthesis of bio-oil or any biofuel from algal biomass follows the following steps: culturing (algae), harvesting/dewatering (algae), extraction of oil (algae), purification (bio-oil), and processing of oil (biofuels) (Ganesan et al. 2020). Bio-oil is a very promising and renewable energy source that is acknowledged around the world. Currently, there are two main processes for bio-oil production from biomass:

1. Pyrolysis
2. Hydrothermal liquefaction

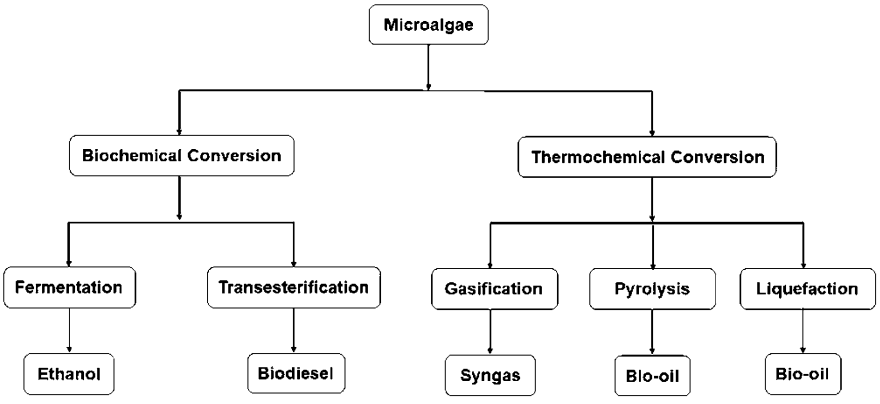

Fig. 5.6 Conversion of microalgal biomass to biofuels

5.5.1 Bio-oil Production Using Pyrolysis

Pyrolysis is a process of chemical decomposition of organic materials at high temperatures in the absence of oxygen. This process occurs at a temperature above 430 °C and under pressure. Pyrolysis is a widely used technique for the production of bio-oil from biomass. Chaiwong et al. (2013) examined biochar and bio-oil produced from *Spirulina* sp. by using slow pyrolysis. They used a thermogravimetric analyzer (TGA) to study the essential components and pyrolytic characteristics of algae. Studies indicated that the temperature for maximum degradation was 322 °C, lower than that of other techniques generally used for bio-oil production. The most suitable temperature to obtained bio-oil was approximately at 550 °C. The components of bio-oil can be identified by using gas chromatography/mass spectrometry that detected a range of saturated functional carbon in the bio-oil, and a total of 24 components were identified. Main groups of aromatic hydrocarbons, phenol, heterocyclic, amine, amide, indole, nitrile, and alkane were found. The boiling point range of bio-oil produced was between 100 and 300 °C. It is observed that the water content of bio-oils obtained from *Spirulina* sp. is comparatively higher than fossil fuel oil. Because of that, the flame temperature and heating value of bio-oil obtained are lower. But higher water content can help decrease viscosity as well as can enhance the fluidity of bio-oil. These properties are good for the combustion and atomization of bio-oil in engines. It was observed that bio-oil from *Spirulina* sp. has a high proportion of oxygen which is similar to wood. Due to that, the bio-oil is not stable and quite reactive compared to fossil fuel. A deoxygenation process is necessary to upgrade obtained bio-oil. This technique could help to maintain the quality of oil during storage. The (ECR) energy consumption ratio of bio-oil was calculated and resulted net energy output was observed positive. An average value of ECR observed was 0.49 at 550 °C; therefore, it can be concluded that pyrolysis of *Spirulina* sp. is a net energy producer (Chaiwong et al. 2013).

High nitrogen content in bio-oil has a negative impact on fuel properties. Du et al. (2012) studied *Nannochloropsis oculata* biomass transformation into an energy-packed bio-oil by using the pyrolysis method. The main problem was the high nitrogen content of this bio-oil that created a challenge for using it directly as fuel. For this reason, hydrothermal pretreatment was used to lessen the nitrogen content in microalgae *Nannochloropsis oculate*. It facilitated in eliminating proteins without needing significant inputs of energy. Then the effects of reaction circumstances on the composition and yield of pretreated algae were studied by changing the reaction time (10–60 min) and temperature (150–225 °C). In comparison with untreated algae samples, pretreated samples were observed to have high carbon contents, and their heating values enhanced under all the reaction conditions. The pretreated biomass showed 6–42% lower nitrogen contents at 200–225 °C temperature which was treated for 30–60 min. The pyrolytic bio-oil obtained from pretreated algae had fewer nitrogen-containing compounds compared to untreated samples. The bio-oil consists mainly of long-chain fatty acids (C_{14}–C_{18}). These fatty acid chains can be

converted into hydrocarbon fuels by using simple catalysts. More than 70% of initial lipids were observed to be retained in pretreated algae. Therefore, by this study, authors proved that hydrothermal pretreatment is an effective strategy for producing high-quality bio-oil by pyrolysis using algal biomass (Du et al. 2012).

Pyrolysis can be used for conversion of whole microalgal biomass as well as for residual biomass left after extraction of metabolites like lipids or proteins used in other applications. (Francavilla et al. 2015) aimed to produce bio-oil and char as end products by pyrolysis using whole microalgal biomass (MA) and residual lipid extracted remaining biomass (R) of *Dunaliella tertiolecta*. The pyrolysis was carried out at a temperature of 600 °C that gave bio-oil maximum (45.13 wt.%) yield (from R). The chemical and physical properties of bio-oil directly have an effect on its application, so in this study, they performed fast pyrolysis experiments in a range of temperature between 450 and 750 °C. The yields of oil produced from R (residue of microalgae) and MA (microalgae) were maximum at pyrolysis temperature of 600 °C. They checked the quality of bio-oil produced from the *D. tertiolecta* biomass. Their composition was then studied, and it was observed that bio-oil derived from residual biomass has low C and higher N and S content compared to bio-oil produced by whole biomass. The higher heating value (HHV) for R-derived bio-oil was detected to be 22.20 MJ/kg, which was lower than bio-oil derived from MA (23.51 MJ/kg). It has resulted in this work that the residue (R) of microalgae *D. tertiolecta*, after extraction of lipids, can be further valorized through fast pyrolysis for bio-oil production with a high yield of 45.13 wt.% at 600 °C. However, the quality of the oil obtained was not suitable to be used as fuel, without further processing (deoxygenation and denitrogenation). So, from this study, it can be concluded that residual lipid extracted remaining biomass (R) can be used for bio-oil production with further processing. That way the use of biomass can be maximized (Francavilla et al. 2015).

In microalgal growth conditions, the nutritional mode also affects the quality of biomass which eventually influences the fuel properties of bio-oil. Miao and Wu (2004) manipulated the metabolic pathway present in microalgae through heterotrophic growth. The heterotrophic *Chlorella protothecoides* cells gave a bio-oil yield of 57.9%, which was 3.4 times higher than autotrophic cells. The bio-oil produced was characterized with a high heating value of 41 MJ kg^{-1}, lower oxygen content, low density (0.92 kg l^{-1}), and low viscosity (0.02 Pa s) which was observed to be lower compared to those of bio-oil produced from autotrophic cells as well as wood. These gained properties of bio-oil were comparable to fossil oil. The manipulation of metabolic pathways that led to heterotrophic growth of *Chlorella protothecoides* gave promising results for bio-oil production. This study can be helpful in developing other microalgae species to produce a high yield of bio-oil (Miao and Wu 2004).

5.5.2 *Bio-oil Production Using HTL*

Hydrothermal liquefaction (HTL) is a thermochemical process (thermal depolymerization process) in which conversion of wet biomass into bio-crude oil takes place. Typically, this process occurs at 200–400 °C temperature and high pressure in between 10 and 25 MPa. To utilize the microalgae biomass completely, the residual biomass can be used (which remains after biodiesel production from lipids) for the production of bio-oil. Shahi et al. (2020) investigated the possibility of crude oil production using the residual biomass of *Dunaliella* sp. by using HTL. According to HTL results, the average yield of bio-oil was 11.81 w/w%, at 350 °C, 60 min of residence time, and pressure of 200 bars. The CHNS analysis results showed that produced crude oil contains 4.82% nitrogen, 21.9% sulfur, 68.53% carbon, and 1% hydrogen, which is found in permitted ranges. However, there is a restricted amount of information present currently about the potential of bio-oil production from microalgae residual biomass after the lipid extraction process. This study helps in analyzing the challenges that come across in this process application. Even though the HTL method is considered good for converting hydrocarbons into biofuel, it still needs to be developed to be used on an industrial scale. The efficiency of this method is reported to be in a range of 50%–60% which is good, but it also depends on the use of homo-/heterogeneous catalysts. Although the HTL method has the potential to extract fuel from biomass, there is restricted information present about the structural effects of microalgae biomass on molecular properties of bio-crude, produced from HTL processes, compared to other thermochemical methods like pyrolysis (Shahi et al. 2020).

Efforts have been made for the production of biofuels from residues of agriculture and other sources, but in comparison, very few investigations on microalgae residue have been performed at that time. Shuping et al. (2010) studied microalgae *D. tertiolecta* cake to find out its ability to produce bio-oil by using the hydrothermal liquefaction process. The main objective of this study was to evaluate the usefulness of *D. tertiolecta* cake to give liquid fuels and to find out the effect of the chemical composition of microalgae *D. tertiolecta* cake on the quality and yield of bio-oil. The various chemical and physical characteristics of bio-oil produced under suitable conditions were studied, and detailed analysis of bio-oil chemical composition was done using an elemental analyzer, FT-IR, and GC-MS. The bio-oil produced was made of fatty acid methyl esters, fatty acids, aldehydes, and ketones with a heating value of 30.74 MJ/kg. The microalgae cake was studied under several liquefaction temperatures, holding time, as well as catalyst dosage. The maximum yield of bio-oil obtained was 25.8% at 360 °C reaction temperature, 50 min holding time, and 5% Na_2CO_3 used as a catalyst. According to results, approximately 26% of the yield was obtained which is average (Shuping et al. 2010).

Kiran Kumar et al. (2018) examined direct (HTL) hydrothermal liquefaction of microalgae to produce bio-oil. They used a high-pressure batch reactor with subcritical water conditions in the production process. Three different microalgae *Botryococcus braunii, Chlorella vulgaris*, and *Scenedesmus quadricauda* were

examined by using hydrothermal liquefaction at different water concentrations in the ratio of 1:6, 1:7, 1:8, 1:9, and 1:10 and temperature range between 200 and 320 °C, pressure of 60 bars, and 30-min reaction time. By liquefaction, the highest bio-oil yield obtained was 18 wt.% at 1:9 ratio of water and 300 °C temperature from *S. quadricauda*. The obtained bio-oil was analyzed for its chemical components using gas chromatography, and the results showed that the bio-oil was made of phenol, furan, acids, and derivatives of ester. It was observed during this study that by increasing the temperature, the yield of bio-oil was increased due to the polymerization reaction that transformed the small components of biomass into heavier molecules. This study has shown a comparison between three different microalgae species with varying conditions like water concentration and temperature. For the application of bio-oil production on an industrial level, this study has a beneficial approach (Kiran Kumar et al. 2018).

5.5.3 *Bio-oil Production Study by the Combination of Techniques*

Traditionally, conversion of microalgae to biofuels is done using oil extraction methods: organic solvents, pyrolysis process, or hydrothermal liquefaction. But there are some problems with these methods like low conversion rate, high-energy consumption, the time requirement for conversion, etc.; therefore, a new conversion route has been developed: microwave-assisted pyrolysis (MAP) process. The new method has many advantages over traditional processes in that it has uniform internal heating of large portions of biomass and is low cost, the process has a simple setup, and it can be easily adapted to large-scale industrial technologies that are currently in practice. Xie et al. (2015) have researched bio-oil production by using fast microwave-assisted catalytic co-pyrolysis along with HZSM-5 as a catalyst. In that, microalgae and scum were used as biomass. Their work was focused on factors like effects of catalyst to feed ratio, co-pyrolysis temperature, and the ratio of microalgae to scum on bio-oil yield and its composition. According to experiments performed, the results showed that temperature had a large impact on the co-pyrolysis process. The temperature of 550 °C was noted as the optimal temperature for gaining the maximum yield of bio-oil as well as at this temperature the highest part of aromatic hydrocarbons that exist in the bio-oil were obtained. The yield of bio-oil is observed to be decreased when a catalyst is used, but aromatic hydrocarbons' product yield was significantly improved when they changed the ratio of catalyst to feed on 1:1 to 2:1. Improvement of the bio-oil and aromatics production can be done by co-feeding of scum. The optimal ratio of microalgae to scum was 1:2 from the viewpoint of the quality of bio-oil. Scum is a good hydrogen supplier that can increase the overall effective hydrogen index value of the feedstock. The effect of synergy between microalgae and scum throughout the co-pyrolysis process

has become significant only when the (EHI) effective hydrogen index of the feedstock was greater than about 0.7 (Xie et al. 2015).

The use of catalysts in pyrolysis causes an increase in the reaction rate, product's yield, and nature of bio-oil. It also improves pyrolysis reaction kinetics by changing the big molecules into smaller molecules like hydrocarbons. Rare earth elements have been found to work as effective catalysts in the pyrolysis and liquefaction processes of biomass production. Xu et al. (2020) worked on the liquefaction and catalytic pyrolysis behavior of microalgae (*Spirulina)* for the production of bio-oil. The results of this study confirmed that the earth compounds, as catalysts, have an important role in accelerating the pyrolysis of microalgae by lowering the activation energy of the pyrolysis process. Ce(II)/HZSM-5 offered the ideal catalytic pyrolysis and liquefaction effect by helping out to cut the molecule chains of microalgae. It has a higher total pore volume, high specific surface area, and higher content of Ce4+ components that reduced activation energy and improved catalytic activities. The maximum yield of bio-oil obtained was 49.71 wt. % at 5 wt.% of the catalyst concentration. The chemical components of bio-oil obtained from *Spirulina* were composed of carboxylic acids, olefins, amides, ketones, esters, ethers, and partially cyclic nitrogen-containing compounds. Even if the combustion of the *Spirulina* bio-oil is not comparable to that of the diesel fuel, it is superior to rice husk bio-oil, indicating a potential application (Xu et al. 2020). Bio-oil production from different microalgae using various techniques are depicted in Table 5.2.

Table 5.2 Bio-oil production from different microalgae, various techniques used, their conditions, and bio-oil yield

Sr. no.	Name of microalgae	Technique used	Conditions	Bio-oil yield	References
1	*Spirulina* sp.	Slow pyrolysis	Temp – 550 °C Time – 60 min ECR – 0.49	~46%	Chaiwong et al. (2013)
2	*Nannochloropsis oculata*	Pyrolysis method	Temp – 200–225 °C Time – 30–60 min	44.9%	Du et al. (2012)
3	*Dunaliella tertiolecta*	Fast pyrolysis	Temp – 600 °C	45.13 wt. %	Francavilla et al. (2015)
4	*Chlorella protothecoides*	Fast pyrolysis	Temp – 500 °C	57.9%	Miao and Wu (2004)
5	*Dunaliella* sp.	Hydrothermal liquefaction	Temp – 350 °C Time – 60 min Pressure – 200 bars	11.81 w/w%	Shahi et al. (2020)
6	*Dunaliella tertiolecta*	Hydrothermal liquefaction	Temp – 360 °C Time – 50 min	25.8%	Shuping et al. (2010)
7	*Scenedesmus quadricauda*	Hydrothermal liquefaction	Temp – 200–320 °C Time – 30 min Pressure – 60 bars	18 wt.%	Kiran Kumar et al. (2018)
8	*Spirulina*	Liquefaction and catalytic pyrolysis	Time – 250–350 °C Catalyst – 5 wt.%	49.71 wt. %	Xu et al. (2020)

5.6 Challenges in Biodiesel Production from Microalgae

Microalgal cultivation is more expensive compared to normal crops because the harvesting of algae needs high-energy input which is almost 20–30% of the total production cost. Algal biomasses are rich in polyunsaturated fatty acids compared to vegetable crops. The higher composition of polyunsaturated free fatty acid affects the fuel stability because it is less resistant to oxidation (Taparia et al. 2016). Biodiesel has been generated from microalgal oil at the laboratory scale, but the challenges remain in scaling up production. Algal lipid extraction requires various cell disruption techniques and lipid extracting expensive solvents which ultimately increase the production cost (Veeramithu and Ngamcharussrivichai 2016).

Supply of CO_2 up to 6–10% can develop algal biomass with substantial lipid content. Because of the mass transfer limitation, the entire dissolution of CO_2 becomes complicated. So, all the CO_2 is not available to algal biomass. Despite several technologies being available for lipid conversion to biodiesel, microalgal biodiesel is very much costly because the cultivation system requires temperature as well as growth-limiting conditions to be controlled. A major bottleneck in algal biodiesel production is the operating cost and high capital. Still several technical gaps are there before taking this process to commercialization.

5.7 Challenges in Bio-oil Production from Microalgae

Bio-oil production from microalgae still has challenges that need to be alleviated. Pyrolysis needs drying of feedstock which results in more energy consumption during the process. Hydrothermal liquefaction is carried out in an aqueous environment that is suitable for wet biomass.

One of the main challenges in bio-oil production is the quality of the bio-oil that we get. It needs to be further processed in order to use it as fuel. The undesirable properties like high oxygen content, acidity, and nitrogen content require upgrading. The other challenges in the process are nutrient sourcing and utilization, management of production of microalgae biomass, harvesting of biomass, extraction of bio-oil and its refining, and residual biomass utilization. Major physical parameters that affect the growth of algae are pH and CO_2 into the medium, illumination, photoperiod for the proper culture maintenance, light intensity that differs the algae growth, and temperature (between 10 °C and 25 °C) needed to maintain because beyond 35 °C it leads to a destructive growth of algae. Seawater or water used for media can have contaminants which can lead to collapse of the culture.

The undesirable properties of bio-oil like high oxygen content, acidity, and nitrogen content require upgrading. Undesired properties for bio-oil application as a fuel also include corrosiveness, water content, high viscosity, etc. Techniques for upgrading bio-oil have their own advantages and disadvantages. Emulsification and solvent addition are physical operations that appear to be temporary approaches for

upgrading bio-oil. Some chemical operations can be used as well, but most methods have requirements of high temperature and high pressure which ultimately result in high cost for the upgrading process. Esterification is another method that can be used to upgrade bio-oil and can be done at normal atmospheric pressure as well as low temperature, but it has no significant effect on the denitrogenation of the product. Hydrotreating looks like a promising approach because it has been used for many years in oil refineries and the process is well established, but again it needs more effort to solve the problem of cocking and catalyst inactivation. There is still research going on for algal bio-oil upgrading techniques (Saber et al. 2016).

A factor that influences the yield of bio-oil can be the design and construction of the reactor used in the production of bio-oil. Not having proper agitation can lead to the nonuniform heat distribution to the reaction mixture resulting in low yield. Another main problem in bio-oil production from microalgae is the amount of nitrogen present in this fuel. If the percentage of nitrogen in the final product is larger than 7%, the problem of NOx emissions will be raised (Shahi et al. 2020). Combustion of some bio-oils is not comparable to the diesel fuel, e.g., bio-oil produced from *Spirulina* (Xu et al. 2020). The quality of the oil obtained from microalgae was not good enough to be used as fuel, without processing it further by deoxygenation and denitrogenation. These extra processing steps incur extra production costs. High temperature is needed for bio-oil production, which requires energy that makes the overall process energy-intensive (Francavilla et al. 2015).

5.8 Conclusion

Microalgae can be considered as promising feedstock for producing biodiesel and bio-oil. In this chapter, many strategies have been discussed regarding bio-diesel and bio-oil production from microalgae. Biomass and biofuel yield is influenced by choice of the cultivation, harvesting, and extraction techniques. Major drawbacks are high cost of nutrients, harvesting biomass, and extraction of oil. Challenges are also there in controlling the photoperiod, temperature, light intensity, pH, and CO_2 supply. Furthermore, research is required to reduce the cultivation cost for large-scale production of biodiesel and bio-oil. Thus, developing efficient harvesting and lipid extraction techniques is significantly important. Focus needs to be given on developing bio-oil production techniques and upgrading of bio-oil to achieve desirable fuel properties.

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Chapter 6
Algae as a Feedstock for Bioethanol and Biomethane Production

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Abstract The current energy demands and depleting fossil fuel reserves have generated a need for searching alternative renewable fuels. This has prompted scientists worldwide on systematically researching third-generation biofuels, bioethanol and biomethane, as a suitable alternative. Algae, a renewable fuel generating biomass, are rich in macromolecules such as carbohydrates, lipids and proteins. Due to the sustainability of the feedstock and environmentally friendly nature, biofuels produced from algal biomass are becoming increasingly popular. This chapter discusses bioethanol and biomethane production from algal biomass using different cultivation, harvesting and extraction techniques. Focus was also given to biochemical and genetic engineering approaches and life cycle assessment studies to increase biofuel production from algae. Overall, the chapter unravels how blending these practices can improve the utilization of algal biomass to develop biofuels and establish it as a promising future energy feedstock.

Keywords Algae · Bioethanol · Biomethane · Pretreatment · Biorefinery

6.1 Introduction

The primary source of global greenhouse gas pollution is fossil fuel combustion, which releases more than 30 billion tonnes of carbon dioxide into the atmosphere annually (Lazarus and van Asselt 2018). Thus, reducing fossil fuel combustion, mainly by reducing their consumption, is a top priority for governments, environmentalists and scientists worldwide. In this regard, the substitution of fossil fuels with biofuels has been identified as the primary means to reduce the global carbon footprint and all other attendant problems associated with petroleum use. Specifically, it is expected that the elevation of biofuel usage over fossil fuels will improve the global economy and environmental sustainability. However, the proponents of

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biofuel use have also identified some drawbacks which have impeded the widespread use of these alternative energy sources.

Biofuels have been classified based on the feedstock source into first-, second- and third-generation biofuels, each with its own merits and demerits. The first-generation biofuels are obtained from crop plants, especially cereals, tuber and root and oil crops. Specifically, these include but are not limited to corn, wheat, sugar cane, sugar beet, sunflower oil, palm oil, rapeseed oil and even animal fats, which have all been important sources of nutrition for humans and animals (Hirani et al. 2018). Second-generation biofuels, however, are based on non-crop high-energy plants (*Iris meda*, *Jatropha curcas*, *Lilium ledebourii*, *Pongamia pinnata*, *Simarouba glauca*, etc.) and lignocellulose biomass (Amobonye et al. 2020; Westensee et al. 2018). Both the first- and second-generation biofuels are dependent on agriculture, which needs high reserves of cultivable land, significant involvement of human resource and irrigation facilities and is greatly influenced by fluctuations in rainfall patterns and other weather conditions as well as biotic variables such as pathogen infections (Westensee et al. 2018; Hirani et al. 2018). Besides, the conversion of lignocellulosic biomass to biofuel has been identified as a challenging process mainly due to the complex plant cell structure and its high lignin content (dos Santos et al. 2019). Thus, considering the drawbacks of the first- and second-generation biofuels and the ever-decreasing energy demand, the use of algae as an alternative source of biofuel (third-generation) emerges as a more viable option.

Algae are members of a distinct group of predominantly aquatic plantlike unicellular and multicellular organisms with diverse biotechnological potentials. They range in size from the microscopic, *Micromonas* species, to giant kelps that grow as much as 60 m in length (Fleischman et al. 2019). Their photosynthetic mechanisms are more varied in comparison to plants. Collectively, they can absorb different light wavelengths, resulting in higher light-use efficiency, more efficient carbon capture and increased valorization of marginal land and waste water (Vecchi et al. 2020). Ecologically, algae contribute a significant share to the earth's oxygen cycle and are also at the bottom of the aquatic food chain serving as the food base for almost all aquatic life. Furthermore, some algal species can utilize either atmospheric inorganic carbon (CO_2), reducing greenhouse gases, or organic carbon from the environment (Freeman et al. 2018). Through any of these processes, algae can synthesize carbohydrates, lipids and proteins within a short period, which can then be valorized into different products (John et al. 2011).

The current uses of algae include human foods, human medicine, animal feed, fertilizers and cosmetic ingredients (Jalilian et al. 2020; Thiyagarasaiyar et al. 2020). A lot of effort is currently placed on using algae as economical feedstocks to produce different biofuels, considering their many advantages over the first- and second-generation biofuel feedstocks. These different biofuels include biodiesel from algal oil (Rajak et al. 2020; Saengsawang et al. 2020), bioethanol produced by fermentation (Tan et al. 2020; Yu et al. 2020), biohydrogen by photobiological fermentation (Goswami et al. 2020; Margareta et al. 2020), bio-oil obtained from thermal conversion (Xu et al. 2020; Lee et al. 2020) as well as methane from anaerobic digestion of the algal biomass (Nassef et al. 2020; Rokicka et al. 2020).

Algae do not need agricultural land for cultivation, unlike terrestrial crops, as they can proliferate in freshwater, brackish water or saltwater, thus making their cultivation relatively easier. Furthermore, the potential biomass yield of algae per unit area is always higher as compared to terrestrial plants. For example, brown seaweeds have been recorded to achieve a yield of ~13.1 kg dry weight (dw) m^{-2} $year^{-1}$ 'under cultured conditions' which is higher than the ~10 kg dw m^{-2} $year^{-1}$ yield from sugar cane (Milledge and Heaven 2014). These undeniable benefits have directed to growing research interest in using algae as biofuel sources compared to other feedstocks as they have immense potential to meet both current and future energy demands. Their high cellular carbohydrate content has also been observed as an added advantage as many algal cells contain easily fermentable sugars with less hemicellulose and no lignin. There are many algal classification schemes, including ones established on their pigmentation and size; however, they are mainly classified as microalgae and macroalgae for their biotechnological potential as biofuel feedstocks.

6.1.1 Microalgae Vs Macroalgae

Algae are divided into two categories according to their size; the larger group is the macroalgae called 'seaweeds', while the smaller microalgae are microscopic single-cell organisms, usually less than 1 mm in size (Pourkarimi et al. 2019). Microalgae grow in any aquatic habitat, whereas most macroalgae species can only be found in the marine environment. Macroalgae may belong to one of the several groups of multicellular algae, viz. the red algae, green algae and brown algae. They have been used mainly as food for humans/animals as well as fertilizers and soil conditioners (Piwowar and Harasym 2020). Typically, macroalgae are structured into the thallus, the lamina, sorus, the stipe and fucus. The stipe and blade are collectively known as the frond and they are the major parts of interest for biomass to energy conversion (Sudhakar et al. 2018). On the other hand, microalgae contain a widely diverse group of unicellular algae, with about 50,000 species identified, representing an extensive reservoir of untapped resources (Suganya et al. 2016). They either exist individually or in chains or groups. About half of the atmospheric oxygen is generated by microalgae which simultaneously utilize the carbon dioxide, a greenhouse gas, to grow photo-autotrophically. Microalgae grow at an alarming rate, with some species doubling in size within 4–24 h, while some can double their biomass as much as eight times within the same period during the exponential growth phase, accumulating significant amounts of cellular polymers in the process (Gautam et al. 2015). In particular, the cultivation of microalgae provides the highest average atmospheric carbon fixation rate of 1.83 kg CO_2/kg biomass and the fastest biomass productivity, 40–50% higher than terrestrial plants (Shahid et al. 2020). Although recent research has focused more on microalgae than macroalgae, the macroalgal production industry is estimated to be a hundred times bigger in wet tonnage terms than the microalgal industry. Macroalgal species from *Eucheuma*, *Gracilaria*, *Laminaria*,

Porphyra and *Undaria* genera produce more than three-quarters of the total tonnage of cultured macroalgae (Milledge and Harvey 2016). In contrast, microalgae including *Botryococcus braunii*, *Chlorella* sp., *Crypthecodinium cohnii*, *Dunaliella primolecta* and *Nannochloropsis* sp. produce the large quantities of hydrocarbons and lipids (Medipally et al. 2015).

6.1.2 Overview of Global Algal Production

Besides their high potential use as biofuel feedstock and their long-established use in human/animal nutrition, algae have been noted to be a source of diverse high-value compounds such as carotenoids (beta-carotene, astaxanthin, lutein, zeaxanthin), phycobiliproteins, omega-3 fatty acids, sulphated polysaccharides, etc. (Hu 2019). They also serve as sources of various base materials useful in bioplastic production such as polyhydroxybutrate (Abdo and Ali 2019). Thus, in the light of its many industrial uses, it has been noted that systematic algal cultivation is the only means by which their current and future demand can be met. However, because of the difficulties and technicalities involved in microalgae production, there is no harmonized study to evaluate their global production, unlike macroalgae.

Approximately 80% of microalgae produced are used as human food, medicine and nutritional supplements, while the remaining portion is used as animal feed additives, fertilizers and bioenergy applications (Sudhakar et al. 2019). Until recently, microalgae production was done on a small scale in facilities of below surface area of 10 ha. Presently, large-scale facilities in the 200 ha and above range are in operation (Acién et al. 2017). Most of the largest microalgae cultivation facilities were located in China, which was also tagged as the biggest global microalgae producer, accounting for more than 60% of global production (Chen et al. 2016). These facilities with surface areas greater than 200 ha can produce up to 3000 tonnes/year of microalgae with *Spirulina*, *Chlorella*, *Dunaliella* and *Haematococcus* being the most cultivated genera (Acién et al. 2017). A study which evaluated the global productivity potential of microalgae estimated an annual biomass yield of between 21.24 and 32.16 cubic metres/ha/m (Moody et al. 2014). However, recent projections have estimated the global microalgae market to grow up to $10 billion in the nearest future (Beal et al. 2018).

Contemporary macroalgal cultivation began in China in the middle of the twentieth century with the production of *Laminaria* juveniles using the growing-on in raft cultivation model (Tseng 2004). About 7000 tonnes of macroalgae (dry weight) are currently produced globally, with a market value estimated at 3.8–5.4 billion USD, thus underscoring the rising profile of algae in different industries around the world (de Mendonça et al. 2021). China accounts for almost two-thirds of the global production of macroalgae, with Indonesia, the Philippines and the Korean Republic following at a far distance (Sudhakar et al. 2018). Currently, the European algal industry is based on wild harvesting, while the Asian industry mainly relies on systematic cultivation. Comparatively, Asian production of macroalgal biomass was

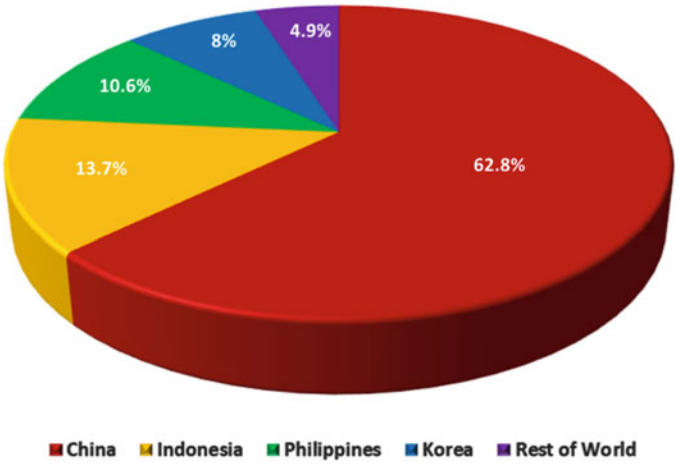

Fig. 6.1 Global macroalgal production estimates (Adapted from Sudhakar et al. 2018)

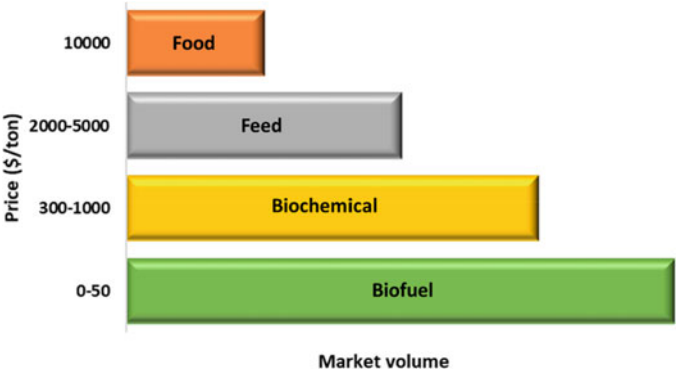

Fig. 6.2 Algal market value across the major industries (Adapted from Sudhakar et al. 2018)

estimated at 27 million tonnes (wet weight), which is multiple folds of the few hundred thousand tonnes produced in Europe (Bak et al. 2018). It has been observed that the cultivation methods applied effectively in Asia have not been found effective and profitable in other parts of the world (Zuniga-Jara et al. 2016). Production is still considered to be at its infancy in Africa, Europe and the Americas (Buschmann et al. 2017). The global production of macroalgae is depicted in Fig. 6.1, while the market potential is shown in Fig. 6.2.

6.1.3 Bioethanol Vs Biomethane

The high carbohydrate and lipid contents of algae have made them valuable feedstock for producing different biofuels including biochar, bioethanol, biomethane, biodiesel, etc. (Kumar et al. 2020a). Even though there has been an advancement in first- and second-generation bioethanol production, there are still numerous challenges of using food crops and lignocellulosic biomass as bioethanol feedstock. Hence, it is believed that the utilization of algae for bioethanol production will overcome these major drawbacks as highlighted in Sect. 6.1, which are common for biofuels in general, to a very large extent.

Bioethanol is a renewable liquid fuel which is mainly produced by yeast fermentation of different feedstocks. Recent statistics have projected the global bioethanol production to reach 134.5 billion litres by 2024 (Bušić et al. 2018). Some of the properties of bioethanol that have endeared it to scientists and engineers alike include its high-octane number, estimated at 108, indicating its high antiknock value (Niphadkar et al. 2018). Furthermore, the high oxygen content makes its combustion cleaner; hence, lower concentration of gaseous pollutants, such as carbon monoxide and nitrogen oxide, is emitted. Though bioethanol has 68% lower energy content than petrol, it produces 80% less CO_2 emission, which is a great advantage (Krylova et al. 2008). However, the sequential processes involved in converting biomass to bioethanol (pretreatment, hydrolysis, fermentation and distillation) have been shown to affect the quality of bioethanol produced in each batch. The effect of the pretreatment method, biomass feedstock and fermenting microbe on the efficiency of bioethanol production has been highlighted in many studies (Zabed et al. 2017). Industrial processes have since been well developed for the production of ethanol by fermenting beet or cane sugars, molasses and sugars from grains such as maize and wheat. However, the intricate technologies currently used in bioethanol production have made the cost of bioethanol production to be higher than fossil fuels.

Currently, Brazil and the United States are the two leading ethanol producers accounting for more than 60% of the global output, with sugar cane and corn serving as the feedstock, respectively (Proskurina et al. 2019). Although there has been a shift towards algal bioethanol and biodiesel production, some studies have demonstrated that algal biomethane production is comparatively less complicated and more cost-effective (Perazzoli et al. 2016). Furthermore, biomethane can be a value-added product from algal residues after initial bioethanol/biodiesel production, as it was noted that as much as 65% of the microalgal biomass is left after biodiesel production (Zhu et al. 2018). Biomethane is usually produced through anaerobic digestion or biomass gasification, each process with its distinct characteristics. Being indistinguishable from natural gas and having the added advantage of lower heating value (estimated at 36 MJ/m^3), biomethane can be used effectively for electricity generation. Furthermore, it is also fully compatible with vehicles that run on natural gas (Barragán-Escandón et al. 2020). Hence, biomethane has been projected to account for a large share of renewable energy in the nearest future.

6.2 Economics and Limitations of Algal Biofuel Production

It is important to understand the economy of algae biofuel to maximize the cost-effectiveness and to enhance its competitiveness with fossil fuels. Algal biofuel is believed to be a competitive alternative in the future and could be a viable substitute for fossil fuel, mainly because of the high oil content, high production rate and less demand for land for its cultivation. However, the market feasibility of biofuel production from algae has been observed to be dependent on different government subventions and the prices of fossil oils such as petrol and diesel (Gallagher 2011). Recently, the ability of algae to grow in domestic and municipal waste water has been noted as a means of minimizing the energy input and reducing the cost of cultivation, as waste water is a readily available nutrient source required for microalgae proliferation (Bhatia et al. 2020). However, as it currently stands, algal-biofuel production is still too expensive for effective commercialization, being far higher priced than petroleum-based fuels. The production cost of biofuel from algal cells depends on various factors, including the yield of biomass from the cultivation system, the content of oil/carbohydrates, the size of production systems and the recovery of final products from algal biomass. Furthermore, the bioprocessing technologies of biofuel from algae are still at its infant stage, making the technologies relatively expensive, which adds more to the cost burden.

The production cost of biofuel from algae was estimated to be $10.50 per gallon without the addition of other accessory costs, including distribution and marketing costs and taxes, which is more than double the price of petroleum-diesel (~ $4.2 per gallon) (Chisti 2007; Medipally et al. 2015). Furthermore, the transformation of algal biomass to a single biofuel product is noted to be economically unviable, especially in terms of the energy input because of the very low net energy return on investment ratio (ERoEI) to fossil fuels. Therefore, the biorefinery approach is a promising alternative to optimize the bioenergetic potential of algal biomass, which will ensure the conversion of biomass into an array of biofuels or other value-added products through various integrated bioprocesses. The integration of anaerobic digestion with ethanol fermentation into a biorefinery platform could be a more efficient choice that could allow all algal biomass components, including cellulose, hemicellulose, protein, fat, etc., to be converted into different types of biofuels, thus improving the economic competitiveness of algal biofuel. Pretreatment has been estimated to account for more than one-third of algae biofuel's total production cost, which is cost-ineffective. Current pretreatment methods have been noted to expend expensive chemicals and demand immense energy. Other barriers to the commercialization of biorefinery algae are related to the logistics of feedstock and extensive water use during the production of algae and biofuel processing. Industrial microalgae cultivation for biofuels has been documented to require a large amount of water, estimated at ~3.4 litres of water per litre of bioethanol produced (Chia et al. 2018). The harvesting process of algae, especially microalgae, has also been observed to be relatively costly. Microalgae farming is more expensive and difficult compared to traditional agricultural practices. However, global focus has been shifted towards

developing new technologies/innovations for increasing the economic value of algal-derived biofuel in future energy markets. For instance, the design of a sustainable bioreactor for large-scale production of algae would go in a long way in reducing the production costs. Heterotrophic cultivation of algae in conventional bioreactors used for bacteria cultivation has been noted to be an economically viable option, as bioreactors are expensive to design. Furthermore, the feasibility of microalgae biofuel can be enhanced by developing cost-effective biomass harvesting and drying technologies and improving molecular strategies for further biomass and macromolecule production (Medipally et al. 2015). Although algae biofuel has been acknowledged to alleviate the many shortcomings of its predecessor biofuels, the potential of algae as a well-accepted biofuel feedstock is based on long-term economic benefits.

6.3 Algal Composition

It is imperative to know the composition of algal biomass to gain insights into how the fundamental structure of the biomass affects biofuel production and how the biomass can be affected by the various treatment methods. Proximate analysis of different algae under diverse conditions has been carried out using various methods (Dong et al. 2016; Mehrabadi et al. 2017; Irkin and Erduğan 2017). All the methods have highlighted the presence of carbohydrates, lipids and proteins in the biomass. Some studies went further to determine the ash content, which estimates the salt and inorganic contents, while others determined the antioxidant/phenolic content. A summary of the biochemical composition of some micro- and macroalgae is presented in Table 6.1. It was noted that the composition of different organic polymers within algal cells varies with the developmental stage of the cells. For example, in microalgae *Nannochloropsis granulate*, out of the three principal components, the protein concentration was shown to be at the highest at the early growth stage, while lipid concentration was lowest. The lipid content increases as algae age, contributing to the largest percentage of the polymers at the late growth stage, while the protein is at the lowest at this stage (Dong et al. 2016; Mehrabadi et al. 2017). Hence, the growth stage of algae is significant in choosing the optimum harvesting stage required for the target biofuel product in downstream processing. Furthermore, the effect of seasonal changes on the composition of algae has been the focus of many studies (Gerasimenko and Logvinov 2016; Irkin and Erduğan 2017). Different carbohydrates serve both structural and metabolic functions in all organisms. Being the first photosynthesis products, they are usually the starting point for synthesizing the other biochemicals.

Various algae have been shown to commonly produce agar, carrageenan, cellulose, hemicellulose and laminarin, while some synthesize specific types of polysaccharides (Wei et al. 2013). For instance, green algae such as *Tetraselmis suecica* produces starch, consisting of both amylose and amylopectin, similar to higher plants as an energy store. Red algae have been shown to produce a carbohydrate

Table 6.1 Biochemical composition of different micro- and macroalgae

Microalgae	Carbohydrate (%)	Lipid (%)	Protein (%)	Ash (%)	Phenolics (Mg GAE g^{-1} DW)*	Reference
Acutodesmus dimorphus	38.6	18.8	28.1	14.5	6.4	Tibbetts et al. (2015)
Botryococcus braunii	18.5	34.4	39.9	7.2	13.2	Tibbetts et al. (2015)
Chlorella spp.	25.2	15.7	53.3	5.8	7.7	Tibbetts et al. (2015)
Chlorella vulgaris	38.00 ± 0.36	23.14 ± 0.19	15.2	5.3	ND	Dong et al. (2016)
Chlorella vulgaris	8.08 ± 0.09	13.32 ± 0.07	47.82 ± 0.05	6.30 ± 0.02	ND	Tokuşoglu and Üunal (2003)
Coelastrum sp.	30.0 ± 3.4	30.4 ± 0.9	28.1 ± 1.4	ND	ND	Mehrabadi et al. (2017)
Desmodesmus sp.	23.0 ± 1.0	40.2 ± 0.9	27.1 ± 3.0	ND	ND	Mehrabadi et al. (2017)
Isochrysis galbana	16.98 ± 0.05	17.16 ± 0.04	26.99 ± 0.08	16.08 ± 0.03	ND	Tokuşoglu and Üunal (2003)
Micractinium pusillum	29.2 ± 5.7	46.3 ± 3.6	16.8 ± 0.4	ND	ND	Mehrabadi et al. (2017)
Mucidosphaerium pulchellum	27.8 ± 6.9	48.2 ± 1.8	13.5 ± 1.5	ND	ND	Mehrabadi et al. (2017)
Nannochloropsis granulata	10.89 ± 0.11	57.33 ± 0.09	9.4	5.1	ND	Dong et al. (2016)
Nannochloropsis spp.	40.4 ± 0.39	21.0 ± 1.84	22.2 ± 0.46	8.66 + 0.09	ND	Rebolloso-Fuentes et al. (2001)
Nannochloropsis granulata	36.2	23.6	33.5	6.7	8.0	Tibbetts et al. (2015)
Neochloris oleoabundans	37.8	15.4	30.1	16.7	9.8	Tibbetts et al. (2015)
Pediastrum boryanum	28.5 ± 2.1	22.1 ± 0.4	47.4 ± 4.6	ND	ND	Mehrabadi et al. (2017)
Phaeodactylum tricornutum	25.2	18.2	39.6	17.0	20.2	Tibbetts et al. (2015)
Porphyridium aerugineum	45.8	13.7	31.6	8.9	6.5	Tibbetts et al. (2015)
Scenedesmus acutus	39.42 ± 0.08	38.55 ± 0.38	7.8	2.2	ND	Dong et al. (2016)

(continued)

Table 6.1 (continued)

Microalgae	Carbohydrate (%)	Lipid (%)	Protein (%)	Ash (%)	Phenolics (Mg GAE g^{-1} DW)*	Reference
Spirulina platensis	15.81 ± 0.07	8.03 ± 0.06	61.32 ± 0.02	10.38 ± 0.05	ND	Tokuşoglu and Üunal (2003)
Spirulina spp.	22.2	14.2	55.8	7.8	10.7	Tibbetts et al. (2015)
Tetraselmis chuii	25.0	12.3	46.5	16.2	20.0	Tibbetts et al. (2015)
Macroalgae						
Acanthophora spicifera	12.4	11	12.6	ND	ND	Suganya et al. (2016)
Cladophora fascicularis	49.50	15.70	15.53	ND	ND	Suganya et al. (2016)
Caulerpa cupressoides	51.75	10.97	7.43	ND	ND	Suganya et al. (2016)
Caulerpa cupressoides	26	7.9	3.9	62.2	ND	Dromard et al. (2017)
Ceramium cf. nitens	51	2.7	10.6	35.6	ND	Dromard et al. (2017)
Codium decorticatum	50.63	9.00	6.08	ND	ND	Suganya et al. (2016)
Codium tomentosum	32.8	3.6 ± 0.2	18.8 ± 0.1	35.99 ± 0.48	920 ± 84 catechol equiv./g	Rodrigues et al. (2015)
Gracilaria gracilis	46.6	0.60 ± 0.01	20.2 ± 0.6	24.8 ± 0.03	228 ± 14 μg catechol equiv./g	Rodrigues et al. (2015)
Grateloupia turuturu	60.4 ± 2.3	2.6 ± 0.1	22.9 ± 2.0	18.5 ± 0.6	ND	Denis et al. (2010)
Osmundea pinnatifida	32.4	0.9 ± 0.1	23.8 ± 0.6	30.62 ± 0.25	337 ± 22 catechol equiv./g	Rodrigues et al. (2015)
Sargassum muticum	49.3	1.45 ± 0.07	16.9 ± 0.2	22.94 ± 0.06	499 ± 32 catechol equiv./g	Rodrigues et al. (2015)
Saccorhiza polyschides	45.6	1.1 ± 0.1	14.44 ± 0.1	28.15 ± 0.01	224 ± 13 catechol equiv./g	Rodrigues et al. (2015)
Ulva lactuca	29.6	1.4	24.9	44.1	ND	Dromard et al. (2017)
Ulva rigida	65.93	3.1	26	ND	ND	Balar et al. (2019)

GAE Gallic acid equivalent, *ND* Not determined

polymer known as floridean, mostly composed of amylose (Williams and Laurens 2010). It has been observed that macroalgae are generally rich in carbohydrates compared to microalgae. This is due to their need for extracellular carbohydrates necessary for support and protection (Debiagi et al. 2017). Like carbohydrates, lipids play both energy and structural roles in the cells, with the simple fatty acid triglycerides serving as energy reserves, while the more complex phospholipids and glycolipids are found in the membranes (Kumari et al. 2013). Via de novo synthesis and recycling of fatty acids to preserve membrane characteristics, algae can easily adapt to new environmental conditions, such as temperature changes (Barkina et al. 2020). Also, shifts in lipid composition occur over the different stages of growth. Many studies have shown the higher dominance of unsaturated fatty acids in algae, with 50% of the fatty acids having a carbon number less than 18 (Williams and Laurens 2010). This high unsaturated fatty acid content of extracted lipids will be an essential fuel quality determinant. Microalgae have higher lipid content when compared to macroalgae as they can accumulate a significant quantity of triglycerides. These triglycerides are more favourable for biodiesel generation than other lipids, such as glycolipids and phospholipids, due to their higher fatty acid content. Besides, they are considered more important because of the absence of nitrogen, phosphate and sulphur in their structures, which usually inhibit the fermentative process required for biofuel production (Mondal et al. 2017). Proteins also possess both structural and metabolic functions in both micro- and macroalgae. As enzymes, they are the catalysts for cell metabolism and by implication cell growth and development. As structural components, they provide the scaffold on which chlorophyll is assembled in the light-harvesting complexes of the chloroplast. They are often integrated into the lipid membranes, where they play a similar structural function and a metabolic role. Interestingly, lignin which has been noted to be a major inhibitor to the hydrolysis of second-generation biofuel feedstock is rarely found in algae. However, some studies have shown lignin or lignin-like compounds in algae at a very low amount, which can be considered negligible (Yaich et al. 2015; Nunraksa et al. 2019).

6.4 Algae Cultivation and Harvesting Systems

6.4.1 Macro- and Microalgae Cultivation

The major factors affecting the economical production of algal biofuel are the high cost of cultivation and harvesting. Some of these undesirable costs are mainly as a result of the engineering and technical barriers. Thus, cost-effective cultivation and harvesting technologies with lower energy input are considered important to improve the economic feasibility of algae in the bioenergy industry (Kim et al. 2013). In addition, to maximize biomass yield during cultivation, it is imperative to increase productivity without competitive growth from foreign bacteria and fungi (Lee et al. 2015). It must be clarified that a significant share of the challenges with

cultivation and harvesting are observed in microalgae, rather than in macroalgae. The most industrially sought microalgae are from the groups Bacillariophyceae (including the diatoms), Chlorophyceae (green algae), Chrysophyceae (including golden algae) and Cyanophyceae (blue-green algae). Particularly, *Arthrospira*, *Chaetoceros*, *Chlorella*, *Dunaliella and Isochrysis* genera are the most commercially cultivated (Rajkumar et al. 2014). Although both micro- and macroalgae grow naturally in different freshwater and marine environments, relative to macroalgae, microalgal cultivation is more technical and demanding.

Three farming types are commonly used in microalgal cultivation, including the land-based ponds, nearshore coastal farms and offshore farms (Roesijadi et al. 2010). Microalgae biomass cultivation is usually done through the batch, semi-batch and continuous culture systems (Lutzu 2012). The optimum biomass production of microalgae in these culture systems is affected by different abiotic and biotic factors such as light, temperature, pH, salinity, O_2, CO_2, nutrient stress, toxic chemicals, pathogens and competition (Lu et al. 2020). Microalgae cultivation is also influenced by operational factors such as depth, dilution rate, harvest frequency, mixing shear and external supplements (Medipally et al. 2015). Furthermore, microalgae growth and composition are significantly dependent on the type of cultivation, usually the phototrophic, heterotrophic, mixotrophic and photoheterotrophic methods (Amaro et al. 2011; Cruz et al. 2018).

In phototrophic cultivation, microalgae efficiently utilize light, especially sunlight, as source of energy, and use CO_2 as a source of inorganic carbon for growth and biomass proliferation (Bolatkhan et al. 2019). This method is the most commonly used and has been acclaimed to be environmental-friendly as it contributes significantly to carbon sequestration while achieving a favourable energy balance at the same time. The phototrophic method can be applied in open ponds and enclosed bioreactors. The open pond system has been identified as the cheapest method for large-scale microalgae cultivation as it does not compete with food crops for land since they can be established in minimal crop production areas. They are relatively not technical and have low maintenance and low-energy requirements (Moreno-Garcia et al. 2017). However, contamination from the atmosphere and surrounding land surfaces is a major disadvantage of the open pond cultivation system. In this regard, the system is usually adapted to special conditions such as high alkalinity and high salinity (Lam et al. 2018). On the contrary, the enclosed photobioreactors are generally carried out in bags, plates or tubes made up of glass, plastic or other transparent materials. The microalgae in these systems are supplied with adequate light, nutrients and CO_2 (Amaral et al. 2020). However, they are limited by their relatively reduced scale of operation and their high cost of construction and maintenance. Some commonly used photobioreactor designs include annular, tubular and flat-panel reactors, with large surface areas. In addition to process control, they possess the added advantages of system efficiency and algal purity (Posten 2009).

Under heterotrophic cultivation, microalgae utilize organic carbon for their growth and development. Usually, in the absence of light, heterotrophic algae, such as thraustochytrids, are solely cultivated in the heterotrophic mode for lipid production (Nagarajan et al. 2018). With this mode, the problem of optimum light

supply can be circumvented. Furthermore, increased cell densities can be achieved, making biomass harvesting more feasible; however, its main disadvantage is in the generation of greenhouse gases (Hu et al. 2018). In mixotrophic cultivation, algae such as *Spirulina platensis* and *Chlamydomonas reinhardtii* can drive both photoautotrophy and heterotrophy, thus utilizing both inorganic and organic carbon sources, respectively (Zhan et al. 2017). Photosynthesis, which is influenced by light conditions, fixes inorganic carbon, while organic compounds are obtained by aerobic respiration, which is affected by the availability of organic carbon (Chen et al. 2019a; Wang et al. 2014). The less known photoheterotrophy cultivation has been noted to be closely related to mixotrophy. The only difference between the two is that while mixotrophy can use organic compounds and light as the energy source, photoheterotrophy is solely dependent on light. In this regard, photoheterotrophic cultivation requires both organic carbon and light at the same time (Zhan et al. 2017).

Although more than 200 species are currently of importance to humans, the majority of macroalgal biomass is sourced from five major genera: *Eucheuma*, *Gracilaria*, *Laminaria*, *Porphyra* and *Undaria* (Milledge and Harvey 2016). More than 90% of the seaweed consumed by humans is sourced from various cultivation activities, while the remaining portion is sourced from natural stocks (Ghadiryanfar et al. 2016). Generally, it has been observed that macroalgae cultivation can be divided into two stages, which are the production of juvenile algae and the propagation of the juveniles to produce biomass (Milledge and Harvey 2016). Although both stages can be combined to reduce cost, some algae such as *Laminaria* may not thrive under this combined state (Wei et al. 2013).

The cultivation methods for macroalgae are grouped into two broad classes: extensive and intensive cultivation methods. In extensive macroalgae cultivation, seaweeds are grown in natural water bodies while utilizing only naturally available light, heat, energy and nutrients (Zollmann et al. 2019). The exploitation of natural seaweed bed, as well as large-scale commercial mariculture, falls under this class. While the exploitation of natural seaweed bed involves the wild harvesting of macroalgae from natural fields and seabeds, commercial cultivation integrates the growth of seaweeds on the bottom and artificial substrata suspended in water while maximizing the usage of natural resources (Titlyanov and Titlyanova 2010). These extensive commercial methods are usually done in seabeds, lines, ropes or nets (Hurtado et al. 2019). On the other hand, the different intensive algal cultivation methods include cultivating one or different algal species in tanks using artificial or natural light, nutrients and plant hormones. It also involves cultivation in small natural water bodies (lakes, lagoons and ponds), using organic and inorganic fertilizers and applying agronomic practices such as regulating light and water motion, as well as weeding and reducing epiphyte growth (Cole et al. 2016). Furthermore, integrated mariculture which uses the tanks or pond systems supplied by water enriched with inorganic and organic nutrients is another intensive method. The nutrients may be supplied through effluents from animal, crustacean, fish or mollusc cultivation and even from public waste water (Ge and Champagne 2017). The beauty of this integrated method is in the ability of the macroalgae to utilize the dissolved nutrients in the waste water and subsequently transform them into its

organic matter, thus purifying water, which can be used later in other cultivation systems. However, though less complicated than microalgae farming, many attendant problems have been observed with macroalgae cultivation. This includes the activity of epiphytes and fouling algae, grazing animals, diseases as well as the negative effects of macroalgae on benthic ecosystems (Titlyanov and Titlyanova 2010; Hurtado et al. 2019).

6.4.2 Algal Harvesting Systems

The cost of harvesting algal biomass has been identified as one of the major contributors to their total production cost. In some cases, harvesting costs have been estimated to be close to half of the total production cost of biofuel and other value-added products from algae. Naturally growing seaweeds are harvested from shallow water or the subtidal zone, and the harvesting methods vary with the species. Basically, due to their large sizes, macroalgae can be harvested by manual or mechanized methods. Manual harvesting is a labour-intensive method done by hand-picking or cutting subtidal thalli; it involves rudimentary devices such as forks, nets and sickles. It is an uneconomical process; for instance, approximately 7% of the total cost of carrageenan production from macroalgae in Indonesia is attributed to manual harvesting labour cost (Milledge and Harvey 2016). Thus, different mechanized harvesting methods have been devised and applied to reduce harvest costs of seaweeds. The common mechanized harvesters include dredging cutters, rotating blades or suction, which require boats, ships, bulldozers or tractors to operate. However, most mechanical harvesting equipment has been adapted for wild harvest rather than harvesting seaweed cultivated intensively. Although mechanical harvesting of macroalgae is cost-effective, manual harvesting has been shown to perform better regarding biomass quality and consistency as it allows for a greater degree of onsite removal of contaminants (Ghadiryanfar et al. 2016). In addition, there have been environmental concerns with regard to mechanical harvesting of wild macroalgae as it causes irreversible disruption to the ecosystem (Fernand et al. 2017). Thus, there is a need for improving the efficiency of macroalgal harvesting which would ultimately result in lower production costs and a more sustainable environment.

In comparison to macroalgae, microalgae harvesting is more tedious, energy-demanding, expensive and less efficient. This is mainly due to their small size, low density and low concentration in the culture medium (Roy and Mohanty 2019). There are currently different microalgae harvesting methods, including centrifugation, filtration and gravity sedimentation and flocculation (Ferreira et al. 2020). The method of choice has been noted to be a function of the type of microalgal species, the culture medium and the desired end product. Gravitational sedimentation is the least expensive and simplest method of microalgae harvesting, the downsides being that it is time-consuming, has low biomass recovery and has high probability of biomass deterioration (Suparmaniam et al. 2019). Though expensive, centrifugation

has been the most used method on a laboratory scale; it is fast and hygienic and offers a high recovery rate. Hence, it is usually applied in the production of high-value products (Muhammad et al. 2020). However, significant damage of microalgal cells due to high shear forces has been observed with the centrifugation method. Filtration, both micro- and ultra-, has been identified to be effective for the harvesting of microalgae with smaller sizes such as *Coelastrum proboscideum*, *Spirulina* and *S. platensis*; however, the periodic fouling of filtration membrane is a major drawback (Zhao et al. 2020). Flocculation has been identified as the most viable harvesting method of microalgae on a commercial scale due to the low-energy demand and reduced cost. Hence, various flocculation methods have been used to agglomerate the microalgal cells to increase the effective 'particle' size and facilitate sedimentation. These include physical, chemical and bio- and self-flocculation (Malik et al. 2020). However, the coupling of flocculation with other harvesting methods will go in a long way in maximizing the harvesting process as each method will compensate for the weakness of the other.

6.5 Processing of Algae to Bioethanol and Biomethane

6.5.1 Pretreatment

Macroalgae, owing to their large polysaccharide and protein content, are mostly used for human consumption (Yoo et al. 2015), while microalgae, rich in carbohydrate and lipid content, are most preferred for biofuel production. However, algal biomass requires a pretreatment step to make the polysaccharides readily accessible for conversion into biofuel (Velazquez-Lucio et al. 2018). The diverse pretreatment techniques currently being employed to treat the algal biomass are described below.

6.5.1.1 Mechanical Methods

Mechanical pretreatment is the simplest and most widely used processing technique for reducing both macro- and microalgal biomass and subsequently release cell components, including macromolecules such as carbohydrates, proteins and lipids. Various mechanical pretreatment methods include grinding, bead and ball milling, cavitation and high-pressure homogenization (Sambusiti et al. 2015). Grinding and milling involve abrasive forces for cell disruption, while cavitation methods such as ultrasound induce electroporation (discussed elsewhere in detail). However, in high-pressure homogenization, algal cells are pressurized and subjected to high-pressure gradient creating a viscous shear force on cells causing their disruption (Anto et al. 2020; Velazquez-Lucio et al. 2018). The efficacy of mechanical pretreatment method can be validated by a previous report where *Laminaria saccharina* and *L. hyperborea* biomass was ground with modified Hollander beater, which resulted in increased biomethane yield (53%) as compared to untreated samples (Tedesco

et al. 2014). In another study, ultrasonic cavitation induced maximum *Ankistrodesmus fusiformis* cell disruption efficiency of 100% which was achieved using an ultrasonic processor for 60 min. However, in the same study, ultra-homogenizer showed ~93% cell disruption efficiency at 24,000 rpm and 70 min of processing, suggesting their efficiency for subsequent biofuel production (Skorupskaite et al. 2017).

6.5.1.2 Ultrasound Pretreatment

Ultrasound works on the principle of sound waves' mediated cavitation in cell structures resulting in the release of cell constituents. Interestingly, it also facilitates the disintegration of cell organelles and large macromolecules, resulting in significant size reduction and solubilization, thus increasing the surface area and accessibility of the respective hydrolytic enzymes (Velazquez-Lucio et al. 2018). The use of ultrasonication for the pretreatment of algal biomass has shown an increase recently due to its proficiency in disrupting the cells, biomass solubilization and increased biofuel production (Anto et al. 2020). As in *Scenedesmus obliquus* biomass which was treated with ultrasound with energy consumption of 4.79 KJ, it yielded around 91% of sugars with subsequent enzymatic saccharification (de Farias Silva et al. 2020). Similarly, in another study, ultrasound pretreatment on *Desmodesmus* sp. reported protein, carbohydrate and lipid yields of 97%, 89% and 73%, respectively (González-Balderas et al. 2020a). However, irrespective of numerous reports citing the significance of ultrasound pretreatment of biomass, its practical application is limited due to its high-energy consumption. For example, though specific methane yields (SMY) of *Scenedesmus* sp. was increased by ultrasonic pretreatment, it required an equal amount of input energy (Bose et al. 2020).

6.5.1.3 Pretreatment by Irradiation

Irradiation-based processes effectively solubilize biomass by polarizing their macromolecules with subsequent hydrolysis of the cell organelles (Passos et al. 2014). Gamma-ray irradiation is a penetrating form of short wavelength electromagnetic waves, while microwave irradiation is a form of electromagnetic irradiation with mobile electric charges (Li et al. 2014; Velazquez-Lucio et al. 2018). The advantage of irradiation treatment is the improved starch digestibility in algae which further extrapolates efficient enzymatic degradation (Anto et al. 2020). In addition, microalgal biomass pretreated with irradiation shows improved biomass thickening and dewatering, which is beneficial for subsequent extraction process (Passos et al. 2014). This was corroborated in a study where *Undaria* biomass treated with gamma irradiation showed improved saccharification with the release of significantly high reducing sugar (Yoon et al. 2012). Similarly, microwave-assisted pretreatment of *Laminaria japonica*, *Microcystis wesenbergii* and *Microcystis aeruginosa* showed higher saccharification efficiency (Cheng et al. 2014; Yin and Wang 2018).

Numerous factors were advantageous for microwave-assisted pretreatments, such as process automation with low-energy input and rapid heating rate, which significantly promoted its application in biomass conversion (Chen et al. 2019b). However, irrespective of its substantial maintenance cost and high heat generation, it is hugely recommended for industrial-level processing (Anto et al. 2020).

6.5.1.4 Hydrothermal Pretreatment

This method involves applying heat in either neutral, acidic or alkaline conditions, where water under high pressure induces disruption of the cell wall and solubilization of cell components (Biller and Ross 2012). The operating temperature for hydrothermal pretreatment usually ranges from 60 to 200 °C for short intervals of 0–60 min (Pirwitz et al. 2016; Velazquez-Lucio et al. 2018). Use of acid and alkaline hydrothermal methods has their own merits where acid treatment helps in degrading the cellulosic matrix and starch into soluble sugars. In contrast, alkaline treatment employs a solvating effect on the cell wall, forming pores along with starch solubilization (Velazquez-Lucio et al. 2018). However, due to the problems associated with the acidic and alkaline medium's disposal, treatment at neutral conditions is preferred (Anto et al. 2020). Application of hydrothermal treatment at 100 °C for 0 min on *Dunaliella salina* biomass showed 80% saccharification efficiency (Pirwitz et al. 2016). Similarly, solar-driven hydrothermal processing of *Chlorella pyrenoidosa* resulted in 7.4 times more carbohydrates than the untreated biomass with a 57% increase in biomethane production (Xiao et al. 2019). However, in another study, hydrothermal fractionation of *Schizocytrium* sp. at 115.5 °C for 46.7 min resulted in 19.4% of oligomeric sugar with subsequent production of 11.8 g-ethanol/L (Kim et al. 2012).

6.5.1.5 Chemical Pretreatment

Use of different chemical pretreatments in lignocellulosic biomass is well documented. Similarly, chemical pretreatment showed significant results for algal biomass (Jędrzejczyk et al. 2019; Li et al. 2014). The most commonly used chemical pretreatment method on algae involves using an acid or alkali that was subsequently neutralized and the treated biomass exploited for the synthesis of biofuels. Acid pretreatment could be directly used to hydrolyse the biomass to simple sugars or acid pretreatment followed by enzymatic hydrolysis could be employed for more prominent results (Jędrzejczyk et al. 2019). Generally, acids such as HCl and H_2SO_4 are used either in a concentrated or dilute form; however, use of concentrated acids usually gives maximum sugar yields. But concentrated acid treatments are associated with various drawbacks such as hazardous processing, the requirement of high-cost reactors and expensive post-recovery and recycling processes, hence less studied (Li et al. 2014). On the other hand, dilute acid hydrolysis has been extensively studied and considered one of the best methods of pretreatment with a wider

application (Sivagurunathan et al. 2017). For example, the efficiency of H_2SO_4 (2%) combined with hydrothermal treatment on algal bloom containing *Microcystis wesenbergii* and *Microcystis aeruginosa* species yielded 299.88 mL CH_4/g total volatile solids (Cheng et al. 2019). In another study, defatted *Chlorella* biomass treated with 0.25 N HCl at 121 °C for 15 min exhibited the highest yield of fermentable monosaccharides (Yun et al. 2020). Similarly, *G. amansii* and *K. alvarezii* treated with H_2SO_4 (0.1 M) released 0.51 and 0.56 (g sugar/g biomass) with subsequent bioethanol fermentation efficiency of 31% and 33%, respectively (Mushlihah et al. 2020).

In contrast, alkaline treatment involves using bases such as ammonium, calcium, potassium and sodium hydroxide, which are majorly involved in lignin solubilization. However, due to the compositional difference in algae with other lignocellulosic biomass such as the lack of lignin content, pretreatment of algal biomass using an alkaline medium is very limited (Li et al. 2014). In an earlier study, *Chlorococcum infusionum* biomass treated with NaOH (0.75% w/v) at 120 °C for 30 min showed a 35% glucose release with subsequent bioethanol yield of 0.26 g ethanol/g algae (Harun et al. 2011), while *C. vulgaris* pretreated by thermo-alkaline pretreatment showed improved methane yields from 4% to 26% (Zhang et al. 2019).

Irrespective of the beneficial effect of the chemical pretreatment on algal biomass such as the increase in the enzymatic digestibility of the treated biomass, different inhibitory compounds produced from the degradation of sugars, such as aldehydes, ketones and phenolic acids, are considered as the main disadvantage of this method (Solarte-Toro et al. 2019).

6.5.1.6 Ozone Treatment

Ozonolysis is another interesting technique that involves using ozone to oxidize, solubilize and degrade the cell wall and components (Mushlihah et al. 2020). Ozonolysis has not been studied in detail for most biomasses; algal biomass is not an exception. However, its low operation cost, ambient pressure and temperature requirements and lack of inhibitory compound generation encourage its usage on an industrial scale (Travaini et al. 2016). In a recent study, *G. amansii* and *K. alvarezii* treated with ozone (400 mg O_3/L) yielded 0.41 and 0.52 (g sugar/g biomass) with subsequent bioethanol fermentation efficiency of 76 and 92%, respectively (Mushlihah et al. 2020). However, in another study, *Scenedesmus obliquus*, when treated with ozone (27 mg O_3/L) in combination with ultrasound, solubilized proteins, lipids and carbohydrates with a yield of 91%, 89% and 63%, respectively (González-Balderas et al. 2020b). Similarly, *Desmodesmus* sp. treated with ozone (45 mg O_3/L) released 84% of carbohydrates (González-Balderas et al. 2020c).

6.5.1.7 Enzymatic Pretreatment

The saccharification of biomass is a critical phase in bioethanol/biomethane production, where complex carbohydrates are transformed into simple sugars. Enzymatic saccharification offers a greener route, as the processes are eco-friendly and efficient requiring less energy, and could be operated at ambient environmental conditions without forming any inhibitory by-products (Amobonye et al. 2020; Kumar et al. 2016). Algal cell walls are rich in carbohydrates, in proteins and with minute lipid moieties. However, the cell wall composition significantly varies with algal species, different phases of growth, growth media composition and concentration (Gojkovic et al. 2020). Enzymatic pretreatment of biomass could be performed using a single enzyme or mixture of enzymes; however, the enzyme mix is always preferred due to its better performance (Okuofu et al. 2020). The most commonly used enzymes for saccharification belong to a group of carbohydrases which include amylases, cellulases, hemicellulases and pectinases, while the enzyme mix consists of different combinations of carbohydrases along with other hydrolytic enzymes such as laccase, lysozyme and protease (Anto et al. 2020). Both macroalgae and microalgae have been intensively studied for enzymatic pretreatment and showed good saccharification potential for subsequent biofuel production. For example, deoiled algal biomass (DAB) dominated with *Chlorella* sp. and *Scenedesmus* sp. treated with α-amylase (400 IU/g) and cellulase (10 IU/g) in combination with physicochemical treatment resulted in higher sugar solubilization (0.590 g/g DAB) with subsequent bioethanol production (0.145 g/g DAB) (Kumar et al. 2020b). However, in another study, *Porphyridium cruentum* biomass treated with cellulase (>700 EGU/g), protease (>16 units/g) and a carbohydrase mix (>100 FBU/g) showed improved biomass solubilization along with higher biomethane yields (Çakmak and Ugurlu 2020). These recent reports confirm the efficacy of enzymatic saccharification; however, more prominent results could be obtained by the amalgamation of enzymatic pretreatments with numerous other pretreatment methods (Amamou et al. 2018; Dar and Phutela 2019).

6.5.1.8 Other Treatment Methods

Certain other treatments such as freezing/thawing and steam explosion have been studied for algal biomass saccharification; however, these pretreatments face severe disadvantages due to the requirement of high energy, tedious and costly practices and high maintenance (Li et al. 2014). Use of ionic liquids, organosolv and deep eutectic solvents is either less studied or not studied at all for algal biomass saccharification; however, owing to their success in lignocellulosic biomass pretreatments, they could show similar efficiency for saccharification of algal biomass (Jędrzejczyk et al. 2019; Okuofu et al. 2020).

6.5.2 Bioethanol Production

Bioethanol production using fermentation technology is well documented where ethanol can be produced by various fermenting microorganisms such as *Candida shehatae*, *Clostridium thermocellum*, *Pachysolen tannophilus*, *Zymomonas mobilis*, *Scheffersomyces stipitis* (*Pichia stipitis*) and *Saccharomyces cerevisiae*. However, most commercial-level ethanol production is carried out using *S. cerevisiae* (Karagoz et al. 2019). To enhance bioethanol production, various parameters, such as isolation of robust strains, physicochemical optimization and genetic manipulation with minimum feedback inhibition, are equally important (Kumar et al. 2020a).

Algal biomass is an extraordinary alternative to existing biomass in terms of bioethanol productivity (Table 6.2). Algae ethanol productivity is estimated to be ~two times higher than sugar cane biomass and ~ five times higher than maize biomass (Sirajunnisa and Surendhiran 2016). High starch-containing microalgae can produce 140,000 L/ha/year of bioethanol, which is comparatively very high compared to other liquid fuels. Microalgae are rich in carbohydrates and contain 40–50% of average sugar to their biomass, while some algal species such as *Grateloupia* and *Ulva* contains more than 50% sugar (Balar et al. 2019; Denis et al. 2010; Kumar et al. 2020a).

Carbohydrate-rich algal biomass after pretreatment can be consumed by fermenting microorganism which subsequently produces an equimolar concentration of ethanol and carbon dioxide. Two well-known fermentation mechanisms for bioethanol production are separate hydrolysis and fermentation (SHF) and simultaneous saccharification and fermentation (SSF) (Sirajunnisa and Surendhiran 2016). The SHF is carried out in two separate fermenters, wherein the first fermentor biomass is hydrolysed, while in the second fermenter, anaerobic fermentation prevailed (Li et al. 2014). Advantage of this process is that separate optimal conditions can prevail for both hydrolysis and fermentation along with the continuous mode operation of the systems. However, a major challenge of this process is the separation of wet biomass after hydrolysis, which subsequently confines the bioethanol yield (Offei et al. 2018). In contrast, the second system, SSF, has a simpler mechanism where both hydrolysis and fermentation are carried out in a single fermenter. This system is more beneficial over SHF as it limits the production cost, processing time and product inhibition. In addition, the process is simple where glucose from concurrent hydrolysis is immediately consumed and converted to ethanol, thereby improving the yield. Furthermore, single fermenter reduces the cost along with reduced contamination (Li et al. 2014). However, an uncontrolled production system is the main disadvantage of SSF. Furthermore, concurrent saccharification and fermentation limit both substrate and enzyme reuse, making the process ineffective at commercial level (Kumar et al. 2020a). Another disadvantage of SSF system is the ethanol mediated denaturation of yeast cells; however, genetically engineered ethanol-tolerant yeasts have successfully encountered this issue (Sirajunnisa and Surendhiran 2016).

Table 6.2 Bioethanol and biomethane production from algal biomass

Algae	Pretreatment conditions	Reducing sugar (g/L)	Organism (fermentation/ anaerobic digestion)	Yield	Reference
Saccharina japonica	40 mM H_2SO_4 (121 °C for 60 min), Novozyme (Termamyl 120 L)	45.6	*Pichia angophorae* KCTC 17574	0.16*/ 33.3†	Jang et al. (2012)
Kappaphycus alvarezii	0.9 N H_2SO_4 (100 °C for 60 min)	51.9	*S. cerevisiae* NCIM 3523	0.42*/ 82.36†	Khambhaty et al. (2012)
Ulva pertusa	High thermal liquefaction, amyloglucosidase and cellulase (pH 4.8, 50 °C, 150 rpm for 24 h)	26	*S. cerevisiae* ATCC 24858	0.48*/ 93.51†	Choi et al. (2012)
Enteromorpha intestinalis	Thermal acid hydrolysis (13% w/v of solid contents, 75 mM of H_2SO_4 for 60 min), Celluclast 1.5 L and Viscozyme L (55°C, 120 rpm for 54 h)	40.4	*S. cerevisiae* KCTC 1126	0.21*/ 41.74†	Cho et al. (2013)
Gracilaria sp.	H_2SO_4 (0.1 N,121 °C for 30 min), commercial enzyme (pH 4.5, 50 °C)	11.46	*S. cerevisiae*	0.42*/ 82.80†	Wu et al. (2014)
G. verrucosa	373 mM H2SO4, Celluclast 1.5 L and Viscozyme L (pH 5, 45 °C, 150 rpm for 72 h)	20.4	*S. cerevisiae* KCTC 1126	0.48*/ 94†	Nguyen et al. (2017)
Gelidium sesquipedale	Mechanical milling, haliatase cocktail (pH 5.5, 37 °C for 72 h)	70.9%	*S. cerevisiae*	0.03*/ 68.7†	Amamou et al. (2018)
U. lactuca	Mechanical milling, haliatase cocktail (pH 5.5, 37 °C for 72 h)	126.3	*S. cerevisiae*	0.05*/ 64.3†	Amamou et al. (2018)
Ulva sp.	0.1% H_2SO_4 (100 °C for 60 min), cellulase 22,119 (pH 4.8 at 45 °C for 36 h)	20.6	*S. cerevisiae*	0.45*/ 88.24†	Hebbale et al. (2019)
Chlorella sp.	Hydrothermal pretreatment, 1.5% H_2SO_4 (117 °C for 20 min), α-amylase (pH 5.7, 85 °C), glucoamylase (pH 4.5, 65 °C)	302.1	*S. cerevisiae* TISTR 5339	0.48*/ –	Ngamsirisomsakul et al. (2019)
Chlorella sp.	1% H_2SO_4 (121 °C for 15 min), α-amylase, cellulase (pH 5.8, 50 °C, 120 rpm for 72 h)	490	*S. cerevisiae*	0.145*/ 78†	Kumar et al. (2020b)
Chlamydomonas reinhardtii	*T. harzianum* strain 78 spent medium (55 °C for 16 h)	22.4	*S. cerevisiae*	0.51*/ 100†	Bader et al. (2020)

(continued)

Table 6.2 (continued)

Algae	Pretreatment conditions	Reducing sugar (g/L)	Organism (fermentation/ anaerobic digestion)	Yield	Reference
C. vulgaris	Onozuka R-10, Macerozyme R-10	ND	Anaerobic inocula (municipal waste water treatment plant)	414‡	Wieczorek et al. (2014)
C. pyrenoidosa	Solar-driven hydrothermal pretreatment	269.7	Anaerobic slurry (countryside biogas digester)	348‡	Xiao et al. (2019)
Porphyridium cruentum	Cellulase, viscozyme, protease: 24 h	90.56	Mesophilic anaerobic inocula (municipal waste water treatment plant)	270‡	Çakmak and Ugurlu (2020)
Chlorella sp.	Hydrothermal pretreatment	ND	Anaerobic sludge	434.38‡	Wu et al. (2020)

ND Not defined
*Ethanol yield (g/g)
†Theoretical yield (%)
‡mL CH_4/g VS

Interestingly, plenty of earlier studies have shown higher production of bioethanol from SSF than SHF. For example, in *Porphyridium cruentum* fermentation for ethanol production, the yield from SSF (80.3%) was more than SHF (77.5%) (Kim et al. 2017). Similarly, in another study, *Gelidium amansii*-pretreated biomass showed maximum bioethanol yield with SSF (3.78 mg/mL) than SHF (3.33 mg/mL) (Kim et al. 2015). A few reports have shown that SSF system could be incorporated with a cofermenting organism such as *Pichia stipitis* to ferment five-carbon sugars that usually few yeasts cannot ferment. This enhances ethanol production, where both pentoses and hexoses are consumed and converted to ethanol (Li et al. 2014).

6.5.3 Biomethane Production

Biomethane is a green natural gas substitute and is primarily produced by pretreated organic biomass anaerobic digestion (AD), which subsequently produces a mixture of methane-dominated gases (50–70%) along with carbon dioxide (30–50%). However, AD is not the only biomethane production method; a complementary gasification technique has also been developing rapidly (Kumar et al. 2020a). Several previous studies have focused on lignocellulose as a potential organic biomass for biomethane production. Interestingly, algal biomass is also gaining importance as an alternate source to the first- and second-generation biomass for advanced biomethane production (Bose et al. 2020).

Biomethane production from pretreated/whole-cell algal biomass utilizing AD has become an alluring and sustainable approach (Khoo et al. 2019). A typical anaerobic digester comprises the following four stages: hydrolysis, acidogenesis, acetogenesis and a rate-limiting step of methanogenesis. Firstly, the hydrolysis process involves the conversion of whole cells and high complex macromolecules to simpler forms. In contrast, in acidogenesis those soluble monomers are converted to various metabolic products, followed by acetogenesis, which converts them into acetic acid and hydrogen. Finally, methane is produced in the methanogenesis step (Koonaphapdeelert et al. 2020). Considering these facts, the choice of algal cells is a very crucial factor for biomethane production. Algal cells rich in lipids have a rigid cell wall that makes them less feasible for AD process. In contrast, algal biomass rich in carbohydrates and proteins is preferable for biomethane production (Klassen et al. 2017). A typical production yield of algal biomethane ranges either from 0.2 to 0.4 m^3 CH_4/kg or 0.024 to 0.6 L CH_4/g VS (volatile solids) which significantly varies depending on the algal species and physicochemical conditions (Kumar et al. 2020a).

On the contrary, gasification process involves the partial oxidative conversion of carbon-rich feedstocks into syngas in the presence of air steam at high temperatures (100–1000 °C), where syngas is a mixture of gases, comprising CO_2, CO, H_2 and CH_4 in different combinations (Raheem et al. 2018). Gasification is a complementary technology for AD where feedstocks that are impossible to digest by AD alone such as woody biomass and polluted organic waste can be converted to biomethane

(Koonaphapdeelert et al. 2020). However, due to the easily digestible nature of algal biomass, gasification is deemed redundant over AD.

Recovery of biomethane from the mixture of gases by removing CO_2 and other impurities is accomplished by well-known biogas upgrading methods. Cryogenic upgrade utilizes the diverse boiling/sublimation points of the different gases. In contrast, the use of a natural enzyme, carbonic anhydrase, is also an interesting mechanism which catalyses CO_2 and water into bicarbonate and protons through a reversible reaction (Koonaphapdeelert et al. 2020). However, use of CO_2 desorption column to solubilize and remove CO_2 from CH_4 is a well-established process where CO_2 is absorbed by an alkaline solution with carbonate medium which removes ~95% of CO_2 along with other contaminants. This CO_2-rich carbonate medium subsequently supports the growth of algal biomass and hence reused as a feed for fresh algal biomass which establishes the efficacy of this process (Bose et al. 2020).

Various algal species have been exploited for biomethane production, suggesting their efficiency as an ideal substrate for this process (Table 6.2). For example, *Chlorella pyrenoidosa* biomass with solar-driven hydrothermal pretreatment yielded 348 mL CH_4/g VS of methane production (Xiao et al. 2019), while enzymatically pretreated *Porphyridium cruentum* biomass yielded 270 mL CH_4/g VS (Çakmak and Ugurlu 2020). In another study, hydrothermally pretreated *Chlorella* sp. showed a biomethane production level of 434.38 mL CH_4/gVS (Wu et al. 2020).

6.6 Algal Biorefinery Concept

Environmental sustainability and energy crises are the two major challenges confronting humans in the twenty-first century. Huge emission of greenhouse gases due to the immense consumption of fossil fuels has made exploring alternative renewable energy resources a priority (Khoo et al. 2019). Due to the emergence of food versus fuel feuds, consumption of first-generation edible feedstocks such as maize and sugar cane is reduced (Jambo et al. 2016). Interestingly, feedstocks such as forest and agricultural residues of second-generation lignocellulosic biomass have the ability to produce more vibrant products such as bioethanol, biochar, bio-oil and syngas. However, the limiting factor in this case, the intensive and costly pretreatment methods, gave rise to third-generation biomass, 'algae' (Khoo et al. 2019). It defies all the limitations of first- and second-generation biomass and has the ability to produce a variety of products cascading an ideal biorefinery process. The algae biorefinery concept embodies the conversion of algal biomass by integrating upstream and downstream processing to generate biofuels and value-added products (Kumar et al. 2020a).

6.6.1 Upstream Processing

Upstream processing involves selection and growth of algal strain which is a critical factor influencing generation and recovery of the product of interest, during downstream processing. Generally, the selection is favoured for fast-growing algal strains with robust nature, capable of handling enough shear stress in the medium for multiple cycles (Rodolfi et al. 2009). Selection of strain based on its physicochemical characteristics is the key factor where macromolecular contents of species are considered for specific products. For example, high carbohydrate-containing species are more suitable for bioethanol and biomethane production, while species rich in lipid content favourited for biodiesel production (Rempel et al. 2019; Shakya et al. 2017). However, secondary factors such as tolerance of algae towards microbial contamination (Canton et al. 2019), species with good separation ability subjugating requirement of high energy for cell disruption (Günerken et al. 2015) and highly flocculating nature of species reducing the chances of adhesion of cells on the wall of the reactor are added advantages (Mubarak et al. 2019). In addition, recent advancement involves selecting appropriate algae through primary screening for the above factors followed by genetic engineering-mediated changes in selected strains to suit the targeted biorefinery routes (Khoo et al. 2019).

6.6.2 Downstream Processing

Depending on the cost-efficiency and sustainability of the operation, there are various routes recorded to date for the conversion of algal biomass into ranges of biofuels and value-added goods (Fig. 6.3). The viability of products from algal biomass is mainly controlled via efficient extraction methods. However, a successful extraction process should be more inclined to extract particular bioproducts while minimizing impurities at the same time (Kumar et al. 2020a).

The transformation of algal biomass to biofuels and biochemical products requires different biorefinery processing technologies (Khoo et al. 2019). Whole-cell biomass can be converted to numerous value-added products such as syngas, bio-oil, biochar, hydrochar and biomethane following the chemical conversion routes of pyrolysis, gasification, hydrothermal liquefaction and carbonization and anaerobic digestion, respectively (Anto et al. 2020; Li et al. 2014). In addition, elaborative processing techniques followed by extraction and purification can yield more refined products such as pigments, sterols, vitamins, proteins, lipids and carbohydrates which can be then be used in the pharmaceutical products, functional foods and bionutrients or converted into biodiesel, bioethanol and biomethane, respectively (Bhushan et al. 2020; Khoo et al. 2019).

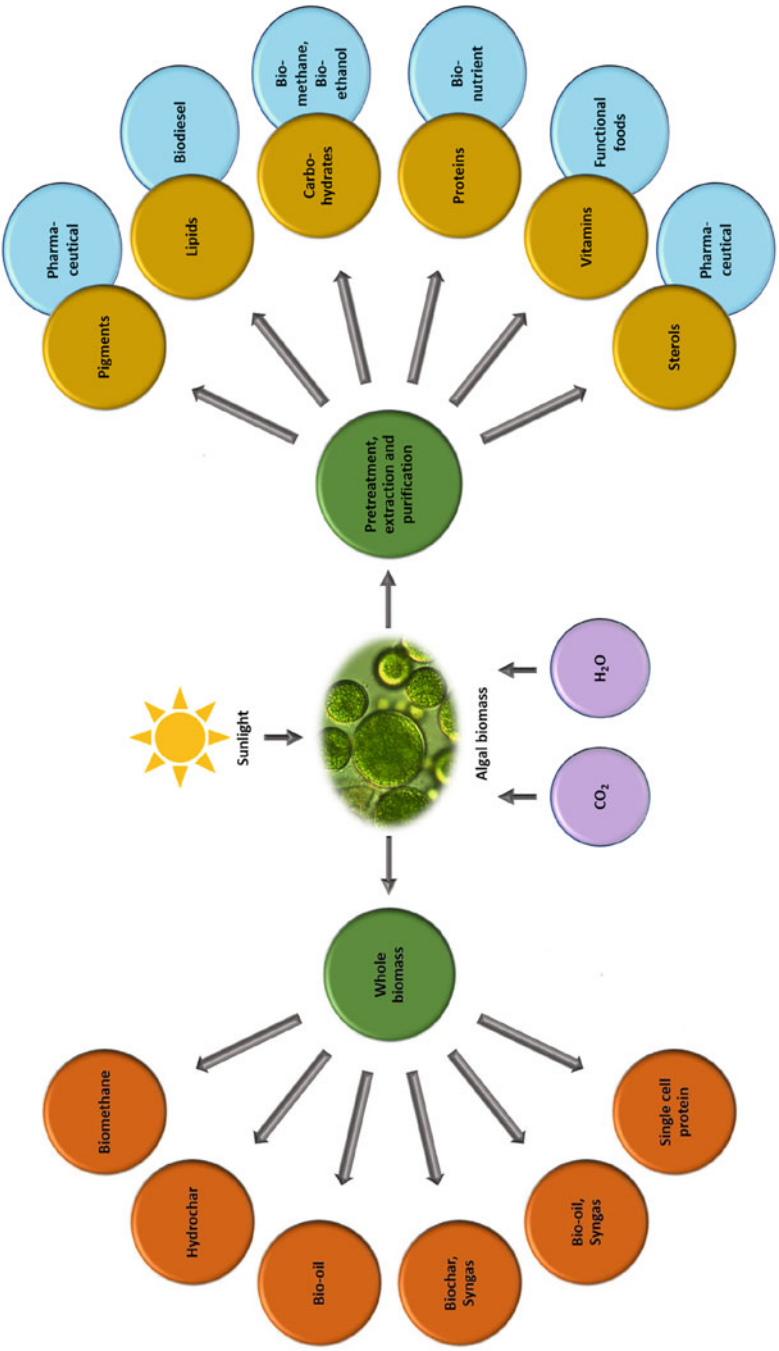

Fig. 6.3 Algal-based biorefinery: conversion of algal biomass into biofuels and value-added products

6.7 Biotechnological Strategies to Improve Algal Biomass and Biofuel Production

The economic viability of algal carbohydrates for biofuel production could be achieved with its improved productivity. Numerous factors have been investigated and reported to improve the carbohydrate content in algal biomass, which could be accomplished by applying techniques such as medium composition and physicochemical parameter optimization along with genetic engineering modifications (Fayyaz et al. 2020; Surendhiran and Sirajunnisa 2019).

6.7.1 Optimization of Physicochemical Parameters

The build-up of various macromolecules such as carbohydrates, lipids and proteins in algal biomass is dependent on medium composition and other environmental conditions such as CO_2 content, light, pH, salinity and temperature. Growth of algae is highly dependent on above-listed parameters suggesting their critical requirement in the production. However, minerals such as magnesium, nitrogen, phosphorus, potassium and sulphur are vital for algal growth. Micronutrients such as iron and manganese are required in minute quantities (2.5–30 ppm). In contrast, trace elements such as boron, cobalt, copper, molybdenum and zinc are reportedly added in very little amounts of 2.5–4.5 ppm (Banerjee et al. 2020; Juneja et al. 2013). These physicochemical alterations subsequently result in varied biochemical composition of algal biomass, contributing to enhanced carbohydrate, lipid or protein accumulations depending on algal strains (Lage et al. 2018).

6.7.2 Effect of Different Physicochemical Parameters

Algal growth is mainly dependent on light and intensity, a critical parameter for its overall growth and accumulation of macromolecules (Agarwal et al. 2019). Previously, microalga grown with varied light conditions exhibited significant changes in carbohydrate accumulation; however, light parameter beyond optimum level results in photoinhibition, which subsequently reduces biomass productivity (Chen et al. 2013). Interestingly, around 500% increase in starch content was reported for *Chlorella vulgaris* when light intensity was varied from 215 to 330 μmol/m^2s (Brányiková et al. 2011). Temperature and pH are also one of the important limiting factors affecting the growth of algal biomass. Temperature is known to influence the starch content of algal cells. At elevated temperatures, α-amylase and α-glucan phosphorylase mediate the degradation of starch; when under cold stress, the accumulation of carbohydrates is increased due to activation of gluconeogenesis and the pathway of starch biosynthesis (Banerjee et al. 2020). Inversely, in some

Spirulina species, increased carbohydrate content with increased temperature has been reported (Ogbonda et al. 2007). The pH also plays a detrimental factor in the growth of algae; however, it is highly species-specific (Surendhiran and Sirajunnisa 2019). For example, a freshwater chlorophycean microalga *Scenedesmus acuminatus* over the pH range of neutral to alkaline (9.0) showed more than 50% increase in biomass and carbohydrate production. However, maximum biomass and carbohydrate content was obtained at pH 6.69 for another species, *S. obliquus* (Chandra et al. 2020; Singh et al. 2019). Nutrient stress is also one of the inducing factors responsible for considerable change in the biochemical composition of algal biomass (Surendhiran and Sirajunnisa 2019). Nitrogen depletion conditions have been previously reported for elevated levels of carbohydrates in the algal biomass where around 50% increase in carbohydrate content was observed for *Microcystis* under low nitrogen content (Huang et al. 2019). In contrast, in another study, nitrogen loading of 0.02 g $N/m^2/d^1$ decreased the *Microcystis* biomass by 56.2% with a parallel decrease in carbohydrate content (Huang et al. 2020). A similar phenomenon is also observed for phosphorus deficiency, which reportedly increased carbohydrate content in algal cells (Labry et al. 2020). The carbon concentration mechanism improves the carbohydrate content in algal biomass during CO_2 starvation where algal cells accumulate and retain inorganic carbon from its extracellular environment (Banerjee et al. 2020).

Alteration of physicochemical parameters reportedly improved biomass and macromolecule accumulation in algal biomass; however, these widely adapted approaches have certain limitations that can affect the overall productivity. In closed photobioreactors (PBR), certain parameters such as light, pH or temperature can be regulated; however, it is certainly impossible to maintain these parameters in outdoor cultivations (Surendhiran and Sirajunnisa 2019). In addition, the mechanism of nutrient starvation enhances the concentrations of various macromolecules (carbohydrates, lipids and proteins). However, many reports suggested that this ends up in distorting photosynthetic efficiency subsequently resulting in reduced chlorophyll content affecting overall algal biomass after repeated cultivations (Banerjee et al. 2020; Lauritano et al. 2019; Takahashi et al. 2020). These obstructions could be successfully overcome by employing genetic manipulation techniques where targeted enzyme modification can improve the accumulation of carbohydrate content without conceding the levels of algal biomass (Banerjee et al. 2020; Surendhiran and Sirajunnisa 2019).

6.7.3 Genetic Engineering-Mediated Metabolite Improvement

Irrespective of extensive research on algal fuels since the 1970s, not a single commercially viable strain has been reported to date for industrial production (Surendhiran and Sirajunnisa 2019). Therefore, genetic engineering-mediated

manipulation of the algal genome to enhance carbohydrate accumulation is considered necessary for algal biofuel production. New genome editing tools such as CRISPR/Cas9, RNAi, TALENs and ZNFs have been used in recent years to improve the quality and quantity of a range of products. This has enabled researchers in constructing transgenic algal strains with increased biomass yield and high-value compound accumulation efficiency (Fayyaz et al. 2020). Around 30 different algal species have been genetically engineered till date, most of them with a stable expression while very few with a transient expression. However, transient expressions can be stabilized through the appropriate use of codon and species-specific endogenous promoter's intron sequences (Banerjee et al. 2020).

A tremendous amount of work has already been done for enhanced lipid accumulation, hydrogen (H_2) production and pigment production using genetic engineering of different algal strains; however, very limited reports are available on carbohydrate accumulation through genetic manipulation (Banerjee et al. 2020; Fayyaz et al. 2020).

Starchless mutants of *Chlamydomonas reinhardtii* (*sta6-sta7*) transformed with WT *sta7* gene showed enhanced starch accumulation (Work et al. 2010). These transformed mutants with enhanced starch content can be used for either bioethanol or biomethane production. Similarly, the overexpression of transcription factor Pi Starvation Response1 (PSR1) in *C. reinhardtii* showed increased starch content and reduced neutral lipid content which is beneficial for bioethanol or biomethane production (Bajhaiya et al. 2016). In another study, to enhance and extract fermentable carbohydrates simultaneously from algal biomass, *Thermotoga neapolitana* amylase gene was transformed in *C. reinhardtii*, where transformed algae expressed thermostable amylase production subsequently reducing additional pretreatment process suggesting the suitability of transformed algal biomass for biofuel production (Wang et al. 2015).

Autotrophic organisms convert atmospheric inorganic carbon to organic compounds with the help of carbon fixation. RuBisCo (ribulose-1,5-bisphosphate carboxylase/oxygenase) is a key enzyme involved in the fixation of carbon dioxide (CO_2) into the Calvin cycle (Feller et al. 2008). Engineering RuBisCo to enhance its catalytic efficiency and selectivity is a spectacular way to improve carbon fixation (carbohydrate accumulation); however, due to the inherent problems of RuBisCo, it fails to enhance selectivity and velocity of enzyme altogether (Whitney et al. 2011). Interestingly, heterologously overexpressing the catalytically active RuBisCo, in particular from red algae, which is more effective than mutation of RuBisCo gene, can overcome it (Ng et al. 2017). In addition, thioredoxin-regulated enzymes such as fructose-1,6-bisphosphatase (FBPase), ribulose-5-phosphate kinase (PRKase) and sedoheptulose-bisphosphatase (SBPase) are key enzymes which can be genetically manipulated to accelerate carbon fixation (Fayyaz et al. 2020).

Due to the poor tolerance of algal cells to the alcohol generated, the production of alcohol-based fuel from algae is unfavourable. *Chlamydomonas* hybrid cluster protein 4 (HCP4) is highly regulated during anoxia conditions, where nitrogen utilization and fermentation pathways are significantly regulated. The respective protein was subjected to gene silencing, where the developed mutant ami-HCP

downregulated several enzymes during the course and enhanced ethanol secretion (Olson and Carter 2016). In addition, presence of both acetyl-CoA and acetaldehyde pathways for the production of ethanol and the known localization of the phosphotransacetylase (PTA) and acetate kinase (ACK) genes have rendered *Chlamydomonas* a simple host for genetic modification for enhanced ethanol production (Banerjee et al. 2020). In another study, in silico metabolic engineering simulations allowed the identification of specific genes and pathways in *Synechocystis* sp. which can be knocked down or upregulated for better production of biofuels (Montagud et al. 2010).

Recently, numerous studies reported successful alteration of the algal genome; however, genetically modified (GM) algal strains' stability and their use in an open environment remain a major challenge. A critical hazard assessment is required before releasing GM organism prior to its commercialization (Surendhiran and Sirajunnisa 2019). Except for a handful, including *Prototheca wickerhamii*, a pathogen responsible for protothecosis disease in cattle, cats and dogs and even in humans, green algae are usually harmless to the environment (Kumar 2015). In addition, any antibiotic resistance marker gene used in GM studies can be passed to a wild-type strain, resulting in a novel antibiotic-resistant strain that may be encountered by eliminating such marker genes prior to commercial cultivation (Shao et al. 2014).

6.8 Life Cycle Assessment

Life cycle assessment is an environmental management methodology used to evaluate a product's life cycle based on the ISO 14040 and 14,044 framework (Finkbeiner et al. 2006). It evaluates the various impacts of the production processes on the ecosystem, irrespective of its production aim. Life cycle assessment relies on four distinct principles comprising goal definition, inventory analysis, impact assessment and interpretation. Every minute step involved in the production, starting from raw materials, the numerous treatments given, different purification methods involved, the final yield of the product, energy consumption and waste generation, is considered and analysed for a life cycle assessment (Rebello et al. 2020).

Irrespective of the immense potential of algal-based biofuels such as bioethanol and biomethane, their energy-intensive production significantly limits their applicability compared to cost-effective conventional fuels (Chisti 2007; Medipally et al. 2015). However, excessive depletion of fossil fuels coupled with increased greenhouse gas emissions, and resultant climate change, demands newer alternate sources that are environmentally sustainable (Khoo et al. 2019). Algal sources have gained much importance in this situation owing to the presence of well-digestible macromolecules (carbohydrates, lipids and proteins), no requirement of cultivable land and rapid growth rate with the absence of recalcitrant structures (lignin) that adds to its utility in sustainable biofuel generation. However, at various stages of algal growth, including harvesting, pretreatment and bioconversion to fuels, the production cost of

algal biofuels (which is ~10 to 100 times more than petroleum-based products) should be considered, analysed and reduced (Rebello et al. 2020).

The choice of algal strain significantly affects the life cycle of bioethanol/biomethane yield owing to its difference in macromolecular construction. Carbohydrate-rich algal strains are usually favoured for bioethanol production (Yoo et al. 2015). High lipid-containing algal strains enhance biomethane production; however, it also makes cell wall rigid, while biomass rich in carbohydrates and proteins show faster biogas production rates (Ohemeng-Ntiamoah and Datta 2018). Promising sources for the synthesis of bioethanol/biomethane could be natural high carbohydrate-containing strains of *Caulerpa*, *Cladophora*, *Grateloupia*, *Ulva*, etc. and even potent carbohydrate yielding strains produced using genetic engineering (Balar et al. 2019; Denis et al. 2010; Fayyaz et al. 2020; Suganya et al. 2016). However, sometimes the optimization of physicochemical parameters suffixes the required yield of algal macromolecules (Banerjee et al. 2020; Surendhiran and Sirajunnisa 2019). Further, to this, wastewater-based units could be favourable for the growth of algae, thereby reducing the production cost (Rebello et al. 2020). However, owing to contamination problems in wastewater-based units, other economically viable options such as flat-plate PBR and open raceway pond (OPR) are preferable (Khoo et al. 2019).

The pretreatment strategies vary with the type of biomass and product of interest. A variety of pretreatment systems such as chemical, enzymatic, hydrothermal, mechanical, etc. are used to treat the algal biomass, which occupies the major production cost. Data analysed from various life cycle investigations suggests that most of the cell harvesting techniques consume intense power. Dewatering using conventional thermal process is very costly (75% of production cost) which could be overcome by cost-effective and better extraction techniques such as electroporation, hydrothermal liquefaction, jet engine extraction, wet extraction, etc. Interestingly, solar-based dryers can be a very cost-effective alternative for dewatering, though these strategies are pragmatically not applicable worldwide, and can be applied in tropical countries (Khoo et al. 2019; Rebello et al. 2020). Selection of pretreatment is also critical for the assessment of the production cost. Various novel techniques such as irradiation, solar-driven hydrothermal processing, ozonolysis, etc. are found to be cost-effective pretreatment methods for algal biomass and paving their way for industrial-level processing (Anto et al. 2020; González-Balderas et al. 2020c; Xiao et al. 2019). In addition, enzyme pretreatment has been found to be highly efficient giving better productivity; however, more emphasis on large-scale optimization of such methods is a dire need of the hour (Zabed et al. 2019).

6.9 Conclusion and Prospects for Future Research Works

First- and second-generation biofuels certainly failed to meet the global requirements, while algal biomass represents an emerging feedstock for biofuel production in a more sustainable manner. Globally, researchers are engaged in cutting-edge

research to develop cost-effective and environmentally sustainable methods for the development of algal biofuels. The current technology for the production of biofuels from these biomass needs further development to reach industrial-scale processes, despite extensive research on algal biofuels in past decades. This chapter contributes significantly to the pragmatic future growth of algal biofuel production in this scenario. And the following are the recommendations for future research works based on the current state of the art:

(a) Selection of algal strain is the most significant factor which determines the fate of the algal biofuels. Physicochemical optimization can help up to a certain extent; however, genetically modified algae with improved metabolic pathways may result in enhanced production/accumulation of biofuels.
(b) Industrial-scale production of algal biomass can be achieved by wastewater-based units, thereby reducing the production cost. However, flat-plate PBR and open raceway pond (OPR) are also economically viable options owing to contamination problems in wastewater-based units and should be studied for scale-up investigations.
(c) Separation of microalgal biomass from liquid culture is very challenging and cost consuming. Costly thermal dewatering processes can be replaced with a cost-effective solar-based dryer, which could be exploited in tropical countries. In contrast, the development of drying methods using other natural renewable energy sources remains unexplored and should be exploited.
(d) Pretreatment techniques used to release polysaccharides from harvested algal biomass have a direct impact on biofuel production. Coupling of multiple pretreatment methods is an alluring approach and needs to be studied for industrial-level scale-up.
(e) The LCA analysis for algal biomass pretreatment is relatively less studied than other stages of biofuel processing. There will be no definitive findings in a systematic evaluation of different algal production strategies; however, LCA research must focus on a single form of feedstock in order to obtain practically validated results. However, available LCA analysis research containing various production strategies may help to establish sustainable biofuel synthesis routes.

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Chapter 7
Aquatic Weeds as Bioenergy Feedstock

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Abstract The decline in fossil fuels instigates the search for more economical and sustainable bioenergy feedstocks. The curbs in the first- and second-generation biofuel switch the focus on nonedible alternative feedstock. The transformation of invasive aquatic weeds into bioenergy has emerged as an intriguing potential feedstock due to their fast growth, ability to adopt in various habitats, high energy content, and low land requirements. Recent advantages in bioenergy conversion techniques surge the alteration of the aquatic weed biomass into biodiesel, biohydrogen, biomethane, biomethanol, bioethanol, and bio-oil. Various conversion techniques are assessed because of energy, environmental, and economic aspects. The main emphasis of this chapter is to evaluate the competence and feasibility of aquatic weed as a feedstock and to enhance public understanding of bioenergy for sustainable development.

Keywords Third-generation biofuel · Aquatic weeds · Feedstock · Bioenergy conversion methods · Eco-friendly biofuel

7.1 Introduction

The unsustainable development with an accelerated rate of industrialization and high need for transportation result in the demand for fossil fuels in the twentieth century. The high consumption of fossil fuels, a traditional source of energy, results in the atmospheric load of CO_2 and other greenhouse gases that led to a massive change in

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the global climate. The diminution of fossil fuel resources with high emission of anthropogenic gases into the atmosphere hastens the exploration for alternative energy resources globally (Kaur et al. 2018). Even though several techniques are developed to capture CO_2 and convert it into high-value products, the exploration of new alternative energy resources is in demand (Kathi 2016). The reason behind these is the noncompatibility of CO_2 capture techniques with the high emission rate of CO_2 through extreme usage percentage of fossil fuels (Kaur et al. 2018).

Even though various alternative energy resources like wind, solar, and water are available to harness alternate energy, biomass emerges as a more reliable source of renewable energy. Biomass is the only alternative energy source that delivers straight liquid fuels for transportation (Ullah et al. 2014). The most beneficial feature of biofuel is its high compatibility with existing engine technologies (Mubarak et al. 2021). Alternative energy resources such as hydrogen, solar, and fuel cells need modification in the current vehicular engine technology (Mubarak et al. 2021). The eco-friendly nature, easy extraction from biomass, carbon dioxide cycle-based combustion, etc. tremendously increase the popularity of biofuels among other alternative energy resources (Capareda 2019). The increased demand for biofuels directly depends on agriculture production. It is reported that around 30 billion liters per annum of biofuel are used in Europe, North America, and South America. Based on IEA estimation, around 10% of the world's transport fuel demand will be fulfilled by bioethanol and biodiesel by 2025 (IEA 2008).

The first-generation biofuels are acquired from edible crop plants. The second-generation biofuels, bioethanol, and biohydrogen are obtained from agricultural by-products or waste (Wang et al. 2018). The requirement of fertile lands and investments for the cultivation of food crops as a feedstock for the first-generation biofuels instigate the conflict between food and fuel (Naik et al. 2010). This fact shifts the focus of research on nonedible feedstock like lignocellulosic biomass and algal biomass (Kaur et al. 2018; Shanab et al. 2018). Among these biomasses, aquatic weeds or macrophytes emerge as the prominent feedstock for the third-generation biofuels due to their fast growth in a short period of time; nurture in varied habitats, either in marine or fresh waters; and low land requirements (Gaurav et al. 2017).

Although aquatic weeds or macrophytes are a vital component of the aquatic ecosystem, their intense growth instigates various negative ecological and anthropomorphic effects (Borah et al. 2019a, b). The fast growth of aquatic weeds in all types of water accompanied by the formation of dense plant cover on water bodies has been recognized as a major catastrophe in freshwater, irrigation projects, hydroelectric dams, and aquaculture systems (Dale et al. 2011; Kaur et al. 2018). The thick covering of aquatic weeds or phytomass reduces light infiltration and oxygen in water bodies and blemishes the aquatic ecosystem (Feng et al. 2017). The control of this growth becomes a big challenge (Yew et al. 2019). The development of physical and chemical eradication process for aquatic weed species demands huge investments. Instead of this, the unwanted fast-growing aquatic or marine algal biomass emerges as a superior feedstock for the production of bioenergy. The low lignin content in aquatic weeds compared to other lignocellulosic biomass makes them

more suitable feedstocks. Various studies were focused on aquatic weeds as a feedstock for the production of biofuels, fertilizer, medicines, enzymes, and high-value products (Rezania et al. 2015). A new concept of the photosynthesis fuel in the biofuel field has thrived in recent years. In this concept, plants and algae consume atmospheric CO_2 and locked energy in biomass which is further extracted through various processes in the form of bioenergy (Gaurav et al. 2017).

Even though the stipulation for the production of bioenergy shifts from edible to nonedible feedstock, this copious untapped potential feedstock is barely studied for its bioenergy potential (Nawaj Alam et al. 2021a, b). Therefore, the focus of this chapter is to evaluate the potential and feasibility of aquatic weed as a feedstock for various biofuels and to enhance public understanding of bioenergy for sustainable development. It also describes the techno-economic challenges and prospects of the utilization of aquatic weed biomass as bioenergy feedstock.

7.2 Potential of Aquatic Weeds as Bioenergy Feedstock

Aquatic weeds or macrophytes are considered invasive plants due to their ability to grow in all types of water with the stipulated speed as compared to terrestrial plants. Aquatic weeds do not possess roots and stems. They are mainly composed of a thallus (leaf-like) structure. The height of aquatic weeds varies from a few inches up to 60 m. The stipulated growth of aquatic weeds occurs in the presence of available sunlight and nutrients and they do not require any additional fertilizer. The growth of aquatic weeds remarkably increased in phosphorus- and nitrogen-enriched water through anthropogenic activities.

The impulsive growth rate of aquatic weeds instigates glitches in the aquatic ecosystem, regulates oxygen balance, disturbs nutrient cycles, blocks water channels, reduces water quality, and creates heavy metal buildup (Rajkumar et al. 2014). The eutrophication of water bodies with aquatic weeds badly affects fisheries, becomes a breeding habitat for mosquitoes, and causes silting in the water bodies (Ahmad et al. 2011).

Their high cellulose content with low lignin content elevates aquatic or marine weeds as the most potential third-generation bioenergy feedstock (Das et al. 2016; Masto et al. 2020). Enriched amounts of biomolecules like carbohydrates or lipids can be efficiently converted into biofuels (Wi et al. 2009). Rajkumar et al. (2014) reported that carbohydrates present in various macroalgae vary from 31.6% (*Padina*) to 64.9% (*Enteromorpha*) (Rajkumar et al. 2014). The thermochemical techniques are generally used for the conversion of lignin biomolecules of aquatic weed into bio-oil and combustible gases (Boudet et al. 2003; Clairmont et al. 2016; Kathi 2016). Fermentation is an enormously efficient technique employed for the bioconversion of carbohydrate components of aquatic weeds into various bioalcohols (Clairmont et al. 2016; Ganguly et al. 2018). The protein and fat biomolecules of weed are easily processed into biogas like biohydrogen and biomethane (Gusain and Suthar 2017).

It was reported that algal species like *Laminaria japonica* (brown alga) and *Gelidium amansii* (red alga) are the vital feedstock for biohydrogen by anaerobic fermentation (Nong et al. 2020; Park et al. 2011). Various species of aquatic algae such as *Ascophyllum nodosum*, *Ulva rigida*, *Enteromorpha intestinalis*, *Fucus spiralis*, *Saccorhiza polyschides*, *Codium tomentosum*, *Sargassum muticum*, etc. contain triglycerides that significantly portrays them as a potential biodiesel feedstock (Vidya Sagar and Kumari 2013; Balina et al. 2017; Singh et al. 2020). The capability of these weeds to add up oxygen in water indirectly shrinks the CO_2 concentration in water (Gusain and Suthar 2017). Some aquatic weeds species such as reptans and *Trapa natans* contain a high percentage of unsaturated fatty acids beneficial for fish breeding business (Ingle et al. 2020). The high capacity of aquatic weeds to accumulate heavy metals makes it best option for the phytoremediation of industrial effluents and sewage wastewater. Aquatic plants can be used for biological treatment and stabilization of contaminated water bodies (Kathi 2016). Apart from bioenergy production, various studies highlighted the use of algal biomass for the production of various value-added products, i.e., fertilizers, enzymes, polymers, protein, paper pulp, pigments, plastics, fish and animal feed, medicinal compounds, etc. (Ruan et al. 2016; Alalwan et al. 2019; Alam et al. 2021a, b).

On the whole, the damage caused by the impulsive growth of aquatic weeds can be controlled by using them as a feedstock for not only bioenergy production but also for other high-value products (Wilkie and Evans 2010). The other significant factors of aquatic weeds like non-requirement of deforestation and land clearance for cultivation, low harvest cost, ability to overcome seasonal constraints, and absence of conflict between food and fuel give them the edge on various traditionally used terrestrial biofuel feedstocks. In this context, these distinctive factors prefer the usage of aquatic weeds for sustainable bioenergy production.

7.3 Approaches for Bioenergy Production from Aquatic Weed

Their unique composition is a vital factor to signpost aquatic weeds as a potential feedstock for bioenergy production in a sustainable way. Due to their low protein and lipid proportion with a high share of carbohydrates, aquatic weeds can be simply transformed into numerous bioenergy products like biodiesel, biogas (biohydrogen and biomethane), bioalcohols (biomethanol, bioethanol, and biobutanol), and bio-oils. Around 3–12% mass of aquatic weeds are composed of lignin which can be easily converted into biofuel (Kaur et al. 2019). A major proportion of carbohydrates are present in the cell wall in the form of starch and cellulose (Balina et al. 2017). The conversion into bioenergy proceeds through anaerobic digestion, pyrolysis, liquefaction, fermentation, and transesterification (Fig. 7.1). These techniques are generally categorized as biochemical conversion, thermochemical conversion, and chemical conversion. The selection of proper conversion technique is based on

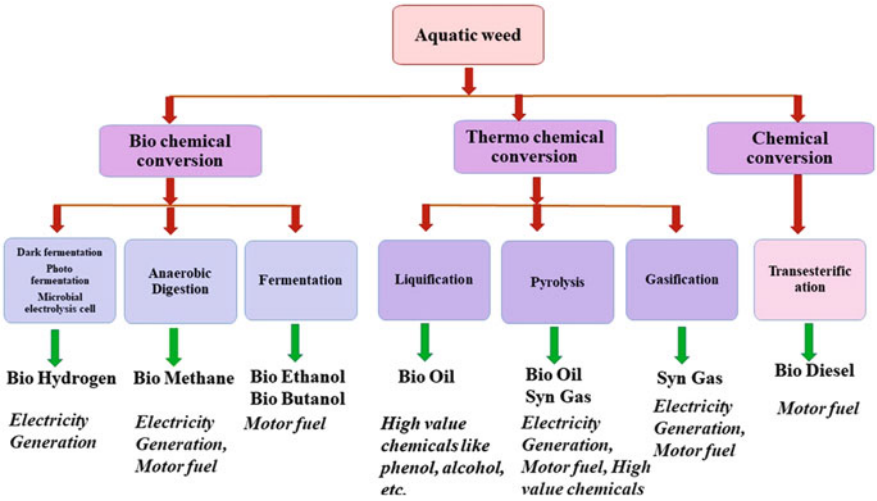

Fig. 7.1 Different techniques for the production of bioenergy from biomass feedstock

feedstock's composition and properties, required form of bioenergy, and economical suitability.

7.3.1 Biochemical Conversion

The biochemical transition of aquatic biomass into biofuels is generally grounded on the microbial and enzymatic processes. It involves the production of sugars from biomass and its conversion into alcohol and other chemicals. Biochemical conversion embraces three key processes mainly anaerobic digestion, alcoholic fermentation, and dark fermentation (Kaur et al. 2018).

7.3.1.1 Anaerobic Digestion

Anaerobic digestion method is an extensively employed economical technique for biogas production using biological components like microbes or enzymes. This method comprises hydrolysis, fermentation, acetogenesis, and methanogenesis stages. The aquatic weed with low lignin and proper C/N ratio is the best feedstock for anaerobic digestion (Lee and Lee 2016; Sirajunnisa and Surendhiran 2016). The efficiency of this process depends on high carbohydrate concentration and the C/N ratio. The C/N ratio between 20 and 30 is more favorable for anaerobic digestion. The low C/N ratio leads to ammonia formation which is incompatible with methanogen bacteria and reduces the efficiency of biomethane formation. In this

case, the C/N ratio is increased by adding carbon-enriched biomass like fallen leaves or straw.

7.3.1.2 Acidogenic Fermentation

Acidogenic (dark) fermentation is recognized as the most potential, sustainable process for the transformation of biomass into economical biohydrogen (bio-H_2). Acidogenic fermentation is a midway stage of anaerobic digestion that directs the conversion of biomass into volatile fatty acids (VFAs) which on further processing convert into biohydrogen. Dark fermentation is accomplished by fermentative H_2-producing microorganisms, such as facultative and obligate anaerobes. The most commonly utilized bacterial species for biohydrogen production are *Clostridium* and *Enterobacter*. This process is carried out under mild operation conditions. In this process, feedstock as an organic substrate (mainly glucose) acts as electron donors and protons serve as electron acceptors. The proton was then reduced to hydrogen. Acidogenic fermentation is a more beneficial method as it produces volatile fatty acids (VFAs) during ATP generation along with biohydrogen (Dahiya et al. 2015). Easy conversion of volatile fatty acids (VFAs) into various chemical products on an industrial scale along with the biohydrogen production put forward this method as the most vital and economical process (Eryildiz and Taherzadeh 2020).

7.3.1.3 Microbial Electrolytic Cell

Some new biochemical conversion techniques, microbial fuel cells (MFC) and microbial electrolysis cells (MEC), are developed in recent years. These techniques are employed for biohydrogen generation and wastewater treatment. The mechanism of MFC is based on a bio-electrochemical process in which microorganism triggers the oxidation of chemical compounds and generates electricity, whereas coupled MFC with electrolysis are used in MEC for the production of biohydrogen (Saravanan et al. 2020). Saravanan et al. (2020) reported that electrodes, membrane, reactor design, substrate, and microbial population are major parameters controlling the H_2 production. Among these operating parameters, MEC rector design is a significant factor for the upsurge of hydrogen production (Kadier et al. 2016). The applied electrical voltage is comparatively less than required for water electrolysis (Pasupuleti et al. 2015).

In microbial electrolytic process, primary and secondary metabolites of organic matter present in aquatic weeds are first released and then converted into biohydrogen (Bais et al. 2006). It is the most energy-efficient method used for biohydrogen production methods. The overall efficiency and quality of generated biohydrogen are enhanced by amalgamating microbial electrolysis with the traditional dark fermentation process. These coupled systems emerge as a milestone for the uplifting of biorefineries and framing it as a unified part of the water-energy

nexus focus area (Borole and Lewis 2017). In general, microbial electrolysis cell offers a bright future for high-quality biohydrogen production along with maximum yield and low energy requirement.

7.3.2 Thermochemical Conversion

In thermochemical conversion process, a high-temperature (<430 °C) and chemical catalyst in oxygen-deficient and pressurized environments is employed to convert aquatic weed biomass into bio-oil, biochar, and gaseous products. It includes direct combustion, gasification, liquefaction, and pyrolysis processes. The heating of biomass produces syngas comprised of hydrogen and carbon monoxide. In the next step, the prepared syngas is further treated to form other gaseous or liquid products (Lee et al. 2015; Chen et al. 2015b)

The selection of a suitable thermochemical conversion process is mainly grounded on the physicochemical properties of the desired products. The pyrolysis technique can produce solid, liquid, and gaseous products, whereas the only liquid product is obtained during the liquefaction of biomass.

7.3.2.1 Pyrolysis

Among thermochemical conversion techniques, pyrolysis is extensively used to produce gas, bio-oil, and a solid residue called biochar through the thermal disintegration of the biomass in an inert atmosphere (Jahirul et al. 2012). The pyrolysis method emerges as the most researched process for the production of liquid fuel products due to its benefits in storage, transport, and versatile applications (Jahirul et al. 2012). Biochar is extensively utilized for soil nutrient enrichment to increase plant production and for long-term carbon sequestration. It can be effectively used in agriculture as fertilizer (Qian et al. 2014). Biochar is also beneficial for the removal of heavy metals, dyes, and other pollutants (Pourkarimi et al. 2019). Various studies highlighted the conversion of aquatic algal mass into bio-oil with 40–50% yield (Sarkar et al. 2015; Biswas et al. 2017; Kaur et al. 2018). The production rate and properties of each pyrolysis product are controlled by various operating parameters such as temperature range, reactor design, type of catalyst, heating rate, residence time, etc. Gaurav et al. (2020) classified the pyrolysis process based on process parameters, namely, temperature, residence time, particle size, pressure, and heating rate, into slow type, fast type, flash type, intermediate type, vacuum, and hydropyrolysis.

Pourkarimi et al. (2019) simplified biomass pyrolysis mechanisms in three main steps. In the first stage, % moisture and volatile matter are reduced along with the breaking of bonds that leads to free radical formation. This free radical formation further led to the formation of primary charred residue. During the second stage, exposure of primary charred residue to high temperature facilitates the formation of

gases, tar, and secondary char products through secondary reactions like cracking, dehydration, and polymerization. In the final stage, further thermal treatment of secondary char product yields gases and char product. High-temperature conditions and high cracking of big molecules lead to the formation of more gaseous products. The volatile product fractions are condensed into bio-oil. Maximum bio-oil yield is reported at a temperature range between 350 °C and 500 °C.

7.3.2.2 Hydrothermal Liquefaction

During hydrothermal liquefaction, the exposure of wet biomass to moderate temperature and high pressure produces crude oil (bio-oil) that is directly used in heavy engines. Further, after processing, bio-oil can be used as straight-run fuel. The energy density of bio-oil is twofold as compared to the oil produced by pyrolysis. Hydrothermal liquefaction emerges as a promising technique as it reduces fuel consumption required for the drying of aquatic weed and fast production rate of products (Duan et al. 2012). Singh et al. (2015) reported the production of bio-oil yield with a conversion rate of 23–30% for different aquatic weed species using hydrothermal liquefaction (Singh et al. 2015).

Jena and Das (2011) reported a 41% yield of bio-oil for *Spirulina* species using hydrothermal liquefaction. Similarly, Vardon et al. (2012) experimentally converted *Scenedesmus* biomass into bio-oil with 24–45% output. Alba et al. (2011) successfully recovered 75% of energy from the microalgal biomass in the form of bio-oil.

7.3.2.3 Gasification

Among various thermochemical processes, gasification is the most potent method for the transformation of aquatic macrophytes biomass for the production of clean bio-liquid fuels or biogas (Alauddin et al. 2010). From the last decade, gasification emerges as the most potential method for large production of biohydrogen. It is generally carried out at 700–900 °C in the presence of air, oxygen, or steam or a mixture of gases (Reddy et al. 2014). In a biomass gasifier, macrophyte biomass exposed to strong heat under controlled amounts of O_2, air, and steam leads to the partial oxidation of feedstock (Das and Hoque 2014; Dasappa et al. 2011). The mixed gaseous products CO, CO_2, H_2, CH_4, and N_2 formed collectively are called producer gas or syngas based on relative composition. Producer gas is employed for power production, whereas syngas gets transformed into fuels and high-value chemical products (Naik et al. 2010). Steam gasification is the most viable and potential technique for the production of the best quality biohydrogen (Duman et al. 2014).

The gasification process proceeds via drying, pyrolysis, oxidation, and reduction processes. The design and working of the gasifier are based on the type of fuel used. Based on the mode of air blast created in the gasifier, it is categorized into updraft type, downdraft type, fluidized bed, cross draft gas producer, etc. (Kathi 2016).

Various gasification process parameters like gasifying medium, the height of the reactor bed, fluidization velocity, temperature, pressure, % moisture in feedstock, air steam ratio, type of catalysts used, etc., affect the efficiency of gasification. Various studies highlighted that the diverse biogas yield obtained during gasification depends on the composition of feedstock (Börjesson and Berglund 2006). The type of digestion process like digestion, batch or continuous, and one- or two-phase digestion, and pretreatment process adopted in the transformation of aquatic weed into biogas also affects the production capacity of gasification (Börjesson and Berglund 2006). Kaewpanha et al. (2013) reported the high temperature and excessive amount of steam during increased biohydrogen production. Cherad et al. (2013) highlighted the use of alumina-supported ruthenium (Ru/Al_2O_3) catalysts in gasification that results in a twofold increase in the production rate of H_2 and CH_4 from *S. latissima* macrophyte.

Supercritical water gasification is the latest gasification method technology successfully employed for recycling of wet biomass (moisture 70–95%) for the preparation of synthesis gas. The high yield of biohydrogen signifies this method has more potential than the other traditional gasification techniques. The rate of water gas shift reactions and methanation reactions that take place mainly controls the efficiency of supercritical water gasification. In the case of aquatic weed, this method is the more beneficial and best-suited technique as feedstock is directly charged in a gasifier without drying (Matsumura 2002).

7.4 Bio Energy Production from Aquatic Weeds

Protein- and carbohydrate-enriched aquatic weeds with low lipid content emerge as a potential feedstock for bioenergy production since the last decade. The potential of aquatic weeds for biofuel production is examined extensively to meet the current energy requirements. Aquatic weeds are utilized to produce biomethane, bioethanol, biodiesel, and biohydrogen through proper processing technologies. This section intends to give an account of the existing bioenergy potentiality and feasibility of aquatic weeds as bioenergy feedstock for sustainable development. Table 7.1 covered some of biofuels produced from aquatic weeds.

7.4.1 Biomethane

Biomethane is considered as a clean, easily adaptable sustainable fuel produced through anaerobic digestion process from a wide variety of feedstocks. Easy storage, transportation, and distribution through pipeline and usage of by-product as a fertilizer portray biomethane as a promising biogas (Alam et al. 2021a, b). Several reports were published and signify the potential of aquatic weeds as a raw material for biogas production. The anaerobic fermentation of organic materials creates

Table 7.1 Biofuels produced from aquatic weeds

Biofuels produced	Aquatic weed used	Pretreatment method	Experimental conditions	Product yield	Reference
Biomethane	*Eichhornia crassipes* (water hyacinth)	–	Batch mode at 2:1 inoculum to feedstock ratio over a period of 60 days using cow dung as an inoculum	552 L/ kg VS (methane content 62%)	Mathew et al. (2014)
	Duckweed (*Lemna* sp.)	–	Two liter batch fermenters were incubated at room temperature, specific mesophilic (35 °C), and thermophilic (50 °C) conditions for 45 days	Biogas yield 7863.69 ml/l in room temperature, 10376.59 ml/l in mesophilic temperature, and 9981.08 ml/l thermophilic temperature, while the total biogas yield of 10,377 ml with maximum methane content of 64.47%	Ramaraj and Unpaprom (2016)
	Pistia stratiotes	Leaves and roots dried at 60 °C and then powdered to 350 μm mesh	Batch digestion system at mesophilic conditions for 45 days	Total gas yield 9667.33 mL	Pantawong et al. (2015)
	Water hyacinth, *Cabomba*, and *Salvinia*	Physical	A set of four pilot-scale, batch digestions were undertaken	Hyacinth yielding 267 L biogas/kgVS, *Cabomba* yielding 221 L biogas/kgVS, *Salvinia* yielding 155 L biogas/kgVS (methane content 50%)	O'Sullivan et al. (2010)
	Five submerged macrophyte species	Mechanical	Batch anaerobic digestion was performed at mesophilic temperature of 37 ± 1 °C	Varied from 161.2 to 360.8 mL/gVS depending on species	Koyama et al. (2014)
Bioethanol	*Eichhornia* sp. and water lettuce	Alkaline/oxidative	*Saccharomyces cerevisiae* stain used	0.14–0.17 g/g dry water hyacinth and 0.15–0.16 g/g dry water lettuce	Mishima et al. (2006)
	Eichhornia sp.	Acid and alkali	*S. cerevisiae* and *Pachysolen tannophilus*	0.21 g/g dry biomass	Mukhopadhyay and Chatterjee (2010)

	Wolffia globosa	Enzymatic	Simultaneous saccharification and fermentation using the enzymes and dry yeast	170 g/kg	Soda et al. (2015)
	Landoltia punctata	Enzymatic	The fermentation experiments carried out in 250 mL flask containing 100 g mash in batch model and inoculated with 10% v/w yeast inoculum and the reaction mixture was incubated at 30 °C and 220 rpm	30.8 g/L	Chen et al. (2012)
Hydrogen	Duckweed *Spirodela polyrhiza*	Acid	At 35 °C and an initial pH of 5.5	75.3 mL/g	Xu and Deshusses (2015)
	Alternanthera philoxeroides	Enzymatic and steam-heated acid pretreatment	The batch cultivations were performed aerobically in an incubator shaker (180 rpm) at 37 °C for 6 h	89.8 mL/g VS	Song et al. (2020)
	Eichhornia crassipes	Microwave-heated alkali	Dark fermentation pH 6.0 ± 0.1 HRT-6 35^0 C	63.9 mL/g TVS	Lin et al. (2015)
	Duckweed	Acid hydrolysis	Dark fermentation pH- 7 35^0 C	169.30 mL/g	Mu et al. (2020)

biogas by methanogenic microorganisms that can be used directly for heating or producing electricity and a nutrient-rich slurry. Day et al. (1990) observe biogas as a safe energy source that can improve the environment on a large and small scale, e.g., deforestation and smoke reduction in kitchens.

Mathew et al. (2014) carried out anaerobic digestion of water hyacinth and *Salvinia* in batch mode at 2:1 inoculum to feedstock ratio for 60 days using cow dung as an inoculum. The biogas yield from water hyacinth was 552 L/kg of volatile solids (VS) and *Salvinia* produce 221 L/kg of volatile solids. Ramaraj and Unpaprom (2016) practically examined the potential of duckweed for biogas production. The yield of biogas at room temperature, mesophilic temperature, and thermophilic temperature is 7863.69 ml, 10376.59 ml, and 9981.08 ml, respectively, and the methane content of the total biogas yield was 64.47%. Pantawong et al. (2015) focused on the probability of biogas production from water lettuce; *Pistia stratiotes L.* in powdered form after drying was used in mesophilic batch reactors in laboratory-scale digesters with the addition of inoculum. The total gas yield was 9667.33 mL with digestion period of 45 days with enriched methane content up to 66.35%.

The potential of *Eichhornia crassipes* in the form of slurry for biomethane production was explored by Clairmont et al. (2016). The authors conducted an experiment in which chopped biomass of water hyacinth was mixed with different combinations of manure. Bio-methanation was conducted with retention time of 6 weeks between mesophilic temperature ranges. The results showed high biomethane production from the co-digested water hyacinth and manure (as inoculum); particularly 25:75 ratio of weed and manure mix resulted in high biogas yield. O'Sullivan et al. (2010) examined the anaerobic digestion potential of three aquatic weeds, water hyacinth, *Cabomba*, and *Salvinia*. The pilot-scale digestions showed that both water hyacinth and *Cabomba* by anaerobic digestion could be recommended.

Koyama et al. (2014) used five submerged macrophyte species as a substrate for anaerobic digestion, and methane yield varied from 161.2 to 360.8 mL g-VS-1 depending on species. The CH_4 conversion efficiency of *C. demersum*, *El. nuttallii*, *E. densa*, *P. maackianus*, and *P. malaianus* was 57.1, 61.4, 60.6, 33.9, and 72.2%, respectively. The results showed that *C. demersum*, *El. nuttallii*, *E. densa*, and *P. malaianus* are feasible for anaerobic digestion due to high methane recovery, whereas *P. maackianus* was not preferable for anaerobic digestion.

7.4.2 Bioethanol

Bioalcohol is a potential source generally expended as a fuel through traditional methods. Bioethanol is the most used bioalcohol yielded by fermentation of carbohydrate-enriched feedstock. Bioethanol is produced from aquatic biomass through biological method that is composed of acid hydrolysis pretreatment, followed by fermentation with suitable fermenting organism (Rodionova et al.

2017). The production of bioethanol is carried out in three stages: pretreatment, chemical reaction, and fermentation (Wilkie and Evans 2010). The enzymes hydrolyze the cellulose portions of the weeds into glucose sugar that are fermented to bioethanol (Ganguly et al. 2012).

Borah et al. (2019a, b) illustrate the use of mixed invasive weeds, viz., *Arundo donax* (AD), *Chromolaena odorata* (CO), *Mikania micrantha* (SS), *Lantana camara* (LC), *Eichornnia crassipes* (EC), *Ipomoea carnea* (IC), *Parthenium hysterophorus* (PH), and *Saccharum spontaneum* (SS), as the feedstock for production of ethanol. The acid hydrolysis of mixed biomass followed by alkaline delignification and enzymatic hydrolysis is composed of pentose-rich and hexose-rich hydrolyzates, with bioethanol yields of 87 and 133 g/kg, respectively. Awasthi et al. (2013) have focused on how water hyacinth can prove to be a valuable source for bioethanol production. Various acids and alkalis were used as reagents for pretreatment. Still, among them, sulfuric acid provides the best result for the yield of sugars compared to other acids and alkalis. Kumar et al. (2009) investigated ethanol production from *Eichhornia crassipes*. The dilute acid treatment has been applied to utilize the full hemicellulosic content of the water hyacinth using *Pichia stipitis*, and 72.83% of xylose was converted to ethanol productivity of 0.176 g/L/h. Aquatic plants, water hyacinth, and water lettuce were investigated for their use in ethanol production (Mishima et al. 2006). Water lettuce had lightly higher starch contents and lower contents of cellulose and hemicellulose. The ethanol yield per unit biomass was 0.14–0.17 g/g of dry water hyacinth and 0.15–0.16 g/g of dry water lettuce.

Mukhopadhyay and Chatterjee (2010) utilized the fast-growing aquatic weed water hyacinth for ethanol production via enzymatic hydrolysis and fermentation. Significant enhancement of concentration (8.3 g/L) and yield (0.21 g/g) of ethanol was obtained through a pre-fermentation hydrolysis-simultaneous saccharification and fermentation process. With its high growth rate and high starch content, the duckweed can be an excellent renewable feedstock for the production of ethanol. Soda et al. (2015) pretreated the duckweed biomass and produced ethanol. The ethanol yield of *Wolffia globosa* biomass in the simultaneous saccharification and fermentation using the enzymes and dry yeast was 170 g/ kg of dry mass.

7.4.3 Biohydrogen

The hydrogen production using aquatic weed biomass is a zero emission process. It can be directly applied as a straight-run fuel for IC engines or as a power fuel cell for electricity generation (Rodionova et al. 2017). The energy content value of biohydrogen (122–142 kJ/g) is highest among all biofuels like biomethane (56 kJ/g) and biodiesel (37 kJ/g). The biohydrogen generation technique is the most beneficial process as it is carried out under normal temperature and pressure and releases only water as by-products, not any toxic pollutant (Voloshin et al. 2020).

Several studies highlighted the benefits of the use of aquatic weeds for sustainable production of biohydrogen (Kaur et al. 2018; Alam et al. 2021a, 2021b). Lay et al. (2013) reported the production of biohydrogen using water hyacinth and beverage wastewater. The optimal combination ratio was 1.6 g water hyacinth and 2.4 g beverage wastewater with C/N ratio of 42. C/N ratio acts as an essential parameter for selecting appropriate feedstock mixture while developing a low-cost hydrogen production process. According to Xu and Deshusses (2015), small aquatic plants such as duckweed can be a suitable substrate for biohydrogen production. They investigated the effects of pretreatment and fermentation conditions on biohydrogen production from duckweed. The mild acidic thermal pretreatment was more effective and resulted in biohydrogen production of up to 75 mL/per g dry duckweed in 7 days, and hydrogen concentration obtained was 42%. Song et al. (2020) studied the biohydrogen production from *Alternanthera philoxeroides* and found the biohydrogen yield to be 89.8 mL/g. Lin et al. (2015) investigated microwave-heated alkali pretreatment for hydrogen production from water hyacinth and obtained a hydrogen yield of 63.9 mL/g. Mu et al. (2020) developed a cost-effective and environmentally friendly method of biohydrogen production from duckweed through dark fermentation. The results implied that acid hydrolysis was more appropriate for the pretreatment of duckweed biomass and gave hydrogen production of 169.30 mL/g dry weight.

Among all methods, steam gasification is more advantageous in terms of quality and yield of hydrogen. Duman et al. (2014) expended two seaweed species biomasses for two-stage steam gasification in a dual-bed microreactor in the presence of catalysts 10% Fe_2O_3–90% CeO_2 and red mud (activated and natural forms). The study reported maximum hydrogen yields of 1036 cc/g for *Fucus serratus* and 937 cc/g for the seaweed *Laminaria digitata*.

7.4.4 Bio-oil

Bio-oil is produced by subjecting biomass to pyrolysis or hydrothermal liquefaction. Hydrothermal liquefaction is more beneficial than pyrolysis as bio-oil obtained contains low oxygen and moisture content and is more stable. Hydrothermal liquefaction of macrophyte is now extensively researched as drying of biomass is not required (Milledge et al. 2014). Generally, bio-oil is composed of various organic compounds and further upgraded to refined fuels (Alam et al. 2021a, 2021b). Furthermore, bio-oil is subjected to processing to obtain various pure chemicals like phenol, alcohol, organic acids, aldehyde, etc. (Asadullah et al. 2007). Xiu et al. (2010) subjected duckweed to thermochemical liquefaction over a temperature range of 250–374 °C, and catalyst loading of 0–10 wt% with retention time varies from 5 to 90 min. in a high-pressure reactor. The study signifies the role of temperature in controlling bio-oil production. The highest oil yields with average heating value of 34 MJ/kg are obtained at a temperature of 340 °C and a retention time of 60 min.

without any catalyst. Bach et al. (2014) reported maximum 79% yield through hydrothermal liquefaction of *Laminaria saccharina* at 350 °C.

The pyrolysis process is an efficient approved method of bio-oil generation from aquatic biomass. During pyrolysis process, thermal disintegration of biomolecules through complex simultaneous and successive reactions takes place from 350 °C to 800 °C in a nonreactive atmosphere. Long-chain biomolecules break into gases, oils, tars, and solid charcoal. During pyrolysis, the cellulose biomolecule transformed into bio-oil and lignin converts into solid residue, namely, biochar. Yang and his team highlighted that bio-oil is mainly derived from the cellulose component of biomass (around 500 °C), whereas biochar is derived from lignin (Yang et al. 2006).

Mullen and Boateng (2008) studied the composition of bio-oil produced through fast pyrolysis of switchgrass and alfalfa stems using GC-MS and HPLC analysis. Fast pyrolysis of biomass was performed at 500 °C under inert atmosphere in fluidized bed reactor. Muradov et al. (2014) exposed duckweed grown in wastewater to pyrolysis and recorded a 36% yield of bio-oil. The obtained bio-oil is then further processed through the catalytic hydrodeoxygenation to produce gasoline and diesel. The biochar produced as a by-product can be efficiently used for soil amendment and carbon dioxide sequestration. Liu et al. (2011) investigated the fast pyrolysis of three plants *Alligator weed*, *Oenanthe javanica*, and *Typha angustifolia* in a vertical drop fixed-bed reactor. Liu and team achieved maximum bio-oil yield around 42.3%, 40.2%, and 43.6% for *Alligator weed*, *Oenanthe javanica*, and *Typha angustifolia*. The analytical result of GC-MS analysis of the obtained bio-oil reported that bio-oil is mainly comprised of nitrogenous compounds, phenols, and oxygenates.

Saikia et al. (2015) synthesized bio-oil from *Ipomoea carnea* using thermal pyrolysis at around 350–600 °C with a heating rate of 10 °C/min and reported the highest yield to be 41.17% at 550 °C as pyrolysis temperature. A similar type of study carried out by Biswas et al. (2017) signifies the generation of bio-oil containing high percentage of aliphatic functional groups and phenolic, ketones, and nitrogen-containing groups. Biswas and team carried out the pyrolysis of *Azolla, Sargassum tenerrimum*, and water hyacinth in a fixed-bed reactor at different temperatures in the presence of nitrogen. The highest yield of liquid product from *Azolla*, *Sargassum tenerrimum*, and water hyacinth was 38.5, 43.4, and 24.6 wt.% obtained at 400 to 450 °C. Gulab et al. (2019) optimized the operating parameters of catalytic pyrolysis of water hyacinth biomass like temperature, time, particle sizes of biomass, and Cu and Al catalyst for the production of bio-oil. A 31.6% of the bio-oil yield was obtained at temperature range 150–450 °C with Cu catalyst 5%wt of biomass with reaction time 60–100 min.

7.4.5 Biodiesel

Since the last two decades, biodiesel is materialized as an excellent biofuel for diesel engine due to its reduced emission, cost efficiency, and high compatibility with diesel engines. Various studies reported production of cost-effective first-generation

biodiesel fuel from both edible and nonedible oil-bearing crops. Various drawbacks like consumption of edible feedstock, high carbon deposition, the coking and trumpet formation, thickening and gelling of the lubricant, etc., divert attention of researchers towards the search of nonedible feedstock for biodiesel generation (Annam Renita et al. 2010). As compared to the first- and second-generation biodiesel, algae-based third-generation biodiesel is more advantageous in terms of high energy and oil content and sustainability (Vidya Sagar and Kumari 2013). To recognize biodiesel as a prospective, economical, and sustainable biofuel, it is essential to exploit its benefits for diesel engine in terms of performance, combustion, and emission characteristics. In relevant to this fact, Alagu et al. (2019) signify that blended diesel with biodiesel has high cetane number, emits emission with reduced CO and smoke, and also reduces ignition delay (Alam et al. 2021a, b).

Chemically, biodiesel is monoalkyl esters of long-chain fatty acids extracted from feedstock material via catalytic transesterification reaction of triglycerides with methanol (Singh et al. 2020). The pathway of transesterification proceeds via the preparation of fatty acids (FA) as precursors followed by the catalytic transformation of acetyl-CoA into malonyl-CoA through acetyl-CoA carboxylase enzyme. Around 16–20 different types of fatty acids are included in the synthesis of triacylglycerols (Alalwan et al. 2019). Nowadays, two methods, that is, catalytic and supercritical methanol transesterification, are generally employed for biodiesel production (Semakula and Inambao 2015).

Water hyacinth (*Eichhornia crassipes*) was extensively studied as a vital feedstock for sustainable biodiesel production because of its vast availability and high biomass yield (Rezania et al. 2015; Feng et al. 2017; Gaurav et al. 2020). Shanab et al. (2018) used water hyacinth as a feedstock and reported a biodiesel yield of 3.22–6.36% with variable lipid contents of 6.79–10.45% via transesterification.

Brouwer et al. (2015) examined *Azolla filiculoides* as a biodiesel feedstock and observed that $3.2 \pm 1.0\%$ of fatty acid methyl ester (FAME) formed after methanol aided conversion. The formation of fatty acid methyl ester is very important for properties like cetane number, iodine value, and cold filter plugging point (CFPP). *Salvinia molesta* species is also examined as alternative feedstock for biodiesel production due to its high growth rate and lipid content (Mubarak et al. 2016). Ameka et al. (2019) evaluate the potential of marine macroalgae, namely, *Caulerpa taxifolia*, *Chaetomorpha antennina*, *Chaetomorpha linum*, *Ulva fasciata*, and *Ulva*, for biodiesel generation.

Other studies carried out used various macroalgae species like *Chaetomorpha linum*, *Ulva lactuca*, and *Enteromorpha (Ulva) compressa* for biodiesel production and pointed out the low yield as compared to the total dry feedstock due to low lipid content in macroalgae (Suganya and Renganathan 2012). Suutari et al. (2015) highlighted that high metal concentrations in macroalgae may have inhibited the processes. Suutari and team concluded that elevated sulfur and nitrogen contents in green algae instigate problems in the biodiesel generation. Hence, macroalgae would not seem to be an appropriate alternative feedstock for the generation of biodiesel.

7.5 Other High-Value Commercial Bioproducts

It is being envisaged that aquatic weed can be effectively employed in wastewater treatment, as a fish and animal feed, heavy metal and dye remediation, food supplements, antioxidants, medicines, paper pulp making, bricks, herbicides, etc. This section discusses the different value-added products which can be produced from aquatic weed.

Aquatic weeds can be turned into compost used in farming as an organic fertilizer because of the increased demand for organic foods in the advanced world. The fertilizer prepared from aquatic weeds conserves soil moisture and restores nutrients. Even after drying, aquatic weeds retain most of the nutrients, minimizing our need for chemical fertilizer (Gunnarsson and Petersen 2007). The composting process is only 30 days compared to other crop plants, which takes up to 2–3 months. Thus, conversion of these weeds into compost to be used as a nutrient-rich fertilizer is the solution which will improve the chemical, physical, and biological properties of soil (Alam et al. 2021a, b).

The aquatic plants have proved to be a good quality protein source for animal feed (Igbinosun et al. 1982). The lack of animal protein with the increasing cost of food production necessitates searching for nonconventional sources. Due to their high nutritional values, aquatic weeds can be used as a food source for animals and fish. Various studies are being conducted to identify a cost-effective supplementary feed for fish as fish food is costly. For weed control, the fish can eat up to 18–40% of its weight in a single day. Aderibigbe and Brown (1995) have demonstrated that the nutritive value and digestibility of aquatic weeds are enhanced in dried condition. The dried, crushed aquatic weeds can be used in mixtures of various percentages (2.5–10%) as ordinary feed for pig, chicken, ducks, cow, and rabbits.

Researchers have worked on the phytoremediation potential of aquatic weed for the removal of nutrients and pollutants from wastewater. It emerges as an ecologically compatible and economically viable technique to clean a variety of pollutants. The diversified macrophytes have been proven to remove nutrients and harmful compounds like heavy metals from the wastewater (Prabhakar et al. 2017). The different stages of water treatment like secondary, tertiary, and complex processes are costly and need high energy demand and skilled workers (Vymazal 2010). Instead of these treatment processes, the use of aquatic weeds for phytoremediation emerges as the most effective method. In a treatment plant, clean, healthy aquatic plants are introduced into water clarifiers and help remove little flocs and materials, resulting in a decrease in turbidity and removal of flocs and a slight reduction in organic matter in the water. Aquatic weeds offer an appropriate atmosphere for the aerobic bacterium to grow. The reports available in literature reveal that aquatic weeds efficiently reduce nutrient load and other compounds from water, thereby reducing the pollution load. Hence, aquatic plants are recognized as one of the best suitable eco-friendly ways of wastewater treatment (Boyd 1970). Various aquatic weed species such as *Pistia stratiotes*, *Eichhornia cras*sipes, *Rapa natans* L., etc. are successfully employed as a hyperaccumulator, to remove heavy metals, organic

compounds, and radionuclides from water bodies (Alam et al. 2021a, b). Mustafa and Hayder (2021) studied the efficiency of *Salvinia molesta* and *Pistia stratiotes* species in phytoremediation and concluded that stoichiometric homeostatic index and resource pulse effects are the most essential factors in wastewater treatment.

ThiripuraSundari and Ramesh (2012) illustrated the preparation of cellulose nanofibers from water hyacinth aquatic weeds. The cellulose microfibers were extracted from the weed plant through bleaching, alkaline, and sodium chlorite reactions. Further, crude nanofibers were processed using cryocrushing in liquid nitrogen followed by sonication to get nanofibers of 20–100 nm diameter. These nanofibers are economical and potential raw materials for the synthesis of nanocomposites. Abdulqahar et al. (2021) reported the synthesis of silver nanoparticles through biological pathways from two aquatic weeds *Eichhornia crassipes* and *Ceratophyllum demersum*. The study highlighted the antibacterial activity of synthesized silver nanoparticles for food factory wastewater (Abdulqahar et al. 2021).

Additionally, aquatic weeds can be utilized in papermaking and grease-proof paper production (De Groote et al. 2003). The application of aquatic weeds in the paper industry can relieve the environmental concern of deforestation, as the aquatic weeds could replace wood obtained from trees. Aquatic weeds with low lignin content are an awe-inspiring possible fiber source for papermaking industry. Bidin et al. (2015) conducted the stability of the aquatic weed fiber as potential raw material for handmade papermaking.

Aquatic weeds serve as a renewable energy source to replace a conventional type of fossil fuel. Briquettes can be manufactured from aquatic weeds as they play an essential role in power plant generation energy sector (Nasrin et al. 2008). The briquette obtained through densification can be used as solid fuels.

Some aquatic weeds own unique biological features and are recognized as suitable for their inherent medicinal use. Aquatic weeds, particularly macrophytes, may contain phytochemicals such as flavonoids, steroids, saponin, lipophilic compounds, alkaloides, tannin, phenols, etc. (Al-Amin Sarker et al. 2016). These phytochemicals can be extracted from aquatic weeds and used in medicinal formulations. They have been proved to have pharmacological properties such as anti-inflammatory, antimicrobial, antioxidant, anti-diabetic, etc. (Al-Amin Sarker et al. 2016).

Due to their availability and fast growth, aquatic weeds can be beneficial for any mainstream manufacturing process when used as raw materials. Furthermore, they are being investigated for their application in other sectors such as soap manufacturing, mushroom cultivation, polymer production, etc. There are several different applications of aquatic weeds, such as handicrafts, which local people from ancient times have prepared (Al-Amin Sarker et al. 2016).

7.6 Major Challenges and Future Prospective

Aquatic weeds are an alternative and next-generation biofuel biomass resource for bioenergy production, restraining the constraints faced by first- and second-generation biofuel resources. Despite many advantages, the generation and utilization of aquatic weeds also meet numerous challenges that require to be solved for efficient resource utilization. Aquatic weeds are fast growing and propagate at a tremendous rate and also create a number of environmental problems (Hatcho et al. 2018). These properties of aquatic weeds necessitate to be given proper consideration when developed for their possible application for generation of biofuel and different products (Chen et al. 2015a).

Considering the significant moisture content of aquatic weeds, an effective and inexpensive collection, storage, and transport operation is the principal challenge for advertising it as a bioenergy feedstock. Also, practical and cost-effective dewatering methods must be evaluated to ease the downstream process of biofuel generation from aquatic weed. The second significant difficulty is the ineffective and costly harvesting of aquatic weeds, which is tedious. On a small scale, aquatic weed harvesting can be done manually, and skimming or mechanical harvesters can easily collect floating weeds. But for harvesting emergent and submerged aquatic weeds, new methods or machines are required to be created. As with the currently available harvesters, only a portion of aquatic weeds is removed.

Nevertheless, wastewater-grown aquatic weeds could be dangerous for their application as compost, medicinal uses, and other applications and may lead to environmental and health problems. So, wastewater-grown aquatic weeds need thorough risk assessment studies. Biohydrogen and biodiesel production and other value-added products from aquatic weeds are still in early developmental stages. Bioethanol, biomethane, and fertilizer production have been studied in recent history. However, there are still research gaps such as recognizing suitable feedstock species and conversion technology, process optimization, and improving the final product's efficiency and cost-effectiveness. The hurdles at large-scale generation of biofuels from aquatic weeds may involve harvesting, drying, transport, and a cost-effective conversion system. These challenges require consideration.

Aquatic weeds have a complex structure that raises difficulties for efficient extraction and transformation to biofuels. The biofuel production from aquatic weed needs process intensification through pretreatment by various physical, chemical, and biological methods to ease this difficulty; yet, there are minimal studies in this area. To maximize biofuel output, the extraction of carbohydrates and lipids from aquatic weeds necessitates further study. Few aquatic weeds like water hyacinths can have higher sulfur content which can cause results or produce some corrosive substances reducing fuel efficiency (Mullen and Boateng 2008). Biological pretreatment is one of the safest and most environmentally beneficial treatment methods involving the use of microorganisms and enzymes that can degrade various compounds that easily extract energy (Zabed et al. 2019). Therefore, recognizing suitable microorganisms and developing integrated approaches such as combined

strategies can improve the overall production. For example, in some cases, using microwave and sonication techniques helps to solubilize and separate compounds, improve sugar recovery, and reduce the loss of essential compounds (Dai et al. 2017).

Aquatic weed has shown massive potential for biofuel production and other applications, but challenges still need to be addressed before its implementation (Alam et al. 2011) (Alam et al. 2021a, b). It is still in its early stage due to economic viability and feasibility concerning the commercial production of aquatic weed-based biofuel, so techno-economics and life cycle assessment of biofuel production need to be studied. Moreover, detailed lab-scale investigations are required to identify and characterize potential aquatic weeds and discover the consequences of heavy metals accumulated in them. Economically feasible, cost-effective, and straightforward methods to biofuel conversion are also needed. After biofuel production, their waste residues, i.e., fertilizers and compost, need to be assessed to decrease the production cost and enhance operational performance. Further new genetic engineering technology needs to be investigated to reduce the requirement for pretreatment processes during bioenergy production. There is a necessity to generate a positive vision for increasing investments in using aquatic weeds, which would reduce our reliance on fossil fuels. A primary cost-benefit analysis is also needed to assure the feasibility of feedstock in terms of its financial viability (Kaur et al. 2018). Another point to be considered for using aquatic weeds for energy generation could be their potential to spread into unaffected ecosystems. Hence, it should not be included in systems for biofuel generation where they are not presently found.

7.7 Conclusion

Aquatic weeds have a negative ecological and anthropomorphic effect. It is more promising to eliminate the consequences of aquatic weed by transforming it into beneficial bioenergy and high-value products. Furthermore, the unique composition of feedstock elevates these weeds as the most potential third-generation bioenergy feedstock. Various conversion methods are also evaluated based on feedstock's composition and properties, end products, economic feasibility, and environmental suitability. The chapter also highlighted the various operating conditions employed in conversion technologies and the need for extensive research demanded a more promising conversion method. The production and compatibility of various biofuels are also discussed to prove aquatic weed biomass as the best eco-friendly and economical solution for bioenergy production. It is observed that there is a scope for extensive research in uplifting the productivity of biofuel to make the process more commercial. Based on the discussion, it is concluded that large-scale biofuel generation is a necessity to meet today's energy demand with fewer environmental corollaries.

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Chapter 8
Wastewater and Solid Waste as Feedstock for Energy Production

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Abstract Due to robust economic growth with expeditious urbanization, industrialization, and amplification of demography across the world, wastewater and solid waste generation and demand for food and drinking water have increased exponentially. It has been theoretically and experimentally proven that carbohydrates, proteins, and lipids present in wastes have the potential to be transformed into several types of fuels such ethanol, diesel, methane, hydrogen, electricity, and other advanced biofuels through chemical and biochemical routes. Solid waste mainly consists of carbohydrate, lignin, protein, and lipids and their chemical composition varies with its origin and source. Solid wastes and wastewater also include carbohydrate and protein. In the present chapter, we review the potential of solid waste and wastewater to serve as a cheap and sustainable feedstock for the production of fuel and energy.

In the first section of the chapter, an introduction is made to present a scenario on energy production, and the first, second, third, and fourth generations of feedstock for biofuel production are discussed to analyze the need for solid waste and wastewater feedstock and technology for bioenergy production. Energy can be recovered in the form of biodiesel, bioethanol, biohydrogen, biogas, and electricity from different types of solid wastes generated from diverse sources including industrial, municipal, agricultural, or forestry wastes. We need a sufficient supply of treated feedstock for the production of biofuel on a commercial scale to fulfill the market demand. Renewable agricultural, industrial, and urban wastes, such as municipal solid wastes, flowers, vegetables, and other market wastes, slaughterhouse wastes, agricultural residues, and industrial/sewage treatment plant wastes, industrial effluents, sewage, and non-sewage wastewater, have been reviewed in the next section as ideal candidates for producing biofuel.

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The chapter further covers an overview of pretreatment of solid waste, conversion of solid biomass to energy through nonbiological technology, solid waste and wastewater as feedstock for second- and third-generation biofuel production, feasibility, and economics of wastewater and solid waste as a sustainable potential feedstock for biofuel production. The advantages and disadvantages of different generation biomass feedstocks along with their challenges and opportunities for commercial-scale biofuel production are also included.

Keywords Solid waste · Wastewater · Municipal solid waste · Agriculture waste · Bioethanol · Biodiesel · Biogas · Biohydrogen · Bioelectricity · Pretreatment

8.1 Introduction

Since time immemorial, the sustenance of an entire civilization was wholly dictated by its potential in retaining the supplies of energy that fuels the processes for all necessary activities of that civilization. The situation has not been much different even today. Human dependence on fossil fuels such as oil, coal, and natural gases as the prime sources of energy in the current era is very risky as it is anticipated that these sources will deplete within the next 40–50 years. The expected environmental damages like global warming, acid rain, and urban smog have tempted us to reduce carbon emissions by 80% (v/v) and utilizing a shift towards a variety of alternative energy resources such as solar, wind, biofuel, etc. that are less environmentally harmful in a sustainable way (Vohra et al. 2014; Loizidou et al. 2021).

The situation of total primary energy consumption in the world for the year of 2020, the year of Covid as enumerated in accordance with the proportion of various sources, is likely 31.2% coal and natural gas 24.7% followed by hydro (renewable 6.9%), nuclear (4.3%), and others (renewables) 5.7% (Looney 2021). It has largely been forecasted by several reports including the IEA report 2020 that the demand for renewable energy will rise exponentially in the years to come. Currently, the global consumption of oil is about 100 million barrels per day (Prisecaru 2021). The other metrics hint towards a global demand of oil and natural gas to increase by 29% by 2050 which will account for supplying 28% of total energy consumed (IEA 2021). To meet the energy consumption of this ever-growing population, energy demand coming from emerging markets and developing countries is shooting up (Vohra et al. 2014). With this boom in global demand, it has been forecasted that the world would need twice as much energy as it produces today to sustain (Bouckaert et al. 2021).

Besides the increasing demand, another challenge that the global energy market faces is the absence of safe and low-carbon, cheap large-scale energy alternatives to fossil fuels. Thus, it becomes pertinent to unravel and explore all those alternatives available to us. The energy problem that has lately been paid much attention to and the matter of concern has been the link between energy access and greenhouse gas emissions. Thus, energy from alternative sources is the only way out to meet the energy needs in a sustainable way. Renewable energy or the bioenergy from various sources of biomass has immense potential to substitute the fossil fuels and fulfill the

market demand (Gray et al. 2021). Furthermore, solid, liquid, and gaseous fuels that are produced from biomass, known as biofuels, refer to a fuel that is produced from biomass material. This biomass also serves as feedstock for many industries. Biofuels include bio-alcohols, biodiesel, biogas, and hydrogen-based fuels derived from different feedstock and require exclusive studies made to devise the best possible way to exploit it to its fullest potential.

In the present chapter, we discuss the second and third generation of solid and liquid wastes as feedstock used for energy production. In the Introduction, we look in detail the different generations and explain how second and third generations of biofuels have tremendous scope for sustainable production. Nonbiological and biological technology available for extraction of energy from these feedstocks and suitability of wastewater and solid waste as feedstock for production of different types of biofuels such as bioethanol, biogas, hydrogen, microbial fuel cell, and biodiesel is reviewed. The chapter further explores the advantages and disadvantages of different generation biomass feedstocks along with their challenges and opportunities for commercial-scale biofuel production.

Feedstock of the biomass employed to produce biofuels can be categorized into different types. The Intergovernmental Panel on Climate Change (IPCC) recognizes three classes of biofuels that include first-generation, second-generation, and third-generation biofuels (IPCC 2011; Ahmed et al. 2021). They are said to belong to four generations synonymous with biofuels. These generations can be distinguished from each other based on the type of biomass used and the processing technology adopted. The four generations of feedstocks and their characteristic features are summarized in Fig. 8.1. The first-generation feedstocks, i.e., those that are obtained from edible biomass, are reviewed extensively because they are a threat to biodiversity (Wright and Wimberly 2013). Production of first-generation biofuels utilizes processing technologies like fermentation (for ethanol) and transesterification (for biodiesel). Also due to the high dependency of first-generation biofuels on subsidies and its incapability of being cost-effective on par with the existing fossil fuels such as oil, it has not been well accepted till date. It has even been estimated that considering the factors of emissions from production and transport, life cycle assessment of first-generation fuels is not sustainable as it frequently exceeds those of traditional fossil fuels.

With the ever-growing population, it is more reasonable to use human food feedstock by-products, rather than food directly, and these were known as a second-generation feedstock. The second-generation feedstocks are obtained from non-food sources like lignocellulosic plant biomass (switchgrass, poplar) and non-edible oilseeds (*Jatropha*) through conventional method mentioned above and by thermochemical routes (for the production of liquid "synthetic biofuels"). The second-generation feedstock holds the scope for sustainable production because they include non-food crops (cellulosic feedstock) and mainly focus on waste materials generated from first-generation feedstock (e.g., waste vegetable oil). Some of the widely used second-generation feedstocks include wood and short-rotation crops such as poplar, willow or miscanthus (elephant grass), wheat straw, bagasse, corn-cobs, palm fruit bunches, and switchgrass.

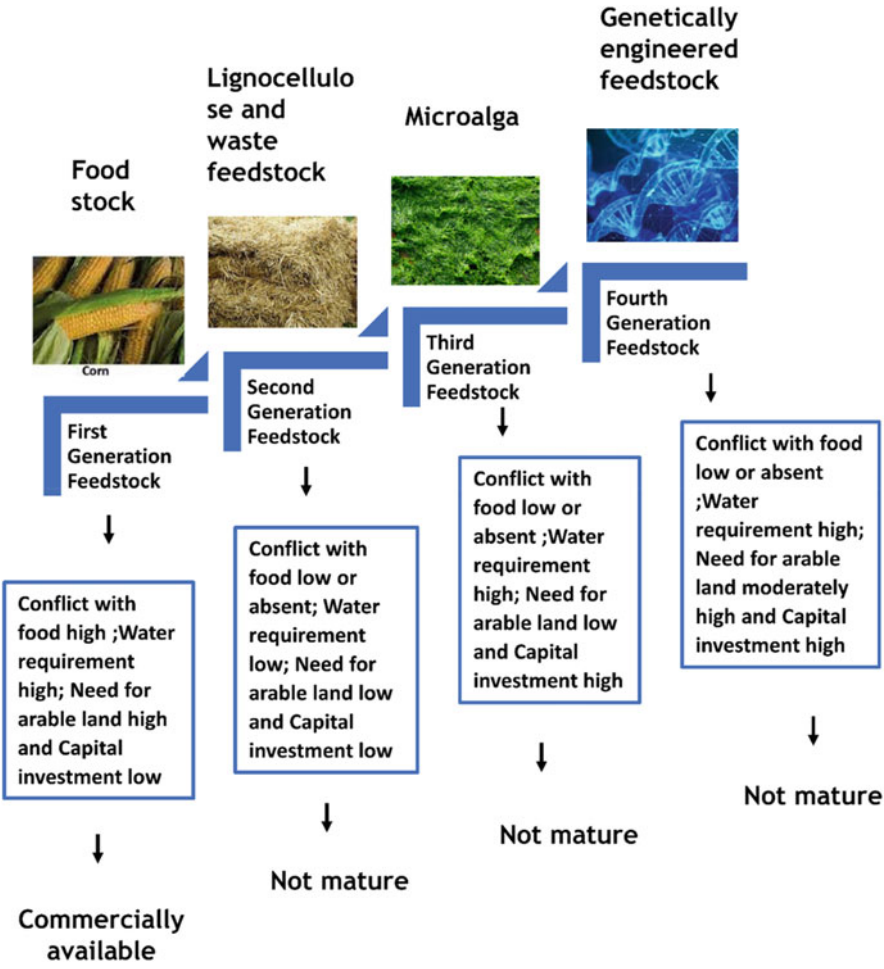

Fig. 8.1 Summary of four generations of biofuels explaining the stage of technology available for each of them

While third-generation feedstock utilizes similar production methods and biomass sources as that of second-generation biofuels, the distinguishing feature is that in third-generation feedstock, the methodology is applied on specifically designed or "tailored" bioenergy crops (often by molecular biology techniques) to improve biomass-to-bioenergy conversions. An example is the development of "low-lignin" trees, which reduce pretreatment costs and improve ethanol production, or corn with embedded cellulase enzymes. Third-generation feedstock has also been reported to be efficiently produced from biomass which is derived from algae and seaweed. Seaweeds, a group of algae, are autotrophic with some exceptions relying on external food materials. They mostly inhabit shallow water and do not require fertilizers, pesticides, herbicides, or land, thus making it a high-contender feedstock

for third-generation biofuel. The second and third-generation feedstock or biofuels are sometimes referred to as the next-generation or advanced biofuels.

There is another generation of feedstocks recognized, i.e., fourth-generation feedstock which is derived from genetically modified plant biomass. Genetically engineered feedstock which consists of genetically synthesized microorganisms such as cyanobacteria is the one which is considered a fourth-generation feedstock. The key point of bioenergy produced from fourth-generation feedstock is "carbon capture and storage," both at the level of the feedstock and the processing technology. Here, the feedstock is tailored not only to improve the efficiency of the processes involved but also to capture more carbon dioxide. The primary processing methods, like thermochemical processes, are coupled with "carbon capture and storage" technologies which funnel off the carbon dioxide into geological formations (geological storage, e.g., in exhausted oil fields) or through mineral storage (as carbonates). This attributes bioenergy produced from fourth-generation feedstocks to be a better alternative to reduce GHG emissions. It is comparatively more carbon neutral or even carbon negative than the other forms of bioenergy produced by the erstwhile generation of feedstock.

A clear understanding of four generations of biofuels and their limitations and the stage at which technology is available is very important in the development of sustainable technology and solving the global energy crisis.

8.2 Solid Waste as a Potential Feedstock

Solid waste, generated from various sources including agricultural, municipal, or forestry sources, is a matter of grave concern. An unlimited amount of annual global waste is being generated, much of which is not managed in an environmentally safe manner. The IEA reports that annual global waste being generated rounds up to 2.01 billion tons, out of which 33% waste is not managed in an environmentally safe manner, and the figures are expected to reach 3.40 billion tons by 2050. The contribution varies according to country or region. The estimated generation of municipal solid waste will increase by 70% and become 3.4 billion metric tons in 2050. The projected figure however varies according to the region (Fig. 8.2) (Govani et al. 2021).

Based on its origin, solid waste is categorized into four main types, i.e., industrial solid waste, municipal solid waste, agriculture solid waste, and forestry solid waste (Fig. 8.3). Municipal solid waste refers to domestic waste which comes from household usage of vegetable and fruit waste, paper, wood, etc. Agricultural wastes include crop residues, agro-industry wastes, livestock wastes, food and feed industry processing wastes, and fruit and vegetable wastes apart from other agriculture-based wastes. Industrial waste is not lignocellulose biomass; it includes cafeteria garbage, dirt and gravel, masonry and concrete, scrap metals, trash, oil, solvents, chemicals, weed grass and trees, wood and scrap lumber, and similar wastes. Point-wise comparison of four types of solid waste materials along with cited references is

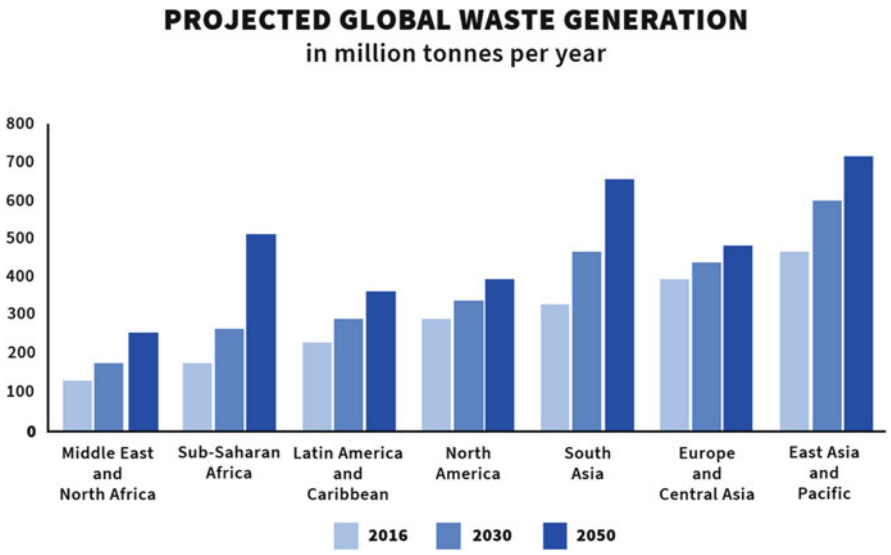

Fig. 8.2 Projected estimation of waste generation in 2016, 2030, and 2050 in million tonnes per year source (World Bank 2018) (Prepared by taking data from IEA report)

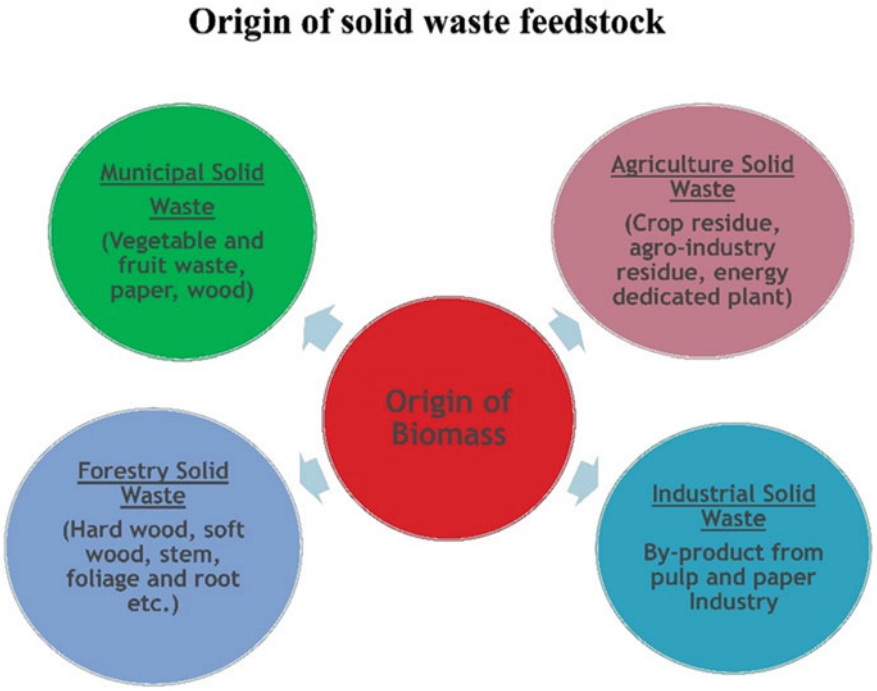

Fig. 8.3 Different types of solid waste biomass based on their origin

given in Table 8.1. Only agricultural industrial waste is considered in the comparison. Industrial waste should be included in the municipal waste itself (Parashar et al. 2020). Industrial waste from paper and pulp industries is investigated with huge potential as an energy source (Rizaluddin 2021). Forestry solid waste stock includes plant parts and litter. According to a report huge amounts of municipal solid waste (1.7 billion tons), agriculture solid waste (2 billion tons), forestry solid waste (0.2 billion/m^3), and industrial waste (9.1 billion tons) were generated in 2016 itself (Millati et al. 2019).

The physical and chemical composition of solid wastes varies according to local conditions, environment, population, demand, and economics. For example, in one study physical composition of solid waste constituted glass 9.59%, metal 2.74%, paper 25.83%, plastics 3.87%, compostable organic matter 57.48%, and other wastes 0.44% and moisture content 16.392% at the discarded density of 150.489 kg/m^3 and solid waste generation rate of 25.94 tonne/day (Yusuff et al. 2014). In another study, a typical waste composition in China is reported to be composed of 55.9% food residue, 8.5% paper, 11.2% plastics, 3.2% textiles, 2.9% wood waste, 0.8% rubber, and 18.4% non-combustibles (Zhou et al. 2015). The physical and chemical composition of these solid wastes determines the treatment technology that is required which will allow the generation of biofuel and value-added products.

The ability of solid waste to function as a biofuel feedstock is determined by the amount of biomass present. Biomass is a material that is derived from living or recently living biological organisms that make up this waste. In the context of energy, it also includes the by-products and waste from livestock farming, food processing and preparation, and domestic organic waste. Biomass is carbon-based and is composed of a mixture of organic molecules containing hydrogen, usually including atoms of oxygen, often nitrogen, and small quantities of other atoms, including alkali, alkaline earth, and heavy metals. Biomass can be used to produce renewable electricity, thermal energy, or transportation fuels commonly known as biofuels. In a nutshell, biomass encompasses all living things (Hendriks and Zeeman 2009). Lignocellulosic biomass can be obtained from municipal solid, agriculture solid waste, and forestry waste. Biomass obtained from different sources can be categorized based on its chemical composition into lignocellulose biomass, starchy biomass, and oily biomass. Table 8.2 shows the composition and relative proportion of cellulose, hemicellulose, lignin, and starch biomass from waste feedstock from various sources in different studies.

8.2.1 Municipal Solid Waste

Municipal solid waste (MSW) is diverse and contains a variety of organic and inorganic materials (Zhou et al. 2014). The composition of a typical municipal solid waste is shown in Fig. 8.4. However, the composition of municipal sewage waste changes according to the lifestyle followed by the people of the country and their socioeconomic status. The estimate indicates that solid waste generation will

Table 8.1 Comparison of different types of solid waste

Compared factor	Municipal solid waste	Agriculture solid waste	Forestry solid waste	Industrial waste (agricultural)
Origin	Households, offices, hotels, shops, schools, and other institutions	Agricultural activities	Forest	Businesses from an industrial or manufacturing process
Composition	Grass and leaves, debris and weeds, bones, bread, muffins, cake, cookies, pies, and dough, coffee, tea bags, eggs and eggshells, fruit and vegetable peelings, chicken, and fish, nut shells, pasta and rice, sauces and gravy, solid dairy products, table scraps and plate scrapings, etc. (David 2013)	Bedding/litter, animal carcasses, damaged feeders, and water trough, food and meat processing agricultural solid wastes including hoofs, bones, feathers, banana peels, crop residues, husks, vaccine wrappers or containers, disposable needles, syringes, wastes from prunings and grass cuttings, wood processing, paper manufacturing, etc.	Hardwood, softwood, and other by-products such as wood chips and sawdust	Cafeteria garbage, masonry, trash, oil, solvents, chemicals, weed grass and trees, wood and scrap lumber, and similar wastes
Lignocellulosic biomass	~25%	Varies, e.g., corn fiber 14.3%, sugarcane bagasse 43.1%, barley hull 37.2%, corn pericarp 4.7% (Shao et al. 2019)	Varies according to plant type	10–25% lignin, 20–30% hemicellulose, and 40–50% cellulose
Pretreatment methods	Mechanical pretreatment, mechanical, biological, treatment and gasification	Milling, steam explosion, chemical pretreatment, biological pretreatment	Milling, steam explosion, chemical pretreatment, biological pretreatment	Chemical pretreatment, enzymatic pretreatment, and physical pretreatment
Biofuel production	Heat, electricity, gaseous, and liquid biofuels	Bioethanol	Biogas	Biogas or syngas
Advantages	Reduces the landfill land	Compositing/ organic manure, substrates for edible fungi cultivation, non-conventional	Waste reduction	Waste reduction, cleaner environment

(continued)

Table 8.1 (continued)

Compared factor	Municipal solid waste	Agriculture solid waste	Forestry solid waste	Industrial waste (agricultural)
		feed ingredient, traditional soap making, and production of silica		
Disadvantages of waste	Not always cost-effective, product formed has short life, treating site dangerous	Health-related issues, eutrophication and soil pollution, etc.	–	Causes water pollution and other environmental pollutions
References	Eriksson and Reich (2005)	Singh et al. (2009)	Teghammar et al. (2014)	Siciliano et al. (2019)

Table 8.2 Composition of waste biomass observed in various feedstocks

Feedstock	Lignocellulosic composition wt%				References
	Cellulose (%)	Hemicellulose (%)	Lignin (%)	Starch (%)	
Crop residue	29.2–52.3	19.8–37.8	3.1–22.3	–	Pattanaik et al. (2019) and Adhikari et al. (2018)
Agro-industrial waste	21.2–63	7–33.5	4–50	23–85	Pattanaik et al. (2019) and Anwar et al. (2014)
Livestock waste	32.5	24.5	42.8	52–62	Pattanaik et al. (2019)
Forest waste	45–55	24–40	18–35	65–75	Pettersen (1984) and Yousuf et al. (2020)
Municipal solid waste	–	–	11.15–41.22	27–53	Meor Hussin et al. (2013)
Food waste	–	–	–	58–85	Yu et al. (2017)
Vegetable waste	17–66	11.38–32	2.6–32	15–35	Sazzad et al. (2017) and Wang et al. (2016a, b)

increase but unsustainable management of these wastes will be done mainly by landfilling.

A large amount of municipal solid waste out of total solid waste generated in East Asia and the Pacific Region and China is accountable for generating a maximum stake of more than 15% of municipal solid waste. MSW composition in India is approximately 40–60% compostable, 30–50% inert, and 10–30% recyclable. The chemical composition of MSW consists of nitrogen component of $0.64 \pm 0.8\%$, phosphorus of $0.67 \pm 0.15\%$, potassium $0.68 \pm 0.15\%$, and carbon to nitrogen ratio (C/N ratio) 26 ± 5 (Joshi and Ahmed 2016). Total urban waste production in India is around 62 million tons of solid waste (450 g/capita/day) in 2015; an approximately 82% of waste collected was segregated as MSW and the remaining 18% was

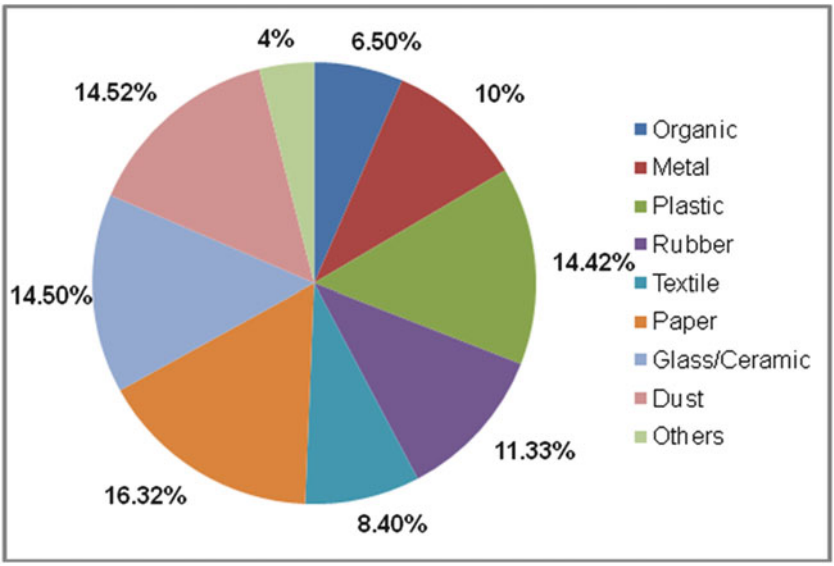

Fig. 8.4 Shows the global municipal solid waste composition [Prepared from data IEA Report (Zhou 2015)]

categorized as litter, out of which the MSW treated was only 28% of the collected waste, and the remaining 54% was openly dumped.

Such a large amount of solid waste management is a difficult task for most urban local bodies due to financial disabilities and inadequate infrastructure. The challenges faced by these local bodies are source segregation of waste, doorstep collection, options for recycling and reuse, technologies for treatment, land availability, and disposal competence (Agamuthu and Fauziah 2010). Waste collection efficiency ranges between 70 and 95% in major metropolitan cities, whereas in several smaller cities, it is below 50% (Sharma and Jain 2020). Low-income countries have collected about 48% of waste in cities, but this proportion drops drastically to 26% outside of urban areas. Across regions, sub-Saharan Africa collects about 44% of waste, while Europe and Central Asia and North America collect at least 90% of waste (Huang et al. 2021). At least 50–55% of municipal solid waste is also a valuable reserve as lignocellulosic biomass which can be converted profitably to biofuel chemical products using different technologies.

8.2.2 Agriculture Solid Waste

Agricultural wastes are the residues obtained from the production and processing of agricultural products such as crops, fruits, vegetables, meat, poultry, and dairy products (Vnwakaire et al. 2016). The global annual estimated production of crop

Table 8.3 Composition of various agricultural residues

Biomass	Cellulose (%)	Hemicellulose (%)	Lignin (%)
Sugarcane bagasse	32–48	19–24	23–32
Corn stover	35–42.6	17–35	7–21
Corn stalk	39–47	26–31	3–5
Barley straw	33.3–42	20.4–28	17.1
Rice husk	31.3	24.3	14.3
Rice straw	28–36	23–28	12–14
Wheat straw	33–38	26–32	17–19
Sorghum straw	32.4	27	7
Groundnut shell	35.7	18.7	30.2
Oat straw	37.6	23.3	12.9
Coconut shell	29.7	NA	44.0
Cotton waste	80–95	5–20	–
Softwoods	45–50	25–35	25–35
Hardwoods	40–55	24–40	18–25
Meadow grass	39.2	19.8	20.3
Clover	24.9	17.0	9.2
Cattle manure	14.2–27.4	12.2–21.4	6.1–13.0
Poultry manure	7.7–12	16.4–21.5	4.1–7.2
Newspaper	40–55	25–40	18–30
Algae (green)	20–40	20–50	NA

Source: Rastogi and Shrivastava (2017) and Paudel et al. (2017)
NA Not available. Composition is represented in percent weight on the dry weight of the samples

residues is 2802 million tons (Zabed et al. 2016). Crop waste residues generated from direct agricultural production at the field level are mostly crop residues like leaves, stovers, straws, and seed pods. Agricultural residues obtained from crop residues are the most abundant and cheapest organic waste, which can be easily transformed into different value-added products. There are mainly three major crop residues that are being used for bioethanol production, that is, rice straw, wheat straw, and corn stover. In Table 8.3, the composition of typical agricultural wastes is compared.

Sugarcane bagasse is one of the major agro-industrial wastes obtained from sugar industries after extraction of juice (Duque-Acevedo et al. 2020). The composition of agricultural waste depends on the system and type of agricultural activities, and they can be in the form of liquids, slurries, or solids. Agricultural waste otherwise called agro-waste is comprised of animal waste (manure, animal carcasses), food processing waste (only 20% of maize is canned and 80% is waste), crop waste (corn stalks, sugarcane bagasse, drops, and culls from fruits and vegetables, prunings), and hazardous and toxic agricultural waste (pesticides, insecticides, herbicides, etc.) (Pattanaik et al. 2019). The generation of agricultural waste was estimated to be about 998 million tonnes yearly (Vnwakaire et al. 2016). Organic wastes can amount up to 80% of the total solid wastes generated in any farm, out of which manure production can amount up to 5.27 kg/day/1000 kg live weight, on a wet weight basis.

8.2.3 *Forestry Solid Waste*

Forestry residues are obtained from the government forestry region that did not require any kind of agricultural land, and hence forestry feedstocks are much away from cultivation land competition. The European Forest Institute in 2014 stated that utilization of forestry solid biomass waste as feedstock for the production of advanced biofuel can bring structural change in the economy (Ullah et al. 2021). The composition of wood in the forest is lignin 18–35% and carbohydrate 65–75%. Overall component-wise composition stands at 50% carbon, 6% hydrogen, 44% oxygen, and trace amounts of several metal ions (Pettersen, 1984). Lignocellulose biomass from forestry residue is the extensive source of cellulosic and hemicellulosic components to produce various bioenergy and fuels and chemical products.

Forest solid waste is easily available and a source of feedstock reduces competition for agronomic feedstock, although the composition of forestry waste requires heavy pretreatment procedures due to complex structures. Forest waste residue serves as a better feedstock than forestry feedstock as the composition is the same but later causes environmental impact (Belyakov 2019).

8.2.4 *Industrial Waste*

Industrial solid waste is generated by businesses from an industrial or manufacturing process or waste generated from non-manufacturing activities that are managed as a separate waste stream and as solid waste. The cost for disposal of industrial waste is the same as for other types of solid waste.

8.3 Wastewater as Feedstock

Globally, wastewater is extensively generated in urban areas and varies majorly in their composition, based upon the source of effluent and treatment process. These wastes are deposits of many valuable components like organic mass, energy, and nutrient materials that may be major contributors to bioeconomy through their effective utilization for the production of value-added components (Mateo-Sagasta et al. 2015). In many instances, wastewater may replace the use of freshwater in the agricultural field due to its composition and provides an alternative to limited freshwater supply. According to a report about 10% of the world's food is produced using wastewater (WHO 2019, 2020). Based upon their source of generation and discharge site, wastewater is classified into various categories and their composition majorly changes based upon being domestic or industrial and then specifically belonging to which particular industry.

Wastewater is majorly 99.9% water with 0.1% being other components. This small percentage comprises microbes and inorganic and organic matter. Millions of microbial species are present in the remains which flourish utilizing organic and inorganic supplements present in the wastewater environment. This gives a clear insight into the presence of materials that may be potentially converted to value-added products. These aerobic bacteria and other species use organic matter for their survival and lead to the decomposition of complex molecules into simple forms and lead to the formation of a slime layer (Almond et al. 2020). Slime in water leads to the production of harmful gases like hydrogen sulfide whose extraction and processing can be converted to biofertilizers and biofuels. These microbes can also be exploited for the conversion of available waste to numerous beneficial components. Since blooming in toxic conditions, this microflora also includes a pool of pathogenic microorganisms, of which few prominent species include *Cholera vibrio*, *Salmonella typhi*, *Shigella*, *Coliform*, *Streptococci*, *clostridium*, certain enteric viruses, and enteric nematodes (FAO 1992, 2020).

Major inorganic components of domestic wastewater include nitrogen, phosphorus, chloride, calcium carbonate, and grease. Those from industrial effluents have a wider pool of elements and salts inclusive of sodium, calcium, magnesium, potassium, chloride, sulfate, carbonate, bicarbonate, ammonia, nitrate, manganese, phosphorus, zinc, etc. The presence of inorganic matter increases its utility in irrigation and agricultural practices.

Organic matter in wastewater like vegetable peel, fruit peel, paper, and cloth provides support to the growth of bacteria. The growing bacteria break this organic matter into simpler units like carbon and nitrogen in the water. Wastewater from industrial and agricultural resources includes major organic pollutants like polychlorinated biphenyls (PCBs), pesticides, herbicides, phenols, polycyclic aromatic hydrocarbons (PAHs), and aliphatic and heterocyclic compounds (Zheng et al. 2013). Broadly, wastewater from farmland has high content of pesticides/herbicides. The wastewater from coke plants is rich in PAHs and chemical industries are a good source of various heterogeneity compounds polychlorinated biphenyls (PCB) and polybrominated diphenyl ether (PBDE), and those coming from food industries include complex organic pollutants with a high concentration of suspended solid and biological oxygen demand.

Major wastewater sources used as feedstock for biofuel include sludge and sewage waste, paper and pulp industry disposals, chemicals waste, etc. Sludge and sewage contain variable phases as dispersive and nondispersive materials, composed of solid waste and gaseous components indicating great matter of concern due to the presence of dry mass, hydrated volume, and elements like Ni, Mg, Zn, and Cd (Agoro et al. 2020). On one hand, their disposal is a matter of concern, and on the other, sludge can be beneficially exploited to be used as biofertilizers in fields and encourages the phytoremediation process. Usage of sludge for biofuel production has the potential of being environmentally beneficial and is a promising source of alternate energy.

Wastewater serves as a platform for algal blooms, leading to toxicity and destruction of marine life. An attempt to extract biofuels from these resources, using

anaerobic digestion (biogas), transesterification (biodiesel), carbohydrate fermentation (bioethanol and biobutanol), and thermodynamic methods of conversion of algal blooms (bio-oils), presents great scope for production of various metabolites, fertilizing components, and energy alternatives (Craggs et al. 2011). One of the most promising approaches is production through the microbial system, by hydrolysis of complex molecules towards production and transformation to significant components (Alawsy et al. 2018). The substantial amount of insignificant cellulose-rich sludge released in the environment through pulp and paper industries is a global issue, and bioprocessing of the same for the development of sustainable products like biofuel and other related components through cost-effective methodology is the demand of the hour. Conversion of wastewater and other available substrates can be effectively carried out by enzymatic usage (Vignesh and Barik 2019).

Various carbohydrates molecules, few oils, fatty acids, and lipids are present as complex forms in industry effluents and are being processed effectively, having the potential to produce a significant amount of cost-effective biofuel, to be further used in blending with the fossil fuel. This would also help to control pollution and carbon emission because of low ash content. Sugar sources are mostly exploited for the production of bioethanol, and lipid sources are used for biodiesel formation through transesterification followed by the generation of products like glycerol (Tawalbeh et al. 2020). Chemical industry effluents with low sulfur content and high biodegradability, along with applications in the bioenergy sector, also find great implications in cosmetics and pharmaceuticals.

Other than the classification based on degradability, wastewater can be classified as sewage waste and non-sewage waste. Sewage wastewater comes from domestic activities that involve household activities, public and hotel wastes, and so on. The wastewater generated from these activities is very high. Sewage water usually contains blackwater and gray water (United Nations University 2013). The characteristics of sewage depend on origin and source; raw and untreated sludge has many pathogens, the major portion of water, and high biochemical demand. It also contains essential nutrients for plants like nitrogen and phosphorus and shows the quality of fertilizers. Carbon content on stabilization shows a potential effect on soil texture as it improves the soil structure for roots (Aghalari et al. 2020; Shrivastava 2020). Transformation of materials to energy resources through incineration, anaerobic digestion, pyrolysis mechanism leads to the production of biofuels such as methane-rich biogas, bio-oil, and syngas. Non-sewage wastewater involves all the types of wastewaters that involve water from agricultural activities, stormwater, and water from commercial sources. Wastewater from agriculture comes as effluent after agricultural practices and from the soil after field activities. Effluents rich in phosphorus are highly valuable for agricultural land and can face the issues of crop gap due to depletion of phosphorus that can make the soil deficient from nutrients and lead to ineffective yields (Kok et al. 2018). Managing the wastewater and implementation of technology that helps in the recovery of phosphorus from water can improve the condition of the soil and provide a solution to deal with the crop yield gap.

Industrial wastewater is highly variable in quality and quantity and the characteristics of water depend on the type of industry. These effluents differ in their biodegradability and are composed of organic and inorganic substances including various heavy metals as well. Wastewater under anaerobic conditions leads to the production of CH_4 by utilizing the metals and the compound present in the water. Methane as effluent generally is released from the paper and pulp industry, beverages industry, and organic chemicals (Krishna et al. 2017). Discharge of wastewater to the environment is a major pollution cause and demands zero discharge process which is ideally difficult to achieve and thus rational usage of conversion of recyclable components is suggested.

Another type of wastewater is stormwater runoff. It leads to significant pathway of microplastic introduction in water bodies (Werbowski et al. 2021). Stormwater comprises tire waste and road wear particles that lead to the introduction of microplastics in water bodies and increase the toxicity that leads to harm to aquatic life. Managing stormwater leads to the control rate of pollution in oceans.

8.4 Feedstock to Energy Conversion Techniques

With the deteriorating picture of the environment and economic prospects, recycling and energy-saving alternatives have resorted to bioenergy-efficient techniques. The technologies which allow the conversion of waste to energy have been extensively studied to find feasible ways to curate renewable sources of energy. There have been several technologies that have been suggested to be effective for biomass energy conversion. The technique selected depends on the component of biomass selected (Fig. 8.5). For example, for feedstock rich in fatty and oily biomass, transesterification is done to obtain biodiesel. There are two other general techniques as well: thermochemical and biochemical conversion which are used for the conversion of lignocellulose biomass. Thermochemical conversion is the technique that incorporates the decomposition of organic constituents in biomass using heat (Zhang et al. 2010). Biochemical conversion on the other hand is essentially about utilizing microorganisms or enzymes to convert biomass or waste into useful energy. For conversion of sugars and starches, enzymatic hydrolysis and fermentation are done to produce bioethanol (Fig. 8.5).

8.4.1 Thermochemical Route for Biomass to Energy

High-temperature chemical reformation process is the working principle behind the biomass energy conversion technique of thermochemical conversion. In this process, high temperature is applied to break and reform the bonds of the organic matter present in the waste into biochar, which is a solid or synthesis gas or oxygenated bio-oil which is the liquid form. Depending upon an array of factors, ranging from

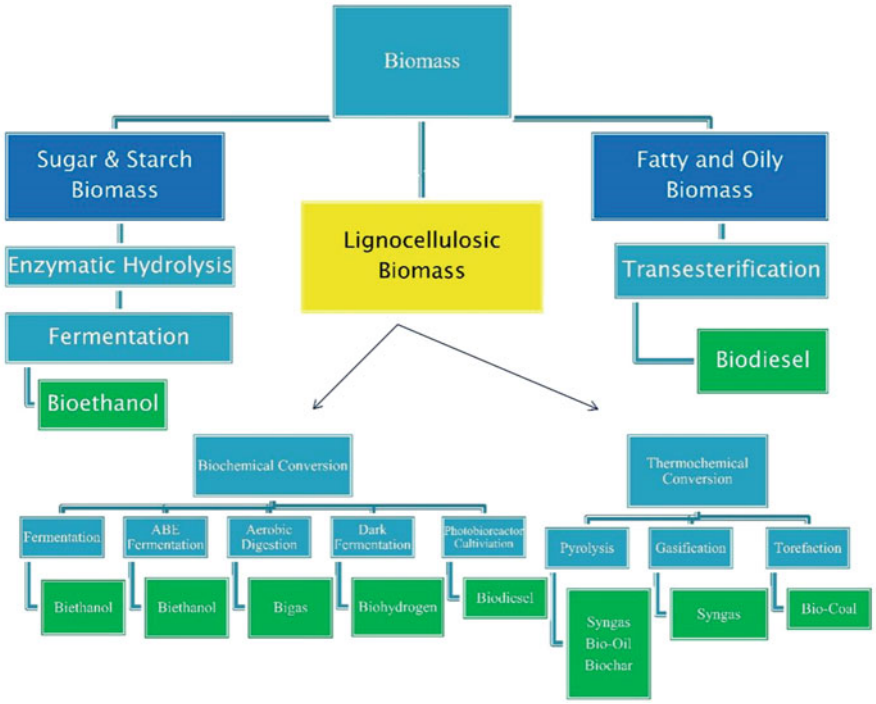

Fig. 8.5 Biomass to energy conversion techniques

the type of biomass feedstock, the necessities of the end-product financial infrastructure, or any other project-specific details, the various options available under this technique are gasification, pyrolysis, and liquefaction (Li et al. 2017). The thermochemical conversion technique has lately gained popularity due to the availability of industrial infrastructure which essentially optimizes the yield of the final products. This technique has been attributed by several studies with prominent qualities of lesser water usage, short processing time, and the most important of it, the independence of the technique from environmental circumstances for production purposes (Pandey et al. 2015). The processes which fall under thermochemical techniques of biomass energy conversion are pyrolysis, gasification, liquefaction, and combustion. Several examples of studies involving these methods and operation conditions and fuel output are given in Table 8.4.

8.4.1.1 Gasification

The working basis of this process is carrying out chemical reactions in an oxygen-deficient environment. In this, biomass is subjected to extreme conditions like high temperature (500–1400 °C) and extreme atmospheric pressures up to 33 bar in

Table 8.4 Energy generation from waste through thermochemical conversion route

S. No	Methods	Bioenergy obtained	Type of waste used as feedstock	Composition and biofuel yield	Pretreatment	Operating conditions	References
1.	Gasification	Syngas	Municipal solid waste	H_2 content (54.22%) and syngas yield (1.75 N m^3/kg)	Air-dried moisture content 10.2%, dried at 393 K for 8 h and particle size 5 mm and mixed for homogenous composition of feed	Catalyst type (NiO/γ-Al_2O_3 or calcined dolomite, Temperature—900 °C, Steam supplied	Siyi Luo et al. (2012)
		Syngas	Municipal solid waste (MSW) & municipal solid waste with bottom ash	Lower Heating Value of syngas are 4.4 $MJ/m^3_{N;db}$ and 5.9 MJ/m3 $N;_{db}$, Gross power generation 403 kWh/t-MSW and 673 kWh/t-MSW +ash, respectively, their gross power generation efficiencies were 18.9 and 23.0%	Direct melting system (DMS) no pre-treatment is required	Process—co gasification Temperature—400–1800 °C Oxygen—33% oxygen rich air	Tanigaki et al. (2012)
		Hydrogen gas	Wood industry waste	Maximum hydrogen yield reaches 45.16 g H_2/kg biomass Maximum lower heating value of fuel gas reaches 11.11 MJ/N m^3 for biomass oxygen/steam gasification	Physical pre-treatment into solid size 27 cm^3	Equipment- Downdraft gasifier, operational temperature—600–900 °C, Steam supplied	Lv et al. (2007)
		Hydrogen gas	Agriculture waste (coconut coir and palm kernel shell)	Hydrogen gas, 67 mol %	Air dried for 2–3 days, crushed	Equipment—Fluidized Bed gasifier,	Wan Ab Karim

(continued)

Table 8.4 (continued)

S. No	Methods	Bioenergy obtained	Type of waste used as feedstock	Composition and biofuel yield	Pretreatment	Operating conditions	References
						temperature—700–900 °C, air supplied	Ghani et al. (2012)
2.	Liquefaction	Bio crude oil	Municipal sewage waste	Energy content of 35.6 MJ/kg	Bioorganic and bio inorganic portion separated, sieved and shredded	Hydrothermal liquefaction temperature—300–350 °C	Mahesh et al. (2021)
		Bio crude oil	Food waste	Maximum HTL bio-crude oil yield (27.5 wt. %), energy recovery 49%	Separated organic waste fraction mixed with deionized water to create a slurry and then frozen at −20 °C prior to HTL	Hydrothermal liquefaction Temperature—240 °C Reactor purged with nitrogen to remove traces of air	Bayat et al. (2021)
		Biocrude oil	Energy crop waste Jatropha curcas cake	High yield of bio-crude oil (32.87 wt%)	Dried at 110 °C for 48 h, grounded and sieved through 350 mm sieve	Sodium carbonate (Na_2CO_3) as catalyst in hydrothermal liquefaction Temperature—600 °C Pressure—120 MPa Nitrogen gas was purged three times into the reactor	Alhassan et al. (2017)
		Bio diesel	Industrial food waste spent coffee grounds, soybean, and rapeseed cakes	Spent coffee grounds contained 16.8% bio-oil, whereas soybean and rapeseed cakes contained only approximately 0.97%	Direct extraction, no pre-treatment	Dimethyl Ether extraction, 1.5 wt % KOH as a catalyst was used. Extraction temperature and pressure were 20 °C and 0.51 MPa,	Sakuragi et al. (2016)

				and 2.6% Bio-oil, respectively		transesterification at 80 °C	
3.	Pyrolysis	Bio char, bio gas and liquid rich in phenolic and aromatic compound	Agricultural waste (oat straw)	Yield of gas increased from 13.1% at 300 °C to 16.3% at 600 °C & yield of solid and liquid waste showed a decreasing trend with increase in temperature	No pre-treatment was done	Semi-batch vertical reactor and the analysis tool compromises of thermal analysis, Temperature—300–600 °C Nitrogen was purged into reactor	Agata Mlonka-Mędrala et al. (2021)
		Biochar, biooil and biogas	Hog fuel secondary woody residue produced from mill byproducts such as sawdust, bark and shavings	Yield obtained of bio-oil (61% yield), biochar (24%) and biogas (15%)	Size 5 mm thick and 30 mm long, grinded, 1.8 mm dust, dried to moisture content from 44 to 5.9%	Laboratory-scale pyrolysis unit used for extraction Temperature—680 °C	Carrasco et al. (2017)
		Bio oil	Food waste (vegetarian as well as non-vegetarian) samples	Maximum bio-oil yield of 30.24 wt.%	Homogeneous mixture of food waste, dried in electric oven for 24 h, at 105 °C, pulverized size less than 1 mm	Microwave assisted pyrolysis. Temperature—400 °C	Kadlimatti et al. (2019)
		Syngas	Food waste Meat (20 wt%), rice (35 wt %), and vegetable leaves (45 wt%)	Bio-gas yield and the syngas concentration increased to 70.34 wt% and 61.50 mol%	Mixed and stirred with 3 g catalyst for 10 min, and loaded with a quartz container for microwave pyrolysis	Microwave assisted pyrolysis, catalyst used Fe_2O_3 and Fe_3O_4 used as catalysts, temperature—1000 °C, carbon dioxide supplied	Lin et al. (2021)

Abbreviations: *LHVs* lower heating value, $MJ/m^3_{N.d.b}$ millijoule per cubic meter of dry base biomass, *HTL* hydrothermal liquefication

anoxic conditions. The product yield from this process is a combustible gas mixture. The carbonaceous constituent of the biomass is converted into syngas which essentially is made of hydrogen, carbon monoxide, carbon dioxide, methane, higher hydrocarbons, and nitrogen. It is this syngas which supplies energy or energy carriers like biofuel, hydrogen gas, heat, etc. Syngas has been reported to be the most efficient way of producing hydrogen gas from biomass (Joshi and Ahmad 2016).

In comparison to other known options of combustion and pyrolysis processes, gasification has been reported to recover more energy and higher heat capacity from biomass. It is comparatively a similar process that allows catalytic methanation of carbon monoxide and carbon dioxide of syngas to synthetic natural gas (Pandey et al. 2015). Gasification is considered to be the ideal process for enabling feasible routes for biomass-energy conversion of diverse feedstocks of varying wastes of agriculture, industrial, kitchen, food, and farm. The decisive components which decide the composition of the gas thus produced in the gasification process are gasifier, gasification agent, catalyst type, and size of the particle. Gasifiers are essential and of four different types: fixed bed, fluidized bed, entrained flow, and plasma. It is these factors that finally dictate the composition of the gas formed (Basu 2010).

There have been several studies made on optimizing the procedure of gasification with promising results. A study by Salimi et al. (2018) on lignocellulosic wastes of canola stalks with bimetallic catalysts like nickel (Ni), ruthenium (Ru), copper (Cu), and cobalt (Co) resulted in a greater generation of H_2, CO_2, and CO yields, high catalytic activity, and stability. With a high heating value of 6.81 ± 0.34 MJ·Nm^{-3}, another study by De Oliveira et al. (2018) explicates the feasibility and behavior of fuel gas produced by the gasification process of coffee waste. Novel mechanisms like the plasma gasification method have also been widely explored recently and have provided promising yields of gas with concentrations of 69.6 (Messerle et al. 2018) and 71.1 (Mazzoni and Janajreh 2017) vol.% from biomedical waste (bonny tissue) and household waste, respectively.

8.4.1.2 Liquefaction

The liquefaction process involves the production of bio-oil at low temperature and elevated pressure with or without catalyst in the presence of hydrogen. Hydrothermal liquefaction (HTL) is termed as anhydrous pyrolysis and is also a prominent process established for conversion of biomass into bio-oil at medium temperatures ranging from 250 to 374 °C and operating pressure from 40 to 220 bar (Anastasakis et al. 2018). Feedstock which comprises higher moisture content like algae-based biomass, woody biomass, and municipal solid waste has great potential to be converted by the method of HTL. Dry tons of biomass feedstocks have immense potential to be used as bioenergy as has been reported by the US Energy and Agriculture Department, which says 700 million dry tons of biomass feedstocks can be used for biofuel production. Forestry and agricultural resources contribute up

to 350 million dry tons, thus making us consider the potential of such easily available feedstock for the production of bio-oil (Langholtz et al. 2016). Lignocellulosic woody biomass consisting of cellulose (30–50%), hemicellulose (15–35%), and lignin (20–35%) has been considered as a suitable feedstock for HTL.

The parameters of whether the catalyst is being used or not and on the type of solvents determine the yield of bio-oil from the given biomass. Dimitriadis and Bezergianni (2017) have reported 17–68 wt% of bio-oil from woody biomass by means of HTL. In an attempt to extract biocrude from algae, Costanzo et al. (2016) performed a two-stage HTL process consisting of first low-temperature followed by high-temperature HTL coupled with hydrodenitrogenation and hydrodeoxygenation catalyst. Sewage sludge has been found to have immense potential to be processed into bio-oil by HTL process. The only challenge in the production of bio-oil using sewage sludge is its high moisture content. There have been several studies made to reduce moisture content using a dry straw (Li et al. 2015), co-liquefaction (Biller et al. 2018), etc. According to the reports of a study made by Yang and colleague, pretreatment of sludge by cationic surfactant-non-ionic surfactant (fatty alcohol polyoxyethylene ether AEO9) SCW on the HTL of sludge allowed high production of bio-oil which accounts for up to 47.6%.

8.4.1.3 Pyrolysis

Pyrolysis is the most studied biomass conversion method. Thermal decomposition of biomass in anoxic conditions with an operating temperature range of 350–550 °C is the basis of the process of pyrolysis. Organic matter in biomass is decomposed into solid, liquid, and gaseous mixtures by the process of pyrolysis. The contrasting feature of pyrolysis than that of gasification is that in the former, liquid fuel known as pyrolysis oil/bio-oil is produced. This bio-oil can be straightaway sorted without stringent transportation or storage norms (Dhyani and Bhaskar 2018).

There are three types of pyrolysis processes that differ according to their operation conditions, namely, slow, fast, and flash pyrolysis. Fast pyrolysis is often studied as a process due to its advantage of yielding high amounts of pyrolysis oil, 75 wt.% from cost-effective technologies which are not just efficient but environmentally friendly as well (Bridgwater 2012; Jahirul et al. 2012). Several studies have reported promising results in obtaining pyrolysis oil. The bio-oil yield of better amounts was witnessed with the introduction of several strategies like hydrodeoxygenation up-gradation, also known as hydrotreatment. This process is the removal of oxygen from oxygenated hydrocarbons via catalytic reaction at high pressure (up to 200 bar), hydrogen supply, and moderate temperature (up to 400 °C). It improves py-oil quality and energy density by several folds (Zhang et al. 2013).

8.4.2 Biochemical Route for Biomass to Biofuel

Biochemical means of conversion is utilizing microorganisms or enzymes to convert biomass or waste into useful energy. The biological processes for biofuel manufacturing are complex processes and the single process itself involves numerous processes that result in an immeasurable number of biochemical reactions taking place simultaneously. These biological processes provide us with a cost-efficient system for the generation of biofuel. This includes several processes like anaerobic digestion, alcoholic fermentation, and photobiological reaction. The challenges faced during anaerobic digestion are associated with the availability of raw material, pretreatment, fermentation, providing favorable conditions for conversion reactions, etc. There is a constant need to understand and increase the efficiency of these processes by better understanding the microbial digester. Understanding of microbial digester is important as the immeasurable amount of the reactions occurring may produce some toxic and inhibiting compounds as well that affect the efficiency or biofuel production (Chung 2013). Several examples of biochemical conversion are included in Table 8.5.

8.5 Pretreatment Methods for Biomass

Pretreatment is essential for the processing of lignocellulosic compounds to produce biofuels. Lignocellulosic compounds are mainly comprised of cellulose, hemicellulose, and lignin which have a complex association with each other that hinder the activity of the enzymatic action by microbes or chemicals in degradation or hydrolysis. Pretreatment helps in increasing the porosity of the biomass that increases surface area and lessens the activity of cellulosic moiety and decomposition of hemicellulose, thus increasing the efficiency of the treatment divulged upon biomass to be treated for biofuel production (Agbor et al. 2011). There are four types of pretreatments: physical pretreatment, physiochemical pretreatment, chemical pretreatment, and biological pretreatment.

8.5.1 Physical Pretreatment

The primary step in biofuel production is the conversion of biomass into a powder-like physical form which includes processes like wet milling, dry milling, ball milling, and compression milling which help in the size reduction of the initial biomass (Zhu and Pan 2010). Another physical method of biomass treatment includes radiation treatment where biomass is exposed to different types of radiation such as microwave radiation and electromagnetic radiation, which weakens the interaction between the lignocellulosic compounds. These radiation techniques can

Table 8.5 Biochemical route for biofuel production

Biogas, Bioethanol and Biohydrogen production								
Methods	Bioenergy produced	Feedstock used	Composition of feedstock	Pre-treatment of feedstock and condition	Microbes used for fermentation/ digestion	Operating conditions of fermentation/digestion	Bioenergy Yield/ composition	References
Anaerobic digestion	Methane gas	Sewage sludge	TCOD—169 g/L SCOD—1.7 g/L TVFAs—0.8 g/L T-N–1.1 g/L S-N–0.1 g/L T-P—0.8 g/L S-P–0.1 g/L T-protein–536.7 g/L S-protein–232.4 g/L T-carbohydrate—2347.1 g/L S-carbohydrate—102.7 g/L	Thermal hydrolysis of sewage sludge	*Methanobacteria* *Methanomicrobia*	Thermal hydrolysis at 180 °C, time 76 min	Maximum CH_4 yield of 273.2 ± 5.6 mL CH4/g	Choi et al. (2018)
	Biogas	Food waste bread, rice, cooked noodles, tea bags, various fruits, vegetables and *Parthenium hysterophorus* (co-substrate)		Physical pretreatment ground, subjected to irradiation and high pressure steam at 121 °C for 20 min	Cattle manure inoculum	Anaerobic co-digestion at 30–31 °C. pH—7.8–8.0, retention time 60 days	Maximum biogas production rate: 559 ml L^{-1} d^{-1} Accumulative biogas 5532 ml L^{-1} after 60 days	Tayyab et al. (2019)
	Biogas	Food waste and wood chips (in ratio 0.5).	Food waste composition: Moisture content: 87.2% Total solids:12.8% Volatile solids :11.5%	Physical pretreatment ground and sieved 4 * 4 mm), soaked in 4% SO_2 aqueous solution for 2 h, added mixture	–	Anaerobic digestion at 35 °C, 125 rpm, retention time 15 days	20 ml/g of methane and 13.9 ml/g of hydrogen	Oh et al. (2018)

(continued)

Table 8.5 (continued)

			Density: 1.1g/ml Wood chips: Moisture content: 2.7% Total solids: 97.3% Volatile solids: 64.8% Density: 0.4 g/ml	of glucosidase, protease, and lipase to chips (10 g of the enzyme mixture per 100 g of the chips), dried at 100 °C for 90 min				
Anaerobic fermentation	Bio ethanol	Microalgae *Chlorella* powder	Carbohydrates–40–70% Proteins—10–20%	Enzymatic hydrolysis α-amylase from *Bacillus licheniformis and* amyloglucosidase from *Aspergillus niger*	*Saccharomyces cerevisiae* For fermentation	Fermentation at 32 °C, at 150 r.p.m, retention time 84 h. pH adjusted to 5.5 using NaOH	Highest ethanol yield 0.28 g/g	Phwan et al. (2019)
	Bio ethanol	Sugar cane bagasse, wheat straw and rice straw	Sugarcane molasses sucrose (32%), fructose (16%), glucose (14%) Wheat straw and rice straw mainly cellulose, hemi cellulose and lignin content	Mixture powder (size 2 mm) pretreated with 3% H_2O_2 + 2% NaOH, steaming at 130°C for 60 min, and hydrolyzed by commercial cellulase enzyme	*Saccharomyces cerevisiae* (for fermentation)	Submerged fermentation at 30 °C for four days of incubation	Ethanol production from sugar bagasse—77 g/L Rice straw—62 g/L Wheat straw—44 g/L	Irfan et al. (2014)
	Bio ethanol	Biomass of *Microcystis sp.*	Carbohydrates 11–50% in form of starch and cellulose	Physical pre-treatment (Solar dried and milled) followed by treatment by 0.5 N H_2SO_4 for 4 h at temperature 120 °C	*Saccharomyces cerevisiae* (immobilized)	Fermentation at 30–32 °C Bio ethanol separation at 70 °C	Bioethanol yield 18.57 g/L	El-Mekkawi et al. (2019)
Biophotolysis	Bio Hydrogen		50% glycerol with COD of	No pretreatment	*C. vulgaris*, *C. prototheocoides,*	Biophotolysis condition at	Maximum hydrogen production of	Sengmee et al. (2017)

		Crude glycerol as exogenous carbon source	1,884,600 mg/L, total nitrogen of 1149 mg/L, phosphorus of 336 mg/L and pH 10.27		*Chlorella sp.* and *Chlamydomonas sp.*	48 micro mol photon m^{-2} s^{-1} light intensity	10.31 ± 0.05 mL/L in serum bottle and 11.65 ± 0.65 mL/L in 1 L bioreactor along with lipid content >40%	
Anaerobic fermentation	Bio Hydrogen	Sewage Sludge	Sewage sludge rich in polysaccharides and proteins	Alkaline pretreatment of sludge by slow addition of alkali of 4 M sodium hydroxide at pH 12, 30 min, 25 °C and 24 h	*Eubacterium multiforme* and *Paenibacillus polymyxa*	Fermentation at pH 11, 36 °C and 150 rpm	Hydrogen production yield of 9.1 mL of H_2/g from raw sludge, 16.6 mL of H_2/g from alkaline pretreated sludge	Cai et al. (2004)
Bio electricity production								
Process	Bio energy	Biomass	Biomass analyte composition	Biomass Cultivation condition	Inoculum in Microbial Fuel Cell	Bioenergy yield		References
Microbial electrolysis	Bioelectricity	*Chlorella regularis* green algal biomass	proteins (46%), carbohydrates (22%), lipids (~20%), no lignin and cellulose, sole electron donor	Media BG 11, Temperature—32 °C, speed 150 rpm	Anaerobic sludge	Power density was 0.86 W/m^2 at a current density of 2.3 A/m^2 and the columbic efficiency reached ~61.5% (biomass)		Ndayisenga et al. (2018)
	Bioelectricity	Household waste –	Coconut wastewater Boiled Rice drain water Milk	–	Cow dung	Electrical potential generated from coconut water 700 mV Boiled rice drain water 335 mV, Diluted Milk- 389 mV, after hours		Sreedharan and Pawels (2016)
Bio diesel production								
Cultivation process	Bio energy	Algal Biomass	Feedstock for cultivation	Photo-Fermentation condition	Lipid extracted/ process	Transesterification	Biodiesel yield	References

(continued)

Table 8.5 (continued)

Photo fermentation	Biodiesel	*Chlamydomonas polypyrenoideum*	Dairy wastewater	Temperature 28–30 deg C, light intensity 10 W/.m^2 (12 light and dark cycle)	Lipid 42% (w/w)	Chemical method	Bio diesel yields of 1.3 and 1.0 ml on 10th and 15th day	Kothari et al. (2013)
Photo fermentation	Bio diesel	*Chlorella vulgaris*	–	–	Crude lipid 22.08% after Sub-critical water pretreatment	Non-catalytic in-situ transesterification	Fatty acid methyl esters (FAMEs) Content 78.45%.	Felix et al. (2019)

Abbreviations: *TCOD* total chemical oxygen demand, *SCOD* soluble chemical oxygen demand, *TVFAs* total volatile fatty acids, *T-N* total nitrogen content, *S-N* soluble nitrogen content, *T-P* total protein content, *S-P* soluble proteins content, *T-C* Total carbohydrate content, *S-C* Soluble carbohydrate content

be used to enhance the effect of the pretreatment of cellulosic compounds, for example, ultrasound-assisted alkali treatment is helpful in cellulosic and lignin digestion (Das et al. 2015). Pyrolysis can also be used as a pretreatment method to char the cellulosic moiety at a temperature greater than 300 °C. These methods are helpful in the depolymerization of lignin (Kumar et al. 2009).

8.5.2 Physiochemical Pretreatment

The physiochemical pretreatment methods include steam explosion, subcritical water, supercritical CO_2, and ammonium fiber explosion (APEX). Steam explosion involves pretreatments of biomass with increased temperature and pressure that increases the xylan yield up to 43–55%. Subcritical water (temperature 170 °C to 230° C, pressure 5 bar) or subcritical CO_2 (temperature more than 31.1 °C and pressure more than 73 bar) is also used for the pretreatment of biomass. APEX is somewhat similar to subcritical water pretreatment, but it also decomposes the lignin component of the biomass and is found to increase efficiency up to 98% in glucose saccharification of corn stover (Onumaegbu et al. 2018).

8.5.3 Chemical Pretreatment

This kind of pretreatment utilizes mineral acids and alkali such as hydrochloric acid, sulfuric acid, peroxyacetic acid, sodium hydroxide, potassium hydroxide, etc. These mineral acids are used to dissolve lignin in biomass. The chemical pretreatment method is expensive as well as hazardous for the environment; hence, it is not much used for pretreatment purposes. There are different innovative processes in chemical pretreatment for various substrates such as organosol process in the paper and pulp industry, ozonolysis for agricultural waste pretreatment, and ionic pretreatment procedure (Aftab et al. 2019).

8.5.4 Biological Pretreatment

Biological pretreatment relies mainly on microorganisms and enzymes for the decomposition of hemicellulose and lignin components in the biomass to increase yield by hydrolysis. Some agents of biological pretreatment are fungal consortium which removes lignin from corn stover and amplifies the yield by seven times. Microbes like *Strobilurus ohshimae*, *Phanerochaete chrysosporium*, and *Trametes versicolor* increase the yield of reducing sugar from the straw, corn stalk, and rice husk by partially or fully removing lignin (Zabed et al. 2019).

8.5.5 Detoxification

Detoxification plays an important role in the biofuel production of bioethanol and biomethanol. It is used for the removal of lignin and cellulosic compound by-products which act as an inhibitor for the microorganism and enzymes during the fermentation process. By-products formed during biofuel production that act as inhibitors are furfural, 5-hydroxymethyl furfural (HMF), and phenolic and aliphatic acids, such as acetic, formic, and levulinic acid. These are formed by degradation of hexose and xylose and partial degradation of lignin and partial digestion of HMF or deacetylation of hemicellulose, respectively (Liu and Blaschek 2010).

Several treatments have been developed to lower the influence of the inhibitors by the use of either chemical or biological agents such as alkali $Ca(OH)_2$, NaOH, and NH_4OH; reducing agents like dithionite and dithiothreitol; and microorganisms, viz., *Coniochaeta ligniaria*, *Trichoderma reesei*, *Reibacillus thermosphaericus*, *Ureibacillus thermosphaericus*, and *Saccharomyces cerevisiae*. Other than delivering the inhibiting treatment, special care in choosing of feedstock can also be used to reduce the effect of the inhibitor and keep the process cost-effective and efficient (Moreno et al. 2015).

8.5.6 Hydrolysis

Hydrolysis can be done either by an acid or enzyme. Acid hydrolysis could be applied through two ways: (1) dilute acid at high temperature and high pressure for a short duration and (2) concentrated acid at low temperature for a longer duration. The former procedure utilizes sulfuric acid at lower concentration and acts on hemicellulose and cellulose for a short duration at a temperature range of 120–160 °C, whereas the latter utilizes acids like HCl, trifluoroacetic acid, and H_2SO_4 with longer reaction time, and this is more efficient in the conversion of cellulose and hemicellulose than the former process. The major limitation of acid hydrolysis is that it causes corrosion of equipment and is hazardous for the environment (Akhtar et al. 2016).

Enzymatic hydrolysis utilizes the mixture of biological organisms and enzymes to degrade the biomass. The main enzymes utilized are cellulase, hemicellulose, and ligninase. Cellulase is produced by an array of microorganisms such as *Bacillus*, *Clostridium*, *Microbispora*, *Streptomyces*, and *Thermomonospora* (bacteria), *Trichoderma*, *Penicillium*, *Humicola*, and *Schizophyllum* (fungus) (Wan 2012). Hemicellulose-degrading enzymes are endoxylanase or endo-1,4-β-xylanase, α-4-methyl glucuronosidases, acetylxylan esterase. Some of the lignin-degrading enzymes are lignin peroxidases, manganese peroxidases, and laccases (Amiri and Karimi 2018; Rocha-Martín et al. 2017). Enzymatic hydrolysis mainly depends upon two factors: (1) enzyme-associated factors (including type, source, and efficiency of enzyme) and (2) substrate-associated factors (composition and structure of

feedstock, degree of cellulose crystallinity, particle size, and porosity) (Aguilar et al. 2018).

8.6 Solid Waste and Wastewater as Feedstock for the Production of Second-Generation Biofuels

Solid wastes are used as second-generation feedstocks. Forestry solid waste is used as a second-generation feedstock that includes hardwood of higher plants, softwood of lower plants, and other plant products such a wood chips and sawdust (Badgujar and Bhanage 2018). Agricultural solid wastes as second-generation feedstock represent feasible alternatives for bioethanol production due to their wide distribution, abundance, and low cost, and they are not competitive with food and feed crops. Solid wastes are used for the production of ethanol, hydrogen, methane, and biodiesel. The pathways involved are summarized in Fig. 8.6.

As compared to fossil fuels, lignocelluloses are almost equally distributed on the earth which provides security of supply throughout the year for biofuel production (Bušić et al. 2018). The compositional variety of lignocelluloses is both advantageous in terms of availability of a broader range of feedstocks as well as more by-products, and also adds a disadvantage for they need a large range of technologies for their effective conversion to biofuels. The heterogeneous structure of lignocellulosic biomass necessitates the use of advanced chemical processes to achieve

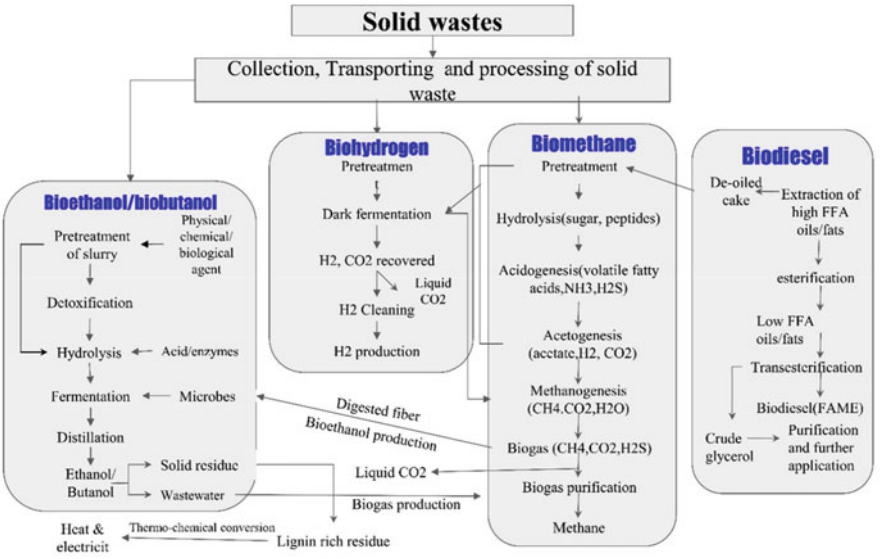

Fig. 8.6 Summary of pathways involved in the synthesis of ethanol, hydrogen, methane, and biodiesel from solid wastes

targeted fermentable sugars (Dale and Kim 2005). Furthermore, harvesting of lignocellulosic crops is usually not possible throughout the whole year; therefore, biomass stabilization for long-term storage is necessary to ensure continuous work of biorefineries (Cherubini and Strømman 2011). In the section on Introduction, we already discussed the different generations of feedstocks and biofuels and explained how the second and third generations of biofuel have tremendous scope for sustainable production of energy. Here we will assess the production of different biofuels from different second-generation waste solid and water feedstocks.

8.6.1 Bioethanol

Bioethanol production is being explored from many microbial sources, through varied approaches including wild and reconstructed strains ranging from prokaryotes to lower eukaryotes (including bacteria, yeast, fungi, and microalgae). Production of bioethanol has been explored from these strains from numerous agricultural and municipal wastes followed by pretreatment methodologies. The most accepted pretreatment process includes enzymatic hydrolysis for maximal saccharification of residues to obtain reducing/mono-sugars for their efficient conversion to bioethanol. Enzymes like cellulases, beta-glucosidases, xylanases, amylases, etc. from a plethora of bacterial and fungal cells (*Escherichia coli*, *Trichoderma reesei*, *Aspergillus niger*, *Thermomyces lanuginosus*, etc.) have been widely used for saccharification with efficiency up to 92% achieved with treatment of beta-glucosidase from *Escherichia coli*, cellulase and amyloglucosidase from *Aspergillus niger*, and alpha-amylase from *Bacillus licheniformis* (Onay 2019), obtaining 57% yield of glucose, with exclusive use of cellulases from *Trichoderma reesei* (Shokrkar et al. 2018) and ethanol production ranging from 38 to 80% in efficiency through combined microbial assimilation of fungal and algal biomass (Kumar et al. 2018; Rempel et al. 2019; Sulfahri et al. 2020; Qarri and Israel 2020). Recombinant strains of *Saccharomyces*, *Clostridium*, *Fusarium*, *Trichoderma*, etc. expressing target genes for enhanced ethanol production in the controlled physicochemical environment have been reported with yield ranging from approx. 2.4–25 g/L, which makes 75% of the theoretical maximum (Rastogi and Shrivastava 2017; Tian et al. 2016).

8.6.2 Biogas

Wastewater and solid waste represent the second-generation feedstock that can be exploited to the utmost level to produce cleaner and efficient biogas. They do not jeopardize agricultural and other interests. By the action of cellulolytic, ligninolytic, amylolytic, pectinolytic, proteolytic, lipolytic, and other enzymes of mesophilic and thermophilic bacteria such as *Clostridium stercorarium*, *Clostridium thermocellum*, etc., complex biomolecules like fats, carbohydrates, and proteins present in the

wastewater and solid waste are broken into its simpler forms of fatty acids, simple sugars, and amino acids (Ferdeş et al. 2020; Zverlov et al. 2010). As a result of the entire process, organic macromolecules are broken down into simpler molecules leading to the generation of biogas which mainly consists of methane. The methane thus generated is further used for heating, cooking, and steam production.

There are different types of wastewaters that have been reported to produce optimal levels of biogas. Lipid-rich feedstock has a higher methane potential than carbohydrate-rich and protein-rich feedstocks (Li et al. 2017). When agricultural wastes are taken into consideration, it has been reported that the methane potential for various types is quite promising which accounts for 302, 290, and 338 L/kg volatile solids (VS) for rice straw, wheat straw, and corn stover, respectively (Pattanaik et al. 2019). In another study, Li et al. (2015) reported a comparative yield of biogas from the animal waste of two types: pig manure and cattle manure which produced 495 mL/g VS with 70–80% CH_4 and 398 mL/g VS with 55–75% CH_4, respectively. Biogas production from MSW and domestic sewage has been reported to produce 0.36 m^3/kg of VS per day at an organic feeding rate of 2.9 kg of VS/m^3/day. The feedstock fed into a 5-l batch type reactor consisted of more than 50% organic matter followed by all sorts of assorted wastes like paper, stones, etc. (Elango et al. 2007). Similar research was conducted by yet another group of researchers, wherein MSW was the primary feedstock. The composition was essentially garden waste, paper, vegetable waste, cooked meat, etc. The maximum heating value measured for the produced biogas was 21.2 MJ/m^3 (Al-Zuahiri et al. 2015). Optimal levels of biogas production have been reported from olive oil mill wastewater which is rich in phenol and organic matter. With a hydraulic retention rate of 19 days, the methane production rate was reported to be steady, 0.91 L CH_4 or 250.9 L CH_4 at standard temperature and pressure conditions (STP) per kg (Dareioti et al. 2010). A biogas composition of 72.98% CH_4, 19.76% CO_2, and 0.9% O_2 was reported from recycled paper mill wastewater by Bakraoui et al. (2020).

The dry anaerobic digestion technique is fit to treat any organic wastes including the organic fraction of municipal solid waste, but owing to its complex functioning and low performance, it could not be brought into practice at present. It is needed to optimize against inoculum, to percolate recirculation and bed characteristics for batch processes, to process start-up, and to understand localized inhibition mechanisms. To make the production of biofuel profit-making, it is required to understand the chemical complexity of feedstock, inhibition kinetics, and mechanism and process operation.

Wet anaerobic digestion is the most preferred technique to produce biogas due to the requirement of low-cost equipment and a more efficient process. The wet digestion process has several problems like removal of inert, acidic, and unwanted material from the substrate which will deviate the biological metabolic equilibrium, the requirement of a large quantity of water to keep substrate dispersed, and distribution of low total solid content digestate (Maile and Muzenda 2014; Murto et al. 2004; Popescu and Jurcoane 2015). One more important problem is associated with the release of biogas and efficient mixing due to foam formation and surface

crust. Wet digestion does not require the crushing of the substrate to make its fine size.

Wet and dry anaerobic digestion are distinguished by the total solid (TS) content, with a value of less than 15% indicating wet anaerobic digestion and more than 15% indicating dry anaerobic digestion. Due to the greater moisture content of the straw (total solid of dry corn stover >80%; total solid of silage corn stover >30%), wet anaerobic digestion has lower operating efficiency and higher costs (Li et al. 2011).

The formation of hazardous by-products or unwanted volatile fatty acids generated during the hydrolysis process has been reported by many researchers to be a rate-limiting step (Khalid et al. 2011; Ma et al. 2013; Vavilin et al. 2008; Neves et al. 2006). Methanogenesis, on the other hand, is said to be a rate-limiting step for easily biodegradable substrates (Gavala et al. 2003). Commonly, the organic fraction of the municipal solid waste is added for co-digestion with sewage sludge for anaerobic digestion. Many researchers have reported on the use of fats, oils and greases, and algae as sludge co-substrate.

When single feedstock is used for the production of biogas, there is low biogas yield either due to lower degradability or high content of protein or presence and formation of inhibitory compounds. To overcome the problem of co-digestion of different feedstock/substrates through optimizing C/N ratio, inhibitor concentration, biodegradability, and pulp density can be considered. At optimized conditions, there is a lesser formation of ammonia and inhibition due to a higher concentration of ammonia. Zeshan and Visvanathan (2012) reported that maintaining C/N ratio of co-digested feedstocks to 32 leads to depletion in ammonia concentration by 30%. Several studies have reported the effect of mixing different feedstocks on biogas yield. It is observed that there is a significant increase in biogas yield by 43% due to the synergetic effects of mixing agro-industrial residue or fruit and vegetable waste, cattle manure, or slaughter waste (Díaz et al. 2011). Callaghan et al. (2020) observed enhancement in CH_4 yields with the co-digestion of fruit and vegetable waste and cattle manure in a ratio of 1:1. The co-digestion method is very much suitable to augment biogas production from 25 to 400% in comparison to single substrate anaerobic digestion using the same feedstocks (Cavinato et al. 2010; Shah et al. 2015). Several studies found that methane yield will increase both the quantity and quality of methane. Pretreatment and co-digestion of the solid substrate are suitable techniques to enhance biogas yield quantity and quality. However, it is required to understand the interaction mechanism of different substrates and microbe substrates and optimization of C/N ratio. Nasr et al. studied the efficiency of single-stage and two-stage digestion systems and found that an 18.5% higher energy yield was achieved through two-stage systems (Nasr et al. 2012). Thus, given the variety of wastewater and solid wastes that can be used as a feedstock for biogas production, it not only addresses the issue of bioenergy production but at the same time it provides a better option to tackle wastewater management.

8.6.3 Hydrogen Production

Several processes have been successful to produce hydrogen industrially. Reformation of steam and combining natural gas with high-temperature steam accounts for the majority of hydrogen production. Other accepted methods include dark fermentation of cheap substrates, biophotolysis, or electrolysis of water (Benemann 1998). The biological production pathway that is put into action for hydrogen production includes dark fermentation, photofermentation, and biophotolysis.

The feedstock for hydrogen production ranges from lignocellulosic materials to wastewater generated from a variety of sources. Organic waste provides both environmentally and economically viable results for producing hydrogen fuel (Ni et al. 2006). The feedstock determines the overall cost of the final product to a large extent. The studies by Das et al. (2015) conclusively proved and emphasized how the processing cost of the initial feedstock for hydrogen production is enormously high, thus hugely influencing the production of hydrogen as a biofuel. There is an immediate need for low-cost methods of planting, collecting, transporting, and pre-processing feedstock.

There are a wide variety of wastewater feedstocks that have been widely reported to produce hydrogen by biological processes. The categories usually consist of sugar-rich wastewater (sugars as glucose, sucrose, and other carbohydrates), protein-rich wastewater (protein and lactose sugars), toxic wastewater (inhibitory compounds), and industrial effluents (wastewater discharged from industrial setups (Kumar et al. 2017)).

The dark fermentation method is predominantly utilized for hydrogen production from wastewater. This method is largely influenced by a variety of factors including complex processes wherein the inoculum condition like pretreatment and enrichment of substrate and substrate types, along with environmental parameters of pH, temperature, and substrate concentration, regulates the metabolic pathway of hydrogen-producing bacteria (Ghimire et al. 2015). Dark fermentation is a process that works under the principle of the acidogenic stage of the anaerobic digestion process to produce biohydrogen in the absence of light. It is the fermentation of the substrate by hydrogen-producing bacteria (HPB). HPB like *Thermoanaerobacterium* spp., *Bacillus* spp., etc. are responsible for producing hydrogen by fermenting the substrate. [NiFe]-hydrogenase and [FeFe]-hydrogenase are generally involved in the process of H_2 formation (Sarangi and Nanda 2020).

The first step is that of pretreatment followed by fermentation and separation. The pretreatment step is to enrich hydrogen-producing bacteria in the bioreactor. Thus, the inoculum needs to be pretreated before the process is continued further to reach the fermentation stage. There are four types of pretreatment: heat-shock pretreatment, acid pretreatment, alkaline pretreatment, and repeated-aeration pretreatment. It is the appropriate condition of acid and heat-shock pretreatment that ensures the best yield of hydrogen from wastewater (Oh et al. 2003).

The next step is fermentation which is carried out by a spectrum of hydrogen-producing microorganisms. It has been widely seen that both mixed and pure

cultures efficiently cause the fermentation of wastewater to produce hydrogen. Pure cultures of *Clostridium* species, such as *Clostridium butyricum*, *Clostridium acetobutylicum*, and *Clostridium acetobutyricum*, are effective in primarily sugar-rich wastewater (Chong et al. 2009). As wastewater usually has an existing population of several microorganisms, a competition for carbon sources or inhibition of hydrogen-producing microorganisms might occur. At this outset, it is the mixed cultures that have an edge when it comes to the fermentation of wastewater. Several types of research have provided conclusive results of efficient hydrogen yield attained by fermentation with mixed cultures (Hsiao et al. 2009; Vatsala et al. 2008; Yokoi et al. 1998).

Using beverage industry wastewater, Sivagurunathan et al. (2014) were able to report peak hydrogen production rate (HPR) at 2250 mL/L/day. Statistically optimizing the variables of pH and substrate concentration, 3096 mL/L-day HPR was achieved as well, which hints towards the economic feasibility of the process. The authors could successfully report the usage of facultative anaerobes belonging to Enterobacteriaceae, namely, *E. coli* and *E. cloacae*, to bioaugement hydrogen production. Mixed cultures of *E. coli* XL1-Blue and *E. cloacae* DSM 16657 were mixed and found to provide the best results achieved at 2.25 L/L/day as HPR as peak production performances. Krishnan et al. (2016) with 75 kg COD m^3 day^{-1} organic loading rate and a hydraulic retention time of 2 days conclusively reported a 15 L H_2/$kgCOD^{-1}$ hydrogen yield from palm oil mill industrial wastewater. *Thermoanaerobacterium* species population was found to be responsible for the amount of hydrogen produced.

It has been observed that biohydrogen production was reported over a range of factors, namely, substrate concentration of 0.25–160 g COD/L, pH 4–8, temperature 23–60 °C, and HRT 0.5–72 h. This was observed and contrasted with various types of reactor configurations as well. Finally, one of the most efficient hydrogen productions has been reported at an organic loading rate (OLR) 320 g COD/L/day, substrate concentration 40 g COD/L, HRT 3 h, pH 5.5–6.0, and temperature 35 °C in a continuously stirred tank reactor system which used wastewater with condensed molasses fermentation (Lin et al. 2012).

Household solid waste is rich in carbohydrates and has been lately reported to be an efficient feedstock for hydrogen production. A study by Okamoto et al. (2000), which comprised of seven varieties of typical municipal organic solid waste including rice, cabbage, carrot, egg, lean meat, fat, and chicken skin, reported carbohydrate content to produce maximum hydrogen. Zahedi et al. (2013) studied hydrogen production from municipal solid waste under the influence of thermophilic acidogenic conditions. A variety of conditions were observed to enumerate a list of conclusions which included nine different organic loading rates (OLRs) (from 9 to 220 g TVS/L/day) and hydraulic retention times (HRTs) (from 10 d to 0.25 d). The maximum hydrogen content was 57% (v/v) at an OLR of 110 g TVS/L/day (HRT = 0.5 d). Several other researchers have proven the efficiency of solid wastes for hydrogen production (Liu et al. 2006; Han et al. 2005; Mohan and Sarkar 2017).

8.6.4 Microbial Fuel Cells from Wastewater and Solid Waste

Microbial fuel cells (MFCs) are a certain type of treatment device which uses several microorganisms, essentially bacteria, to catalyze bioelectricity generation from diverse organic wastes (Jia et al. 2013). Exoelectrogenic bacteria, either in pure or mixed cultures, degrade the organic matter present in the wastewater or solid waste and transfer the electrons to the anode, thus generating electricity in MFCs (Logan 2009; Logan et al. 2006). Even though several substrates can be utilized in MFCs for electricity generation, it is the organic fraction of municipal solid waste which has the least fraction of lignin (<2.4%), thus making it a good option for feed for electricity generation (Maroun and El Fadel 2007). More than 60% of the wastewater generated in developing countries constitutes an organic fraction of municipal solid waste and hence is a valuable source of feed for MFCs (El-Chakhtoura et al. 2014).

An ideal MFC has two (anode and cathode) separate chambers made up of different materials like glass or polycarbonate. Each chamber has its respective electrodes essentially of graphite, carbon paper, carbon cloth, etc. It is the anodic chamber that is filled with an organic substrate like wastewater or solid waste and is further metabolized by the microorganisms present, generating both electrons and protons. The cathodic chamber is filled with a high potential electron acceptor which thus closes the circuit and successfully generates electricity. Ideally, the substance in the cathodic chamber should be of the nature that does not interfere with the microorganisms and is also at the same time least toxic. Oxygen has been predominantly used as an electron acceptor in most MFCs.

Usually, mixed cultures of microorganisms are used in MFCs for anaerobic digestion of the chosen substrate, thus generating electricity efficiently. MFCs essentially exploit the metabolic potential of microbes for converting organic substrates to electricity by transferring electrons from within the cell to the circuit. Karluvali et al. (2015) performed experiments with an MFC reactor construction which consisted of a cylindrical glass tube (4 cm diameter, 25 cm height) and two tubular electrode assemblies. An organic fraction of municipal solid waste (OFMSW) obtained from a recovery facility was used as a substrate. A maximum current density of 355.4 mA/m^2 was obtained after subsequent optimizations of several factors like temperature. The spectrum of microorganisms responsible was studied and reported as *Geobacter*, which played an important role in transferring electrons to the electrodes, and *Bacteroides* and *Clostridium*, which contribute to fermentation.

In another such study by El-Chakhtoura et al. (2014), electricity generation by OFMSW-fed air-cathode MFCs inoculated with wastewater sludge or cattle manure was tested, and 116 ± 29 mW m^{-2} power density was reported in wastewater-seeded MFCs. Microbial community analysis showed that the dominance of the phylum Firmicutes was at the anode; thus, the role played by the members of this phylum in electricity generation was huge. OFSMW was obtained from households. For the enrichment phase, glucose was used as a substrate for a while. Subsequently, MFCs were inoculated with wastewater and manure. Along the processing line,

several optimization techniques were made in batch fermentation and the MFCs were run in fed-batch mode at room temperature: 25 ± 1 °C. Acidogenic fermentation of organic MSW and the bioelectricity production potential were studied by Cavdar et al. (2011); 2 kg of MSW underwent acidogenic fermentation in a 6 L anaerobic leach-bed reactor under mesophilic conditions (30 °C). A maximum power density up to 8.6 W/m^3 was witnessed along with an exponential increase in coulombic efficiency from 6 to 22% obtained by a series of optimization experiments.

8.7 Wastewater and Solid Waste as Feedstock for Third-Generation Biofuel (Biodiesel)

The third-generation biofuel has emerged as an alternative technology comprising microalgae and seaweeds for biofuel production. Macroalgae and seaweeds are extensively used for wastewater treatment as they grow easily on contaminated and brackish water but their application in biodiesel production is not feasible due to very low lipid content. They can be utilized in anaerobic digesters, thermal liquefaction, and pyrolysis for biogas and biofuel production, but yield and cost-wise the technique has less acceptance. Oleaginous microorganisms carrying lipids and fats more than 20% are the most focused bioresources for biodiesel production (Vasistha et al. 2021).

Scientists are exploring the seaweed biorefinery technology where the biomass cultivated on solid and liquid wastes helps in nutrient removal and the potential biomass could be used for multiple product generation (Michalak 2018). Microalgae grow faster than other plants and do not require an arable or large land area to grow. Hence, as a result of this, there is no competition with the agricultural industry, human residential land, or animal farms (Chew et al. 2018). Numerous researches have been conducted to explore the application of microalgae in the biotechnology sector; carbon consumption is one of them. Microalgae aid in the removal of 40% of total CO_2 produced globally (Pierobon et al. 2018). Microalgae store high cellular lipids, generally ranging from 10 to 50% of dry cell weight (Wu et al. 2014; Sun et al. 2018); but in some genera, such as *Botryococcu*s and *Dunaliella* sp. the lipid content can reach 60–90% of dry weight (Metting 1996; Salbitani et al. 2019). Their fast growth rate, high carbon consumption, and large oil content make these microorganisms better than terrestrial oil crops, and their cultivation does not compete for resources used in conventional agriculture (Katayama et al. 2020).

Microalgae cultivation, on the other hand, necessitates a lot of freshwater and nutrients, which can drive up the cost of production; therefore, it's critical to create a low-cost alternative media for microalgae cultivation. One option is to use food waste and wastewater as the potential cultivation medium, as they are rich in many nutrients which the microalgae can utilize for growth (Wang et al. 2016a, b). In an integrated biorefinery model, combining different types of liquid and solid wastes

serviced the dual function of waste management and microalgal biomass generation for biofuel. Phycoremediation technology uses microalgae or macroalgae as a potential feedstock for harmful chemicals and pollutant removal from wastes along with the production of improved biomass (Emparan et al. 2019). Algae are capable of consuming organic and inorganic carbon, nitrogen, sulfur, phosphorus, and other heavy metals from the solid or liquid wastes (Mohsenpour et al. 2021). This strategy has the potential to be cost-effective since it replaces valuable freshwater and expensive synthetic nutrients in growing media with low-cost industrial and municipal wastes (Karemore and Sen 2015).

Open pond systems (OPs) and closed photobioreactors (PBRs) are the two types of systems used to treat municipal, industrial, household, agro-industrial, and other forms of wastewater using microalgal cultivation (Xiaogang et al. 2020). Raceway ponds have a theoretical production potential of roughly 60 g/m^2/day, but in practice, 10–20 g/m^2/day productivity is difficult to achieve (Pires, 2015). MaB-floc sequencing batch reactors were commercialized to a 12-m^3 outdoor raceway pond in an aquaculture wastewater treatment facility in Northwest Europe. Scaling up, on the other hand, lowered nutrient removal potential (1–3 times) and volumetric biomass output (10–13 times) (Van Den Hende et al. 2014). Process control is difficult with OPs, which have a significant risk of contamination, high evaporation losses, high CO^2 losses, and low biomass productivity. PBRs provide a solution to the shortcomings of OPs, such as ease of maintaining operational parameters, contamination prevention, lower initial investment and ongoing costs, and lower energy consumption (Xiaogang et al. 2020). Nevertheless, PBRs eliminate the possibility of evaporation and contamination while achieving excellent biomass productivity. PBRs, on the other hand, necessitate a higher initial investment, problematic scaling, and huge shear stresses. It's worth investing in the development of economically efficient PBR designs with better operations because of high biomass production and regulated limiting variables in PBRs (Dahmani et al. 2016). Even after extensive research, a full-proof PBR design for large-scale microalgal culture is still required.

8.8 The Economics of Biofuels

Biofuels are energy requisite of the current time and are of considerable calorific value and economic benefits. Bioethanol finds potential applications and contributes majorly to the Government of India's mission on its blending with petrol. Production of bioethanol takes place by fermentation of sugars present in wheat, sugarcane, and corns and the process includes fermentation of sugar, distillation, dehydration, and drying. It can be used as a replacement for gasoline and mixing with other fuel increases its efficiency. With globalization and increased environmental issues, the use of bioethanol has increased worldwide. Its viability indicates that it has all potential to replace conventional sources of energy like petrol and replacement leads to sustainable development and conservation of fossil fuels. Brazil and the USA are pioneers in the bioethanol industry with advanced technologies implied for

production. Over the years this industry has also shown tremendous growth in Asia and Europe. Despite worldwide keen interest in the development of sustainable and alternate energy, only a few countries, namely, Brazil, the USA, Germany, France, etc. are important contributors at the international level, wherein Brazil and the USA together account for 99% of global ethanol production, and on the other hand Germany and France contributed 69% of global biodiesel production.

The characteristics of fuel make it a suitable option that leads to the control of pollution with high-performance capacity. To achieve growth and use of fuel, several countries developed diverse policies that aim to develop a domestic ethanol program that promotes the use of bioethanol in numerous industries. Ethanol fuels are very common and used in blended form to control pollution and increase the efficiency of gasoline. The future of bioethanol appears to be bright as countries around the globe shifted their technology to bioethanol and begin the production of fuel (Akbar et al. 2019). The major requirement of the present time is to achieve bioenergy security by creating an enterprise in biotechnology that is equipped with viable green and clean technology. Overload on fossil fuels and its crisis thereof has forced the search for an alternative fuel, of which biofuel is intended to be a major contributor. Worldwide bioenergy has drawn attention as a sustainable energy source for coping with rising energy prices, energy demand, and concern over global warming and domestic energy security. It is believed that with proper technology development,the rich agriculture of developing countries with tropical climates may be potential second-generation ethanol producers. The first significant large-scale push for the production and use of biofuels occurred in Brazil and the USA, as a response to the 1973 oil export embargo imposed by the Arab members of OPEC (Organization of the Petroleum Exporting Countries) against Japan, the USA, and Western European countries.

India is the world's fifth-largest energy consumer and is believed to become the third-largest consumer by 2030, overtaking Japan and Russia, and holds only 0.4% of the world's proven oil reserves, is projected to run out of coal in another 40 years, and has limited domestic natural gas reserves. The Indian policymakers' approach to biofuel is the use of non-food feedstocks to be raised on degraded or wastelands that are not suited to agriculture, thus avoiding a possible conflict of fuel vs. food security. An analytical target of 20% blending of bioethanol and biodiesel by 2020 and further mandates have also been proposed. The current need is to develop advanced sustainable technologies based on renewable resources for enhancing biofuel production.

Each country has its trade regulations that increase the cost of the product. To promote green technologies in different countries and promote the use of bioethanol and its related technologies, countries loosen their trade barriers that allow the feasible exchange of technology in developing countries. Developing countries face the issues of cost while establishing a plant for biofuel production. By means of trading with developed countries, developing countries tend to implement technology from developed countries which further favors growth here. Along with these, the spread of fuel trade leads to the transfer of various technologies that make the relation between different countries more cordial and robust. The development

and demand of bioethanol lead to the increased use of technology that attracts government and consumers to get involved in the use of bioethanol. For example, after seeing the interest and demand for green technology in the year 2003, Volkswagen released its first fuel flex vehicle capable of running on any fuel blended with bioethanol. Recent researchers have tried a way that leads to the cost-efficient production of fuel; the use of waste crop materials like corn stalk, sugarcane leaves, and wheat husk leads to the substitution of main crops and produces fuel from waste that leads to the reduction in the manufacturing cost and makes the fuel more affordable in developing countries dealing with environmental issues; bioethanol is the future as it provides a way towards sustainable growth and promotes the conservation of fossil fuels. With development, the global issues also get increased that involve pollution and depletion of reservoir resources (VanithaKani et al. 2019; Rastogi and Shrivastava 2020). The use of bioethanol provides a solution for the global energy crisis and leads to the road of development that makes economic and ecological growth.

Microalgae require plenty of nutrients for appropriate growth and biomass production. Hence, suitable media is required which further adds to the cost of biodiesel production, inhibiting the commercialization of the energy (Maizatul et al. 2017). Therefore, by cultivating it in wastewater, microalgae could use the available organic carbon and absorb the N and P components, encouraging its growth and maintaining energy metabolism. The environmental benefit of cultivating the microalgae in wastewater is the reduction of the pollutants as they are usually released improperly into the environment. In a study by Molinos-Senante et al. (2010), the elimination of N and P from wastewater by the treatment system would increase the economic feasibility advantages by 8.06 and 30.94 €/kg, respectively (Molinos-Senante et al., 2010). Additionally, algae aided treatment of 1 m^3 of wastewater per day, yielding profit values of 250 and 235 US$/year, respectively, for the ST-N&P and ST-N cultures (Ansari et al. 2021). It is estimated that the production cost of third-generation biodiesel derived from microalgae depends on the cost involved in cultivation, harvesting, dewatering, and drying processes. The highest cost of biodiesel production from algae was observed in bubble column PBR that is about $125.08/L. On the other hand, the tubular PBR system shows the lowest production costs mainly due to the high productivity of biomass that leads to a reduction in the cost of feedstock. The minimum culture cost expenditure was estimated for the open pond system but this system requires a high supply of water added to the operational cost of the system (Aron et al. 2020). Hence, it is concluded that with the increase of world population, waste generation, water demand, and energy crisis, there is a need to develop an integrated solid or liquid waste treatment system with improved algae biomass production in third-generation biofuel. Waste utilization along with potential algae biomass production is one of the best ways to fulfill the future energy supply globally.

8.9 Future Prospects and Challenges

Rapidly increasing prices of crude oil led to the requirement for a cheap, alternative option that led to the same performance under budget. The production and utility of biofuel make it a cost-effective option that can be used as an eco-friendly vehicular fuel (Baig et al. 2019). The use of biofuels will help the country in many ways and also provides an additional benefit that involves control of gas emissions in the atmosphere as combustion of crude oil leads to the release of various harmful gases and black soot that make the environment hazardous.

The scope of converting the content of solid waste and wastewater into enhanced sources for energy production has huge potential. Despite that, due to the immature level of technical expertise present in today's date, there are certain challenges associated with the same which cannot be overlooked. In developing and underdeveloped countries, proper solid waste management is not available which leaves an impact on the poor people of society and also leads to a mountain of waste dumped in different areas. A huge amount of various forms of solid organic waste, i.e., municipal solid waste, agricultural waste, forestry waste, and industrial waste, is generated from different sources in developed and developing countries. All forms of organic wastes are managed by landfilling, burning, and gasification to either dump or generate heat which causes severe pollution and greenhouse gas emission in developing and developed countries for a long period of time.

The collection and transportation of municipal solid waste from every house is a critical component in municipal solid waste management in using feedstock for sustainable biofuel production. Therefore, it is essential to develop a proper management system for the collection, segregation, and transportation of municipal solid waste from each door and supply it to the location of biofuel plants efficiently. It has been reported that the increasing trends of municipal solid waste generation and waste biomass-derived cellulosic materials have the potential to produce ethanol potentials due to socioeconomic development across 173 countries. It has been observed globally that up to 82.9 billion liters of wastepaper-derived cellulosic ethanol can be generated worldwide. It has substituted for 5.36% of gasoline consumption, with accompanying GHG emission savings of between 29.2 and 86.1%. The biorefinery approach for the conversion of municipal solid to energy will make the process sustainable and cost-effective. There is huge scope to supply waste as sustainable feedstock directly or after their pretreatment to produce second-generation biofuel, i.e., bioethanol, biobutanol, biogas, and electricity.

The use of bioethanol leads to the process towards carbon neutrality. The carbon-neutral process means that carbon released during fuel combustion gets reabsorbed in and gets balanced by plants and supports plant growth. It is the same as the recycling process that returns the amount of carbon to the plants. Promoting a carbon-neutral process leads to an increased level of carbon and other greenhouse gases that make global warming and lead to ozone depletion. For a sustainable growth and route of development, bioethanol will make a country able to recover their economy and provide trade opportunities and options for technology transfer

and thus provides an immense option for conversion of numerous wastes to biofuel. The implementation and establishment of bioethanol technology will also lead to a solution for agricultural waste and promotes the use of green technology through eco-friendly approach.

A large amount of renewable feedstock in the form of animal manure, crop residues, and organic food municipal solid wastes (OFMSWs) generated from different sources and locations in developing countries is employed to produce biogas using successful technologies at cost-effective manner. Anaerobic digestion of animal manure and other biogenic wastes has a positive impact for the society at large besides contributing to biogas production. These benefits are enhancement in fertilizer value of the digestate, inactivation of pathogens, and reduction of problem to some extent.

Biogas is a clean renewable fuel that is employed for multiple end applications. Development of integrated plants for biogas production together with other objectives such as waste management, fertilizer production, and clean energy production through dry anaerobic digestion is an ideal idea for high-cost benefits and revenue. The widespread application of biogas technology in developing nations has tremendous potential in reducing GHG emissions, thereby creating new possibilities for carbon trading in the global market. The revenue generated through carbon trading could be deployed for further research, development, and dissemination of biogas technologies domestically.

After the establishment of drawbacks of chemical and thermochemical processes, scientists have been eager to research the biocatalysis approach. Following this, whole-cell biocatalysis is an emerging trend that holds the potential to revolutionize the large-scale production of biofuels. Not only does whole-cell biocatalysis provide a cheaper alternative to isolated, pure enzymes but also has an additional advantage of inherent stability due to the outer cell structure. Even though there are a few disadvantages associated with microbial whole-cell catalysts like mass transfer limitation, these can be overcome by engineering tools to modify outer cell structure and other permeabilization treatments. Whole-cell catalysts are widely accepted as an alternative to the most prominent enzymatic pathways. Several researchers now have readily studied and proposed solutions to tackle challenges to take this field to the next level of industrial application. Microbial whole-cell catalysis, arrested at the resting stage or immobilized by efficient techniques, allows a continuous reaction mode which makes it unusually attractive as an alternative for commercial application. With further advancements made in the field of omic studies and bioengineering tools, a huge spectrum of microorganisms can be studied and applied with promising results at a large scale.

Biohydrogen production is the most challenging area with respect to environmental problems. Due to the energy potential of hydrogen, new processes are developed for sustainable hydrogen production using biological methods. This review emphasized the cost of raw materials as a major limitation for biohydrogen production, showing that the utilization of some carbohydrate-rich or starch-containing solid wastes or some industry wastewaters is an attractive approach for

biofuel production. The productivity and yield of hydrogen from any of the processes explained above was low for commercial application.

However, the third-generation fuels fit into achieving the sustainable development goals. Many studies have concluded that cultivating microalgae with a single goal in mind, namely, biodiesel production, is not a viable option because the expense of supplements and other materials is insufficient to make this biodiesel cost-effective and feasible in comparison to commercial biodiesel. As microalgae production necessitates a considerable amount of freshwater for dwelling, it may generate conflicts with human needs. Therefore, by cultivating the microalgae onto disparate waste effluents and solid waste, the cost of nutrient supply could be cut down, but along with that arises the associated challenges like:

- Other contributors to wastewater, such as high organic content pollutants and biological organisms, have a negative impact on microalgal growth.
- The heavy metals in the waste could lead to the generation of a high concentration of reactive oxygen species, which are detrimental for the cells.
- There is commercial inviability of the technology, due to the expensive pretreatment and downstream processes involved in obtaining the energy product.
- There is still a lack of scale-up studies to realize the practical success of the process.

Hence, future research in third-generation biofuel should have put more emphasis on the pilot and industrial-scale experiments involving solid and liquid wastes with techno-economic evaluation. An integrated model production line, from the processing of biowastes to the purification of biofuel, is proposed as a guide for future work towards a highly efficient production system. Microalgae biorefinery is also a future solution towards economic biofuel production on waste feedstock.

The United Nations (UN) Commission on Sustainable Development (CSD) has included basic energy services in the list of essential elements of sustainable development. Every country has to form a policy for basic energy services as per the UN. The development of a waste management system for collection and transportation of municipal waste and the segregation of organic biomass to employ as a sustainable feedstock for biogas production. Energy production is very well dependent and interrelates market demand, circular economy, employability, climate mitigation, health, improvement in quality of life. Given the possibility of sustainable supply of feedstock as all forms of unlimited waste biogas technology has the perfect potential to make every person, community, society, village, city, and country self-sustained with respect to all necessities required for a quality life.

New policies and subsidies by government and major investments by both private and public are needed to be displayed in the next decades to achieve the objectives, and all sectors (electricity, heat, transport, fertilizer) need to work together and subscribe to common sets of principles and practices. In this context, retail-based operation and subsidies are important tools, but the policy has been framed for allotment of subsidies towards heat. Renewable energies are given around 80% of

the subsidies to the power sector and only 1% to heat. Fertilizer is also not given any subsidy.

At present mature technology is not available to produce several forms of biofuel such as bioethanol, biobutanol, biodiesel, biogas, and biohydrogen and generate electricity with a high yield and fast rate from solid waste and wastewater. Lots of research are required to be carried out by both research institutions and industry on process optimization, the kinetics of the process, and bioreactor/biodigester design. Besides new strains of microorganism/novel enzymes have to be developed/synthesized according to the specific problems exploiting advanced metabolic and genetic engineering and technology for futuristic goals.

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Chapter 9
Agricultural Lignocellulosic Waste for Bioethanol Production

Deovrat Begde

Abstract The ever-increasing energy demands and rapidly depleting resources of fossil fuels have perplexed both the automobile and the petroleum industry. Global over-exploitation of such natural resources to meet the fuel demands has led to concerns regarding fuel price inflation and environmental pollution. Alternative fuel resources as the clean, safe and sustainable energy deliverables have been looked upon as the future of this industry. Yearlong cyclical production of enormous agricultural waste useful as a potential feedstock for biofuel/ethanol production has spurred a ray of hope through technological advancements in the fields of metabolic engineering, bioprocess technology and new age biorefinery models. Bioethanol production from agricultural waste essentially rich in lignocellulosic biomass (LB) presents an interesting multifaceted delivery system even for lignin valorization to obtain valuable phenolic co-products along with ethanol which is based on a next-generation zero-waste biorefinery concept. The chapter makes the reader dive deep into technological advancements in the field, providing a sufficient detail of steps involved in LB-based bioethanol production.

Keywords Valorization · Recalcitrance · Cellulase · Consolidated bioprocess · Biorefinery

9.1 Introduction

The dwindling natural resources of petroleum products and extensively time-consuming process of fossil fuel synthesis have been major points of concern to satisfy the exorbitant global energy demand. In 2019, 84% of total energy demand was met by conventional sources of energy, viz. coal, oil and gas, which essentially needs attention, not only because their natural reserves are scarce or rapidly depleting but also due to the negative impacts that their excess use has on climate (Luderer

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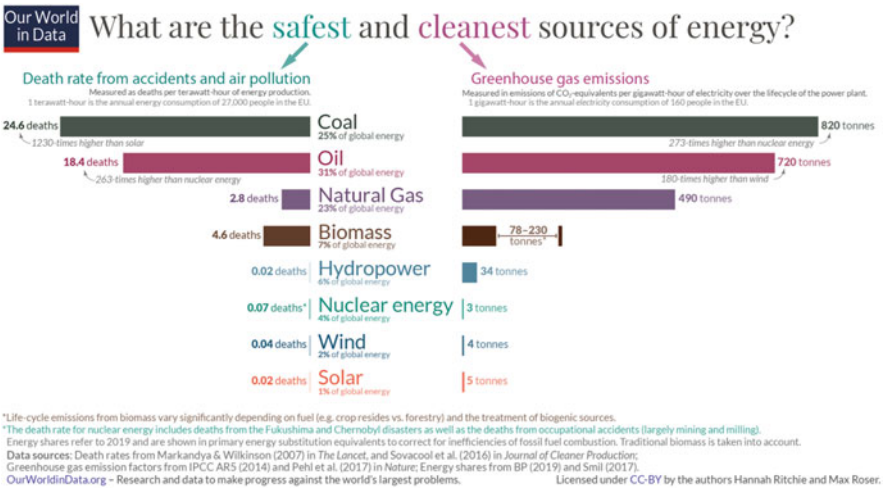

Fig. 9.1 Statistics on worldwide consumption of energy resources scaled on their safety aspects and environmental impact assessment updated as per the records till 2019

et al. 2019). All the conventional sources of energy produce carbon dioxide (CO_2) on combustion and also are the major contributors to air pollution, human health problems and even premature deaths (Fig. 9.1) (Lelieveld et al. 2019). In decreasing order coal, oil and natural gas combustion have been recorded to lead to health ailments and greenhouse gas emissions, respectively (Turconi et al. 2013; Pehl et al. 2017). With careful examination of the safety aspects and environmental impact of fuel sources, it appears that there is no substitute for nuclear and renewable sources of energy (Fig. 9.1). A contemplation of biomass as a safer alternative due to its balanced impact on climate and animal health can be considered at least till challenges associated with explicitly cleaner forms of energy do not get resolved (Sovacool et al. 2015; Sovacool et al. 2016).

Agricultural waste is generated cyclically throughout the year during the harvesting of agricultural products and processing of the crops. This may serve as a promising renewable source of biomass rich in lignocellulosic content that often gets neglected as a potential substrate for bioethanol production (Dawson and Boopathy 2007; Ravindranath et al. 2011). Biofuels such as bioethanol can substantially reduce the negative impact associated with petroleum products and perhaps may also counter economic burden due to the ever-increasing demand of conventional fuels (Su et al. 2020). Saccharification of lignocellulosic biomass (LB) preferentially obtained from agricultural feedstock can serve as an attractive alternative substrate for the production of second-generation bioethanol (Soccol et al. 2010). There has been some extensive research conducted in this direction to substantiate the claim that lignocellulose, if treated in a proper manner, can serve as a rewarding precursor for bioethanol production (Rezania et al. 2020; Sharma et al. 2020). This chapter gives a comprehensive mechanistic view of steps involved in bioethanol production, describing crucial steps involved in pretreatment of LB considering the conventional versus the advanced strategies, ways to harness the

agricultural feedstock to maximize bioethanol yields, management of agricultural wastes by biorefineries and overall environmental impact assessment of the entire process.

9.2 Chemistry of Lignocellulosic Biomass (LB): Challenges and Prospects

The production of bioethanol from plant waste rich in lignocellulose might sound appealing but has its own challenges. Lignocellulose molecule is itself a "tough nut to crack" and hence its natural degradation takes decades. Lignocellulose chemically is a complex mixture of polysaccharides (cellulose and hemicelluloses) and an aromatic polymer called lignin. The monomeric composition and structural details can be seen in Table 9.1. This combination of polymers makes LB extremely inaccessible for natural microbial enzymes to carry out its complete decomposition. One strategy proposed for its use in bioethanol production advocates some pretreatment mechanisms by which LB can be restructured for improving its amenability to enzymatic hydrolysis (Zhao et al. 2012a, b; Kumar and Sharma 2017). The major purpose of this restructuring is facilitation of conversion of LB polysaccharides into fermentable sugars. However, variables influencing LB recalcitrance are firmly interconnected and hard to separate (Zhao et al. 2012a, b; Bichot et al. 2018). The structural challenges include but are not limited to cellulose crystallinity, degree of polymerization, cellulose specific surface area and pore size and volume, whereas chemical factors are related to composition and content of lignin, hemicelluloses and acetyl groups in different sources of LB (Zoghlami and Paës 2019) (Fig. 9.2). Several studies present arguments on the strategies to enhance the susceptibility of different LB feedstocks to enzymatic hydrolysis, describing the impact of the aforesaid factors on the yield of fermentable sugars but often lead to conclusions with contradictory findings (Silva et al. 2012; Meng and Ragauskas 2014; Peciulyte et al. 2015; Vaidya et al. 2016; Kim et al. 2018; Kruyeniski et al. 2019; Yu et al. 2019). It thus becomes obligatory to extensively review the physical/structural as well as chemical attributes of lignocellulose as a biomolecule to understand the challenges associated with its potential utilization in bioethanol production.

9.2.1 Cellulose

The supramolecular assembly features of cellulose are very well characterized describing a proportion of crystalline and amorphous regions within the molecule. Hydrogen bonds are principally involved in holding the crystalline cellulose fibres together making them almost 3–30 times inaccessible to the enzymatic hydrolysis

Table 9.1 Biochemistry of lignocelluloses with description component molecular entities such as cellulose, hemicelluloses and lignin

	Monomeric units	Bonds between monomers	Degree of polymerization	Chemical composition	Polymeric units
Cellulose	D-Glucopyranose	***β***-1,4-Glycosidic bonds	100–10,000	Three-dimensional, linear molecule composed of the crystalline and amorphous region	***β***-Glucan
Hemicellulose	L-Arabinose, galactose, glucuronic acid, D-xylose, mannose	***β***-1,4-Glycosidic bonds in main chains; ***β***-1,2-, ***β***-1,3-, ***β***-1,6-glycosidic bonds in side chains	< 200	Three-dimensional, non-homogeneous molecule with small crystalline region	Polyxylose, galactoglucomannan (Gal-Glu-Man), glucomannan (Glu-Man)
Lignin	Guaiacylpropane (G), syringylpropane (S), P-hydroxyphenylpropane (H)	***β***-O-4 Ether bond	4000	Amorphous, non-homogeneous, non-linear three-dimensional polymer	G lignin, GS lignin, GSH lignin

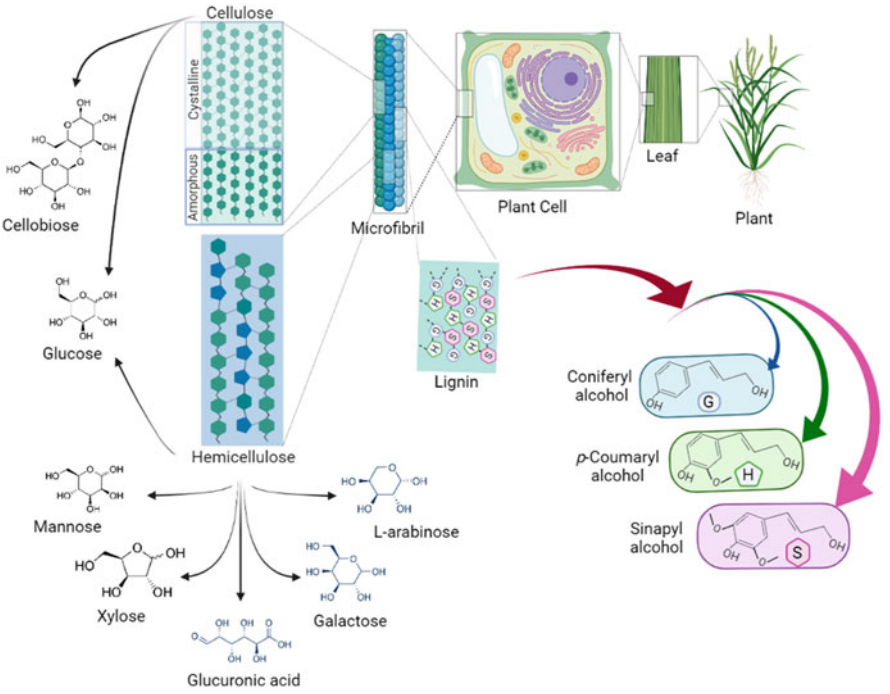

Fig. 9.2 Structural elucidation of organizational chemistry of lignocellulose in agricultural crop waste. The complex arrangement of fibrous cellulose microfibrils is intertwined with hemicellulose fibres and bonded with each other with lignin. The chemical composition of lignocellulose on complete hydrolysis of all these three fiberous biopolymeric components releases glucose preferentially from cellulose, a variety of pentose and hexose sugars from hemicelluloses and phenylpropanoid derivatives from lignins. The figure gives a summary of monomeric subunits of each of the polymers present in the lignocellulosic biomass

compared to those in the amorphous regions. This structural feature warrants for substantial impact of crystallinity on hydrolysis of cellulose, but the study results conducted in this direction appear contradictory and varying depending on LB source. Investigators document a negative correlation for enzymatic hydrolysis and crystallinity in the case of pretreated wheat straw (Pihlajaniemi et al. 2016), corn stover (Liu et al. 2015; Xu et al. 2019) and hybrid polar, switchgrass and bagasse (Chang and Holtzapple 2000). However, some studies argue that other physical features such as degree of polymerization (DP), i.e. the glucose content, pore volume, accessible surface area and particle size, are more limiting to hydrolysis than crystallinity (Mansfield et al. 1999; Ioelovich and Morag 2011; Aldaeus et al. 2015; Meng et al. 2016; Auxenfans et al. 2017a; Zhang et al. 2018). The major limitation of most of the studies conducted on cellulosic saccharification yield is essentially the use of pure cellulose which cannot account for natural LB cellulose, thereby explaining the discrepancy in the documented results. For example, reports on the influence of DP affecting enzymatic hydrolysis are limited and at the same

time contradictory. Although logically higher DP should be associated with greater saccharification yield just on the basis of glucose content, the data predicting its influence on enzyme action is obscure. Both positive and negative correlations of DP with the extent of enzymatic hydrolysis have been documented by different groups (Sinitsyn et al. 1991; Ioelovich and Morag 2011; Meng et al. 2016; Lu et al. 2019) and thus the area is still open for investigation.

The factors other than those inherent to cellulose (viz. crystallinity and DP) like pore volume, accessible surface area and particle size as listed above essentially rely on the mechanical or chemical preprocessing of LB and can profoundly affect enzymatic hydrolysis. Although technically pore size can be considered an inherent feature of cellulose fibres as it is dependent on length and extent of hydrogen bonding, dilute acid pretreatment has been predicted to influence the pore size and the enzyme access. In lieu of pretreatment it is found that only the pores larger than the size of the cellulase enzyme (around 5.1 nm) can allow enzyme access and thereby affect saccharification yield (Grethlein 1985). But as this cellulose feature can vary depending on the source of LB, even the dilute acid pretreatment strategies need to be optimized accordingly (Meng and Ragauskas 2014). There is also an argument that if the lignin content is less than 15%, pore size has negligible effect on cellulase activity (Vaidya et al. 2016). Despite this report being restricted only to pine LB, it certainly hints towards the influence of lignin content on pore size, thereby justifying the inclusion pore size into a feature external to cellulose structure (Kruyeniski et al. 2019). Other external factors such as accessible surface area and particle size essentially depend on the mechanical deconstruction of lignocellulose increasing the availability of this otherwise compact structure for enzymatic digestion. The process of milling and grinding has been predicted to significantly influence the enzyme activity upon cellulose and in segregation of lignin from intimate lignocelluloses (Pang et al. 2018; Yu et al. 2019). Ball milling has been found useful to reach smaller particle size and enhance the specific surface area of a variety of feedstocks and has been positively correlated with glucose yield (Pang et al. 2018).

9.2.2 Hemicelluloses

Higher content of glucose, as the naturally preferred carbon source for fermentation, and often cellulose, because of its higher content in lignocellulose, are the primary focus during the restructuring pretreatment of LB. However, another fairly abundant source of natural sugars is the heterogenous group of polysaccharides; accounting for almost 30% of LB are hemicelluloses (Chandel et al. 2018). Hemicelluloses are rich sources of heterogeneous monosaccharides, viz. of D-xylose, D-mannose, D-glucose or D-galactose and other glycosyls (McKendry 2002). The DP value of hemicelluloses is much lower in comparison to cellulose and generally in the range of 100–200 offering substantially low yields of glucose (Mota et al. 2018). But the most heartening aspect of hemicelluloses is that they are amorphous with low physical strength; thus, unlike cellulose, these are readily hydrolyzed by dilute

acids or bases, as well as specific hydrolyzing enzymes (Isikgor and Becer 2015). Therefore, LB pretreatment can easily remove hemicelluloses which often create a physical barrier for the cellulase access to cellulose (Auxenfans et al. 2017a; de Oliveira Santos et al. 2018; Herbaut et al. 2018). Extensive acetylation of hemicelluloses is the reason behind the effectiveness of this physical barrier. Acetylation not only fiddles with the cellulose hydrophilicity but also creates steric hindrance for the catalytic domain of cellulases for its action (Pan et al. 2006; Zhao et al. 2012a, b). Deacetylation pretreatment can substantially overcome this physical hindrance. Additional evidence suggests equal propensity of removal of hemicelluloses and lignins during mild acid or base or steam explosion pretreatment regimes essential for effective availability of cellulose for enzymatic digestion (Yoshida et al. 2008; Kumar and Wyman 2009; Leu and Zhu 2013; Lv et al. 2013). Having said that there are arguments about whether the removal of lignin or hemicelluloses is more effective for enzymatic hydrolysis of cellulose (Guo et al. 2014; Kruyeniski et al. 2019). Nevertheless, ease of hemicellulose degradation for saccharification certainly increases the hopes about decreasing the LB recalcitrance.

9.2.3 Lignin

Biopolymer which is most abundantly found in LB after cellulose is an extremely complex heteropolymer of phenylpropanoid monomers, called lignin (Agbor et al. 2011; Ragauskas et al. 2014). Although amorphous, its hydrophobicity and rigid structure glue the hemicellulose and cellulose fibres together in the cell wall. DP for lignin in terms of glucose content is zero. Also, its proportion in LB negatively correlates with accessibility of the enzymes for cellulose degradation (Meng et al. 2016). In addition to being a physical barrier to enzymes, it may also reversibly bind and inhibit enzyme activity through its hydrophobic structural features (Pihlajaniemi et al. 2016; Kruyeniski et al. 2019). The latter also depends on the structural composition and total lignin content of LB (Yang and Pan 2016; Yao et al. 2018b; Yu et al. 2019). Chemical blocking of the phenolic hydroxyl groups of phenylpropanoid monomers to hydroxypropylation derivatives has been found to significantly decrease the lignin's inhibitory effect (Yang and Pan 2016). This, it implies that the variability of lignin content is strongly correlated with LB recalcitrance and is one of the major limiting factors of saccharification of cellulose especially through enzymatic hydrolysis. With this understanding recently there have been much investigations and technological advances that can be employed to effectively predict the content of lignin and other limiting factors described above for optimizing the pretreatment strategies and to maximize the source-dependent saccharification yield from LB (Auxenfans et al. 2017a, b; Chabbert et al. 2018).

9.3 Available Techniques for Physicochemical Analysis

To gauge and predict the recalcitrance property of LB from different sources, the most reliable and cost-effective technique is the different forms of quantitative spectrophotometry. For instance, fast Fourier transform infrared (FT-IR) spectroscopy and Raman scattering spectroscopy are two techniques which complement each other to precisely predict the effectiveness of pretreatment strategies used (Hou and Li 2011; Sills and Gossett 2012; Lupoi et al. 2014; Bekiaris et al. 2015), whereas near-infrared (NIR) spectroscopy (Huang et al. 2012) and fluorescence spectroscopic methods are useful in quantifying the polymer composition of LB (Auxenfans et al. 2017b; Huang et al. 2017). Due to the rigid aromatic nature of lignins, they are inherently fluorescent, so are some of the monomeric components of hemicelluloses, viz. ferulic acid and cinnamic acids, which can be exploited effectively by fluorescence spectroscopic analysis to determine the content of these polymers in different LB sources (Auxenfans et al. 2017b). Furthermore, fluorescence lifetime (FL) and fluorescence recovery after photobleaching (FRAP) techniques can be used to rapidly determine or predict saccharification efficiency and LB accessibility, respectively (Chabbert et al. 2018; Herbaut et al. 2018). Finally, an indirect yet handy and comparatively simple method that can give a fair estimate of LB hydrolysis rate is the measurement of water retention value (WRV) and water contact angle value (WCAV) (Noori and Karimi 2016; Crowe et al. 2017; Williams et al. 2017; Paës et al. 2019). Whereas WRV is positively correlated with enzyme accessibility, WCAV is a predictor of lignin hydrophobicity in LB (Li et al. 2015). Thus, the choice and effectiveness of pretreatment strategy can be determined based on these simple and inexpensive methods. Additionally, investigators have also proposed some high-throughput methods like NMR, HPLC, GC-MS, SEM, TEM and atomic force microscopy, each with their specific advantages as well as limitations (Karimi and Taherzadeh 2016).

9.4 Bioethanol Production: An Overview

Bioethanol production has shown a gradual progress through the last few decades from the first generation of bioethanol principally produced from traditionally grown food crops (viz. corn, sugarcane, sugar beet, etc.) serving as a direct source of sugars for fermentation (Jobling 2004; Linoj et al. 2006; Cardona and Sánchez 2007), to the second generation depending essentially on non-edible renewable biomass, mostly the agriculture waste (Dawson and Boopathy 2007; Talebnia et al. 2010). The first-generation bioethanol production posed competitive pressure over food crop yield and their demand for consumption, thus warranting the evolution of the second generation of bioethanol production to avoid compromising the situation of food scarcity (Pimentel et al. 2008; Sims et al. 2008; Nonhebel 2012; Muktham et al. 2016). Whereas the first-generation strategies were much straightforward and

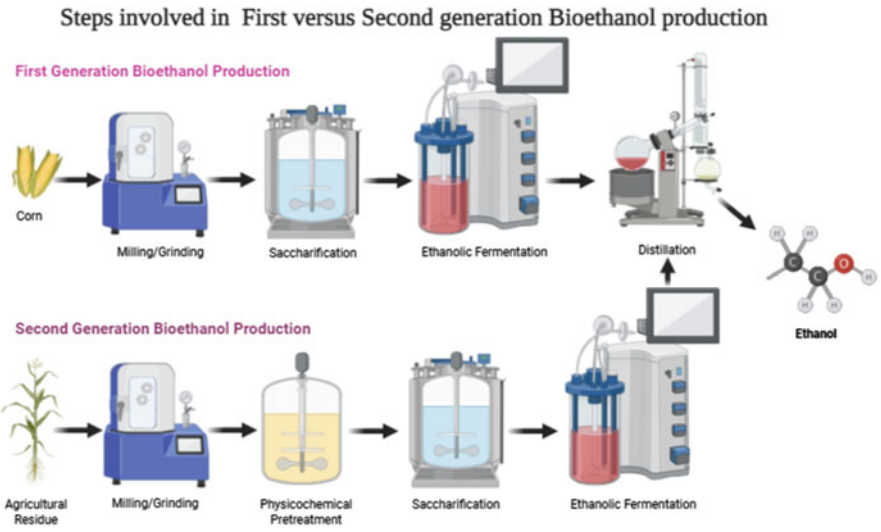

Fig. 9.3 A schematic depiction of the stepwise progression of raw material during first- and second-generation bioethanol production. The pretreatment of LB is the most essential component of second-generation bioethanol production and the efficiency of this step decides the cost-effectiveness of the ethanol produced

demanded minimal pretreatment, the raw material used in the second generation was more challenging in terms of saccharification for fermentation (Fig. 9.3) (Soccol et al. 2019). The pretreatment strategies needed more attention for obtaining substantial yield during second-generation ethanolic fermentation (Soccol et al. 2010). Essentially, both the fermentation strategies can be broadly divided into three steps: pretreatment, fermentation and distillation.

9.4.1 Pretreatment Process

The first-generation pretreatment process is extremely simple which involves washing, grinding, pressing and juice filtration that can be directly subjected to fermentation. A minor variation in this process includes enzymatic/chemical hydrolysis of starchy raw material for saccharification followed by fermentation (Lima and Marcondes 2002). Essentially the plant material which is discarded as the non-fermentable waste material during first-generation bioethanol synthesis is used in the second generation. More precisely non-conventional and extremely difficult to hydrolyze LB from agricultural waste is the principal material of choice in the second-generation fermentation process (Sun and Cheng 2002). The choice of the pretreatment process is therefore of utmost importance here. Broadly, the pretreatment strategies include mechanical, physicochemical and enzymatic pretreatment processes (Cheng and Timilsina 2011). The chemical composition of

the agricultural residue decides the proportion of utility of each of these processes during the pretreatment plan (Wi et al. 2015; Chen et al. 2017). The overall aim of this step is to enhance the amenability of LB for improved sugar yield, making it ready for microbial assimilation during the fermentation process. Let us summarize briefly each of these pretreatment processes.

9.4.1.1 Mechanical Processing

The aim of this process is to increase the surface area with particle size optimization depending on the agricultural raw material being processed. This can be achieved routinely through milling, grinding or chipping. Although least particle size increases the surface area, the attempt to achieve minimal particle size often requires high energy input during milling which is reported to have a negative influence on downstream pretreatment processes at least in the case of wheat straw (Cadoche and López 1989; Talebnia et al. 2010). Also, it may require heavy-duty expensive machinery to bear the load. Therefore, mechanical processing must operate on the principle of minimal energy input to attain optimal particle size for downstream processes.

9.4.1.2 Physicochemical Pretreatment

The fundamental objective of this step is to cost-effectively improve the availability of the polymers for their enhanced biodegradability (Sun and Cheng 2002; Fernandes et al. 2009). The process attempts to minimize the negative aspects of complex polymers in terms of their availability for saccharification. This requires extensive optimization of each step depending on the variety of the feedstock.

Steam Explosion

Grounded LB is often subjected to high-pressure (0.69–4.83 MPa) saturated steam treatment at temperatures ranging from 160 to 260 °C for some time followed by an immediate drop in pressure. Such treatment leads to explosive decompression of the material, transformation of lignin and hemicellulose degradation and facilitates exposure of cellulose for subsequent hydrolysis (Rabemanolontsoa and Saka 2016). The parameters of the process like presoaking time, inclusion of acid or alkali as catalyst and their concentration and material exposure duration and temperature can be optimized to improve the effect of this process (Öhgren et al. 2007). Other variants of this method like ammonia fibre explosion, ammonia recycle percolation and carbon dioxide explosive process are also popular and are being explored extensively (Hu and Ragauskas 2012).

Hydrothermal Pretreatment

This process also sometimes referred to as vapocracking can be considered as a variant of steam explosion technique where the material is treated with liquid water heated under isochoric conditions (Cybulska et al. 2019). Water is believed to acquire some enhanced physical and chemical properties including autocatalysis leading to biomass hydrolysis and improved availability of digestible cellulose without any chemical treatment. Chemically this process triggers an interesting cascade of reactions that engages hydronium ions in hydrolysis of glycosidic linkages as well as acetyl groups (Carvalheiro et al. 2008; Carvalheiro et al. 2009). The acetic acid thus formed further catalyzes the hydrolysis of hemicellulose improving the accessibility to cellulose (Carvalheiro et al. 2008). Lignins are also found to get hydrolyzed in a similar manner but phenolic monomers may repolymerize to a certain extent and deposit back on cellulose called pseudolignin (Negro et al. 2003). Nevertheless, generation of accessible cellulose with minimal crystallinity, as achieved in this process, is exceptional and has been validated over and over again (Cybulska et al. 2019).

Acid and Alkaline Hydrolysis

Both dilute and concentrated acids are routinely used in the pretreatment of LB and this process is known to enhance the degradation of hemicellulose, lignin-polysaccharide disruption and amorphous cellulose conversion. The main parameters that influence acid pretreatment are solid loading, acid concentration, temperature and residence time. Dilute acid treatment often accompanies high-temperature conditions to enhance the pretreatment outcome (Shi et al. 2011). Phosphoric acid, acetic acid and sulphuric acid commonly used during acid pretreatment may lead to inhibitor accumulation affecting downstream treatment and it often involves high cost of acid recovery (Taherzadeh and Karimi 2007; Sannigrahi et al. 2011). As opposed to acid pretreatment alkaline pretreatment is considerably reliable and less toxic for downstream enzymatic hydrolysis of LB. The most commonly done sodium hydroxide pretreatment is documented to selectively remove lignins without compromising sugar and carbohydrate loss, increasing surface area and porosity, and thus is the most suitable for enzymatic hydrolysis (Chen et al. 2013; Kim et al. 2016). Delignification of LB due to alkyl-aryl linkage degradation of lignin readily happens under alkaline conditions, thereby enhancing the availability of cellulose for enzymatic saccharification (Rastogi and Shrivastava 2017). The only disadvantage of this method is the extended reaction time that can vary from several hours to days depending on LB chemical complexity (Bali et al. 2015).

Green Solvent Versus Organic Solvents

Ionic liquids or large cationic organic salts with small anions usually liquid at room temperature are considered green solvents due to their chemical properties and recyclability (Rabemanolontsoa and Saka 2016; Rastogi and Shrivastava 2017). Some examples of these include imidazolium-based ($[(C3N2)Xn]^+$), pyridinium-based ($[(C5N)Xn]^+$), pyrrolidinium-based ($[(C4N)Xn]^+$), ammonium-based ($[NX4]^+$), phosphonium-based ($[PX4]^+$) and sulfonium-based ($[SX3]^+$) solvents (Zhang et al. 2017). These are proven to be most effective in delignification and lignin extraction to impart cellulase stability and maintain its activity. Also, these solubilize LB the best with increasing sugar and carbohydrate yield (Elgharbawy et al. 2016). No matter how attractive these may appear the high cost and extensive energy input required for their recycling have limited their use in pretreatment plants (Zavrel et al. 2009). Furthermore, there are debates about their toxicity and biodegradation potential (Rabemanolontsoa and Saka 2016). Organic solvents on the other hand serve as attractive alternatives due to their cost-effective recovery; best fractionation potential for obtaining high-purity cellulose, hemicellulose and lignins; and production of high-value co-products during LB pretreatment (Zhao et al. 2009; Zhang et al. 2016). A variety of organic solvents ranging from alcohols to dioxane and amines are used for this purpose with or without catalysts (Shuai and Luterbacher 2016). Alcohols are the most cost-effective of all with the best recovery potential (Rezania et al. 2020).

Enzymatic Hydrolysis

Pretreated LB with increased availability of cellulose – the principal carbohydrate required for saccharification – is subjected to enzymatic hydrolysis to produce sugars required for bacteria or fungal ethanolic fermentation. Cellulases are the principal class of enzymes involved in performing hydrolysis of cellulose in a multi-step manner in a heterogeneous environment. Initially cellulose at the solid-liquid interface is digested by the synergistic action of endoglucanases (EC 3.2.1.4) and exoglucanases/cellobiohydrolases (EC 3.2.1.91). Thus, the generated soluble cellulo-oligosaccharides and cellobiose units are then acted upon by β-glucosidase (EC 3.2.1.21) to form β-D-glucopyranose sugar residues for fermentation (Andrić et al. 2010). This is a much cost-effective method for saccharification without any requirement of solvent recovery or toxicity reduction. The process principally involves three steps: association of cellulases with cellulose, cellulose hydrolysis and cellulase release. The first step of cellulase association with its substrate cellulose is a rate-limiting step governed by the complexity of LB (lignin and hemicellulose content) and percentage of amorphous versus crystalline cellulose in biomass (Han and Chen 2010).

9.5 Enzymes and Microbes for Second-Generation Bioethanol Production

Complete enzymatic hydrolysis of cellulose to glucose is carried out by a group of enzymes (viz. endo- and exoglucanases and β-glucosidase) collectively called as cellulases. The entire process of enzymatic digestion of cellulose experiences a strong inhibition by accumulation of cellobiose, an intermediate product of this catalytic pathway (Zhang et al. 2006). Additionally, LB characteristics such as lignin and hemicellulose content and cellulose structural attributes are limiting factors affecting the activity of cellulases (Kuila et al. 2016). It is often observed that researchers have paid more attention to the investigation on the biological attributes of cellulases in which hemicellulose and lignin-degrading enzymes are often neglected (Sun and Cheng 2002). Despite cellulose being the carbohydrate of choice yielding glucose for fermentation, the monosaccharide units released from hemicellulose digestion can also be used by microorganisms during fermentation. Apart from this, the enzymes degrading hemicellulose and lignin not only improve the access of cellulases to cellulose but also yield valued co-products, thereby improving the overall economic outcome of the process (Keshwani and Cheng 2009).

Unlike cellulose, hemicellulose, also known as xylan, is a heteropolysaccharide and hence needs a multienzyme system for its complete hydrolysis (Dos Reis et al. 2003) (Table 9.1). Also, the final hydrolysis product is a mixture of a variety of monosaccharide units xylose, arabinose, galactose, mannose, fucose and rhamnose, along with glucose (Keshwani and Cheng 2009). The inclusion of xylanases in addition to cellulases can thus improve the overall productivity of the bioethanol production if simultaneous fermentation reaction is planned. The utilization of isolated enzymes although being a popular method presents some technical challenges like the one described previously of product inhibition or each enzyme having its own optimum conditions of activity. Therefore, using live microorganisms serving as a source of the enzymes described above is gaining more interest recently. These microorganisms can be aerobic, anaerobic, mesophilic or thermophilic and include several naturally occurring bacterial and fungal species. The bacterial genera like *Clostridium*, *Cellulomonas*, *Bacillus*, *Thermomonospora*, *Ruminococcus*, *Bacteroides*, *Erwinia*, *Acetovibrio*, *Microbispora* and *Streptomyces*, while fungi such as *Sclerotium rolfsii*, *P. chrysosporium* and species of *Schizophyllum* are well known to produce cellulases. As a wide variety of bacteria and fungi such as *Trichoderma* spp. (Filho et al. 1991; Wong and Saddler 1992; Haltrich et al. 1996), *Penicillium* spp. (Filho et al. 1991; Jørgensen et al. 2003), *Talaromyces* spp. (Filho et al. 1991; Tuohy et al. 1993), *Aspergillus* spp. (Dos Reis et al. 2003) and *Bacillus* spp. (Virupakshi et al. 2005) are capable of producing both xylanases and cellulases, they are more effective in complete saccharification of the entire carbohydrate content of LB.

As opposed to saccharification of LB which can be achieved either by isolated enzymes or microorganisms, the fermentation process for bioethanol production is done essentially only by microorganisms and cannot be achieved by using individual

enzymes. Conventionally the organisms popularly employed in bioethanolic fermentation were *Saccharomyces cerevisiae* and *Zymomonas mobilis* which favoured glucose for fermentation (Keshwani and Cheng 2009). However, investigators have isolated yeasts such as *Pichia stipitis*, *Candida shehatae* and *Candida parapsilosis* which are naturally inclined towards xylose fermentation (Katahira et al. 2006). Identification of genes specific to xylose metabolism in these organisms helped the researchers to generate a recombinant form of *S. cerevisiae* capable of metabolizing both glucose and xylose (Tian et al. 2008). Recombinant *S. cerevisiae* has subsequently emerged as a popular organism of choice for bioethanol product due to its robust characteristics and its fairly optimal performance despite varying fermentation parameters such as temperature range, pH range, alcohol tolerance, growth rate, productivity, osmotic tolerance, specificity, yield, genetic stability and inhibitor tolerance (Tran et al. 2020).

Despite the robustness and popularity of yeasts in fermentation bacteria were found to demonstrate distinct advantages over yeasts. Investigators claim that bacterial rapid rates of fermentation are unmatched to traditional yeast fermentation (Hayes 2009). Also gram-negative bacteria like *Z. mobilis* are naturally equipped to utilize sugars such as glucose, fructose and sucrose and can produce bioethanol not only at higher rates but also have been recorded to have a theoretical yield of 97% surpassing traditional *S. cerevisiae* by a wide margin (Gunasekaran and Raj 1999; Sáez-Miranda et al. 2006; Abril and Abril 2009). Subsequently recombinant xylose-utilizing variant of *Z. mobilis* was also produced with considerable advantages such as minimal nutrient requirements, growth at low pH and high temperatures, and it's generally recognized as safe (GRAS) status (Abril and Abril 2009; Yang et al. 2018; Xia et al. 2019). Compared to *Z. mobilis,* other bacteria like *E. coli* and *K. oxytoca* were found to naturally metabolize arabinose; additionally their ethanologenic strains could ferment all lignocellulose-derived sugars (Hahn-Hägerdal et al. 2006). Eventually generation of metabolically engineered *E. coli* revolutionized the field of bioethanolic fermentation providing a selective advantage of being ready for direct industrial use (Atsumi et al. 2008; Atsumi and Liao 2008; Clomburg and Gonzalez 2010). The extensive application of *E. coli* however showed some disadvantages, questioning its broad-scale utility in ethanolic fermentation due to a narrow pH range required for its growth and being less robust than conventional yeast (Dien et al. 2003; Lin and Tanaka 2006; Tomas-Pejo et al. 2008). Equipped with all the advantages of *E. coli, K. oxytoca* was found to grow even at pH as low as 5 and also tolerate higher temperatures up to 308 K (Dien et al. 2003). This spurred the investigators to look for thermotolerant anaerobic bacteria with potential for bioethanol fermentation which included organisms like *Thermoanaerobacter ethanolicus* (Avci and Dönmez 2006)*, Clostridium thermohydrosulfuricum* (Cook and Morgan 1994), *Thermoanaerobacter mathranii* (Larsen et al. 1997), *Thermoanaerobium brockii* (Lamed and Zeikus 1980), *Clostridium thermosaccharolyticum* (Baskaran et al. 1995), etc. These organisms were found to have exquisite capabilities to ferment a wide variety of feedstocks with compatibility for heat pretreatment of LB (Arora et al. 2015; Di Donato et al. 2019). But these were found to have limited industrial utility due to their low bioethanol

tolerance (Georgieva et al. 2007). Considering all the advantages and disadvantages of different microorganisms suitable for bioethanolic fermentation, and with recent advances in genome engineering, there is an immediate need to produce a hardy strain of industrially suitable organism for broad-scale applicability (Liu et al. 2018; Prasad et al. 2019).

9.6 Advances in Pretreatment Strategies

Realizing the drawbacks of conventional pretreatment strategies and with the technological advances, some optimizations are performed on the existing strategies and additionally some new strategies were also developed (Cheah et al. 2020; Rezania et al. 2020). The focus of all these newly researched techniques was to improve carbohydrate availability for enzymatic saccharification along with harvesting of valued co-products for having an improved biorefinery setup with maximized utilization of the LB feedstocks. The three major processes which have been researched heavily in the past decade are hydrothermal pretreatment (Hamraoui et al. 2020), ionic solvent pretreatment (Hou et al. 2017; Asim et al. 2019; Usmani et al. 2020) and biological pretreatment strategies (Wagner et al. 2018). Additionally, the other methods described above have also seen some minor advances. Amongst the physical methods improvement in milling techniques, inclusion of microwave and ultrasound assistance have been found to improve the pretreatment outcome significantly (Amin et al. 2017; Baruah et al. 2018). Let us now review each of these pretreatment advances briefly to gather their advantage over conventional methods.

9.6.1 Physical Methods

9.6.1.1 Milling

Advances in milling technology have allowed the workers to reach up to a particle size as less as 0.2mm and thus reach a maximum exposed surface area for enzymatic degradation of the biomass. Various milling techniques include ball mills, attrition mills, centrifugal mills, colloid mills, hammer mills, vibratory mills, pin mills and extruders (Amin et al. 2017). Ball milling has been recorded to be most beneficial in the case of empty fruit bunch and dead frond fibre of palm oil biomass with a maximum of glucose (87%) and xylose (~82%) recovery (Zakaria et al. 2014). The rate-limiting factors of milling technique however include biomass water content, feeding rate, machinery and initial biomass size (Jędrzejczyk et al. 2019).

9.6.1.2 Freezing

This has comparatively recently emerged and is cost-effective, environmentally friendly and free of inhibitor technique of biomass pretreatment (Rooni et al. 2017). The volumetric changes of water (biomass moisture) that take place when biomass is subjected to repeated freeze-thaw cycles lead to the rupture of cell walls and are predicted to give significantly high glucose yields (Rooni et al. 2017; Li et al. 2019; Li et al. 2020).

9.6.1.3 Microwave

This choice of pretreatment has been found to be most effective in pretreatment of switchgrass and miscanthus. But there have been different combinations researched wherein microwave pretreatments have proven to be useful even in agricultural residue. Like alkali and acid when included in microwave pretreatment has been found to improve sugar yield by 12-fold compared to alkali acid alone (Kumar and Sharma 2017). The major advantage here is the penetration of microwaves to even the rigid structures of LB, decreasing its recalcitrance. The combination of glycerol or $CaCl_2$ -assisted microwave treatment was found to significantly decrease the recalcitrance of corncob and corn stover LB and increase the hemicellulose saccharification (Li and Xu 2013; Zheng et al. 2015).

9.6.1.4 Ultrasound

Delignification and surface erosion are two conceptual pillars of this pretreatment procedure (El Achkar et al. 2018). Its operation at low temperatures, minimal chemical requirements and short time processing have made this method pretty attractive for further research on its outcome (Bussemaker and Zhang 2013; Luzzi et al. 2017). Although presently there are limited studies on its applicability, similar to microwave technique this method can also be improved using some combination strategies of pretreatment.

9.6.2 Physicochemical Methods

9.6.2.1 Hydrothermal Pretreatment

It is one of the most extensively researched techniques in the last decade with promising advances, optimizations and outcomes. During the evolution of this technique considering the target product, it has been categorized into three classes: carbonization, liquefaction and gasification. Here the focus is on the type of the products yielded and accordingly the class of the technique is decided, and other

physical parameters, essentially temperature characteristics, are varied. For instance, carbonization is performed between 200 and 270°C to obtain carbon-rich solid tar as the product, whereas liquefaction is carried out between 270 and 400°C range to obtain products like water-soluble constituents, bio-oil, char, etc. (Toor et al. 2011). Lastly, gasification is conducted at temperatures beyond 400°C and the method is dedicated for obtaining fuel gases as the product. Extreme temperature conditions employed during gasification are believed to enhance the rate of reaction, whereas liquefaction has been tried along with certain organic solvents which have been found to improve bio-oil energy density and minimize the char formation, more suitable for further fermentation. A more recent study records that ethanol addition during hydrothermal liquefaction allows solvent penetration in rigid LB and enhances bio-oil yields (Wu et al. 2019). Thus, inclusion of organic solvents during hydrothermal pretreatment is attracting good research attention and the combination of different pretreatments is being actively studied (Torres-Mayanga et al. 2019). With such encouraging advances in this pretreatment process, the only area which needs active investigation associated with improvements is scale-up technologies.

9.6.2.2 Ionic Liquid (IL) Pretreatment

The advantages of this technique in terms of minimal toxic gas emission, ionic liquid green properties, thermal stability and inconspicuous vapour pressure have increased research interests (Wang et al. 2017). Imidazolium, pyridinium, ammonium, phosphonium or morpholinium cations with anions have been found to dissolve cellulose due to their effective hydrogen bonding with the hydroxyl groups, effectively decreasing the cellulose crystallinity and segregating hemicellulose and lignin from cellulose (Li et al. 2010). High viscosity and expensive nature of ILs had restricted their use at industrial scale, but certain recent investigations have tried to alleviate this problem by integration of ILs with other physicochemical methods to minimize their use and reduce the associated drawbacks. A 1:1 IL and water and IL combination not only reduces its viscosity but also decreases the expense to half and has been reported to improve enzymatic hydrolysis of cornstalk by 81.68% (Hu et al. 2018). Another such combination of IL pretreatment with microwave assistance was found to enhance the delignification and substantially decrease the LB recalcitrance in case of Eucalyptus sawdust (Hou et al. 2019). Also, previous to these observations some studies have reported organosolv (aprotic solvents) combination with IL to be more efficient in terms of cellulose solubilization and higher rate of reaction at comparatively very low operating temperatures (Rinaldi 2011; Xu et al. 2013). With these encouraging results, more such investigations of IL combination in improvement of pretreatment strategies are warranted.

9.6.2.3 Biological Pretreatment

A wide variety of bacteria and fungi are naturally equipped with LB modification and digestion enzymes. The use of cellulolytic and hemicellulolytic enzymes of bacterial origin has been discussed in the previous section. However, fungi (viz. white-rot, brown-rot and soft-rot fungi) are also known to produce lignin-digesting enzymes like phenol oxidase, lignin peroxidase, versatile peroxidase and manganese peroxidase (Waghmare et al. 2018; Sharma et al. 2019). The advantage of utilizing microorganisms equipped with LB digesting properties is envisioned for simultaneous pretreatment and alcoholic fermentation, with an additional byproduct of biomaterials including various enzymes, lactates, acetates and organic acids (Sharma et al. 2019). Apart from bacteria and fungi, other organisms including insects (Varelas and Langton 2017), worms (Devi et al. 2020) and gastropods (Trincone 2018) have also been tested for their natural ability to digest LB in biological pretreatment. Isolated gut microflora of certain insects, gastropods and ruminant mammals has also been put to test for finding its application in biological pretreatment of LB (Yao et al. 2018a, b). Although they are attractive and cost-effective, biological pretreatment strategies need close monitoring, as these are bound to be affected by changes in physical, chemical as well as biological environment, creating a profound impact on the overall outcome of the process.

9.7 Agricultural Lignocellulosic Waste Feasibility Assessment for Bioethanol Production

Several factors are to be considered when the suitability of the feedstocks is to be judged for bioethanol production. This includes biomass quality issues like (1) geographical location and cultivation practices, (2) availability of land and land use practices, (3) chemical composition of the biomass, (4) injection of pesticides, (5) absorption of minerals to water and soil, (6) water requirements and water availability and (7) emission of greenhouse gases, acidifying gases and ozone depletion gases. Also there are economic and environmental concerns like (1) energy balance, (2) use of resources, (3) contribution to biodiversity and landscape value losses, (4) farm-gate price of the biomass, (5) logistic cost (transport and storage of the biomass), (6) direct economic value of the feedstocks taking into account the co-products, (6) creation or maintenance of employment and (7) soil erosion (Balat 2011; Saini et al. 2015). Although the economic and environmental issues require a separate platform for discussion, when the overall yield of bioethanol is assessed, these factors do play a vital role in determining the feasibility of LB for bioethanol production. Here however major emphasis will be given to the issues inherent to biomass.

When the feasibility of the plant material is done to test its usefulness in second-generation bioethanol production, two important features are to be considered:

whether the woody biomass belongs to the hardwood or softwood category. The processing strategies for hardwood are different from softwood species and there are not very encouraging studies describing successful saccharification for fermentation of woody biomass due to its high recalcitrance (Soccol et al. 2019).

Common assumptions about agricultural crop residues is that it only includes materials such as stalks and stubble (stems with roots), leaves and seed pods, left in the agricultural field post-harvesting, also collectively known as field residues. However, in addition to field residues there are also processing residues, including husks, seeds, bagasses and roots, which are the leftovers post-processing of the crop into a usable resource (Pandey et al. 2000a, b). Thus, agricultural waste has to include both field and processing residues. Traditionally these residues were cut, dried and used as fodder for farm animals, fetching minimal revenue against this potentially important bioethanol generating raw material. Furthermore, harvesting of cereals, vegetables and fruits often generates a huge amount of LB. LB from agricultural waste thus stands a better chance to be useful as a renewable raw material in second-generation bioethanol fermentation (Pandey et al. 2000a, b).

The agricultural produce can also be classified on the basis of type of carbohydrate available in it for further processing as follows: sucrose-containing feedstocks (e.g. sugar cane, sugar beet, sweet sorghum and fruits), starch materials (e.g. corn, milo, wheat, rice, potatoes, cassava, sweet potatoes and barley) and lignocellulosic materials (e.g. wood, straw and grasses) (Balat 2011). Apart from the LB biochemical composition, economic feasibility assessment is also important which is decided by the overall sugar yield post-processing of the feedstock and the total bioethanol production. The four major factors that have impacts during economic feasibility assessment are (1) the extent of biomass resistance to breakdown, (2) the different forms of sugars released after complete hydrolysis of hemicellulose and cellulose polymers, (3) the requirement of genetically engineered organisms for efficient fermentation of these sugars and (4) financial assessment of expenses required for collection and storage of low-density lignocellulosic materials (Saini et al. 2015).

When the raw material is chosen considering the above points and it qualifies to be suitable for bioethanol synthesis, there are a few last concerns to be addressed before these are subjected to processing. Apart from the chemical composition of biomass, the cultivation practices are also to be paid attention to. Pesticide/herbicide/insecticide infusion in the feedstock might adversely affect the overall yields. Some of these might undergo some chemical modifications during preprocessing steps leading to accumulation of certain inhibitory compounds undesirable for optimal enzymatic action. Traces of these chemicals could restrict the growth of microorganisms during fermentation. Therefore, a proper judgement of content of such compounds in the raw material is also important.

9.8 Industrial Fermentation with Lignocellulosic Biomass

Scale-up strategies for fermentation and distillation of bioethanol have attracted considerable research attention recently (Gupta et al. 2019). The major delay in utilization of LB for industrial fermentation is perhaps due to absence of best-suited microorganisms which can efficiently ferment all the forms of sugars produced by a variety of feedstocks. There is an extensive demand for genetically engineered microorganisms that can utilize a wide range of substrates for bioethanol production and that can withstand high temperatures without compromising their ethanol productivity. *S. cerevisiae* is still quite a popular organism of choice for industrial fermentation. *S. cerevisiae* strains with an explicit ability to simultaneously co-ferment the two most prominent products of cellulose and hemicellulose hydrolysis, viz. sugars glucose and xylose, have recently attracted much scientific attention. Researchers have successfully produced recombinant *S. cerevisiae* using revolutionary metabolic engineering strategies which through isomerase-based pathway can simultaneously co-ferment glucose and xylose, recording a remarkably high ethanol yield even at high temperatures (Tran et al. 2020)

Conventionally there are two proposed methods of fermentation: (1) simultaneous saccharification and fermentation (SSF) and (2) separate hydrolysis and fermentation (SHF). Despite theoretical advantages of SSF, SHF has been found to be commercially and economically more viable (Tengborg et al. 2001). Recent investigations have improved the SSF applicability and thus it is slowly becoming more popular due to its inherent advantages of higher ethanol yield and comparatively simple arrangement (Olofsson et al. 2008; Bertilsson et al. 2009). As explained previously there are a variety of microorganisms available today that can be utilized for their cellulolytic abilities for the release of glucose from LB. To restrict the glucose so released from being consumed by the same microorganisms, fermentative microorganisms can be added to convert the surplus glucose to ethanol; this process is called SSF. The prominent advantage of SHF over SSF is that each enzymatic step can be performed at its specific optimal conditions.

To make LB bioethanol economically and commercially viable, its concentration during the production process should exceed 40 g/L. To achieve this there are four major challenges faced by the industries working in this direction. The formidable challenge is associated with optimization of pretreatment for maximizing fermentable sugar digestibility from LB with low inhibitor concentration and economic feasibility. Strategies for efficient hydrolysis of pretreated LB leading to maximized saccharification yield are the second challenge. The prevailing third challenge is the lack of an industrially applicable microorganism capable of fermenting a variety of sugars and their derivatives released from hydrolyzed LB. And lastly, the expense involved in bioethanol distillation which has an obvious correlation with the efficiency of all the process challenges listed above impacts the commercialization of LB bioethanol. Considering all the above-mentioned challenges, there have been some recent trends and advances demonstrating economically attractive, integrated practical industrial technologies for LB bioethanol production, which include:

1. Selective-fractionation technology based on steam explosion pretreatment
2. Synergistic enzymatic hydrolysis system
3. Industrial fermenting yeast strains
4. Pre-hydrolysis and simultaneous saccharification and co-fermentation
5. Consolidated bioprocess (CBP)

9.8.1 Selective-Fractionation Technology Based on Steam Explosion Pretreatment

Enzymatic efficiency of hydrolysis largely depends upon the porous structure, essentially the pore-size distribution of LB, that can be successfully improved by steam explosion pretreatment optimized for the specific LB. Steam explosion pretreatment of corn stover has been recorded to achieve 80% hemicellulose hydrolysis and recovery with more than 90% cellulose residue in biomass. This component recovery success rate was found to be consistent in laboratory as well as industrial application (Zhao and Chen 2013). Even a two-step steam explosion strategy has been proposed in order to reduce the inhibitor conversion by 33% with intermediate separation of fibre cells improving the efficacy of enzymatic hydrolysis as quantified by the hydrolysate to over 12.82 % (Zhang et al. 2012). Thus, based on steam explosion process optimization at the core, selective-fractionation technology has been included in the integrated industrial technologies (Alvira et al. 2010; Zhang et al. 2012). This technology was developed with an intention to retain the original macromolecular features, optimize and maximize biotransformation of components suitable for enzymatic hydrolysis and improve the value of intermediate products for larger commercial utility of LB bioethanol.

9.8.2 Synergistic Enzymatic Hydrolysis System

This method was developed for efficiency improvement during enzymatic hydrolysis of pretreated LB in addition to cost-effectively produce the cellulase on-site with supplementation of other hydrolytic enzymes to optimize the synergistic hydrolytic mechanism (Park et al. 2012; He and Chen 2013). Corn cell wall proteins (CWP) were found to exhibit many cellulase synergistic activities despite no inherent cellulose hydrolytic activity of their own (Han and Chen 2007). The approach has been predicted to be so rewarding that the same group proposed supplementation of corn stover along with CWP as an additive along with *T. viride* cellulase for optimal cellulose hydrolysis (Han and Chen 2007). Similarly, laccase from *Sclerotium* sp. was found to exhibit exquisite synergy with cellulase enzyme improving its hydrolytic potential by partially degrading the surface lignins in LBs and increasing cellulase accessibility to the feedstock (Qiu and Chen 2012). Another enzyme,

feruloyl esterase (FAE), can hydrolyze the feruloyl ester bonds between hemicellulose and lignin in lignocellulose, and break covalent linkages, thereby increasing the hydrolytic rate of cellulase action through improved cellulose access (Zeng and Chen 2009). Thus, inclusion of CWP, *Sclerotium* sp. laccase and FAE along with cellulase has been recognized as the integrated industrial technology that can cost-effectively improve the hydrolytic yield of LB.

9.8.3 Industrial Fermenting Yeast Strains

Recombinant *S. cerevisiae* expressing cellulases of heterologous origin, either bacterial and fungal, have been tried extensively in the last two decades to get enhanced industrial application (Lee et al. 2017; Smekenov et al. 2020). The secretory form of cellulase has a high expression compared to membrane-localized forms. However, the membrane-localized cellulase displayed on the cell surface has some distinct advantages over the secretory form. All the three forms of cellulases, endo- and exoglucanases and β-glucosidase are placed in close proximity on the cell surface for enhanced cellulose-degrading ability; there is no irreversible adsorption of crystalline cellulose limiting ethanol production and such yeast can be easily recycled between batches of fermentation without any additional enzyme expense (Oh and Jin 2020). Several other efforts in metabolic engineering of yeast and improving yeast tolerance to fermentation inhibitors were done in the recent past to improve its industrial viability. Metabolic engineering to enhance intracellular glutathione synthesis and acetic acid degradation pathways has been found to promote anti-stress mechanisms for strengthening robustness and ethanol productivity in recombinant yeast (Qin et al. 2020; Walker and Basso 2020). Utilizing adaptive stress responses in mitigating adverse outcomes and enhancing tolerance and productivity of industrial yeast have also been popular approaches (Walker and Basso 2020). Two industrial Brazilian *S. cerevisiae* strains, PE-2 and SA-1, evolved from parental strains AMY35 (SA-1) and AMY12 (PE-2), were found to demonstrate much better thermotolerance compared to the respective parental strains, emphasizing the importance of adaptive laboratory evolution mechanism for improved industrial application of older industrial yeast strains (de Melo et al. 2020). As many as seven lignocellulolytic enzyme expressions have been successfully attempted till date in industrially important yeast strains (Oh and Jin 2020). Still more efforts and research to maximize cellulase activity in *S. cerevisiae* are required, which calls for elucidation of underlying mechanisms involved in the secretory pathway and protein translocation machinery. Also some additional strategies for detoxification of fermentation inhibitors in lignocellulosic hydrolysates may make it more feasible for industrial applications (Oh and Jin 2020).

9.8.4 *Pre-hydrolysis and Simultaneous Saccharification and Co-fermentation*

With well-documented advantages of SSF over conventional SHF, there has been an evolution of a simultaneous saccharification and co-fermentation (SSCF) process which further combines the hexose and pentose fermentation steps by one or more microorganisms as in SSF. Central to all these processes of fermentation, be it SHF, SSF or SSCF, are the cellulases, and the cost-effectiveness of these is also regulated by the expense and efficiency of cellulase (Oh and Jin 2020). As described above in order to be useful for SSCF, the saccharification step has to be followed by fermentation of all the sugars released from LB. With the emergence of engineered microorganisms, this has become quite feasible but at the same time SSCF is being phased out by another advanced method of consolidated bioprocessing (CBP) described below.

9.8.5 *Consolidated Bioprocess (CBP)*

This is a comparatively recent strategy which truly integrates cellulase enzyme production for saccharification and simultaneous co-fermentation of every sugar to produce ethanol using a single organism. CBP technology actually eliminates the use of commercial enzymes. It includes simplification in the biorefining of lignocellulose, enhancing the bioprocess efficiency and minimizing total costs (den Haan et al. 2015). In addition to the engineered yeast, studies have identified certain wild-type microorganisms with CBP potential, viz. anaerobic bacteria like *Clostridium thermocellum* and filamentous fungi like *Fusarium oxysporum*, to highlight a few (Van Zyl et al. 2007; Liu et al. 2018). Even microbial consortiums like *Aspergillus niger* co-cultured with *S. cerevisiae* have been predicted to be useful in CBP (Liu et al. 2018). The CBP process however seems to be more rewarding with engineered strains rather than wild-type organisms due to some unwanted byproducts produced by the latter. Both bacteria and yeast and non-yeast organisms are thus being extensively tested and engineered to optimize and improve their application in the CBP process of integrated industrial technology (Liu et al. 2018).

9.9 Biorefinery for Management of Agro-waste

The recovery of bioethanol and valued co-products post-fermentation is the last step in determining the qualitative and quantitative success of the entire pathway. During alcoholic fermentation, accumulation of the end product might inhibit further ethanol production. The capacity of yeast to continue the process of fermentation is reported to be blocked as ethanol concentration reaches beyond 20% (w/v) (Garhyan

and Elnashaie 2004; Utama et al. 2016). Therefore, continuous or intermittent removal of ethanol produced is important to improve the efficiency of the pathway. The conventional distillation technique of alcohol recovery suffers from the drawback of yeast inactivation due to heat treatment (Vane 2008). There is an estimated record which states that around 40–80% of bioethanol cost is decided by the extraction technique used for alcohol recovery; thus, much attention is currently on devising new methods improving the efficiency of this final process of bioethanol production (Le et al. 2011). Traditional distillation although has high ethanol recovery efficiency suffers from the drawbacks of high temperatures and energy requirements for operation impacting overall cost and fails in effective drying of the product (Lei et al. 2003). Considering the disadvantages associated with distillation, some non-conventional recovery processes are recently attracting interest (Vane 2008). Alternative methods of product recovery like pervaporation separation, vacuum fermentation, adsorption, gas stripping, solvent extraction and other hybrid processes were developed as early as in the 1970s with an intention to minimize fossil fuel consumption but still have not achieved the level of industrial optimization (Serra et al. 1987; Offeman et al. 2005; Vane 2008; Zentou et al. 2019). Cost-effective production and commercialization of bioethanol not only require an efficient extraction process but also demand the development of a biorefinery-based approach for integral use of LB (Tao et al. 2011). As has been seen earlier, a rational choice of pretreatment process is key for maximizing bioethanol production, and so is true for the establishment of a biorefinery since it impacts subsequent steps of recovery of valuable co-products during ethanol synthesis (Fermoso et al. 2018). The overall revenue efficiency of second-generation bioethanol can be positively influenced by recovery of highly valued co-products obtained from lignin which not only requires improvements in pretreatment, fermentation and product separation efficiencies but also a careful planning of the biomass supply chain and valorization of residual biomass to establish a viable biorefinery plant (Özdenkçi et al. 2017; Gonzalez-Contreras et al. 2020). The current approach of integrated green biorefineries tries to combine LB bioethanol production with energy generation, lignin valorization to valuable phenolic compounds, reduced greenhouse gas emission, recovery of commercially viable enzymes and biomolecules and recycling of solvents (if any) to attain zero-waste multiproduct sustainable model (Talekar et al. 2018; Carrillo-Nieves et al. 2020; Islam et al. 2020; Clauser et al. 2021).

A successful industrial-scale application of biorefinery requires an executable process design, viable techno-economic assessment and determination of socioeconomic impact at regional and local levels. Conventionally the concept of biorefinery was only limited to integration of units performing the process of pretreatment, saccharification, fermentation and distillation. However, mounting on the design principles some new processes such as evaporation, size reduction and chemical and solvent recycling can be incorporated to improve the green and sustainability aspects of second-generation bioethanol biorefineries (Keijer et al. 2019). Extensive energy and water requirements during the pretreatment, substrate conditioning and product recovery are some major concerns hindering the techno-economic success of these prodigious attempts made in the development of sustainable biorefineries. Although

water is the greenest solvents for biorefineries, its excessive use demands high energy consumption during heating, evaporation and distillation (Dong et al. 2019). A comparative assessment of greenhouse gas or CO_2 emission during different pretreatment processes done previously (Prasad et al. 2016) has indicated liquid hot water pretreatment being the least contributor but suffers from the drawback of incomplete fractionation of LB (Sabanci and Buyukkileci 2018). A recently proposed simulation-based model predicted the efficiency of catalytic hydrogenolysis of lignin obtained from ionic liquid pretreatment of LB from diverse feedstocks for eugenol and other phenolic production, being a cost-effective approach. The authors claim a net reduction of 78% in greenhouse gas emission accounting to around 21g CO_2-eq/MJ ethanol under specified conditions (Martinez-Hernandez et al. 2019). The same article asserts that a biorefinery sized for an intake of 3000 t/d of biomass and diverting 80% of lignin stream to phenolic production can yield a minimum ethanol selling price lower than the current average US FOB ethanol price. Furthermore, if cost-effective strategies for recycling of ionic liquids and isopropanol can be devised, the efficiency of the entire process can be taken to the next level to improve product yields impacting the overall outcome of an ideal biorefinery. Some methods for effective structuring and design assessment of biorefineries have also been published to determine the success of any biorefinery depending on its biobased economy (Aristizábal-Marulanda and Cardona Alzate 2019).

Recent years have seen development of many biofuel- and chemical-producing biorefineries in the USA, Europe and Asia (Hassan et al. 2019), despite prevailing challenges pertaining to the development of highly efficient and cost-effective pretreatment technology to fractionate LB (Zhao et al. 2020). Well-articulated research studies for the development of low-temperature and cost-effective delignification technology to improve LB valorization will give us hope that integrated multiproduct biorefineries from agricultural wastes are not a distant dream.

9.10 Environmental Impact Assessment and Future Directions

Optimization of LB bioethanol production at industrial levels has recently raised several environmental concerns, the dominant of it being the emission of greenhouse gases during the process. Right from the pretreatment process till ethanol recovery, the chemicals and solvents involved along with the combustion or degradation of the biorefinery products impact upon the carbon footprint and thereby the green index of the biorefinery. Prasad et al. (2016) have given a comprehensive life cycle analysis (LCA) of greenhouse gas emission during the generation of 1 Kg of fermentable sugars from corn stover by comparing four different pretreatment processes (Prasad et al. 2016). The environmental impact of any alternative products or systems can be assessed today using the method of life cycle assessment (Amelio et al. 2014). Even

the European Commission has found it as a useful method and included it in its Waste Framework Directive (Directive 2008/98/EC as amended by Directive 2018/251) as a resource-efficient way for the management of waste (Cobo et al. 2018). The choice of waste management strategy has a great impact on the green index of the biorefinery, and a variety of studies have been conducted in the direction analysing its environmental tradeoffs pertaining to biowaste management, but with limited emphasis on life cycle assessment of biowastes generated during ethanol production. A recent report based on LCA method indicates that biorefinery systems for biowaste-derived ethanol production have a very good environmental impact recording the highest net benefits in the impact categories "freshwater eutrophication" and "human toxicity-carcinogenic", while the highest net burdens are recorded for the categories "ecotoxicity" and "marine eutrophication". The net green index in terms of carbon footprint is estimated to be −15 kg CO_2eq/ton biowaste. Another less popular method for green index determination is the EHS method which is a preliminary screening process for prediction of possible hazards of solvents in a chemical process (Sheldon 2018). The choice of pretreatment solvent plays a crucial role in the environmental impact assessment process as organic solvent utilization is associated with toxicity, flammability, exclusivity and difficulty in their degradation. The utilization of green solvents is thus encouraged for improvement of green indices of biorefineries and water is the preferred solvent of choice during bioethanol production.

The entire process of bioethanol production from LB is a water-intensive process and it has been predicted by many investigators that energy requirements of the overall process are positively correlated with water requirements (Pan et al. 2015; Dong et al. 2019). Anaerobic treatment of high-strength organic wastewater from LB-based biorefineries is proposed to yield a mixture of methane and carbon dioxide, commonly known as biogas, which might satisfy the energy demands of the plant (Bories et al. 1988; Wilkie et al. 2000). Anaerobic reactors however may not be a cost-effective alternative to suit the overall economics of LB biorefineries. But integration of wastewater treatment with the biorefinery can prove beneficial and reduce ecotoxicity (Haryanto 2012; Tobin et al. 2020). Newer ways of integration and recycling of wastewater from second-generation LB-based biorefineries have shown some promise and the concept of zero wastewater generation is becoming popular (Tobin et al. 2020; Zheng et al. 2020). The success of the wastewater treatment strategy largely depends upon the upstream processes used during the production of bioethanol from LB; therefore, techniques either utilizing less water (Wang et al. 2019) or minimizing inorganic addition may prove rewarding (Tobin et al. 2020). Challenges still prevail and more studies are needed to devise an effective techno-economic strategy to reduce the environmental impact associated with wastewater.

The ever-increasing demands of food and energy resources with limited land for cultivation have raised concerns of food security versus biofuels due to the negative impact recorded by biofuels over food prices (Soto et al. 2015). Keeping in mind this conflict, the second-generation bioethanol production was developed. But these

concerns raised are criticized by some recent reports which highlight the fact that issue on food versus fuel is more of a public emotion rather than a reality and also implies upon the importance of second-generation bioethanol to neutralize this debate (Filip et al. 2019; Goswami and Choudhury 2019; Martínez-Jaramillo et al. 2019).

Complete recovery of value-added co-products with least energy expenditure and zero wastewater generation to have a wholesome and sustainable establishment of a second-generation LB-based ethanol biorefinery is a challenging goal to achieve. Balancing the ever-increasing energy demands with eco-friendly strategies and popularizing the use of LB-derived bioethanol however might seem difficult can be achieved in the near future due to the technological advances that biotechnology and engineering have seen in the recent past. Researchers are putting in focused efforts to minimize the costs involved in the process and maximize the gains by using novel approaches to valorize lignin (Martinez-Hernandez et al. 2019; Miliotti et al. 2019). Soon complete utilization of agricultural wastes to generate economic bioethanol in a sustainable multiproduct zero waste biorefinery can be made possible with all the criticisms associated with second-generation bioethanol put to rest.

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Chapter 10
Food Wastes for Biofuel Production

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Abstract At the center of energy transitions are biofuels. Biofuels play an important role in the low-carbon economy. Currently, supported by various policies, the demand for biofuels is increasing across the globe. Agricultural products also used for food purposes are the primary feedstock used for the production of biofuels. Unfortunately, this has led to an ethical debate known as food-versus-fuel that endures until today. However, converting food wastes into biofuels may be the solution to end this fierce debate. This chapter provides an overview of the production of various types of biofuels from food wastes. Food waste valorization by microalgae for biofuels and the progress of microalgae biofuel commercialization are also addressed.

Keywords Wasted resources · Non-fossil fuels · Microalgae · Biomass

10.1 Introduction

Converting food wastes into ecological fuels would have an impact on global threats, such as petroleum shortages and food wastage. Besides that, it would minimize the competition between fuel production and food production. Using food residues that would be incinerated or would end up in landfills, contributing to climate change, is a step in the right direction toward sustainable biofuel production (Matsakas et al. 2014).

Recent studies warn that global food wastage can increase significantly if the governments, businesses, and consumers do not act. Globally, each year, one-third of the food produced for consumption is lost or wasted. The food wastage upstream and downstream is balanced, representing around 54% and 46% of total wastage, respectively. The causes of food wastage fit for consumption are many, and the environmental cost is enormous. The carbon footprint and blue water footprint of

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food produced and not consumed are estimated at 3.3 Gtonnes of CO_2 equivalent and about 250 km^3; besides occupying in vain 30% of the world's agricultural area, this represents 1.4 billion hectares (FAO 2013).

Undoubtedly, reducing food wastage is a global priority that needs the commitment and collaboration of various actors. Actions to reduce food wastage need to be supported and implemented. Encouragement of the reuse of food in the human and animal food chain is advantageous and is within reach of all. In cases where reuse is not possible, recycling and recovery may be the best option. Food waste that is not edible can be processed and generate various new products such as bioplastic, biofertilizer, and bioenergy. In particular, various types of non-fossil fuels can be produced from food waste, such as biomethane, biohydrogen, biodiesel, bioethanol, and biobutanol (Kannah et al. 2020a, b; Engelberth 2020).

The opportunity to generate biofuels from food residues, besides benefitting the environment, can alleviate concerns related to food production for biofuel production. Discordantly, while some treat biofuels as an outlet to contain climate change, others see them as a threat that might decrease the land availability to cultivate food. In this sense, given the concerns related to food wastage and the use of food for bioenergy, in recent years, the researchers have put the bet on the use of low-cost food waste as feedstock for biofuel production (Girotto et al. 2015).

This chapter provides an overview of the production of various types of biofuels from food waste. Food waste valorization by microalgae for biofuel production and the progress of microalgae biofuel commercialization are also addressed.

10.2 Food Wastes as Feedstock for Biofuel Production

Food wastes (FW) are considered as the final product discarded or not used for other purposes, derived from different sources, among which stand out the food processing industries, services food, household waste, and agricultural residues, among others (Ezejiofor et al. 2014; Henz and Porpino 2017). However, the problem does not end only with the waste of food and the financial problems that this can generate through its treatment. FW have negative consequences for the environment, since the consumption of resources for food production involves the use of water, energy, and work (Li and Yang 2016; Tonini et al. 2018). Besides that, more than 90% of this waste ends up being converted into methane, carbon dioxide, and other greenhouse gases (GHGs) (Lin et al. 2013; Deng 2016).

Most of the FW are derived mainly from plant and animal sources in which they present several nutrients, rich in carbohydrates, proteins, oils and fats, organic acids, and other compounds of low composition (Deng 2016; Socaci et al. 2017). The exploration of these compounds in a secondary alternative of valorization is in perfect harmony with the concept of green chemistry, which aims to reduce the generation of substances that harm the environment. At the same time, it develops more environmentally friendly products or processes (Isah and Ozbay 2020). In this

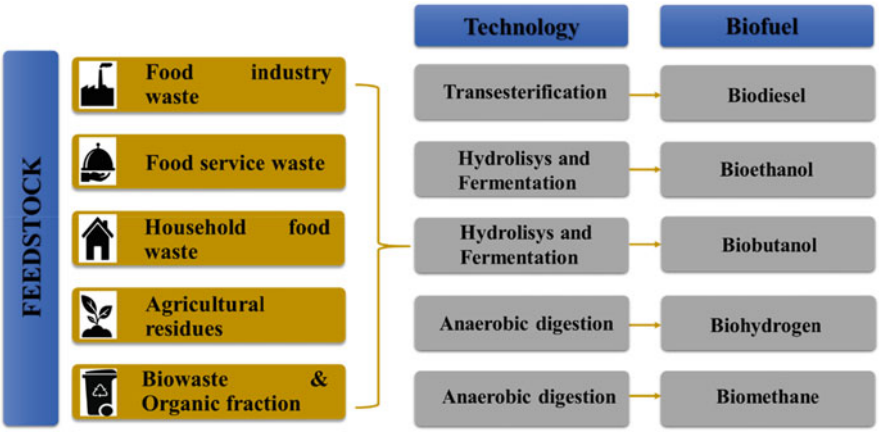

Fig. 10.1 Main biofuels produced by food waste and their production technologies

case, FW are used as a raw material for the development of valuable chemicals and co-products.

The FW can be ideal and low-cost substrates for high productivity of oleaginous microorganisms, such as microalgae, yeasts, and bacteria (Cho and Park 2018). This type of process has advantages in the production of lipids in microorganisms and in reducing pollutant emissions from waste to be treated (Cho et al. 2017). Hydrolysis of carbohydrates in FW can also result in the breakdown of glycoside bonds, facilitating fermentation to biofuel production (Li and Yang 2016). In addition, the use of food waste for microbial growth has benefits in terms of savings mainly in the cost of raw materials for the production of biofuels, and it is probably one of the most sustainable ways to produce bioenergy and other bioproducts (Isah and Ozbay 2020).

Recently, it was reported that the market demand for biofuels is much higher than for other chemicals, besides being more profitable (Khan et al. 2018). Therefore, we focus on the generation of biofuels and their main production processes from various food residues (Fig. 10.1). In the following sections, we highlight a more detailed study of some specific biofuels. These include biodiesel obtained from the chemical reaction of lipids, oils, or fats; bioethanol and biobutanol obtained by fermentation carbohydrates; and biohydrogen and biomethane produced by anaerobic digestion of food waste (Vieira et al. 2014).

10.2.1 Biodiesel Production

Biodiesel is a fuel similar to conventional diesel in many of its properties. It is considered a clean and renewable fuel (Yaakob et al. 2013; Karmee and Lin 2014). According to the literature, there are different processes applied to decrease the

viscosity of oils for use in diesel engines. These processes include mixing, esterification, transesterification, micro-emulsification, and pyrolysis (Knothe et al. 1997; Demirbas 2009; Vijayaraj and Sathiyagnanam 2016). The raw materials must meet a series of specifications, respecting the attributes of biodiesel established by ASTM International (ASTM D6751), and the laws required by each country (ASTM 2002).

The quality of this biofuel is influenced by the fatty acid profile, the makeup of the raw material, production, and storage process. The properties associated with the fatty acid profile and composition of the raw material include iodine value, viscosity, cloud point, cetane number, and phosphorus content. On the other hand, properties related to the output process include free and total glycerin, carbon residue, ester content, methanol content, and flashpoint, while those related to storage include oxidative stability, acidity value, and water tenor (Cavalheiro et al. 2020; Lobo et al. 2009).

Taking into account only the chemical reaction for the output of biodiesel, the most suitable raw material is refined vegetable oils; with this raw material the transesterification reaction occurs in a more complete and short form (Caetano et al. 2018). In Europe and in the USA, biodiesel is most commonly produced from rapeseed oil and soybean, respectively; in Malaysia and Indonesia, palm oil is the most significant source of biodiesel, while in India and Southeast Asia, the jatropha tree is the most important source for the output of biodiesel (Demirbas 2009). The most explored vegetable oil sources are soybean, canola, palm, and rapeseed, while the animal fat sources are beef tallow, lard, chicken fat, and fish oils (Demirbas 2008).

Taking into account the social, economic, and environmental aspects, a major problem related to the use of these raw materials is that they end up contributing to the reduction of the supply of basic foods to the population (Caetano et al. 2018). As a solution to this problem, food waste can serve as an excellent source of biofuel production. Unfortunately, food waste occurs in almost all phases of the food industry and in significant quantities (United Nations Industrial Development Organization 2012).

Food waste is not just about financial loss. It also has a major impact on climate change, as food production consumes diverse resources (Zorya et al. 2011). The use of these food residues combined with the cultivation of microorganisms and other processes can generate biodiesel with the properties and quality according to the requirements of the legislation and according to studies already carried out previously (Mata et al. 2014.

According to Fonseca et al. (2019), Jenkins et al. (2016), and Canakci (2007), food residues such as frying oil residues, restaurant fat, animal fats, and coffee beans are all low cost and potential sources for obtaining biodiesel. They are a key component in reducing the cost of this product. Most of the residues present in their composition possess free fatty acids, high moisture content, and some contaminants, such as NaCl, which contribute toward reducing the efficiency of the transesterification method. Two-step processes from acid-catalyzed esterification followed by traditional basic catalyzed transesterification are generally reported as

an effective way to avoid the undesirable effect of free fatty acids, without significantly increasing production costs (Cai et al. 2015; Dias et al. 2013).

A study using catalyzed calcined egg shells by Tan et al. (2015) reported that pretreatment using esterification is essential for the biodiesel production process. However, the pretreatment must be optimized due to the fact that the various raw materials have different compositions (de Almeida et al. 2015). A spin-off company located in Cordoba in Spain (SENECA Green Catalyst SL) was able to produce biodiesel using oils used in a single container, with steps of esterification and subsequent transesterification, obtaining a value of approximately 3–5 tons of biodiesel per day (Lin et al. 2013).

A UK company (Brocklesby Ltd.) developed another highly efficient approach that gave about 98% yield. Where the food waste was subjected to heating, with subsequent disruption of the cells, to release oils contained in the cells, then the fat is separated by centrifugation process, obtaining about 2000 tons per year of residues rich in triglycerides, for the production of biodiesel (Liu et al. 2014).

The production of alternative biofuels from food wastes presents many challenges. Biodiesel is one of the best replacement alternatives, where many efforts are being made to produce it at a lower cost and with excellent properties. The transesterification reaction is the best method for producing and modifying biodiesel. The type of raw material is the most important factor in the production of this biofuel and in the case of the use of microorganisms; the cultivation conditions and the choice of the strain are decisive. Thus, the exploration of methods to increase the quality and the profitability of biodiesel for applications in diesel engines without modifying the properties of the engine comes as an emerging technology of exploration (Fonseca et al. 2019).

However, for a biodiesel production plant, we must take into account several aspects beyond the selection of the raw material, such as the engineering aspect, which is directly linked to the life cycle of each raw material, including soil availability, logistics, and costs of transport and storage, energy use, greenhouse gas (GHG) emissions, and contribution to biodiversity losses. Other important factors have to do with taxes, policies, subsidies, and legislation in force in each country (Mata et al. 2014; Hajjari et al. 2017).

Considering a production plant, it integrates a multidisciplinary process, which involves the composing of the raw material and its physical-chemical properties and the different stages of the process, such as the definition of the processing system, design, equipment sizing, heating, mass balances, chemical requirements, utilities, and waste management, among others (Mittelbach 2009).

Regarding the processes already mentioned above, they can be used to make biodiesel and to decrease the viscosity of natural oils. Pyrolysis is the process where a substance is converted into another substance by the action of heat in the presence of a catalyst in a simpler process. Dilution is the process in which oils from different sources are mixed with diesel oil to reduce viscosity and also to apply straight to the engine. In micro-emulsification, there occurs the shaping of microemulsions where it becomes the potential solution for solving the problem of high viscosity of vegetable oils (Mofijur et al. 2013; Abbaszaadeh et al. 2012).

Fig. 10.2 Transesterification reaction resulting in the production of biodiesel and glycerol. Adapted from Caetano et al. (2018)

Transesterification is the most used process for the output of this biofuel, as it is more efficient for the production of fuels with properties that meet the required biodiesel standards. The transesterification procedure occurs in three consecutive reversible reactions between oil or grease and alcohol (Fig. 10.2) (Mahmudul et al. 2017).

The transesterification output occurs through three consecutive reactions that can be reversible between oil or fat and alcohol. It involves a triglyceride (TAG) that reacts with short-chain monohydric alcohol plus a catalyst at an elevated temperature that will form alkyl esters of fatty acids. This conversion of TAGs into biodiesel is a gradual process in which alcohol initially reacts with TAG to produce alkyl esters of fatty acids (FAAEs) and diacylglycerols, which subsequently react with alcohol (alkoxide) to release another FAAE molecule and generate monoacylglycerols (MAG). Finally, glycerol and FAAE are produced from MAG; these combined FAAEs are collectively known as biodiesel (Mahmudul et al. 2017). Approximately three moles of FAAE and one mole of glycerol are obtained for each mole of TAG that undergoes complete conversion (Moser 2012; Sajid et al. 2018).

10.2.1.1 Non-catalytic and Catalytic Transesterification Process

Non-catalytic transesterification is a thermochemical process carried out in tubular or bubble column reactors developed by Joelianingsih et al. (2012), at high temperature (250–500 °C). The transesterification principle that occurs in this bubble column reactor is similar to reactive distillation, where the products of this reaction in the gas phase are removed from the reactive zone, while the reagent is retained in the reactive zone. The production of this biofuel by non-catalytic transesterification could achieve efficiencies of up to 95–99%. Despite the high yield, the disadvantage is that the biodiesel degradation can occur above 270 °C, and the process bears a high production cost related to energy due to the necessary high temperature requirement (Kwon et al. 2014; Tran et al. 2017).

Catalytic transesterification could be performed using homogeneous and/or heterogeneous catalysts (acids or alkalis) or biocatalysts. The usage of biocatalysts is more advantageous as it requires lower temperatures (<70 °C), leading to a lower production cost. However, the usage of these biocatalysts in transesterification is of limited use, as it has an inhibitory effect on alcohol and sensitivity to reaction parameters, such as temperature, pH, and alcohol/oil molar proportion, among others. They can be deactivated by alcohol when the molar relation of alcohol to

triglycerides exceeds the stoichiometric ratio for transesterification (Ghaly et al. 2010; Liu et al. 2012).

These reactions can occur under supercritical conditions, both non-catalytic and catalytic, through application of chemical catalysts or biocatalysts. In supercritical conditions, alcohol is used at pressure and temperature above its critical levels. The alcohol most commonly used in the output of biodiesel is methanol. The critical temperature reported was 512.2 K and the critical pressure reported was 8.1 MPa (Demirbas 2005).

The usage of microwave technology accelerates the transesterification; its efficiency is due to heterogeneous heating and its dependence on the thermal conductivity of the materials. The energy supply occurs through irradiation (in the lengths of waves ranging from 0.01 to 1 m and the corresponding frequency range from 0.3 to 300 GHz) (Gude et al. 2013; Tran et al. 2017). The usage of ultrasonic irradiation is also a technology applicable to the output of biodiesel, where at high frequencies (2–10 MHz) or low frequencies (20–100 kHz) irradiation creates molecular cavitation, which promotes the generation of local heat in a shorter time compared to conventional heating (Tran et al. 2017).

Biodiesel output using membrane technics can work in batch, semi-batch, or continuous flow. The membranes can be made of organic or inorganic material and can be used together with catalysts. In the output process, the emulsified oil is dispersed in alcohol; at the interface between the oil and methanol phases, the transesterification output takes place. As the reaction products (biodiesel and glycerol) are soluble in alcohol and the pores of the membrane are larger than the size of the formed molecules, the alcohol and biodiesel pass through the membrane, and the unreacted oil is retained (Dubé et al. 2007; Cheng et al. 2010; Baroutian et al. 2011; Tran et al. 2017).

Free fatty acids and water are harmful in the biodiesel output because, in addition to consuming the catalyst, it decreases the transesterification yield, forming soaps and unwanted products in the process (Demirbas 2005). Before transesterification, we will have the processing of alcohol to output biodiesel and water, where the water content can be removed by flash evaporation under vacuum after esterification. The efficiency of this process can be increased using either microwaves, supercritical conditions, ultrasounds, or membranes (Kombe et al. 2013; Caetano et al. 2018).

At the industrial level, biodiesel is normally produced by the homogeneous catalyzed transesterification process using methanol. The purification of biodiesel is a crucial step before storage and marketing to meet the standard specifications. The biodiesel purification methods are based on balance, affinity, membrane technology, reaction, and solid-liquid separation processes. However, in addition to the search for cheaper and more productive raw materials, another challenge is to design low-cost catalysts capable of producing excellent quality diesel oil in a one-step process (Tran et al. 2017).

10.2.2 Bioethanol and Biobutanol Production

The demand for ethanol has grown worldwide, due to the wide variety of potential applications. Ethanol can be used as a transport fuel, fuel for power generation, and important raw material in the production of polyethylene and other materials, with commercial appeal (Li and Yang 2016). With a global production of over 95 billion liters in 2015, ethanol is predicted to remain with more growth demand and best cost-effective biofuel for the coming decades, with prices close to those of gasoline (USDOE R F 2016; Gomez-Flores et al. 2018).

Traditionally, bioethanol is developed from agricultural crops such as corn, rice potatoes, and sugarcane (Kiran et al. 2014). It is worth mentioning that the first-generation biofuels, produced from food crops, have been seriously criticized for triggering food versus fuel competition and the consequent increases in the price of food (Jouzani and Taherzadeh 2015). For this reason, the FW has been an attractive substrate for the sustainable production of bioethanol, since it is composed mainly of low economic value products (Gomez-Flores et al. 2018). The conversion of these raw materials into bioethanol also contributes to the reduction of environmental damage, since most of them would certainly be discharged into landfills or in places without adequate treatment.

Despite the challenges of using FW for bioethanol production, due to their high perishability and rich composition of complex structures, many studies have been presented beneficially (Li and Yang 2016). Ethanol production from FW with high solid content (35%, w/w) reached an ethanol titer of 144 g/L in 72 hours of fermentation (Huang et al. 2015). Meanwhile, Matsakas et al. (2014) and Matsakas and Christakopoulos (2015) found 55.12 and 19.27 g/L of ethanol from household food waste, in just 21 hours of fermentation, respectively.

Although ethanol fermentation from FW presents high-performance processes, there are a number of disadvantages associated with bioethanol as a fuel (Gomez-Flores et al. 2018). For example, ethanol can only be mixed effectively with gasoline to a proportion up to 10% for use in common engines (Thakur et al. 2017). Furthermore, the transportation of the gasoline-ethanol mixture is done exclusively by tanker trucks, since the existing pipelines cannot transport it due to the high miscibility of ethanol with water, which generates even more costs for its production (Swana et al. 2011). Thus, recent research has shown an appropriate redesign of the existing bioethanol plant for transformation into biobutanol (Isah and Ozbay 2020).

Biobutanol has an energy density closer to that of gasoline amounting to 90% than any other alternative fuel used today, such as bioethanol (60%). While bioethanol has a lower heating value (LHV) of 21.3 MJ/L, the LHV of butanol is 27.8 MJ/L, closer to the value of that of gasoline (32.3 MJ/L) (Wu et al. 2008). For this reason, biobutanol can be mixed in any concentration with gasoline and can be transported through existing pipelines (Swana et al. 2011). Consequently, biobutanol presents advantages over bioethanol as liquid fuel with high hydrogen to carbon ratio, high saturation of carbon bond, and greater stability and low solubility in water (Isah and Ozbay 2020). However, a general comparison between the input and

output of nonrenewable energy is still needed for the production of biobutanol and bioethanol to support the preference for the use of biobutanol.

Commonly, the bioalcohol production process, such as bioethanol and biobutanol, from food waste, involves a chemical or physical pretreatment, followed by hydrolysis and, finally, fermentation (Kannah et al. 2020a, b). In general, the pretreatment aims to break the hydrogen bonds in cellulose, remove lignin, and, finally, increase the cellulose porosity and surface area so that polysaccharides are more accessible to the enzymes in the hydrolysis (Banu et al. 2019). Different pretreatment technologies, such as physical pretreatment (crushing, grinding, microwave, and extrusion), chemical pretreatment (alkali, acid, ionic liquids), physicochemical pretreatment (steam explosion, hydrothermal, ammonia explosion), mechanical pretreatment (ultrasonic), and biological pretreatment (using enzymes or fungal), were developed (Banu and Kavitha 2017; Kannah et al. 2018). However, each pretreatment has its specificity, advantages, and disadvantages, and the choice of the appropriate method depends on the composition of the biomass used and the final desirable characteristics (Jouzani and Taherzadeh 2015).

In the hydrolysis stage, the molecules break down, transforming them into fermentable sugars (Kannah et al. 2020a, b). Hydrolysis processes can be acidic, enzymatic, or fungal. The enzymatic reactions tend to be more efficient due to the high specificity, favoring the conversion of cellulose into sugars without degrading them. Enzymatic hydrolysis is carried out through the presence of highly specific enzymes that operate under mild conditions of pH (4.5–5) and temperature (50–60 °C) (Jouzani and Taherzadeh 2015). After the hydrolysis step, fermentable sugars will be available to be converted to alcohol. The biological process that transforms these sugars into bioethanol and biobutanol is carried out by microorganisms through fermentation (Flores et al. 2018).

Although diverse bioprocessing methods have been proposed, the main one obstacle in converting FW into biofuels is still the lack of economic technologies. Recently, diverse combined methodologies have been developed to surmount the trouble associated with individual methods. The combination of pretreatment methods such as alkaline and ionic liquid pretreatments (Nguyen et al. 2010), dilute acid and steam explosion pretreatments (Chen et al. 2011), supercritical CO_2 and steam explosion pretreatment (Alinia et al. 2010), microwave-assisted dilute acid pretreatment (Chen et al. 2011), and ionic liquids and ultrasonic pretreatment (Ninomiya et al. 2015) was developed. Still, the integration of hydrolysis and fermentation such as simultaneous saccharification and fermentation (SSF); simultaneous saccharification, filtration, and fermentation (SSFF); and simultaneous saccharification and co-fermentation (SSCF) is employed as a way of economically producing biofuels from FW (Jouzani and Taherzadeh 2015; Kannah et al. 2020a, b).

10.2.3 Biohydrogen and Biomethane Production

Hydrogen is used as a compressed gas and has been highlighted as a carrier of clean, carbon-free energy and zero emissions of greenhouse gases (Sivagurunathan et al. 2017). Its biological production is considered more environmentally friendly and lower energy intensive compared to other biofuels, with energy yield (142 kJ/g) of almost 2.8 times that of gasoline (Deng 2016).

Recently, some emerging techniques for the production of biohydrogen have been presented. Although hydrogen can be produced by photolysis and microbial electrolysis cell, these processes involved are not economically viable (Osman et al. 2020). For example, hydrogen can be produced by electrolysis of water only in places where cheap electricity is accessible. Therefore, the use of microorganisms in fermentation from food waste has great potential to expand the use of biohydrogen as a biofuel (Deng 2016).

The production of fermentative biohydrogen depends on the chemical composition of the raw material involved, pretreatment methods, inoculum type, and process configurations (Abreu et al. 2019). Higher temperatures, pH ranging from 5.2 to 8, and pretreatment with dilute acid have been widely used as they are considered more efficient and economical with regard to fermentation of FW (Nissila et al. 2014; Mishra et al. 2019). In general, the highest biohydrogen production rates are obtained by dark fermentation processes carried out in thermophilic conditions (Osman et al. 2020). In addition, biomasses rich in carbohydrates have a higher production of biohydrogen than in the presence of higher lipids and proteins in their composition (Bharathiraja et al. 2016). In fact, several authors report that the majority of FW, like processing industries, services food, and household waste, are essentially composed of specific carbohydrates, reaching more than 60% of the total dry mass content (Wang et al. 2008; Vavouraki et al. 2014; Huang et al. 2015).

Another advantage of the production of biohydrogen by fermentation from food waste is that a second stage can be coupled to the process, in which the final fermentation products, rich in volatile fatty acids (VFA), can be converted into methane by biological process divided into four stages (hydrolysis, acidogenesis, acetogenesis, and methanogenesis), the so-called anaerobic digestion (AD) (Monlau et al. 2012; Mishra et al. 2019). Conversion into biomethane under control not only reduces GHG emissions but also can be used directly as a renewable fuel or fed into the natural gas network, replacing fossil fuels (Abreu et al. 2019).

The efficiency of the anaerobic digestion process for biomethane production is mainly related to the configurations and type of reactor used (Li and Yang 2016). The continuous stirred tank reactor (CSTR) seems to have an optimal performance in short-term operations; however, they are more used in laboratory conditions due to their low biomass concentration. Fluidized bed reactor (FBR) and other fixed bed systems, such as packed-bed reactor (PBR), were more stable in long-term operations, but produced less biogas (550 L of biogas/Kg volatilize solid) when compared to CSTR (670 L of biogas/Kg volatilize solid) (Kastner et al. 2012; Li and Yang 2016). In the single-stage AD, where all reactions take place in the same reactor, they

are also widely used due to low investment costs; however, this technique ends up generating less biogas production (Deng 2016).

In contrast to these systems, several studies report better system performance when adding the two-stage process. For example, Massanet-Nicolau et al. (2013) compared single- and double-stage anaerobic fermentation systems in the production of biogas from FW. Methane production in the two-stage phase has been enhanced by 37%, and the process has been more stable and with lower periods. Han and Shin (2004) studied the two-stage process to produce biogas from food waste and the result was a loading rate of 3.63 L/L/day and 1.75 L/L/day, for biohydrogen and biomethane, respectively. Lee and Chung (2010) also showed that the two-stage process has a substantially higher energy recovery potential, in addition to being more economical by including two systems of production together (biohydrogen/biomethane).

Finally, we can note that the generation of biofuels still has some challenges to be met. Although its production has already been proven technically feasible, the whole process involved is not yet mature. However, efficiency and economy could be improved through the valuation of cheap raw materials, such as FW, and also through the use of microorganisms with high performance.

10.3 Microalgae as a Feedstock for Biofuels: Food Waste Valorization

The energy crisis of the 1970s boosted research on fuel production from microalgae. However, the technology and the cost associated with the production discouraged its commercial development. The idea of using microalgae in the production of renewable energy was diverted mainly to food products. However, the continuous research efforts intensified over the past 20 years are, fortunately, allowing the commercial potential of microalgae to shift to the production of bioenergy products (Ganesan et al. 2020).

However, although this is true, the biofuel production from microalgae is not yet profitable (Deprá et al. 2018). The high cost of nutrients is one of the bottlenecks associated with microalgae cultivation (Lowrey et al. 2015). In particular, heterotrophic, mixotrophic, and photoheterotrophic microalgae depend on sources of carbon, nitrogen, and phosphorus of high cost. An opportunity to reduce this cost is through the nutrient recovery from food wastes (Ryu et al. 2013; Haske-Cornelius et al. 2020).

The composition of the food waste makes them a promising feedstock for microalgae cultivation. Nutrients from food waste can be easily recovered by physical, chemical, mechanical, or biological methods (Kannah et al. 2018). Pleissner et al. (2013) reported the recovery of glucose, free amino nitrogen, and phosphate from food waste for microalgae culture. Studies such as those by Pleissner et al. (2013) indicate that the valorization of food waste in microalgae processes may

present a new paradigm, more sustainable and economical, for the production of microalgae biofuels.

10.3.1 Challenges of the Bioconversion of Food Wastes to Biofuels

Low value-added products, such as biofuels, establish cost as a fundamental factor in the development of new processes and technologies for obtaining it. In a worldwide transition to a bioeconomy, aimed at reducing the emission of carbon dioxide and the production of waste, the use of microorganisms has become an emerging technology. However, a major barrier to be overcome in industrial-scale production is the economics. Intracellular components such as lipids and carbohydrates play a fundamental role in the analysis of economic viability by exploring new emerging technologies for obtaining biofuels through alternative raw materials to conventional fossil raw materials (Sydney et al. 2019; Bilal and Iqbal 2019).

The processes from microalgae are unique, as they associate the fixation of CO_2 with the production of several biofuels concomitantly with the use of effluents and food residues (Fig. 10.3). However, there are some challenges for economic viability and its applicability to occur significantly. The three main challenges are (a) exploring improvements in biomass production and improving cultivation and genetic exploration practices; (b) reducing the proportion of energy in processes such as dehydration, drying, and oil extraction; and (c) adding value to biomass components through the use of biorefinery concept (Kumar et al. 2020; Cuevas-Castillo et al. 2020).

The high cost of producing large-scale crops faces the main challenge of high energy demand, to carry out the processes of sterilization, mixing, aeration, lighting, gas exchange, and others (Peng et al. 2015, 2016b; Borowitzka 2013). Sterilization

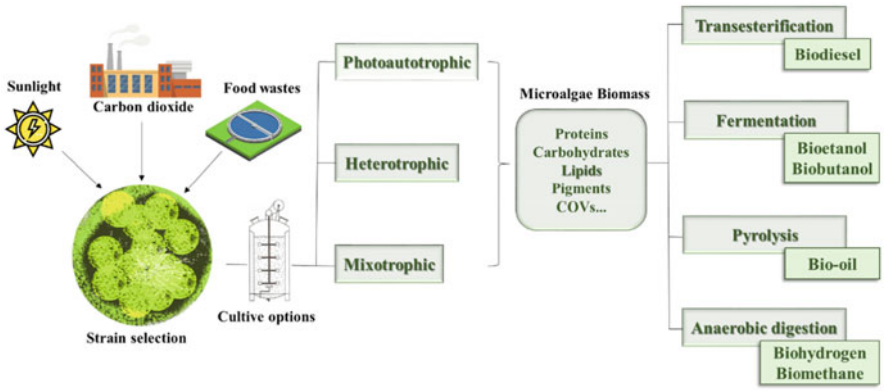

Fig. 10.3 Microalgae process for a different biofuel production. Adapted from Peng et al. (2020)

processes applied to a large volume of cultures and maintenance of this sterility (efficient lighting, deoxygenation) are expensive. Therefore, the importance of selecting and exploring the metabolic routes for the cultivation of microalgae, in the quest to reduce the cost of cultivation, and at the same time, obtaining a high concentration of biomass and lipid productivity is essential (Peng et al. 2016a).

First, it is necessary to explore strains that have desirable characteristics, are uniform, and produce low-cost nutrients or that use food residues or wastewater for the production of these constituents, thus improving the design of the reactor choice to obtain a suitable cultivation process. The second issue comes against the intensification of the processes. In addition, the third is linked to new strategies for process intensification projects, taking into account the economic aspect through the biorefinery approach (Peng et al. 2019; Cuevas-Castillo et al. 2020).

According to algaebase.org, there are more than 158,300 strains registered. Due to the large number of species available, each one with different characteristics, it becomes a critical step to choose the strain, and a robust selection must be made; however, the available information becomes limited on a large number of microalgae and cyanobacteria (Borowitzka 2013; Aravantinou et al. 2013; Borowitzka 2018).

There are several types of microalgae that are already being investigated for the production of biofuels. In general, the choice of species takes into account criteria such as ease of adaptation, high growth rate, lipid content, resistance over a wide pH range, temperature, irradiation, tolerance to oxygen, and nutrients present in the waste in the case of photoautotrophic, heterotrophic, mixotrophic, and/or photoheterotrophic cultivation (Cuevas-Castillo et al. 2020). Considering the production of biofuels from food residues, the search for strains resistant to nutrients present in the residues, especially those that in high concentrations can become toxic and inhibit growth, and high productivity of lipids becomes a decisive parameter (Queiroz et al. 2011; Griffiths and Harrison 2009; Osundeko et al. 2019).

Food waste contains essential nutrients for the growth of microalgae, such as nitrogen, phosphorus, traces of metals, and carbon. However, it should be noted that the concentrations of these nutrients vary according to each residue, including other factors such as climate. This double frontier has been well discussed as a possible way to increase the sustainability and economy of the microalgae biofuel strategy (Pittman et al. 2011; Olguin 2012; Osundeko et al. 2019).

Another point that must be taken into account in the bio-valorization of food residues for the production of biofuels is the pretreatment. Several pretreatment methods are adopted for food residues to dilute the solution efficiently (nutrients and carbon), for the valorization of microalgae (Hao et al. 2015; Zhang et al. 2016; Banu et al. 2012; Razaghi et al. 2016; Banu et al. 2011; Guo et al. 2014; Kavitha et al. 2013; Yang et al. 2015). Effective pretreatment can maximize the efficiency of the process, which will depend on the choice of microalgae applied.

Finding everything that is sought in a single lineage is another challenge. Therefore, an option is the genetic modification of these strains through the use of mutagenesis and genetic engineering, where it is possible to modify the gene and program the necessary metabolomics. The use of these techniques is essential in the use of industrial residues, aiming at greater robustness of the crop, which meets the

industrial viability of low added value products, such as biofuels based on microalgae (Bharadwaj et al. 2020).

The next step to consider is the cultivation system. Although microalgae have metabolic versatility, the most used route is photoautotrophic, but they can assume other types of metabolism, such as heterotrophic, mixotrophic, and photoheterotrophic. Large-scale cultivation of microalgae usually occurs in open tanks or closed, raceway ponds are examples (Brennan and Owende 2010; Maroneze and Queiroz 2018).

Control of metabolic pathways is completed by nutritional and environmental regulation in bioprocess engineering (Park et al. 2019). Through growth conditions, photoautotrophic cultivation refers to the supply of energy through captured light, and sources of inorganic carbon (usually CO_2) are added, converting it to chemical energy through the process of photosynthesis. When food residues are used in this type of cultivation, the use of open lagoons is generally applied, providing the nutrient sources of these residues and sunlight as an energy source. This type of cultivation is widely applied, as it is considered ecologically correct and with good performance for low-cost products such as biofuels, as they are composed of low-cost facilities and easy operation (Suparmaniam et al. 2019; Severo et al. 2019).

There are several configurations of closed system photobioreactors; these offer greater temperature control, low risk of contamination, and high cell concentrations. However, they are very difficult to expand and sanitize; over the years there may be a loss of light penetration by the material of the tubes; they need to be degassed, and they are also more expensive to build and operate than open tanks; its use is more indicated to obtain products with high added value (Suparmaniam et al. 2019; Severo et al. 2019; Jacob-Lopes et al. 2018).

Considering obtaining organic carbon and other nutrients from industrial waste, where these carbon sources can support the growth and production of cell oil and offer an economical alternative for microalgae cultures, with parallel effluent treatment, heterotrophic cultivation becomes a viable alternative (Francisco et al. 2014). Not all species have the ability to develop using this type of metabolic route; however, when viable, heterotrophic cultures can be efficiently grown in conventional fermenters, such as bubble column bioreactors, which in general are low cost and simple sizing to maintain large-scale production (Perez-Garcia et al. 2011; Francisco et al. 2014).

Mixotrophic cultivation, a variant strategy of heterotrophic cultivation, comes to overcome the limitations of the cultivation methods shown above, by combining its advantages in the search for the promotion of a high growth rate and accumulation of intracellular compounds of interest. In this case, the microorganisms simultaneously assimilate organic and inorganic carbon and use photoautotrophy and heterotrophy, thus facilitating the engineering of reactor construction and facilitating large-scale production. One of the advantages of this cultivation method is that it allows the use of food residues, wastewater, and carbon dioxide from the combustion gases generated by the industries (Meng et al. 2020).

As already mentioned, food residues are an important source of nutrients and carbon to support the growth of microalgae and cyanobacteria, but they still present

some challenges that must be considered. Depending on the origin of the waste, inhibitors such as heavy metals, nitrogen oxides, sulfur and ammonium oxides, viruses, fungi, and bacteria, among others, may be present (Osundeko et al. 2019). To overcome these barriers, application of chemical herbicides and pesticides has been a viable solution (McBride et al. 2014). Other options with great potential are the application of genetic and metabolic engineering techniques to improve the resistance of microalgae strains (Bagwell et al. 2016).

The understanding of cellular metabolisms, for the production of biomass, is a critical aspect of the manipulation of culture. The production of microalgae biomass to obtain different biofuels is considered a promising alternative. To guarantee the cost-benefit of obtaining microalgal biomass, the implementation of farming systems integrated with the use of food waste is an alternative approach to overcome this problem (Meng et al. 2020).

The use of waste generated by the industry not only reduces the cost of production, but it can also reduce the pollution caused by the industry, being environmentally sustainable. The development must also focus on other by-products obtained from a single crop applying a biorefinery approach. In addition to obtaining biofuels, there should be full use of microalgal biomass for the production of other products, so that value is added and more profit is obtained in the use of microalgal biotechnology (Meng et al. 2020; Peng et al. 2019).

10.4 Progress of the Commercialization of Microalgae Biofuels

The issue of microalgae as an alternative fuel source appeared in the mid-1970s in response to concerns about climate change stemming from the overuse of fossil fuels and the need for energy security (Khan et al. 2018). Nearly three decades after, the number of research, developments, and innovations (R&DIs) programs in the field of microalgae biofuels increased dramatically. Many university departments, technological institutes, organizations, and research centers are now working in partnership with nascent companies or those that have already consolidated market share to produce alternative fuels. Today, it is estimated that around 200 projects by microalgae biofuel companies are in operation around the world (Oncel 2013), including both demonstration-scale projects and pilot plants, as summarized in Table 10.1.

The USA is the leading country in microalgae biofuel projects. Several countries in Europe have also invested in this area. The Cellana LLC company, for example, a joint venture formed by Royal Dutch Shell and HR BioPetroleum, was founded in 2004, headquartered in Hawaii, has an area of 2.5 hectares, and produces about 8 tons of biomass and uses it to test its combustion properties as a fuel. Sapphire Energy Inc., started operating in 2007, in San Diego, California. The company has a project where it integrates the entire biofuel production chain, manufacturing about

Table 10.1 Main microalgae-based biofuel companies. FBR: photobioreactor

Company	Location	Cultivation system	Reference
A2Be Carbon Capture LLC	USA	Closed PBR	https://www.algaeatwork.com/
Algae Floating Systems, Inc.	USA	Closed PBR	http://www.algaefloatingsystems.com/
AlgaeLink N.V.	The Netherlands	Closed PBR	https://www.dnb.com/business-directory/company-profiles.algaelink_nv.40fb3e38c58eff81985528745ad89e4a.html
Algaewheel	USA	Rotating wheels (open and closed systems)	https://algaewheel.com/
AlgaEnergy S.A.	Spain	PBR at laboratory, pilot and industrial scale	https://www.algaenergy.es/
Algaecake Tech. Corp.	USA	Closed PBR	http://www.oilgae.com/ref/cap/
Algenol	USA	Different outdoor closed systems	http://www.algenolbiofuels.com/
AlgalOilDiesel, LLP	USA	Open and closed systems	http://algaloildiesel.wikifoundry.com/
AlgoSource (Alpha Biotech)	France	Raceway ponds and PBR	https://algosource.com/
Aquaflow Bionomic Corporation	New Zealand	Outdoor systems	http://www.aquaflowgroup.com/
Aurora Algae, Inc.	USA	Open ponds	http://aurorainc.com/
Biofuel Systems	Spain	Outdoor systems	https://biopetroleo.com/
Bodega Algae	USA	Closed continuous-flow PBR	http://www.bodegaalgae.com/
Cellana, LLC	USA	Different large-scale outdoor systems	http://cellana.com/
Carbon Trust	UK	Closed PBR	https://www.carbontrust.com/
Chevron Corporation (in collaboration with US-DOE NREL)	USA	Closed PBR	https://www.chevron.com/
Dao Energy, LLC	China	Closed PBR	http://www.opportunitycycle.com/
Eldorado Biofuels	USA	Open ponds	http://eldoradobiofuels.com/
ExxonMobil	USA	Different outdoor and indoor closed and open systems	https://corporate.exxonmobil.com/Research-and-innovation/Advanced-biofuels
Evodos	The Netherland	Outdoor closed and open systems	https://www.evodos.eu/
Global Green Solutions	USA	Open and closed system	http://globalgreensolutions.co.uk/

(continued)

Table 10.1 (continued)

Company	Location	Cultivation system	Reference
Green Star Products, Inc.	USA	Closed system	http://www.greenstarusa.com/
Imperium Renewables	USA	Closed PBR	https://www.regi.com/
Inventure Chemical	USA	Outdoor closed PBR	https://inventurechem.com/
Joule Unlimited, Inc.	USA	Closed PBR	http://www.jouleunlimited.com/
Kent Bioenergy	USA	Closed PBR	https://openei.org/wiki/Kent_BioEnergy
LiveFuels, Inc.	USA	Closed PBR	https://www.agriculture-xprt.com/companies/live-fuels-inc-35673
Manta Biofuel	USA	Closed PBR	https://mantabiofuel.com/
Organic Fuels	USA		
OriginOil	USA	Raceway ponds	http://dev2014.originoil.com/why-originoil/algae
PetroAlgae	USA	Raceway ponds	http://www.petroalgae.com/
PetroSun Biofuels	USA	Outdoor open systems	https://www.petrosun.us/
Phycal LLC	USA	Raceway ponds	http://www.phycal.com/
Sapphire Energy, Inc.	USA	Raceway ponds	http://www.sapphireenergy.com/
Seambiotic Ltd.	Israel	Outdoor open systems	http://www.seambiotic.com/
Solazyme, Inc.	USA	Open and closed systems	https://www.solazyme.com/
Solix Biofuels, Inc.	USA	Outdoor open systems	http://solixbiofuels.com/
Synthetic Genomics	USA	Different outdoor and indoor closed and open systems	https://syntheticgenomics.com/
Varicon aqua Solutions Ltd.	UK	Closed PBR	https://www.variconaqua.com/
W2 Energy	Canada	Closed PBR	https://w2energy.com/

100 barrels of crude oil per year on a commercial scale. The product is called "Green Crude" with low CO_2 emissions. Algenol, an industrial biotechnology company, produces known biofuels such as bioethanol, gasoline, bio-jet fuel, and biodiesel through patented algae technology. The production volume covers about 8000 barrels of liquid fuel annually (IEA 2017). In 2009, the great project of ExxonMobil entrepreneurs together with Synthetic Genomics developed advanced microalgae fuels. This partnership aims to obtain 10,000 barrels of biofuel by 2025 through genetic modification techniques for some strains to increase lipid yield to over 40% (Tang et al. 2020).

These microalgae biofuel projects attracted investments of hundreds of millions of dollars from the public and private sectors. The promise would be to produce

millions of gallons of fuel in the short to medium term at competitive costs with petrodiesel (Su et al. 2017). However, to date, virtually none of these companies claims to have attained commercial profitability. Thus, many of them changed their research goal in the near-term market to achieve financial returns. The current industrial landscape shows other products with high-added value and low volume, such as speciality chemicals, including food, feed, pharmaceuticals, and nutraceuticals (Severo et al. 2020). Examples of this are pigments, which have sale prices of around USD 790/kg (β-carotene) and 2500/kg (astaxanthin). These products could balance the production costs of microalgae biofuels (Severo et al. 2019).

Most studies indicate that the bioeconomy of producing microalgae biofuels essentially depends on biomass productivity, cultivation systems, and downstream steps. In theory, these factors appear to be efficient and would have reasonable costs. However, in practice, the scenario is quite divergent. By way of example, biomass and lipid productivity varies widely from one microalgal species to another. Some strains have high oil content, while others have high biomass yields, indicating that the productivity of both products is not precisely correlated, which makes it difficult to select the most suitable strain for this purpose. This is without mentioning the techniques of genetic modification and omics approaches, which still need more studies to unveil the mechanisms involved in the synthesis of biofuel precursors in different lineages (Misra et al. 2016). In addition, several other aspects must be considered for the production of biofuel, including the culture parameters and operational factors (Deprá et al. 2018).

In contrast, many researchers assert that the hottest point about the successful production of microalgae biofuels is due to cultivation systems. As far as is known, biodiesel mass production, for example – the most popular microalgae fuel – requires huge production volumes to meet global demand with a significant reduction in expenses to manufacture it (Singh and Gu 2010). According to Severo et al. (2019), estimates indicate that the selling price of a liter of microalgae biodiesel ranges from USD 0.42 to 22.60. That is, this barrier depends solely on the robust design of a bioreactor that can handle large-scale workloads.

Beyond cultivation, the processing and conversion steps into biofuel are responsible for approximately 70% of the total production costs. These stages are crucial in microalgae-based processes, but they are exceedingly energy-intensive. They still need to be studied on a laboratory scale to analyze their application, efficiency, and harmony with the entire production chain in order to reduce the costs of the final fuel (Oncel 2013).

Even in face of undeniable progress of the commercialization of microalgae biofuels throughout history, the future prospects for these commodities are currently more challenging than ever. Its short-term feasibility of production is still unclear and the technical, economic, environmental, and political limitations remain quite pronounced (Oltra 2011; Ganesan et al. 2020). In view of this scenario, these bioprocesses will need to adopt an integrated and intensified biorefinery strategy, where the extraction of co-products with higher added value may aid to boost the circular bioeconomy of third-generation biofuels.

10.5 Conclusion

Food waste is a promising feedstock for the production of low-carbon biofuels. The generation of renewable fuels from food waste would reduce dependence on petroleum and decrease a resource that contributes strongly to climate change. The idea of creating renewable fuels from non-food biomass is not new. There are technological routes already applied commercially with pros and cons and emerging technological routes in the development and demonstration stage. At this time, it is important that research efforts focus on simpler, cleaner, and lower-cost technological routes. Thus, we could aim to move toward a more sustainable society.

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Chapter 11
Animal Fat-Derived Biodiesel and Nano-Technology Applications

Pranta Barua, V. Dhana Raju, Manzoore Elahi M. Soudagar, and Nazia Hossain

Abstract Animal fat is being considered waste in many areas across the globe nowadays due to the health complexity caused by animal fat consumption; therefore, this animal fat is mostly dumped into an open environment. To utilize this animal fat waste into value-added product, the fat can be converted to a form of biofuel, viz. biodiesel. Due to the interruption with human food and feed chain, vegetable oil lost popularity for biofuel production. Therefore, animal fat is deemed as a potential resource to produce second-generation biofuel such as biodiesel. The main objective of this chapter is to focus on the viability of biodiesel extracted from various animal fats. This chapter also discusses whether animal fat-based biodiesel could be competitive in the commercial market compared to other upcoming renewable resources or not. The possibility of commercial success of animal fat-based biodiesel has been highlighted in this chapter. This chapter also reviews the current global biodiesel scenario from animal fat, different methodologies of extracting biodiesel from fat, and its application in the transportation and electricity generation sector. Besides biofuel generation, the environmental benefits of animal-fat-based biodiesel have been outlined in this chapter. Animal fat-based biodiesel can contribute towards a significant share among the total produced biodiesel all over the world. With the proper management and collection system this waste can be used as a potential raw material for biodiesel production.

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Keywords Animal fat · Biodiesel · Transesterification process · Slaughterhouse waste

11.1 Introduction

The by-products of animals are very high in quantity, especially from the poultry industry's meat and processing chain. Besides, because of health consciousness, people avoid animal skin as food materials (Zalouk et al. 2009). The maximum waste comes from more than 328 million sheep, pigs, goats, and hamburger and dairy steers and 6 billion chickens, turkeys, and other poultry butchered each year in Europe (Zalouk et al. 2009). Every day 12 million tons of meat supplies worldwide produced massive slaughtering-based waste (Toldrá et al. 2016a, b). This massive amount of animal waste can be the raw material for liquid biofuel such as biomass and biodiesel (Rosson et al. 2020). Biodiesel production from waste animal fat presents itself as a source of alternative fuel and contributes to waste management. Additionally, animal waste comprises the organic matter produced from meat preparing industries that come after human consumption. Biodiesel is a long chain that includes mono-alkyl esters of unsaturated fats extracted from fat or oil. The utilization of vegetable oil for biodiesel production incurs a high cost. It provoked the utilization of animal fats as a feedstock for biodiesel production. Biodiesel also presents an appealing option since it enhances the fuel properties over diesel and is non-toxic and recyclable. It likewise possesses a high flash point and a standard cetane number (CN). Biodiesel is a sustainable green fuel solution by diminishing the carbon impression because of lower CO_2 outflow contrasted with fossil diesel fuel (Nigam and Singh 2011).

Current research outcomes present some promising results on high biodiesel yield using animal fat with various catalysts. A study on biodiesel from animal fat demonstrated that low-cost biodiesel could be produced using a mixture of soybean oil and animal fat (50% volume) (Canoira et al. 2008). Various methods have been proposed in the literature to convert animal fats to energy assets (Fig. 11.1). Biodiesel from vegetable oils was a popular method because of its excellent ignition properties (cetane number and heating value), but it clashes with food and feed chain that makes the system competitive and less attractive for commercial purposes (Teixeira et al. 2010; Popescu and Ionel 2011; Mittelbach 2015; Punsuvon et al. 2015; Lazaroiu et al. 2017). The pretreatment steps of biomass for biodiesel also made the process quite expensive (Banković-Ilić et al. 2014; Barua et al. 2020a, b). Animal fats have some processing disadvantages, such as higher content of proteins, phosphoacylglycerols, and high moisture content. Besides, animal fat contains a low pH value and is acidic (> 2 mg KOH/g), which restricts soluble catalysts for biofuel production (Behçet 2015). Figure 11.1 showcase the entire procedure and products derived from waste animal fat until biodiesel production.

Various animal fats are being considered for biodiesel synthesis like chicken, beef, tallow, fish, pork, etc. A case study on chicken fat-derived methyl ester and fish fat-derived methyl ester shows that a good quality biodiesel can be derived through

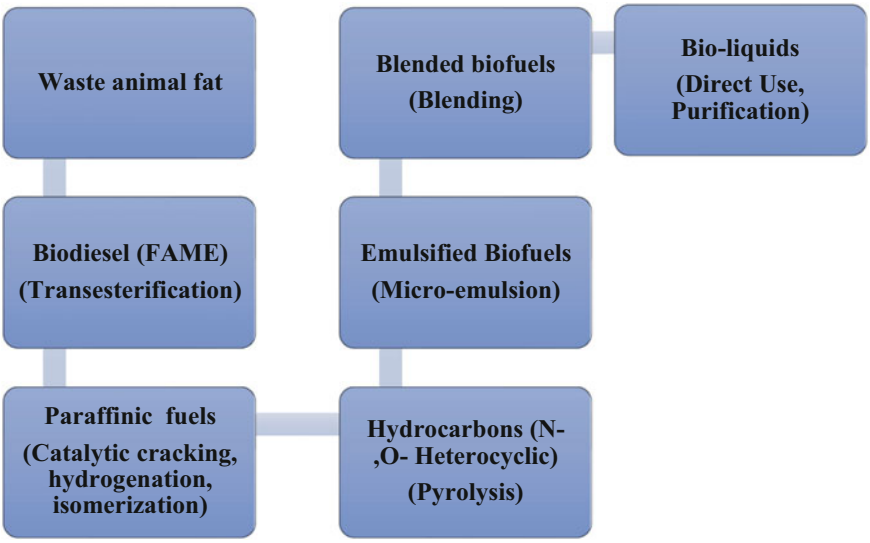

Fig. 11.1 Procedures and products obtained from waste animal fat

the transesterification process from chicken and fish fat (Encinar et al. 2011). Methyl esters from these sources are utilized as a fuel in a direct infusion, solitary chamber, cooled (air) diesel motor, and four-stroke engine; and the effects and energies on motor execution and fumes or emanations are nearly researched with diesel (D2) that maintains standard fuel property. Tests of motor highlight that limitations of biodiesel don't vary significantly from those of the diesel fuel. The exhaust gas, HC (hydrocarbon), and CO (carbon monoxide), and emission of combustion of fish fat and chicken fat methyl ester as fuel were lower than those of diesel fuel (Encinar et al. 2011). An experiment on biodiesel extraction considering three types of animal fat shows a significantly high fatty acid content of around 90% (Ravikumar et al. 2020). Another study presented that the extracted biodiesel from animal fat is mixed with diesel at different mixing ratios 30%, 20%, and 10% in volume measurement. The mixed properties of fuel like fire point, density, kinematic viscosity, flash point, and carbon content were varied for each mixture. It was also observed that the heating value of biodiesel is near to that of mineral diesel.

The mixed fuels have been experimented for contamination and execution boundaries utilizing a combustion engine motor test. The outcomes have been examined and compared to diesel fuel for the same conditions of the test. The lower calorific value of the mixture has resulted in more energy to create equal force in engines. Decreases in HC (hydrocarbon) tailpipe and NOx (nitrogen oxide) emanation are an empowering perception. B20 (mixture of 80% diesel and 20% biodiesel) in the diesel-biodiesel blend is considered ideal when contrasted with varying proportions of mix (Attia et al. 2014). All of the experiments showcased that animal fat-derived biodiesel will be more effective than other biofuels considering carbon emission prospects, fuel prices, and fuel properties. The chapter focuses on

different prospects of animal fat-derived biodiesel synthesis and its application in diesel engines and highlights a review on the future of nano-additives in biodiesel synthesis and its application in the diesel engine.

11.2 Conversion Techniques

Biodiesel is a promising substitute for petroleum due to its biodegradability, clean combustion characteristics, a wide range of raw material sources, sulphur-free atomic structure, superior lubricity property, and others. Animal fat oil contains higher viscosity and low volatile nature over petroleum diesel. Literally, Rudolf Diesel, who patented the diesel engine, had demonstrated his engine with peanut oil on 10 August 1893. Depending upon the climatic conditions, different types of vegetable oils and animal fat oils were used as alternative fuels in many countries. Many conversion techniques are widely used to convert the crude vegetable/animal fat oil into biodiesel, such as transesterification, esterification, and enzymatic production (Dhana Raju et al. 2018). Recently, various enzymes like *Candida rugosa* lipase are used as efficient catalysts in transesterification. Nonetheless, the low yield rate and response time could forestall this technique in industrial and commercial use. Novel approaches like supercritical alcohol are drawn with greater focus on biodiesel production due to its lower energy requirement and faster reaction process. However, the pressure and temperature required in these methods were like 350–600 bar and 250–400 °C, respectively, as reported by Toldrá-Reig et al. (2020a, b). Table 11.1 delineates the factors affecting the transesterification technique.

11.2.1 Transesterification

Biodiesel is a liquid fuel derived from vegetable seeds and animal fats and other lipid-bearing sources. It is recyclable, is non-toxic, and has fewer discharges than diesel, essentially during combustion. Rudolf Diesel designed the awareness of employing vegetable oil as a fuel. He utilized peanut oil as a biofuel to run the engine. So, biodiesel is a promising substitute fuel for fossil diesel. The way of

Table 11.1 Factors affecting the production of biodiesel through transesterification

Parameters	Range	Reference
Molar ratio	3:1 to 40:1	Toldrá-Reig et al. (2020a, b)
Reaction time	1–360 min	Fadhil et al. (2017)
Reaction temperature	50–400 °C	Pollardo et al. (2018)
Reaction pressure	1–600 bar	Pollardo et al. (2018)
Catalyst	0.5–5%	Encinar et al. (2011)

Table 11.2 Typical composition in primary fatty acids of beef, pork, poultry, and mutton tallow

Fatty acid	Chemical structure	Beef tallow	Pork lard	Poultry fat	Mutton tallow	Reference
Myristic	C14:0	1.7	1.6	0.4	2.1	Toldrá et al. (2004)
Palmitic	C16:0	24.4	25.2	21.3	24.1	Pattarkine & Pattarkine (2012)
Stearic	C18:0	18.7	14.6	10.4	14.5	Pattarkine & Pattarkine (2012)
Oleic	C18:1	36.5	36.5	33.6	38.4	Toldrá et al. (2004)
Linoleic	C18:2	12.5	17.5	28.4	17.2	Pattarkine & Pattarkine (2012)
Linolenic	C18:3	2.3	1.1	2.4	1.1	
Saturated fatty acids	SFA	49.0	39.5	29.2	40.3	Pattarkine & Pattarkine (2012)
Monounsaturated fatty acids	MUFA	41.0	39.6	33.1	47.2	Toldrá et al. (2004)
Poly unsaturated fatty acids	PUFA	10.1	20.9	37.6	12.4	Pattarkine & Pattarkine (2012)

converting this oil to biodiesel is called transesterification. The catalyzed transesterification produced almost all biodiesel. The catalyzed transesterification process has shown a higher yield of biodiesel at lower temperature and pressure conditions. A study reported that triglyceride and alcohol's chemical reaction generates esters and glycerine as by-products (Venu et al. 2019). In the process of transesterification, the most commonly used alcohol is either methanol or ethanol. The catalyst commonly used in the base-catalyzed process is potassium hydroxide or sodium hydroxide. Biodiesel has closer characteristics to petroleum diesel, and it also provides sufficient lubrication for the engine. Neat biodiesel can be used or may be blended with diesel at concentration up to 20% without any modification in the engine. The quality of biodiesel blend is assessed by the ASTM (American Society for Testing and Materials) standards D6571. In transesterification, animal fat oil containing triglycerides is chemically changed into fatty acid methyl esters called biodiesel. Transesterification decreases the viscosity of biodiesel without influencing the calorific value of the oil. The fatty acid composition of beef, pork, poultry, and mutton tallow is presented in Table 11.2.

11.2.1.1 Chemical Catalyst Production

The diverse nature of catalysts has been used for the production of biodiesel from animal fat oils. The selection of catalyst mainly depends upon the free fatty acid (FFA) content in the crude animal fat oil (Fadhil et al. 2017). Alkali transesterification is a well-known technique across the world for the production

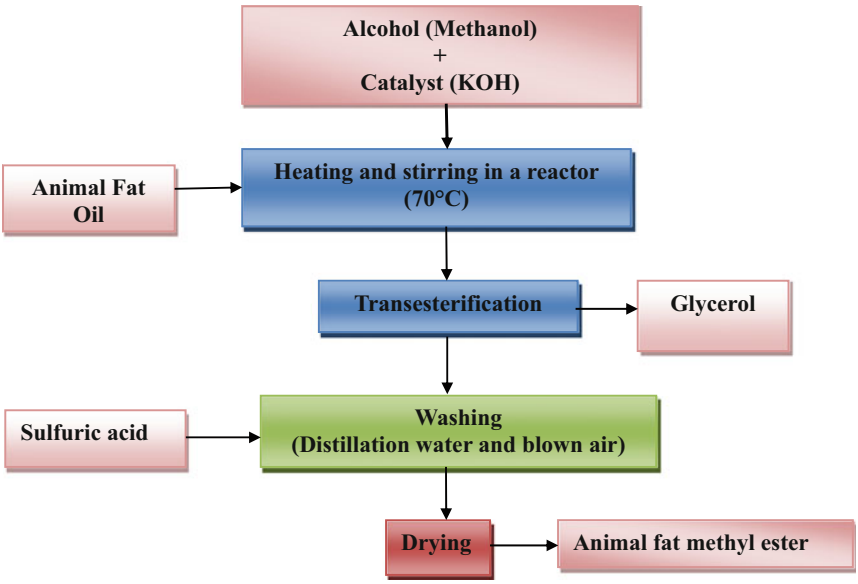

Fig. 11.2 Flowchart of the transesterification process (Fadhil et al. 2017)

of biodiesel. This technique is utilized when the FFA content is restricted to 1% in the raw fat oil. For more than 1% of FFA in fat oil, the acid catalyst method is applied. The catalysts, like sodium hydroxide and potassium hydroxide, are popularly used as the homogeneous base catalyst. The flowchart of the transesterification process is delineated in Fig. 11.2.

The major problem with the use of crude oil extracted from vegetable/animal fat is higher viscosity. The transesterification process is the best approach to lower the viscosity of raw oil obtained from animal fats. In this method, the raw oil is first heated by adding methanol up to 65–70 °C in an electrical heater. The catalyst, potassium hydroxide, is added to intensify the reaction process. They were then allowed to cool up to room temperature without disturbing for 24 hours. Then, two layers are formed, glycerin at the bottom and biodiesel at the top. Then oil is separated from glycerin and treated with water to remove any soap content. This process is repeated 2–3 times to remove the soap and dissolve salt contents. The various physicochemical properties of animal fat biodiesel are shown in Table 11.3.

11.2.1.2 Non-catalytic Production

The use of raw animal fat oils for diesel engine application is not recommended due to their higher viscosity and lower volatility than diesel fuel. These fuels do not undergo complete combustion and form deposits in the diesel engine's fuel injectors. Biodiesel is also extracted from different animal fat oils such as chicken fat, meat, tallow, lard, and fish oils. The transesterification of animal fat oils through

Table 11.3 Properties of animal waste fat biodiesel

Properties/ animal fat	Density at 15 °C	Viscosity at 40 °C	Cetane number	Acid value (mgKOH/g)	Iodine value (g/100 g)	Flash point (°C)	Pour point (°C)	Reference
Poultry fat	877	6.86	52	0.55	78.9	172	−7	Pollardo et al. (2018)
Chicken fat	883	4.92	58	0.22	75.6	171	−6	Alptekin and Canakci (2011)
Pork lard	870	4.71	56	0.23	66.8	175	−5	Encinar et al. (2011)
Mutton tallow	856	5.1	59	0.65	125	178	−5	Bhatti et al. (2008)
Beef tallow	871	5.35	57	0.21	45	171	6	Pollardo et al. (2018)

supercritical methanol, propanol, butanol, and ethanol has already demonstrated an efficient, viable process for biodiesel production. In recent times, biodiesel's non-catalytic production from animal fats with supercritical methanol (SCM) has been used to generate more biodiesel yield (Demirbas 2006). Biodiesel yield in the supercritical process is mainly influenced by factors like temperature, reaction time, and pressure on the solvent's multiple properties in the process.

The range of temperature, pressure, molar ratio, and reaction time used in biodiesel's non-catalytic production from animal fat oils are 523–573 K, 10–25 MPa, 1:6–1:30, and 7–15 min, respectively, as reported by Demirbas (2006). It is also revealed that the increasing reaction temperature, particularly in the case of supercritical temperatures, had a significant effect on biodiesel yield. In the supercritical transesterification process, biodiesel yield enhanced from 60% to 95% in 10 min.

11.2.2 Esterification

The chemical treatment of alcohol with a carboxylic acid to produce the esters is referred to as the esterification process. It is also referred to as the process of changing the fatty acids into esters. Esterification is one of the efficient methods used for the production of biodiesel. It works to reduce the FFA level in animal fat oils. An acid-catalyzed transesterification method will eliminate most of the FFA from animal fat oil. Whenever the level of free fatty acid is high, then the esterification process is used. It is a two-step process. A high temperature or faster stirring rate could drive the acidic esterification to transform FFA to methyl ester. An experimental study by Adewale et al. (2015) suggested using H_2SO_4, HCl. and H_3NSO_3 as catalysts and methanol as alcohol for the pretreatment of animal fat oils. The esterification process could lead to lower soap formation and produce a higher yield of alkyl esters.

In this method, a acid, like sulphuric acid, is mixed with methanol and stirred until the sulphuric acid is dissolved in methanol. Later, the solution is added to the animal fat oil in the required molar ratio and again stirred to combine with the animal fat oil solvents. This solution was heated at 60 °C for a specific reaction time. Then, the mixture was settled over time, and two phases were identified after pretreatment. The combination of methanol, sulphuric acid, and water is noticed in the upper grade. Water is noticed in the upper stage, where animal fat and esterified FFAs are at the lower stage. The upper phase solution is removed. Further, the lower phase was again thermally treated, subjected to heating of 110 °C for 60 min to eliminate the alcohol and water content (Chakraborty et al. 2014). Finally, the acid value of the fat-ester mixture was measured.

11.2.3 Enzymatic Production

The conventional technique for biodiesel production involves the use of acid and base catalysts. This process involves high processing costs and environmental issues. In recent times, it is found that enzymes like lipase can be used to catalyze the transesterification by immobilizing them on an appropriate support. The reaction process can be operated at a temperature of 50 °C, which is low compared to the other methods. Further, the enzyme can be reused. In this context, the enzymatic production of biodiesel is one of the viable approaches. Also, the enzymatic technique is eco-friendly and treated as a green reaction (Pollardo et al. 2018). The enzymatic transesterification process produces high-purity biodiesel and enables quick separation from the by-product, called glycerol.

Figure 11.3 represents the enzymatic production of biodiesel. The enzyme that is found to be capable of catalyzing methanolysis is lipase. It is extracted from the sources like micro-organisms such as *Candida antarctica*, *Mucor miehei*, *Pseudomonas cepacia*, and *Rhizopus oryzae*. Enzymatic production is obtained by the use of extracellular and intracellular lipases. The enzyme is immobilized and reused in these two methods, eliminating the later process like separation and recycling. Overall, biodiesel production by applying extracellular and intercellular immobilized enzyme processes is highly efficient compared to using free enzymes.

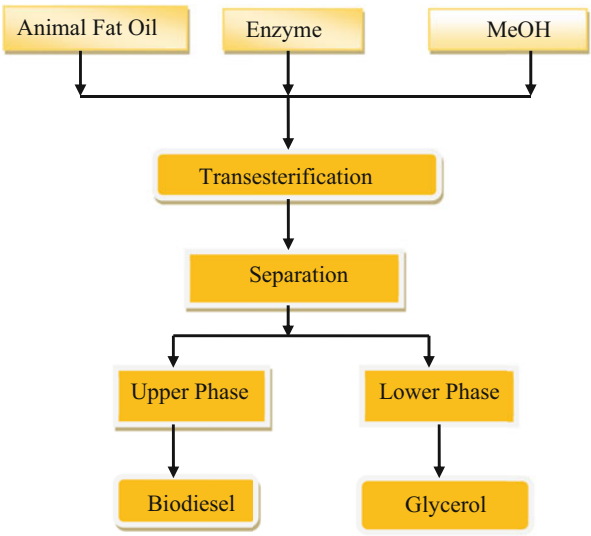

Fig. 11.3 Enzymatic production of animal fat biodiesel (Pollardo et al. 2018)

11.3 Application of Animal Fat-Derived Biodiesel in Diesel Engine

In 2014, approximately 328 million farm animals (cows, herd, pork, and goats) and 6 billion poultry livestock (especially turkeys and chicken) were slaughtered in Europe for meat consumption (Zalouk et al. 2009). A substantial sum of slaughtered livestock generates massive quantities of animal waste, including fats to be processed to minimize eco-pollution or reprocessed to provide additional value (Toldrá et al. 2016a, b). Figure 11.4 shows the flowchart for fat processing in slaughterhouses. The fats present in the animal waste contain 16 to 18 carbon chains, which is ideal for biodiesel production (Öner and Altun 2009). Biodiesel is synthesized using an acid or base catalyst by transesterification reaction of a fat with a short-chain alcohol (Soudagar et al. 2018a, b, Soudagar et al. 2020a, b). The use of methyl ester derived from waste animal fats has significant benefits: reduction in the emissions of polycyclic aromatic HC by 75–90% and UHC (unburned hydrocarbon) produced from animal fats by 90% compared with traditional diesel fuel (Carraretto et al. 2004). The SOx (sulphur oxides) and CO emissions are lowered when animal fats are used as biodiesel in a diesel engine (Shaghaghi et al. 2020), and a reduction in PM (particulate matter) and NOx emissions was also reported (Alptekin and Canakci 2011, Ramos et al. 2019). The CN (cetane number) of biodiesel is derived from animal fats, owing to its high concentration of saturated fats (40%), reduced carbon concentration, and higher oxygen content more than 50 and more than that of the biodiesel synthesized from the plant oils (Cernat et al. 2019). Animal fat methyl esters have a flash point of <150 °C, thereby enhancing transport and storage

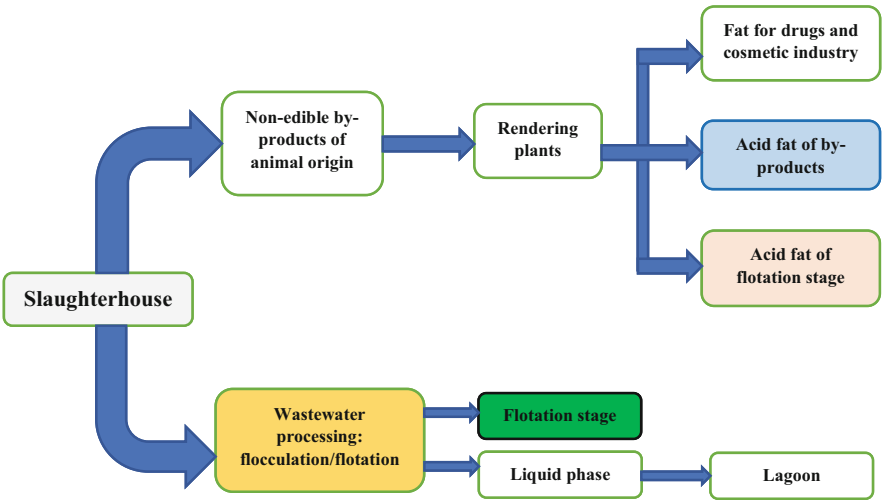

Fig. 11.4 Fat processing in slaughterhouses (Feddern et al. 2011)

security. Biodiesel offers better lubricity that maintains an extended diesel engine life (Basha et al. 2009).

11.3.1 Animal Fats: Engineering Applications

During the manufacturing process of various products from the non-edible fats derived from animal, biodiesel (methyl esters, (BD)) is a significant application in the manufacturing industry. The products of non-edible animals are classified into three groups in the European Union (EU), identified according to their hazard to animals' or human beings' health. Category (Cat.) 1 carries the maximum risk; likewise, Cat. 2 has the higher risk, whereas Cat. 3 carries minimum risk. Risk is ideal for food intake for humans, even though it is not commonly used for the nutrition of human beings due to some of its inedible content or for commercial purposes. The primary use of products in Cat. 3 is feed for pet care. Fats from all three groups will, in any case, be used to produce BD, and few stakeholders have indicated that Cat. 3 offers an enhanced efficiency to produce BD. For cattle, almost 26 of kidney and cod and 81% knob, caul, lung, and channel fats are recommended for biodiesel processing. For sheep, caul fat (88%), lung fat (43.3%), and knob and channel fat (67.1%) are recommended to produce BD. In 2019, BD synthesis from fats derived from animals and vegetable oil feedstocks surpassed 13 million tons in Europe (Walsh 2014, Toldrá-Reig et al. 2020a, b, Haye 2016).

11.3.1.1 Energy

New markets had to be established due to the ban on the feed, removing a few animal goods from the feed chain. As a source of energy for the steelworks and incineration, the fats are used to produce steam for these industries, and the animal fats were used as a fuel in the rendering industry in steam-raising boilers. Before the biodiesel market emerged, the only application for fats in the EU was for utilization in the thermal boilers. Burners were substituted in thermal boilers to comb coal, mineral oil, and fat grease. The use of their fat rendered power plants self-sufficient for producing energy. Nowadays, animal fats are used extensively in boilers to substitute mineral oil and gas due to its inefficiency and lack of development to be used as a fuel in automobiles (European Committee for Standardization n.d., Park et al. 2020).

11.3.1.2 Biodiesel

The production of methyl esters from fats derived from animal and vegetable oil feedstocks has become increasingly widespread in the European Union since the late 1990s and soon after in the United States. Biodiesel is a methyl ester of fatty acids

and can be synthesized from any plant oil or animal fat. It has the same ignition properties and can be mixed at all concentrations as mineral diesel. This suggests that it could be directly utilized in diesel vehicles and trucks. This has ended up in the biodiesel industry in recent decades, becoming the fastest growing single market for fats. Sustainability tests of various biofuels were demanded and measured after the 'food or fuel' debate in the European Union arose. Default values for the most common biofuels have been issued in the Renewable Energy Directive. Biodiesel derived from animal fats and used cooking oil (UCO) has an 81% saving capacity for GHG emissions, which is almost 2.5 times higher than soybean-derived biodiesel (31%). This is because the fats from animal meat waste are not processed for this reason but are a derivative of the meat chain. Therefore, the entire upstream chain is also not included in this default value. In addition to this excellent benefit, the EU supports the production of surplus and waste biofuels. Biodiesel from animal fats, which can only be utilized for technical, non-feed, or food uses (Categories 1 and 2 and UCO), is double the biofuel limit (Kubiak 1947; Toldrá et al. 2004; Toldrá-Reig et al. 2020a, b). Barua et al. (Barua et al. 2020a, b) reported that in Bangladesh, biodiesel synthesized from waste chicken skin fats is cost-effective with the smallest carbon footprints compared to biodiesel derived from vegetable oils. The biodiesel produced from waste chicken fats is half the cost of diesel fuel production, with a yield of 95% for the specific catalyst. Also, it fulfils the need for energy in faraway places and rural communities where there is no access to energy and electricity. The processing and synthesis of biodiesel from fats of animal waste is more complex and expensive than the synthesis of BD from vegetable oil. This is due to familiar elements such as salts, arsenic, sulphur, and plastics that cannot be eliminated entirely in the preceding phase. However, because animal fat is merely a by-product of the meat chain, its use for biodiesel is still related to the processing of meat and cannot, as such, be increased. This implies that it can only be a part of the solution. Fats (with a low melting point) or UCO have also been documented to be used directly in the engine of heavy vehicles like trucks. Table 11.4 shows outcomes on the investigation of animal waste fat applied in compression ignition (CI) engine and depicts the future of animal fat-derived biodiesel.

11.4 Technological Advancement of Biodiesel Synthesis with Nano-Additive

11.4.1 Nano-Additives for Biodiesel Production and Synthesis

Nano-additives have been playing a lead role in biodiesel production and synthesis recently. Different nano-additives, e.g. cerium oxide (CeO_3), alumina (Al_2O_3), zinc oxide (ZnO), titanium oxide (TiO_2), cobalt oxide (CoO), iron oxide (Fe_2O_3), copper oxide (CuO), and carbon nanotubes (CNT), are recently being used in biodiesel

Table 11.4 Preceding investigation on the influence of waste animal fat-based biodiesel on compression ignition (CI) engine

Names of mixed fuels derived from biodiesel	Density (kg/m^3)	Flash point (°C)	Kinematic viscosity (cm^2/s)	Heating value (kJ/kg)	Cetane number	Reference
B100	873	85	4.1	39,500	–	Gardy et al. (2017)
B100A30C30	874	83	4.1	40,200	–	Gardy et al. (2017)
B20	843	55	2.58	41,700	–	Gardy et al. (2017)
Synthesised biodiesel and $TiO_2/PrSO_3H$	898	171	4.8	–	–	Harsha Hebbar et al. (2018)
B20A30C30	844	52	2.59	42,200	–	Harsha Hebbar et al. (2018)
BCME	875	155	4.78	40,320	–	Yuvarajan et al. (2018)
Diesel	840	60	2.63	42,500	–	Yuvarajan et al. (2018)
MOMET100	884	–	4.34	37,854	54	Yuvarajan et al. (2018)
MOMET200	891	–	4.38	37,652	57	Yuvarajan et al. (2018)
JB2025GNPs	850.1	–	4.05	41,160	52.3	Yuvarajan et al. (2018)
JBD50CNT	897.9	81	5.33	39,780	57	Yuvarajan et al. (2018)
JBD25AO25CNT	895.2	81	5.36	39,990	57	El-Seesy et al. (2018)
JBD	895	85	5.25	38,880	53	Yuvarajan et al. (2018)
Diesel	830	50	2	42,300	46	Basha and Anand (2013)
D70C10E20	820	11	2.35	39,000	44.6	Basha and Anand (2013)
E15	890	–	11.4	36,160	–	Yang et al. (2013)
Diesel	850	–	2.8	45,000	–	Yang et al. (2013)
E10	880	–	8.8	38,250	–	Yang et al. (2013)
HOME25CNT	898	166	5.7	34,560	–	Tewari et al. (2013)
HOME	880	170	5.6	36,016	–	Tewari et al. (2013)

(continued)

Table 11.4 (continued)

Names of mixed fuels derived from biodiesel	Density (kg/m^3)	Flash point (°C)	Kinematic viscosity (cm^2/s)	Heating value (kJ/kg)	Cetane number	Reference
HOME50CNT	900	164	5.8	34,560	–	Tewari et al. (2013)

HOME Honge oil methyl ester, *CNT* carbon nanotube, *E* ethanol, *JBD Jatropha* biodiesel, *MOME* mustard oil methyl ester, *MOMET* mustard oil methyl ester with TiO_2 nano-additive, *BCME* n-Butyl carbamic acid methyl ester

synthesis. Unconsumed oil-based biodiesel is outstanding among other sources of energy. The expansion of nano-metal has added substances to biodiesel by different structures that essentially modify the properties with improvement, adding upgraded execution with decreased discharges. Different types of nano-additives are being addressed with other properties and activities like antioxidants such as PG (propyl gallate), TBHQ (tert-butyl hydroquinone), BHT (butylated hydroxytoluene), BHA (butylated hydroxyanisole), and PA (pyrogallol); oxygenated additives (methanol, ethanol, dimethyl carbonate, biodiesel, dimethyl ether, diethyl ether, diethylene glycol, sorbitan monooleate, and others); additives to improve cold flow behaviour (glycerol ketals, phthalimide glycerol acetates, ethylene-vinyl acetate copolymer, and succinimide copolymer); and additives to improve cetane number (nitro-alkanes, nitrates, peroxides, nitro-carbonates, and others) (Brennan and Owende 2010; Aalam et al. 2015; Arockiasamy and Anand 2015; Shaafi and Velraj 2015; Aalam and Saravanan 2017).

Nano-additives can be doped with biofuel using different methods like plasma arcing, sol-gel, ball mill, and others (Kannan et al. 2011; Ramesha et al. 2019). Alumina (Al_2O_3), carbon nanotubes (CNT), aluminium (Al), cerium oxide (CeO_2), silver (Ag), and graphene nanoparticles blended with B100 are responsible for the reduction of flash point and improvement of the value of density and viscosity. CNT and graphene contribute to increasing the value of the flash point (Ribeiro et al. 2007; Shams et al. 2013).

11.4.2 Nano-Additive Application for Diesel Engine Implementation

In diesel engine implementation, nano-additive in the diesel-biodiesel blend is added to improve diesel engine fuel properties, for instance, reducing the exhaust emission, ignition delay time, and flash point; improvement of oxygen concentration and viscosity index; and others (Debbarma and Misra 2018). Various metal nano-additives are now applied in the diesel-biodiesel blend for the diesel engine to improvise the fuel property. For example, for reduction in NOx production, cobalt is used. Magnalium particles are famous as they act as heat sink through reducing temperature (Keskin et al. 2018; Soudagar et al. 2018a, b). Manganese particle takes part in reducing GHG emissions. Another study found out that the TiO_2

nanoparticles help in hydrogen production from water because of the catalytic photoelectric effect and can contribute to activation of molecular bond in the emulsion of water-diesel (Ichikawa 1997; Shaafi and Velraj 2015).

Different research and investigations showcased that biodiesel blending in diesel engines causes an increase in greenhouse gas emissions; most importantly, NOx emission is at its maximum level, which is alarming and disappointing. Different studies and experiments are performed on injecting. Different studies and experiments are performed on injecting a mixture of nano-additives, which brought a noteworthy change and modification in the diesel engine. $TiO_2/PrSO_3H$, multi-walled carbon nanotubes (MWCNT), Graphistrenght C100, Zn/CaO, and others are applied in diesel-biodiesel blend in a diesel engine, which reduces health hazard and GHG emission (Aneggi et al. 2006; Mohan et al. 2008; Selvan et al. 2009; Berner et al. 2013; Gumus et al. 2016). The combination of carbon nanotube and the silver nanoparticle plays a significant role in reducing CO emissions compared to neat diesel. Minimum reduced CO was measured to be 25.17% in B20 blend diesel engine, whereas, in neat diesel engine, the percentage was 22.48% (Khalife et al. 2017). Improper oxygen supply causes incomplete combustion, which can be resolved by oxygenated nano-compounds that increase A/F ratio (Dec 1997; Shi et al. 2006; Prabu 2018). The mixture of nano-additives in the diesel-biodiesel fuel blend brings about different fuel property changes. Table 11.5 highlights the modification of fuel properties in a pure diesel engine or diesel-biodiesel combination.

The literature and reviews available show extensive progress in the addition of nano-additives in diesel engine applications. Metal nano-additives showcased a reduction in the viscosity, density, and flash point despite improving the oxygen content. However, some antioxidant additives increase the cetane number and the flash point. In current studies, the oxygenated additives are applied because of their low price and available synthesis devices.

11.5 Conclusions

Animal fat-derived biodiesel is a promising green fuel among the other renewable resources. This type of biodiesel is not commercially viable due to multiple limitations such as low viscosity, density, and inadequate calorific value. Lab-scale research is ongoing to enhance the physicochemical characteristics of pure animal fat-based biodiesel and mixture with other fuels for practical diesel engine applications. Based on earlier experimental outcomes on biodiesel production from various animal fats, catalyst application during the conversion process, and realistic implementation for fuel purposes, this chapter concludes that animal fat is the more standard and environment-friendly raw material for biodiesel. A brief discussion on the implications of nano-additives in diesel engines in this chapter highlights the potentiality of animal fat biodiesel in upcoming decades. After reviewing all discussions and studies, it is reported that biodiesel extracted from waste animal fat can be considered for more research to overcome the limitations and shortcomings to make it more feasible in the diesel engine in the near future.

Table 11.5 Fuel properties after the mixture of nano-additives in the diesel engine application

Biofuel blends	Biodiesel	Engine names	Results	Reference
B20 and D100	Chicken waste fat oil	SC, 5 HP, 4-S, CI, ECD, WC, CR 17.5: 1, Kirloskar	• Enhancement in calorific value (CV) and flash point • Improvement in brake thermal efficiency (BTE) compared to diesel • Increase in brake-specific fuel consumption (BSFC) owing to lower heating value of biodiesel	Kinnal et al. (2018)
B10 (DCFME10), B20 (DCFME20), B30 (DCFME30), B40 (DCFME40), D100	Waste chicken fat	SC, 180 bar IP, CR 17.5, 1500 rpm, 23°BDTC, ECD, Kirloskar	• For DCFME30 fuel, BTE reduced by 4% compared to diesel, but better than other fuel blends • BSFC increased by 26% • EGT reduced by 4.9% • CO, HC, and smoke reduced by 24.4%, 22.9%, and 14.4% compared to D100 at max. load; NOx enhanced by 11%	Barik and Vijayaraghavan (2020)
B20, D100	Chicken fat oil	SC, VCR, 4-S, CI, ECD, WC, CR 17.5: 1, Kirloskar	• Enhancement in CV and flash point • 3% rise in BTE compared to diesel • B20 fuel blend reduces the BSFC • Lower HC and CO emissions in comparison to diesel	Yusuff et al. (2017)
B10 (PYD10), D100	Chicken slaughter waste	SC, VCR, 3.5 kW power, DI, 4-S, CI, CR 17.5: 1, Kirloskar	• CO and HC for B20 blend reduce by 20.83% and 9.68% compared to diesel at max. Load • CO_2 and NOx emissions for B20 blend reduce by 8.19% and 19.07% at max. Load • BTHE increases by	Janarthanam et al. (2020)

(continued)

Table 11.5 (continued)

Biofuel blends	Biodiesel	Engine names	Results	Reference
			9.19% • BSFC reduces by 10.52% compared to D100	
B5, B25, B50 and B75, D100	Beef tallow	SC, 220 bar IP, CR 17.5, NA, WC, 5.2 kW power, 1500 rpm, 23°BDTC, ECD, Kirloskar TV1	• BSFC increases in comparison to D100 for all blends • Slight drop in BTE; NOx slightly increases for all fuel blends; B100 showed lower NOx • CO and HC reduce by 24.7% and 32.5% for B100 compared to D100 • Smoke reduces in comparison to D100 for all blends	Selvam and Vadivel (2012)
B10, B20, B30, D100	Tallow	SC, VCR, 3.5 kW power, DI, 4-S, CI, CR 17.5: 1, EGR, IC engine software, Kirloskar	• Ignition delay and combustion duration reduced for tallow biodiesel • BTE slightly reduced by 5% compared to D100; SFC increased by B10 (2.85), B20 (4.28), and B30 (7.14%) • MGT, HRR, BP, and output torque were elevated for D100 with max. variation of 4.4%, 15.06%, 1.7%, and 8%	Gautam and Kumar (2020)
B25, B50, B75, B100, and D2	Waste anchovy fish oil	SC, AC, DI, 10 HP, 18/1 CR, Rainbow-186 diesel	• BSFC for all fuel blends increases in contrast to D100 • Slight decline in BTE for all BD blends • NOx slightly increases for all fuel blends compared to D2; B100 showed the highest NOx • CO and HC lowered by 21.35%	Behçet (2011)

(continued)

Table 11.5 (continued)

Biofuel blends	Biodiesel	Engine names	Results	Reference
			and 33.42% for B100 compared to D2 fuel • Smoke reduced by 19.02% and 22.33% for B75 and D100 compared to D2	
B10, B20, B30, D100	Lard oil	SC, VCR, 4-S, CI, ECD, WC	• CO, HC, NOx, and smoke reduced by 20%, 4%, 14%, and 3% for B20 fuel blend compared to diesel • Brake thermal efficiency (BTE) and brake-specific fuel consumption (BSFC) reduced slightly by 3% compared to D100	D'Souza et al. (2015)
B20 (pork lard methyl ester-PLME20), D100	Pork lard	SC, AC, 4.4 kW power, 19–23°BDTC, DI, 4-S, CI, CR 17.5:1, 200–240 bar IP, Kirloskar TAF1	• PLME20 fuel illustrated higher BTE and lower brake-specific energy consumption (BSEC) at 240 bar and 21° before top dead Centre (bTDC) • At 240 bar HC emissions reduced by 14%, while at 21° bTDC reduced by 23.5% • Brake-specific carbon monoxide (BSCO) emissions reduced by 30% for PLME20 fuel at 240 bar retard injection timing (IT) • At 21° bTDC retards IT and 200 bar injection pressure (IP), brake-specific oxides of nitrogen (BSNOx) reduced by 9.1	Ashok et al. (2019)

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Chapter 12
Potential Microorganisms for Power Generation via Microbial Fuel Cells

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Abstract Bio-electrocatalyst (microbes) is the most important component of microbial fuel cells (MFCs). Microbes act as a biocatalyst to generate reducing/oxidizing power. Primarily extremophiles are used as biocatalyst to obtain the desired redox reactions. They show electrocatalytic action by oxidation-reduction process via metabolic reaction to transport the electrons. The strategy involves formation of electrochemical cell with electrode chambers that may or may not be separated by the semi-permeable membrane. Electroactive biofilm formation takes place over these electrodes to harness the reducing power (reduction involves generation of NADH and $FADH_2$). Concomitantly, metabolic reactions are necessary for the production of high-energy electron (e^{-1}) for generation of electricity in MFCs. Microbes for the above purpose are isolated from highly polluted areas such as wastewater, lake sediment, and soil. These microbes range from algae, bacteria, cyanobacteria, fungi, eubacteria, etc. Microbial selection is based on their ability to consume a wide range of substrates, such as fatty acid, alcohols, gases, cellulose, etc. in MFCs. Microbes contain specialized protein carrier which help in redox on respective electrode. This chapter discusses the variety of biocatalysts (microbes) that are applied in MFCs.

Keywords Microbial fuel cells · Biocatalyst · Electrode · Redox · Biofilm

12.1 Introduction

Microbial fuel cells (MFCs) bear similarities with chemical fuel cells which generate energy through fuels via chemical reaction without involving combustion. Like them, MFCs have anode-cathode, electrolyte, and catalyst. While other components of MFCs are like their chemical counterparts, the catalysts are of microbial origin. Electrocatalytic microorganisms generate H^+ and e^{-1} from their metabolism and transport them to suitable redox agent (Logan and Rabaey 2012). Electrode

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compartments may or may not be partitioned by semipermeable membrane in MFCs (Butti et al. 2016). The large range of electroactive microorganisms comes from eubacteria (Liu et al. 2002), archaea (Dopson and Holmes 2014), fungi (Fernández de Dios et al. 2013), algae (Chandra et al. 2012), photobacteria (Venkidusamy and Megharaj 2016), and cyanobacteria (Lea-Smith et al. 2016). They are isolated from a wide range of environments and have the capability to oxidize a wide variety of organic molecules to carbon dioxide and water (Venkata Mohan et al. 2014a, b). Yet understanding the relation between electroactive microbes and electrodes is a major challenge in developing efficient MFCs. This could be accomplished by understanding the basic biochemistry of biocatalyst fixing, electrode interactivity, and electrode charge transport. Apart from power generation, MFCs can be applied to synthesize a variety of useable products while addressing the problem of sewage treatment, bioremediation, eutrophication, biomass generation, etc. The application and efficiency of MFCs greatly depend on the nature of microorganism (biocatalyst) used and this chapter focuses on them. It is important to note that these biocatalysts are majorly of bacterial origin.

12.2 Working of MFCs

Electroactive microorganisms oxidize a variety of organic substrates and generate power via extracellular electron transfer (EET) when grown in electrochemical cell with electrodes. The real challenge in establishing MFCs is to discover microbes that metabolize organic matter while transferring the electrons to the anode (Debabov 2008). The approach for isolating microbes from different sources that can contribute to power generation is by collecting environmental samples and selecting them based on their ability to colonize electrode surfaces, i.e., they should be able to reduce/oxidize minerals, while the enrichment steps involve their colonization of the electrode surface in the form of biofilm (Jung and Regan 2007; Kim et al. 2007; Lee et al. 2008).

12.3 Biocatalysts of Prokaryotic Origin

12.3.1 Sulfur-Reducing Bacteria

Bacteria used in MFC can survive on oxygen-rich or oxygen-insufficient condition by using different e^{-1} acceptor. Oxygen acts as terminal e^{-1} acceptor in aerobic bacteria during energy generation, while anaerobic bacteria can use NO^{3-}, SO^{4-}, metals, etc. as a terminal e^{-1} acceptor (Venkata Mohan et al. 2014a, b). Few bacterial species are also able to modify the oxidation state of metals. These electroactive bacteria can modify the oxidation state of metals and hence are used for immobilization of radionucleotides to prevent their spread and degrade pollutants

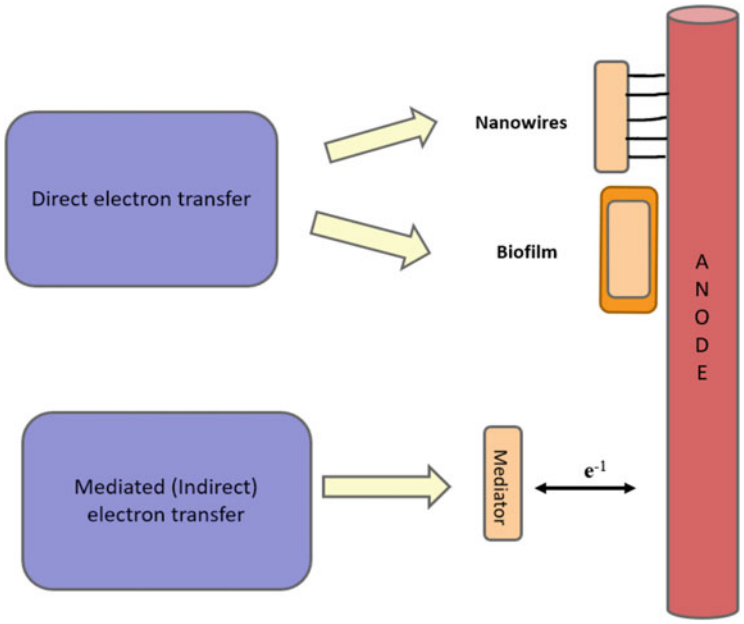

Fig. 12.1 Direct and mediated electron transfer in MFCs [Adapted from Cao et al. (2019)]

present in a wide range of environments such as sea, aquifers, lake, etc. (Kumar et al. 2017). *Geobacter sulfurreducens* and *Shewanella oneidensis* represent the example of electroactive bacteria that not only use oxygen and nitrates as terminal e^{-1} acceptors but can also use iron, manganese, sulfur, and nickel. From the large community of electroactive bacteria, only few have been isolated. Once isolated they are analyzed for growth, their role in the environment, and their gene markers. These microbes can be applied in biochemical cycle and pollutant removal process (Ilbert and Bonnefoy 2013). *Geobacter sulfurreducens* and *Shewanella oneidensis* are the most investigated electroactive bacteria. Though many bacteria engage in redox reactions, the mechanism is far less well known on the biocatalyst side.

The transport of electrons in *Geobacter sulfurreducens* and *Shewanella oneidensis* is facilitated by specialized electrical canals that carry the electrons through cell membranes. But still these bacteria face several challenges while acting as extracellular e^{-1} acceptors or donors during metabolism (Lovley 2012; Shi et al. 2016). To exemplify, neutrophilic bacteria need to avoid accumulation of insoluble ferric and ferrous form on their surface (Bond et al. 2012). Bacteria in MFC, which are placed near the electrode, have the greatest accessibility to terminal e^{-1} acceptor but have lesser reach to nutrient. On the contrary, those placed on the surface of biofilm are in lesser contact with electrode surface but have higher accessibility to the nutrients. Surface-based biofilm bacteria have various ways of transporting electrons, i.e., direct and meditated (indirect) mechanisms, from the biofilm surface to the electrode surface (Fig. 12.1) (Cao et al. 2019). Direct electron transfer, as the

name suggests, is mediated by e^{-1} transporting cytochromes (Bond et al. 2012) and by pili which possess nanowires to transfer e^{-1} from the surface to electrode. The main advantage of it is that it can conduct electricity to longer distance (Kracke et al. 2015). Direct electron transport is mediated by MtrAB porin cytochrome complex which exists in e^{-1} active bacteria such as *Geobacter sulfurreducens* and *Shewanella oneidensis* (White et al. 2016).

Comparatively, less challenging is the e^{-1} transfer between electrode and bacteria through soluble redox mediators. The cellular membrane is crossed by the mediators facilitating e^{-1} transport between electrodes and electroactive bacteria. The examples of mediators are flavins, phenazines, and siderophores. These three mechanisms work in accordance with environmental pressures (Richardson et al. 2012; White et al. 2016).

The genetics of the external e^{-1} transfer is well studied in *Shewanella oneidensis* MR 1. Metal-reducing (Mtr) pathway present in it uses iron and manganese as a terminal e^{-1} acceptor (Shi et al. 2012). C type cytochrome complex oxidizes quinol present in cytoplasmic membrane, which is shuttled to insoluble e^{-1} acceptors present outside via periplasmic membrane through channel proteins (Pawar et al. 2022).

Nanowires in *Geobacter sulfurreducens* are physically attached to the electrode by pili-like organelle. The e^{-1} transfer mechanism through nanowires is under examination and two hypotheses are being put forward to explain it. According to first hypothesis, aromatic amino acid allows delocalization of e^{-1} by π stability (Malvankar et al. 2015), while the second hypothesis proposes "super exchange model," according to which e^{-1} transfer happens by direct transfer to redox proteins (Shi et al. 2016). This system also possesses multiheme C type cytochrome along with other channel proteins which assist in donating an e^{-1} to external electrode (Pawar et al. 2022). *Geobacter sulfurreducens* form thick biofilm and superior extracellular e^{-1} transport which make it one of the best electroactive bacteria.

12.3.2 Pseudomonas

For cell-to-cell communication, one of the vital signaling mechanisms in bacteria is quorum sensing. This mechanism is a universal regulator in accordance with cell density, and the advantage of this mechanism is that individual cells show coordination as a single unit with the surroundings. In simpler words, quorum sensing aids in collective decision making like pathogenesis and biofilm generation. In previous decades, quorum-sensing proteobacteria *Pseudomonas* were tested in MFC for bioelectricity generation (Yong et al. 2011). Electricity generation by these bacteria is mediated by the synthesis of phenazines, which transport e^{-1} between bacteria and electrode. Synthesis of phenazines is coordinated by quorum-sensing pathway, while PqsE can upregulate phenazine production in the absence of quorum-sensing system (Wang et al. 2013). Extracellular polymeric substance (EPS) reduces the conductivity of biofilm resulting in lesser generation of energy and therefore

suppression of quorum sensing to downregulate EPS production in biofilm while enhancing power generation (Wang et al. 2013).

12.3.3 Gram-Positive Bacteria

Gram-positive bacteria rarely display extracellular e^{-1} transport as indicated by very few literature reports perhaps due to the presence of a cell wall, consequently preventing porin exposure to milieu (Carlson et al. 2012). The mechanism of extracellular e^{-1} transport in gram-positive bacteria is yet to be understood. Nevertheless, *Thermincola potens* JR is a gram-positive bacterium forming anode biofilm and is efficient in power generation (Wrighton et al. 2008). Numerous C type cytochrome coding genes are investigated from this gram-positive bacterium. Thermophilic gram-positive bacteria mostly help in the reduction of insoluble iron and this is mediated by cytochrome and conductive pili (Gavrilov et al. 2012). Other gram-positive bacteria as biocatalyst tested for power generation via MFC are *Clostridium butyricum*, *Clostridium beijerinckii*, and *Clostridium cellulolyticum* (Cao et al. 2019).

12.3.4 Photosynthetic Bacteria

Researchers are looking forward for using photosynthetic bacteria as biocatalyst in MFC owing to two reasons. First, they are more beneficial compared to other microorganisms as they help in deducting carbon dioxide from environment (He et al. 2009). Additionally, mutualistic relation between photosynthetic and heterotrophic bacteria can enhance power generation without the involvement of supplementary e^{-1} acceptor or oxygen supply (Xiao and He 2014).

The photosynthetic biocatalyst applied in MFC includes purple non-sulfur-reducing bacteria and cyanobacteria. Photosynthetic purple non-sulfur-reducing bacteria like *Rhodopseudomonas palustris* DX-1 have shown promising result as biocatalyst in MFC (Xing et al. 2008). The other photosynthetic purple non-sulfur-reducing bacteria investigated for MFC application and have shown promising results include *Rhodospirillum*, *Rhodobacter*, and *Rhodovulum* (Qi et al. 2018).

Cyanobacteria represent another group of photosynthetic bacteria which has been applied as biocatalyst in MFCs. High bioelectricity output is demonstrated by *Synechocystis* in a microfluidic cell (Bombelli et al. 2015). *Nostoc* immobilized on anode, fabricated with carbon nanotubes, has shown light-dependent energy generation in MFC (Sekar et al. 2014). As far as mechanism of power generation is concerned, *Microcystis aeruginosa* is a cyanobacteria species that produces reactive oxygen species helping in the production of electricity (Cai et al. 2013), while in another report it has shown a dual role of power generation and waste water treatment (Ali et al. 2020). Evidence suggests that the mechanism of cyanobacteria

to generate electricity is different from the other electroactive organisms (Pisciotta et al. 2010).

12.4 Biocatalysts of Eukaryotic Origin

12.4.1 Fungi

Yeast species such as *Arxula adeninivorans* (Haslett et al. 2011), *Hansenula anomala* (Prasad et al. 2007), and *Candida melibiosica* (Hubenova and Mitov 2010) can be used in MFC as biocatalyst. The advantages of using yeast is that they are non-pathogenic, easy to manage, and have the ability to take up maximum substrates for growth. Moreover, they can manage to survive in diverse condition (aerobic or anaerobic). Initially, yeast biocatalyst showed lower output in comparison to bacteria. It could be due to lower catabolic rate owing to complication in accessing e^{-1} transfer mediators. Further, internal redox mediators are not present in *Saccharomyces cerevisiae*. Therefore, microorganism which is used in fuel cell requires exogenous or external mediator for external e^{-1} transfer. In an investigation, methylene blue/ferricyanide was used as the e^{-1} transfer mediator (Gunawardena et al. 2008) which showed improved output at the same growth rates. Similarly, improvements were also observed for *Candida melibiosica* (Hubenova and Mitov 2010). Subsequent improvement in electrode fabrication also increased the output in *Candida melibiosica* yeast MFCs (Hubenova et al. 2011).

Filamentous fungi like *Trametes versicolor* are also investigated for their application in MFC. It possesses long thread-like structure known as hyphae (Fernández de Dios et al. 2013). The hyphae cannot help directly as biocatalyst as it does not make e^{-1} exchange with electrode but provides connectivity between them. Fungi can survive in diverse condition and produce oxidative enzymes which provide reasonable opportunity to act as co-biocatalyst in MFC applications which can be coupled with wastewater treatment contaminated with harmful chemicals (Fernández de Dios et al. 2013).

12.4.2 Algae

Few eukaryotic members of algae that are known to power MFC include *Chlamydomonas reinhardtii* and *Chlorella* (Cao et al. 2019), and a major advantage of using these biocatalysts is that their biomass can be harvested for biofuels. The electricity is generated by introduction of *Chlorella pyrenoidosa* at anode of MFC, where it acts as an e^{-1} donor and electricity is generated by adjusting the oxygen content, light intensity, and algal cell density without the need of externally adding substrates (Xu et al. 2015).

12.5 Conclusion

The interest in MFCs is not limited to power generation and has diverse applications like wastewater treatment, desalination, biomass generation, etc. which hold the key in achieving circular bioeconomy. Moreover, MFCs can aid in reducing carbon footprint globally. Hence, there have been consistent efforts to improve MFC efficiency and they range from electrode fabrication, electrode compartmentalization, and biocatalyst development. It is important to note that these biocatalysts have been isolated from a wide range of environments (halophiles, psychrophiles, thermophiles, acidophiles, etc.) and have been co-cultured or in consortia to enhance the efficiency of MFC. We haven't covered these aspects in this chapter and a discussion on a variety of biocatalyst is also far from over. But the above discussion will aggrandize readers' knowledge on MFCs and their biocatalyst. In spite of their promising advantages and efforts to improve them, they are marred by low power generation capacity. However, we expect that interdisciplinary research will improve the efficiency of MFCs to the application level. Advancements in engineering and synthetic biology tools hold the key of the future of MFC along with knowledge of biocatalyst.

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