Clean Energy Production Technologies

Series Editors: Neha Srivastava · P. K. Mishra

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Status and Future Challenges for Non-conventional Energy Sources Volume 2

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.

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Preface

The balance between sustainable supplies of our energy sources and global concern related to emission of greenhouse gases (GHG) is quite critical for us humankind and our planet. The volatility of oil prices (and both demand and supply) in recent years and issues related to increased GHG emissions due to the use of fossil fuels have motivated almost all countries to change the way they will produce, transport, and distribute energy (sources), which is also the driving force for many countries to invest in alternative nonconventional energy sources, derived with the help of biological techniques. Some of the success could be seen with bioethanol and biodiesel for the electricity and transport sectors. Ongoing research and renewed interest in next-generation feedstock, hydrogen, and microbial fuel cells will surely prove them to be some of the promising nonconventional future energy sources. However, the bottleneck for such nonconventional energy sources and renewable energy industry is "cost v/s applicability," with fossil fuels, and also (poses a doubt) to prove as a sustainable business model. However, awareness of environmental benefits of such nonconventional biological technologies is rapidly increasing, boosting the global renewable energy capacity, which is quite encouraging. Even though such next-generation energy sources are becoming more affordable, they still face the criticism that they cannot meet the demand of providing power to our homes, communities, and industries. However, renewable energy now accounts for almost a third of our global power capacity, according to the data released by the International Renewable Energy Agency (IRENA), and the emerging and developing economies lead the transition.

This book, "Status and Future Challenges for Non-conventional Energy Sources: Volume 2," highlights recent advancements in such an important topic, through contribution from experts demonstrating different applications in "day-to-day" life, both existing and newly emerging biological technologies, and thought-provoking approaches from different parts of the world, potential future prospects associated with some frontier development in nonconventional energy sources. Initial chapters cover different aspects of cellulosic and lignocellulosic biomass: Current State of the Art on Lignocellulosic Biomass: Future Biofuels; Cellulosics Biorefinery: Concepts, Potential, and Challenges; and Biorefinery Technology for Cellulosic Biofuel Production. Algal fuels and biodiesel-related aspects are covered by Life Cycle Assessment of Algal Biofuels; Biodiesel: Features, Potential Hurdles, and Future Direction; Solid-State Fermentation: An Alternative Approach to Produce Fungal Lipids as Biodiesel Feedstock; and Metabolic Engineering Approach for Advanced Microbial Fuel Production Using *Escherichia coli*. Microbial fuel cells are touted as a future of energy sources and are covered by Microbial Fuel Cells (MFC) and Its Prospects on Bioelectricity Potential; Biocatalysis of Biofuel Cells: Exploring the Intrinsic Bioelectrochemistry; and Bioelectric Fuel Cells: Recent Trends to Manage the Crisis on Resources for Conventional Energy. One chapter covers Bioethanol: Substrates, Current Status, and Challenges. Nanotechnology for bioenergy is covered by Progress and Perspectives of Nanomaterials for Bioenergy Production and Nanofarming: Nanotechnology in Biofuel Production. One chapter also covers the potential of extremophiles in bioelectrochemical systems and biohydrogen production. We believe that this book will be able to address potential energy applications and challenges in this particular area. We also hope that the chapters of this book will be novel to readers and can be readily adopted as references for newer and further research. Moreover, since this book contains information related to different applications, we assume that international readers, especially students and researchers, will also find this book valuable for reference purposes.

Last, but not the least, we are thankful to all the researchers, expert academicians, and leading scientists whose contributions as authors and reviewers have enriched this book. We also express our deep sense of gratitude to our family members for their kind understanding and unconditional support during the course of such scholarly academic activities. While we strived to make sure that this book is free from any misleading or erroneous information, any such mistakes are completely unintentional, and pardon us. We are also thankful to Springer Nature for giving us this opportunity, and especially the editorial support team members, Ms. Aakanksha Tyagi and Ms. Veena Perumal, for their relentless support throughout the publishing process. We would also like to sincerely thank our universities for extending the facilities and encouragement for such activities. We thank them from the core of our heart.

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Contents

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Chapter 1
Current State of the Art of Lignocellulosic Biomass: Future Biofuels

Renu Singh, L. R. Pooja, Ritu Tomer, and Ashish Shukla

Abstract Abundance and renewable nature of lignocellulosic biomass (LCB) make it a viable candidate for future biofuel production. However, LCB cannot be directly converted to biofuel due to its complex lignin structure leading to sustainable biomass process and value addition is a global challenge and requires pretreatment. Recent economic and social-political factors have stimulated the development of biofuels and bioenergy. Not only this but also the vital need of energy security as well as growing environmental concerns are the main drivers behind the growth in this sector. EU directive issued in 2009 specified that 20% of all energy in the EU-27 should come from renewable energy resources of which particular emphasis is on replacing 10% energy used in the transportation sector through the use of biofuels. However, this would not be possible if we are not able to use diverse range of available biomass for future biofuel production. This includes lignocellulosic biomass and this has further challenges before it can be transformed into the value product. This book chapter focuses on the current state of the art of lignocellulosic biomass (LCB) and its role in shaping future biofuels.

Keywords Lignocellulosic biomass · Lignin · Bioenergy crops · Delignification

1.1 Introduction

The overexploitation of resources, suffering from climate change, and greenhouse gas emissions have increased pressure on policy makers and researchers to develop novel and carbon-neutral resources to meet our energy needs. This makes it today's

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necessity to have sustainable and environmentally friendly energy based on renewable sources to tackle the ever-increasing requirement of energy consumption. In this perspective of renewable energy resources lignocellulosic biomass (LCB) continues to attract worldwide interest as a clean and sustainable alternative to fossil fuels. It can be used to generate second generation of biofuels and other value-added products (chemicals) without adversely impacting the environment or influencing the food security. These LCB include a wide range of biomass such as agricultural waste such as rice straw, short-rotation crops or dedicated crops such as miscanthus, forest residues, and so on. LCB is a sugar-rich sustainable feedstock and is globally available. However, in order to convert LCB into the biofuels and other value products an appropriate processing is required. Several authors have reported that sustainable biomass process and value addition are global challenges (Adsul et al. 2009). LCB is made of lignin, cellulose, and hemicellulose creating a very complex assembly of polymers very hard to go through enzymatic conversion. This is the reason that the use of lignocellulosic biomass for biofuel conversion requires further pretreatment prior to any enzymatic conversion. These pretreatment methods will make cellulose much more reachable by changing physical or chemical structure of LCB and help in conversion into fermentable sugars. This requires answers to few key questions such as economic efficiency, environmental capability, social sustainability, and ease of the process. Several authors have reported insight into processing lignin. Abdel-Hamid et al. (2013) provided detailed investigations on degradation of lignin and its potential industrial applications. Biochemical characterization is another focal point for researchers. One of the key methodologies on how to extract value from LCB is the use of biotechnology (Iqbal et al. 2013). Authors have reported six key value-added products derived from LCB (Fig. 1.1).

Current, growing environmental concerns on consumption of fossil fuels and associated greenhouse emission make valorization of LCB more relevant and timely. LCB's natural availability and abundance make it a competing candidate to create green and sustainable replacement for fossil fuels. This book chapter focuses on the structure of LCB, their classification, key pretreatment methods in the use, and strategies for future biofuel production.

1.2 What Is Lignocellulosic Biomass?

Lignocellulosic biomass (LCB) is a form of organic matter derived from plants, produced through photosynthesis where the solar energy is stored in chemical bonds. Rice straw, maize stubbles, and pulse husks are best examples of LCB which acts as a renewable source of liquid biofuels. Biofuels produced from these residues have the potential to contribute for about 40% of the country's fuel requirement (Fatma et al. 2018). It is mainly composed of cellulose, hemicelluloses, and lignin which creates a complex polymer framework that is inherently intransigent to enzyme hydrolysis. Proper hydrolysis of these biomass yields in biofuel-producing sugars and various other compounds like organic acids, phenols, and aldehydes. Hence

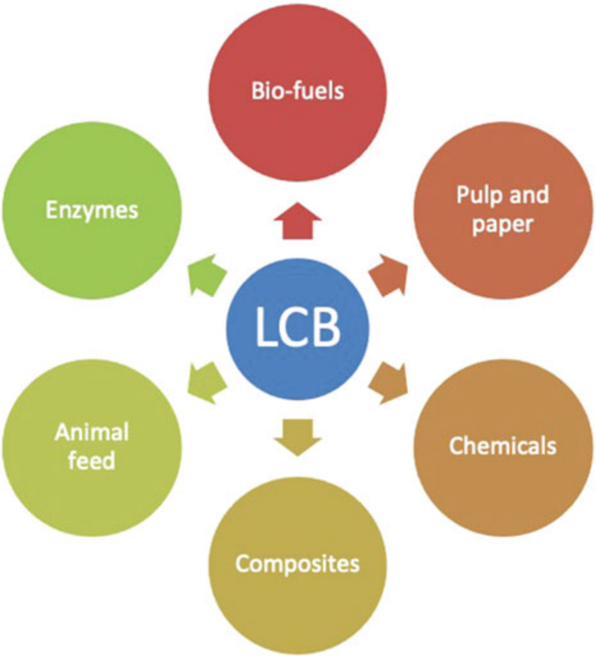

Fig. 1.1 Conversion of LCB into value-added products

pretreatment measures are much required to make cellulose more available by modifying its physical and chemical properties. It has high potential to produce second-generation biofuels as an alternative to polluting fossil fuels and ensure healthy environment. A concentrated approach to tackle climate change via legislation would reinforce the role of renewable technologies and protect the biofuel industry of the second generation (Fig. 1.2).

1.3 Structure of Lignocellulosic Biomass

As shown in the figure the LCB's structure is mainly composed of lignin, cellulose, and hemicellulose along with some amount of pectin, proteins, and ash. Cellulose and hemicellulose are polysaccharides held through hydrogen bonds and lignin which is an aromatic polymer held by covalent bonds (Magalhães et al. 2019). The composition of lignin, cellulose, and hemicellulose varies depending on the source of biomass. In most of the agricultural wastes and residues the rough compositions by percent dry weight of cellulose, lignin, and hemicellulose are 37–50%, 5–15%, and 25–50%, respectively (Fatma et al. 2018). These bonds make LCB more resistant to different treatment processes. Depending on the technologies used, the same form of biomass will produce various green liquid biofuel (Fig. 1.3).

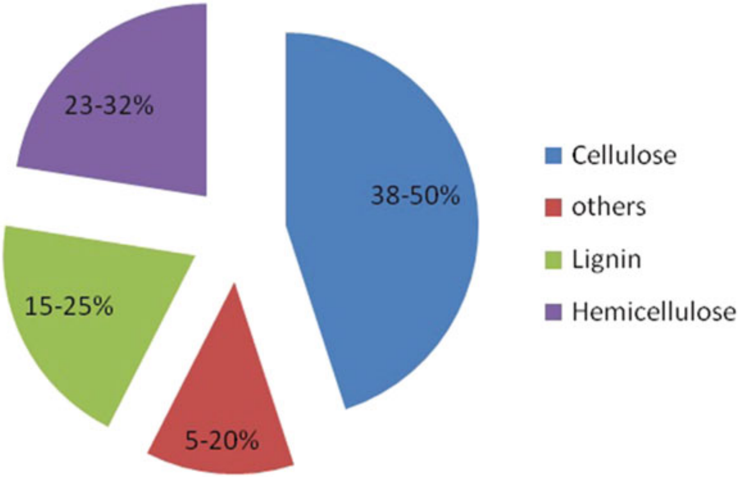

Fig. 1.2 Relative composition of lignocellulosic biomass adapted from Javed (2012)

1.3.1 Cellulose

It has the major share in the LCBs contributing up to 40–60% by weight. The major component of cellulose is ß-D-glucopyranose; each unit is connected through ß-(1, 4) glycosidic bond along with cellobiose as basic unit. Each chain of cellulose contains 500–1400 units of D-glucose, combining to form microfibrils and then fibrils of cellulose. It is insoluble in water except at exceptionally low or high levels of pH. It has beneficial properties such as biocompatibility, stereoregularity, hydrophilicity, and active hydroxyl groups and functions as a flexible provisional resource for thiolated products such as fabrics, films, composites, fuels, and chemicals.

1.3.2 Hemicelluloses

It is a heterogeneous biopolymer composed of monosaccharide forming xylans, mannans, glucomannans, and others contributing up to 20–25% of biomass produced. The components of chain are connected by xylan β (1/4)-linkages (90% D xylose and 10% L-arabinose). The hemicelluloses present in softwood LCB are composed of mainly glucomannans whereas hardwood LCB are made up of mainly xylans. It establishes a strong bond between cellulose and lignin in plant cell wall. Unlike cellulose, owing to its poor degree of polymerization and noncrystalline nature, hemicellulose is easily degradable to monosaccharides and is thus commonly used in industrial purposes like drug delivery systems, hydrogels, and formulations.

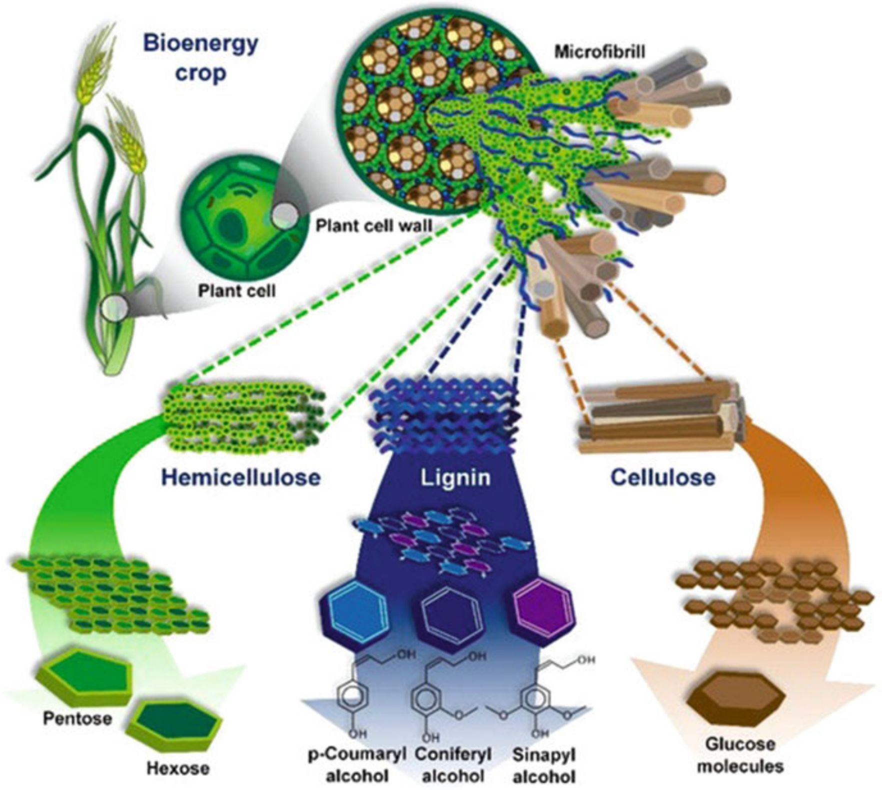

Fig. 1.3 Structure of lignocellulosic biomass containing lignin, cellulose, and hemicellulose (Hernández-Beltrán et al. 2019)

1.3.3 Lignin

It is an amorphous heterogeneous polymer which has second most share in biomass weight by contributing 15–40%. It is considered to be a noncarbohydrate polymer which is not suitable for biofuel production and it hinders the availability of polysaccharides and enzymatic degradation of cellulose. But it is very much essential for plant survival as it gives them structural rigidity and hydrophobicity. It is possible to remove or reduce lignin content through various delignification processes, thereby enhancing the biofuel production.

1.4 Classification of Pretreatment Methods Used

Lignocellulosic biomass (LCB) is resistant to biological and chemical breakdown and this is called biomass recalcitrance. There are several factors which are responsible for this biomass recalcitrance such as crystalline structure of cellulose, structural heterogeneity, degree of lignification, and complexity of cell-wall components. This challenge must be met for any value addition and utilization of lignocellulosic biomass. Considering this context the pretreatment process is a key step in biorefinery process. The main objective of the pretreatment process is the recalcitrant structure of the lignocellulose so that it can break lignin layer, degrade hemicellulose, and further reduce the crystalline structure of cellulose. A range of pretreatment techniques have been developed and researched by scientists in the last several decades as given in Fig. 1.4. However, the most suitable pretreatment method varies with the variety and type of used biomass resource. This will depend on the composition of lignin, cellulose, and hemicellulose in the used lignocellulosic biomass (Fig. 1.5).

Biofuels are the best alternatives to gasoline and other fossil fuels but due to the complexity in the structural and chemical properties of the potential LCB which restricts the cellulose hydrolysis to sugars from which biofuel is produced, we are unable to meet the requirement. LCB, known for its biomass recalcitrance, is chemical and biological breakdown resistant. The biomass recalcitrance that should be resolved for the beneficial use of lignocellulosic fuel sources is accountable for many variables, such as the crystalline structure of cellulose, the degree of lignification, and the structural heterogeneity and complexity of cell-wall constituents. To overcome this barrier we need to adapt suitable pretreatment methods in which hydrolysis rate is enhanced by changing LCB physicochemical and structural properties. These pretreatment methods improve the production of sugars through hydrolysis processes and reduce loss of carbohydrates and fermentation processes. The main pretreatment methods include physical, chemical, biological, physicochemical, and electrical methods which are briefed below.

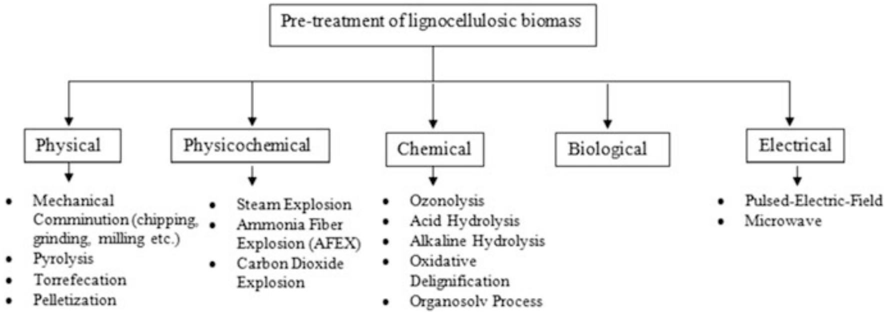

Fig. 1.4 Classification of pretreatment methods

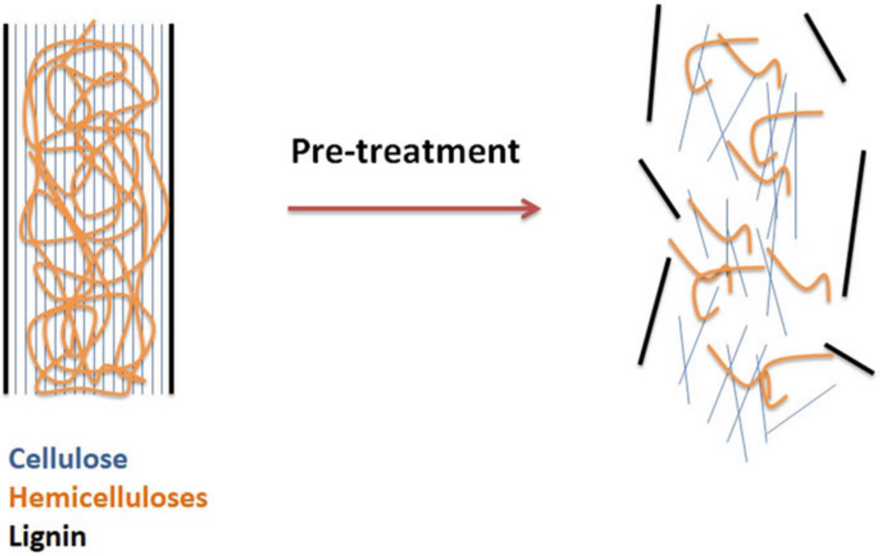

Fig. 1.5 Process of pretreatment of lignocellulosic biomass

1.4.1 Physical Pretreatment Methods

Physical pretreatment methods are required most of the time before applying any other pretreatment methods. The main purpose of physical pretreatment method is to primarily reduce the particle size. In this method of pretreatment, only physical properties of LCB are changed like increasing of the porosity and surface area and reduction in crystalline nature and polymerization degree (DP) without using any sort of chemicals. Due to the physical pretreatment method the other subsequent process (Chen et al. 2017) becomes easier and more effective. Physical treatment methods used for LCB are environment friendly and rarely generate any toxic residues (Shirkavand et al. 2016). However, major concern or drawback for physical treatment methods is their high energy-intensive nature. However, energy use will depend on the variety and type of lignocellulosic biomass used. Rajendran et al. (2018) found that reduction of size of some softwoods, e.g., maize stover and switchgrass, needs 11.0 and 27.6 kWh/metric ton of energy accordingly. However hardwoods, e.g., pine and poplar chips, require 85.4 and 118.5 kWh/metric ton of energy, respectively. Some of very popular physical pretreatment methods include extrusion, milling or mechanical size reduction, ultrasonication, and microwave treatment. The section below explains some of these methods in length.

Mechanical Size Reduction Method It involves milling, chipping, or grinding of LCB which reduces the particle size, increasing the biomass surface area and reducing the crystallinity of cellulose. Milling will minimize the size of the particles to 0.2 mm. Pretreatment with fresh LCB for anaerobic digestion suggested that decreases in particle size have greatly improved the bioavailability of the substrate, thus increasing the kinetic reactions. The significant downside of this method is that it needs a lot of energy and it does not remove lignin content of LCB. Chang et al. (1997) reported that the size (<0.4 mm) of biomass particle has no significant outcome on the degree and hydrolysis yield. Various types of motorized equipment operated in the various milling techniques are two-roll milling, rod milling, hammer milling, ball milling, vibratory milling, colloid milling, and wet disk milling. The method used, time consumed, and variety of lignocellulosic biomass used for milling determine the reduction in the size of the particles and crystallinity (Kumar and Sharma 2017). Another very popular physical pretreatment method is wet disk milling because of its reduced energy use (Zakaria et al. 2015; Kim et al. 2013).

Irradiation This method involves high-energy radiations like gamma, UV rays, electron beam, and microwave heating for the treatment of LCB. It improves the surface area and reduces the DP and cellulose crystallinity. This method also involves high energy and cost for lignin degradation and is not environment friendly. One of the very popular methods among irradiation is microwave irradiation. It is a nonconventional heating method that has been used by researchers and is dated back to 1984 when first reported by Ooshima et al. (1984). The microwave pretreatment method has been very popular because of its several underlying reasons such as energy efficiency, easy operation, and high heating capacity and requires less amount of time (Tayyab et al. 2018).

Pyrolysis It is a thermochemical process, where LCB are treated at more than 300 °C temperature producing bio-oil, biochar, and some gaseous products. The degradation process is very low at a reduced temperature. There are various types of pyrolysis processes: slow torrefaction, slow carbonization, and intermediate and fast pyrolysis. Among these processes the most followed is slow torrefaction, in which LCB is treated with mild temperature of about 200–300 °C enhancing energy density by drying. With a lower acid concentration in the smoke and a higher heating benefit, torrefaction provides cleaner burning fuel and improves the easy handling.

Bai et al. (2018) reported pyrolysis efficiency for wheat straw by hammer milling and rod milling pretreatment methods. They have found that rod milling at an optimum duration of 1 h provided significant reduction in size and crystallinity. This led to successful size reduction and crystallinity degradation leading to improved surface area and volume of pores in wheat straw. Further, authors concluded that due to lower thermal degradation during rod milling with respect to hammer-milled wheat straw, the efficiency of pyrolysis increased. However, an important aspect to consider is that both milling processes rely heavily on high use of energy and cost of equipment for milling process. Gu et al. (2018) reported the impact of planetary ball milling. These authors have used pre-milled wood fiber and observed significant energy use efficiency (Table 1.1).

Table 1.1 Energy required for reduction of LCB to ideal particle size (1.6 mm) through pyrolysis process (Baig et al. 2019)

Lignocellulosic materials	Calorific value (MJ/kg)	Energy requirement for reducing particle size to 1.6 mm (kWh/ton)
Maize straw	16.8	14
Other cereal straws	15.0	42
Hardwoods	17.5	130

1.4.2 Physicochemical Pretreatment Methods

Steam Explosion (SE) This is among the very much used effective pretreatment method (Chen and Liu 2015; Singh et al. 2015; Vivekanand et al. 2013; Verardi et al. 2018). In the steam explosion technique biomass is exposed to high levels of saturated pressure stream at high temperature (160–290 °C). This enables water molecules to penetrate the substrate structure of lignocellulosic biomass. In this method pressurized steam which has a high temperature (160–290 °C) for a short duration is used, and after that LCB is decompressed at atmospheric pressure. In two stages, SE is achieved. The first is the step of auto-hydrolysis, and the second is the step of explosion after high-pressure steam penetration into the walls of the plant cell. The stage of hydrolysis includes the breakdown and gradual degradation of hemicellulose, whereas lignin becomes solubilized and recondensed but often subdivided into low-molecular-weight units of lignin. This method is cost effective and helps in separation of individual fibers in LCB but the drawback of this method is incomplete delignification of lignin-cellulose complex and also that it forms some inhibitors. Steam explosion technique has several advantages, e.g., use of limited chemicals, low environmental impact, high energy efficiency, no recycling cost, and high sugar recovery in comparison to other pretreatment methods (Pielhop et al. 2016). Kim (2018) reported that steam explosion uses 70% less energy in comparison to conventional mechanical pretreatment methods to gain similar particle size of lignocellulosic biomass.

Ammonia Fiber Explosion (AFEX) It is a physicochemical process where liquid ammonia (1-2 kg NH_3/kg of biomass) is used under high temperature (90 °C) and pressure for a certain period of time (30 min) and then there is a quick reduction in pressure. In this method very meager amount of biomass is solubilized; it means no lignin and hemicellulose are degraded so this method is not effective for higher lignin-containing LCB.

1.4.3 Chemical Pretreatment Methods

Acid Pretreatment In this method both dilute and concentrated acids are used to disrupt lignocellulose complexes to produce simple sugars like glucose, and it also hydrolyzes hemicelluloses (Lloyd and Wyman 2005; Kärcher et al. 2015; Zu et al. 2014). But these concentrated acids corrode walls of equipment, and cause production of some inhibitors and also loss of produced sugars. In certain studies, sulfuric acid is of much importance as it is affordable and productive at concentrations typically around 4% by weight. Dilute H_2SO_4 was used for the commercial processing of furfural from cellulosic materials.

Alkaline Pretreatment Various alkaline solutions like sodium hydroxide and ammonia are used for delignification of lignocellulosic complexes at low temperature and pressure. Among the above alkaline solutions sodium hydroxide is most preferred. Alkaline reactions produce less sugar oxidation compared to acid processes, and much of the caustic salts could be extracted or revived. All through the alkali pretreatment process, a saponification reaction happens that induces the intermolecular ester bonds between hemicellulose and lignin to cleavage. This helps in the solubilization of the components of lignin and hemicellulose in the alkali solution and even in the activity of enzymes with cellulose. In this method, lignin structure is changed with less production of inhibitors but it is a very time-consuming processes and requires neutralization monitoring.

Organosolvent Pretreatment In this treatment many organic solvents like ethanol, methanol, and glycol are used for hemicellulose hydrolysis which are found in LCB. Multiple simultaneous reactions, such as the primary depolymerization processes of cellulose, hemicellulose, and lignin, are part of biomass conversion reaction. Pure lignin can be recovered as a by-product, with less sugar degradation, and it limits cellulose loss to less than 2% but requires expensive chemicals for removal of solvent and some inhibitors are generated. For health, safety, and environmental enforcement purposes, the selection of organic solvents should be carried out deliberately.

1.4.4 Biological Pretreatment Methods

Microorganism-Mediated Pretreatment Microorganisms like bacteria and fungi are applied to treat LCBs for the degradation of lignin content. This method is cheaper than other pretreatment methods having less production of inhibitors, less consumption of energy but very time consuming. Usually it takes 10–14 days to reduce the DP of cellulose and hemicelluloses and loss of sugars are also observed as part of it is consumed by microorganisms for their growth and development that eventually accelerates the degradation rate.

1.4.5 Electrical Pretreatment Methods

Pulsed Electric Field Pretreatment (PEF) In this treatment the LCB is kept between the two electrodes and sudden flash of high-voltage current is passed to produce an electric field. Due to this phenomenon a critical electric potential is developed around cell membrane which increases membrane permeability and rupture of plant tissues. It also makes structural changes in plant cell wall and membrane. This process still needs a lot of research work to be done.

Microwave Irradiation It is a nonconventional form of heating often used under an applied electromagnetic field for the pretreatment of LCB. Due to its many benefits, such as simple processing, energy consumption, minimal inhibitor production, as well as rapid heating power in a short span of time, this approach has indeed been retained convenient. As a possible tool for pretreatment of different lignocellulosic materials, microwave pretreatment technology has been commonly used. As it heats the target material directly by applying an electromagnetic field to dielectric molecules, microwave heating is favored over conduction/convection heating. Not only does it break down lignin and hemicellulose, but also enzymatic hydrolysis can even be increased. In an experiment to improve the enzymatic digestibility of rice straw, microwave-assisted H_2SO_4 is used for pretreatment. Processed rice straw samples were first subjected to microwave therapy, followed by chemical treatment with H_2SO_4. Using a microwave power range of 70–700 W, a chemical concentration of 0.1–2.0% was used for a treatment period of 1–5 min. Within ideal circumstances, e.g., reaction time, strength, and chemical concentration, the maximal sugar reduction achieved by microwave-assisted H_2SO_4 is 1376.9 μg/mL. The XRD analysis showed that perhaps the microwave-assisted H_2SO_4 rice straw sample crystallinity index is substantially high (61.36 percent) relative to untreated samples (52.2%). The morphological analysis conducted with the scanning electron microscope (SEM) reported that perhaps the surface of the microwave-assisted H_2SO_4-treated samples was more broken. The silicon waxy layer was also weakened or broken down by ether linkages among lignin and carbohydrates (Singh et al. 2013).

1.4.6 Other Delignification Treatment Methods

Delignification is the method of removing lignin from LCB using various processes. The goal is to deteriorate the lignocellulosic material into its fibrous elements.

1.4.6.1 Hot Water Pretreatments

It is better known as solvolysis, hydrothermolysis, and aqueous fractionation. In this process a liquid hot water under certain pressure and temperature (170–230 °C) is made to flow on biomass under certain flow rates. Based on the position of biomass and liquid flow rate it can be of two types: (1) co-current type and (2) countercurrent type. In the first method, flow of liquid hot water and placement of biomass are in the same direction for a certain period of time and in the second method flow of liquid hot water and placement of biomass are in the reverse direction. This method has successfully inculcated in various LCB treating industries.

1.4.6.2 Enzymatic Delignification

In this process some common ligninolytic enzymes, for example laccases, lignin peroxidase, manganese peroxidase, and aryl alcohol oxidase, are used to degrade lignocellulosic complexes. This process avoids physicochemical pretreatment making it more cost effective and eco-friendly. To delignify the biomass using enzymes they mainly use two methods: one using cell culture supernatant and second one using a solution containing single isolated and concentrated enzyme (Baig et al. 2019).

1.4.6.3 Ozonation

Ozone is a gas formed by elevated electrical voltage exposure to O_2. Lignocellulosic complex is degraded by ozone injection. It mainly works well for lignin degradation and acts poorly on hemicellulose degradation. Various studies have found that up to 40% of lignin can be removed from the wheat straw by ozonation alone.

1.4.6.4 Biological Treatments

Not only thermochemical treatments but also microorganisms do the job of delignification. These are eco-friendly processes, which give high product yield with fewer side effects and using less energy. It is found that white-rot basidiomycetes delignify the biomass very quickly and substantially. They secrete numerous ligninolytic enzymes that catalyze the reaction. A fascinating method of solid-state fermentation is that biological delignification is carried out because it imitates the existing world of fungi that degrade lignin. Various processes of delignification on different lignocellulosic biomass are given in Table 1.2.

1.5 New Strategies for Future Biofuels

Fossil fuel nearly contributes more than 40% of total primary energy use globally and is adding greenhouse gases at every point of use. Considering continuous rise in energy use and fossil fuel consumption it becomes rather important to curb the energy use and develop alternatives to replace fossil fuels. Till now, a series of

Table 1.2 Various processes of delignification on different lignocellulosic biomass

Lignocellulosic biomass	Delignification process	Type of biofuel	Results	Other products (simple sugars)	References
Sugarcane bagasse	Steam explosion 1.3 MPa, 190 °C, 15 min	Bioethanol	7.9 ± 9.1% lignin solubilization	–	Karp et al. (2013)
Wheat straw	Steam explosion 220 °C, 3 min	Bioethanol		86.46% glucose conversion	Cui et al. (2012)
Sugarcane bagasse	Alkaline pretreatment NaOH 1.0% (w/v)	Bioethanol	92.7 ± 3.9% lignin solubilization		Karp et al. (2013)
Sugarcane bagasse	Organosolv 30% (v/v) ethanol with NaOH, 60 min, 195 °C	Bioethanol		67.3% (w/w) glucose	Karp et al. (2013)
Sugarcane bagasse	Solid-state fermentation *Phanerochaete chrysosporium*, 2 weeks	Bioethanol	Reduction of lignin content from 26.5% to 21.3%	–	Karp et al. (2013)
Prosopis juliflora and *Lantana camara*	Solid-state fermentation *Pycnoporus cinnabarinus*, 2 weeks	Bioethanol	6–10% delignification	–	Gupta et al. (2011)
Bambusa bambos	Enzymatic delignification laccase from *Pleurotus sp*. at 400 IU/mL, 8 h	Bioethanol	84% delignification	–	Kuila et al. (2011)
Bagasse and cane leaf residue	Ammonia fiber expansion 2 kg ammonia +1.5 kg water/kg dry bagasse, 140 °C, 30 min	Bioethanol	–	85% glucan conversion by cellulases and 95–98% xylan conversion by hemicelluloses	Krishnan et al. (2010)
Canola straw	Biological, fungus *Trametes versicolor* (cellobiose dehydrogenase deficient) 84 days, 62% moisture	Bioethanol	90% enzymatic digestibility (compared with 45% for untreated material)	–	Canam et al. (2011)
Barley straw	Mechanical (glass reactor 2 L)	Biogas	Methane yield of 370 mL/g VS at particle size 5 mm	–	Menardo et al. (2012)

(continued)

Table 1.2 (continued)

Lignocellulosic biomass	Delignification process	Type of biofuel	Results	Other products (simple sugars)	References
Rice straw	Physiochemical (hydrothermal)	Biogas	Methane yield increased by 204%	–	Luo et al. (2019)
Corn straw	Chemical (alkaline hydrogen peroxide)	Biogas	Up to 115% increase in methane yield	–	Song et al. (2014)

various approaches have been investigated for converting biomass to biofuels. Among these technologies, gasification is relatively mature, whereas approaches to thermochemical pretreatments involve three vital components: (1) pretreatment to break the lignin, (2) hydrolysis to simple sugars, and (3) converting these simple sugars to fuel. Several approaches have been adapted to carry out these three steps depending on the source of feedstock and its character. However, sustainability, economic efficiency, and complete life cycle are still not clear.

Next-generation biofuel strategies are being developed with the hope that they will avoid several issues such as environmental concerns, land use, food scarcity, and human rights of workers specially in developing countries where these crops will be grown. Another focal point is that these biofuels should be made from every kind of biomass such as inedible woody crops, trees, grass, agricultural residue, municipal solid waste, and sources from sea beds or rivers such as algae. In order to achieve this, researchers have used a range of chemical and biotechnology methods including future plant breeding technologies, GM (genetic modification), and synthetic biology. These advances in current times in biofuel technology have given hope to more sustainable generation of future biofuels leading to reduction in fuel poverty and greener future.

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Chapter 2
Cellulosic Biorefinery: Concepts, Potential, and Challenges

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Abstract A large amount of energy is required worldwide to support and run industries, society, and community centers. The present energy distribution is directly reliant on the accessibility of nonrenewable energy reserves, which results in rising energy demands, atmospheric carbon dioxide accumulation, and an increase in greenhouse gases. It elevates the concept of the circular bio-economy of "the sustainable utilization of the lignocellulosic waste into a range of by-products and biofuels" generally recognized as biorefinery as defined by the International Energy Agency (IEA). The essential target for biorefineries is to obtain both high-volume biofuels and high-value structural chemicals. A lignocellulosic biorefinery is a second-generation system that mainly depends upon the utilization of lignocellulosic crops and non-crop feedstock, and different industrial waste such as sugarcane bagasse via thermochemical conversion. At the same time, various challenges are also aligned with the development of the lignocellulosic biorefineries such as seasonal availability, land usage, yields, and accumulation of various inhibitors during the pretreatment steps. Improved strains, streamlining of the regional substrates, integrated bioprocess, and compatibility to the existing petrochemical infrastructure will hold the key to the circular bio-economy-based biorefineries.

Keywords Sugarcane bagasse · Bio-economy · Lignocellulose · Lignin · Pretreatment

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2.1 Introduction

The awareness of the production of alternative energy has been energized due to the swift deterioration in fossil fuel reserves. The global scenario of rising population and energy consumption deteriorates environmental health and is not far short of reaching the limit of fossil fuel availability on earth. A huge amount of energy is required globally to support and run industries, society, and lifestyle (Sharma and Saini 2020). The existing energy distribution directly depends on the availability of nonrenewable energy reserves, which results in rising energy demands, atmospheric carbon dioxide accumulation, and an increase in greenhouse gases. All such apprehensions are the driving vigor for curt efforts being taken for the development of technologies for the sustainable approaches for converting lignocellulosic feedstock into biochemicals, biopolymers, biofuels, and other bio-based materials (De Bhowmick et al. 2018). It raises the concept of the circular bio-economy of "the sustainable utilization of the lignocellulosic waste into a range of by-products and biofuels" generally recognized as biorefinery as defined by the International Energy Agency (IEA).

As petrochemical refineries, biorefineries can offer multistage separation of chemicals by fractioning agricultural feedstock into various intermediate products such as carbohydrates, proteins, and triglycerides which can be further transformed into value-added by-products (Guo et al. 2018). It is working in a multistep reaction where every step is considered as a "cascading phase" (Fig. 2.1). The efficient conversion of agricultural biomass can offer an advantage by decreasing the impressions on the environment, due to fewer emissions of CO_2 and other pollutants. Besides, biorefineries are projected to attain the following goals:

1. Supply the biofuels, structural chemicals, and biomolecules.
2. The conception of new job opportunities for rural areas.
3. Valorization of agriculture feedstock and industrial waste.
4. Attain the goal of reducing greenhouse gases.

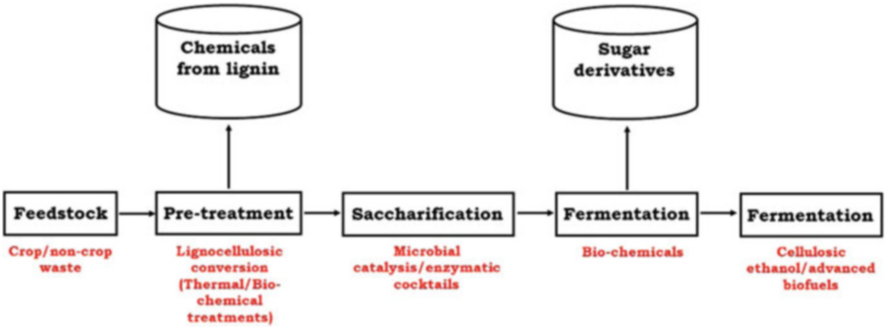

Fig. 2.1 Schematic depiction of lignocellulosic bioethanol

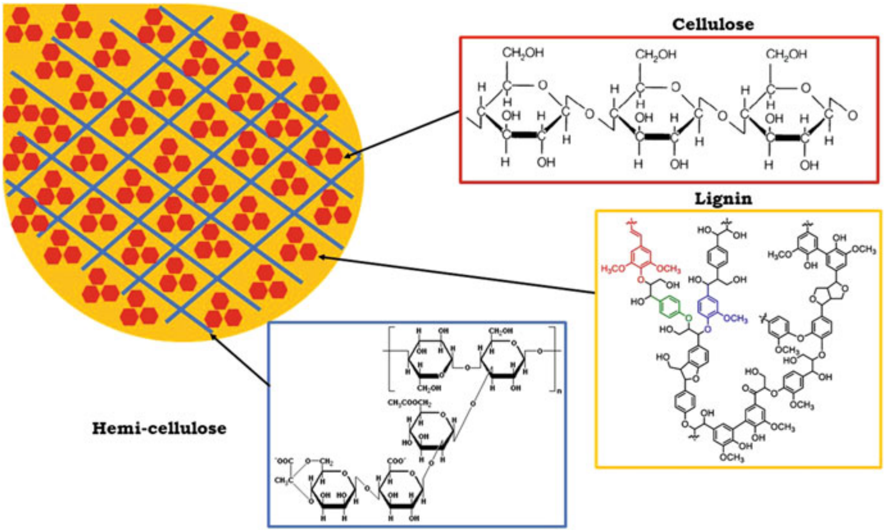

Fig. 2.2 Depiction of structural constituents of lignocellulosic waste

Substantial advantages have been recognized in the lignocellulosic biorefinery technology and its incorporation into the circular bio-economy. The biorefinery technology provides a solution for profitably the option of obtaining and recovering all the biomolecules and at the same time diminishing waste production (Ubando et al. 2020). Now it is a trend in current society to move ahead from the idea of "take, make, and dispose of" to "reuse and recovery" of earth resources to attain the millennium goal of "soil and human and socioeconomic wealth."

The essential target for biorefineries is to obtain both high-volume biofuels and high-value structural chemicals. Biorefineries have been regarded as the key for access to integrated systems that are playing a progressively foremost role in the world economic system, with the capabilities to at last substitute petroleum refineries as the conventional approach of fuel generation (Ahmad et al. 2020).

Lignocellulose biorefinery establishes a sustainable model, offering clear value advantages from the same site, where the associated units exchange raw feedstocks/ intermediate by-products, energy, and peripherals such as steam boiler, cooling units, and air compressor (Tran et al. 2020). If a suitable approach of conversion and waste valorization is accessible, an extensive range of agroforestry feedstock, food processing, and other subsidiary waste obtained from plants are established as feasible feedstock for biorefinery (Alzagameem et al. 2019).

A lignocellulosic biorefinery is a second-generation system that mainly depends upon the utilization of lignocellulosic crops and non-crop feedstock, and different industrial waste such as sugarcane bagasse via thermochemical conversion. Such conversions comprise gasification, fast pyrolysis, and liquefaction progressions (Balat et al. 2008). Lignocellulosic biorefineries convert biomass to bioethanol through intermediates, which include cellulose, hemicellulose, and lignin (Fig. 2.2).

Various efforts have been made to achieve the most advantageous biorefinery strategies keeping in mind the three phases of processing, explicitly:

- *Biorefinery Phase I*: Single feedstock, single process, and one major product.
- *Biorefinery Phase II*: Single feedstock, multistage processes, and multiple end products.
- *Biorefinery Phase III*: Multiple feedstock, multiple processes, and multiple end products.

Lignocelluloses are composed of a natural mixture of cellulose, hemicellulose, and lignin. Lignocellulosic biorefinery has been considered as Phase III biorefinery concepts which are categorized by the capability to utilize several resources by the multistage process to generate multiple end products (Schmetz et al. 2019). The lignocellulosic biorefinery profitability mainly depends upon the technology employed to change the structural composition of lignocellulosic feedstock to obtain high-value by-products from its major fractions such as cellulose, hemicellulose, and lignin (Sharma and Saini 2020).

The lignocellulosic biomass is a mix of the various constituent biopolymers that exhibit different hierarchical structural arrangements which in turn have huge impacts on biorefinery steps. The agricultural feedstock obtained from natural resources such as forests, agricultural residues, and aquaculture largely encompasses cellulose, hemicellulose, lignin, and pectin (Sanchis-Sebastiá et al. 2019). But in non-lignified feedstocks, the breakdown is much easier to deconstruct via enzymatic saccharification using efficient cocktails as compared to the lignin-containing highly resistant feedstock comprised of cellulosic nanofibrils, branched hemicellulose, and lignin material.

The cellulose is the most abundant biopolymer present in the plants mainly composed of linear, parallel, and unbranched arrangements of β-(1,4)-linked D-glucose monomers compacting an extremely crystalline structure (Dietrich et al. 2019). On the other hand, hemicellulose is mainly composed of β-(1,4)-linked glycans, showing a complex mixture of glycosyl substituents, like D-xylose, D-mannose, D-galactose, D-glucose, L-arabinose, 4-O-methyl-D-glucuronic acid, D-galacturonic acid, and D-glucuronic acid or, rarely, L-rhamnose and L-fucose, and simply hydrolyzed by acid and alkali treatments. Lignin is a heterogenous phenolic mixture that interlaces with cellulosic microfibrils and hemicelluloses, providing mechanical support to the plant, and supports the xylem-water transport system. At the same time, lignin is composed of three chief phenylpropanoid major subunits, as 4-hydroxyphenyl (H), guaiacyl (G), and syringyl (S) subunits, which are the major bottleneck in the economic utilization of feedstocks (Yuan et al. 2013). Such a type of resistance of lignocellulosic feedstocks leads to hindrance in different conversion technologies, which is often termed as “biomass recalcitrance,” pretending as a barrier in feasible biorefinery methods. The lowering of the lignin content or increasing of the syringyl and guaiacyl S/G ratio were established to have a positive interconnectedness with the maximal yield of fermentable sugars and ultimately provide the profitable value of lignocellulosic feedstock for commercial requirements.

Lignocellulosic feedstocks are majorly derived from corn stover, barley, wheat, and rice straw stalks of sorghum, husk obtained from coconut, sugarcane processing waste such as bagasse, and forest wood (Pattanaik et al. 2019). Lignocellulosic feedstocks are chiefly of three types like softwood, hardwood, and grasses. Pine, spruce, and fir have belonged to the softwood group which is predominantly available in the northern hemisphere. Hardwood mainly consists of guaiacyl and springyl with xylan as primary hemicellulose. However, grasses have completely different pore structures, with the thick outer wall that keeps against compression and tension while the inner soft pitch positioning the foam core. Perennial grasses are always in high demand in biofuel conversions due to their low lignin content and hemicellulose degradation (Pattanaik et al. 2019).

However, the issue remains whether this lignocellulose feedstock can effectively meet the primary mandate for material, animal feed, and secondary consumption for biofuels and structural chemicals. However, excessive production rates and less biomass expense confirm the potential of lignocellulosic feedstocks as a substantial source for by-product generation and energy proliferation.

2.2 Biopolymer Fraction of Lignocellulosic Biomass: Cellulose, Hemicellulose, and Lignin

Lignocellulosic biomass has three major constituents: the polysaccharide fraction of the plant cellulose and hemicellulose, collectively considered as holocellulose, other than the pectin, lignin, and inorganic content (Kumar et al. 2017). The composition ratio varies from plant to plant and the efficacy of the biorefinery.

Most of the lignocellulose content, that is, 30–50%, is comprised of cellulose, hemicellulose, and lignin with a smaller fraction of 20–40% and 15–25% separately (Cherubini 2010) (Fig. 2.2). The lignocellulosic is embodied by glucopyranosyl monomeric monomers connected by 1–4-β-glycosidic bonds, thereupon molding its structure into flat sheets which enables the packing of different cellulosic constituents as crystalline fibrils. Cellulose is typically found in two forms, explicitly amorphous and crystalline, and has the highest extent of polymerization between all 03 key constituents of lignocellulose, due to which its flexibility is very low, with the inability to get simply dissolved in water and other solvents. The higher the crystallinity of cellulose, the more recalcitrant the biomass, thus hindering the process of degradation in biorefineries (Lee et al. 2014).

Hemicellulose has a complex, heterogeneous, web of noncellulosic sugars that associate directly with the cellulosic fibrils and comes out as a gel-like matrix (Mokhena and John 2020). In comparison to cellulose, there is a huge structure heterogeneous in nature and comprised of pentoses such as xylose and arabinose along with six-carbon sugar like glucose, galactose, and mannose. Various other functional groups like acetyl, methyl, cinnamic, glucuronic, and galacturonic acids are also present (Mathew and Abraham 2004). So, the degree of polymerization of hemicellulose is relatively lower than cellulose, but such added dissimilarity and complexity in the structure make room for a prerequisite for the obtainability of diverse hydrolytic enzymes essential for the destruction of hemicellulose for further processing.

One more highly inconstant polysaccharide present in the lignocellulosic cell wall is pectin, which contains a backbone packed with galacturonic acid bound by α-(1,4) bonds. As compared to the cellulose and hemicellulose structures, the various pectin structures are not distinct molecules; likewise, they belong to a similar domain comprising homogalacturonan, xylogalacturonan, apiogalacturonan, rhamnogalacturonan I, and lastly rhamnogalacturonan II (Park et al. 2017).

The 03 universal and major monolignols are p-coumaryl, coniferyl, and sinapyl alcohol which turns into p-hydroxyphenyl (H), guaiacyl (G), and syringyl (S) remain when combined into the lignin polymer, and vary in softwood, hardwood, and grass diversity. Such differences in configuration have a considerable influence on the delignification and degradation of feedstock. Lignin acts as a vital molecule in plant growth, development, cell physiology, and evolution in plant life (Achyuthan et al. 2010).

Out of all 03 key ingredients, hemicellulose is the most simple to hydrolyze as compared to cellulose and lignin to obtain ethanol and other by-products. The non-carbohydrate phenolic polymer, i.e., lignin, makes the feedstock more recalcitrant to enzymatic degradation as it hampers enzymatic conversion to carbohydrates and inspires nonproductive bonds with cellulases, thus curtailing hydrolysis efficacy (Barakat et al. 2012). Also, the irregularity of lignin is a key challenge for immeasurable enzymes, which are largely utilized on specific structures and/or bonds. The development of various pretreatment methods to remove lignin before the enzymatic deconstruction is one significant solution (Alvira et al. 2010).

It is established that sugars remain inaccessible as only a small portion of cellulose is accessible to cellulase for degradation; thus the development of new strategy for the degradation of unapproachable polysaccharides is more important. The major conversion steps associated with the conversion methods are very expensive. But there is a huge shift in the approach towards lignin valorization and its recent implementation in some industrial applications such as the production of wood adhesives and bioplastics which has played a major role.

2.3 Lignocellulosic Substrates

2.3.1 First Generation (Food Crops)

The first generation of agricultural feedstocks relied on easily available and edible components of any food crop, intending to produce bioethanol. Bioethanol is produced from the biomass rich in sugar fractions present in the sugarcane bagasse, sugar beet waste, and sorghum crop residues and starch obtained from the corn and cassava crops (Duku et al. 2011) (Fig. 2.3). But at the same time, the debate about

Fig. 2.3 Different generations of lignocellulosic feedstocks available

food vs. fuel rises in various developing and under-developing countries. In the last decades, grave criticisms have been raised about competition in land practices that have to get up as a direct consequence of promotion and encouragement of energy and oil crop feedstocks at the cost of food crops (Sharma and Saini 2020). The bioethanol and edible crop price deliberation includes diverse views and is a long-standing, debatable issue in the current world situation. Various commodities such as corn and sugarcane can be utilized moreover as food and animal feed, or to generate bioethanol. In 2006, in the United States, some portion of the agricultural land use has been changed to grow the maize for bioethanol. Approximately, 25% of the total corn production is destined for bioethanol production only (Eckert et al. 2018). But such practices may lead to grave concern about food production in developing and under-developing countries. And the debate on fuel vs. food will be a huge challenge and apprehension in such global areas.

2.3.2 *Second Generation (Nonfood Crops and Lignocellulosic Wastes)*

Fermentation economics, country-specific policies, sustainability aspects, and rising debate of “food versus fuel” encouraged the production of the second generation of biomass based on lignocellulosic residues. Nonfood crop and lignocellulosic residues include Switch and Napier grasses, agricultural waste and pruning (sawdust, sugar crops, husk, rice bran, wheat straw), and agro-industrial processing wastes (e.g., fruit pulp, potato and orange peel, tea, and coffee leftover) (Kumari and Singh 2018) (Fig. 2.3).

Two primary conversion strategies are typically utilized for the valorization of lignocellulose residues in the biorefining process and may be categorized as biochemical and thermochemical. Biochemical conversion technology involves the degradation of carbohydrates to soluble sugars, i.e., saccharification and subsequently ethanol fermentation, or non-fermentative anaerobic digestion (Cesaro and Belgiorno 2015). Thermochemical conversion involves direct combustion, pyrolysis degradation, gasification, and torrefaction (Sanz et al. 2017).

2.4 Production for Ethanol from Lignocellulosic Biomass

Ethanol can be produced from lignocellulosic biomass in different approaches using common steps: saccharification of cellulose and hemicellulose to simpler sugars, subsequent fermentation, and downstream processing. The major differences lie in the hydrolysis part, which can be achieved by dilute acid, concentrated acid, or enzymatic degradation (Sharma and Saini 2020; Galbe and Zacchi 2002).

2.4.1 Pretreatment

A pretreatment stage is required to solubilize the lignocellulosic material using different approaches. The pretreatment of lignin removes and de-crystallizes cellulose and can be accessible to the hydrolytic enzymes. The pretreatment is a vital stage in the lignocellulosic conversion technology as it impacts the quality and concentration of the sugar (Balat et al. 2008). Pretreatment strategies can be categorized as physical, physicochemical, biological, and electrical, or a blend of more than one (Kumar et al. 2009; Alvira et al. 2010). The target of any pretreatment method is to change or remove compositional and structural obstacles to hydrolysis to enhance the rate of enzymatic reaction and increase the concentration of fermentable carbohydrates from feedstocks. Moreover, pretreatment stage conditions must be customized following the structural constituents of the different, and variable, bases of lignocellulosic feedstocks.

In the last decade, the idea of an emerging pretreatment approach under “green processing” has attained increasing curiosity as a possible method to the challenge of evolving a viable biorefinery impression. The concept of more efficient utilization of the lignocellulosic feedstocks eradicates waste and avoids the utilization of toxic and hazardous pollutants. Various emerging methods such as ultrasound, microwave, hydrostatic pressure, pulsed electric field, and high-pressure homogenization are available to attain maximal sugar recovery. However, the more extensive acceptance of such strategies by the fuel industry, with expected reductions in initial asset cost and enlarged scale of industrial operation, may inspire uptake for pretreatment of lignocellulosic feedstock (Fig. 2.4).

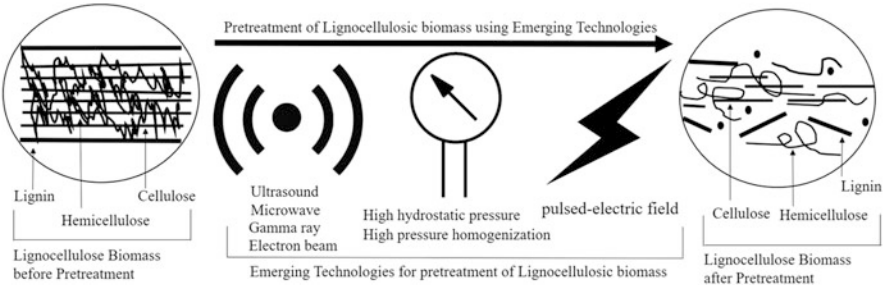

Fig. 2.4 Different advanced strategies in pretreatment technologies (courtesy by Hassan et al. 2018)

2.4.2 Acid Hydrolysis

Acid hydrolysis is advantageous as it can penetrate lignin deprived of any preliminary pretreatment process of feedstock, and thus saccharification of cellulose and hemicellulose molecules to form fermentable sugar molecules. Various acid solutions have been used to attain acid hydrolysis such as several types of acids, concentrated or diluted, such as phosphoric, sulfuric, nitric, and hydrofluoric acid (Galbe and Zacchi 2002; Lenihan et al. 2010). A high hydrolysis yield (approx. 90% of theoretical concentration) can be attained with 10–30% of acid during the process (Iranmahboob et al. 2002). But the use of concentrated acid leads to the large accumulation of residual acids resulting in corrosion. The utilization of the diluted acid during the processes has an advantage as only low level of acid is required (2–5%) (Kootstra et al. 2009). Improvisation in acid hydrolysis with temperature has been established as an efficient technology as depicted in technology developed by BC International (1999). It is a two-step reaction where the two-step reaction is as follows: in the first stage mild hydrolysis at 170–190 °C has been performed to hydrolyze hemicellulose; subsequently, the second stage has more stringent conditions to hydrolyze cellulose at 200–230 °C (Wyman 1999) (Fig. 2.5).

2.4.3 Enzymatic Hydrolysis

Enzymatic pretreatment of lignocellulosic biomass into fermentable sugar has developed as the utmost efficient biomass conversion strategy. Synergistic hydrolysis of lignocellulosic feedstock constituents is attained using an enzyme consortium integrating various enzymes such as cellulases, hemicelluloses, lytic polysaccharide monooxygenases (LPMOs), laccases, and cellobiose dehydrogenases (CDHs), in amalgamation with other proteins, which is essential to break down the plant structure. The key obstruction in lignocellulose biorefining is essential for more competent, vigorous, and low-cost enzymatic conversion technologies (Silva et al.

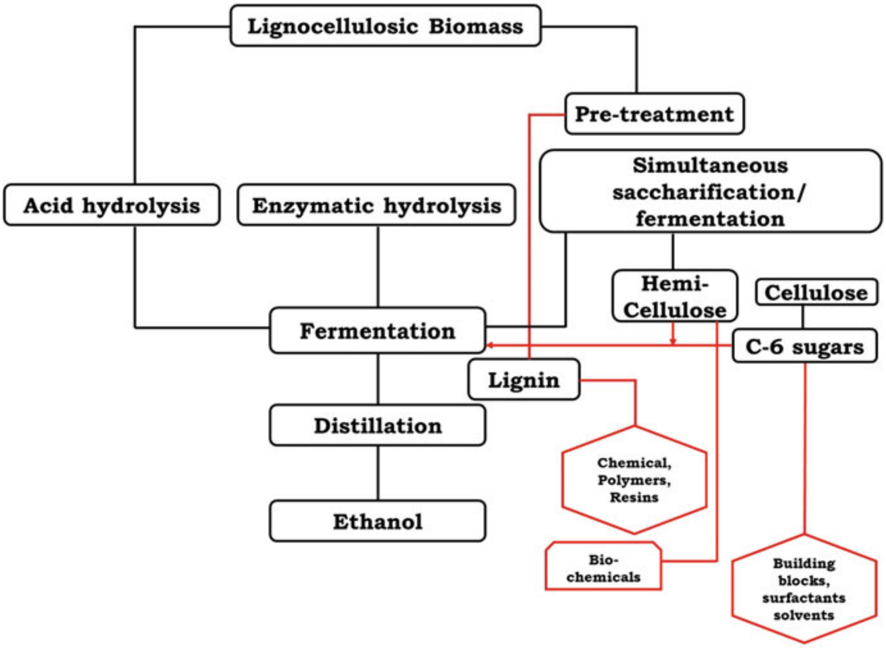

Fig. 2.5 Process for producing ethanol in a lignocellulosic biorefinery

2018). It is predictable and even established in some cases that the lignocellulosic ethanol biorefineries' enzyme epitomizes about 28% of the inclusive cost of producing cellulosic ethanol (Johnson 2016). However, the current aim is to make lignocellulosic biorefineries cost competitive. Such number is 28% utilizing off-site technologies and decreases to 22% with on-the-spot technologies, and, unpredictably, it is decreased to 10% when enzyme synthesis is coupled with the process. Such developments epitomize a 64% decrease in total costs associated with enzyme use. In specific, the cumulative pretreatment approaches are studied for improving the enzymatic hydrolysis of lignocellulose biomass and apprehending the inclusive utilization of lignocellulosic feedstocks.

2.4.4 Bottlenecks of Pretreatment Strategies and Prospects

- The major bottlenecks linked with the lignocellulosic biomass-based biorefinery can be elaborated as (1) selection and seasonal availability of selected biomass; (2) efficient pretreatment technologies; (3) capital investment such as industrial setup, reactors, chemicals, and bioprocess control; (4) inhibitory by-products accumulated during different pretreatment approaches; (5) post–pretreatment approaches; and (6) waste accumulated and environmental hazards.

Therefore, while selecting for various pretreatment methods it should follow the below-mentioned conditions, i.e.:

- Capable of hydrolysis of three-dimensional compositions of lignocellulosic feedstock LCB.
- Alters the extent of polymerization and diminishes cellulose crystallinity which ultimately improves the performance of enzymatic hydrolysis output.
- Obtaining pretreated pulp with high cellulose and hemicellulose conversion yields, which ultimately increases the fermentability of sugar up to 90% or more.
- Decreased levels of organic acids and inhibitory agents and phenolics result in the minimizing fermentation process.
- Recovery of the lignin to produce value-added compounds.
- Substrate-oriented reactor design.
- Low chemical and water requirement or reuse of water and chemicals utilized in the bioprocess.

2.4.5 Hydrolysis and the Fermentation of Lignocellulosic Biomass

After the efficient pretreatment stage, the potential conversion of lignocellulosic biomass takes in the degradation of structural polymers and fermentation of the sugar. There are two types of fermentation process commonly used nowadays as separate hydrolysis and fermentation (SHF) and simultaneous saccharification and fermentation (SSF). SSF strategy is commonly recognized as more useful than SHF approach, for different reasons as limited steps, limited end product inhibition impact due to the fast conversion of sugar into ethanol (Viikari et al. 2007), and lower incidence of process contamination by unwanted strains due to the ethanol accumulated gradually (Elumalai and Thangavelu 2010).

Temperature plays a crucial role in enzymatic hydrolysis and fermentation. It is generally observed that the optimum temperature range for the enzymatic breakdown of lignocellulosic biomass is classically higher than that required in fermentation. In such comparison of SHF, the optimum temperature for the enzymatic breakdown can be optimized separately from the fermentation temperature; however, a conciliation must be found in SSF fermentation (Olofsson et al. 2008). In precise, the enzymatic hydrolysis could be worked out in two phases: an initial step with thermostable enzymes at elevated temperatures for the liquefaction of feedstock (SHF); in the second stage, efficient saccharification of the lignocellulosic feedstock could be worked out at lower temperature range by utilizing the SSF process (Olofsson et al. 2008).

For optimal and continuous production SHF process has ease in scale-up operations as compared to the SSF process by recirculating and reprocessing the producing strain due to the existence of the solid material obtained from the enzymatic hydrolysis. In an ideal case, to attain high ethanol yields, with high gravity mediums.

In particular, 4–5% of the ethanol yield can be attained with solid feeding of pretreated feedstocks up to 30% (w/w) that is regarded as a brink level for an efficient and sustainable distillation technology. However, by raising the volume of solid mass in a bioreactor, the hydrolytic efficiency of the enzyme cocktail leans towards getting worse. The increase in the initial concentration of the substrate increases medium viscosity as well as affects the homogeneity distribution of the hydrolytic enzymes (Sassner et al. 2006).

2.5 Case Study: Sugarcane Bagasse as a Potential Feedstock for Biorefineries

Sugarcane bagasse is a lignocellulosic feedstock that comprises cellulose, hemicellulose, and lignin from an intractable structure. The major sugar present in the cellulose is glucose whereas hemicellulose contains pentoses and hexose sugar mainly glucose, galactose, mannose, arabinose, and xylose. Xylose is the major fraction available as the most abundant sugar (approximately 90%) of total hemicellulose content. Therefore, major fermentable sugar in sugarcane bagasse is composed of cellulose and hemicellulose fractions.

The sugarcane bagasse is mainly composed of cellulose (32–55%), hemicellulose (26.7–32%), lignin (19–24%), and waxes (0.8–6%). The method of conversion of sugarcane bagasse to bioethanol needs strains which have the potential of utilizing different sugars. For decades several strains including bacteria, fungi, and yeasts have been utilized to ferment bagasse. However, filamentous fungus particularly white-rot fungus belonging to basidiomycete has been majorly utilized by the bioethanol industries. The only limitation in the utilization of diverse strains is that they cannot ferment xylose.

Mandegari et al. (2017) demonstrated multicriteria analysis for sugarcane bagasse-based biorefinery. In their modeling, alternative lignocellulose biorefineries appropriated to a distinctive sugar mill waste were studied to co-produce ethanol, lactic acid, and generation of electricity, converting bagasse and a constituent of harvesting brown leaf residues as feedstock. In different scenarios studied, ethanol as the sole end product from glucose and xylose has been obtained as scenario 1, production of the lactic acid from glucose and xylose was the scenario 2, ethanol production from glucose and lactic acid from xylose was scenario 3, and ethanol from xylose and lactic acid from glucose was scenario 4, all of which were linked with a certain level of electricity production. Economic evaluation, energy consumption assessment, and life cycle assessment on environmental aspect were carried out on the developed simulations, in the multicriteria assessment of the appeal of each scenario. Scenario 2 was economically best suitable with the maximal internal rate of return of about 31.1%, while scenario 1 had the least internal rate of return of 10.0%. Scenarios 2 and 4 were economically the most vigorous, with minimal sensitivity to discrepancies in the key economic factors, i.e., ethanol, lactic acid, and production of enzymes. The life cycle assessment recommended that lactic acid-producing scenarios presented environmental burdens that were slightly higher than scenario 1, due to maximal consumption of processing chemicals. Inclusively, scenario 4 was established as the most required biorefinery scenario, by balancing economical and environmental suitability. The results of such a study would assist as strong benefits for the sugar mill to diversify their contribution through investment and extension in this sector, particularly through the production of ethanol and lactic acid and energy co-generation. However, the production of high-value by-products other than lactic acid should also be explored.

Sugarcane bagasse is low in nutritional benefits; that is why the utilization of biomass in developing countries impedes apprehensions about the food vs. fuel dispute.

The development of sugarcane biorefineries for the existing sugarcane processors would be more beneficial to valorize the waste streams as well as generate ethanol (Bezerra and Ragauskas 2016). The key problem in the utilization of sugarcane bagasse via a biochemical method is the establishment of inexpensive pretreatment strategies to make the residues able to further conversion, simple and inexpensive, effective biomass hydrolysis, and fermentation of sugar to ethanol.

2.6 Opportunities and Challenges in the Lignocellulosic Biorefining

Lignocellulosic biorefineries not only yield ethanol or other biofuels but also produce new adhesives, eco-friendly plastics, green surfactants, and different plastics and biopolymers. The diverse products obtained in lignocellulosic biorefineries with required characteristics that are not easily executed by refineries are predominantly promising targets (Arevalo-Gallegos et al. 2017). Therefore, low-cost pressure would occur initially for such biorefinery products. However, the efficient strains for the production of green and other by-products are far from being completely exploited. The cost and availability of lignocellulosic residues remain one of the key points for the success of the bioprocess design and production (Balat 2011). However, available bioprocess economic prospects and environmental essentials make the utilization of lignocellulosic biomass and biorefinery not only viable but also highly striking from many perspectives. In any lignocellulosic biorefinery, we now need to recognize by-products that have economic viability and expand the process to a scale where such technologies can be developed cost-effectively. By keeping such things in our mind, various opportunities and challenges need to be discussed in lignocellulosic biorefineries.

- *Biomass diversity and collection*: The success of the lignocellulosic biorefinery depends upon the type and diversity of the biomass. Biomass varies in physical characteristics, chemical constituents, seasonal availability, and cost depending on the types, sources, and transport. The diversity in the lignocellulosic biomass posed a challenge to establish a route of feedstock supply systems and advanced conversion processes to yield biofuels. The challenge is to reduce a huge range of biomass and work out the regional solutions (Cherubini 2010). The transport of the lignocellulosic feedstock particularly switchgrass, straws, and corn from farm to biorefinery is enormously expensive. The accessibility of low-cost pilot-scale biorefining systems is thus vital to decrease the expensive logistics of agricultural biomass. The minimal processing of the lighter biomass at production or farm site will allow conversion of the feedstock to easy-to-transport high-density residues. The high-density feedstock or primarily condensed products can then be easily transported and fermented at large production sites or biorefinery. Such problems can be addressed using different approaches such as developing clusters of local suppliers located near feedstock source, large contractual farming or plantations where the production industry is situated, or adjacent area and regional agricultural feedstocks like sugarcane bagasse in India and Brazil that can collect a huge amount of biomass (Sharma and Saini 2020).

- *Seasonal variation and land usage*: The seasonal variation in fermentable sugar and feedstock (particularly agricultural residues) is seen generally in seasonal crops which restrict the accessibility around the year due to the seasonal time frame. The storage of such biomass for a longer duration with commercial storage facilities will be an alternative solution but a huge storage space is required. Additionally, the spoilage of such agricultural feedstock requires attention during storage. The cultivation of fast-growing crops can minimize the challenge of sacrificing arable lands (Maity 2015). The use of forestry residues and the utilization of leftover crops can reduce the challenge of utilizing arable land.
- *Industrial compatibility with existing infrastructure*: The industrial compatibility of lignocellulosic biomass biorefinery with current fuel refinery and petrochemical processes is a huge factor and can minimize the capital investment to establish a dedicated biorefinery. The compatibility with existing petrochemical infrastructure will also enable the rapid establishment of biorefinery (de Jong and Jungmeier 2015). Minor modifications to existing systems can also accommodate lignocellulosic biofuel efficiently or concentrated liquids like sugar hydrolysates will increase its synergy with present industrial setups.
- *Integrated biorefinery and economic viability*: Integrated biorefinery process with an option to fractionalize all the other by-products will add merit to commercialization. The production cost of the lignocellulosic biofuel can only be comparable when by-products are having economic viability. Approximately, 85–90% of the total capital expenses are in refinery production and about 10–15% are being utilized for the production of biochemicals (Moreira 2008). The lignocellulosic biorefinery in principle should also yield a significant amount of fuels and biobased chemicals to match precisely with consumer demands.
- *Life cycle assessment*: The life cycle assessment must be cautiously modeled and observed for various biomass to comprehend the economic, environmental, and social impressions of lignocellulosic biorefineries. Life cycle assessment of different agricultural biomass and other residues will provide a sustainable approach to understand its impact on the local community, environment, and

practices (Prasad et al. 2020). Only a handful of life cycle assessments have been however documented so far utilizing agricultural feedstocks.

2.7 Conclusion

The recent developments in the lignocellulosic biorefineries provide considerable progress in the direction of improved economic and environmental sustainability. However, despite significant industrial research and development, substantial challenges persist for minimized pretreatment, fermentation, and production of the by-products. Explicitly, optimizations of feedstock pretreatment, saccharification, and fermentation of the accumulated sugar and fractionation of lignin and biochemical products could critically accelerate the extensive distribution of modern biorefineries. An integrated approach to recovery and life cycle assessment of the lignocellulosic biomass would be a future line of research and industrial developments.

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Chapter 3
Biorefinery Technology for Cellulosic Biofuel Production

Poulomi Ghosh and Saprativ P. Das

Abstract A sustainable biomass processing into a wide range of merchandisable products along with energy is biorefining. Several biofuel candidates are anticipated to replace fossil fuels eradicating the obligation of energy areas. Among these emerging biofuel molecules, biodiesel and bioethanol produced from algae and lignocellulosic biomass have attracted the greatest attention of the world as potential substitute. Various technological platforms have been designed to convert biomass into biofuels which include sugars derived from lignocellulosic biomass, chemical conversion deploying pyrolysis or gasification of lignocellulosic and algal biomass, biological conversion utilizing microbes with subsequent production of bioethanol, rich chains of biodiesel from the neutral lipids and biogas through anaerobic degradation. Cost-effective biological conversion of the lignocellulosic biomass into biofuels is, however, slowed down by noteworthy obstacles, viz., absence of specifically developed energy crops, lesser enzymes' activity in the breakdown of lignocellulosic biomass, difficulty in designing and assembling operational metabolic pathways, and inhibitory effect of secondary and tertiary products on biofuel producers. Therefore, liquid biofuel generation from biomass is suffering from various limitations such as high cost along with less productivity and sustainability. This chapter describes an integrated conversion process to produce multiple products from different range of biomass components through biorefinery approach, aiming toward sustainable biofuel generation, with emphasis on using new or improved processes to derive products, viz., ethanol, diesel, isosorbide, polylactic acid, 1,3-propanediol, and other new chemicals.

Keywords Biorefinery · Bioethanol · Biodiesel · Biogas · Pyrolysis

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3.1 Introduction

Energy necessities of the industrial and domestic sectors are in large part met through fossil-based fuels. The limited fossil fuels' reserves, united with environmental troubles, inclusive of greenhouse gases have encouraged mankind to search for the option of viable resources to fulfil the growing call for energy. Biomass is one of the limited resources having the capability to satisfy the demanding situations of sustainable and green electricity systems. Biomass is the matter derived from plant and animal sources with photosynthetic energy trapped inside, and is non-geologic in nature. Like a petrol refinery, the biomass through a "biorefinery" concept may be utilized to produce an array of beneficial chemical compounds and fuels (Table 3.1).

As defined by National Renewable Energy Laboratory (NREL), "a biorefinery is a one-stop solution uniting conversion procedures and equipment to provide fuels, electricity and chemical substances from biomass." To reach the aims of sustainable development, biorefineries must play a leading position in the upcoming millennia. This chapter has attempted to study the developments and improvements of biorefineries so far, and the possible future directions.

Implementing sustainability on the humanity timescale is an assurance to our lifestyles. Prior to the 1900s, agriculture and forestry were the principal sources of raw materials for electricity, food, and an extensive variety of everyday

Table 3.1 Refinery vs. biorefinery

Topic	Refinery	Biorefinery
• Feedstock	• Feedstock moderately homogeneous • Lessened oxygen content • The weight of the item (mole/mole) for the most part increments with preparing	• Feedstock heterogeneous with respect to mass parts, e.g., carbohydrates, starches, lignin, proteins, oils • Greater oxygen content • The heaviness of the item (mole/mole) by and large declines with handling • It is imperative to see the usefulness in the beginning material
• Building block	• Primary structure blocks: Ethylene, methane, toluene, propylene, benzene, and xylene isomers	• Primary structure blocks: Glucose, xylose, unsaturated fats (e.g., oleic, stearic, sebacic)
• Chemical (bio)process	• Entirely synthetic processes • Outline of heteroatoms (O, N, S) • Comparative homogeneous cycles to show up to building blocks: Steam breaking, synergist changing • Wide scope of transformation sciences	• Blend of substance and biotechnological measures • Evacuation of oxygen • Comparative heterogeneous cycles to show up to building blocks • More modest scope of transformation sciences: Hydrogenation, dehydration, aging
• Intermediate product	• Many	• Scarce, but accumulating (e.g., furfural, bioethanol, biodiesel, mono-ethanol glycol, lactic acid, succinic acid)

merchandises; the human civilization depended completely on renewable substances. Humanity becomes constrained by utilizing the defensible supply of incompetent harvest from the biomass, drawing power from the sun. The commercial revolution has introduced a soar within the human civilization. Mass manufacturing of products through machines dominates our everyday life. The commercial revolution further matured through the use of combustion engines and subsequent improvement of fossil energy and chemical industry. Besides the doubling of beneficial biomass manufacturing/harvest, mankind has an increasing impact of exploitation into the huge reservoirs of fossil energy. In the beginning, the fossil chemical substances had been deemed as waste, and hence any usage became welcomed. It quickly became the most economical, chemical, and power resource towards escalating commercialization. As a consequence, our dwelling standards have witnessed a visible leap with no turning back to the basic way of life in near future. However, as the fossil power and chemical resources are exhausting, there may be a crucial desire to an alternate of the present-day enterprise and human civilization in a sustainable way, promising that our current lifestyle endures at the direction of development post-diminution of fossil assets. Our manners of lifestyles happen handiest if sustainability is retained on a timescale now not as much as than our life span. In a biorefinery, lignocellulosic biomass (LCB) is converted to chemical substances, materials, and power that run on the human civilization, restoring the prerequisites of biogas, coal, petroleum, and other nonrenewable power and chemical assets. Lignocellulosic biomass (LCB) is renewable as revealed in Fig. 3.1, in which plant produces chemical compounds expending solar, carbon dioxide, and water from the environment whilst releasing oxygen.

Biomass combustion yields energy, carbon dioxide, and water. Consequently, biorefinery performs a significant role in confirming the cycle of biomass manufacturing and that consumption is protected pleasing human wishes for strength and chemicals. A biorefinery incorporates a ramification of transformation processes to supply numerous product streams, viz., transportation liquid fuels, steam/warmth, power, and chemicals from lignocellulosic biomass (LCB). Biorefinery has been recognized as the most encouraging direction to the establishment of a sustainable bio-primarily based economy. Biorefinery is a group of critical technology to convert organic uncooked substances into quite a number of industrially beneficial intermediates. By generating a couple of merchandise, a biorefinery exploits the value derived from a lignocellulosic biomass feedstock. A biorefinery could produce one or more low-volume excessive-cost chemical merchandise together with a low-value, excessive-volume liquid transportation fuel while producing energy and technique warmness for its personal use and/or export. There are fundamental categories or techniques in the biorefining process—biochemical and thermochemical techniques. In biochemical techniques, the lignocellulosic biomass is usually broken down to individual components thoroughly for required conversions that are subsequently performed. The simple technique is primarily based on a systematic breakdown and transformation to preferred chemical substances. The biochemical tactics depend closely on separation and/or physical fractionation of the intermediates, along with the ultimate favored products. Organic conversions are favored over

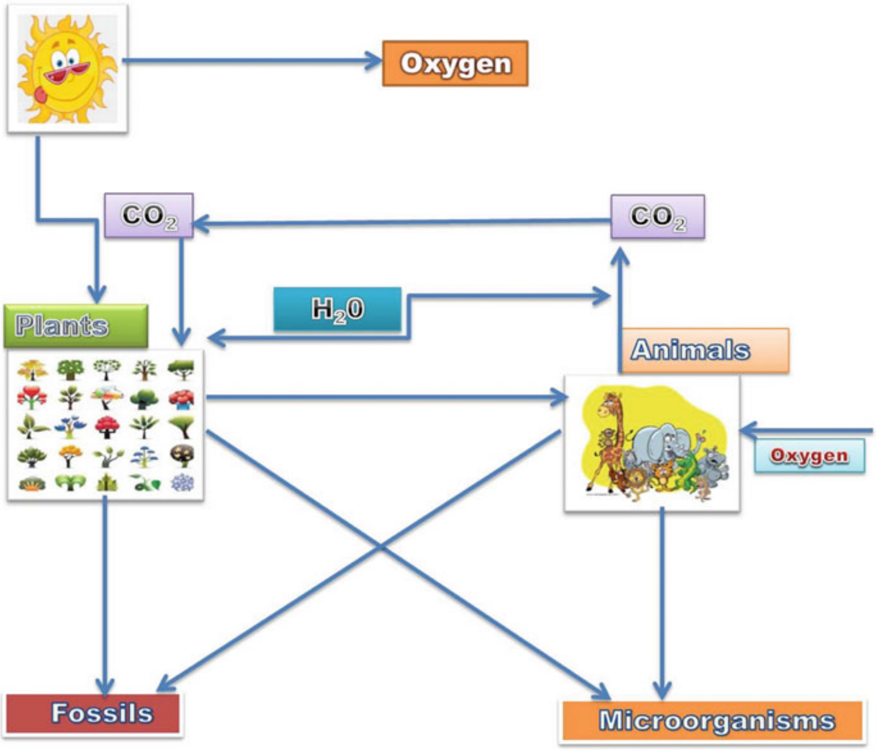

Fig. 3.1 Renewability of lignocellulosic biomass

chemical conversions owing to their green chemistry principles. But due to the complicated structure of the lignocellulosic biomass, a large number of organic methods are mandatory for most advantageous operations. The biological reactions also are very sluggish and hence require larger facility footprints. Pyrolysis can be labeled as systematic disassembling process with regulations on the produced products. Gasification is an extreme facet of transformation era, wherein the lignocellulosic biomass is broken down to the basic constructing blocks for hydrocarbons H_2 and CO after which it congregates to preferred products as favored, viz., Fischer-Tropsch process can turn CO along with H_2 into better alcohols, alkenes, and lots of other products. Synfuel (along with air aggregate of O_2 and N_2) is likewise the initial point for the production of ammonia, from which nitrogen fertilizers and many different products are formed. Though all thermochemical procedures are affected by selectivity, at some point of the disassembling manner, “coke” or “carbon” is formed specifically at excessive temperatures and as a consequence lessens the conversion efficiency if H_2 and CO are the favored intermediates. Contrastingly, biorefinery has the potential to satisfy the human essentials producing platform chemical substances besides the very basic building blocks of CO and H_2. For instance, glucose may be fermented to ethanol by using yeast anaerobically, and lactic acid may be formed by using lactic acid bacteria (LAB). A lot of these chemicals are examples of crucial platform (or intermediate) chemical compounds, along with the commodity chemicals, viz., ethanol, which is widely recognized for its use as a liquid transportation gasoline; it can be dehydrated producing polyethylene or dehydrogenated and dehydrated toward artificial rubber production and better alcohols and alkenes.

3.2 Biorefinery Concept

The International Energy Agency (IEA) currently coined a formal description for biorefinery, as "the sustainable processing of biomass into a wide range of bio-based products (food, feed, chemical substances, and/or materials) and bioenergy (biofuels, electricity, and/or heat)." Almost all types of biomass can constitute a possible feedstock for use in a biorefinery till effective transformation techniques and valorization are available—aquaculture (algae and seaweeds), biomass from forestry and agriculture, waste residues from households and other natural residues derived from plants and animals; a really perfect biorefinery has to be a close-to-zero-waste method, using sequential methods of fractionation along with extraction, followed by means of an aggregate of biochemical along with thermal processing, with chronic recycling of energy and waste streams (Fig. 3.2).

Therefore, a biorefinery accounts for the aggregation of interdisciplinary knowledge comprising chemical engineering, biology, biochemistry, chemistry, and biomolecular engineering. While biomass has been utilized as a premise of warmth and development substances, contemporary substances from a biorefinery comprise fluid

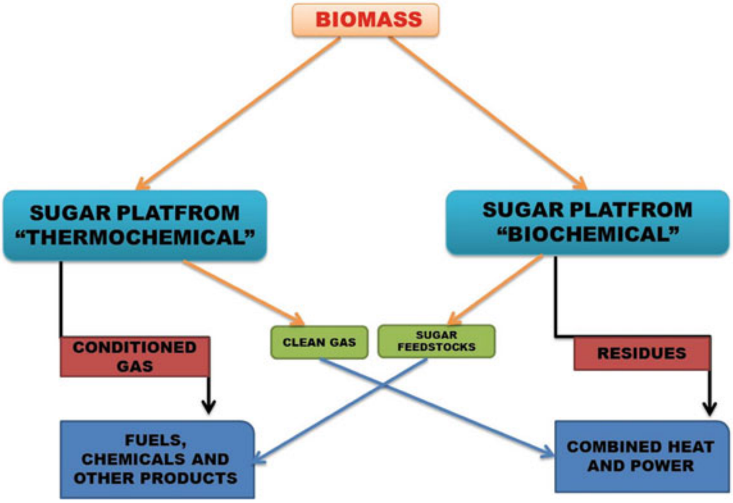

Fig. 3.2 The biorefinery concept

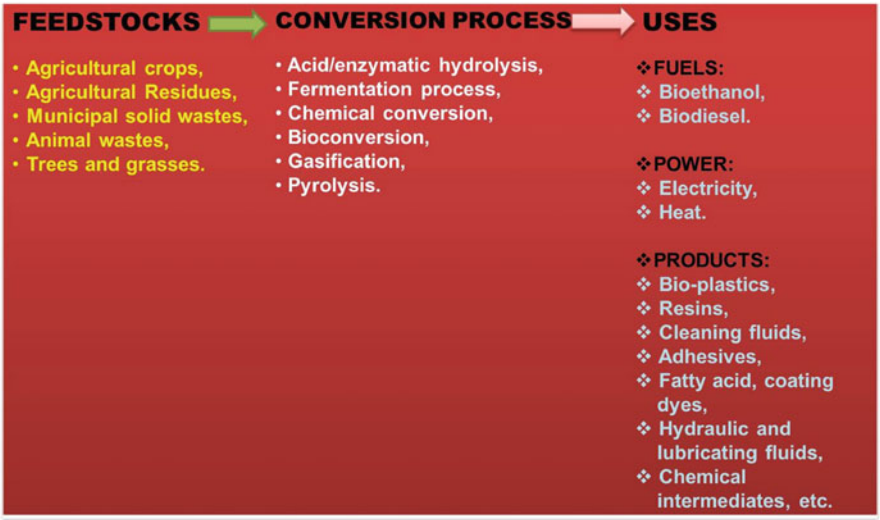

Fig. 3.3 Feedstocks and conversion process toward biorefinery

transportation fills and product synthetic compounds. The transport of these bio-based synthetics and products will give financial and natural endowments; notwithstanding, specialized difficulties regardless obstruct the goliath modern selection of the biorefinery idea. The legitimate biorefinery thought is a characteristic sign of the principles of “green science” for the explanation that it is focused on maintainable, synergistic change of inexhaustible feedstocks while making progress toward molecule and energy execution at insignificant disservice to the climate. Huge innovative work endeavors are as yet required in order to make the lignocellulosic biorefinery a reasonable and monetarily conceivable certainty. To this point, various stages of the biorefinery idea have been conceptualised in writing through the utilization of Clark and Deswarte thoughts as:

- Stage I biorefinery (single feedstock, single cycle, and single significant product).
- Stage II biorefinery (single feedstock, a few strategies, and numerous significant products).
- Stage III (a few feedstocks, more than one cycle, and numerous transcendent products).

The idea of manufacturing merchandise from agricultural biomass is a predefined process. However, expending biomass as an input to supply a chain of consumer merchandise, the usage of complex processing techniques, a methodology just like a petroleum refinery where fossil fuels are used as input, is rather new. Biomass consists of carbohydrates, lignin, proteins, fat, and to a lesser extent various other chemical compounds, consisting of vitamins, dyes, and flavors (Clark et al. 2012). The intention of a biorefinery is to change such abundant organic substances into beneficial products consuming an aggregate of technology and methods. Figure 3.3

defines the factors of a biorefinery wherein biomass feedstocks are used to provide various beneficial merchandises along with gasoline, power, and chemicals using organic and chemical conversion tactics. The principal aim of a biorefinery is to provide high-value low-volume (HVLV) and low-value high-volume (LVHV) merchandise—the use of a sequence of unit operations. The operations are aimed at maximizing the valuable extractables while curtailing the waste streams by altering LVHV intermediates into strength.

The high-value merchandise increases the effectiveness, while the high-volume fuels help to fulfil the worldwide power demand. The power created from a biorefinery additionally helps to diminish the overall value. In contrast to a petrol refinery, a biorefinery utilizes renewable sources producing fuels and chemicals lessening environmental pollution.

3.2.1 Background

The majority of the compound enterprises' products come from fossil sources, from both oil- and biogas-processing plants. Around 5% of the oil and gas produced each year goes to the preparation of the multitude of petrochemicals of our general public. Uncooked materials from fossil resources are highly vital because they have up to now been the least high priced, easily and readily obtained, and the maximum appropriate for the manufacturing of a restricted set of platform chemicals, from which many chemical substances and derivatives are acquired. Enhancements in conversion efficacies and optimization of product synthesis have been executed through a few years of research and development, which have brought about a production of chemical substances with specific homes, appropriate for multiple packages and uses. In the beyond, petrochemicals have had a dramatic impact on our meals, chemical, clothing, and production industries—artificial substances had been designed for specific and customized residences, and are higher applicable for specific programs in place of natural products. In addition to eco-friendly issues resulting from fossil refinery methods (together with toxicity and degradability), it is a not unusual notion that the stop of the reasonably priced fossil technology is just across the nook, and that crude oil prices, transportation fuels, and petroleum-derived chemical compounds are probable to progressively upsurge within the future years (Greene et al. 2004; Bentley et al. 2007). These problems can be overcome by using step-by-step shifting from a fossil gas-based society closer to a brand-new economic system wherein renewable sources, domestically to be had in many countries, impart a dominant function. This alteration has both power and material perspectives which must be considered. Sustainable assets for the strength and transportation fuels' creation had been to a great extent inspected by the established researchers, with recognition on innovative cycles, market potential, and techno-financial audits (Azar et al. 2003; Hamelinck et al. 2005; Bai et al. 2008; Börjesson and Mattiasson 2008; Dale and Kim 2008; Demirbas 2009b; Gassner and Maréchal 2009). In comparison to bioenergy studies, fairly slight interest has been paid to the opportunity of changing current petrochemicals with chemical substances constituted of renewable substances. While the manufacturing of renewable power can depend on an extensive range of options (wind, hydro, solar, and many others), biomass is the only renewable carbon-rich material accessible on the earth, besides fossil assets, for manufacturing of chemical commodities (Kamm et al. 2008a, b; Cherubini 2010). The sustainable usage of bio-based carbon completely indicates incorporated production in biorefineries. The biorefinery concept holds a widespread variety of technologies able to isolate biomass resources (wood, grass, corn) into their building blocks (carbohydrates, proteins, fats) which could then be transformed to consumer merchandise, biofuels, and chemical compounds.

A biorefinery is a facility (or a network of centers) coordinating biomass transformation innovations to create transportation biofuels, power, and synthetic substances from biomass. Research dealing with the likelihood of manufacturing valued chemical substances from biomass exists already. Haveren et al. examined the probability of changing petrochemicals from the refinery placed in Rotterdam with bio-based chemicals (van Haveren et al. 2008). Their studies focused upon a short-term substitution capacity of 10–15% of oil-derived chemical substances with biochemicals, particularly for oxygenated bulk chemical substances (ethylene glycol, propylene glycol, acetone, and many others), at the same time as for the alternative of oxygen-free and N-containing chemical compounds, a medium-time period angle is important. Werpy and Petersen recognized 12 constructing-block chemical compounds which could be constituted of sugars through organic or chemical conversions; these constructing blocks (or platform chemical compounds) may be consequently transformed into a variety of high-price bio-based materials and substances (Werpy and Petersen 2004). Holladay et al. and Tuomi et al. provided an overview of lignin’s role as a raw material toward the production of energy, substances, and chemical compounds, specifically aromatics. Mäki-Arvela et al. carried out an analysis of the synthesis of fine and specialty chemicals from wooden and different biomass resources via heterogeneous catalytic procedures (Mäki-Arvela et al. 2007). Bos et al. supplied a varied collection of chemical products that may be made from carbohydrates, and then evaluated their existing and prospective markets. Marquardt et al. insightfully assessed the “biorenewable possibility,” discovering subsequent-era procedures and product systems (Marquardt et al. 2010). Explicitly, Herchinger et al. designed newer fuels and synthesis routes keeping in mind the molecular assembly of desired product properties. Other research has centered at the production of chemical substances from gasification generation (Spath and Dayton 2003; Consonni et al. 2009), different lignocellulosic biomass treatment methods (Hayes et al. 2008), green biorefinery (Kamm et al. 2010; Mandl 2010), and biorefinery market penetration eventualities and destiny traits (Laser et al. 2009; Sokhansanj et al. 2009; Cherubini 2010; Marquardt et al. 2010). Yet others have explored the amalgamation and transformation to subsidiaries of explicit, biomass-inferred platform chemical substances, including levulinic acid, furfural, succinic acid, and others (Bozell et al. 2000; Ezeji et al. 2007; Kamm et al. 2008a, b; Jem et al. 2010).

3.3 Types of Biorefinery

Three sorts of biorefineries recognized as stage I, II, and III have been portrayed by Kamm et al. and Van Dyne et al. A stage I biorefinery plant has stable preparing abilities and grain as a feedstock. A dry factory ethanol plant is an illustration of a stage I biorefinery which yields a fixed measure of ethanol, other feed items, and carbon dioxide and has practically no handling mobility. A cycle including present-day wet processing innovation could be viewed as a stage II biorefinery which utilizes grain feedstock as information like dry processing. Be that as it may, it has the capacity to deliver different final results and unquestionably additionally preparing flexibility relying on an item interest, costs, and contract commitments. Types of biorefinery are depicted in Table 3.2.

The commonplace items are high-fructose corn syrup, corn oil ethanol, and starch. Stage III, the most created, biorefinery utilizes a blend of biomass feedstocks and produces a variety of items utilizing expertise conglomerate (Van Dyne et al. 1999; Kamm et al. 2008a, b). It permits a blend of rural feedstocks, has the capacity to utilize different sorts of handling strategies, and has the ability to create a blend of higher esteem synthetic substances while coproducing ethanol (Tyson et al. 2004). It depends on both the HVLV and LVHV standards. Phase III biorefineries, in particular the entire harvest, green, and lignocellulose feedstock (LCF) biorefineries, are still in exploration and development.

3.3.1 *Entire Crop Biorefinery*

An entire crop biorefinery measures and devours the whole yield to get valuable items. Crude materials, viz., wheat, triticale, rye, and maize, can be utilized as a contribution to the feedstock in the unit activities of a whole-crop biorefinery as

Table 3.2 Types of biorefinery

Biorefinery type	Features	Products
Lignocellulosic biorefinery	Uses crops and agricultural residues	Bioethanol (lignocellulosic)
Agricultural biorefinery	Uses all types of waste generated from agricultural purpose	Methane, hydrogen, chemical by-products, and other materials
Industrial or municipal waste biorefinery	Uses all types of waste generated from municipal and industries	Methane, hydrogen, fuels, energy, and chemical by-product
Green biorefinery	Uses green plants and grasses	Bioethanol
Cereal biorefinery	Expends starchy crops, sugar crops, and grains	Bioethanol
Oilseed biorefinery	Expends oil plants and oilseed crops	Biodiesel, vegetable oil
Forestry biorefinery	Uses all types of forestry residues, sawdust, pulping liquors, and fibers	Fuels, energy, chemical by-product

portrayed in Fig. 3.3. The cycle of changing over biomass into energy is started by mechanical detachment of biomass into various segments that are at that point treated independently. Biomass is the beginning substance for the creation of syngas where syngas can be utilized as the fundamental material for the amalgamation of energies and methanol utilizing the Fischer-Tropsch (FT) process (Kamm et al. 2008a, b). Corn can be either utilized straightforwardly in the wake of granulating to supper or changed over to starch. Further preparation can be completed as (1) separating, (2) plasticization, (3) compound change, and (4) biotechnological transformation through glucose.

3.3.2 *Green Biorefinery*

A green biorefinery signifies a multiproduct framework which grips its processing plant trimmings, item, and divisions as per the related plant material physiology as portrayed by several scientists (Fig. 3.4). A green biorefinery utilizes common wet feedstocks obtained from untreated items, for example, green plants, grass, or green harvests.

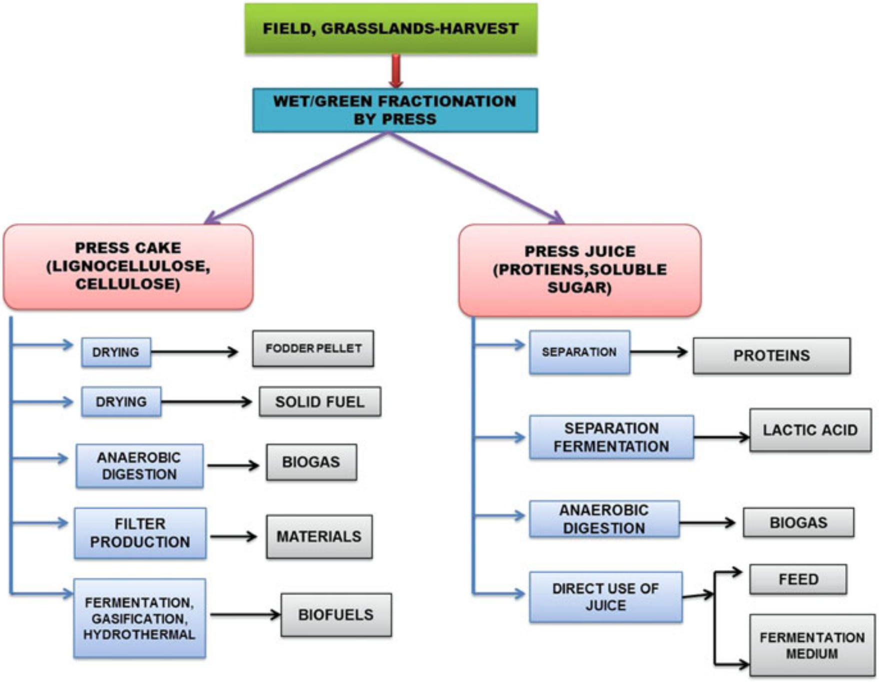

Fig. 3.4 Green biorefinery

The initial step of the processing plant is the treatment of the green biomass substances in their characteristic structure utilizing wet fractionation producing a fiber-rich press cake along with a supplement-rich green juice. The press cake comprises cellulose, starch, important colors along with shades, crude medications, and different organics; the plant extracts contain proteins, free amino acids, natural acids, other natural substances, colors, catalysts, hormones, and minerals.

The squeezed cake can be likewise utilized toward the creation of natural feed pellets, as a crude material for the creation of synthetic compounds such as levulinic acid and for transformations to syngas and engineered fuels (Kamm and Kamm 2004).

3.3.3 Lignocellulose Feedstock (LCF) Biorefinery

LCF comprises three essential synthetic divisions: (1) hemicellulose, a five-carbon sugar polymer; (2) cellulose, a six-carbon glucose polymer; and (3) lignin, phenol polymers. A LCF biorefinery as portrayed in Fig. 3.5 utilizes hard fibrous plant materials from leftovers of cuttings and other sources. At first, plant material is washed and separated into the three portions (hemicellulose, cellulose, and lignin) through compound processing or enzyme-based saccharification. Hemicellulose along with cellulose can be created by soluble (harsh pop) and sulfite (acidic, bisulfite, basic, and so on) process. Lignin present in plant materials is separated

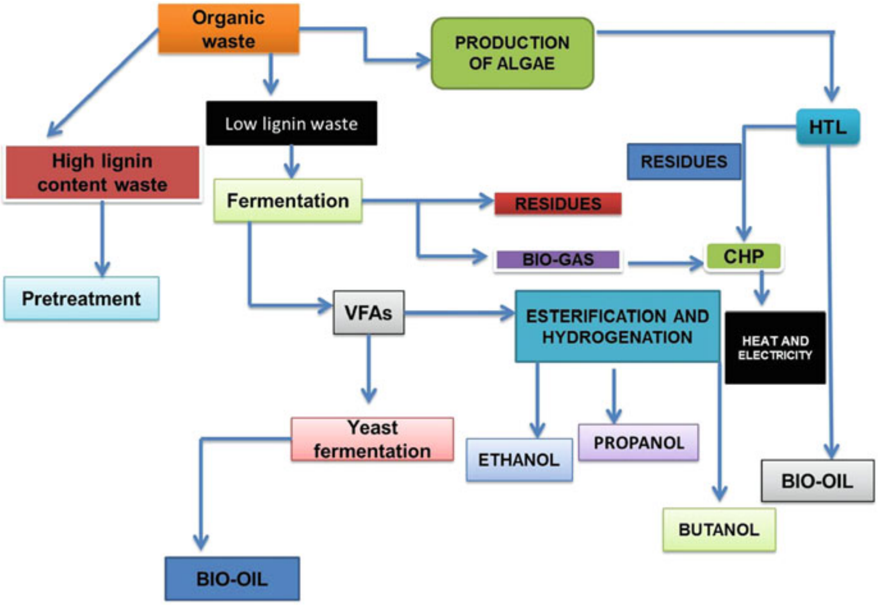

Fig. 3.5 Incorporated biorefinery

with proteins by the action of ligninases, lignin peroxidases, laccases, and xylanolytic proteins. The sugar polymers (hemicellulose and cellulose) are broken down to their monomeric segment sugars through hydrolysis (Fig. 3.5). On account of hemicellulose, it comprises little, profoundly spread chains of sugars. Unlike cellulose, which is a polymer of glucose, a hemicellulose is a polymer of five-carbon sugars (typically D-xylose, L-arabinose), six-carbon sugars (D-galactose, D-glucose, and D-mannose), and uronic acids. The hydrolytic cycle of hemicellulose sugars along with the accompanying substance responses give an overall diagram of the changes that occur in a LCF biorefinery. The xylose part from hemicellulose is significant in light of the fact that it tends to be changed over to furfural, a beginning material for nylon. Moreover, furfural has numerous utilizations: it can be utilized in the refining of engine oils, as an antecedent of certainty. The saccharification of cellulose to glucose can be accomplished by enzymatic or synthetic processing with the production of ethanol, butanol, succinic acid, and other aging items. In spite of the fact that the hemicellulose and cellulose portions have various utilization, it is not exactly the situation for lignin. As of now, lignin has restricted uses, for example, a glue or folio and as a fuel for direct ignition. In any case, the lignin framework has huge potential to create different types of monoaromatic hydrocarbons, which, whenever detached in a monetarily productive way, could increase the value of the essential LCF measure. It should be seen that there are no self-evident, normal chemicals to part the normally happening lignin into its essential monomers as effectively as is feasible for normally framed polymeric starches or proteins. The LCF plant in Missouri yields around 28,800 tons and 33,269 tons of ethanol and furfural, respectively, every year from day-by-day feedstock utilization of 4000 tons. A significant microbial glucose alteration can be completed utilizing an elective highway (Zeikus et al. 1999; Willke and Vorlop 2004) for the petrochemically delivered substances, viz., hydrogen, methane, propanol, and acetone.

In a current methodology, the U.S. Division of Energy and NREL have portrayed transformation advancements for extended biomass dependent on the “stages” on the grounds that the fundamental innovation would create base or stage synthetics from which industry could yield a wide scope of energies, synthetic substances, materials, and power. Out of the five stages in sugar-stage biorefineries (SPBs), the four recommended stages are thermochemical or syngas stage, biogas stage, carbon-rich chain stage, and plant item stage. The “sugar stage” centers around the sugars’ maturation extricated from biomass feedstocks. The goal is to organically measure the sugars to create fuel, for example, ethanol, or then again other structure block synthetic substances. SPBs are firmly identified with LCF biorefineries in the traditional classification. The thermochemical or syngas stage centers around the gasification of the biomass feedstocks. This methodology changes over the strong biomass into vaporous and fluid fills by blending it with restricted oxygen before ignition. Different parts delivered through this cycle can be isolated into fills or important synthetics. NREL’s primary spotlights are on the SPB and “syngas” stages. The biogas stage, a broadly utilized innovation, especially in agricultural nations is for delivering cooking gas. This stage decays biomass with normal microorganisms in anaerobic digesters. The cycle yields methane along with carbon dioxide. The carbon-rich chain stage utilizes plant oils, for example, soybean, palm, corn, and canola oils, which are as of now utilized for food and substance creation. Transesterification of the vegetable oil or creature fat harvests unsaturated fat methyl esters, ordinarily known as biodiesel. Biodiesel is now being used as a significant business air discharge-lessening added substance or substitute for oil diesel. Specific rearing and hereditary designing can be utilized to create plant strains which produce more noteworthy measures of alluring feedstocks, synthetics, or even mixes that the plant does not normally yield. The aim is to play out the biorefining in the natural plant itself instead of in a modern plant. This approach is recognized as the plant item stage.

3.3.4 Incorporated Biorefinery

The biorefinery types mentioned in earlier sections are based on single-conversion stage providing different chemicals. A biorefinery is a capital in-depth challenge, and while it is primarily established on simply one conversion technology, as is the case for the previously described biorefineries, it will increase the value of productivities (or products) produced from such biorefineries.

As a result, numerous conversion technologies (biochemical, thermochemical, etc.) are mixed collectively to decrease the overall value, as well as to have an added mobility in product generation and to offer its personal energy. Figure 3.5 presents a schematic of an included biorefinery.

Three unique designs, explicitly thermochemical, sugar, and non-stage or present innovations, are incorporated. A joined biorefinery produces various items, which include power-involved thermochemical and bioproducts from the combination of sugar and other current change innovation frameworks. An arising idea inside the biorefinery region is transformation of bio-oil, the item from biomass pyrolysis, which might be steered through a regular petrochemical processing plant to create assorted synthetic substances. The advantage of this bearing is that the entirety of the indispensable foundation for the detachment and refinement of items created is as of now in area. This thought can be misused as most petrol treatment facilities are appropriately prepared to deal with variable feedstock with the suspicion that no groups of raw petroleum are equivalent. Tables 3.3 and 3.4 provide the composition of bio-oil compounds. Bio-oil chemical source ranges with the feedstock but woody biomass normally produces a combination of 30% water, 30% phenolics, 20% aldehydes and ketones, 15% alcohols, and 10% miscellaneous compounds. A procedure referred to as hydrodeoxygenation (HDO) might be carried out substituting oxygen via hydrogenation of the raw bio-oils. After numerous HDO treatment steps the bio-oil could be converted into a liquid hydrocarbon with sources similar to those of petroleum crude oil. The deoxygenated bio-oils can probably be refined in prevailing petroleum refineries, with minor changes for practical use to the modern petroleum enterprise refinery infrastructure set up for hydrodesulfurization (HDS) techniques (Bridgwater and Cottam 1992). HDO treatments of bio-oils with metal catalysts, along with sulfated Co, Mo, W, or Ni, were approved from the petroleum industry (Baker and Elliot 1988; Bridgwater and Cottam 1992; Oasmaa and Boocock 1992; De La Puente et al. 1999; Ferrari et al. 2002; Zhang et al. 2003). It has been proven that a two-degree method is needed (Baker and Elliot 1988; Gagnon and Kaliaguine 1988). The primary stage applies a slight hydrogenation at rather low temperatures underneath approximately 270 °C. Complete HDO of bio-oils necessitates temperature beyond 300 °C which ends up in polymerization of the fairly oxygenated compounds in uncooked bio-oils (Bozell et al. 2000). It is moreover crucial to regulate the quality necessities of biorefinery products at the commencement of this generation to diminish variability. Such standardization will assist the exploration studies to achieve products with high quality. For example, it could be useful for bio-oil researchers to understand the minimal features to target if bio-oil is to be routed thru a petrol refinery. Perceiving these base capacities is a task, particularly in view of the multidisciplinary idea of the worry, and should be refined in close coordinated effort with petrol engineers, bioenergy designers, scientists, and researchers. Likewise with petrochemical processing plants, the fundamental target of the essential bio-oil-based biorefinery is to give items comprising significant compound substances, notwithstanding energy. Subsequently, it is vital to have a look at the cost-effective chemical compounds made out of the incorporated biorefinery, which economically and technically help the manufacturing of gasoline and energy comprised of those refineries. NREL and PNNL (Pacific Northwest National Laboratory) researchers accomplished a comprehensive examination to discover precious sugar-derived chemical compounds and substances that would serve as a financial driver to the included biorefinery (Elliott and Neuenschwander 1997). Raised efficiency, declined fabricating worth, and execution might be finished by utilizing activities that decline the general strength profundity of the biorefinery's unit and pressing factor down all creation charges through amplifying the use of all feedstock segments, side effects and waste streams, economies of the scale, basic preparing tasks, substances, and contraption. Data of some of the essential value added compounds have been assessed, as reported elsewhere.

3.4 Major Platform Chemical Substances in Present-Day Fossil Refinery

Unrefined petroleum is a combination of various normal hydrocarbon compounds. The initial phase in petroleum treatment facility is made in the evacuation out of water and debasements. The unrefined petroleum is then refined into its different portions, viz., gas, diesel, naphtha, lamp oil, greasing up oils, and pavements. The overall volumes of the parts framed depend on the preparing circumstances and the synthesis of the unrefined petroleum. The naphtha portion is at last utilized as feedstock for the creation of a couple of stage synthetic substances from which all the crucial item compounds are sometimes determined. A basic element of the naphtha feedstock is that, as opposed to biomass, it is exceptionally low in oxygen content. The greatest imperative synthetic compounds from oil and flammable gas-processing plants are displayed in Fig. 3.1, alongside their worldwide creation in 2009. This figure shows that the present synthetic industry methodologies' sources are a little assortment of stage substance compounds from which a broad range of auxiliary synthetic mixtures are created. These compounds have a few projects in practically all the areas of our general public, for example materials, plastics, pitches, and food and feed-added substances. The stage materials from which the overall population of item synthetic substances might be created are ethylene, propylene, butadiene, and the aromatics BTX (benzene, toluene, and xylene).

3.4.1 Lignocellulosic Biomass as Unprocessed Materials

3.4.1.1 Biomass vs. Fossil Resources

The structure of biomass is less homogeneous than oil. The extent of biomass inside the feedstock consists of natural arrangements of C, H, and O (in addition to other minor segments comprehensive of N, S, and a couple of mineral mixtures). Concerning lignocellulosic biomass, it has three key components: cellulose, hemicellulose, and lignin. Cellulose and hemicellulose are made of sugars, particularly glucose and xylose, which are procured after depolymerization through hydrolysis. Sugars have the advantage of being transformed into a wide range of substances by biochemical methods or compound improvements. The processing of sugars to ethanol is demonstrated as accessible and available lately; over 90% of the world's ethanol creation is derived from biomass feedstock, even as a definitive 10% is made out of oil- or gas-processing plants (Altaf et al. 2007; Jem et al. 2010). The one-third segment, lignin, comprises haphazardly stretched phenylpropenyl (C9) units. Today, lignin is utilized as an inventory of warmth and force for handling vegetation (particularly in mash and paper enterprises), and all its bleeding-edge business utilizes exploiting its polymer and polyelectrolyte houses (as dispersants, emulsifiers, folios, and so forth). Regularly, lignin is utilized in these bundles with practically no change besides sulfonation or thio-hydroxymethylation. In contrast to petrol, biomass by and large has too little hydrogen, an unreasonable measure of oxygen, and a lower part of carbon. The compositional assortment in biomass feedstocks is both a reward and a disadvantage. The preferred position is that it is allowed in biorefineries making additional classes of items than petrol-processing plants, in a manner having the option to rely on a lot more extensive scope of crude substances. The disadvantage is that an enormously bigger assortment of preparing innovations is needed, and the limit of those innovations is still at a budding stage (Dale and Kim 2008). Another distinction with petrol sources concerns the occasional changes taken into account by utilizing biomass providers; collecting is now generally not practical throughout the year. An exchange from petrol to biomass may likewise require a trade in the capability of synthetic ventures, with a need to create the materials and compound substances occasionally. Maybe like all the compounds considered, biomass likewise ought to be balanced out before extensive term stockpiling to have the option to guarantee a constant, all-the-year activity of the biorefinery (Nurmi 1999; Wihersaari 2005; Rentizelas et al. 2009).

3.4.1.2 Biomass Processing in a Biorefinery

In the broad sense, biomass has a measure of carbon which must be held throughout the chain, not at a point of sufficient hydrogen, which needs to be brought, and a lot of oxygen, which must be dismissed along the edge of other undesirable variables (which incorporates nitrogen and sulfur) (Cherubini and Strómman 2010). Hydrogen can be presented for the utilization of water (H_2O) as a help, despite the fact that this involves the expansion of even additional oxygen, which should be dismissed. The expansion of hydrogen as H_2 is more noteworthy, engaging, and productive (the use of right steel impetuses), however, oppressed by the reality that essential hydrogen is absent in nature and that energy should be contributed to give it. Oxygen is dismissed both as CO_2 and as H_2O. In the two cases, there are natural issues: inside the principal case, each mole of oxygen disposes of 1/2 a mole of carbon (accordingly diminishing the carbon proficiency), while inside the second case, one mole of oxygen eliminates two moles of hydrogen (which, oppositely, wishes to be presented). It would be absolutely best to dismiss oxygen as O_2, yet this is certifiably not a standard yield of any accessible measure of biomass change. The diverse undesired variables, for example sulfur and nitrogen, are commonly dismissed of their oxide organization (SO_2 and NO_2, separately), consequently adding to dismissal of additional oxygen. The improvement of biorefinery edifices calls for both a customary, year-round supply of biomass sources and a variety of compound strategies to follow up on nonhomogeneous substrates. It is likely that this cannot be traded, so advances should be developed to precondition biomass feedstocks, accordingly rendering their sources and reactivity styles more prominently strong, normal, and uniform (Hatti-Kaul 2010). The different biomass-added substances must be isolated ahead of schedule in biorefinery activities, a decent method to have an effect among individuals expending energy uses and individuals bound to synthetic bundles (requiring inordinate degrees of virtue). The biomass stream committed to the assembling of transportation biofuels and synthetic substances wants to be depolymerized and deoxygenated. Deoxygenation is fundamental because of the presence of O_2 in biofuels bringing down the warmth content material of particles and regularly giving them an extreme extremity, which obstructs mixing with existing petroleum products (Lange 2007). Synthetic applications may furthermore require significantly less deoxygenation, for the explanation that the presence of O_2 regularly is an important phenomenon. Depolymerization might be attempted beneath harsh conditions (gasification, pyrolysis), in the event that we need to totally crush down the first carbon chains into C1 compounds, or milder circumstances (for example biochemical change), in which the key unique construction is safeguarded.

3.5 Biorefineries: Scenarios and Challenges

The recourse of action of whole economies to natural uncooked substances as wellspring of cost increment requires totally new fundamental strategies in investigations, improvement, and business execution. From one perspective, normal science includes a main capacity inside the time of future enterprises of the twenty-first century. Then again, new techniques for cooperative energy of rural, organic, physical, compound, and specialized sciences ought to be explained and set up. The need for biorefinery improvement in Europe and Germany is distinctive in the “strategies of bioeconomy”; the comparing explanations on the significance of the overall population advancement and scale-up endeavors can be situated through various methods. In 2012, the German specialists distributed the “Biorefineries Roadmap,” for example, notoriety quo and potential outcomes in a period collection of 2030. In accordance with the compounds and fuel creating industry, the lignocellulosic crude substances as nonfood biomasses have the best need to arise as a real chance to supplant the forefront fossil resources along with their immensely green petrochemical assembling of synthetic materials and energies. The improvement of advanced treatment facilities of economically developed crude materials and the assembling of antecedents and products of essential processing plant alongside sugars, lignin, and extractives, e.g., proteins and inorganic materials, are imperative. Clinical comprehensions in different fields, particularly actual partition procedures, biotechnological strategies, biocatalysis, economical science, and combination of methods, are basic (Fig. 3.1). The assumption is ought to be a judicious and natural relationship of the product of the biomass-handling plant at the rural improvement places with the discretionary fractionation at the district of compounds manufacturing industry. This could be the reason for procedures of new biorefinery/bioeconomy endeavor styles. All biomass usage ways, which incorporate each setup and progressive strategy to biomass change, are viewed as fundamental and will be the agenda of improved examinations and advancement endeavors inside the issue. This is genuine in an assessment both with a fossil reference gadget and with different ideas for biomass use pointed toward decoupled utilization of biomass or potentially biomass-added substances without concurrent assembling of a few other fabric and additionally energetic items. Bio-based substances are ready for a financially feasible programming by an important blend of different procedures and methods (physical, substance, organic, and warm). It is, thus, fundamental that biorefinery premise innovation, especially lignocellulosic feedstock biorefinery, cereal complete harvest biorefinery, and green biorefinery, must be advanced. Biomass-predecessor sugars, lignin, lipids, and proteins might be the beginning materials for the stages. Those building blocks will be sometimes changed over to some of the excessively valued bio-based synthetic substances (Fig. 3.2).

3.5.1 Lignocellulosic Biomass Through Aliphatic and Aromatic Stage Compound Creation

Lignocellulose feedstocks are biomasses, predominantly composed of complicated two structural carbohydrates, cellulose (38–50%), hemicellulose (23–32%), and phenolic lignin (15–25%). Depending on the corresponding physical or chemical composition, lignocellulose feedstock can be divided into the following subgroups: cellulose, hemicellulose (polyoses), lignin, extractive substances, and ashes, as illustrated in Fig. 3.3.

Cellulose adds to around 44.5% of wood's dry weight. Cellulose is made of direct chains of D-glucose associated through β-1,4-glycosidic bonds with polymerization between cycles of 9000 and 15,000. Intra- and intermolecular hydrogen securities are formed through hydroxyl associations inside the C2, C3, and C6 positions of the D-anhydroglucopyranose ring; they harden the chain and sell assortment of around 100 cellulose molecules into uniform glass-like structures called micelles, microcrystallites, or principal fibrils. Roughly 20 micelle structures are protracted, threadlike constructions referred to as microfibrils; these microfibrils have high ductile force and are around 20–30 nm in measurement. In any case, the frameworks of microfibrils are not totally uniform as far as the arrangement of the cellulose macromolecules. The zones of nonuniformity of the micelles inside the microfibrils are known as indistinct locales; therefore, microfibrils have an undefined glass-like structure. Microfibrils are packaged of all things considered to shape macrofibrils. Cross-connecting these macrofibrils through hemicellulose by means of hydrogen holding offers the underlying spine of the plant cell divider. Hemicellulose is a shapeless, stretched heteropolymer that varies in the creation relying upon the sort of plant material. Monomer-added substances of hemicellulose are xylose, mannose, galactose, glucose, arabinose, and methylglucuronic acids. In delicate wood, hemicellulose is basically established of mannose contraptions, and extreme wood comprises xylose. The polymerization degree is around 100–200 and is subsequently lower than that of cellulose. The composite structure of translucent cellulose is setting up in miniature and macrofibrils and undefined hemicellulose are encased through lignin, a three-dimensional polymer of phenylpropane units. In wood, the spaces between strands are almost made out of pure lignin and are named the middle lamella. Lignin is viewed as the paste or encrusting substance of wood and gives mechanical strength and firmness. Lignin organization is a totally safe complex contrary to hydrolytic or bacterial attack. Besides green biomass, lignocellulose feedstock is the most extreme basic uncooked texture for biorefinery strategies. The fine recognized assets are the resulting wood, fast-developing timbers, old backwoods and wood, recuperated paper, and straw. In Table 3.1, lignocellulosic assets are summed up and classified into four organizations. Each of the four stock organizations assumes an important part inside the stockpile of LCF. Be that as it may, sizable territorial varieties exist. Organizations 2 (expedient developing ranches) and 3 (lignocellulose squander from farming, ranger service, and industry) turn into the speediest creating sections because of significant resources (e.g., fast-developing woods and straw). In Europe, establishment 1 will profit additional interest because of sizable changes in horticultural legislative issues. The LCF biorefinery has a brilliant capacity for making genealogical item trees as exhibited in Fig. 3.4. The utmost increase of the biorefinery idea is that home-grown frameworks and construction factors are safeguarded totally or as a base part of the way. Besides, crude materials are sensibly evaluated and numerous item types are feasible. Be that as it may, extraordinary endeavors are needed inside the turn of events and improvement of these advances, extraordinarily for the partition of cellulose, hemicellulose, and lignin. Lignin science should open up the chance of lignin usage as an uncooked surface for the substance business as an option in contrast to its present use as a solid fuel. Hemicellulose will be a beginning material for C-5 structure blocks, comprising furfural and C-6 structure blocks of cellulose, incorporating levulinic acids and 5-hydroxymethyl furfural (HMF) (Fig. 3.5). Levulinic acid is as of now delivered on a limited scale of around 450 tons every year (Hayes et al. 2008).

Levulinic acids might be made out of hexoses in acidic media or from furfuryl liquor through ring beginning. Furfuryl liquor is acquired from furfural decrement. Furfural is exclusively produced using hemicellulose contained in sugar-rich bagasse, corncobs, rice, and oat frames at a business scale with a yearly worldwide assembling of 2,00,000–3,00,000 tons; roughly 70% are delivered in China. A total creation of furfural and levulinic acids from LCF has all the earmarks of being conceivable through the Biofine framework. The current creation of 5-hydroxymethyl furfural happens by utilizing hydroxymethylation of furfural with formaldehyde. A mechanical technique for its production through the sugar course has not, at this point, been progressed on the grounds that arriving at sensible yields requires the use of solid acids and characteristic solvents that could require expensive balance and division measures. Also, glucose utilized as a beginning texture shows outstandingly low reactivity, simultaneously as more responsive fructose (1000 €/t) and inulin (500 €/t) would involve a commercial center cost of 5-HMF at any rate of 2500 €/t; the expense is excessively high for a mass-scale synthetic accumulation. 5-HMF auxiliary product comprises 2,5-furan dicarboxylic acid, 2,5-bis (hydroxymethyl) furan, and a couple of 5-furandicarboxaldehyde as monomers for polyamides, polyesters, and polyurethanes. These reagents should refresh petro-artificially determined mixtures, for example, terephthalic, isophthalic, and adipic acids, that would be utilized for the assembling of purchaser plastics and will highlight as a beginning texture for the blend of recommended medications. In this manner, 5-HMF has a high potential that businesses call for and is known as "a resting monster."

3.5.2 Green Biomass as Crude Material for Proteins and Chemical Synthetics

The green cropland development in Europe (establishment: 15 part states without new part states on record) amounts to 45 million tons and therewith to 35% of the horticultural harvest land dependent on a middle yield of ten parcels dry matter with regard to hectare and year, and 450 million tons of dry matter is delivered yearly with the guide of the 15 EU part states. In Europe the most significant scavenge crop is horse feed (Lucerne) as a result of its ability to absorb nitrogen from air and to supplement it inside the dirt. Hay is developed on around 32 million hectares inside the referenced 15 EU part states. In the USA top-to-bottom examinations inside the control of biorefineries have been occurring during the most recent 10 years. The Alfalfa New Product Initiative (ANPI), which has a place in 5 of the states, targets on the increase of the development and utilization of alfalfa. In this manner perceived innovations, applied at every scale just in France, similar to parchedness and fractionation, are used. The extreme protein content and the valuable amino acids make horse feed somewhat fascinating by water-solvent proteins which may be around 15% of the normal protein content material; 40–60% of the dissolved water, additionally called white proteins is RuBisCo (ribulose 1,5-bisphosphate carboxylase/oxygenase). RuBisCo is articulated to address about 65% of the whole water-solvent proteins in hay leaf juice. In popular terms, the situation of meadow as feed provider transforms into a less significant factor considering the way that compelled fabricating costs and expanding efficiency are key to diminished domesticated animals sooner rather than later in Europe. Subsequently, the field may be progressively available for material usage. Particularly the harvest of "nature wet" grass and the quick use of precisely crushed fresh squeeze are energizing the biotechnological undertaking. Existing rural frameworks of field development like green harvest drying vegetation give appropriately potential outcomes to the usage of unpracticed biorefineries. Along these lines notice should be taken about the reality that the forefront warm drying is halfway outdated and current methods inside the feed creation must be executed. It may be useful to take moderate advantage of the predominant farming designs inside the field of horticulture employing certain methods for the creation of semifinished items like press squeeze and press cakes. Present-day unpracticed yield drying vegetation might appear as horticultural crossing points inside the grass and unpracticed harvest agribusiness.

Green biorefineries posses multi-item designs utilizing the varied union of the plant material produced through nature. Further to the overall biorefinery idea, GBRs are firmly founded on manageable standards (reasonable land use, supportable uncooked substances, mellow advancements, autarkic energy convey, etc.). Existing horticultural constructions of the unpracticed harvest handling venture, as unpracticed yield drying vegetation, offer careful opportunities for the execution of biorefinery advances to help defeating power inside and out and to some extent obsolete innovations like the warm drying of feedstock. A fundamental piece of those harvests is dried in biomass or multi-natural product drying verdure and is accessible as pellets or bunches. Inside the future such drying vegetation will play an essential role as rural business crossing point inside the business preparing of biomass. Inside the edge of guide biorefinery in Germany, various unpracticed biorefinery activities are thought about. One idea envelops the setup of a horse feed-based green biorefinery for the assembling of stage synthetic mixtures lysine lactate, lactic acids, proteins, and biogas creating digestate as a co-item. The idea fuses a method of activity with both snowy climate and summer season activity. For frostiness activity, grass cuttings are ensiled, creating silage. In the primary handling step, silage is extricated and squeezed in a screw press. The final product is silage press juice (green juice) and silage press cake (green fiber). Lactic corrosive and acidic corrosive are chromatographically isolated from the silage press juice, and the contrary juice-added substances are acquainted with the lysine aging medium. The silage press cake is exposed to enzymatic hydrolysis to have the option to hydrolyze the sugars (saccharification); all in all with the leftover stage, this is then combined in an entire aging mode for lysine producing. The lysine is put away for summer season activity. For late spring activity, shining grass is removed and squeezed in a screw press. The squeezed juice is partially warmed and the white proteins are layer isolated from the unpracticed proteins. This results in a fluid lingering section (earthy-colored squeeze) that is conveyed to the mechanism for lactic corrosive aging. The snap cake is exposed to extraction and enzymatic hydrolysis to monomerize the contained carbs (saccharification); all things considered with the remaining segment, this is then combined in a full maturation mode for lactic corrosive assembling. Balance with lysine (from the colder time of year activity) gives lysine lactate. The remaining biomass can be converted to biogas. The subsequent digestate co-item is utilized as manure. The passage of biorefinery de GmbH to the press juice line for first-class white protein creation will be determined and may be independently overhauled in the wake of undertaking realization by the extension of mission working. The association has its own patent aptitude for the formation of bio-based regular blends what's more polymers (proteins, polymer electrolytes) similarly as market access (remedial, food, and biotechnological industry). Considering the white protein concentrates research establishment Biopos e.V. in a joint venture with mechanical associates will investigate the protein centers around their physically manufactured properties and make useful water-dissolvable proteins uniquely designed for unequivocal applications as emulsifiers, film engineers, gelling, or foam building experts in the food and cosmetics industry.

3.5.3 Industrial Outlook

The manufactured business is experiencing a vital move as cost-genuine bio-based-stage intensification is becoming a business reality. A cutting-edge guide of the energetic bio-based industry is the association Cargill Dow LLC (today Nature Works). Since 2002, the association has been producing bioplastics out of grain of maize. This thing can be manufactured into plastic packaging, for instance, foil or cups, and even into T-shirts—everything biodegradable. Around 140,000 tons of bioplastics can be made by the biorefinery in Blair, Nebraska, reliably. Subsequently the cornstarch is diminished enzymatically into glucose syrup. By then it is changed over by maturing into lactic derivatives and sometimes later misleadingly changed into polymer polylactic compounds. This polymer can be thermoplastic created into foil, formed bodies, and strands. Breathable pieces of clothing out of PLA are mainly sold in Southeast Asia. In Europe biodegradable squeezing for food is available. DuPont has gone into an alliance with Diversa in a biorefinery to make sugar from husks, straw, and stovers, and to make cycles to co-produce bioethanol and regard added manufactured mixtures, for instance, 1,3-propanediol. Through metabolic planning, an *Escherichia coli* K12 microorganism produces 1,3-propanediol (PDO), in a clear glucose maturing cycle made by DuPont and Genencor. In a pilot plant worked by Tate & Lyle, the PDO yield shows up at 135 gL^{-1} at a speed of 4 gL^{-1} h^{-1}. PDO is used for the formation of PTT (polytrimethylene terephthalate), another polymer which is used for the making of extraordinary strands with the brand name Sorona. The association Braskem, one of the fundamental Brazilian ethanol creators, set up another polyethylene office reliant on sugar stick. Ethanol is made from sugar by development and changed over to ethylene by drying out and finely ethylene is polymerized. The plant was dispatched in the year 2011. The plant size is commensurate with present-day petrochemical-based polyethylene plants. Polyethylene is publicized as versatile mass-made plastic in the key zone structure, e.g., pipe systems, connections and conductors, and vehicles in addition to upmarket packaging. Other current plants like the formation of 3-hydroxypropionic destructive as starting material for methacrylic destructive and 1,3-propanediol (Perstorp, Sweden), 1,4-butanediol (Genomatica, USA), and succinic destructive (BASF, CSM-Purac) are being worked on. Succinic destructive fills in as dicarboxylic destructive part for direct aliphatic polyester or can be the starting material for mix things like 1,4-butanediol, succinediamid, 1,4-diaminobutane γ-butyrolactone, and maleic destructive. Further petrochemically conveyed substances can likewise be made by liberal microbial change of glucose, for instance, hydrogen, methane, propanol, acetone, butanol, and itaconic acid. A couple of business-scale formation of cellulosic ethanol is a work in progress in the USA, for instance, in Hugoton, Kansas, or Thomaston, Georgia. As far as possible up to two million gallons/year of ethanol is to be produced.

3.6 Current Status

Biorefineries, as the name demonstrates, have up to now been conceptualized around power and biofuels. A biorefinery is an office, fairly practically equivalent to the petrol-processing plant, which incorporates biomass transformation strategies and innovation to create fills, strength, and substance compounds. Biorefinery frameworks have arisen as an appealing way for power age, looking like mixed warmth and force (CHP) and biofuels, along substance fabricating, with stunning guarantee for decreased ecological impact (Kamm and Kamm 2004; Cherubini et al. 2009). In the event that all the bioresources inside the biorefinery structure are effectively utilized, comprising the natural extras from a course change system procedure, an almost zero-waste assembling is finished. The worldwide biorefinery idea remarkably comprising ground biomass along with greenery has recently been devoted to green growth (Balina et al. 2017). It has been recorded that, in first and second generation, lignocellulosic biomass (LCB) is specifically utilized for fluid biofuel production. The comprehensive production of bioethanol, biodiesel, biogas or biomethane worldwide created a colossal place for principal innovation of biofuels towards the start of the 2000s (Kim and Dale 2005). Food-waste management has also sparked interest to second generation biofuels' production. The second generation biofuels are remarkably harmless to the ecosystem. Deployment of lignocellulosic biomass is specifically noteworthy, to restrict the effects on the food and grain markets. Marine macroalgae or kelp has a high potential to mostly supplant earthly biomass. With current examinations occurring on this region it is miles previously announced that green growth is a third feed blossom, nor is utilizing hot spots for their blast (Balina et al. 2017). Developing endeavors are being made to decrease the reliance of the field economy on raw petroleum as a modest regular asset. Quite possibly the most encouraging methodology is to expand measures that license the use of biomass as an elective common guide base. In this way, the biorefinery idea arises as an option simply like petroleum-processing plants, in which biomass is fractionated into conveyed cost items with special yields and disseminations. Besides, these appropriations range extensively depending on the physical and substance nature of the uncooked fabric notwithstanding the monetary side interest of the methodology (Demirbas 2009a; Lyko et al. 2009; Moncada et al. 2013a, b; Posada et al. 2013). This gauge comprises biorefinery items that would refresh fossil-based items which can be artificially equivalent (for example ethylene from bioethanol can supplant ethylene from home-grown gas) and those that would be a substitution for items having comparable usefulness (for example polylactic corrosive can trade polyethylene terephthalate utilized for plastic jugs) (Martinez-Hernandez et al. 2013). Innovative and high-end strategies are constantly implemented for feasible utilization of biomass. Bioenergy and biofuels make use of surplus materials from other creation techniques. This strategy will build asset productivity and adds far and away superior cost to utilized biomass, which is a piece of round economy. Duplicated asset productivity is additionally saving the uncooked fabric convey, in light of the fact that biomass might be utilized over and over. With this standard normally the issue is that extra biomass should be shipped from one region to another to keep up with the ensuing advance of assembling, yet biorefinery thought addresses this difficulty for the explanation that thought should be actualized in a solitary region (Balina et al. 2017). As an outcome, GHG emanation markdown, fossil energy saving, and financial capacity of biorefinery stock have ended up being fundamental estimates that make a biorefinery a useful option in contrast to raw petroleum treatment facilities (Brehmer et al. 2009).

3.6.1 The Role of Biorefinery in Industry

The biorefinery thought process has been refined extensively inside the corn processing plant, for improved financial matters and ecological supportability (Lynd et al. 2005). The verbal trade at the Lead Commercial Centre Initiative (COM (2007) 860 last) showed six areas as lead markets: eHealth, ensuring materials, feasible development, reusing, bio-based product, and sustainable power sources that permit to settle on an appropriate item portfolio; the accompanying five norms had been figured (Lynd et al. 2005): high hypothetical item yields from substrate; market diversion inside the item as a finished result or as a mechanically imperative halfway; high assembling amount (bleeding edge or potential); nonfood utilization of the item; and potential to be organically integrated from the regular sugars obtained from different kinds of biomass. IEA Bioenergy adventure 42 as of late produced an appropriate definition for biomass into a range of bio-fundamentally based products (food, feed, synthetics as well as substances) and bioenergy (biofuels, strength, which implies that a biorefinery can be a thought, an office, a method, a plant, or even a bunch of focuses that clearly requires the reconciliation of numerous exceptional locales of skill incorporating compound designing, science, science and natural chemistry, biomolecular designing, and different fields). Along these lines, biorefining can offer a maintainable way to deal with valuable products that can likewise improve biomass preparing financial matters notwithstanding natural impression (e.g., GHG monetary reserve funds) if a totally incorporated thought is advanced. The reconciliation of bioconversion and reasonable substance advancements is significant for the effective interpretation of treatment facility standards to fluid changes. On account of synthetic advancements and compound assembling, this implies the utilization of green science strategies comprising heterogeneous catalysis and the product of unpracticed science thoughts (Clark et al. 2012). The rule focal point of biorefinery frameworks with an end goal to come into activity inside the ensuing years is on the assembling of transportation biofuels. The decision of the most intriguing biofuels depends on the chance that they can be blended in with fuel, lamp oil, diesel, and home-grown fuel, mirroring the essential advantage of utilizing the generally present framework inside the transportation territory, along with the expenses incurred towards transportation commercially viable (de Jong and Jungmeier 2015).

Biorefineries ensures new capabilities along with business sectors' development to be relied upon for industrialized and global ecological areas. The monetary cost of biomass is chosen by methods for the deals from the different items available and the assembling charges (e.g., capital and activity costs) of the assorted items (de Jong and Jungmeier 2015). The financial cost of biomass feedstocks terribly surpasses the worth related with their caloric worth (that is, just 3/GJ). They may establish estimations of up to 75/GJ, outfitting that added substances can be obtained in an unadulterated structure. Expecting a biomass yield of 10–20 tons of dry load in sync with hectare steady with year and that the biomass will simply be utilized for its caloric expense would address a cost of 450–900/ha as indicated by year, values which may be excessively low for ranchers in Western Europe to make a worthy notable abiding. Matters would be explicit on the off chance that we should isolate biomass into divisions that can be utilized to give food, feed, bio-based items (compound substances, materials), or potentially bioenergy (energy as well as warmth). As seen above, isolated biomass parts can produce financial returns surpassing their caloric worth. Expecting that 20% of biomass is suitable to give synthetic substances, 40% to supply biofuels, and the rest to supply power and warmth, a biomass yield of 10–20 tons dry includes a number with regard to hectare biomass yield possibly ought to produce 2000–4000/ha, adequate for ranchers to make a worthy mainstream of staying (de Jong and Jungmeier 2015). Regardless of this, addressing detachment of deftly LNG markets is currently not expected to rebalance sooner than the mid-2020s. The financial creation of transportation biofuels is generally a venture. The coproduction of synthetic mixtures, substances, suppers, and feed can create the essential presented esteem. Nowadays a paper that becomes distributed featuring all bio-based synthetic compounds with the chosen stock either shows solid commercial center increment or has huge undertaking interest in progress and exhibition programs (de Jong and Jungmeier 2015). Table 3.2 offers a framework of the principal resemblances and dissimilarities of petrochemical treatment facilities and biorefineries.

3.7 Biomass-Biorefinery-Bioeconomy

Biorefineries are a promising included procedure for the coproduction of both value-added items (biomaterials, biochemicals, bioplastics, food, feed) and bioenergy (biofuels, biogas, heat, or potentially energy). The bio-based financial framework incorporates the entire scope of characteristic and inexhaustible natural sources (land and ocean resources, biodiversity, natural substances (plant, creature, and microbial), and natural methodologies). For this situation, a bio-based economy is the same old thing in itself, as before the modern upset, economies have been for the most part bio-based completely. Biomass is as of now utilized as feedstock for instance wooden substances, mash and paper fabrication, biomass-determined filaments, and biofuel feedstock (from oil harvests, starch, and sugar vegetation). However, the progress toward a current-day bio-based economy suggests difficulties, for example, the maintainability of biomass uncooked material, execution in biomass use, and monetary arrangement of scales in biomass activation (Dumeignil 2012). Biorefinery can utilize different combinations of feedstock and change advances to deliver a dissemination of produces. Notwithstanding, the vast majority of the current biorefinery standards' utilize limited feedstocks along with innovation, and solely produce bioethanol or biodiesel (Scarlat et al. 2015). Inexhaustible compound substances are often associated innovatively to the biorefinery for the creation of supplementary feed materials and products (Kamm and Kamm 2006). Such a stage could coordinate the synthetic endeavor into the so-known bioeconomy. The essential longings are to deliberately decrease the reliance on raw petroleum and to relieve the nursery gas outflows as pleasantly on the grounds that the awful effects on the environmental factors brought about by the handling and in the end the consuming of fossil carbon assets. All through these energizing patterns it is miles basic now not to dismiss the ozone-depleting substance-related effects, issue for which supports a decent arrangement of the explanation for biorefinery and bioeconomy achievements up until this point. The current worldwide market size for ethylene bills for 127 Mt./a with a portion of 0.2% (0.25 Mt./a) coming from biomass assets (E4tech, Re-Cord and Wur 2015).

3.8 Biorefinery Concept: Future Prospects

As per the Energy Information Administration (EIA 2015), utilization of petroleum products causes ozone-harming substances' outflow, particularly carbon dioxide (CO_2). Power period will blast our reliance on petroleum product increments. It has been found in numerous prescience researches. EIA (2015) information of creation of power by 2040, indicates that a yearly assembling of around 144×10^6 m^3 and a worldwide extent of over 3% dependency on power content material anticipates biofuels of playing a broad capacity inside the vehicle fuel production zone. The potential biorefinery commercial center all through the total biomass value chain universally has been projected as $295 billion during 2020. The necessary destinations for the utilization of sustainable force in vehicle have supported the use of biofuels achieving 564 PJ in 2012. The NREAPs (National Renewable Energy Plan) estimated a utilization of biofuels of around 1216 PJ Mtoe in 2020, addressing more noteworthy than 90% of the sustainable force anticipated to be utilized in transportation (Banja et al. 2013). The NREAPs gauge that around 100 and 5 PJ lignocellulosic biofuels will be used in vehicle in 2020; anyway this relies upon their business accessibility and costs.

As per negative forecasts, with regard to UNEP, 35.7 million ha was utilized for biofuel fabricating in 2008 and an expected 80 million ha is to be used by 2020. The assembling of biofuels and energy is used by a lion's share of the wellsprings of the timberland. Wooden pellets comprised 7% of power and transport in 2010. USA, Canada, and Russia are anticipated to be the main exporter of biomass. The USA and Brazil are foreseen to lead ahead in biofuel creation. The acceptable alternatives have all the earmarks of being energizer of things to come, particularly power obtained from microalgae. Changing all the conveyance fuel devoured inside the USA with biodiesel would require 0.53 billion m^3 of biodiesel yearly at the cutting-edge cost of admission. Oil crops, squander cooking oil, and creature fats cannot practically satisfy this interest. For example, gathering a large portion of the present US transporting fuel wishes by methods for biodiesel would require impractically huge development districts for principal oil plants. As a matter of fact, oil plants cannot significantly make a commitment to changing oil determined fluid fills inside the predictable predetermination. These circumstances change while microalgae are utilized instead of plants. Somewhere in the range of 1 and 3% of the by and large US editing district would be adequate for producing algal biomass that fulfils half of the vehicle fuel prerequisites. Microalgae have different oil content as their species. Each unreasonable stage oil substance and considerably less land require they will be least complex wellsprings of biodiesel that can uproot crop.

3.9 Conclusion

The chapter has outlined the idea of biorefineries, exceptional sorts of biorefineries, future rules, and related specialized difficulties. The biorefinery idea keeps on being in its outset. It is critical to define necessities for the merchandise procured from the biorefineries, if not accessible, starting from the beginning of the period all together that the assortment of the moderate items is the least to smooth out with current innovation. One issue that wants basic contemplating is whether present-day biorefineries ought to be equipped nearer to creating a completely new line of synthetic substances/items, which incorporate stage compound substances which are antecedents to high-esteem synthetic substances, or to supply crude material that might be starting feedstock for existing treatment facilities or synthetic plants. Biorefinery outcomes are relied upon to push upward the utilization of compound substances. High-stage oil content material availability by quick and substantial production of microalgae supply a future for clean fuel. Owing to the rise in oil prices, biorefineries will be the period of 2020.

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Chapter 4
Life Cycle Assessment of Algal Biofuels

Neelima Kumari, Km Swapnil Singh, and Pratham Arora

Abstract Algal biofuels have the potential to effectively replace conventional crude-based fossil fuels. The fact that the algae growth process sequestrates a considerable amount of carbon dioxide can lead to substantial greenhouse gas (GHG) emission reduction. In the recent past, several life cycle assessment (LCA) studies have been conducted to demonstrate the benefits of algal biofuels. However, considerable differences are observed in the predicted environmental benefits from these studies. These differences arise from a variety of factors such as choice of the functional unit, system boundary, algae species, and conversion processes. Furthermore, most studies focus only on GHG emissions, thus neglecting other potential environmental impacts of algal biofuel production. This chapter aims to provide a review of the LCA studies for algal biofuels, which have been conducted in the recent past. The basics of algal biofuels and life cycle assessment have been thoroughly discussed. The differences in the assumptions, and their possible effects on the final results, have been highlighted. Some of the recent advances, as well as future directions in LCA of algal biofuels, have also been discussed. The chapter aims to underline the state-of-the-art practices to enable the decision makers to make informed choices related to the sustainability of algal biofuels.

Keywords Algal biofuels · Greenhouse gas emissions · Life cycle assessment · Sustainability

Abbreviations

TEI	Total energy input
FFC	Fossil fuel consumption
AD	Abiotic depletion

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ET	Ecotoxicity
FAETP	Freshwater aquatic ecotoxicity potential
GWP	Global warming potential
HT	Human toxicity
MT	Marine toxicity
HTP	Human toxicity potential
MAETP	Marine aquatic ecotoxicity potential
TETP	Terrestrial ecotoxicity potential
PMF	Particulate matter formation
ODP	Ozone depletion potential
SFP	Smog formation potential
AP	Acidification potential
EP	Eutrophication potential
TA	terrestrial acidification
MET	Marine ecotoxicity
FET	Freshwater ecotoxicity
TET	Terrestrial ecotoxicity
CC	Climate change
PM	Particulate matter
TE	Terrestrial eutrophication
FE	Freshwater eutrophication
ME	Marine eutrophication
EROI	Energy return on invest
NREC	Nonrenewable energy consumption
FEC	Fossil energy consumption
ULO	Urban land occupation
ALO	Agricultural land occupation
WD	Water depletion
MD	Metal depletion
FD	Fossil depletion
RD	Resource depletion
IR	Ionizing radiation
PCOF	Photochemical oxidant formation
NLT	Natural land transformation
LU	Land use
EU	Energy use
MFRD	Mineral, fossil, and renewable resource depletion
DALY	Disability-adjusted life years
WSF	Water scarcity footprint
CED	Cumulative energy demand

4.1 Introduction to Microalgae

Microalgae are single-cell living organisms that can perform photosynthesis to convert carbon present in various forms such as CO_2 and carbonates into an organic form. Algae are composed of chlorophyll, which enables the algal cells to produce carbohydrates in the presence of sunlight or any artificial light, using water and CO_2 by the photosynthetic process. The efficiency of photosynthesis is higher in the case of algae as compared to terrestrial plants. This efficiency improvement is because the structure of the algal cells is comparatively simpler. This makes microalgae highly productive. The photosynthetic efficiency is referred to as the portion of light energy that can be converted into a chemical form of energy during the photoautotrophic growth mode. The amount of sunlight varies spatially and temporally, which is problematic, but it is rather economically superior to an artificial source of illumination. Also, the environmental performance of artificial illumination depends on the local energy mix. Since they have high photosynthetic efficiency, it provides an opportunity to develop algae-based bioenergy, which can potentially reduce fossil fuel consumption.

Apart from the autotrophic growth mode, some microalgae do not depend on sunlight for their growth. This mode of growth is known as heterotrophic growth mode, where the algae grow heterotrophically on organic substrates (e.g., acetate or glucose). The energy for cell growth comes from carbon and carbon skeletons. Besides glucose and acetate, carbohydrates such as sucrose, lactose, fructose, and starch are other carbon sources for the growth of algae. An influencing factor is the carbon/nitrogen (C/N) ratio that affects the content of cellular lipids. This ratio controls the lipid and protein synthesis switch. When nitrogen is deficient in the culture media, i.e., the C/N ratio is high, it leads to the accumulation of lipids. According to some researchers (Kumar et al. 2015), the technical feasibility of the heterotrophic method is more in comparison to photoautotrophic production. In some algae, heterotrophic production may lead to higher accumulation of lipid and production of biomass. Algae should possess the following features for heterotrophic growth:

- Capability to metabolize and divide in the absence of light.
- A tendency of growing in cheaper media.
- Small or no delay period when new media is provided.
- Potential to sustain hydrodynamic stresses present in various equipment.

The heterotrophic mode of culture does not require light and can increase cell productivity and density immensely. Hence, it could be more cost effective when compared to autotrophic mode. Even if the heterotrophic mode is viable, the energy needed to grow an organic carbon source for algae growth can negate the advantages. Hence the process of organic carbon cultivation source must be considered to attain maximum possible benefits.

Some algae have the potential to photosynthesize as well as synthesize carbohydrates and lipids heterotrophically. This is known as mixotrophic growth. Some examples include *Chlamydomonas reinhardtii and Spirulina platensis*. It facilitates the combination of heterotrophic and photosynthetic components during the day as well as during conditions of limited light availability. It helps in the reduction of loss of biomass during respiration in the absence of light and also reduces the quantity of organic matter used during growth.

4.1.1 Algae Cultivation

Photosynthetic transformation of CO_2 in the presence of sunlight (or artificial light) and other nutrients to form carbohydrates, protein, and lipids is known as phototrophic cultivation or growth. It can be done either using open or closed production systems, where the main goal is to achieve high productivity of algal biomass. The management of nutrients and water is done according to the biochemical composition of the algal biomass being produced.

Another production outline involves heterotrophic cultivation, where the inputs are sugars and air (rather than sunlight and CO_2). Fermenters (under highly controlled conditions) are used for the growth and increase of mass concentrations of algae. With the ability to modify cell's biochemistry, the lipid content is high in heterotrophically grown algae; hence it is a commercialized method of algae production.

The successful production of biofuel from algae depends upon many factors. The choice of a proper algal strain is essential. A suitable strain and algal species should possess a definite dominance as compared to others and must:

- Have more productivity of lipids
- Cope with the stresses commonly present in photo-bioreactors (PBRs) and open ponds.
- Have more capacity for CO_2 absorption
- Have restricted nutrient needs
- Be able to tolerate a wide range of temperatures arising from seasonal variations and diurnal cycle
- Result in useful co-products
- Have a rapid cycle of productivity
- Have high efficiency of photosynthesis
- Have self-flocculation properties

These are extremely challenging situations, and no algal strain can meet all of these prerequisites. However, some species have some advantages over others, which cannot be disregarded. Species having more lipid concentration or productivity are more viable for the production of biofuel as costs corresponding to infrastructure, water requirements, and nutrients are nearly the same but inputs such as the energy required for per unit production of biofuel are reduced. Hence the choice of the suitable species must be made according to the biomass composition under a given culture system, growth mode, nutrient availability, and final product required.

Mainly two methods are used to cultivate algae: open pond systems and PBRs. Normally, a raceway pond is 0.25–0.4 m deep and consists of a paddle wheel, which helps in water and nutrient circulation in the pond. PBRs consist of closed plates or tubes made up of transparent material, and it also has degassing and cooling systems, which helps in maintaining the environment suitable for algae growth.

Open Ponds It is the easiest method for the wide-scale cultivation of microalgae. This method uses shallow ponds for algae cultivation. The cultivation is carried out under conditions similar to their natural surroundings. The raceway arrangement is used for designing the pond, which consists of a paddle wheel for mixing and circulating nutrients and algal cells. These raceways can be constructed in different ways, like a pit having concrete spurted over it or having a plastic lining to block the liquid from seeping into the ground. For maximum utilization of the space, baffles are used to control the flow in the channel and near the bends. Generally, a continuous mode of operation is used; that is, the new feedstock is added along with the phosphorus, nitrogen, and other inorganic compounds at the head of the paddle wheel. After the complete circulation, the algal broth is collected in the back of the paddle wheel. Different types of wastewater resources can be utilized for algae cultivation based on the nutrients needed by the species. For example, water having more salinity or seawater is better for the cultivation of marine microalgae.

Enclosed Photo-Bioreactors To maximize the use of natural light, such systems are manufactured from transparent materials and are kept outdoors. The containers used generally have more surface area than volume. In open ponds, the oxygen produced through photosynthesis goes back to the atmosphere but here, it is confined to the system. To avoid the poisoning of algae due to excess oxygen, it is periodically degasified. Due to excess utilization of CO_2, the carbon level may reduce, thereby increasing the pH level. Hence, for the successful cultivation of microalgae, it is necessary to feed CO_2 in the system. PBR temperature must be controlled by some means like heat exchangers, during the day as well as night. The most commonly used PBR design is tubular type; it has many transparent tubelike structures that are normally aligned in the direction of the sunrays. These PBRs are manufactured from plastic tubes or glass. These are either vertically or horizontally aligned and are supplied with CO_2 and other nutrients through a central system having sensors and pumps. Tubular PBRs are widely used from laboratories to large-scale production systems. PBRs have a high level of purity and efficiency. The high-quality production of biomass, i.e., more concentrated biomass, makes the process more energy efficient. Some other types of PBRs include Christmas tree PBR, flat plate PBR, foil PBR, and porous substrate PBR.

4.1.2 Algal Biomass to Biofuel Conversion Technologies

Different energy extraction technologies have been instituted for the conversion of algal biomass to biofuel conversion. The possible pathways include gasification, thermochemical liquefaction, pyrolysis, direct combination, biophotolysis, transesterification, fermentation, anaerobic digestion, and hydroprocessing (Fig. 4.1). Algal biomass, both dry and wet, can be processed into biofuels through the abovementioned pathways. Wet algal biomass can be converted to biofuels by hydrothermal liquefaction (HTL), fermentation, and anaerobic digestion. The higher upstream energy return on investment (EROI) values of these extraction pathways help achieve the desired biofuel without any requirement of biomass drying. Moreover, biofuels can be extracted from dry algal biomass through pathways such as direct combustion, pyrolysis, gasification, and transesterification. These algae conversion pathways are briefly discussed in the following section.

Gasification To produce syngas, the gasification pathway involves partial oxidation of algal biomass at high temperatures (800–1000 °C) in the presence of oxygen and steam. Syngas is generally a mixture of gases, namely carbon monoxide, hydrogen, carbon dioxide, nitrogen, and methane. Owing to its low calorific value (4–6 MJ m^{-3}), syngas is well suited as a gas engine or a gas turbine fuel. This combustible mixture gas can also be burned directly.

Thermochemical Liquefaction Thermochemical liquefaction, a catalytic pathway, is employed to obtain bio-oil from algal biomass. To produce the desired product, this process needs low temperature (300–350 °C) and high pressure (5–20 MPa) operating conditions together with hydrogen. This process is readily utilized in the extraction of energy from wet algal biomass. This wet biomass is decomposed into liquid fuel of high energy density without using any energy investment for drying the biomass. The fuel-feed systems and thermochemical liquefaction reactors show complex execution and are hence expensive.

Pyrolysis Petroleum-based fuels could be replaced by biofuels through the preferable potential pathway pyrolysis. The process involves high to medium temperature requirements of 350–700 °C for the conversion of algal biomass into products like syngas and bio-oil specifically. Charcoal is also obtained at lower temperatures and slower heating rates. The pyrolysis oil extracted is acidic and viscid, and contains dissolved chemicals. This processed oil is unstable and therefore requires refinement. The up-gradation of processed oil includes pathways like hydrogenation and catalytic cracking.

Direct Combustion Direct combustion is a pathway that comprises conversion of biomass to hot gases. The stored chemical energy of the biomass is transformed into gaseous energy at a temperature greater than 800 °C in the presence of air. This gaseous yield produced in a boiler or furnace is utilized for heat and steam power generation. The prerequisite for the conversion is that the algal biomass should not consist of moisture content more than 50% dry weight. Demerits of direct

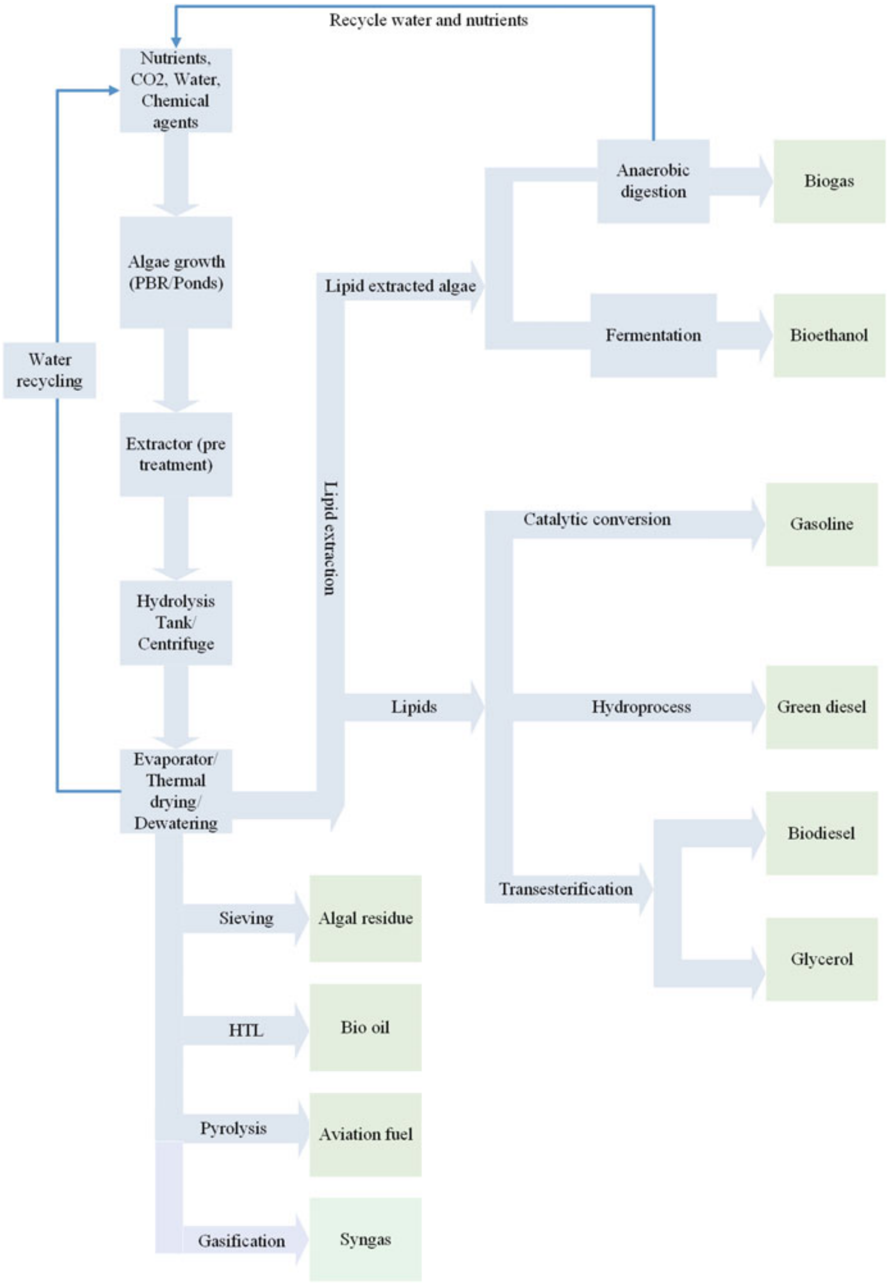


Fig. 4.1 Major algal biofuel production pathways

combustion include additional energy demand for pre-conversion processes like drying and grinding. Additionally, the heat generated must immediately be used since there is no provision of heat storage.

Biophotolysis The photolysis of water undergoes the production of electrons and two by-products as hydrogen ions and oxygen. The electrons released are utilized in the electron transport chain. The hydrogen ions are converted to hydrogen (H_2) as in natural photosynthesis. This requires the utilization of hydrogenase enzyme as a catalyst, but the production of oxygen hampers its functioning. Biohydrogen production is also generated through direct and indirect photolysis of microalgae. Direct photolysis involves the recombination of H+ and e^- to yield hydrogen in the presence of sunlight and oxygen. Indirect photolysis is accomplished under anaerobic aspects. Biophotolysis deals with the production of hydrogen that has the highest energy content on a mass basis when compared to other fuels. Additionally, hydrogen is a clean fuel and does not impose a threat to the environment as it is an outcome of biomass cultivation.

Transesterification The transformation of a glycerol-based ester to mono-hydric alcohol-based ester via derivation from biomass-based lipids is known as transesterification. The final product formed is identified as fatty acid methyl ester (FAME) or biodiesel. Here, lipid extraction is followed by transesterification of algal biomass utilizing methanol (alcohol) and a catalyst. Technological challenges include dewatering and drying of biomass algae.

Fermentation Microalgae such as *Chlorella, Dunaliella, Chlamydomonas, Scenedesmus, and Spirulina* contain cellulose and glycogen. This form of carbohydrate is effectively used in the alcoholic fermentation of algal biomass to yield ethanol. The complex carbohydrates undergo enzymatic hydrolysis by a process in which yeast and water combine with simple sugars. This process occurs in large tanks called fermenters at a comparatively warmer temperature. Ethanol is the final product formed as a result of the breakdown of carbohydrates by yeast. The fuel is suitable to run gasoline-based engine vehicles. The solid residue is also utilized as a cattle feed or can be used for gasification. The biofuel obtained needs to be purified as it contains water and other impurities. This process is aided by distillation, in which concentrated ethanol (95% by volume) is extracted.

Anaerobic Digestion In anaerobic digestion, algal biomass is converted into biogas. Biogas is a combustible gas mixture comprising CH_4, CO_2, and traces of H_2S. The energy-intensive process of dewatering and drying of algal biomass is excluded in this process as anaerobic digestion accepts wet biomass. Three stages of conversion can be observed here: hydrolysis and fermentation followed by methanogenesis. Firstly, the complex biomass compounds are broken down into soluble sugars. These soluble sugars are converted into alcohols, acetic acid, and other volatile fatty acids by fermentative bacteria. H_2 and CO_2 gases are also evolved, which on methanogenesis get metabolized into methane (60–70%) and carbon dioxide (30–40%).

Hydroprocessing Algal biomass consists of triglycerides, which are converted into green diesel via hydroprocessing. Hydroprocessing is a two-step process involving hydrocracking and hydrogenation (for producing the chain of C15–C18 hydrocarbons). Green diesel is mainly composed of octadecane and heptadecane (which are hydrocarbons), while biodiesel has an ester composition. For the production of green diesel, the temperature and pressure conditions required are nearly 300 °C and 5 MPa of hydrogen in the presence of a catalyst. Hydroprocessing is sometimes considered better than transesterification because it requires a lesser amount of energy to dry algal biomass.

4.2 Life Cycle Assessment

Most of the impacts associated with the final industrial products do not display the precise environmental performance. Often the impacts associated with various production steps of the final product are not included in the environmental performance. These steps include extraction of raw materials, transportation of the extracted materials, manufacturing technologies used, transportation of final product to the distribution or use point, and finally its disposal. These steps may have detrimental impacts on the environment. Therefore, to get a comprehensive outlook on the environmental impacts, it becomes essential to consider all the processes involved in the manufacturing of any product. Life cycle assessment (LCA) is an important tool for the assessment of a product's environmental performance associated with the production, transportation, use, and disposal throughout its life cycle. The tool works as in the collection and evaluation of interlinked and successive stages of a product's life cycle.

LCA is based on the consideration or estimate of the similarities or dissimilarities of different products, the sources of such products, and the different manufacturing techniques. The most environment-friendly alternative is selected out of the various alternative options available. This tool also helps in highlighting the advancement and enhancement opportunities such that the environmental sustainability of the process is achieved. LCA, therefore, provides a broad outlook regarding the environmental facet in particular. The four characteristics of LCA involve (Fig. 4.2):

1. Definition of goal and scope for the assessment
2. Compilation of life cycle inventory (LCI) data for the energy and material flow and environmental discharges
3. Life cycle impact assessment (LCIA) based on inventory data
4. Interpretation based on significant findings and analyses

The intended applications of the study are elaborated in the goal and scope definition step. The LCI identifies the major mass and energy flows in the various stages of the process. In the impact assessment stage, these inventories are further characterized to estimate different impacts such as global warming potential (GWP) and human health. The interpretation stage is used to forecast the process's impacts while also identifying its shortcomings and making suggestions for the future. According to the flows between the various LCA phases, the entire process will

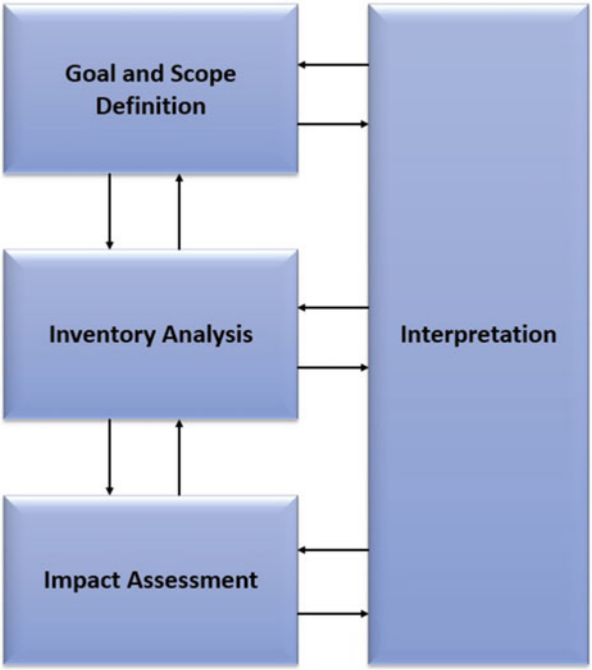


Fig. 4.2 Four stages of life cycle assessment

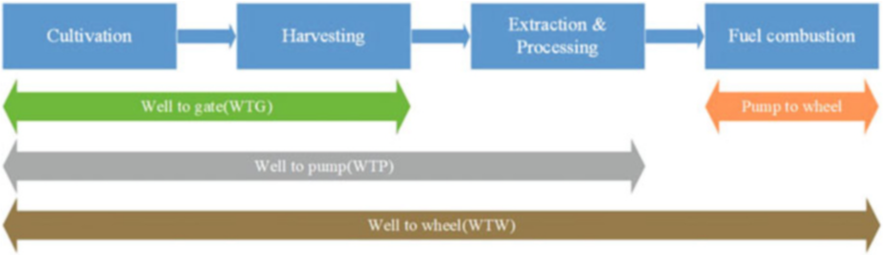


Fig. 4.3 System boundaries for LCA of algal biofuels

require multiple iterations to reconcile the targets, data specifications, usable inventories, impact categories, and characterization models. Some of the major aspects related to the LCA of algal biofuels are presented in the following sections.

4.2.1 System Boundary

The system boundary determines which processes will be included in the LCA and which will be exempted. The prominent types of system boundaries adopted while conducting the LCA of algal biofuels are the following (Fig. 4.3):

1. *Well-to-Wheel*: The LCA inventory includes the entire stages of product analyses that begin from algae cultivation to harvesting of algal biomass. This is followed by the production of biofuels. After that, the biofuel is extracted until it is ready for use and disposal. The LCA inventory includes all of the subsequent phases and levels. The biofuel is then processed until its end use and disposal. All the subsequent steps and stages are included in the LCA inventory.
2. *Well-to-Gate*: This LCA inventory includes only those stages that come before the stage of biomass production only. The biofuel production, end use, and product disposal are not included in the system boundary.
3. *Pump-to-Wheel*: This inventory only holds the assessment of the end use of the product. Other stages are not considered.
4. *Well-to-Pump*: The LCA inventory only includes biofuel processing and does not include end-of-life disposal.

4.2.2 Functional Unit

The functional unit provides an input and output flow reference for any LCA. Various kinds of functional units exist for different comparable studies. These functional units depend upon several factors such as the volume of algal fuel produced (e.g., 1 L of biofuel), energy content of the fuel (e.g., 1 MJ of biofuel), after-combustion energy release (e.g., 1 MJ of energy produced), mass (e.g., 1 kg of biomass produced), and distance travelled (e.g., 1 tonne-km of travel). Four major classes of functional units considered in LCA analyses, particularly to biofuels, are the following:

1. *Input-based unit*: This functional unit determines the energy and mass of the input biomass. Various resources and their consumption patterns of biomass production are studied under this functional unit, e.g., 1 tonne of algal biomass used.
2. *Output-based unit*: This functional unit determines the useful energy generated or total distance in km of transportation in the form of output. Such a functional unit is considered when there is a need to evaluate the provision of a given service, e.g., 1 MJ of energy from algae biofuel generated.
3. *Agricultural land-based unit*: This functional unit takes into account the cultivated land area for the yield of algal biomass feedstock, e.g., 1 ha cultivable land for biomass feedstock cultivation.
4. *Mass-based unit*: This functional unit undertakes the final biofuel production based on the mass of production, e.g., 1 kg of biodiesel produced.

4.2.3 Impact Categories

Various impact categories are taken into consideration to assess the comprehensive environmental sustainability of biofuels. Some of the major impact categories associated with the LCA of biofuels are energy return on investment, GWP, water resources, land-use changes, ozone depletion potential, nutrient needs, eutrophication potential, acidification, and human and ecological health impacts. Economic and social impact categories are not accounted for in the LCA of biofuels. However, several software-based LCA modelling procedures account for these factors as well (UNEP 2009). Some of these impact categories are explained in detail in the following section.

Global Warming Potential (GWP) The life cycle carbon footprint is also known as the GWP. It shows how biofuels contribute to climate change in relation to equivalent carbon dioxide emissions (g CO_2 eq.). GWP provides a relative measure of the energy emissions of the biofuel to that of the emission of carbon dioxide of equal quantity over a specified period. Calculations are usually done for a 100 years' time horizon. It is one of the most important policymaking tools to reduce emissions and compare alternatives for the reduction of emissions across several sectors.

Water Resources The processing of biofuel feedstock into fuel utilizes large quantities of water. Water quality requirements and water consumption indicators are considered to evaluate the environmental sustainability of biomass cultivation. The variations in the algae strain lead to varying water quality requirements for microalgae cultivation. Water sources such as low-grade wastes can be used in the cultivation of microalgae to truncate pressure on natural water resources.

Land-Use Changes Microalgae cultivation necessitates several criteria such as appropriate salinity and chemistry-based water supply, suitable land topography for site selection, favorable climatic conditions, and transportation access. Inevitable changes to land are important to be considered. These are of two types: direct land-use change and indirect land-use change. When forest land is converted to biofuel production land, direct land-use change occurs. The direct GHG emissions generated by shifting from one land use to another are measured by direct land-use change. When agricultural land is replaced by a biofuel production site while agricultural land is increased by deforestation elsewhere, this is known as indirect land-use transition.

Ozone Depletion Potential (ODP) ODP is defined as the reduction potential of the protective ozone layer found in the stratosphere. It is generally expressed as CFC-11 equivalents. Various compounds like freons, carbon tetrachloride, chlorofluorocarbons, and methyl chloroform contribute to ozone layer depletion. ODP provides a relative measure of the impact of the emission of a unit mass of gas on the ozone layer when compared to the emission of an equal unit mass of CFC-11 integrated over a while. It provides a measure of the relative impacts of several gases on the ozone.

Eutrophication Potential Eutrophication potential is defined as the overfertilization of water and soil, leading to an increase in the growth of obnoxious aquatic plants majorly attributable to the nutrients such as nitrogen and phosphorus. This is generally expressed in $PO_4{}^{3-}$ equivalents. Eutrophication leads to several impacts, such as nutrient enrichment causing the death of aquatic animals and reduced biodiversity, increased turbidity and water toxicity, a decline in water quality, and repulsive odors and repugnant tastes.

Acidification Potential Acidification potential is defined as the formation of H^+ ions from potential acidifying pollutants that are majorly airborne. These include NH_3, NO_x, SO_2, HF, and HCl. These pollutants are emitted mainly either from fossil fuel combustion or due to soil nitrification. This impact category is generally expressed in SO_2 equivalents and has detrimental impacts on plants, animals, and structures.

Ecotoxicity (Freshwater, Marine, Terrestrial) Ecotoxicity potential is defined as the potential emission of noxious and malignant substances in freshwater, marine, or terrestrial ecosystem. It is expressed in 2,4-dichlorophenoxyacetic acid equivalents. It involves the toxic effects, exposures, and harmful impacts on the air, water, and soil.

4.3 LCA of Algal Biofuels

A review of life cycle assessment studies of algal biofuels has been presented in Table 4.1. These studies differ on the basis of conversion pathways and technologies used, environmental impacts, functional unit, and system boundaries. The geographical distribution of the studies covered is presented in Fig. 4.4. Multiple conversion pathways compatible with the extraction of both dry and wet algal biomass have been reviewed. The most widespread and common technology utilized in the reviewed studies is transesterification. Transesterification, being a traditional pathway for the production of biodiesel, is most preferably employed in 13 LCA studies selected under review. More recent technology, such as HTL, is compatible with wet extraction and can effectively be utilized in the extraction of biocrude. This biocrude from algal biomass can then be upgraded to the desired liquid fuel. It is observed that seven studies have utilized HTL as the conversion process. LCA studies that have incorporated HTL conversion predict that the GHG emissions of the main fuel produced are significantly lower when compared to petroleum fuels and corn ethanol. Another conversion pathway that has been accounted for in five LCA studies is pyrolysis. This technology is widely employed in the generation of aviation fuels. Fermentation and anaerobic digestion are also the conversion technologies that have been observed in four LCA studies (Hossain et al. 2019; Maga 2017; DeRose et al. 2019; Wu et al. 2019) and two studies (Shi et al. 2019; Axelsson et al. 2012), respectively. Other LCA studies have focused only on microalgal

Table 4.1 Review of LCA of algal biofuels

Author (Year)	Algae species	Final product	Conversion technology	Pond/PBR	Impact categories	Functional unit	System boundary
Guo et al. (2017)	*Spirulina, chlorella, and Isochrysis*	Aviation fuels	Pyrolysis	Pond	TEI, FFC, GWP	1 MJ of aviation fuel	Well-to-wheel
Gnansounou and Kenthorai Raman (2016)	*Chlorella vulgaris*	Biodiesel	Acid esterification and alkali transesterification	Raceway pond	GWP, FFC, land use	1 kg of biodiesel	Well-to-wheel
Togarcheti et al. (2017)	*Scenedesmus dimorphus*	Biodiesel	Transesterification	Raceway pond	AD and ADP elements, AP, EP, FAETP, GWP, HTP, MAETP, and TETP	1 kg of biodiesel	Well-to-gate
Monari et al. (2016)	*Nannochloropsis*	Biodiesel	Transesterification	PBR	GWP and NREC	1 MJ of biodiesel	Well-to-pump
D'Imporzano et al. (2018)	*Chlorella vulgaris*	–	–	PBR	GWP, CC, ODP, TA, FE, ME, HTP, PCOF, PMF, TET, FET, MET, IR, ALO, ULO, NLT, WD, MD, and FD	1 kg of produced chlorella biomass (algae production for food, not for energy)	–
Collet et al. (2014)	*Nannochloropsis oculata*	Biodiesel	Transesterification	Open raceways pond	GWP, CC, ODP, PCOF, HTP, TET, FET, MET, PMF, FE, ME, TA., ALO, ULO, MD, and FD	1 MJ of algal methyl ester (biodiesel)	Well-to-gate

Pragya and Pandey (2016)	*Chlorella vulgaris*	Green diesel	Pyrolysis and hydrothermal liquefaction	Open ponds and PBRs both	GWP	1 h	Well-to-wheel
Soratana et al. (2014)	*Chlorella vulgaris*	Biodiesel	Transesterification	PBR	ODP, GWP, SFP, AP, and EP	2 million gallons of microalgal biodiesel per year or 2.68 x 108 MJ of microalgal biodiesel	Well-to-gate
Jez et al. (2017)	*Scenedesmus obliquus*	Cake and refined oil	–	Open pond	GWP, CC, HTP, TA, FE, ME, TETP, FET, MET, ALO, WD, MD, and FD	Embodied energy (MJ) in 1 kg of oil produced	Well-to-gate
Wu et al. (2019)	*Chlorella vulgaris*	Biodiesel and biobutanol	Transesterification and fermentation	Open raceways pond	GWP, FD, MD, NLT, ULO, ALO, MET, FET, TET, FE, TA, CC ecosystems, IR, PMF, PCOF, HTP, ODP, CC, and human health	100 MJ biofuel produced	Well-to-gate
Hossain et al. (2019)	*Chlorella vulgaris*	Bioethanol	Saccharification, fermentation, and dehydration	Ponds/PBRs both	GWP	89 MJ/gallon of bioethanol	Well-to-gate
Handler et al. (2014)	*Nannochloropsis* sp.	Biofuel/jet fuel	Whole-cell pyrolysis/oil extraction and hydroprocessing	Raceway pond	GWP	1 MJ of biofuel	Well-to-wheel

(continued)

Table 4.1 (continued)

Author (Year)	Algae species	Final product	Conversion technology	Pond/PBR	Impact categories	Functional unit	System boundary
Dutta et al. (2016)	*Microalgae*	Biodiesel	Transesterification	Open ponds	GWP	–	Well-to-wheel
Roostaei and Zhang (2017)	*Chlorella* sp.	Bio-oil	Lipid extraction, microwave pyrolysis, and hydrothermal liquefaction	Open ponds	EU and GWP	50,700 MJ/year	Well-to-gate
Quiroz-Arita et al. (2017)	*Synechocystis* sp.	Bisabolane, heptadecane, ethanol	Vapor compression, distillation, and filtration/chemical hydrogenation (hydroprocessing)	PBRs	GWP	–	Well-to-gate
Collotta et al. (2018)	*Chlorella vulgaris*	Biodiesel	Transesterification	Ponds	GWP, ODP, CC human health, CC ecosystem, HTP, PCOF, PMF, IR, TA and ET, FE, FET, MET, ULO, ALO, NLT, MD, and FD	1 kg of *Chlorella vulgaris*	–
Ye et al. (2018)	*Spirulina platensis*	Edible spirulina tablets	–	PBR	ODP, GWP, smog, AP, EP, carcinogenic, noncarcinogenic, respiratory effects, ET, and FFC	1 kg of algae tablets	Well-to-gate

Sills et al. (2020)	*Desmodesmus* sp.	Biocrude oil	Thermochemical conversion	Both open pond/PBR	GWP, ecosystem quality, nonrenewable resources, human health, and CC	1 MJ of fuel, 1 kg of animal feed, or 1 ha of cultivation	Well-to-gate
Smetana et al. (2017)	*Chlorella vulgaris and Arthrospira platensis*	Microalgae biomass cultivation and processing into food and feed products	–	Open raceways pond, tubular photobioreactor, heterotrophic fermenter	NREC, GWP, CC, ecosystem quality, human health, and respiratory inorganics emissions	1 kg of fresh microalgae biomass sludge, whole dried microalgae biomass used in the form of protein powder (concentrate), 1 kg of bulk proteins, 1 kg of oil	Well-to-gate
Wang et al. (2017)	*Isochrysis sp.*	Aviation fuels	Pyrolysis	Open raceway pond	GWP, TEI, and FFC	1 MJ of algae-based hydrotreated pyrolysisaviation fuel (AHPAF)	Well-to-wheel
Morales et al. (2020)	*Tetraselmis suecica*	Microalgae biomass and algae meal (protein concentrate powder)	–	Pond	GWP, CC, ODP, HT noncarcinogens, HT carcinogens, PM, IR, PCOF, acidification, TE, FE, ME, FET, LU, WD, and MFRD	1 kg of microalgae biomass at 20%-DW, used for animal feed, 1 kg of algae meal at 10% moisture, 1000 WNDS weighted nutrient density score for the actual nutritional value	Well-to-gate

(continued)

Table 4.1 (continued)

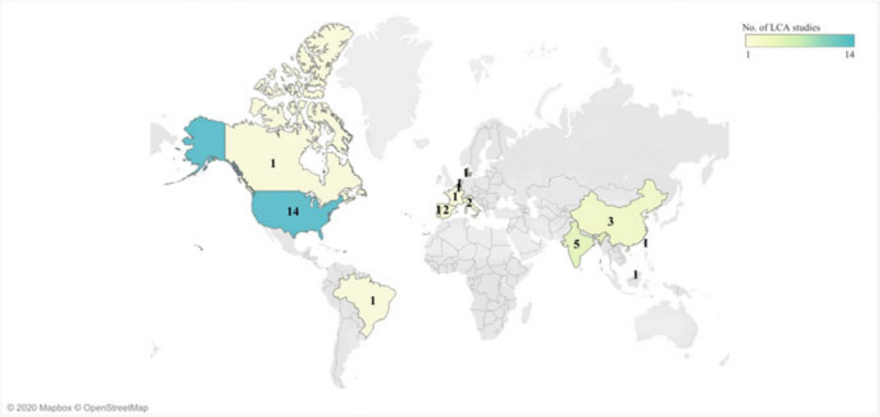
Author (Year)	Algae species	Final product	Conversion technology	Pond/PBR	Impact categories	Functional unit	System boundary
Maga (2017)	*Microalgae*	Biomethane	A pressure-swing-adsorption (PSA) system and fermentation	Open pond	DALY total, EROI, AD, ULO, WSF, loss of species during a year, TA, PMF, ODP, PCOF, GWP100 excl. Biogenic carbon, FE, ME, and FET	1 m3 treated wastewater, 1 MJ CNG (LHV) used in a gas engine	Well-to-wheel
Fortier et al. (2017)	*Microalgae*	Renewable gasoline	Hydrothermal liquefaction	Open ponds	GWP, albedo change impacts, carbon flux change impacts, loss of original biomass impacts, and loss of soil organic impacts	1 GJ of algal renewable gasoline	Well-to-wheel
Fortier et al. (2014)	*Microalgae*	Bio-jet fuel	Hydrothermal liquefaction (HTL)	Pond	GWP	1 GJ of bio-jet fuel produced	Well-to-wheel
Ajayebi et al. (2013)	*Chlorella*	Biodiesel	Transesterification	Raceway ponds	GWP and FD	1 kg of biodiesel produced	Well-to-pump
Rickman et al. (2013)	*Algae*	Biodiesel	Transesterification	Pond	GWP	–	–
Kern et al. (2016)	*Algae*	Biodiesel	Transesterification	Raceway ponds	GWP	10 million gallons of biodiesel fuel annually	Well-to-wheel

Shi et al. (2019)	*Chlorella* sp.	Biogas	Anaerobic digestion	Pond	GWP	GHG results for harvesting technologies were based on 1 kg of dry algae biomass harvested, while results for extraction technologies were based on 1 MJ of lipid oil output	Gate-to-gate algae harvesting and extraction LCAs within the algae biofuel pathway
DeRose et al. (2019)	*Algae*	Fusel alcohol and diesel naphtha	Fermentation and hydrothermal liquefaction (HTL)	Algal turf scrubber	GWP	1 MJ of fuel produced	Well-to-pump
Parsons et al. (2018)	*Microalgae*	Biodiesel	Transesterification	Raceway ponds	Direct and indirect material flow of the product, GWP, RD, and bulk waste	1 kg of biodiesel produced	Well-to-wheel
de Schneider et al. (2018)	*Desmodesmus subspicatus*	Microalgae biomass	–	Raceway ponds	GWP, CC, ODP, TA, FE, ME, HT, and PCOF		Well-to-gate
Axelsson et al. (2012)	*Neochloris oleoabundans*, *Chlorella sorokiniana*, *Tetraselmis suecica*, *Nannochloropsis oculata*	Biodiesel	Anaerobic digestion	Ponds	GWP, eutrophication, and CED	1 kg of biodiesel produced	Well-to-pump

(continued)

Table 4.1 (continued)

Author (Year)	Algae species	Final product	Conversion technology	Pond/PBR	Impact categories	Functional unit	System boundary
Van Boxtel et al. (2015)	*Phaeodactylum tricornutum, Thalassiosira pseudonana*	Microalgal biomass	–	Raceway pond, flat plate, and horizontal and vertical tubular PBR	AD, AP, EP, GWP, ODP, HT, and MT		Well-to-gate
Sarat Chandra et al. (2018)	*Scenedesmus dimorphus*	Microalgal biomass	–	Raceway pond, PBR	AP, EP, GWP, and HTP	1 kg of biomass	Well-to-gate
Beal et al. (2015)	*Staurosira* sp., *Desmodesmus* sp.	Biocrude	Hexane extraction and Valicor's thermochemical conversion	PBRs and ponds	GWP, human health, ecosystem quality, CC, NREC, and WD	1 ha of facility area	–



Countries and LCA publications on bio-fuels

Map based on Countries and the number of LCA publications on bio-fuels. Color shows number of LCA studies for different countries with the maximum number of LCA publications in USA(14), followed by India(5), China(3), Italy(2) and Spain(2), and Netherlands, Belgium, Denmark, Taiwan, France, Brunie Darussalam, Brazil, Canada and Portugal with (1) each.

Fig. 4.4 Spatial distribution of LCA publications on algal biofuels

biomass cultivation, processed food, and feed products and consequently did not employ any of the abovementioned conversion technologies.

The LCA studies under review have selected the functional units based on output energy produced, mass, and land area facility. Energy-based and mass-based functional units were found to be the most common among the reviewed studies. These include a total of 15 studies that have considered an energy-based functional unit (MJ/GJ of biofuel). Fourteen studies have employed a mass-based functional unit (in kg). Three LCA studies have considered the facility area-based functional unit (in hectare). System boundaries of the reviewed LCAs include well-to-gate, well-to-pump, and well-to-wheel. The well-to-gate system boundary comprises the entire processes and emissions until the extraction of biofuel. A total of 14 LCA studies have incorporated a well-to-gate system boundary. The well-to-pump boundary, also known as cradle-to-tank or cradle-to-pump, covers the entire emissions up to the biofuel distribution. Here, the combustion of biofuel is not included. It has been observed that four LCA studies have adopted a well-to-pump system boundary. Well-to-grave includes all the perimeters of the above two system boundaries of well-to-gate and well-to-pump, along with the assessment of biofuel combustion. Twelve LCA studies have involved well-to-wheel system boundaries. Some of the commonly used impact categories in the reviewed LCA studies include global warming potential, eutrophication potential, ecotoxicity potential, land-use changes, acidification potential, ozone depletion potential, climate change, fossil fuel consumption, human toxicity, and nonrenewable energy consumption (Fig. 4.5). The following sections highlight the effect of different essential parameters on the LCA of algal biofuels.

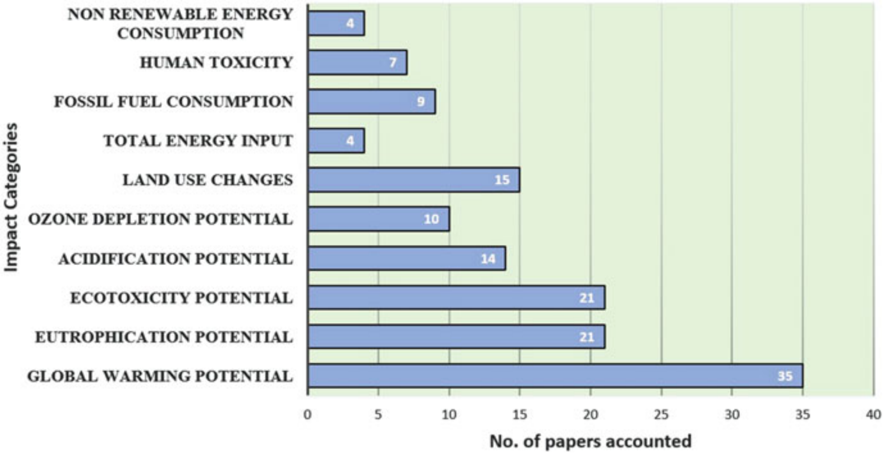


Fig. 4.5 Number of peer-reviewed LCA studies of biofuels by impact categories assessed

4.3.1 Effect of Type of Algae

Phototrophs utilize light energy and use CO_2 for the lipid, protein, and carbohydrate production. On the contrary, heterotrophs utilize simple sugars, i.e., organic carbon, as a carbon source for their growth as well as the production of a much complex organic carbon compound range. There has been increasing commercial use of heterotrophic microalgae to produce a wide variety of products. Despite this, most LCA studies focus on the phototrophic mode of algal growth for biofuel production. Heterotrophs are of growing interest because of their fast algal growth characteristics, high oil productivity, and high cell density. Genus *Chlorella* is the most commonly studied heterotrophic species. Heterotrophic feedstocks include simple sugary compounds as carbon sources such as waste molasses, glycerol, hydrolyzed carbohydrates, acetate, or complex lignocellulosic material. Non-requirement of light leads to simpler microbial oil production in the case of heterotrophic algae. Despite this, a mixed opinion on large-scale cultivation of algae using heterotrophs and autotrophs can be perceived. The current cultivation technology used in both photoautotrophic and heterotrophic production systems is uneconomical—a challenge faced by both the cultivation systems. This is why the utilization of co-products is pointed out to obtain prominent economic value from the process. D'Imporzano et al. (2018) estimated the environmental impacts based on the cultivation mode of algae, i.e., mixotrophic and autotrophic, and defined the conditions under which mixotrophic gives the best results.

4.3.2 Effects of Pretreatment

One of the most important operations in the extraction of biochemicals and the manufacture of biofuels from microalgae is cell pretreatment. Mechanical and nonmechanical processes are used to pretreat algae. Physical, chemical, and enzymatic methods are examples of nonmechanical methods. For microalgae lipid extraction, pretreatment methods utilized are ultrasonic pretreatment, microwave pretreatment, and acid pretreatment. Cell pretreatment can reduce nitrogen content, thus increasing the liquid yield. It also improves the bio-crude quality and oil extraction efficiency by disrupting the microalgae cells. Acid heating pretreatment decreases the oxygen content and ash content due to the inorganic salt solution in water utilized during the process. The pretreatment of algal cells reduces the energy ratio while increasing the energy consumption/energy output ratio. The pretreatment process needs further research to enhance energy and cost efficiencies of microalgae.

4.3.3 Effect of Infrastructure

During the life cycle assessment of microalgae biofuel infrastructure, emissions are often overlooked. Canter et al. (2014) have dealt with the energy consumption and GHG emissions associated with infrastructure materials utilized in the algal biofuel-processing plant. The infrastructure cycle, which involves plastics used to line the ponds and concrete that supports paddle wheel mixing stations, anaerobic digester reservoirs, and CO_2 distributors, contributes to a large amount of GHG emissions. Plastic use was the source of the most associated GHG emissions, followed by the CO_2 delivery system and anaerobic digester. Steel and iron contributed only 1% of the total GHG emissions associated with infrastructure, according to the study. Each material that was sourced from fossil fuels such as coal, petroleum, or natural gas required energy during its manufacturing. The most extensive use of energy is made by plastic during material production. Excavation is followed by plastics, and then concrete. If environmental regulations and soil conditions allow, the first step towards reducing GHG emissions from the infrastructure cycle will be to remove or reduce pond liners. The second step is to reduce the amount of concrete used.

4.3.4 Effect of the Functional Unit

The functional unit affects the comparative performance of modelled systems and hence plays an important role in the life cycle assessment of biofuels. Quantitative specifications of the system under study can be represented using a functional unit. A misleading choice of the functional unit can thereby result in erroneous and imprecise information. Sills et al. (2020) have conducted an LCA for an algal biorefinery that consists of incorporating three functional units: 1 MJ of fuel, 1 kg animal feed, and 1 h of land. The choice of a functional unit can indicate several environmental implications. However, some parameters, such as human health and climate change, are least affected by the choice of the functional unit.

4.3.5 Effect of co-Products

Soratana et al. (2014) compared six dissimilar pathways for co-products produced from microalgal biodiesel to examine the process with maximum environmental impacts and also determine the scope of these co-products in the market. The categories considered for environmental impact assessment are photochemical smog formation, ozone depletion, acidification, global warming, and eutrophication. Due to the chemical and energy-intensive behavior of present harvesting and extraction techniques, the production of microalgal biodiesel is not economically feasible as opposed to other biodiesels. Hence, to make its production viable, valuable co-products can be produced. These co-products can be used in various manners such as glycerol can be used as a source of carbon for algae cultivation or as a source of heat for other processes; biomethane can be transformed into electricity and heat; and lipid-extracted algae can be utilized as feedstock for animals or as a fertilizer or as a raw material for biomethane and bioethanol production. The results showed that the scenario, which utilizes a large number of co-products, including biomethane, bioethanol, recovered CO_2, and recycled nutrients, had the minimum environmental impacts and maximum net energy ratio. However, from an economic point of view, higher income comes from higher quantities and prices of co-products, not merely the variety of co-products. Additionally, the net energy ratio was more for the processes with more co-products. In this context, Sills et al. (2020) studied the method used for the handling of co-products. According to their study, by using a fuel-based functional unit, environmental impacts (ecosystems and climate change) are affected differently by the method used for handling of co-product. For instance, the system expansion method adversely affected the results for climate change, whereas it was beneficial for ecosystem quality.

4.3.6 Ponds Vs. PBR

Raceway ponds have recently been touted as a viable alternative for large-scale commercial microalgae cultivation. The ease in construction, maintenance, and operation has constituted raceway ponds to accomplish the production of microalgae at an enlarged scale. However, they come up with several significant limitations. As a consequence, closed PBRs are employed as an effective alternative to attain higher productivity of algal biomass. The controlled conditions of the concentrated light regime enable a higher photosynthetic efficiency leading to higher favorable biomass productivity. Though PBRs have several advantages, it is highly energy intensive when compared to raceway ponds. In raceway ponds, the net emission ratio of CO_2 (NERCO2), which is known as the ratio of CO_2 sequestered to CO_2 emitted, is higher than that in PBRs. When compared to an open raceway pond, the environmental impacts of PBRs are higher, according to a report by Togarcheti et al. (2017).

4.3.7 Consequential LCA

Brander et al. (2008) describe consequential LCA (CLCA) as an LCA that investigates the consequences of changes to a product's output, including effects both inside and outside the life cycle of the product. Zhang and Kendall (2019) have assessed the consequential impacts on ocean resources created by by-products formed during algal oil production. Fish feed based on captured wild fishes can be swapped with the algae based if the vital nutrients are present in it. An algae-based feed could be utilized in proportion to their market value and nutritional value, and hence, they might potentially bring down the fishing of wild fishes and also the impacts on marine ecosystems. Algae help in resolving some critical issues related to terrestrial crops, for instance, "direct and indirect land-use change," because they can be easily cultivated on poor-quality water resources not suitable for terrestrial crops. Defatted algae biomass (DAB) can act as a raw material for several products like bioenergy, animal feeds, and carboxylic acids. Studies have been implemented to assess the potentiality of DAB to serve as fish-meal substitutes (Zhang and Kendall 2019). These substitutes efficiently provide essential nutrients such as vitamins, energy, lipids, and protein for fish breeding. In some conditions, algae-based feedstock showed improvement in growth, immune system, weight, and health of fishes as well as animals. By using algal-based feedstock, fishing can be minimized, hence conserving the ocean resources but not terrestrial resources. Therefore, a trade-off must be made between terrestrial and ocean resources. Particularly, by reusing waste nutrients and wastewater for algae cultivation, various resources and costs can be reduced significantly.

4.3.8 Uncertainty Analysis

Lima et al. (2020) studied the relationship between LCA and uncertainty analysis for the biorefineries to determine its effect on the results. There are various factors due to which the LCA does not anticipate the possible environmental impacts precisely, one of them being the uncertainty in the data and also in the modelling of the impacts. To increase the results' reliability and also the transparency of the data, the uncertainties must be identified along with their types and sources, which further helps in avoiding erroneous decisions. There are various types of uncertainties such as technological advancements, changes in policies of the government and its incentives, different supply chains having numerous methods of allocation, variations in weather conditions, availability of feedstock, and variations in cost. These uncertainties present in LCA have numerous sources such as distorted or unsatisfactory data, limitations of the system, time duration selected for the assessment of impacts, and hypotheses based on incorrect models. The results indicated that over the last few years, LCA studies' results had been validated predominantly with the help of uncertainty analysis in the scenarios that encompass several sources and a variety of uncertainties, like biorefinery systems. To overcome the uncertainties inherent to the LCA process, researchers have developed specific specialized LCA procedures. Some of these specialized LCA procedures are discussed in the following sections.

4.4 Specialized LCAs

4.4.1 Spatially Explicit Life Cycle Assessment (SELCA)

LCA can be enhanced by coupling geographic information systems (GIS) along with it. In conventional LCA, the spatial data is ignored, and in many cases such as agricultural yield and environmental impacts of emissions, the location is an important factor and needs to be considered. Hence, in such cases, SELCA is more feasible as it represents spatially varying data that has been previously ignored. SELCA thus helps in overcoming limitations related to conventional LCA. SEHR-LCA (spatially explicit high-resolution-life cycle assessment) model has been presented by Roostaei and Zhang (2017) in their work for the production of algal biofuel by using wastewater. An integrated data analysis of LCA, wastewater treatment plants (WWTPs), and GIS has been carried out in this study. Conversion of bio-oil has been modelled using three pathways: hydrothermal liquefaction (HTL), microwave pyrolysis, and lipid extraction. The site-specific and seasonal variations in environmental impacts and productivity of biofuel have been assessed using the model. The results of the model indicate that this biofuel production using wastewater can provide a chance to increase the biofuel output of the USA; that is, it could reach up to 0.98 billion gallons/year. The results of LCA indicate the differences among WWTPs, different locations, and seasons of operation. Although conventional fuels are better than algal biofuel in energy efficiency, biofuel could significantly reduce GHG emissions. Hiloidhari et al. (2017) have used GIS in the following context: (1) agro-residue resource assessment, (2) biomass logistic, and (3) power plant design. GIS and LCA are complementary to each other. GIS is useful in the acquisition, visualization, processing, and storage of spatial data while LCA is not, with reference to the aforementioned concerns. Maintenance of availability, distribution, and physiochemical properties of agro-residues in a spatiotemporal database is important because of the diversified agro-residue varieties. Such a database is maintained by GIS that helps in cost-benefit analysis and effective benefit allocation. Transportation, one of the logistic parameters, can be effectively optimized using

GIS. This optimization results in the shortest pathway that reduces the transport-related emissions significantly.

4.4.2 *Life Cycle Climate Change Impacts of Land Use and Albedo Change*

"Direct land-use change impacts" (DLUC) have been typically ignored by algal biofuel LCAs. DLUC is affected by the reduction in biomass, a decrease in organic carbon present in the soil, change in carbon flux, and variations in albedo, leading to an increase in climate change impacts. Plants and ground have an abundance of carbon and control the water, energy, gases, and momentum trade-off between atmosphere and land. Over time, the land-use pattern has led to considerable radiative forcing associated with albedo. The concern is increasing regarding the advantages offered by afforestation and various measures that reduce the emissions, being nullified by the change in albedo. Efforts are being made to include albedo in the LCA framework so that common ground could be provided for albedo and GHG comparison.

To combine the climate change impacts of DLUC related to open pond cultivation of microalgae and the impacts of topographic variables on the growth of algae, various LCAs have been developed by Fortier et al. (2017) in their study. Two regions with similar climatic conditions have been considered for LCA of renewable gasoline production from microalgae. These two areas were chosen to study the variations in the impacts of DLUC caused due to differences in land cover. The significant contributors to "life cycle climate change impacts" are changes in carbon flux and albedo; and also, these two factors differed for the areas selected for the study. By including albedo change effects into DLUC effects, the life cycle climate change impacts in one location were 10.2%, while in a separate location, it was 36.4%. This technique could be quite useful while assessing the sustainability of biofuels based on geographical conditions and can also help in choosing the site for biofuel production.

4.4.3 *Time-Dependent LCA*

By overlooking the fact that the GHGs cumulatively increase in the atmosphere with time, the calculations related to GHG intensity reduce emissions by equally dividing them over a time period. The conventional LCA method does not consider the time at which the emissions were produced during the life cycle of the product; hence it does not provide accurate results regarding the effects on climate change. So, the time factor must be included in LCA to generate accurate results. In the case of algae growth, the molecular composition and the fractional growth rate are dependent on time and vary accordingly. Similarly, the changes in harvesting time for algae also change its energy requirements like electricity. Hence this may vary the LCA results.

The amount of co-product depends upon the composition of algae during its harvesting. In a study done by Axelsson et al. (2012), the variation in the productivity of protein, lipid, and starch was shown concerning the species, harvesting period, and feeding regime. Four different species of algae were cultivated in the presence and absence of nitrogen and were analyzed at the interval of 3 days. LCA was done using the GREET model (Argonne GREET Publications 2020) to evaluate the best suited growth condition, GHG emissions, cumulative energy demand (CED), and eutrophication. Results indicate that more growth cycle time does not certainly result in better environmental performance; hence biorefinery systems should be designed to optimize lipid and non-lipid contents.

4.4.4 Harmonized LCA

A harmonized LCA is generally a consistent methodological framework that is focused on choices concerning multifunctionality, functional unit, CO_2 balance, and system boundaries approach. Valente et al. (2019) have developed and applied a harmonized LCA for estimation of biodiesel carbon footprint. In order to facilitate robust comparative studies, a harmonized LCA framework has been defined. The contribution of capital goods to the carbon footprint of microalgal biodiesel is significant. Hence, capital goods are included within the harmonized system boundaries. The risk of misinterpretations is avoided when methodological choices in an LCA are harmonized. Tu et al. (2018) in their study have stated that the use of harmonized inventory data could significantly reduce life cycle energy and water consumption as well as GHG emissions of multiple algal biodiesel and renewable diesel process trains.

4.4.5 Integration of Resilience

Collier et al. (2017) have described the modelling of environmental sustainability using LCA while modelling the resilience of the value chain using a decision model that is scenario based. Several factors influence the various stages of the biofuel value chain, including technology investments and other processes that include the processing, transportation, and storage of biofuels. The changing economic, social, and physical environment of value chains must be considered while making investment decisions. LCA and resilient assessment can together assist in the business case for investments in biofuels. Collier et al. (2017) have used the term value chain in preference to supply chain because end users are the ultimate destination, and the demand for biofuels is the fundamental source of value. This value, in the eyes of the consumer, is created by making effective biofuel strategy and planning options.

Biofuel manufacturers, investors, and other stakeholders must ensure that value chain projects are resilient—in other words, the value chain can withstand unexpected and sudden emergent circumstances such that their investments are not compromised. The potential of the value chain to withstand unpredicted future condition lies in the concept of resilience. Biofuel resilience is characterized by five capabilities—diversity, cohesion, transformability, efficiency, and adaptability. There is a need to identify environmentally resilient value chain innovations, specifically in infrastructure, agricultural, technological, and business initiatives prone to uncertainty. This incorporation of relevant stakeholders and their values can move towards more sustainable and resilient end states.

4.4.6 Social LCA

Rafiaani et al. (2020) in their study have assessed the social consequences and impact of an algae-producing company through social life cycle analysis (SLCA) in Belgium. The integration of the social dimension gives the likelihood of effective evaluations resulting in better social, economic, and sustainable environmental effects. Social LCA includes the review of the social impact of the internal assessment and optimization of a company. The possible risks and opportunities are identified and taken into consideration from the social outlook in the initial production and development stage. SLCA also incorporates an enhanced communication between the business partners and supply chain by sharing and providing data sources and developing databases based on social performance. The opportunities and challenges of SLCA are highlighted in this study. Three stakeholder groups—workers, consumers, and local community—have been accounted for by the United Nations Environment Programme (UNEP) and Society of Environmental Toxicology and Chemistry (SETAC) guidelines in SLCA analysis. Three subcategories are associated with each stakeholder group, namely for workers, (1) fair salary, (2) health and safety of workers, and (3) equal opportunities are assured and no discrimination holds; for consumers, (1) transparency of the company and (2) consumers' health and safety are considered; and for local community, (1) safe and healthy living conditions, (2) secured living conditions, and (3) local employment are accounted. The share of women employed is found to be lower when compared to the sector-level data. There is a need for policy makers to identify the social impacts and account together with the economic and environmental consequences while framing interventions for innovation development. This innovation development leads to the enhancement of the demand and reputation of companies resulting from the beneficial sides of society.

4.5 Conclusions

This chapter provides a comprehensive review of the recent studies focusing on the LCA of algal biofuels. The basics of algal biofuels and life cycle assessment have been discussed. The differences in the assumptions, and their possible effects on the final results, have been highlighted. Some leading specialized LCA procedures have also been elaborated. Some of the recommendations include a need for special attention to parameters such as the inclusion of infrastructure, and also the handling of co-products. The inventory data needs to be treated consistently for a comparable LCI between different studies. Impacts other than GHG emissions such as acidification and eutrophication, human toxicity and ecotoxicity, and direct and indirect land-use changes (LUC) need to be given more emphasis. There must be a transparent evaluation of carbon stock changes in the impact category of LUC by incorporating remote sensing and satellite data. Interpretation of results using more than one functional unit is expected to bring more insights into the sustainability assessment of algal biofuels. Fertilizer inputs and water consumption are inventories that must not be undervalued and disregarded. Finally, there is a need for a more transparent and coherent methodology for the selection of boundary conditions, functional units, and impact category metrics.

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Chapter 5
Biodiesel: Features, Potential Hurdles, and Future Direction

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Abstract The need for cleaner and greener fuels has considerably evoked much interest in the worldwide renewable energy scenario. Biodiesel is one such biofuel that is widely used as green fuels and can replace conventional fuels. Over the years several methodologies have been employed to produce biodiesel, and these production processes have been modified over the period utilizing the latest technologies and optimizing the feedstocks. The main difference between conventional diesel and biodiesel is its source. Biodiesels are made of naturally occurring fatty acids or oils. Biodiesel consists of fatty acid methyl/ethyl esters (FAMEs/FAEEs) derived from vegetable oil or animal fats in the presence of alcohol and acid/base catalyst. Recently there have been many modifications in the feedstocks and their generation to optimize the yield. The key and the most promising features of biodiesel are clean burning fuel, no toxicity, reduced emission, and being safer and biodegradable. However, a high methanol/oil ratio, limited conversion, and high operational cost make large-scale production less competitive. Process intensification strategies like microreactors, microstructured reactors, and microchemical technologies could play a vital role to make the process more economical and competitive. Despite several advantages, present biodiesel production processes face issues like high catalyst separation and recovery cost, high alcohol/oil ratio, and less logistic viability. However, some of these issues could be solved using innovative heterogeneous/enzymatic catalyst-embedded systems in the future. This study is a comprehensive outlook of biodiesel in a modern context with production challenges and hurdles. Furthermore, the study also suggests some future process intensification alternatives to overcome conversion and yield issues that make biodiesel production commercially feasible and competitive.

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5.1 Introduction

Energy resources play a vital role in the industrial, technological, and socioeconomic growth of countries. The energy consumption per capita has become a symbol of economic development and a higher standard of living especially after the industrial revolution in the late eighteenth century. International Energy Agency (IEA) (World Energy Outlook 2007) and Shahid and Jamal 2011, reported that the consumption of energy will increase by 50% in 2030 as compared to the present demand. 45% of world energy consumption will be accounted only by China and India. The demand for energy resources in global transportation sectors is expected to increase by an average of 1.8%/year from 2005 to 2035 (International Energy Outlook 2010). Coal, natural gas, petroleum oil, and electricity are the major commercial sources of energy across the world and are widely used as an energy resource in transportation and industries. Fossil fuels are preferred in transportation and industries due to their high energy density, availability, high calorific value, and combustion characteristics (Hossain and Davies 2013). Fossil fuel resources are limited in nature and will certainly be depleted in the next few decades, although energy demand will increase due to population growth, higher living standards, rapid industrialization urbanization, and technological progress. It is intimated that worldwide energy demand will increase five times in the year 2100 as compared to today (Ogunkunle and Ahmed 2019). Indian energy consumption statistics are dominated by fossil fuels like petroleum oils, coal, and natural gas. Nearly 95% of the transportation sector is powered by petroleum oils in which the demand for diesel is five times higher than petrol. India is a country of 135 crore peoples and the fifth major economy of the world but it lies behind many small countries in per capita term. Thus for sustainable 8% average annual economic growth, India needs to generate 2–3 times more energy than the present to support its growing population. In the last decade, the consciousness of countries increased towards climate change and global warming. Thus excessive use of fossil fuels is not encouraged by countries to show commitment with Paris climate accord, 2015. Globally, the governments of countries now discourage fossil fuels and encourage its green alternative to meet ever-growing energy demands. India is one of the five countries and ranks in the fifth position after the USA, China, Russia, and Japan in CO2 emission that creates environmental and ecological imbalances. Several energy alternatives like wind, hydroelectric, solar, and nuclear are used as renewable sources of energy (Ogunkunle and Ahmed 2019; Cintas et al. 2010). Despite the renewable nature of these alternatives, higher initial investment and operational flexibility are major concerns. These are the reasons that limit the use of renewable energies in the transportation sector. Biodiesel, derived from natural oils by chemical transesterification process, is among the best alternatives available for conventional fossil diesel. Biodiesel is fatty acid methyl ester

(FAME) which has very good combustion properties due to higher cetane value than fossil diesel (45–65 compared to 40–55). Biodiesel could be a feasible solution for fossil scarcity and global warming as renewable fuels emit fewer greenhouse gases than fossil fuels (Abed et al. 2018; Mohd Noor et al. 2018). However, biodiesel is the best alternative for fossil diesel as it has a higher viscosity (3.5–5.5 centistokes at 40 °C) than fossil diesel (2–3.5 centistokes at 40 °C), which makes its direct use difficult in a conventional diesel engine (Lapuerta et al. 2008). To overcome the high-viscosity issue alcohols are primarily used in the transesterification process (Van Gerpen 2005). In that case an objective of 100% renewable biodiesel is only possible if alcohols are also derived from renewable sources.

Biodiesel, chemically known as fatty acid methyl ester (FAME), is biodegradable and nontoxic. It has an environment-friendly combustion–emission profile, as it produces much less sulfur dioxide, carbon monoxide, and unburned hydrocarbons compared to fossil diesel. That makes biodiesel a reliable alternative for fossil fuels (Cintas et al. 2010). The production of biodiesel gains strategic importance in several countries due to three main reasons: (1) gaining self-reliance on growing energy demand, (2) creating oil independence, and (3) reducing greenhouse gas emissions. Vegetable oils have become more attractive recently because of their environmental benefits and the fact that these are made from renewable resources. The vegetable oils are much-shouted feedstock for biodiesel production as it comes from natural derivatives. Moreover, biodiesel is a renewable and potentially inexhaustible source of energy and has a higher cetane value with energy density closer than fossil diesel (Živković and Veljković 2017). A variety of biolipids can be used to produce biodiesel. Various feedstocks like rapeseed virgin vegetable oil and soybean oils are most commonly used, although other oil sources like palm, sunflower, mustard, hemp, and even algae could also be potentially used. Feedstocks like waste vegetable oil, animal fats including tallow, lard, yellow grease, and nonedible oils (Jatropha, neem oil, castor oil, tall oil) for biodiesel production through transesterification are under developmental stages. Biodiesel blending with conventional fuels improves combustion properties and cetane number of fuel as reported by several researchers. It is reported through several studies that diesel and biodiesel blends reduce smoke opacity, carbon monoxide, carbon dioxide, unburnt hydrocarbons, and particulates (Agarwal and Das 2001). The biodiesels are advantageous than fossils due to portability, availability, renewability, and biodegradability (Ma and Hanna 1999) and it has a higher cetane number, improved combustion efficiency, lower sulfur, and aromatic content (Mudge and Pereira 1999). Other strategic advantages of biodiesel include reduced import bill, domestic origin, inherent lubricity, and high flash point and it can also reduce dependency over imported petroleum (Mittelbach and Remschmidt 2004).

This book chapter is a comprehensive outlook of biodiesel, feature, and production technologies and it discusses some of the important aspects of biodiesel production, oil feedstocks, technological advancement, synthesis routes, and alternative processes. The later section of this book chapter discusses potential hurdles/challenges and future opportunities available to make biodiesel production competitive and lucrative in terms of industrial and environmental concerns.

5.2 Existing Feedstocks for Biodiesel Production

Over the years there has been enormous transformation and modifications in the feedstocks for biodiesel production. One of the most common techniques of biodiesel is the transesterification reaction of triglyceride with methanol in batch/continuous reactors. Recently nonedible feedstocks like animal fats are widely used as a promising feedstock because of their low-cost structure, environment friendliness also increasing the fuel quality by low NOx emissions, high cetane number, and oxidative stability (World Energy Outlook 2007). Another feedstock that is gaining quite importance is microalgae which are photosynthetic microorganisms with the ability to breed rapidly in a variety of environments because of their molecular structure. These microalgae have the capability of self-reproduction using solar energy and converting it into chemical energy via photosynthesis (Shahid and Jamal 2011). There is also some major class of feedstocks used widely for biodiesel manufacture over the globe which includes soybean oil, rapeseed oil, palm oil, sunflower oil, and other potential sources like sunflower, peanut, coconut, Jatropha, hemp, corn (maize), and mustard. These feedstocks are conventionally used but they give a serious threat to the food chain which has encouraged the use of animal fats and algae and they are the third-generation feedstocks for biofuels. A comparative study for the use of microalgae and palm oil as biodiesel feedstocks shows that microalgae are far better than palm oil considering the food security and environmental aspects (International Energy Outlook 2010). Feedstocks of biodiesel are now moving towards nonedible feedstocks like waste oils and fats and currently, works are going on in the production of microbial oils by the cultivation of microorganisms that can generate high amounts of lipids in their biomass. The major probable feedstocks in this category are yeast, filamentous fungus, bacteria, and microalgae (Hassan and Kalam 2013). Some research work has also been done on the use of vegetable oils in biodiesel production. Vegetable oils have very less sulfur content which helps in the low greenhouse effect (Hossain and Davies 2013). The use of substitute feedstock as bovine fats, microalgae, and waste cooking oils (WCO), for biodiesel production, has several recompenses like cost and emission free. However, these oils contain some contaminants, which can reduce the quality of biodiesel. Transesterification reaction with the base as a catalyst using WCO feedstocks and alcohol was used to produce biodiesel. The quality evaluation from raw materials and final biodiesel was done and it is seen that the above method is quite promising (Cintas et al. 2010).

5.3 Production of Biodiesel from Vegetable Oils

There are four possible pathways to convert oil and fats into biodiesel: (1) direct blending, (2) thermal cracking (pyrolysis), (3) emulsification (microemulsion), and (4) transesterification (Demirbas 2009a, b).

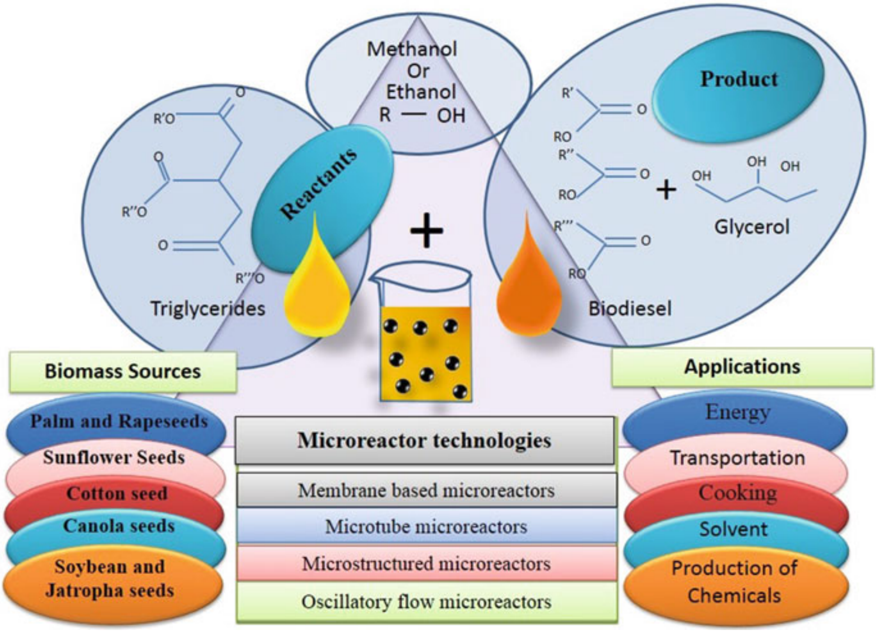


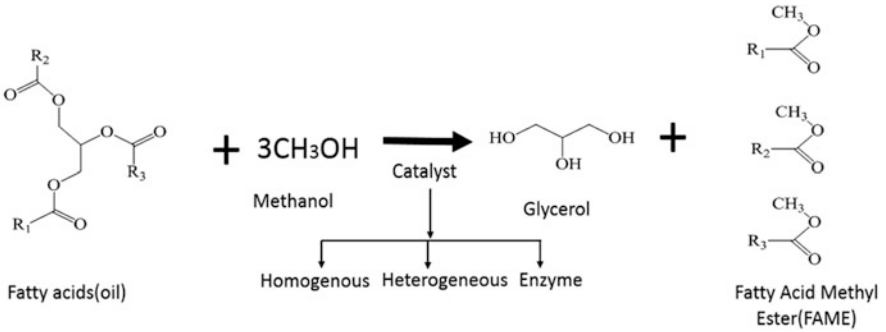
Fig. 5.1 Schematic representation of biodiesel production technologies with biomass feeds and application

The above-given schematic (Fig. 5.1) represents the various biodiesel conversion microreactor technologies with potential biomass feedstocks and applications.

Direct blending of oils in fossil fuels severely damages diesel engine due to its high viscosity, gum formation, free fatty acids, and acidity. Polymerization, oxidation, and lubricant oil thickening also create issue during continuous operation and storage of vegetable oil-blended fuels (Mayvan et al. 2012). The pyrolysis and microemulsion technologies are highly cost intensive and face issues like lower cetane number and incomplete combustion (Borges and Díaz 2012). Therefore, the only convenient method for biodiesel production is transesterification (alcoholysis). In transesterification mixture new bonds between free fatty acids (FFAs) and alcohol are formed while ester bonds between fatty acids (FAs) and –OH bond of glycerol break down. Methanol, ethanol, propanol, butanol, and amyl alcohol are used for transesterification but use of ethanol and methanol is more predominant. Fatty acid ethyl esters (FAEEs) and glycerol are produced if ethanol is used whereas fatty acid methyl esters (FAMEs) and glycerol are produced if we use methanol instead of ethanol (Demirbas 2009a, b).

5.3.1 *Latest/Current Technologies for Biodiesel Production*

Technologies like microemulsion, pyrolysis, direct blends, and transesterification are used for the commercial production of biodiesel. The microemulsion technology has the advantage of low-viscosity product but has a lower cetane number and energy density. Transesterification technology has the advantage of high cetane product, higher conversion, and renewability while the pyrolysis process requires a higher temperature. Among these processes, transesterification is the most adapted biodiesel conversion technique. The conversion of biodiesel from fatty acids is a three-step chemical reaction. Triglycerides are converted to diglycerides in the first steps, and diglycerides to monoglycerides and monoglycerides to glycerol in subsequent second and third steps. Three alkyl esters are produced each in subsequent steps of this process (Sharma and Singh 2008). The biodiesel synthesis reaction from fatty acid to biodiesel is given below.



Most of the conventional transesterification process requires alcohol in large quantities to shift reaction equilibrium towards the product side. Thus higher product yield, unfortunately, requires higher alcohol consumption that eventually increases product cost (Othman et al. 2010). The homogenous acid catalyst is used to reduce alcohol consumption but these are corrosive and require larger holding times for the desired yield. However homogeneous alkaline catalyst could potentially overcome this issue (Vicente et al. 2004). Many researchers are working on the optimization of the reactors for simulating the multiphase reacting system. The supercritical transesterification could potentially solve mass transfer issues but only limited to laboratory scale due to high operating cost (Pinnarat and Savage 2008). Although present technologies have product yield and cost economics-related issues, these issues can easily be overcome by process intensification.

5.3.1.1 Microreactor Systems for Biodiesel Synthesis

The traditional methods for biodiesel production like microemulsification, pyrolysis, and transesterification have certain disadvantages like low efficiency, longer

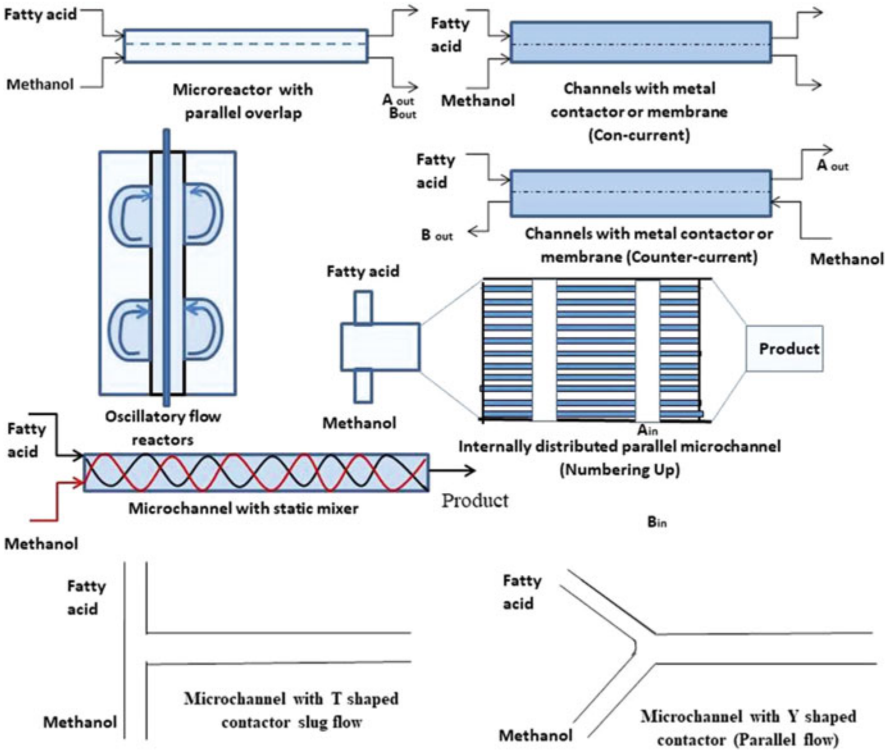


Fig. 5.2 Schematic representation of various flow reactor systems for biodiesel synthesis (modified with permission from Kashid and Kiwi-Minsker (2009) Copyright American Chemical Society)

residence time, high operational costs, and energy consumption. Alcohol and triglycerides are showing multiphasic nature as they are not miscible enough to produce a single phase. The multiphasic nature of transesterification mixture limits contact between reactants and conversely decreases conversion, which eventually increases residence time. To overcome mixing and conversion-related issues miniaturized reactor technologies for process intensification are being developed. One of the promising candidates is microreactor technology which has the advantage of high volume/surface ratio, short diffusion distance, fast and efficient heat dissipation, and mass transfer rates (Ma and Hanna 1999). Microreactor systems are characterized by their geometry, multiplicity, and architecture. Microreactor systems inherently or coherently intensify mixing that enhances overall FAME yield. Laziz et al. (2020) reported torus-shaped internal recirculations through simulation within methanol slug in microchannel that enhances overall mixing of phases. Microreactor systems are classified into four types: (1) membrane microreactor, (2) microstructured reactor, (3) oscillating flow reactor, and (4) microtube reactor. Figure 5.2 depicts a schematic representation of various microreactor systems.

Microtube Reactors

The microtube reactors are the simplest reactor system used for the transesterification of fatty acids. The lower pressure drop and higher interfacial surface area efficiently improve radial as well as axial transport. These microtubes are made of materials like quartz, fluorinated ethylene propylene (FEP), and steel. The low-temperature transesterification takes place at 40–80 °C in the presence of an alkaline catalyst. The conversion temperatures (approx. 300–350 °C) are much higher in the absence of catalysts. The microtube reactor could potentially utilize different architectural shapes (serpentine, helical) for more effective conversion.

Fast transesterification at an industrial scale is possible using microstructured reactors, oscillatory flow reactors, and ultrasonication microreactors. These above-given microreactors facilitate higher interfacial surface area and demonstrated better conversion efficiency for different chemical reactions. A simulation study of COMSOL model of the multiphase biodiesel reaction and to evaluate various 2D microreactor designs is being made for the new research (Agarwal and Das 2001). Various microreactor designs are proposed to enhance mixing for higher product yields. Kashid and Kiwi-Minsker (2009) proposed various contracting schemes for microreactors, namely porous membrane microreactor, microreactors with a static mixer, multichannel microreactor, and microreactor with “T”- and “Y”-shaped mixing element. Sun et al. (2008) studied the synthesis of biodiesel in KOH-catalyzed capillary microreactors with two different IDs (0.25 or 0.53 mm) using unrefined cottonseed oil and rapeseed oil as feedstocks. The effects of alcohol/oil ratio, residence time, reaction temperature, and capillary size were examined in experiments. They found that the production rate of biodiesel was enhanced with better yield by the use of microreactors rather than conventional reactors.

Tanawannapong et al. (2013) used waste cooking oil in a 0.508 mm ID channel of length 1.2 cm with a T mixer for biodiesel production. They observed 91.7% FAME yield by using oil/methanol ratio of 9:1 and 1 wt. % KOH catalyst at 65 °C. They reported shorter residence time with microreactor than the batch system under similar operating conditions. Basiri et al. (2016) investigated the soybean oil methanolysis in ultrasound-assisted (UA) microreactor using KOH as a catalyst. They obtained 97.1% FAME yield at the optimal reaction temperature of 47 °C, 1.29 (w/w %) KOH catalyst concentration, and methanol-to-oil molar ratio of 1:6. They concluded that UA transesterification reactions in microreactors can reduce the reaction time and temperature, alcohol-to-oil molar ratio, as well as energy consumption. Kaewchada et al. (2016) used microreactor technology for palm oil + methanol system in the presence of KOH catalyst. They used a microreactor of ID 0.508 mm and 1.2 m length with a 6:1 oil:methanol ratio. Kaewchada et al. (2016) reported a sharp increase in FAME yield with a small increase in catalyst amount. The pictorial representation of the microtube setup for transesterification of fatty acid is given in Fig. 5.3.

Billo et al. (2015) used microreactor technology with parallel microchannels to improve production capacity. They reported a higher biodiesel yield with lower residence time. They organized thousands of parallel streams of reactant feed and

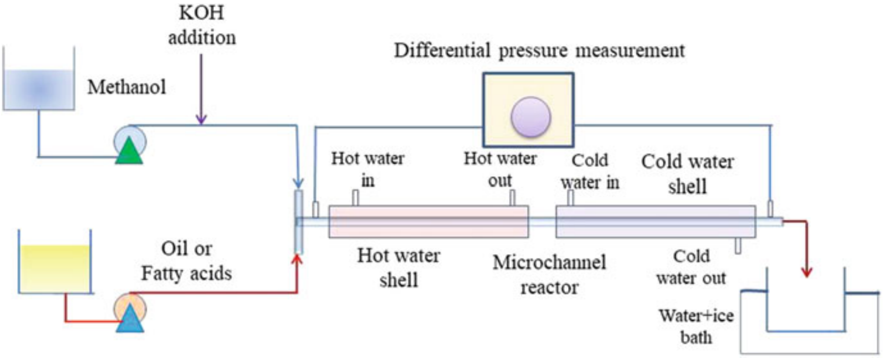


Fig. 5.3 Schematic representation of microtubular reactor system

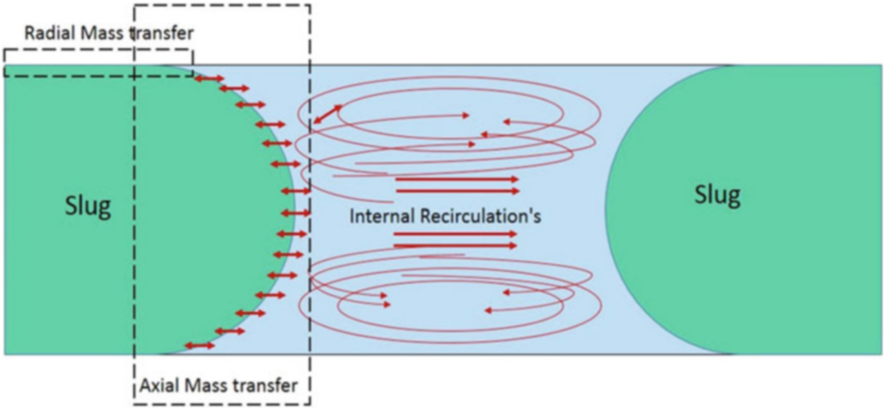


Fig. 5.4 Mass transport phenomenon in slug flow (modified with permission from Kashid et al. (2007) Copyright American Chemical Society)

reported alternate parallel slugs of reactants. The slug flow pattern intensifies mass transfer and conversion efficiency due to its higher surface area-to-volume ratio and lower diffusion distance. Slug flow patterns favor mass transfer between phases and parallel slugs due to both axial and radial mass transport as depicted in Fig. 5.4. Nanoscale movement at axial interface and film mass transport at radial interface intensify overall FAME yield during transesterification and lower residence time.

Membrane Microreactor

Membrane microreactor attributes a larger surface area per unit volume. Membranes are known for their higher selectivity and ability to control the mixing of two components between two phases. Various types of membrane (organic, inorganic, porous, and dense membrane) are used in various reactor designs (extractor, distributor) (Caro 2008). Organic membrane offers higher product selectivity and interfacial area for mass transfer. However, ceramic membranes offer higher chemical, thermal, and mechanical resistance with higher permeability rates. The ceramic membrane could effectively be utilized for transesterification of fatty acid in acidic or basic environments (Barredo-Damas et al. 2010). Achmadin et al. (2010) used a biocatalytic porous membrane microreactor (BMM) for transesterification of triolein and methanol. BMM is designed over an asymmetric polymer membrane (MW of 300 kDa) of diameter 63.5 mm coated with 280 μm enzyme carrier. The mixture of triolein and methanol is passed through the pores of the biocatalytic membrane for enhanced conversion. They reported 80% FAME yield with residence time of 19 min at 35 °C. The biocatalytic membrane made of polyethersulfone has attributed good stability and catalytic activity for at least 12 days of continuous operation without any decay. Baroutian et al. (2010) used 40 cm length ceramic (TiO_2/Al_2O_3) membrane with a pore size of 0.05 μm, and inner and outer diameters of 1.60 and 2.54 cm, respectively. The TiO_2/Al_2O_3 membrane has very high selectivity towards biodiesel and it was able to block the triglycerides. They obtained nearly 94% yield with 157.04 g catalyst/volume of reactor at 70 °C reaction temperature.

Microstructured Reactor

Microstructure reactors consist of internal structures to facilitate efficient mixing. Microstructures like HiPress Slit Interdigital Micromixer (HSIM OR H-SIMM-V2), Caterpillar Split-Recombine Micromixer (CSRM), SIMM-V2 (slit interdigital micromixer), and RIMM (rectangular interdigital micromixer) are efficiently utilized in microreactors for biodiesel production (Canter 2006; Sun et al. 2010; Athar et al. 2020). Various shapes of microchannels like serpentine, zigzag, meandering, and 90 ° bends could efficiently utilize for further enhancement of interfacial surface area and biodiesel yield. Canter (2006) used a microstructured reactor with parallel microchannels for biodiesel synthesis from vegetable oil and methanol/NaOH. Microchannels are cut in a thin plastic plate and oil and methanol fed in a reactor with the help of two syringe pumps. They observed that >90% (40 °C) and 96% (45 °C) of FAME yields could be achieved in 4 and 10 min of residence times, respectively. Avellaneda and Salvadó (2011) synthesized biodiesel by using recycled oil through a helicoidal reactor system. The reactor consists of two longitudinal flow lines (0.218 cm) made of SS-316 conversing at “T”-type mixer that is connected with helicoid tube (ID-6 cm). They reported 89% FAME yield in 13-min residence time with NaOH catalyst.

Oscillatory Flow Reactor

On a similar note, oscillatory flow reactors consist of equally spaced orifice plate baffles that facilitate oscillatory motion within the system as given in Fig. 5.5. Oscillatory motion within the system improves efficient mixing of components;

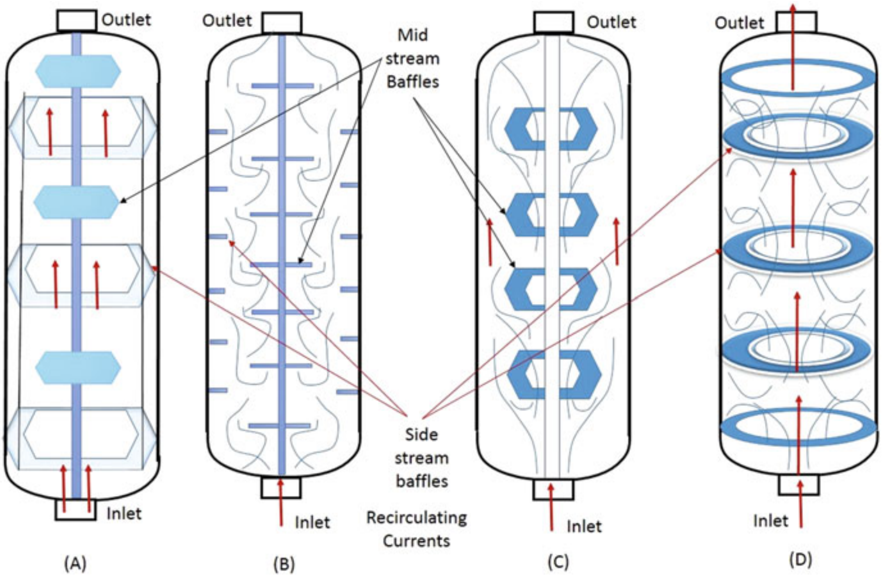


Fig. 5.5 Various types of oscillating flow reactors (OFRs) along with orifice baffles. (**a**) Hexagonal concentric orifice with hexagonal baffled OFR. (**b**) Baffled plate OFR. (**c**) Hexagonal concentric orifice OFR. (**d**) Circular concentric orifice OFR

thus this resulted in higher mass transfer and conversion yields. The controlled oscillatory motion improves the radial mixing of components and equally spaced baffles facilitate long residence time. The microtube technologies come with a higher product yield with lower pressure drop as it does not contain any moving or static system within the reactor. The parallel microchannel reactor system could effectively ramp up production rates and provide flexibility in operation (Avellaneda and Salvadó 2011; Harvey et al. 2003).

The comparative analysis of different biodiesel synthesis systems reported by various researchers is given in Table 5.1. The highest reported mass transfer is achieved in 0.8 mm ID microtube with 4.4% KOH and significantly larger oil: methanol ratio.

5.4 Types of Transesterification

The transesterification process is reaction with slow reaction kinetics. The reaction rates are enhanced by using catalysts, enzymes, or supercritical conditions. The fatty acid transesterification process is classified into three parts depending on the reaction mechanism: (1) catalytic transesterification, (2) enzymatic transesterification, and (3) noncatalytic transesterification.

Table 5.1 Comparison of various biodiesel production technologies

Author	Reactor technology	Catalyst	Experimentation details	Percentage yield (FAME)
Sun et al. (2010)	Microstructured reactor Mixing element: SIMM-V2 PTFE tube 3 mm ID packed With Dixon rings	1% KOH	Methanol:Oil ratio: 8:1 Residence time: 17 s at 70 °C	99.5%
Guan et al. (2009) Sun et al. 2008	1. Microtube reactor with 0.8 mm ID FEP tube Mixing element: T-shaped micromixer 2. Stirred tank with 0.25 mm ID quartz tube	4.5% KOH 1% KOH	Methanol:Oil ratio: 23.9:1 Residence time: 100 s at 60 °C Methanol:Oil ratio: 6:1 Residence time: 5.89 min at 60 °C	100% 99.4%
Wen et al. (2009)	Zigzag microchannels 0.25 mm ID quartz capillary Mixing element: T-shaped micromixer	1.2% NaOH	Methanol:Oil ratio: 9:1 Residence time: 28 s at 56 °C	99.5%
Harvey et al. (2003)	Oscillatory flow reactor 25 mm ID QVF tube with orifice plate baffles	1.2% NaOH	Methanol:Oil ratio: 3:2 Residence time: 30 min at 50 °C	98%

5.4.1 Catalytic Transesterification

The catalytic transesterification process is carried out in the presence of catalytic agent to enhance reaction rates that is present in either homogeneous or heterogeneous state.

5.4.2 Catalyst for Biodiesel Synthesis

The transesterification of fatty acid can proceed in the absence of a catalyst but that requires higher temperature and pressure. However, a long residence time is required at a higher temperature in the absence of a catalyst. The catalyst reduces transesterification activation energy that results in higher FAME yields at lower temperatures.

Various catalyst systems like alkaline, acidic, and lipase (enzyme based) catalysts are used for the transesterification process. The brief classification of catalyst is given in Fig. 5.6. Alkaline catalysts are preferred as they facilitate higher conversion at the mild condition with lower residence time than the acidic catalyst (Kawashima et al. 2009). Homogeneous catalysts are mostly used for commercial processes, while heterogeneous catalysts are under developmental stages.

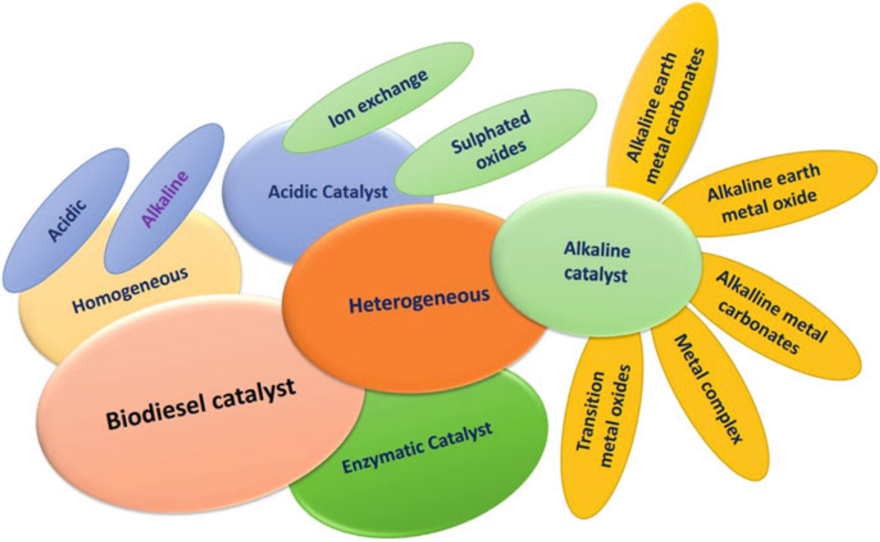


Fig. 5.6 Classification of different biodiesel catalysts based on their nature

5.4.2.1 Homogeneous Catalyst

The homogeneous catalyst for biodiesel synthesis is present in the same state as reactant. The homogeneous catalyst is found in soluble form within reaction mixture. Two distinct classes of homogeneous catalyst used for biodiesel synthesis are acid catalyst and base catalyst.

Acid Catalyst

Hydrochloric acid, sulfuric acid, ferric sulfate, sulfonic acid, and organic sulfonic acid are the most used acid catalysts for biodiesel synthesis. However, sulfuric acid, sulfonic acid, and hydrochloric acid are preferred due to their higher acidity and lower cost. Acid catalysts are favored as they are not sensitive towards free fatty acids that reduce side reactions (saponification) and produce high-purity glycerol as a by-product (Atadashi et al. 2013). Moreover, acid catalysts are sensitive to moisture or water content. Water presence even in small quantities below 0.1 wt. % can adversely affect the overall reaction. The presence of water in larger quantities of nearly 5% can be able to stop the reaction completely (Cerveró et al. 2008). An acid catalyst like sulfuric acid is useful when feedstocks like waste cooking oil, animal fat, and nonedible oils are used that contain free fatty acid (Athar et al. 2020). However, the use of a homogeneous catalyst required additional separators to remove residual inorganic substances (Zullaikah et al. 2005).

Base Catalyst

The commonly used homogeneous catalyst for biodiesel synthesis is the alkaline catalyst that includes metal hydroxides, alkoxides, and organic amines. The acid catalysts like phosphoric acid, sulfuric acid, benzene sulfonic acid, and dihydrochloride acid are used for transesterification. The homogeneous alkaline catalysts like KOH, NaOH, and CH_3ONa are most common for biodiesel synthesis (Borges and Díaz 2012). NaOH and KOH are most commonly used with methanol due to higher solubility. They form sodium and potassium methoxide catalytic agents in transesterification mixture to promote conversion. The NaOH catalysts are only used at lower temperatures to avoid saponification and emulsification of the mixture (Kawashima et al. 2009). Moreover, KOH and CH_3ONa reported higher FAME yield than NaOH. Alkaline catalysts demonstrated nearly 4000 times high catalytic activity than an acid catalyst for transesterification. The problem associated with the alkaline catalyst is emulsification and saponification of methanol + fatty acid mixer at higher temperatures. A homogeneous base catalyst is a lucrative option as it can operate in moderate operational conditions and at a modest cost with exceptional catalytic activity. It is reported that sodium and potassium hydroxide are operating exceptionally well with biodiesel yield (94–99%) and methanol:oil ratio (6:1) even in smaller mass fraction (0.5–1 wt.%) at low temperature (45–80 °C) (Demirbas 2007). However, separation of product from catalyst requires an additional separation unit that enhances overall operational cost and makes the process impractical and unattractive. The base catalyst produces unwanted products (soap) with free fatty acid that not just decreases overall biodiesel yield but also spoils catalyst and makes glycerol separation troublesome. However, the two-step acid-catalyzed and base-catalyzed hybrid process can effectively solve the soap formation issue. The hybrid transesterification unit consists of acid-catalyzed transesterification followed by base-catalyzed transesterification. Researchers reported an overall ester yield increase by 10% in the hybrid transesterification unit (Wang et al. 2007).

5.4.2.2 Heterogeneous Catalyst

The heterogeneous catalyst consists of alkaline oxide or alkaline earth metal oxide supported over zeolite or hydrotalcite. Several heterogeneous catalysts like zinc ethanoate/SiO_2, zinc stearate/SiO_2, WO_3/ZrO_2-Al_2O_3, MoO3/ZrO2, TPA/ZrO_2, WO_3/ZrO_2, and MoO/SiO_2 are studied at a laboratory scale (Atadashi et al. 2013). Heterogeneous catalyst has an advantage like low cost, higher ester yield, larger surface area, high-purity glycerol, and low maintenance. However, cost and energy consumption are considerably higher than the homogeneous catalyst. Heterogeneous catalysts are preferred due to simpler and cheaper separation and lower operational costs (Hamza et al. 2020). The heterogeneous catalyst for transesterification is an environmentally benign approach as it requires a low-effluent water load (Sharma and Singh 2008). The heterogeneous catalysis approach is still under development phases as only one commercially known heterogeneous catalyst is CaO-loaded microcapsule developed. The co-extrusion process is used to encapsulate CaO in amphiphilic alginate. The conversion occurs in pores of microcapsule when fatty acid and methanol are passed through (Fumio et al. 2010). CaO is the most often used heterogeneous catalyst due to high activity, long catalytic life, ready availability, and moderate reaction conditions. It has been observed that a heterogeneous catalyst requires a higher transesterification temperature than a homogeneous catalyst. This increases the overall cost of operation in heterogeneous transesterification. The commercialization of heterogeneous catalysts is easy due to insensitivity to free fatty acids, easy separation, lower corrosion issue, and elimination of purification unit (Borges and Díaz 2012). Heterogeneous catalysts derived from natural resources (lemon/orange peel and eggshell) are low-cost alternatives of CaO catalyst (Tang et al. 2018; Goli and Sahu 2018). Heterogeneous catalysts effectively downsize plants, reduce basic wastewater, and reduce environmental impact. That is why heterogeneous transesterification could scale up to industrial scales with less environmental impact.

5.4.3 Enzymatic Catalyst Transesterification

The use of enzymes for transesterification has several environmental and operational advantages like mild reaction temperatures, higher selectivity, high oil/alcohol ratio, no side reactions, enzyme biodegradability, less enzyme recovery cost, high-quality glycerol, improved stability, and recyclability of enzymes. Despite several advantages, enzymatic transesterification faces issues like lower reaction rates, higher production cost, and catalyst inhibition (Wang et al. 2007). New inventions like recombinant DNA technology open doors for new, inexpensive, and highly efficient enzymatic catalysts that will reduce the overall cost of operation (Wang et al. 2007; Akoh et al. 2007). Various enzymes like *Rhizopus oryzae* lipase (ROL), triacylglycerol lipase (TGL), Pseudomonas cepacia lipase (PCL), and esterases (carboxyl ester hydrolase—CEH) are used for transesterification. TGL converts long-chain fatty acids to glycerol and fatty acid (Al-Zuhair 2005), whereas esterases convert short-chain fatty acids to transesterification products (Bornscheuer 2002). An enzymatic catalyst like Pseudomonas, Candida, and Rhizopus derived from the natural sources like fungi, yeast, and bacteria is mostly used. Alcohol choice, oil/alcohol ratio, reaction temperature, and immobilized/free enzyme ratio are factors that affect biodiesel production apart from the origin and type of enzyme (Wang et al. 2007). Enzyme immobilization is a technique in which enzymes are immobilized or attached over the solid surface using physical and chemical methods. Various techniques like cross-linked enzyme aggregates (CLEAs) and protein-coated microcrystals (PCMCs) are used for enzyme immobilization. Covalent attachment, entrapment, adsorption, and cross-linking are the primary approaches used for stable immobilization of enzymes over solid surfaces (Bornscheuer et al. 2005). The higher biodiesel yield was reported with immobilized lipase (*Thermomyces lanuginosus*, *Pseudomonas cepacia*, and *Candida antarctica*). The immobilized lipase system is highly effective to lower the cost of enzyme recovery and purification units and produce high-purity glycerol. The batch reactor and continuous plug flow reactors are used at an industrial scale with free or immobilized enzyme systems. However, continuous plug flow reactors for biodiesel production have advantages over batch reactors in different aspects like high product quality, lower labor cost, stable operational condition, easy control, and automation (Wang et al. 2007; Christopher et al. 2014; Hama et al. 2018). However low conversion yield and large residence time are important issues that still need to be solved.

5.4.4 Noncatalytic Transesterification

The higher cost of separation and recovery in catalytic and enzymatic transesterification is leading the way towards the tertiary approach. The noncatalytic transesterification techniques like supercritical alcohol and co-solvent (BIOX) are noncatalytic processes that have gained much attention these days (Mayvan et al. 2012). The low solubility of methanol in oils is the major roadblock in noncatalytic transesterification that can be solved by using a co-solvent that is soluble in both methanol and oil. Tetrahydrofuran is the best co-solvent due to close boiling near methanol that enables low-temperature transesterification. The complete separation of tetrahydrofuran is highly necessary from the product stream due to its hazardous and toxic nature (Mayvan et al. 2012). However, single-step recovery of both excess alcohol and tetrahydrofuran can be possible (Demirbas 2008).

Supercritical alcohol process is carried out at higher pressure and temperature conditions. Alcohol dielectric constant decreases as supercritical conditions prevail that consequently increases the solubility of TGs in alcohols. The increase in solubility of TGs in alcohol further enhances the transesterification process. The supercritical transesterification process is insensitive to the presence of free fatty acid and has advantages like fast reaction rates, continuous operation, and no water requirement for washing and recovery (Tan et al. 2009). However, higher pressure and higher methanol:oil ratio increase overall operation cost in supercritical transesterification (Mittelbach and Remschmidt 2004; Farobie and Matsumura 2017). The comparative analysis of different catalytic systems used for biodiesel production is given in Table 5.2.

5.5 Factors Affecting Biodiesel Synthesis

Various factors like residence time, pore size, capillary diameter, temperature, and mixing mechanism affect biodiesel conversion. The yield of FAME increases with the prolongation of holding or residence time. The reported residence times are 10–100 times shorter in the microreactor system than in conventional batch reactors. The efficacy of microreactors increases with the miniaturization of channel

Table 5.2 Comparative analysis of different transesterification techniques with their advantage and disadvantage

Feedstock	Catalyst type	Advantage	Disadvantage
Virgin vegetable oils (low free fatty acid (FFA) content)	*Alkali catalyst*		
	Homogeneous alkali catalyst (yield 96–98%) Example: NaOH, KOH, MoO_3/ZrO_2	1. Higher reaction rate 2. Low cost and readily available 3. Mild reaction conditions	1. High production cost 2. Soap formation
	Heterogeneous alkali catalyst (yield <90%) Examples: ZnO, CaO, Na_2CO_3, MgO, $CaCO_3$	1. Higher reaction rates 2. Mild reaction conditions 3. Easy separation 4. High-purity glycerol	1. Leaching of catalyst 2. Required rectified feedstock
Low-grade feedstocks like waste cooking oils that contain higher FFA	*Acid catalyst*		
	Homogeneous acid catalyst (yield 96–99%) Examples: Sulfuric acid, hydrochloric acid, sulfonic acid, ferric sulfate	1. No saponification or emulsification 2. Insensitive for water and FFA content 3. Suitable for both esterification and transesterification process	1. Slow reaction rate 2. Catalyst separation is difficult 3. Corrosive in nature
	Heterogeneous acid catalyst (yield <90%) Examples: Activated carbon-supported SO3H SBA-15, HPA	1. No saponification or emulsification 2. Insensitive for water and FFA content 3. Easy separation and high-purity glycerol	1. Slow reaction rates 2. Higher residence time required 3. Complicated and expensive
Compatible with refined vegetable oil or waste low-grade oils	Enzyme-based catalyst		
	Lipase-based catalyst (yield 99%) Examples: Lipozyme TL, *Thermomyces lanuginosus*, *Candida antarctica*	1. Mild reaction conditions 2. Simple purification 3. Insensitive with both water and FFA content 4. Environment friendly	1. Slow reaction rates 2. Higher residence time
Feedstock depends on the nature of catalyst	*Carbon-based catalyst*		
	Yield <90% 1. Functionalized carbon (a) Acid	1. Reusable 2. Low-cost synthesis 3. Environment	1. Higher alcohol/oil ratio 2. Catalyst leaching

(continued)

Table 5.2 (continued)

Feedstock	Catalyst type	Advantage	Disadvantage
	functionalized (b) Base functionalized 2. Supported carbon	friendly 3. Higher thermal stability	3. Slower reaction kinetics
Suitable for all feedstocks	*Ionic liquid systems*		
	Yield <90%	1. Easier product separation 2. Can act as both acidic and basic catalyst 3. Can act as both solvent and catalyst	1. Low glycerol purity as it is difficult to separate from product stream 2. Highly expensive 3. Slow reaction kinetics

diameters. The miniaturization is attributed to short diffusion distances, higher surface-to-volume ratios, fast and efficient heat dissipation, and mass transfer. The lowering microchannel size enhances slug flow behavior and efficiently decreases slug length (Biswas et al. 2015a, b; Kumar et al. 2018). Similarly, the use of bends and different geometries in microchannels also demonstrated positive effect on phase mixing that eventually increases mass transfer (Biswas et al. 2015a, b; Kumar et al. 2019). The lowering in slug length eventually increases effective mixing and interfacial surface area that leads to an increase in FAME yields. The miniaturization of the microreactor comes with the issue of a higher pressure drop. The increase in FAME yield was reported as we increased the temperature from 30 ° C to 60 ° C. Further, an increase in temperature over 60 ° C resulted in a decrease in FAME yield due to flow transition of methanol:oil system from slug flow to bubble flow. The decrease in FAME yield over methanol boiling point is due to flow transition and emulsification of alkaline catalyst. However, microstructured reactors showed an increase in FAME yield above 60 °C due to the formation of biphasic gas-liquid slug annular flow of cottonseed oil and methanol (Canter 2006). The mixing mechanism has a strong influence on FAME yield as interfacial forces between phases generate a tubular flow pattern commonly known as slug flow. The axial mass transport along the flow direction and radial mass transport over the capillary wall contribute to the conversion of fatty acids. The various mixing elements like “T”- and “Y”-type micromixers are used to further enhance the mixing of phases in microtube reactors (Fig. 5.7).

The “Y”- and “T”-shaped micromixers are used to introduce biphasic fluid into the microreactor. The parallel flow is preferred in the microchannel but difficult to obtain in the microsized system. Instead of parallel flow the slug flow pattern is mostly obtained as given in Fig. 5.6. Microstructured reactors are equipped with RIMM and SIMM-V2 micromixers, which enhances mixing (Canter 2006; Baroutian et al. 2010). Similarly, the oscillating flow reactor is equipped with an equally spaced orifice baffle that enhances radial mixing through the oscillating flow of liquids.

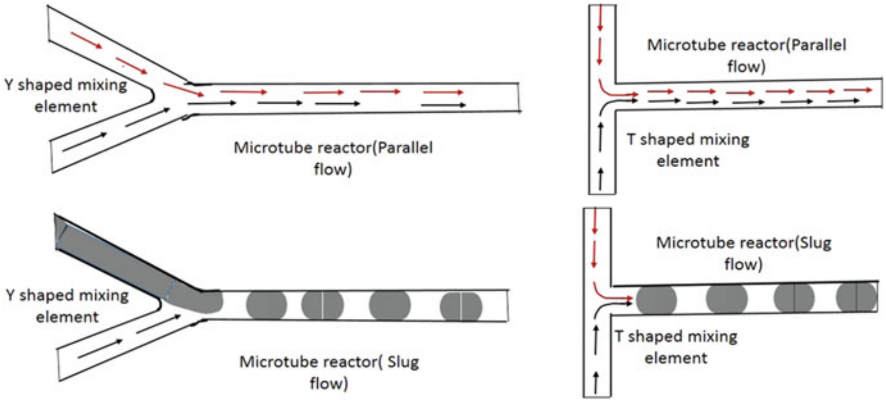


Fig. 5.7 Microtube reactors with Y-shaped and T-shaped mixing elements

5.6 Potential Hurdles

The hurdles in biodiesel production not only include the technological challenges of producing good-quality biodiesel which can be compared to gasoline in terms of both sustainability and economics. The other sprints include important social, economic, and environmental challenges, various government policies on the use of biofuels, etc. The constraints can also be due to economic and market restrictions, health risks, nutrient cycle disruption, resource limitations, climate forcing, water reserves and its demand, and land availability. The poor flow properties at low temperature and poor oxidation stability are due to the presence of highly polyunsaturated fatty acids in biodiesel feedstocks. The low stability and high oxidative nature of biodiesel may create issues in biodiesel storage and cold-weather applications. Moreover, the available technologies faced the issue of pressure drop and decreasing flow rates while keeping other conditions constant significantly decrease FAME yield. Oscillatory flow reactors are currently operated with a polymer-supported catalyst or homogeneous solid catalyst. Further enhancement in mass transfer and conversion efficiency with the heterogeneous catalyst is highly difficult and still not done. Similarly, membrane systems are limited by porosity, pore size, and material that is to be filtered. The higher cost of membrane-based reactors is also a potential hurdle for the future development of this technology at larger scales.

5.7 Future of Biodiesels

The major aim of biodiesel production is to foster rural development, reducing greenhouse gas emission and reducing fossil fuel dependency by disposing of used cooking oil and fatty acids. Currently, biodiesel sold in India contains not only FAME but also several additives that enhance flow properties and oxidation stability of biodiesel. The use of waste plastics like polyethylene and polypropylene with solvents like methanol has shown positive effect on the oxidation stability of biodiesel at laboratory scale (Varatharajana and Pushparani 2018; Arjanggi and Kansedo 2020). Moreover, partial hydrogenation of biodiesel also demonstrated improvement in cold flow properties and oxidation stability of biodiesel. Unsaturated fatty acids have low oxidation stability but improved cold flow properties. Conversely, saturated fatty acids improve cetane number and oxidation stability but worsen cold flow properties. Low heating value and high viscosity of saturated fatty acids make cold-weather application of biodiesel difficult. Partial hydrogenation could easily overcome challenges of cold flow properties and oxidation stability in future (Mensah et al. 2019). Low conversion of fatty acids in PFR and batch reactor could potentially be overcome by the process intensification techniques like microwave irradiation and ultrasound irradiation coupled with PFR (Ding et al. 2018; Xuan et al. 2019; Athar et al. 2020). The waste minimization is a primary concern with biodiesel synthesis. Transesterification process requires water for cleaning and recovery of catalyst. Pure glycerol produced by heterogeneous catalytic route could potentially be converted into biohydrogen by using biophotolysis, photofermentation, and dark fermentation (Haron et al. 2018). The use of new and innovative process intensification techniques (coiled flow inverters (CFI), nanoparticle, microchemical technologies) aiding transesterification could be effective for higher FAME yield in future.

Membrane reactors can combine with both reaction and separation systems simultaneously in the future. The future membranes can effectively block unreacted triglycerides to pass through. Similarly, in modern oscillatory flow reactor systems mixing is no longer dependent on flow rates. Oscillating currents in OFR contribute to radial mixing that allows long batch processes to convert into continuous flow processes. Similarly, microtubes facilitate efficient heat dissipation, higher mass transfer, and conversion yield that allow this technology to operate at a higher temperature for further enhancement in FAME yield. These microtube reactors could potentially operate with a heterogeneous catalyst (nanoparticles) for further enhancement in FAME yield with lowering of residence time.

5.8 Conclusions

Biodiesel is one of the best environment-friendly renewable sources of energy and has attracted several researchers in this field. Although biodiesel is a low-cost substitute for fossil fuel, its commercialization at a larger scale is quite difficult. The use of microreactor technology for higher FAME yield and reduction of residence time make this process more competitive. The microreactor systems not only increase overall conversion but also lower operationalization temperature. The higher FAME yield is obtained in lower residence time at lower temperature and pressure conditions that make microreactor systems more competitive for potential industrialization prospective. However, issues like higher pressure drop and considerable clogging make the continuous operation of microreactor systems more difficult. The use of alkaline and acid homogeneous catalyst could effectively further reduce operational conditions but this can raise product separation and operational cost. The use of heterogeneous catalyst lowers product separation cost but heterogeneous catalytic systems are under development stage. Seeing the present growing demand for biofuels the scale-up production through microchannels is still difficult. However by using the numbering-up approach or parallel microchannels the production rates could scale up according to demand. However, the production of high-purity biodiesel from feedstocks that contain high free fatty acids is still challenging and needs considerable attention in the future. More design and material approaches still need to be developed for effective product separation. Further developments in heterogeneous catalysts to further lower the cost of production are needed today. Microreactor systems with continuous production and separation units by using high product selective material and heterogeneous catalyst systems could meet future demands of biofuels.

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Chapter 6
Solid-State Fermentation: An Alternative Approach to Produce Fungal Lipids as Biodiesel Feedstock

Mahesh Balwant Khot

Abstract The high lipid-accumulating fungi have a unique physiology that makes them promising oil resource of fatty acid-based biofuels and oleochemicals with diverse applications as biodiesel, bio lubricants and in food, feed, cosmetic industries. Fungal lipids specifically intended for biodiesel synthesis constitute a bulk product (high volume, low value) and need to be produced at low cost, unlike the functional lipids such as n-3/n-6 polyunsaturated fatty acids. Several factors (including scalability and minimized costs) give solid-state fermentation a biotechnological advantage over submerged processes. Fungal lipids produced in solid-state cultures do hold a great potential for biodiesel production at an industrial scale. This chapter briefly overviews the agro-industrial residues (renewable carbon sources) and solid-state fermentation as two effective ways to increase the commercialization potential of single-cell oil-derived biodiesel production.

Keywords Biofuels · Biodiesel · Fungal lipids · Solid-state fermentation · Agro-industrial residues

6.1 Biomass and Biodiesel

Our world today is overly reliant on fossil fuel-based technologies. Extensive coal and petroleum utilization has forced reserves of these nonrenewable fuels to near depletion, resulting in such fuel sources becoming increasingly limited and challenging and extremely expensive to acquire. Therefore, there is an acute need for alternative fuels and sustainable methodologies for their synthesis. Ideally, new fuel production processes should be cost effective and environment friendly yet allow high yields of quality end products and desirably co-synthesis of other valuable

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materials. Biofuels and bio-based products are essential for maintaining a sustainable bioeconomy, protecting the environment, and enhancing citizens' health and safety.

The concept of sustainable fuels and green chemistry will continue to be important as research shifts away from heavy reliance on fossil fuels. Among various renewable energy sources being investigated, biomass, and lignocellulosic biomass, in particular, has been observed to hold good promise. Composed of the carbohydrate polymers—cellulose and hemicellulose—and an aromatic polymer—lignin—lignocellulose is recognized as the most abundant biopolymer on earth. Being the major structural component of woody and nonwoody plants, lignocellulosic materials are classified, based on origin, into virgin biomass, energy crops, and waste biomass. Regardless of the form, such as straw, stover, bagasse, chaff, paper, cardboard, garden waste, domestic waste, and waste wood from construction, such waste biomass lignocellulosic materials are perennially available abundantly for free or, at most, for low prices, making them a vital resource capable of being used as a feedstock for the production of biofuels and other commercially valuable biomaterials such as enzymes and platform chemicals. It would be, therefore, advantageous to have adaptive methodologies for the renewable biomass materials co-generating fuels and other valuable products employing biorefinery strategy (Kamat et al. 2013; Khot 2015).

The generation of bio-based products derived from lignocellulosic materials via microbial fermentation has been studied extensively. For the production of these derivatives from lignocellulosic materials to be economically feasible, efficient conversion of all the main carbohydrate constituents of the feed/raw material to biofuels is necessary. However, the complexity of the lignocellulosic material and its resistance to biodegradation have been the greatest challenge for its utilization via microbiological methodologies to produce biofuels and valuable chemicals.

Conventional studies on the conversion of lignocellulosic biomass to biofuel are commonly observed to involve two key steps—first of which is thermochemical pretreatment to remove recalcitrant lignin facilitating the release of fermentable sugars from the hemicellulosic fraction of biomass and second, the subsequent utilization by desirable microbial strain to produce the fuel of interest. Here, the thermochemical pretreatments seen in practice are mostly inefficient and costly, involve harmful chemicals, and release growth inhibitory compounds, making an expensive detoxification intermediary process indispensable.

Furthermore, the biochemical flux in these methodologies favors a single product, limiting the overall process's economic potential. Therefore, there is a critical need to concert the appropriate raw material and suitable biocatalyst (microorganism) and provide the right fermentation conditions (solid-state fermentation) for the biochemical flux to proceed towards valuable fuel products, thereby enhancing the process economics. Such an approach has not been explored in biofuel research, particularly biodiesel (Ravi Kumar et al. 2015).

6.2 Single Cell Oil of Fungi as Biodiesel Feedstock

Lipids accumulated by oleaginous microbes can serve as a renewable intermediate "building block" to produce fatty acid-based fuels such as biodiesel, bio-jet, and oleochemicals. Microorganisms that can accumulate 20% or more of their biomass as lipid (single-cell oil, SCO) have been known from the early 1940s and have been termed "oleaginous." The lower limit has been taken as 20% for the amount of lipid to be accumulated within a cell to be designated as oleaginous for practical purposes of lipid production and extraction. The oleaginous property is limited to a relatively small number of microorganisms and is seen mostly in eukaryotic species. Oleaginous microorganisms are distributed among specific genera and species of microalgae, fungi (yeasts and molds), as well as bacteria (Ratledge 1989).

Triacylglycerols (triglycerides) are the major component of the SCOs, followed by free fatty acids, monoacylglycerols, diacylglycerols, steryl esters, sterols, and polar lipids (glycol-, sphingo-, phospholipids). Lipid accumulation is not observed in oleaginous microbes under balanced nutrient conditions. When there is a limiting substrate required for cell proliferation, available carbon is channelized into lipid synthesis. These limiting nutrients include N, P, K, Mg, S, and Fe. Most of the time, N (nitrogen) is the limiting factor for lipid accumulation. In the absence of new cell production, the existing cells continue to assimilate the carbon available to them and then get converted into triacylglycerol within the cell as discrete oil droplets by specific lipid biosynthetic pathway depending on the carbon source (Ratledge 2004).

Among different oleaginous microbes, the oil-storing fungi are ideal candidates for direct conversion of lignocellulosic sugars into lipid because of their inherent ability to utilize a wide range of renewable carbon sources employing an inbuilt battery of hydrolytic enzymes. Fungi represent the second largest and diverse group among living organisms, with the species number estimated to be 1.5 million on earth (Hawksworth 1991). Fungi are chemoorganotrophs and display simple nutritional requirements compared to bacteria. Considerable diversity is present in their morphological properties and life cycles. Besides yeasts, filamentous fungi (molds) are morphologically distinct types of fungal organisms. Molds show apical growth employing replication of a "growth unit" composed of the apex of the hypha and a short length of supporting hypha. The species of oleaginous molds are more significant in number than yeasts. However, the lipid-accumulating property is limited to the individual fungal strain that may not be present in the whole species or even genus. In oleaginous molds, the major lipid component of the accumulated total lipid has been found to be triglycerides (85–90%). Among different growth conditions, temperature and carbon source are known to affect the entire cellular lipid content.

Fungi possess several advantages for SCO production over their plant and algal counterparts, namely

- Easier to grow in conventional bioreactors with no need for specialized cultivation setup such as photo-bioreactor for microalgae
- Unaffected by light, space, or climatic variations
- Easier to scale up

- Diverse lipid profiles
- Ability to utilize renewable carbon from different sources such as agro-industrial residues by lignocellulolytic enzymes
- Pelleted growth facilitating easier and cost-effective downstream processing by simple filtration
- Can accumulate oils by solid-state cultivation on insoluble substrates

Oleaginous fungi, with their battery of lignocellulolytic enzymes, could be ideal candidates in biodiesel production from cheap renewable carbon sources and will be an essential factor in the overall economics of biomass to fuel strategy (Khot et al. 2012).

6.3 SCO Production from Renewable Carbon

Among different renewable sources available for microbial oil production, lignocellulosic biomass holds good promise. It constitutes the most abundant and local open source of carbohydrates in agro-industrial residues with low transportation costs. As there are still technical challenges in the production of biodiesel using lignocellulose, the process has been restricted to the laboratory stage. The study reported to date involves two key steps to process lignocellulosic substrate for growth medium preparation: thermochemical pretreatment of the substrate resulting in hydrolysates and hydrolysis mediated by enzyme cocktails. The whole process also involves several solid-liquid separation steps, washing, and polishing to remove toxic substances and inhibitors released by pretreatment and the difficulties related to simultaneous fermentation of both pentoses and hexoses. Thus, this approach may suffer from drawbacks such as increased capital and processing costs and wastewater disposal due to hazardous chemicals. There remains scope for simplifying the process configuration by coupling individual unit operations with environmental and economic benefits by employing novel approaches such as consolidated bioprocessing (Olson et al. 2012a, 2012b; Wyman 2007). SCOs of oleaginous heterotrophic microorganisms, especially yeasts and filamentous fungi, have been studied for a long time as a source of functional lipids containing polyunsaturated fatty acids of food and medical interest (Certik and Shimizu 1999; Jang et al. 2000; Fakas et al. 2009). Lipids for biodiesel production constitute a bulk product (high volume, low value) compared to those produced as valuable sources of n-3/n-6 polyunsaturated fatty acids. Therefore, SCO intended for biodiesel must be generated at a low cost. The possibility and potential of commercialization of SCO production based on lignocellulosic biomass as substrates for oleaginous microorganisms have been evaluated (Huang et al. 2013). The fermentation substrate is the key factor involved in industrial scale lipid production. Therefore, the production of SCO intended for biodiesel should be cost effective. The production cost of microbial lipids can be reduced by

- Use of cheap renewable substrates
- Solid-state culture over submerged fermentation
- Lowering of energy input during the production process

Although the SCO production process has not been studied extensively concerning energy input, current research efforts are on developing the bioprocesses from oleaginous microorganisms grown on various renewable, low-cost substrates via different fermentation strategies (Koutinas and Papanikolaou 2011; Koutinas et al. 2014). A large variety of low-cost materials has been used in formulating fermentation media for SCO production to date, which can be grouped into hydrophilic and hydrophobic. The hydrophilic substrates studied and reported in the literature for SCO production include industrial derived sugars (from a sugar refinery plant), molasses, materials from the food processing industry such as starch hydrolysates, tomato waste hydrolysate, hydrolysates of shrimp processing waste, wastewaters (sewage sludge, olive oil mill wastewaters, monosodium glutamate wastewater), whey, and industrial glycerol (Papanikolaou and Aggelis 2011; Maina et al. 2017). A limited number of reports are available on the use of hydrophobic substrates for SCO production and include industrial fats, waste cooking oil, and waste motor oil (Bialy et al. 2011; Katre et al. 2012).

6.4 Solid-State Fermentation for SCO Production from Fungi

Solid-state fermentation has been defined as the bioprocess carried out in the absence or near absence of free water on definite insoluble matrices possessing enough moisture to support the microorganism's growth and metabolic activity. The solid matrix could serve as either the source of carbon (or other nutrients) or the inert support for the microbial growth (with impregnated growth solution). SSF has been again at the forefront of industrial bioprocess developments because of lower energy requirements associated with higher product yields and less wastewater generation. Lizardi-Jiménez and Hernández-Martínez (2017) described the diversity of applications of solid-state fermentation to valorize biomass regarding alternative energy and environmental purposes. Besides, it offers potential ecological benefits by utilizing solid agro-industrial wastes (resides) as the substrate (carbon and energy source). Solid-state fermentation has always been an alternative to submerged cultures for industrial bioprocess development. Several vital factors give SSF an extra advantage; for example, the cultivation costs are much lower and produce less wastewater. Besides, the transport costs are reduced as it can be operated at the source of biomass (farms and agro-industries). Several reviews available in the literature (Hölker et al. 2004; Krishna 2005; Thomas et al. 2013) have cited biotechnological advantages offered by SSF over conventional submerged cultivation, namely

- Simulated natural habitat of microorganisms
- Reduced energy requirement
- Fewer water demands
- Low wastewater generation
- High product yield
- High volumetric productivity
- Low capital cost
- Easy to use at the source of biomass
- Reduced transportation cost
- Lower production costs
- Environment friendly due to the use of agro-industrial wastes as substrates

The filamentous fungi (molds) are the most preferred microorganisms for SSF because of their hyphal growth pattern assisting them in colonization and penetration of the solid substrate resulting in better utilization of nutrients. Molds are also quite well adapted to low water activity present in SSF systems. This facilitates the use of substrate-bound water, allowing their growth in the absence of free water (Cheirsilp and Kitcha 2015). Asadi et al. (2015) highlighted the potential benefits of SSF for the production of SCO by fungi belonging to the genus *Mortierella*. This article discusses the effect of culture conditions and fermentation substrate on the yield of SCO and fatty acid profile under SSF conditions. The reports are available on SCO production from filamentous fungi employed in SSF of both pretreated and untreated substrates such as pear pomace, wheat straw, wheat bran, rice straw, palm by-products (palm empty fruit bunches, palm pressed fiber, palm kernel cake), soybean hulls, and sweet sorghum (Table 6.1). Studies included the direct conversion of lignocellulosic biomass into SCO; for example, endophytic fungi with cellulase activity have been isolated from oleaginous plants by Peng and Chen (2007). The process yielded 19–42 mg SCO/g of the substrate, and yield was further improved up to 74 mg/g dry substrate composed of steam-exploded wheat straw mixed with wheat bran (Peng and Chen 2008). Economou et al. (2010) reported the semisolid-state fermentation process wherein at 92% water content, lab-scale production of SCO was achieved with a yield of 11 g/100 g dry weight of sweet sorghum stems used as substrate. The produced oil of *Mortierella isabellina* was shown to be a suitable feedstock for biodiesel production. The direct conversion of wheat and rice straws into fungal lipids by cellulolytic fungi has also been attempted (Lin et al. 2010; Dey et al. 2011). Soybean hulls without any pretreatment have also been used for SSF by an oleaginous fungus *Mortierella isabellina* to produce lipid up to 47.9 mg/g of the substrate in a much more extended period of 4 weeks (Zhang and Hu 2012).

Aspergillus tubingensis produced lipids up to 88.5 mg/g coupled to cellulase and xylanase release when grown on alkali-pretreated palm empty fruit bunch along with palm kernel cake employing SSF culture conditions (Kitcha and Cheirsilp 2014). Repeated batch fermentation with these lignocellulosic wastes of palm oil mill improved lipid yield when cellulolytic enzymes were extracted from the first SSF batch and reused in the next batch releasing higher enzyme titers (Cheirsilp and

Table 6.1 Solid-state fermentation of renewable biomass sources to produce fungal oils

Fungal sp.	Substrate	Lipid yield	Pretreatment	Application	Reference
Aspergillus sp.	Sweet potato + root meal	3.17–8.71%	–	PUFAs	Abu et al. (2000)
Thamnidium elegans	Spelt wheat flakes + spent malt grain	4.9%	–	PUFAs	Certík et al. (2006)
Microsphaeropsis, Cephalosporium, Sclerocystis, Nigrospora	Wheat straw + wheat bran	19–42 mg/g	Steam	–	Peng and Chen (2007)
Mortierella isabellina	Pear pomace	12%	–	PUFAs	Fakas et al. (2009)
Aspergillus oryzae A-4	Wheat straw + wheat bran	28.5–62.87 mg/g	Steam	Biodiesel	Hui et al. (2010)
Alternaria sp. *Colletotrichum* sp.	Rice straw + wheat bran	60.3–68.2 mg/g	–	Biodiesel	Dey et al. (2011)
Aspergillus tubingensis TSIP9	Palm empty fruit bunch	121.4 mg/g	–	Biodiesel	Intasit et al. (2020b)
Mortierella alpina	Cornmeal mixed with an animal fat by-product	32.1 mg of arachidonic acid/g	–	PUFA	Slaný et al. (2020)
Mortierella wolfii AH12	Sugarcane bagasse	27 mg/g	–	Biodiesel	Hashem et al. (2021)

Kitcha 2015). The fungal strain originally isolated from palm wastes was employed to pretreat biomass wastes and simultaneously produce SCO through non-sterile SSF by Intasit et al. (2020a). Continuous biomass pretreatment and production of fungal oil were achieved in the repeated batch fermentation with 90% substrate replacement. The fungi could grow and produce oils with good biodiesel fuel properties. Such studies demonstrate the low cost and environmentally friendly process to generate renewable energy through a zero-waste strategy. In general, SSF with oleaginous fungal strains attempted different pretreatment strategies, e.g., thermochemical or externally added enzymes or additional substrates. Different co-substrates or supplements have also been used while intended application areas of fungal lipids range from PUFAs to biodiesel synthesis (Table 6.1).

The process economics of both submerged aerobic fermentation and solid-state fermentation have been discussed in biodiesel feedstock production. In a study by Meeuwse et al. (2013), the energy input is estimated for generating biodiesel feedstock on a large scale using solid organic waste material and oleaginous yeast and filamentous fungi. Both submerged and solid-state fermentation processes were assessed, each with its own merits and demerits. Sugar beet pulp was used as a model substrate, while both yeast and filamentous fungus were included in the evaluation. SSF culture system design considered an aerated packed-bed reactor with evaporative cooling and without mixing. Lipid yield was predicted using kinetic models, which were significantly lower in the SSF process than in submerged fermentation partly due to the low specific lipid production rate. Energy yield is estimated considering the biodiesel produced and the energy consumed for all steps, such as pretreatment, fermentation, and downstream processing, expressed as the net energy ratio (NER). The SSF generated low energy on account of the low lipid yield. Even energy use is every day, and it gives a high NER value partly due to the low energy costs of oxygen transfer. A lower production cost is estimated for SSF (50–100 €/GJ) compared to submerged process (200–1000 €/GJ biodiesel). Other factors give SSF an advantage over submerged processes such as less wastewater and feasibility to use at the biomass production sites of farms and agro-processing units, leading to reduced transport costs. With the improved yield and productivity, the fungal SCOs produced by SSF hold the potential to be promising candidates for sources of biodiesel shortly.

6.5 Production of Lipases by SSF for Biodiesel Application

The enzymes responsible for the hydrolysis of triacylglycerols from fats and oils in a lipid-water interface are classified as lipases (triacylglycerol acyl hydrolases, E.C. 3.1.1.3). The end products of lipase catalysis include free fatty acids, mono- and diglycerides, and glycerol. Enzymes, including lipases, have been produced via SSF with various agro-industrial residues as substrates resulting in cost-effective bioprocesses. Lipases are widely applied in food, pharma, cosmetics, nutraceuticals, detergents, and biodiesel (Gupta et al. 2015; Borrelli and Trono 2015). Lipase also catalyzes the transesterification of fats and oils with short-chain alcohols yielding fatty acid alkyl esters; (biodiesel). Lipase-catalyzed biodiesel production is known as a nontoxic, less energy-intensive, eco-friendly process with mild operating conditions (Nelson et al. 1996; Almeida et al. 2021). Despite the advantages over chemical biocatalysts, the high cost of commercially available lipases limits industrial scale processing of enzymatic transesterification for biodiesel production.

SSF has emerged as a promising method for cost-effective lipase application in biodiesel synthesis by offering the direct use of fermented solid as biocatalyst. For example, lipase produced in SSF by *Burkholderia cepacia* was utilized in lyophilized fermented solids for esterification and transesterification reactions in the n-heptane system (Fernandes et al. 2007). Salum et al. (2010) studied the biodiesel synthesis utilizing the dried fermented solid of *B. cepacia* LTEB11 in a fixed-bed reactor. A novel tri-substrate SSF was employed (Edwinoliver et al. 2010) for improving the lipase production in tallow hydrolysis by *Aspergillus niger* MTCC 2594. Liu et al. (2013) evaluated lipase production by *Burkholderia cenocepacia* in SSF of sugarcane bagasse and sunflower seed cake. The whole fermented solid was

Table 6.2 Synthesizing fatty acid alkyl esters using lipases produced by SSF

Substrate/s	Microorganism	Reaction catalyzed	Yield	Reference
Sugarcane bagasse, sunflower seed meal	*Rhizopus microsporus*	Ethanolysis of corn oil	91% Ester yield in 48 h, at 44 °C	Zago et al. (2014)
Sugarcane bagasse and sunflower seed meal	*Burkholderia cepacia*	Esterification of fatty acids obtained from soybean soap stock acid oil	92% Ester conversion in 31 h, at 50 °C In packed-bed reactor	Soares et al. (2013)
Sugarcane bagasse and sunflower seed meal with olive oil as inducer	*Burkholderia cenocepacia*	Ethanolysis of soybean oil	86% ethyl ester yield in 96 h at 45 °C	Liu et al. (2013)
Babassu cake	*Rhizomucor miehei*	Esterification of the acid oil from macauba (*Acrocomia aculeata*) pulp	91% of conversion after 8 h in a solvent-free system at 40 °C	Aguieiras et al. (2018)
Corn bran with corn oil as the inducer	*Burkholderia cepacia*	Esterification of oleic acid and transesterification of corn oil	94% Ester yield in 18 h at 37 °C	Fernandes et al. (2007)

used for biodiesel production by catalyzing the ethanolysis of soybean oil in tert-butanol. This study also demonstrated the potential of SSF in producing lipase with low cost for enzyme-catalyzed transesterification reactions.

SSF of sugarcane bagasse and used vegetal oil was carried out by *Rhizomucor miehei* for the lipase preparation by Ojeda-Hernández et al. (2018). The dried fermented solid was directly applied as a heterogeneous biocatalyst for the ethanolysis of different low-cost used oils, including those with high free fatty acid content.

The process of applying lipase in dried fermented solid as biocatalyst for biodiesel is feasible and economical. SSF facilitates the use of relatively cheap and readily available substrates with minimal water requirement. Fermented solid serve as enzyme support and integrates the steps of lipase extraction, purification, and immobilization. The whole fermented solid from SSF requires only drying before its direct addition to the reaction media of biodiesel synthesis. Such a mode of application allows the lipases to be immobilized naturally and bypasses the expensive downstream processing steps of extraction and immobilization before its use for transesterification (Fernandes et al. 2007). The studies are limited on lipase applications in the form of fermented solids, which catalyze the esterification/transesterification and are given in Table 6.2.

Reports are also available on SSF of fungi to obtain crude enzyme extracts with lipase activity. In a study by Rigo et al. (2010), filamentous fungi, bacteria, and yeasts were isolated from different sources and screened by SSF of soybean meal. The substrate for SSF was obtained as the residue of soybean oil extraction after cold press and solvent extraction. The isolates were investigated for lipase activity in the fatty acid alkyl ester synthesis. Crude enzyme extracts from the fermented solids were frozen (−80 °C) and then lyophilized. A range of alcohols such as methanol, ethanol, n-propanol, and butanol and fatty acids such as oleic and lauric acids were used to evaluate the enzyme specificity. Esterification activities up to 81 U/g of substrate were obtained by fungal isolates in lauric acid. Silva et al. (2011) obtained lipase by SSF of babassu and castor bean cake from *Penicillium brevicompactum*. Crude enzyme extract from fermented solids was concentrated with ammonium sulfate precipitation followed by immobilization with sodium alginate and activated coal. Esterification activity increased to 207.4 U/g using babassu cake as SSF substrate after concentrating the lipase compared to the non-concentrated crude extract. Different alcohols (methanol, ethanol, n-propanol, n-butanol) and fatty acids (lauric, oleic, butyric, 72 h) were assessed to study the specificity of concentrated freeze-dried fungal lipase. The concentrated lipase preferred long-chain alcohols and fatty acids with a chain length similar to that present in SSF substrate.

The crude enzyme preparation offers low cost but suffers from poor stability. Dutra et al. (2008) investigated the profile of lipase production by *Aspergillus niger* in SSF of wheat bran enriched with ammonium sulfate. Vegetable oils, namely castor, soybean, olive, corn, and palm oils, were evaluated as lipase inducers, among which castor oil gave the optimum enzyme activity. Digital image process technique was demonstrated with the potential to monitor fungal growth in SSF and to further correlate biomass production with lipase activity. The crude lipase extract was freeze-dried and later immobilized by physical adsorption on hydrophobic support. The immobilized lipase was used to estimate the esterification activity and catalyze the biodiesel synthesis from soybean oil in the presence of ethanol. The transesterification resulted in poor biodiesel yield with immobilized fungal lipase.

Whole cells have also been used to assess their lipase activity produced in SSF. On account of enhanced stability and feasibility in the application, entire cells are preferred over free purified enzymes to eliminate the extraction and purification steps. Sun and Xu (2008) optimized the production of whole-cell lipase from *Rhizopus chinensis* in a combined substrate of wheat flour and wheat bran. Olive oil was used as a lipase inducer. In downstream processing of intracellular enzyme, the cell disruption was performed by homogenization in a blender and then in a French pressure cell press at 8000–12,000 psi. The cell debris was recovered to obtain the crude lipase extract after centrifugation and treating with triton X-100. Purification of the functional lipase included ammonium fractionation, hydrophobic interaction, and gel filtration. Enzyme validation was carried out by catalyzing the synthesis of ethyl octanoate and ethyl caprate. This study reported synthetic lipase activity of 24,447 U/kg of the substrate and, thus, established SSF as the efficient and economical process in generating lipases for enzyme-catalyzed biodiesel production.

6.6 Downstream Processing for Lipid Recovery from Fermented Solids in SSF

When applied in SSF for lipid production, the oleaginous fungi represent challenges for extracting total lipids from the biomass. Filamentous fungi colonize and penetrate the solid substrate while utilizing the substrate for support and carbon/energy. Fungal hyphae of the mycelium become entangled in the substrate particles affecting the complete recovery of the fungal biomass. Fungi also possess rigid cell walls with high mannan/chitin content making them resistant to pressure and organic solvents. The intracellular storage of lipids in lipid droplets, lipase activity, and coextraction of lipophilic compounds are the crucial factors contributing to the recalcitrance of fungal cells, especially yeasts, to the lysis or cell disruption required for recovering lipids for biodiesel production (Khot et al. 2020). Conditioning of cell mass is an essential step after fermentation to modify the cell wall permeability and assist in lipid extraction protocol. Khot et al. (2020) have reviewed and discussed a range of available pretreatment methods of physicomechanical, chemical, and enzymati nature. Chemical pretreatment methods employ acid or alkali hydrolysis as well as detergents. Bead milling and high-pressure homogenization are the widely used physicomechanical methods of cell disruption based on solid/liquid shear. Recently microwave-assisted lipid extraction has emerged as a suitable method for treating wet oleaginous biomass; however, no reports are available for its application to fermented solids in SSF. The cell lysate obtained after biomass conditioning is used for solvent extraction of lipids following established protocols such as by Folch et al. (1957) and Bligh and Dyer (1959). Few representative examples of lipid recovery methods from SSF of fungi-based processes are given below.

In a study on SSF of lignocellulosic wastes from palm oil mill (Cheirsilp and Kitcha 2015), the whole substrate fermented by *Aspergillus tubingensis* was harvested together with fungal biomass, subjected to water washes, and heat-dried to constant weight. The dry fermented biomass was ground and sonicated in the lipid extraction protocol after adding chloroform:methanol (2:1). The liquid phases containing lipid were pooled, solvent was removed by evaporation, and total lipid was estimated gravimetrically. The lipid yield of SSF processes is generally expressed as milligram of lipid per gram dry substrate (mg/gds). The fatty acids were estimated as their methyl esters (FAMEs) in the SSF matrix of animal fat by-products fermented by *Umbelopsis isabellina* (Slaný et al. 2020). The dry homogenized fermented mass was mixed with dichloromethane containing margaric (heptadecanoic) acid as an internal standard and anhydrous methanolic HCl solution. After sample incubation at 50 °C for 3 h, water was added, and FAMEs were extracted with hexane. The fungal biomass was recovered by adding Tween 80 (0.1%) at 1:10 (w/v) to the fermented palm biomass waste (empty fruit bunch) in the non-sterile SSF process (Intasit et al. 2020a). The cellular lipids were extracted from the heat-dried fungal mass, according to Folch et al. (1957). Sonication was applied to the sample before filtration to obtain the solvent phase containing the fungal lipids.

6.7 Conclusion

The use of renewable carbon sources such as agricultural wastes and agro-industrial residues and a cheaper process such as solid-state fermentation (SSF) over submerged fermentation are two effective ways to reduce the biodiesel production costs from microbial lipids. A wide variety of agricultural wastes from big agrarian economies present an essential carbon and energy source for the "consolidated bioprocessing" by oleaginous fungi via SSF. Oleaginous fungi with hydrolytic enzyme activity are ideal candidates for the one-step conversion of biomass into biofuel without added enzymes or hazardous chemicals. It improves process economics by integrating unit operations and simplifying the process scheme (Brethauer and Studer 2014). These strategies may contribute significantly to cost-effective and sustainable bioconversion of lignocellulosic biomass into biodiesel feedstocks.

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Chapter 7
Metabolic Engineering Approach for Advanced Microbial Fuel Production Using *Escherichia coli*

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Abstract Currently, biodiesel (fatty acid alkyl ester, FAAE) and bioethanol are two major candidates with great commercial importance concerning biofuel industries. Ethanol is the most extensively produced, utilised and collective with biodiesel accounting for 90% of the biofuels market. Fatty acid alkyl ester is synthesised during the transesterification of triacylglycerols obtained from plant-derived material. The major hurdles for the transesterification process are the use of toxic methanol, various cereal/oil crops, agricultural land and higher energy consumption. Hence, the production of free fatty acid-derived biofuels via microbial fermentation is a great strategy to overcome the hurdles. For the fatty acid synthesis, *Escherichia coli* has been demonstrated as a model bacterium due to its higher growth rate, has been well studied concerning metabolic engineering and has been an industrially important microorganism. Several sets of enzymes are engaged in fatty acid biosynthesis, membrane synthesis and fatty acid degradation. By overexpression and deletion of the respective genes, the higher production of free fatty acid (FFA) and biofuel intermediates can be possible in any industrially important strain. Specific strategies such as transcriptional regulation, promoters of the Fab operon, regulation by stringent response and remodelling of the FFA biochemical pathway can be used to exploit the production of biodiesel.

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7.1 Introduction

The quest for renewable energy is intensifying with exponential expansion. Researchers have put efforts into finding alternatives for the existing fossil fuels due to their limited existence and increasing price. Whether it is sunlight, wind, thermal energy and plant material, it restores itself with minimal efforts after its usage. Plant-derived biodiesel and ethanol are an excellent strategy to overcome the dependency on limited resources. However, it has a disadvantage in meeting the global overexpanding fuel demand. For the past 50 years, the idea of regenerative resources has been inspiring researchers to look inside the microbial cell for a global solution, and in the past 10 years, the efforts have been intensified for the next-generation biofuels through the microbial fermentation route. The investigations on microbial fatty acids and derivatives for the replacement of diesel have been the main focus of this chapter. This chapter is focused on the theme of recent trends in the metabolic engineering approach for microbial biofuel production. *Escherichia coli* has been identified as an excellent industrial strain for various advantages such as short doubling time, known genome, growth to higher cell density and higher solvent tolerance, and it carries efficient metabolic pathways of fatty acid biosynthesis and degradation. Various approaches for biodiesel and other advanced fuels are discussed for microbial host *E. coli.*

7.2 Microbial Fatty Acid Biosynthesis and Metabolic Engineering in *E. coli*

Fatty acid synthesis is the most ubiquitous pathway in microbial cells. Fatty acids are import precursors for cellular components such as sterols, phospholipids and sphingolipids, and also act as cell signalling molecules. Overall cellular membrane fluidity and temperature tolerance depend on the saturation of various fatty acids present in microorganisms. Fatty acids also serve as a reserve of surplus carbon and energy. Disintegration of fatty acids generates a good ATP quantity, and reducing equivalents are either prokaryotes or eukaryotes. The cellular fatty acids accumulate as triacylglycerols (TAGs, wax esters) and polyhydroxyalkanoates (PHAs). At the ATP expense, malonyl-CoA is synthesised by carboxylation of acetyl-CoA along with bicarbonate during the first step. Malonyl-CoA is then transformed to malonyl-ACP by exchanging coenzyme A by acyl carrier protein (ACP). ACP saves the synthesising chain of fatty acid from fatty acid degradation and other anabolic pathways. The first cycle of fatty acid biosynthesis is initiated by condensation of malonyl-ACP with acetyl-CoA with the generation of acetoacetyl-ACP, bicarbonate and free coenzyme A. Acetoacetyl-ACP is further converted to 3-hydroxybutyryl-ACP, and converted to 2-butenoyl-ACP by a dehydration reaction.

Further reduction of 2-butenoyl-ACP yields butyryl-ACP that comes in the subsequent phase of the cycle again with the condensation step with malonyl-ACP. After achieving a specific chain length, fatty acid synthesis stops, and the acyl-ACP is diverted towards the cellular membrane synthesis. Despite the general similarity of the pathway in eukaryotes and prokaryotes, different genes and enzymes are involved in various domains. Type I fatty acid synthase is mainly found in animal cells and fungi with few exceptional presence in prokaryotes, while type II fatty acid synthase carried out a similar reaction in bacteria and plant plastids (Harwood 1988). Several sets of enzymes belong to type II FAS enzyme system that has played an essential role in carrying out fatty acid biosynthesis in *E. coli*, such as acetyl-CoA carboxylase (AccABCD); malonyl-CoA:ACP transacylase (FabD); 3-ketoacyl-ACP synthase I, II and III (FabB, FabF, FabH); 3-ketoacyl-ACP reductase (FabG); 3-hydroxyacyl-ACP dehydrogenase; enoyl-ACP reductase (FabI); ACP synthase; and ACP phosphodiester catalyse fatty acid biosynthesis. The overexpression, deletion and regulation of the genes coding for the corresponding enzymes have shown the manyfold increase in fatty acid synthesis in *E. coli* by several researchers. For the efficient fatty acid production via increment of coenzyme A(CoA) synthesis and malonyl-CoA influx by expression of exogenous acetyl-CoA carboxylase (acc), pantothenate kinase (*coaA*) and fatty acid synthase (*fasA*) stimulated fatty acid production by 5.6-fold (Satoh et al. 2020). Tan et al. (2018) have used multi-latitude integration in silico computational and wet laboratory experimental approaches to achieve 45% increase in octanoic acid titre.

7.2.1 Enzymes and Metabolic Strategies for Enhanced Fatty Acid Production

7.2.1.1 Acetyl-CoA Carboxylase

The initiation of de novo fatty acid biosynthesis is carried out by acetyl-CoA carboxylase. Further, acetyl-CoA is diverted and chain elongated by acetyl-CoA carboxylase. The reaction is divided into two sub-steps: (1) biotin is carboxylated, spending ATP with Mg^{2+}-ions as a cofactor, and (2) carboxyl group is transferred to acetyl-CoA, generating malonyl-CoA (Cronan Jr and Waldrop 2002). The acetyl-CoA is a multimeric protein complex coded by the *accABCD*. A very-well-coordinated transcription of *accABCD* was observed in *E. coli*. All the subunits of AccABCD have to be synthesised in equimolar proportion for the stable enzyme. The gene *accB* and *accC* codes for single mRNA translate into AccB and AccC (Li and Cronan Jr 1992). In most cases, AccB autoregulates their transcription, and overexpression of *accB* leads to inhibition of both *accB* and *accC* (Li and Cronan 1993; James and Cronan 2004). The report suggests that deletion of *accB* has minimum effect on the transcription of *accBC* (James and Cronan 2004). Many evidences claimed that the excess *AccB* leads to a decrease in biotin synthesis (Abdel-Hamid and Cronan 2007; Zhao and Beckett 2008). Davis et al. (2000) have demonstrated the overexpression of four subunits of acetyl-CoA carboxylase with different combinations in equimolar concentration. A remarkable increment of about 11-fold was seen by the overexpression of *accBCD*.

7.2.1.2 Malonly-CoA:ACP Transacylase

The malonyl moiety is transferred to ACP by malonyl-CoA:ACP transacylase, which influxes malonyl-ACP towards neogenesis and chain elongation of fatty acid. Overaccumulation of malonyl-ACP leads to cell death. Overexpression of fabD gave alteration in fatty acid composition, and its deletion was found to be lethal in *E. coli* (Verwoert et al. 1994; Zhang and Cronan 1998). Researchers have reported an overall increase in cis-vaccenic acid and decreased palmitoleic acid by overexpression of the fabD gene in *E. coli* (Magnuson et al. 1992). The possible reason behind might be that the increased level of malonyl-ACP pool leads to increased 3-ketoacyl-ACP synthase II activity expressed by *fabF*. The chain elongation of C16:1 to C18:1 is caused by the 3-ketoacyl-ACP synthase II (Garwin et al. 1980).

7.2.1.3 3-Ketoacyl-ACP Synthase I, II and III

The condensation of fatty acyl-ACP with malonyl-ACP generates 3-ketoacyl-ACP that is catalysed by the enzyme 3-ketoacyl-ACP synthase. The initiation of the first cyclic reaction of chain elongation is carried out by 3-ketoacyl-ACP synthase III (FabH) using primary substrates acetyl-CoA and malonyl-ACP during fatty acid biosynthesis. The following elongation steps are catalysed by 3-ketoacyl-ACP synthase I (FabF) and II (FabB). The biosynthesis of fatty acids with a varying number of carbon atoms by 3-ketoacyl-ACP synthase III (FabH) is due to its good compatibility with both substrates propionyl-CoA and acetyl-CoA. The enzymatic reactions of FabF and FabB vary only in some reactions. Both enzymes react with the standard substrate C6–C14 saturated fatty acyl-ACP esters. However, both enzymes have very low specificity with C14:0 and FabF displays lower activity with C16:0 substrate (Edwards et al. 1997).

The condensation reaction of cis-7-tetradecenoyl-ACP, cis-5-dodecenoyl-ACP and cis-3-decenoyl-ACP, each with malonyl-ACP, is catalysed by 3-ketoacyl-ACP synthase II (FabB) during the synthesis of unsaturated fatty acids (Feng and Cronan 2009). The last metabolic product synthesis, i.e. vaccenic acid, is catalysed by 3-ketoacyl-ACP synthase I (FabF) during the elongation step (Edwards et al. 1997). The *fabH* gene (3-ketoacyl-ACP synthase III) deletion has been believed to be deadly (Lai and Cronan 2003). However, a report suggests that there is only a significant reduction in cell size and growth rate after deletion of fabH (Yao et al.

2012). So, the activity of FabH can only comparatively be replaced by additional related enzymes involved in the pathway. The extension of C14:1 to C16:1 is catalysed by 3-ketoacyl-ACP synthase I (FabF) and its deletion leads to a change in temperature sensitivity of *E. coli* (Garwin et al. 1980). Again, overexpression of 3-ketoacyl-ACP synthase I (FabF) is found to be fatal and a significant increase of malonyl-CoA intermediates is reported (Subrahmanyam and Cronan 1998). It is also evident that overexpression of fabF would occupy all available FabD proteins, and consequently the FabD-FabH complex would not be synthesised. In *E. coli*, deletion of *fabB* (3-ketoacyl-ACP synthase II) creates the condition of auxotrophy for unsaturated fatty acids (Cronan et al. 1969). Overexpression is only appropriate if *fabA* (3-hydroxyacyl-ACP dehydrase) is also overexpressed to boost the unsaturated fatty acid share (Cao et al. 2010). Contrarily, a noteworthy improvement in total fatty acid build-up is not achieved even with combined overexpression of *fabA* and *fabB*.

7.2.1.4 3-Ketoacyl-ACP Reductase

By the expense of NADPH and H^+, the 3-ketoacyl-ACP is reduced to 3-hydroxyacyl-ACP in *E. coli*. This reversible reaction is catalysed by 3-ketoacyl-ACP reductase (FabG). The 3-ketoacyl-ACP reductase catalysis in fatty acid biosynthesis cannot be replaced by any other enzyme in *E. coli* (Zhang and Cronan 1998; Lai and Cronan 2004). The two to three times of increases in the levels of C16:0 and C18:0 acids were reported during homologous expression of fabG in *E. coli* (Jeon et al. 2012).

7.2.1.5 3-Hydroxyacyl-ACP Dehydrase

The dehydration of 3-hydroxyacyl-ACP is carried out by enzyme 3-hydroxyacyl-ACP dehydrase expression by *fabZ* and *fabA* genes in *E. coli*. The first step towards the synthesis of unsaturated fatty acids is isomerisation of trans-2-decenoyl-ACP into cis-3-decenoyl-ACP by FabA (Kass and Bloch 1967). These catalytic reactions can be driven in both orders depending on the concentration of substrate and product under different physiological conditions. It was observed that overexpression of *fabA* enhances the saturated fatty acid content in *E. coli* (Clark et al. 1983) and dehydration of saturated 3-hydroxyacyl-ACPs with varied chain lengths carried out by FabA (Heath and Rock 1996). For an enhanced proportion of unsaturated fatty acid production, co-overexpression of both *fabA* and *fabB* is needed in *E. coli* (Cao et al. 2010). The dehydration of 3-hydroxydecanoyl-CoA is seen with a significant activity of 11% compared to 3-hydroxydecanoyl ACP by FabA (Bloch 1971).

7.2.1.6 Enoyl-ACP Reductase

The enzyme enoyl-ACP reductase coded by a single gene *fabI* catalyses the last step in fatty acid biosynthesis by reduction of 2-enoyl-ACP to fatty acyl-ACP at the expense of NADPH and H^+ or NADH and H^+ (Bergler et al. 1994). It completes the final step in every fatty acid biosynthesis cycle, making the gene crucial if no external fatty acids are externally supplemented to *E. coli* (Bergler et al. 1996; Nakashima et al. 2006). Overexpression of the *fabI* results in no change in growth and does not change palmitic acid or stearic acid content and cellular lipid (Xu et al. 2006; Jeon et al. 2012). The drastic inhibition of enoyl-ACP reductase was observed in the presence of even low concentrations of palmitoyl-CoA (Bergler et al. 1996).

7.2.1.7 ACP, ACP Synthase and ACP Phosphodiesterase

The ACP is an acyl carrier protein expressed by *acpP* in *E. coli* belonging to the fatty acid biosynthesis gene cluster. The ACP synthase (AcpS) catalyses the phosphopantetheine group's attachment to a serine of the synthesised apo-ACP to get the physiologically active protein. The ACP plays a crucial role in distinguishing all CoA ester intermediates needed to bind to ACP during fatty acid catabolism in fatty acid biosynthesis. The ACP is the most abundant protein among all soluble proteins expressed in *E. coli* with a weightage of 0.25% (Chan and Vogel 2010). The cellular growth termination was observed upon overexpression of *acpS* in *E. coli*, because of its significant inhibition of the glycerol-3-phosphate acyltransferase (Keating et al. 1995; Rock et al. 1981). This scenario can be partially overcome by the co-expression of *acpH* as apo-ACP which is a prominent inhibitor as compared to holo-ACP. It is also reported that fatty acid biosynthesis inhibition was observed in vitro by the additional supplement with holo-ACP or apo-ACP to cell-free extracts of a FFA production in *E. coli* (Liu et al. 2010).

7.3 Fatty Acid Degradation in *E. coli*

Fatty acids are stimulated to acyl-CoA esters to metabolise fatty acids during initial fatty acid degradation—the exogenous fatty acids are a carbon source attached to FadL, a transporter protein. FadL attachment helps fatty acids in conformational changes, enabling a pore to open, and leads to diffusion of fatty acids into the periplasmic space (Lepore et al. 2011). Interruption of *fadL* expression leads to disability to utilise oleate (Campbell and Cronan Jr 2002). Another carrier protein, FadD, helped the translocation of fatty acid from the periplasm to the cytosol and resulted in the acyl-CoA ester synthesis at the cost of ATP (Weimar et al. 2002). Studies have revealed that FadD utilises free fatty acids released from membrane lipids to make acyl-CoA channelled to the β-oxidation pathway for degradation.

Subsequently, reports also suggest that disruption mutation in *fadD* creates a pool of free fatty acids in the cytosol (Pech-Canul et al. 2011) and on the other hand, *E. coli* is unable to grow on oleate as a sole carbon supplement (Campbell and Cronan Jr 2002). Homologous overexpression of fadD empowers *E. coli* to utilise medium-chain-length fatty acids that boosts the transcription of genes like *fadBA* and *fadE* (Zhang et al. 2006). Using site-directed mutagenesis in *fadD* gene, the affinity of FadD protein was improved for medium-chain-length fatty acids (Black et al. 1997). FadR, a repressor protein, acts as a transcriptional controller to every gene related to the aerobic fatty acid degradation cycle. It enables the transcription of β-oxidation genes upon binding long-chain acyl-CoA (Henry and Cronan Jr 1991, 1992). Additionally, the cyclic adenosine-monophosphate (cAMP) receptor protein-cAMP complex (CRP-cAMP) positively controls the fatty acid degradation cycle in such a way that at least *fadL*, *fadD* and *fadH* are upregulated upon partial availability of glucose (Feng and Cronan 2012).

7.4 Transcriptional Regulation of Fatty Acid Biosynthesis and Degradation in *E. coli*

In *E. coli*, fatty acid biosynthesis- and degradation-related several genes are regulated at the transcriptional level. In fatty acid biosynthesis and degradation, proteins such as FabR and FadR act as main transcription factors. Apart from that, (p)ppGpp, a signalling molecule, also plays a vital role in governing fatty acid metabolism. All the transcriptional regulation of fatty acid biosynthesis and degradation is summarised in Table 7.1. The transcription of all genes related to β-oxidation cycle is repressed by the FadR protein when it is required during regulation (Feng and Cronan 2012). Similarly, the glyoxylate shunt pathway is repressed upon activation of the repressor protein IclR during regulation (Gui et al. 1996; Kumari et al. 2000). FadR binds to the promoter site to achieve repression of fatty acid degradation-related genes, which gene expression is restored by interaction with long-chain acyl-CoA esters upon several specific conditions such as the accumulation of acyl-CoA esters upon external fatty acid absorption by *E. coli* and during the degradation of phospholipids from the membrane (Henry and Cronan Jr 1991, 1992; DiRusso et al. 1992).

Subsequently, FadL and FadD regulate the uptake of fatty acids by the cells, and the equivalent genes are only moderately controlled by FadR during transcriptional regulation (Feng and Cronan 2012). In a particular condition such as low glucose availability, the fatty acid uptake is additionally triggered by CRP-cAMP complex protein. The transcription of fad genes is regulated at a minor level during the availability of glucose in the fermentation medium, irrespective of fatty acid presence (Pauli et al. 1974). Surprisingly, although FadR acts as a negative regulator of genes related to fatty acid degradation pathway, it also positively regulates the transcription of fatty acid biosynthesis genes such as *fabA* and *fabB* and the operon

Table 7.1 Summary of fatty acid biosynthesis and degradation regulation

Sr. No.	Mode of regulation	Pathways	Factors	Activation	References
1	Transcriptional inhibition	Fab	ppGpp	AccABCD, FabD, FabH, FabG, FabZ, PlsX, PlsB, PlsY, FadL, FadD	Tanaka et al. (1989), Verwoert et al. (1994), Podkovyrov and Larson (1996), Zhang and Cronan (1998), Janßen and Steinbüchel (2014)
			FabR	FabA, FabB	Henry and Cronan Jr (1991, 1992), Janßen and Steinbüchel (2014)
			BasR	PlsB	Heath et al. (1994), Durfee et al. (2008), Janßen and Steinbüchel (2014)
		Fad	FadR	FadL, FadD, FadE, FadB, FadA	Fujita et al. (2007), Feng and Cronan (2012), Janßen and Steinbüchel (2014)
2	Enzyme inhibition	Fab	Acyl-ACP	AccABC, FabF	Lu et al. (2008), Lennen et al. (2010), Janßen and Steinbüchel (2014)
			Acetyl CoA	FabD	Magnuson et al. (1992), Podkovyrov and Larson (1996)
			Palmitoyl-ACP, palmitoyl-CoA	FabI	Bergler et al. (1994, 1996)
			Papo-ACP	PlsB	Heath et al. (1994), Wahl et al. (2011), Janßen and Steinbüchel (2014)
			Holo-ACP	PlsB	
			ppGpp	PlsB	
3	Transcriptional activation	FAB	FadR	FabD, FabH, FabG, FabA, FabB	Joshi and Wakil (1971), Podkovyrov and Larson (1996), Janßen and Steinbüchel (2014)
			ppGpp	FabA, FabB	Verwoert et al. (1994)
		FAD	σE	PlsB	Durfee et al. (2008), Traxler et al. (2008), My et al. (2013), Janßen and Steinbüchel (2014)
			Crp-cAMP	FadL, FadD, FadE, FadB, FadA	Pauli et al. (1974), Janßen and Steinbüchel (2014)
			RpoS	FadE, FadB, FadA	Dong and Schellhorn 2009, Janßen and Steinbüchel (2014)

fabHDG (Henry and Cronan Jr 1991, 1992; Campbell and Cronan Jr 2001; My et al. 2013). Hence, it serves as the activator for the synthesis of unsaturated and saturated fatty acids. Besides their regulation by FadR, researchers have observed that apart from FadR, genes of fatty acid biosynthesis such as *fabA* and *fabB* are additionally under the control of the fatty acid biosynthesis regulator (FabR) (McCue et al. 2001; Zhang et al. 2002). It has been found that FabR can sense the cytosolic fatty acid pool as well as its composition. Several experiments have given an understanding of FabR as the primary regulator for *fabB*, while the transcription of *fabA* is dominantly controlled by the activity of FadR (Feng and Cronan 2011). Regulator FabR strongly represses fabA and fabB when in complex with unsaturated fatty acyl-ACP, whereas the attachment is relaxed when FabR binds to saturated fatty acyl-ACP (Zhu et al. 2009).

Biodiesel, consisting of fatty acid methyl, ethyl or propyl esters (FAME, FAEE and FAPE, respectively), is currently utilised in diesel engines at more than two billion gallons per year. Efforts were made for the enhanced FAEE and related fuel derivative production using engineered *E. coli* strain (Steen et al. 2010). Successfully an ethanol biosynthetic pathway has been introduced in *E. coli* host by genetically introducing and expressing an alcohol dehydrogenase (adhB) and a pyruvate decarboxylase (pdc) to the biological conversion of pyruvate into ethanol. For the production improvement of certain biodiesel derivatives, metabolic engineering was found as a promising approach, where a wax-ester synthase gene (*atfA*) was co-overexpressed with the endogenous *fadD* gene to trigger free fatty acids to acyl-CoAs and catalyse the esterification of intermediate to FAEEs (Steen et al. 2010). The metabolically engineered strain reported about 427 mg/L FAEE after 72 h of fermentation incubation (Kalscheuer et al. 2006). Likewise, overexpression of *fadD* and an acyl-CoA reductase (*acr1*) in different host *Acinetobacter calcoaceticus* produced fatty alcohols (Steen et al. 2010).

Certain alkanes and alkenes (C8–C21) are the principal chemical constituents of diesel fuel (Rude and Schirmer 2009). Certain successful efforts were made to introduce the long-chain alkene biosynthetic pathway construct of a three-gene form from *Micrococcus luteus* in a fatty acid-producing *E. coli* strain overproduction of alkene (Beller et al. 2010). The two acyl-CoAs are condensed by heterologous enzymes and followed by a chain of reduction and dehydration enzymatic reactions ultimately giving internal alkenes, mostly of C27:3 and C29:3 variants (Frias et al. 2011; Sukovich et al. 2010). Moreover, the expression of a cytochrome P450 enzyme (OleTJE) from *Jeotgalicoccus* spp. in *E. coli* strain reported production of terminal alkenes (mostly C18–C20) using the catalysis of the decarboxylation of free fatty acids to alkenes (Rude et al. 2011). Furthermore, cyanobacterial genes for biosynthesis of alkane were identified (Schirmer et al. 2010). The reduction of acyl-ACPs by enzyme acyl-ACP reductase creates aldehydes, and it is further transformed into alkanes by an aldehyde decarboxylase. Upon overexpressing two enzymes in *E. coli* host, 300 mg/L of alkane (C13–C17) mixture is produced after 40 h of incubation (Schirmer et al. 2010).

7.5 Next-Generation Biofuel Production Using Metabolic Engineering Approach

For the enhanced production of advanced biofuels and fatty acid-based fuel, various metabolic strategies have been developed for *E. coli*, among which all the major metabolic engineered pathways are represented in Fig. 7.1, showing that biofuel production starts with significant central metabolites such as phosphoenolpyruvate, pyruvate and acetyl-CoA and leads to ultimate synthesis of butanol, isobutanol, isopropanol, farnesol, terminal alkanes, fatty alcohols, FAEEs and FAMEs (Fig. 7.1).

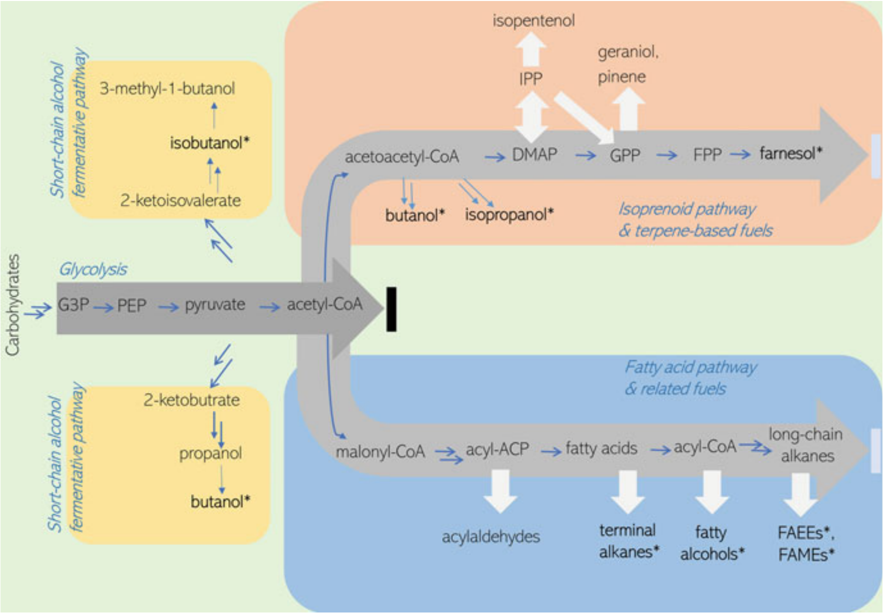

Fig. 7.1 Metabolic engineered pathways for advanced biofuel production in *E. coli*. Grey arrow represents central metabolism. The white arrow represents the final products. The two-way arrow shows the interconversion of metabolites. Fatty acid pathway and related biofuels are shown in the blue compartment. Similarly, isoprenoid pathway and terpene-based fuels are in orange colour, and short-chain alcohol fermentative pathway is in yellow blocks. Double arrows represent conversions catalysed by several enzymes, and single arrows represent conversions catalysed by one enzyme. *G3P* glyceraldehyde-3-phosphate, *PEP* phosphoenolpyruvate, *DXP* 2-C-methyl-D-erythritol-4-phosphate, *ACP* acyl carrier protein, *IPP* isopentenyl-diphosphate, *DMAP* dimethylallyl phosphate, *GPP* geranyl-diphosphate, *FPP* farnesyl-pyrophosphate, *GPP* geranylgeranyl-pyrophosphate, *FAEE* fatty acid ethyl ester, *FAME* fatty acid methyl ester. * represents commercially important biofuels

7.5.1 Fermentative Pathways for Short-Chain Alcohol Production

Butanol and isopropanol are considerably more suitable alcohol fuels as compared to ethanol because of low hygroscopicity and high energy density, and they are less corrosive to engines, industrial reactors and pipelines during transportation (Liu and Khosla 2010; Rude and Schirmer 2009). Isopropanol production has been reported using biosynthetic modifications with genes *thl*, *ctfAB* and *adc* from *Clostridium acetobutylicum* introduced in *E. coli*, which can metabolise acetyl-CoA into acetone through acetoacetyl-CoA and acetoacetate intermediates (Hanai et al. 2007; Jojima et al. 2008). Isopropanol production was demonstrated in *E. coli* host by expressing an *adh* gene which codes for alcohol dehydrogenase enzyme obtained from *Clostridium beijerinckii*. The genetically improvised strain produced 13.6 g/L of isopropanol using glucose as a carbon supplement. Also, gas trapping method was utilised to capture the isopropanol produced from fermentation medium to minimise the toxicity caused by isopropanol to *E. coli*. The metabolic engineering approach resulted in the increment of isopropanol production of 67% (mol of isopropanol/mol of glucose) with 143 g/L after fermentation incubation of 240 h, which is a significant increase from 2 g/L of isopropanol produced by the wild-type *Clostridium* strain (Inokuma et al. 2010). Several efforts were made to engineer the butanol production pathway in *E. coli* by introducing butanol biosynthetic pathway-related genes such as *crt*, *bcd*, *hbd*, *thl*, *adhE2* and *etfAB* from *Clostridium*, resulting in the butanol production of 13.9 mg/L (Atsumi et al. 2008a, b). Researchers have identified and deleted specific genes such as *fnr, pts, ldhA, adhE* and *frdBC* associated with opposing pathways for carbon and reducing cofactor usage to enhance butanol production potential. Using this approach, butanol production was not boosted enough and only 1.2 g/L of yield was obtained after 60 h of fermentation incubation (Inui et al. 2008) compared to 19.6 g/L of butanol in a *Clostridium* strain. For the systematic channelisation of metabolic intermediates into the butanol biosynthetic pathway, two genes were substituted, responsible for the irreversible catalysis in engineered *E. coli* (Bond-Watts et al. 2011). The experiment was carried out by replacing the *thl* gene with the *pdaA* gene obtained from native *Ralstonia eutrophus* strain, which ropes acetyl-CoA condensation as it drives biological polymer polyhydroxyalkanoate (PHA) production. The PHA production pathway was further modified by replacing multiple genes of the *bcd-etfAB* with the *ter* gene from *Treponema denticola*, where the butyryl-CoA dehydrogenase catalyses the permanent conversion of crotonyl-CoA into butyryl-CoA. The modified pathway leads to the production of 2.95 g/L butanol after 72 h. The overexpression of pyruvate dehydrogenase complex (aceEF–lpd) to influx NADH and acetyl-CoA towards butanol production was shown, and production was further enhanced to 4.65 g/L butanol with increment in the yield of 28% using glucose in the genetically engineered *E. coli*.

7.5.2 2-Keto Acid Pathways for Short-Chain and Medium-Chain Alcohols

Researchers have also utilised the tool of metabolic engineering to develop a series of synthetic non-fermentative pathways for the production of short-chain-based alcohols from common precursors (2-keto acids) in *E. coli* via amino acid biosynthesis ultimately. Taking benefit of the natural, high-flux, amino acid biosynthetic pathway (Atsumi et al. 2008a, b), starting with 2-keto acids, it converted into aldehydes by a broad substrate range enzyme 2-keto acid decarboxylase. The resulting aldehydes were then reduced to alcohols by a non-specific alcohol dehydrogenase (Adh) enzyme. The native amino acid biosynthesis in *E. coli* owning high flux for metabolites is driven to amino acid biosynthesis. Furthermore, the adh2 gene (alcohol dehydrogenase) from *S. cerevisiae* and the *kivd* gene from *Lactococcus lactis* were replaced in engineered *E. coli* (Atsumi et al. 2008a, b). The overall configuration of alcohol products solely depends on the 2-keto acid pool's availability in *E. coli*. 2-Keto acid flux increased by overexpression of specific genes *alsS* and *ilvCD*, which increases the production of isobutanol and alcohol via 2-ketoisovalerate biosynthesis in *E. coli*. Several other short-chain alcohols such as 3-methyl-1-butanol (Connor et al. 2010), 2-methyl-1-butanol (Cann and Liao 2008), 1-propanol and 1-butanol (Shen and Liao 2008) have been produced using similar tactics with high specificity and good yields. Moreover, protein structural engineering was used to optimise the substrate-binding pocket of 2-isopropylmalate synthase that performs the chain elongation reaction to accommodate larger substrates in leucine biosynthesis. By site-directed mutation in LeuA, a range of medium-chain alcohols (C6–C8 alcohols) were biosynthesised in *E. coli* (Zhang et al. 2008).

7.5.3 Fuels from Isoprenoid Pathways

Terpenes are also well known as isoprenoids, which are resulting from a five-carbon isomeric unit (C5) called isopentenyl pyrophosphate (IPP) or dimethylallyl pyrophosphate (DMAP). IPP or DMAP is the product of either the 1-deoxy-D-xylulose 5-phosphate (DXP) pathway or the mevalonate (MEV) pathway. After DMAP or IPP is synthesised, prenyltransferases catalyse the condensation of the 5 carbon units to longer carbon-containing form geranyl pyrophosphate (GPP, C10), farnesyl-pyrophosphate (FPP, C15) and geranylgeranyl-pyrophosphate (GGPP, C20).

The terpene synthases catalysed conversion of prenyl-pyrophosphates into monoterpenes (C10), sesquiterpenes (C15) and diterpenes (C20). Leonard et al. (2010) have optimised the production of terpenes by carbon influx towards isoprenoid pathways. Researchers have screened proteomics to identify possible blockages of a pathway formerly established by Martin et al. (2003) using a rational design approach. The idea resulted in increased production of more than 500 mg/L of sesquiterpene amorphadiene (Redding-Johanson et al. 2011). The diterpenes levopimaradiene and taxadiene were synthesised at 700 mg/L and 1 g/L, respectively, using combinatorial approaches such as screening for gene expression levels and enzyme mutations (Ajikumar et al. 2010).

Withers et al. (2007) demonstrated the production of isopentanol in *E. coli* using the expression of pyrophosphatase *nudB* obtained from *B. subtilis*, via the hydrolysis of DMAP or IPP. Fifteen carbon-containing farnesyl-pyrophosphate (FPP) generated farnesene and farnesol that have been proposed as diesel fuels (Renninger and Mcphee 2008). Wang et al. (2010) have engineered *E. coli*, which synthesises 135 mg/L farnesol by enhancing FPP biosynthesis. It was observed that high intracellular FPP levels force endogenous phosphatases to hydrolyse FPP to farnesol non-specifically. Likewise, Song (2006) reported that the expression of a soluble phosphatase in *S. cerevisiae* produces farnesol by downregulation of the squalene synthase, Erg9 (Song 2009). Erg9 catalyses the first catalytic conversion of ergosterol biosynthesis, and its downregulation results in the accumulation of FPP (Ro et al. 2006; Paradise et al. 2008). Certain metabolite end products such as terpinene, pinene, limonene and sabinene and commonly categorised cyclic monoterpene olefins have been identified as precursors to promising aviation (jet) fuels (Ryder 2009). Pinene dimer mixtures possess chemical properties such as appropriate fuel densities, and specific combustion heats imitate the current jet fuel JP-10 (Harvey et al. 2010).

7.6 Conclusion

The never-ending search for improved biosynthetic pathways for advanced biofuel production has shown incredible possibilities of microbial fuels as a commercial renewable resource in energy sectors in the near future. Fatty acid-based biofuels and other derivatives produced through the metabolic engineering approach can further reduce the burden on fossil fuels and related consequences. The recent advancement of enhanced production of various next-generation fuel with a tailormade approach gives an extra edge to creating a parallel sustainability-driven economy. Fatty acid-based biofuels are being produced with greater efficiency after identifying the curial enzymes and developing strategies for regulating the expression of such enzymes involved in Fab and Fad pathways. The *E. coli* has responded well upon constructing an engineerable metabolic pathway or using protein engineering to advance microbial fuel production.

Furthermore, many challenges that are ahead and need to be addressed include utilising cost-competitive agro-industrial waste utilisation for advanced biofuel production, availability of diverse fuel candidates and production time to address microbial fermentation efficiency in maximum applications. To find a practical solution for all these hurdles, research around the globe has put various cutting-edge technology such as metabolic engineering, proteomics, genomics, fluxomics and transcriptomics. To have a microbial fuel at the minimum usage of non-renewable resources for commercial and industrial purposes with a minimum carbon footprint on the environment is the common goal of every researcher working in next-generation biofuels.

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Chapter 8
Microbial Fuel Cells (MFC) and Its Prospects on Bioelectricity Potential

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Abstract Microbial fuel cell (MFC) technology is a viable advancement towards commercial solution to electricity requirements enabling the transformation of chemical energy into electrical energy mediated by the metabolism of microbes that utilize organic matter for their energy requirement. The future threats on the growing need for more power with depleting conventional fossil fuel reservoirs can be managed with additional scope in waste management, biosensors, smart farming, etc. This chapter envisages the basics, importance, and constructional evolution with respect to the existing MFC models and customized designs. Factors that influence the stable bioelectric potential are the only cue that needs to be focused for the drift of commercializing MFC. The stable and bioelectric challenges by the MFC can be achieved with suitable technologies on a wide range of future perspective and prospective.

Keywords MFC · Microbial fuel models · Bioelectricity · Organic matter

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8.1 Introduction

In this modern era, we are in lack of sustainability for all our needs which include money, water, air, electricity, fossil fuels, etc. The scientific interest and research to overcome the scarcity for all these needs have gradually increased. The production of electricity from microorganisms has been identified by scientists wherein microbes are used which have the potential to perform as electrogen that can aid in power generation (An et al. 2019). It is possible by certain potential microorganisms coined as microbial fuel cells, through which the electrons and protons are released which results in the production of electricity (Rahimnejad et al. 2011). It is widely performed both in single and in dual chambers that consist of the anode which acts as a biofilm electrode containing MFC. The concept of MFC not only supports green fuel generation but also with an added advantage of treating the wastewater along with oxidation (digestion) of the substrate (Sekar and Ramasamy 2013). Recently, MFC has also been acting as a biosensor. Not all the microbes have the ability to perform as MFCs; only a few microbes are able to transfer actively generated electrons and protons. This transfer of electrons is produced by the catalytic action of microbes and the resultants are first transferred to the anode followed by cathode in the chamber (Ivars-Barceló et al. 2018).

MFC is a bioelectrochemical system which helps us to produce electricity by converting the organic substance into chemical energy by utilizing the catalytic action rendered by the microbes (An et al. 2019). This chapter highlights the different aspects of deploying microbes for power generation. Studies have also proven that the energy production by mixed consortia is more as compared to pure cultures. ‘Electricigen’ or ‘electrogenic’ is the term for certain microbes that can donate electrons to solid electron acceptor upon certain conditions and only these microbes are employed for MFC-mediated power generation. The production of electron synthesis by using this electrogenic bacteria is termed as “bioelectricity” MFCs; it holds great promise as a sustainable biotechnological solution for the future generation. The power generation in MFCs depends on the growth, survivability, and catalytic action of microbes in the chamber (Parkash 2016). Mostly, organic compounds act as a base for the chemical energy and those electron sources are the value-added products for power generation.

8.2 Concept Invention

The concept of novel MFC stimulated first the key factor (or) key elemental design of the usage of dual-chamber MFCs. This first innovation of MFC in dual chamber had better performance than a single chamber for high power throughput due to internal resistance. Here the anode and proton-exchange membrane play an effective role which describes the design of an effective chamber system (Rojas Flores et al. 2020). Recent studies implied on using consortial microbes instead of using single

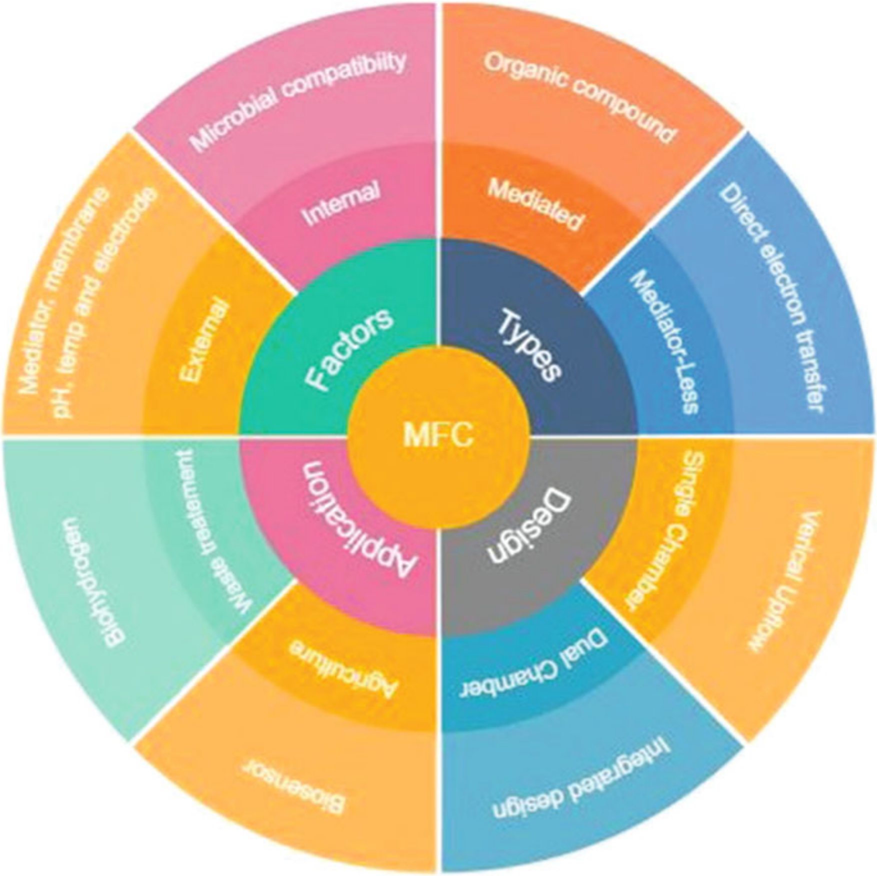

Fig. 8.1 Overall guideline to understand the facts about the MFC

pure culture. The MFCs fed with mixed consortia comprising of more than two cultures enhance the catalytic reaction of power generation (Rabaey et al. 2004). The numbers of microbes in the MFC chamber depict the outcomes of power generation; that is, this power output always depends upon the microbes that are present actively in the MFC chamber under anaerobic condition (Fig. 8.1).

8.3 Materials Used to Construct MFC

In general, the selection of electrodes for MFCs should possess certain attributes which are some of the major craters to choose any electrode for the MFCs, viz., surface area, permeability, electrical conductivity, stability, robustness, price, and accessibility.

8.3.1 Cathode

In MFC, the cathode supports in three ways which act as a diffusion layer, conducting material, and catalyst; likewise, anode-cathode should also contain certain properties as mentioned:

1. Catalytic property.
2. High mechanical strength.
3. High diffusion property.
4. High ionic conductivity.

Albeit, carbon-based materials act as potential cathode, but, at times, owing to poor catalytic action, coupling with an external catalyst is needed aiding the reduction process. The electrochemical oxidation treatment can be used which helps in the active transfer of electrons.

8.3.2 Anode

The selection of conventional anode material is considered as the most important key factor for MFC performance. The anode material significantly impacts the electron transport mechanisms which take place in the microbes and the biofilm formation. "Bio-anode" was commonly used in practice due to the presence of a microbe in this chamber (Pisciotta et al. 2012). In this anode chamber, the catalytic reaction takes place aiding in electron generation. In addition to that, the anode material used for MFC should have biocompatible properties. This property is significant for the adherence of microbe and formation of biofilm at the anode. The microbes are also reported to form a biofilm which adheres to this bio-anode. The reverse engineering of biofilm can be further studied by subjecting the biofilm through phylogenetic analysis. The metagenomic analysis of the different MFCs that can form their own set of biofilms in specific substrates identifies thrust areas to work on.

8.3.3 Proton-Exchange Membrane (PEM)

PEM plays a significant role and is an improvement factor in microbial fuel cells. The function of the PEM is transportation. It acts as a separator to the electrode. The PEM is mainly depended upon the output power density and coulombic force efficiency. It will separate the bio-anode and cathode physically and it also enables the transformation of protons from bio-anode to the cathode. This will result in the production of electric current. PEM on power production will depend on its surface area (Tharali et al. 2016). If the area is smaller compared to the electrodes used in MFCs, it will reduce (decrease) the output power because of its internal resistance.

Nafion is a widely used product for PEM which decides the working efficiency of MFC. It also consists of certain ideal characteristics, primarily cost-effectiveness. Salt bridge, possessing high mechanical strength along with better segregational characteristic is the simplest and widely used from of PEM in MFC.

8.4 Classification of MFCs

Major two types of MFCs include non-mediator and non-membrane MFCs (Jang et al. 2004). Mediator-less depends on exoelectrogens for the electron disposing by oxidation process of substrate. Mediator-less is widely preferred over the usage of mediator in microbial fuel cells as mediators impact the results and can become toxic towards the cells, along with cost increment. It has been proved that the mediator MFCs will produce a low amount of power output when compared to the mediator-less MFCs. Mediator MFCs use certain organic compounds like thionine, methyl viologen, and methyl blue, but the usage of these is costly and toxic. On the contrary, mediator-less MFCs contains redox particles that are active electrochemically on their outer membrane helping in the transfer of electrons to the anode (Mao and Verwoerd 2013).

The mediator types in MFCs were first demonstrated in the twentieth century. From this demonstration, it has been identified that the transfer of electrons is facilitated by mediators towards the anode.

8.4.1 Mediator MFCs

This type of MFCs is not preferred widely. In this type, the electron produced from the catalytic reaction gets transferred to the bio-anode directly, with the aid of organic compounds known as mediators. Employing this kind of mediators, induces change and difference in the power output. It may result in toxicity in the operation. Using some mediating chemicals augment the cost too. They do have an advantage of stimulating the microbes that are not aligned to produce the power density, unlike other potential indigenous MFCs.

8.4.2 Mediator-Less MFCs

This kind of MFCs is commonly used for fuel cell chamber and construction. There is no addition of external mediators which aim to induce the straight way of electron transfer to the anode. Hence, the electron-transporting mechanism takes place naturally due to the catalytic reaction and redox potential.

From this classification, the mediator-less MFCs have their advantage when the microbes are potential and their metabolism does not require any stimulation; there is no requirement of external agents; only catalysts can be added. And, by using mediator-less we can produce high-throughput power. These are the crucial things in MFC construction which are used to build a perfect fuel cell chamber. It is not always the case of mediator-less to have high-throughput power; some conditional requirements are there where the application project is of core importance, like in waste reduction. These applications can be possible only with a certain group of microbes that can act as potential decomposers but do not have the potential to transfer the electron. This may end up in failure to produce energy; such cases can be treated with mediated MFCs.

8.5 Design of MFC

The construction of MFC contains dual chambers which can be separated by mediators. These chambers contain anode and cathode as their electrodes. This anode chamber is maintained under anaerobic condition in which the catalytic reaction takes place to produce electrons that facilitate the production of power density. MFC operation consists of electrodes and salt bridges (PEM). Apart from this, MFC can be constructed as single, dual, stacked MFC and other derived models.

8.5.1 Single Chambered

Single-chamber MFC can be constructed using various ways. The ideal difference in the dual and single chamber is the placement of anodic electrodes and cathodic electrodes. In the case of single-chambered construction, the electrodes (bio-anode and compatible cathode) are placed in a single chamber instead of placing it in dual compartments (Johnson 2018). Such design has no well-defined exchange membranes for proton and cathode compartment. The cathode used should be porous and can occupy one portion of the chamber which is facilitated to utilize oxygen from the atmosphere. There is no specific membrane which helps in the diffusion of oxygen and allows the protons to diffuse. The catalytic reaction takes place by using atmospheric O_2 and producing protons; the porous nature of cathode on one side of the chamber permits the protons' movement resulting in power generation (Wang et al. 2018). The construction of the dual-MFC chamber is difficult as compared to a single chamber because in single-chambered MFC, there is no need to build separate compartments. This whole process is connected by an external wire to harvest energy.

8.5.2 Double-Chambered MFCs

In dual-chambered MFC, the constructional design has two compartments for the electrodes. These compartments are separated by a salt bridge which acts as a proton-exchange membrane (Fig. 8.2). The anode electrode is placed in one compartment where it acts as bio-electrode. In this chamber, the catalytic reaction takes place which results in the production of an electron, as a by-product (Saha et al. 2019). The produced electrons get attracted to the anode. Once, the electrons reach the anode it gets transferred to the second chamber. The proton-exchange membrane acts as a barrier (platform) which helps in the transformation of the electron and has a reduced internal resistance (Tharali et al. 2016). The placement of electrodes in the different chambers helps to produce a high yield of power turnout. The catalytic and redox reaction taking place abruptly releases the protons into the chamber and gets transferred to the cathode via PEM. Compared to a single chambered MFC, dual chamber consists of more salient features and it is more effective in terms of power turnover.

8.5.3 Other Models

With the two basic designs, various other designs are derived by integrating one or few features of the basic designs: (1) vertical upflow, (2) flat plate, (3) anaerobic fluidized bed, and (4) stacked models. The first model, vertical upflow, is designed in such a way so as to favor the waste treatment with a single-chamber model. The design differs in the arrangement of electrode and the inlet of the substrate/medium/

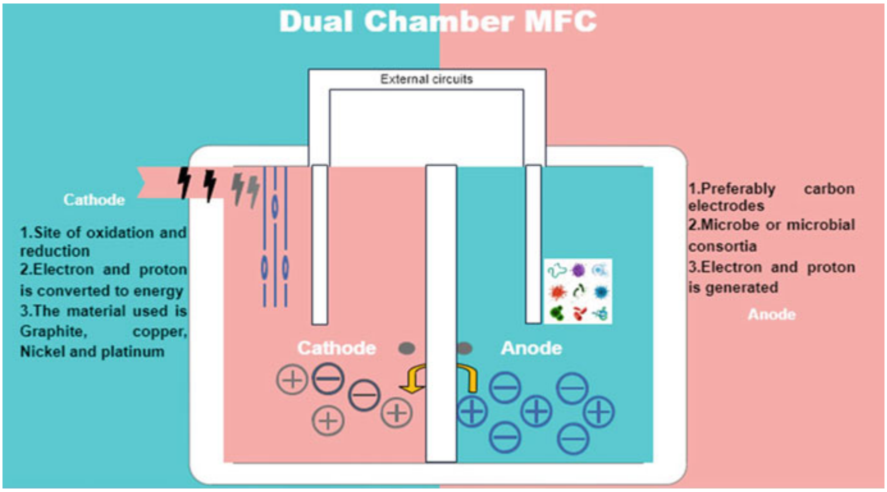

Fig. 8.2 Structure of dual-chamber MFC

waste. The anode is placed at the bottom in the MFC vessel and the cathode at the topnotch near the outlet. The second, flat-plate model is a design with continuous setup where the separation membrane is cationic exchanger. The distance between the electrode is maintained very less. The power density here depends on the efficiency of the membrane. This type of design is reported to favor the pilot-scale and large-scale energy production that overcomes the internal resistance confronted by the other designs. The next model is the anaerobic fluidized bed model derived with continuous operational mode and air cathode for large-scale operation of wastewater. The final model is the stacked design that supports the series of single design connected together. They are widely manufactured for augmenting the power density output to a maximum along with effective reduction of COD values and suspended solids. This design works by connecting multiple number of individual type of designs either single chamber, dual chamber, vertical upflow, or flatbed model (Wang et al. 2017).

8.6 Microbes Used for MFC

8.6.1 Bacteria

As a biocatalyst, microbes play a foremost role in the MFC. The selection of pure culture has its output records based on their substrate and metabolism efficiency. Mixed culture with compatibility issues face minimal power turnover (Shen et al. 2019). Most of the microbes selected for MFC production belong to bacterial species which are archaebacteria, acidobacteria, cyanobacteria and proteobacteria (Cao et al. 2019). Firmicutes are microorganisms which have a high electrogenic property and have the potential to produce the power, known well as electricity-producing microbes. The bacteria belonging to *Archaea* family are able to survive at extreme environmental conditions such as high temperature and pH. This microbial family encompasses greater potential to behave as an electrigen. It has been proved that the microbes which have been selected from bacterial species particularly from *Archaea* including *E. coli* produce higher power as compared to the other bacterial species (Fig. 8.3).

8.6.2 Fungi

Fungi are also one of the microbial species selected and widely used for biofuel and power generation. Fungi can be used as an MFC, in the combination with bacterial culture helping in the production of sustainable energy (Sekrecka-Belniak and Toczyłowska-Maminska 2018). Though fungi have been used as a microbial fuel cell, thorough studies and analyses are not yet well documented. In contrary to bacteria, fungi also metabolize to perform the mechanism of the electron transfer

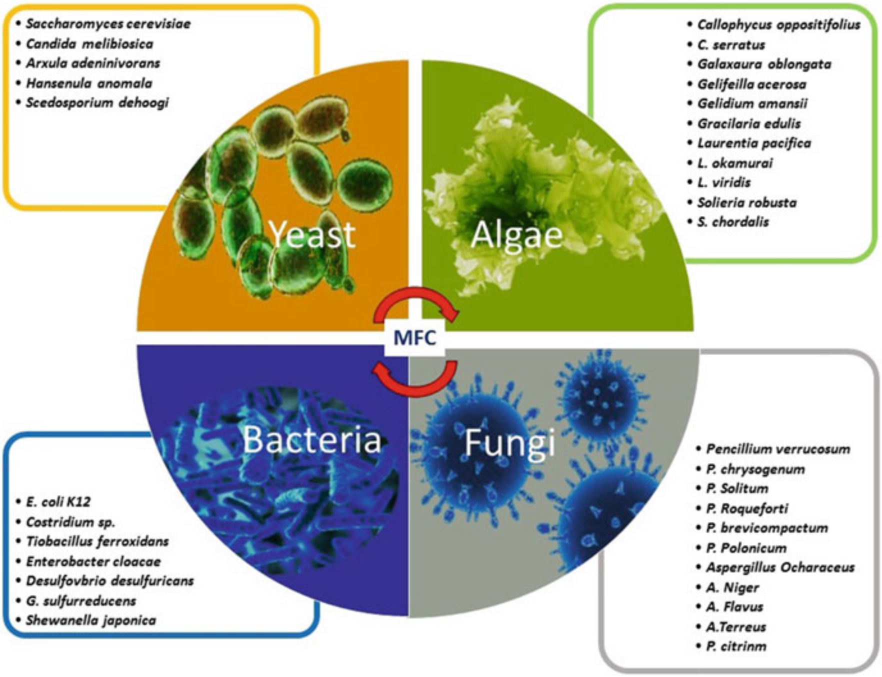

Fig. 8.3 Microbes reported with by far power density

process (Shen et al. 2019). Moreover, the fungus-based electrodes are used for electrochemical reactions and analyses. Fungi act as a biocatalyst in the anode electrode which produces electron via metabolic activity and also works in the cell attachment in the anode which results in the biofilm that can be used for further researches and analyses. The power generation by using fungi as an MFC is depended upon the ambient factors of the anode and cathode material, exogenous mediators and PEM that impacts the energy production by fungi. It also faces certain drawbacks in power production that ensues in low sustainable power in the usage of single- and dual-chambered MFC. Unlike single-chambered MFC, the double chambered one is widely suggested owing to the avoidance of internal resistance and in turn, aiding the fungi to yield a substantial energy output.

8.6.3 Yeast

Like bacterial culture, yeast strains are also widely used as an electrogenic medium for MFC. However, various bacteria are studied and have been employed very recently to replace yeast which is equipped with bifunctional enzymatic supported metabolism for active and complex substrate utilization (Rahimnejad et al. 2011).

Most of the bacteria have been used as catalysts for MFC. Yeast shows a clear and well-distributed genetic pathway; it gives a reliably faster growth rate. Comparing with the bacterial MFC, yeast culture shows and gives less power efficiency, but it also seeks attention in this green approach with unique properties. In the yeast cells, the presence of redox proteins helps in the direct electro-transport mechanism. Yeast was also used to enhance the attachment of cells in the electrode, which helps in biofilm characterization (Singh et al. 2017). The most widely used yeast cultures are *Saccharomyces cerevisiae*, *Candida melibiosica*, *Hansenula anomala* and *Arxula adeninivorans*. Although various strains have been reported, lesser output is being recorded as compared to the bacterial cultures (Fig. 8.3).

8.6.4 Algae

In MFC, diverse microorganisms have the ability to serve with a greater potential for electricity production (Jagadevan et al. 2018). Generally, algal species also exhibits the enhanced peak of power output. Usage of eukaryotic algal species does not utilize any external substrates in MFC, as these algal species serve as a substrate and medium on its own for electricigens in the MFC. Additionally, algae can act as both donor and acceptor of electrons due to the autotrophic nature (Huarachi-Olivera et al. 2018). This eukaryotic algal culture can be placed in the cathode chamber of MFC. It has the capability of utilizing CO_2 which can be used to generate O_2 and helps in the cathodic chamber. Algae contain lot of biopotential in nature and there are enormous number of microalgae which are employed for the various applications due to their capabilities and metabolic activities (Jagadevan et al. 2018). An eukaryotic alga contains greater relative adaptability and is used for quick generation time.

Algae can also be grown in a medium which contains low amount of carbon source and also an energy source. It can also function in dark conditions with an organic substrate that results in functional photosynthesis and it can also utilize the energy from other resources, i.e., (available resource) which are considered as waste. Due to its wide application algal species are used in many studies for generation of biofuel development and commercialization, bio-oil, methane, hydrogen and methane. Though algae are not exoelectrogens, they are documented to produce and perform redox reactions due to its metabolic activities.

8.7 Factors Influencing MFC

8.7.1 pH

There are various factors which influence the power generation in the MFC processing and also in other areas, viz., wastewater treatment plants. In order to obtain higher energy output, it is essential to know about the influencing factors to get desirable and active performance. In the list of various influencing factors; pH, temperature, microorganisms, electrode, mediator, proton-exchange membrane and substrates are mostly considerable to achieve highly efficient MFC.

Ammonia depletion in the organic matter results in the direct influencing factor of pH in the MFC. At higher pH, the microbes in the bio-anode get affected and this affects the power generation; likewise, loss (or) removal of organic nitrogen and phosphorus will make changes in the fuel cell production. It has been identified that the compatible microbial consortium in the anodic chamber is in acidic, neutral, and also alkaline medium.

The microbial fuel cells show optimal power output in the acidic environment compared to the other medium. The performance of most microbes is no longer viable in the acidic medium below 8, but it has been stated that most microbes show better growth in acidic environment during the process of MFC. The lower proton transfer rate results in lower anodic and cathodic consumption and in turn, pH gradient between the chambers. The growth of microbes and transfer (production) of protons reverse the pH that is maintained under anaerobic conditions. pH gradient will also have implication on other factors which affects the effective power production. So, it is considered as the primary essential factor to identify the selective pH range which is suitable for the electrogenic microbes.

8.7.2 Temperature

Temperature is considered to be the greatest influencing factor for the efficient working of MFC as it is dependent on internal resistance. Additionally, the electrode potential and the electrolyte conductivity also get affected with an increase in temperature. Contrastingly, some of the electrigens are capable of adapting themselves at very low temperature. In the operation of microbial fuel cell, the process temperature must range between 10 and 20 °C that has an impact on process operations' cost reduction and decrease in power for heating. Influence in temperature results in the "COD removal" desalination, etc. as witnessed in many studies (Siddique et al. 2017). Due to the negative influence of temperature, there will be reduced power output in the performance of MFC. They do have a strong effect on the biochemical process of the microbial consortia. In order to achieve sustainable power production in MFC, the optimal conditions and factors should be maintained. If the temperature parameters are increased as compared to the optimized range, the power output will drastically decrease which is figured out clearly whether it is above or below the range.

8.7.3 Electrode Material

To scale-up power production using microbial fuel cells, the choice of electrode material is one of the significant factor which influences the performance. Another consideration is of the electrode material; in displaying effectiveness of cell attachment, that is, adhesion of microbes to the anode material and also promotion of electron transfer mechanism for the electrochemical sysnthesis. (Mustakeem 2015). In order to construct a biofuel cell deploying microbes, cost-effective carbonaceous materials exhibiting a constant power output are used. In case of cathode electrode, the selected material should perform the catalytic reactions and also have the catalytic properties. It has been proved that carbon-based electrodes contain strong cell attachment property as compared to other materials. For MFC to produce sustainable output, it is better to choose low-cost material which has high stability and sustainability. Though the anode and the cathode electrodes are different in their material choice, the selection of material should be biocompatible which helps in the adhesion of cell culture in the electrode, especially anode.

8.7.4 Mediators

Based on the mediators, there are mediated MFC and mediator-less MFC. Mediator-less MFCs are most widely used and most preferred for the power generation and biofuel production process, exhibiting enhanced performance as compared to mediated MFC because mediator usage in the form of organic compound may end in toxicity to the microbial strain selected to perform as MFC. Mediated MFC is not considered as cost effective to the recurring need of mediator to perform as electrigens. It also complicates the production process so it is not widely considered for the MFC development. Moreover, mediator-less MFCs have the ability of transferring (disposal) of electrons from substrate oxidation. The major drawback in using the mediator-less MFC is that there is no evolving of the exoelectrogens which are used in agricultural and many other applications (Pisciotta et al. 2012). The mediator MFC produces output power density of about 0.1–1 AM^{-2} which is less as compared to that of mediator-less. The MFC research on various mediators is still in indefinite structure. Search of potential exogenous mediators is an identified area to develop the research element.

8.7.5 Proton-Exchange Membrane (PEM)

Separation of liquid content in the two chambers is the major role of the proton-exchange membrane. PEM separates the electrodes as well as supports the transfer of protons from the bio-anode to the cathode where PEM acts as a transmitter. PEM also reduces the rate of oxygen dispersal in the bio-anode of the living cells. So, it is the greatest factor to be considered during MFC chamber construction. The materials used to build up PEM should contain unique and delicate work function satisfying the cheap and the higher working efficiency.

In exchange to the PEM, cation-exchange membrane (CEM) also came into the research screen and it has also been tested whether CEM can replace PEM by using any fabricating agents such as clay. But compared to PEM, CEM is expensive. Moreover PEM allows only the relocation of protons from anode to cathode and also reduces the rate of oxygen dispersion towards cathode from anode cell. Hydrogen ions pass through Nafion (PEM) to cathode (Holladay et al. 2009). The concentration gradient takes place across the membrane generating current along with concentration of (H^+) ions.

8.8 Application

8.8.1 Biosensor

Sensor is a device that is used for sensing the signals (frequencies) produced. The information that is received by the sensors will be transmitted to the signal; likewise biosensors are now prominently used in the environmental studies. In the biosensors, there will be a biological element which helps in producing a bio-response. In addition to that, MFC-based biosensors are widely used in the research areas (Stein et al. 2010). These are used to identify and calculate the parameters and the factors in the biological process. Specifically, it is used in calculating dissolved oxygen (DO) in the medium, chemical oxygen demand (COD), biological oxygen demand (BOD) and other components in the media and also the microbial kinetics and activities (Cui et al. 2019). Because of these properties, MFC-based biosensors are becoming a promising area in this modern research arena. It can also be used in monitoring temperature, pH and chemical composition in the surroundings. Comparative studies suggest that MFC-based biosensors give greater output in terms of accuracy and it has been used for approximately 5 years. Due to its long-term usage, it earns and captures its attraction towards MFC-based biosensors.

Towards the detection of biological oxygen demand, MFC biosensors can be modified and designed to satisfy the need of power generation in rural areas as MFC-based biosensors have the potential in generating power and reducing the burden of environmental hygiene and adulteration in the rural waterbodies. MFC-based sensors are also used to study the water quality which can be aided by using ANN to the biosensors; that is, with the help of computerized language, the biosensors are used in sensing the water quality treatment. It can also be used as a bio-electron sensor during the redox reaction occurring at cathode. If there is a lack of electrons that have to be transferred to cathode, during that time this sensor is used which is a type of MFC-based BOD sensors (Chang et al. 2005). It has application in sensing the anodic overpotential which mainly occurs during the detection of toxic events. The MFC-based biosensor is used for identifying anodic potential, pH, and also concentration of media and substrates.

8.8.2 Biohydrogen

In MFC during the electron production, i.e., metabolism of the microorganism, there occurs the protons and electrons that are released, which can further be transferred to the cathodic chamber via PEM (or as an external circuit). The protons and electrons perform redox reaction and reduce the oxygen diffusion and also limit (H^+) ion production. Bio-hydrogen produced by the MFC consortia (bacteria) will convert the substrates (i.e., metabolize) to produce CO_2 and hydrogen gas (State and Grot 2005). Then the produced protons and electrons combine with O_2 to form H_2O. In the process of energy generation by MFC, the hydrogen produced can be harvested and can be used for further application of MFCs like supplying biohydrogen fuel which can be achieved by increasing the anodic potential and decreasing the oxygen diffusion in the cathodic chamber.

8.8.3 Agriculture

An MFC is a biochemical process in which the organic wastes are used as a substrate which gets oxidized and metabolized by the single type of microbe or indigenous microbial consortia. The oxidized substrate can be used as a biofertilizer. The most important factor in MFC process is that it has numerous added applications to its process of energy derivatization. Metabolism sequence yields multiple by-products based on their substrates and their environment. Due to its multidisciplinary approaches, it has been used in various applications including agriculture. MFCs perform various actions like help in decomposing (or) degrading the wastes (substrates). MFC in agricultural livestock has a great potential in the electricity and biofuel generation. MFC has been doing a great help for farmers by decreasing the environmental and cultivating costs of farming. The indigenous soil microbial consortium is reported to generate energy which again stimulates farming practices through the electroculture. The MFC in agriculture is the way to promote the green and bio-sources for the production of bioelectricity (green electricity).

8.8.4 Wastewater Treatment

More recent issues on potable water scarcity are probably increasing (Feng et al. 2017). Decreasing the rate of wastewater generation is not possible due to the industrial revolution in this era. Utilizing the wastewater by treating either through physical or biological aspects is the only key element available to increase the water resources (Muga and Mihelcic 2008). The wastewater mass contains certain unwanted organic and chemical substances that influences the well-being of the ecology, ecological problems and sanitation owing to improper removal. This issue is being addressed by many technologies to achieve the quality. Microbial fuel cells are now taking charge and are being used for the treatment process; the sludge produced in the waste management is being prospected and reported positively for its reduction (Feng and An 2014). By using selective MFCs, the sulfur compounds and organic wastes present in the water can be utilized by the microbes where the chemical energy of the wastewater to be treated is converted to electrical energy which is linked to or connected by a circuit. It occurs with the help of electrochemical reaction. Moreover, MFCs are also used for removing the refractive organic pollutants from the effluent.

In the conventional process for treating wastewater, the demerit is the management of solid waste risen due to treatments. Anaerobic digestion is the common process practiced for treatment of refractive organic pollutants (Chaturvedi and Verma 2016). Some physical processes of treating waste consume higher energy. But MFC contains many features like stability, low cost, quality improvement and reusability. Due to these prominent features, MFCs have become an active technology in the wastewater treatment process. Contribution to the green approach in any environment is safe and to that end, MFC is a cost-effective system to accelerate the wastewater treatment and in activated sludge processing (Ismail and Jaeel 2013).

8.9 Recent MFC Design

8.9.1 Biofilm

Biofilm has the crucial role in designing efficient MFCs. Formation of biofilm over the anode surface can increase the MFC potential as in Fig. 8.4. The density of biofilm on electrode depends on the growth of the microbial cells and its metabolic activity. The electrochemical analysis cannot be determined by using the density and availability of biofilm (Krige 2019). Moreover, it can handle with the live and dead cells where the dead cells are formed on the inner layer, i.e., the upper surface of anode electrode. The factors which influence the characteristic of biofilm are nutrient starvation, salinity, pathogen invasion and depletion in the level of nutrients.

The biofilm formed in the anode can be further researched for phylogenetic analysis and derived to a conclusion of compatible microbial community whether bacterial, fungal or actinomycetes-based community depending on single dominant species or not. The tree can be developed based on either 16s rRNA or 18s rRNA gene sequencing of the biofilm extracted from the anode electrode or metagenomics analysis (Ishii et al. 2008).

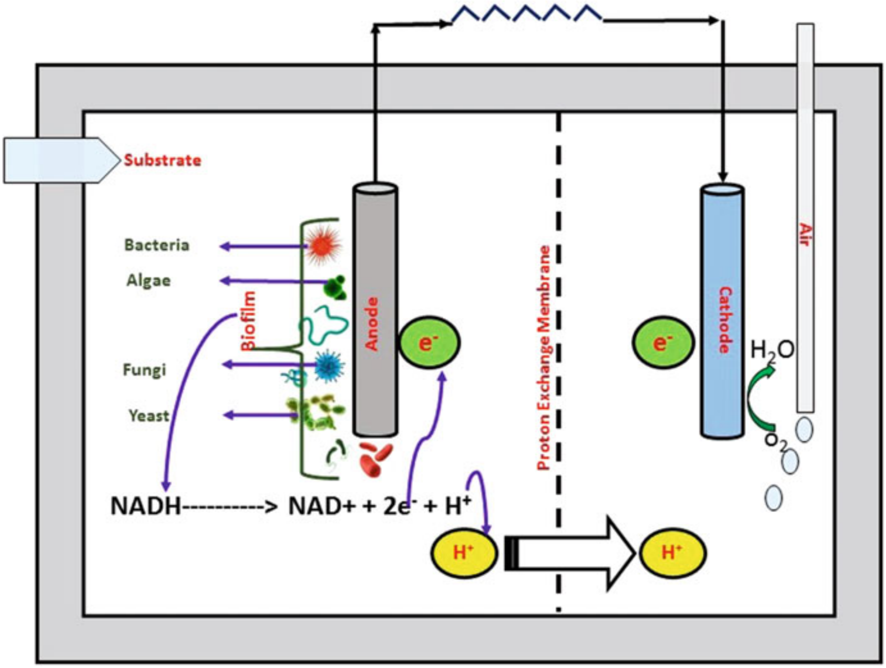

Fig. 8.4 Structure of biofilm MFC

8.9.2 In Silico Method

In recent times, the in silico method has been widely incorporated in the MFC for power generation; in that case the metabolic flux analysis between an MFC and non-MFC is performed by in silico analysis without using any mediators. In this methodology the metabolic activity of microbial consortia and screening of microbes have been recognized, resulting in an improved MFC performance. Also, in silico methodology, there are many tools and applications which are used for the investigation and analysis of the performance (Kenny and Flynn 2014). For example, nested analysis, flux, SEM analysis, and many computational studies can be done to improve the MFC efficiency before the conduct of any real-time experiments. From this, we can find out the gene deletion analysis which shows the genes responsible for producing and increasing the rate of energy. Gene knockout analysis is also done for the strain improvement which leads to upsurge in the rate of power generation. It will support and optimize the production to achieve the nutrient supply, starvation, deletion and selection of the strain and its characteristics. Simulations will reveal the maximum growth rate of the microbial consortia and the required growth medium along with the rate of dilution.

8.9.3 Self-Rechargeable Device

In concern with the usage of power in this modern era, MFCs can be designed to charge the mobile phones and other auxiliary electronic devices. At first, the MFCs are tested in charging the basic mobiles and now it extends to the smart phone. There are enormous developments in the MFC technology. The microbial fuel cells fed with urine are used for charging the smart phone and this is the first time the full charging of mobile has been done with the aid of urine transduction to energy and is mainly concerned with the remote system-type charging. Here, urine is used as a substrate for MFC at first, taking around 42–68 h to charge, thereby, aiding the usage of mobile continuously for 3 h (Walter et al. 2017). Silicon-based nanowires are used with MFC to produce self-rechargeable mobile phones and they convert those energies into usable electrical energies with the support of silicon nanowires and MFCs (Krishna et al. 2012). Self-stratified and super-capacitive MFCs are fed with urine to produce higher output power of about 1.20 ± 0.04 mW (2.19 ± 0.06 mWme^{-1}) for a pulse of 0.01 s (Santoro et al. 2019). MFC-driven energies are used as sensing devices to quantify CO_2 and greenhouse gases and for long-range wireless data communication (Yamashita et al. 2019).

8.10 Future Perspective

The MFC-based application area has been very limited so far. Exploring the MFC for every required electronic device like, self-driven robotics with MFC is in the near vicinity. The alternative MFC construction material, microbe, influencing factors, and design were well studied in terms of maximizing the output. Microbes are the only alternative resources for all problems of depleting natural resources as is proven in many cases of pharmaceutical, waste management, agriculture and for all toxic chemical alternatives. The green or free energy in terms of solar dependency can be combatted by the use of microbial fuel cells with wide implementation of compact MFC design for every house with their own set of substrates or centralized MFC-supporting common substrates. Implementation of MFC can bring commendable changes in the depletion ratio of fossil fuels.

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Chapter 9
Biocatalysis of Biofuel Cells: Exploring the Intrinsic Bioelectrochemistry

Srinithya Ravinuthala and Saprativ P. Das

Abstract Biofuel cells are potential multifaceted solutions to a myriad of environmental concerns of pollution, waste management, and resource depletion. Along with serving as alternative green energy sources, biofuel cells can simultaneously be used for wastewater treatment, bioremediation, and producing valuable by-products, viz., biohydrogen (Bio-H_2). The underlying mechanism of energy production in biofuel cells lies in the catabolism of complex substrates by biocatalysts through fermentation or redox reactions with the subsequent release of electrons as by-products. The power-generating capacity of biofuel cells is dependent not only upon the biofuel system (anode-cathode materials, configuration of the cell), but equally upon the reactions involved in breaking down the substrates to produce electrons along with the electron transfer mechanisms. Despite the complex biochemical and electron pathways involved in electricity generation in biofuel cells, the same property proves biofuel cells to be advantageous over typical chemical fuel cells—a versatile range of substrates can be used for generating electricity. Also, due to the usage of biocatalysts in biofuel cells, the conditions required for electricity generation are milder as compared to typical chemical fuel cells. This chapter explores the types and various biochemical pathways of biocatalysts used in biofuel cells, electron transfer mechanisms, as well as anodic and cathodic reactions, with emphasis on recent advances and future prospects towards improving the performance of biofuel cell biocatalysts.

Keywords Biocatalysis · Biofuel cells · Electron transfer · Bioremediation · Biohydrogen

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9.1 Introduction

Environmental degradation, pollution, global climate change, and resource depletion are among the top environmental concerns the world is facing, due to the high dependency of the global population upon fossil fuels for energy generation. According to the Living Planet Index Report 2020 from World Wildlife Federation (WWF), these deleterious consequences not only affect the environment, but are equally detrimental to biodiversity as well. In the search for alternative cleaner, greener energy sources, there has been a sharp rise in the interest for biofuel cells (BFCs) over the past decade. A biofuel cell (BFC) can be defined as a system in which energy is produced through the catabolism of complex substrates by biocatalysts through a series of oxidation (more specifically, loss of electrons) and reduction (gain of electrons) reactions on the anode and cathode, respectively, following the release of electrons as by-products, causing a current flow. BFCs not only serve the purpose of electricity generation (Cui et al. 2014; Kumar et al. 2017a, b), but can also simultaneously be used for other applications including septic (Thulasinathan et al. 2020) and also industrial wastewater treatment (Mahdi Mardanpour et al. 2012; Mohanakrishna et al. 2018), degradation of antibiotics (Guo et al. 2016) and toxic xenobiotic compounds (Cao et al. 2010; Pontié et al. 2019), water desalinization (Jacobson et al. 2011; Elawwad et al. 2020), monitoring systems (Kim et al. 2003), and implantable power sources (Halámková et al. 2012).

The earliest reports of using biocatalysts for bioelectricity production were in the year 1911, when Potter had used *E. coli* and *Saccharomyces cerevisiae* as the biocatalysts, platinum for electrode material, and substate as sugar solution. From his series of experiments, he observed that the maximum output of voltage was independent of the volume of the container, as well as the thickness of the electrode (Potter 1911). This is theoretically proven as well, and the calculations for the same are discussed later in Sect. 9.4.1.

Cohen Barnett, in 1931, connected a series of bacterial cultures to obtain a battery of potential 35 V, however only 2 mA of current. He also suggested adding potassium ferricyanide or benzoquinone to increase the performance capacities of the cells, which are now referred to as mediators. Bennetto et al., in the 1980s, taking this point into consideration, extensively worked on various classes of mediators for enhancement of BFC performance (Delaney et al. 1984). Later in 1999, Kim et al. discovered that *Shewanella putrefaciens* can perform transfer of electrons lacking the addition of artificial exogenous mediators (Kim et al. 1999).

In 1963, NASA announced a space program to transform human waste to bioelectricity in space flights, which stirred a short-term hype towards micro-bioelectrogenesis. Towards the turn of the century, Habermann and Pommer demonstrated a MFC that could function over a period of 5 years without any maintenance or malfunction, which used sulfate-reducing bacteria. Their work was also a pioneering milestone of using MFCs for simultaneous wastewater treatment and bioelectrogenesis (Habermann and Pommer 1991).

In the current years, there has been a sharp rise in understanding, and has led to the development of technologies and establishment of startups based upon this concept. In 2015, Universidad de Ingeniería y Technology (UTEC), Peru, developed a "Plant Lamp," a plant-based microbial fuel cell (MFC) providing up to 2 h of light in a day through high-illumination LED lamp, expending little power consumption. It especially benefitted the rural people of the Ucayali region, known to have one of the lowest rates of electricity access. Another example is "Plant-e," a Netherland-based startup that uses plant-based microbial fuel cell-generated energy to decorate landscapes with fairytale lights and LEDs. Their recent project is "The Park of Tomorrow," the world's first park with interactive lights based on plant-based MFCs. "JSP Enviro" is a startup based in Chennai, India, using MFC technology to treat wastewater from dyeing, printing, leather, and dairy industries in India and simultaneously generate energy. Compared to the current conventional technologies in the market, this technology proves to be more cost effective and requires low maintenance, making it accessible to small industries as well.

Though BFCs prove to have a plethora of potential applications, the underlying pathways and mechanisms of biocatalysts remain the same. And as with any biological system, we can work towards working of biofuel cells to perform to the fullest only when there is a sound understanding of these mechanisms and pathways. This chapter aims at unravelling the biochemical and molecular workings crucial for the very process of bioelectrogenesis.

9.2 The Essentials of BFCs

The architecture/structure of biofuel cells is an important factor that determines the working efficiency of these cells. Though over the years a diverse range of designs and materials have been used to construct biofuel cells, the basic components do not vary. BFCs can be divided into two types based in their construction—single-chambered and double-chambered BFCs. Depending on the presence and absence of a membrane, they are further divided into membrane and membrane-less. Figure 9.1 represents the typical construction of BFCs.

9.2.1 Biocatalysts

Biocatalysts in the anode break organic substrates down into simpler molecules and generate electrons in the process (oxidative reactions), whereas biocatalysts in the cathode act as electron acceptors (reductive reactions). Electrons released during oxidation in the anodic terminal are transported to the cathode, passing across an external circuit; protons generated in these reactions diffuse through the cell to the cathode, ultimately creating electron flow, and thus an electric current. Biocatalysts operate under milder conditions and can utilize a larger range of substrates as fuel for

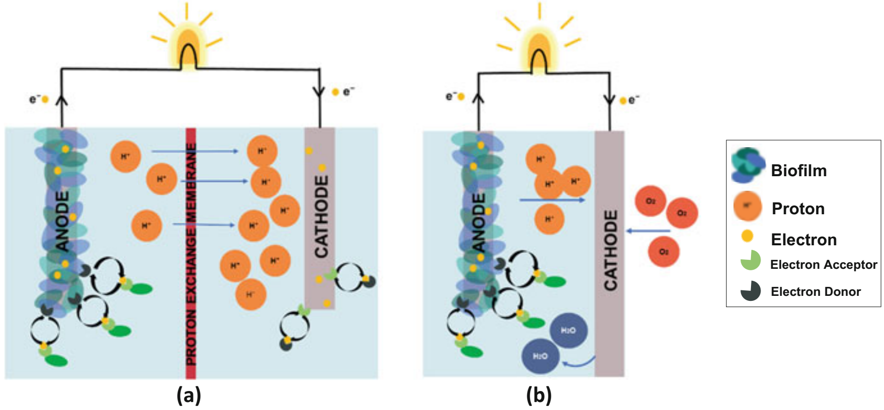

Fig. 9.1 Basic construction of typical BFCs: (**a**) Double-chambered BFC with proton-exchange membrane separating both chambers; (**b**) membrane-less single-chambered BFC with air-cathode (cathode with the terminal electron acceptor as oxygen)

electricity generation as compared to their chemical fuel cell counterparts. Biocatalysts can be broadly divided into the following types—whole-cell, enzymatic, and organelle-based biocatalysts.

9.2.1.1 Whole-Cell Biocatalysts

Whole-cell biocatalysts are microorganisms which catalyze reactions of metabolizing substrates through either oxidation or reduction reactions, either at the anode or the cathode, respectively. Biocatalysts from all three domains—archaea, bacteria, and fungi—have been studied and used for bioelectricity generation. Exoelectrogens are microorganisms with abilities to transfer electrons extracellularly, from their inner cell membrane. Electrotrophic microorganisms have the capacity to accept electrons from extracellular sources. Typically, exoelectrogens are found in the anodic chamber, catalyzing oxidative reactions, and electrotrophs in the cathodic chamber, catalyzing reductive reactions (Logan et al. 2019). A consolidated list of whole-cell biocatalysts identified in various studies is given in Table 9.1.

Bacteria are the most commonly used biocatalysts in BFCs due to their relative abundance and ease of cultivation. *G. sulfurreducens* (class—Deltaproteobacteria) and *Shewanella oneidensis* (class—Gammaproteobacteria) are model organisms studied owing to their exoelectrogenic and electrotrophic properties. Several studies have shown that mixed cultures containing both electrically active and non-active bacteria perform more efficiently and give higher power densities as compared to pure cultures of electrically active bacteria alone. Limitations of pure cultures are that they could be cultured only in a limited range of substrates and require relatively stringent conditions for growth and sustaining (Sharma and Kundu 2010).

Table 9.1 Exoelectrogenic and electrotrophic whole-cell biocatalysts

Exoelectrogens	Electrotrophs
Bacteria *Geobacter sp.* *S. oneidensis* *Proteus vulgaris* *Rhodoferax ferrireducens* *Klebsiella pneumoniae* *C. freundii*	Bacteria *Geobacter metallireducens* *A. faecalis* *Desulfopila sp.* *Desulfovibrio sp.* *Marinobacter spp.* *E. cloacae* *Shigella flexneri* *Moraxella catarrhalis*
Archaea *Methanosarcina acetivorans* *Geoglobus ahangari* *Pyrococcus furiosus* *Candidatus sp.* *Nitrosomonas sp.* *Acidiphilium sp.* *F. placidus*	Archaea *Methanobacterium spp.* *Methanococcus maripaludis*
Eukaryotes *S. cerevisiae* *C. melibiosica* *Arxula adeninivorans* *Hansenula anomala* *Kluyveromyces marxianus*	Eukaryotes *Trametes versicolor* *Ganoderma lucidum* *Galactomyces reessii* *Rhizopus sp.* *Aspergillus sp.* *Penicillium sp.*

Bioelectrochemical systems that use photosynthetic organisms as biocatalysts and light as a power source for water-splitting reactions for bioelectricity production are termed as photosynthetic microbial fuel cells. Cyanobacteria and algae have been of great interest as biocatalysts due to their minimal nutrient requirements and high-value biomass production. As they have both exoelectrogenic and electrotrophic properties, they could serve as both electron donors in the anodic terminal and electron acceptors in the cathodic terminal. In the anode, they could be used for solar-powered water oxidation; the anode chamber containing carbon dioxide can be utilized at the cathode facilitating cathodic reactions. These photosynthetic organisms can also be used to serve as substate for the other biocatalysts in mixed consortiums in the anode.

Archaea have been studied to produce bioelectricity as they could function in extreme environments and could use a completely different range of substrates. Using extremophiles that have electroactivity in extreme conditions like pH and temperature reduces contamination possibly by other microorganisms, and increases growth and metabolism rates, thereby incasing power output. Methanogens and ammonia-oxidizing archaea have been employed to use methane and ammonia gases as substrates, respectively. Another interesting and recently discovered class of archaea is *Candidatus* sp., or the “cable bacteria,” belonging to the Desulfobulbaceae family. They oxidize sulfides of both freshwater and seawater sediments in anaerobic conditions, and have been observed to grow multicellular filaments that are electroconductive. These filaments could grow more than 1 cm, forming cable-like structures for electron transport over long ranges (Logan et al. 2019).

Fungi, particularly yeast, have been extensively used in BFCs due to their fast growth, non-pathogenicity, and ease of culturing. Yeast has the ability to transfer electrons directly and through electron shuttles. However due to respiration taking place in them mitochondria, the membranes being a hinderance for free flow of electrons, cause relatively lower power densities. *Saccharomyces cerevisiae* is the model yeast organism used for exoelectrogenic studies. Other fungi such as *Trametes versicolor* and *Ganoderma lucidum* have been used in the cathode as they secrete laccases, multicopper oxidases that catalyze reduction of oxygen to water (Sekrecka-Belniak and Toczyłowska-Maminska 2018).

9.2.1.2 Enzymatic Biocatalysts

Enzymatic biocatalysts are immobilized on the electrodes of biofuel cells through physical or chemical methods. They generally have higher power densities as compared to the BFCs using whole-celled biocatalysts—the defined biochemical pathways due to the use of specific enzyme(s) help overcome mass transfer resistances, also eliminating the need of using membrane separators. However, most BFCs employing enzymatic biocatalysts use only one enzyme, causing the substrate to be only partially oxidized, whereas in BFCs using whole cells, the substrate is completely oxidized. Another limitation of enzymatic biocatalysts is that they are not as economical as their whole-cell counterparts due to their shorter life spans and expensive extraction processes (Jia and Wang 2010; Pant et al. 2012). Table 9.2 indicates some commonly used enzymatic biocatalysts and their substrates/terminal electron acceptors in BFCs.

According to the position of the active site, enzymatic biocatalysts are classified into three types (Fig. 9.2) (Yu and Scott 2010):

1. Contain cofactors nicotinamide adenine dinucleotide (NADH/NAD+) or nicotinamide adenine dinucleotide phosphate (NADPH/NADP+) as redox centers: These enzymes have diffusible active centers that are feebly bound to the enzyme's protein portion. The active centers serve as acceptors of electrons and are reduced; upon transmission of the electrons to the electrode, they return to

Table 9.2 Enzymatic biocatalysts and substrates

Anodic biocatalyst	Substrate	Cathodic biocatalyst	Terminal electron acceptor
Glucose oxidase	Glucose	Laccase	O_2
Dehydrogenases	Methanol Ethanol Glycerol	Cytochrome oxidase	
Hydrogenase	Hydrogen	Horseradish peroxidase	H_2O_2
Krebs cycle enzymes	Pyruvate	Microperoxidase	

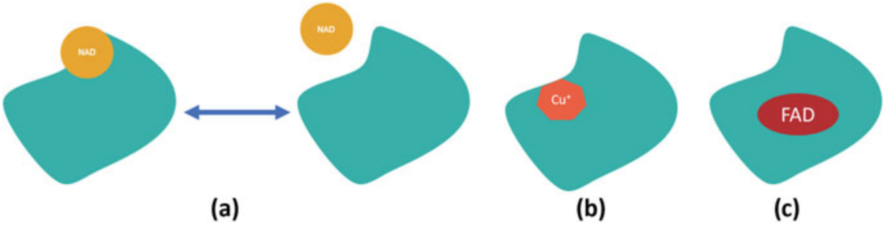

Fig. 9.2 Enzymatic biocatalysts—classifications: (**a**) enzymes with diffusible redox centers; (**b**) enzymes with redox centers near periphery; (**c**) enzymes with redox centers deep inside

their oxidized state, acting as electron shuttles. Oxidoreductases such as lactate dehydrogenase and alcohol dehydrogenase belong to this category.

2. Contain the redox center on or near the periphery portion of the enzyme, allowing direct exchange of electrons: The orientation of the active site is a significant factor that determines the effective functioning of the enzyme. Examples include peroxidases and laccase.
3. Contain strongly bond redox centers deep inside the enzyme protein structure or surrounded by a glycoprotein shell, thus making the diffusion of electrons to the electrode extremely slow or impossible: For electrons to be transferred over this distance of more than 21A, redox mediators are required. The components of the succinate dehydrogenase complex, containing the redox-active coenzyme flavin adenine dinucleotide (FAD), fall under this category.

9.2.1.3 Organelle-Based Biocatalysts

Another class of biocatalysts used relatively lesser in BFCs is organelle-based biocatalysts. Compared to single-enzyme biocatalysts, they prove to be more advantages as a cascade of enzymes are used, hence the ability to completely oxidize a substrate. Mitochondria have been used on the anode to oxidize pyruvate completely through Krebs cycle (Lapinsonnière et al. 2012). In 1980, Janzen and Seibert conducted the first known studies upon anoxygenic subcellular photosynthetic reaction centers as biocatalysts in BFCs using *Rhodobacter sphaeroides*. When light was shined upon the photosynthetic centers, electrons released from the photosynthetic centers flowed into the connected electrodes, thereby a current flow (Janzen and Seibert 1980).

9.2.2 Substrates

Substrates in BFCs are basically the fuels which are oxidized by the biocatalysts. This is the first step towards generating bioelectricity, and therefore the substrate is one of the primary and crucial factors which determine the power generated in a BFC. Biofuel cells have the capacity to utilize a diverse range of both simple and complex substrates, unlike typical fuel cells. The substrate availability and cost of procurement are determinants of a BFC's economic feasibility (Ivase et al. 2020). Substrates used in BFCs may be simple as molecules, viz., glucose, butyrate, acetate, glycerol, lactate, methane, ethanol, methanol, and cellulose, or may be complex mixtures like food wastes, urine, wastewaters from industrial and domestic sources, and marine sediments. Acetate is one of the most preferred substrates as it generates a higher power output as compared to other substates; it is also not prone to fermentation or any other biochemical pathways at room temperature (Pant et al. 2012).

9.2.3 Electrodes

The electrodes in BFCs are the conductive materials that generate a potential and electrical current through harvesting, transferring, and reducing electrons. Along with having high conductivity, electrode material should be biocompatible and chemically stable, to prevent the hinderance of sustainable functioning of the biocatalysts (Gul and Ahmad 2019). The surface area and distance between the electrodes are factors that influence BFC power density. With upsurge in the surface area of anode, there is a decline in power density; increase in cathode surface area leads to increased performance. As the distance between the electrodes increases, BFCs are more prone to ohmic losses (Krieg et al. 2014).

9.2.3.1 Anode

The anode is the site of substrate oxidation and electron harvesting. All whole-celled biocatalysts are either obligate or facultative anaerobes, hence requiring the anode to be placed in anaerobic conditions for optimal performance. Such organisms that oxidize substrates along with the relocation of the electrons to the anode are known by various names—exoelectrogens, electricigens, and electrogens. Bacteria pertaining to this particular class are also known as exoelectrogenic bacteria and anode-respiring bacteria (Mathuriya et al. 2018). Materials used for the anode include graphene, carbon nanotubes, charcoal, stainless steel, etc. Due to its high conductivity, chemical inertness, along with easy availability, graphite is the frequently employed anode material.

9.2.3.2 Cathode

Known as the "electron sink" of BFCs, the cathode is a three-phase interface that occurs at air, liquid, and solid components. The cathode, being an important determinant of the redox potential of the whole BFC, has typically three layers—diffusion layer, conduction-supporting material, and catalyst-reducing agents or electron acceptors (Mathuriya et al. 2018).

Though the materials used in the cathode and anode are usually the same, the cathode is coated by a catalyst layer. Catalysts are required to accelerate the reduction rate, as the cathode is placed in mild temperatures and pH. Oxygen is primarily used as the terminal electron acceptor since it has a high oxidation potential and is abundantly available. The concentration of oxygen availability is directly proportional to the electron flux.

Depending upon the catalyst used to coat the cathodes, they are divided into two types—abiotic and biotic. Platinum is the utmost common abiotic cathode catalyst used owing to its great efficiency of oxygen reduction and low overpotential (Bhargavi et al. 2018). However, as platinum is not economically feasible for larger scale applications, less expensive yet equally efficient alternatives are being searched for. Studies have cited using abiotic cathodic catalysts like carbon nanotube/polypyrrole nanocomposite; silver nanoparticles; nickel-phthalocyanine/MnOx; manganese dioxide; iron and aminoantipyrine (Fe-AAPyr); cobalt oxide; MnO_2; polyaniline; composite catalysts such as manganese/palladium, zirconium/palladium, and nickel oxide/carbon nanotube composite; and biochar derived from bananas.

Cathodes which use biofilms as catalysts are known as biocathodes; the organisms on the biofilm acting as electron acceptors are known as electrotrophs. In aerobic cathodes, also known as air-cathodes, the terminal electron acceptor is oxygen. Some of the species in air-cathodes used include *Alphaproteobacteria, Flavobacterium sp., Marinobacter sp., Roseobacter sp., Bacillus sp., Thiobacillus ferrooxidans,* and *C. vulgaris*. Nitrate and sulfates are used as electron acceptors in cathodes placed in anaerobic conditions. Microorganisms used include anaerobes like *Nitrosomonas* sp., *Desulfovibrio*, Alphaproteobacteria, *Betaproteobacteria*, *Gammaproteobacteria*, and *Flavobacteria*. Another class of biocathodes, known as photo-biocathodes, uses photosynthetic organisms as electron acceptors. The dissolved oxygen is used for the oxygen-reducing reaction (Prakasam et al. 2017). Organisms known to be used in photo-biocathodes include cyanobacteria and blue-green marine algae.

9.2.4 Membrane

Membranes or barriers form a physical separation between the cathodic and anodic regions. They selectively allow cations to pass through to the cathodic region from the anodic region, so that there is a potential difference between the two chambers. Without these membranes, the oxygen diffusing from the cathodic region would compete for the anode, decreasing the efficiency of the cell. A diverse range of membranes have been used in MFCs, right from microfiltration membranes (Sun et al. 2009), ultrafiltration membranes (Zuo et al. 2007), cation-exchange membranes and ceramic membranes (Midyurova et al. 2015) to more cost-effective materials such as cheese cloth (Pamintuan and Sanchez 2019), laboratory gloves (Winfield et al. 2014), eggshells (Ma et al. 2016), glass wool (Venkata Mohan et al. 2008), and J-cloth (Zhuang et al. 2009). Nafion and its modified versions with ceramic or nanocomposite membranes, and salt bridges, are among the most used materials for membranes (Midyurova and Nenov 2016).

9.3 The Mechanisms Behind Bioelectrogenesis

The workings of BFCs are influenced by their environmental factors just as any other biosystem. Factors that influence functioning of a BFC include the pH, temperature, ionic strength, redox potential, etc. Maintaining an optimum, controlled pH helps in producing maximum current and power density along with lessening of internal resistance (Puig et al. 2010). Higher temperature accelerates metabolism of microorganisms. Therefore, at higher temperature there is higher current output along with decreasing resistance. A higher redox potential not only helps in microbial growth but also regulates electron transfer (Larrosa-Guerrero et al. 2010).

9.3.1 Electron Transfer: Types

The flow of electrons from the biocatalysts to the anode, and cathode to the biocatalysts (in biocathodes), has the same basic mechanisms, but opposite directions though the molecules involved may differ. Electron transport takes place by transport of electrons from relatively lower redox potential electron carriers to relatively higher redox potential through a chain of electron carriers. Depending on the requirement of mediators for transport of electrons to the electrode or vice versa, the transferal of electrons can be divided into two types—direct electron transfer and indirect electron transfer (Fig. 9.3).

9.3.1.1 Direct Electron Transfer (DET)

In direct electron transfer, the connectivity of the biocatalysts with the electrode takes place through direct physical contact by forming biofilms on the electrodes or nanowires (modified electrically conductive pili and flagella) (Reguera et al. 2005). Electron transport proteins relocate electrons from the cytoplasm to the electrode. In the case of biofilms, electron transfer to the electrode takes place through transmembrane c-type cytochrome proteins; membrane-bound cytochromes are connected to nanowires which are in direct connection with the electrode (Cao et al. 2019). Many species of metal-reducing bacteria are known to exchange electrons with metals as their terminal electron acceptors. Species such as *Geobacter*, *Rhodoferax*, and

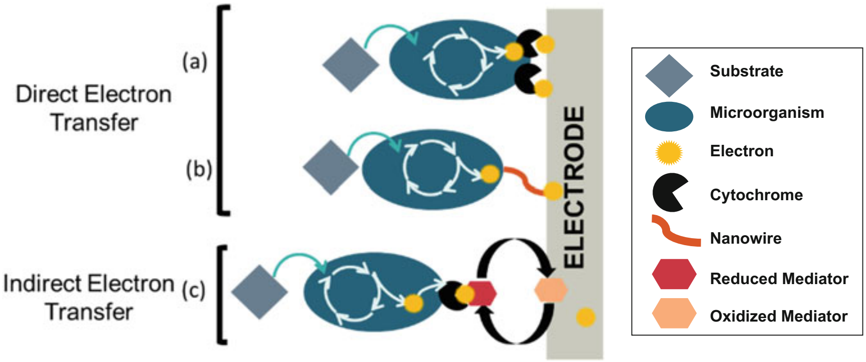

Fig. 9.3 Electron transfer—types: (**a**) direct electron transfer (DET) through cytochrome proteins via biofilm formation; (**b**) DET through nanowires; (**c**) indirect electron transfer via mediators

Shewanella have been known to transmit electrons to the anode, as the terminal electron acceptor (Chaudhuri and Lovley 2003). *G. sulfurreducens*, *Shewanella oneidensis*, *Cyanobacterium synechocystis* PCC6803, and *Pelotomaculum thermopropionicum* are examples of some species that use nanowires for electron transport (Gorby et al. 2006). In bioanodes and biocathodes where DET is the mechanisms of electron transfer, a biofilm upon the electrode is a requisite. Factors that influence biofilm formation, and thereby the efficiency, include surface roughness, surface area, porosity, conductivity, and hydrophobicity (Prakasam et al. 2017).

9.3.1.2 Indirect Electron Transfer

Most biocatalysts are not capable of direct electron transfer as they do not possess the required structural apertures. Instead, they rely upon redox mediators. Redox mediators are low-weight, soluble molecules which can enter the reaction centers of biocatalysts, collect the electrons, and transfer them to the electrode. An effectively functioning mediator must possess an oxidation-reduction potential close to the potential of the biocatalyst, have a fast and reversible electron transfer rate to the electrode, and be chemically stable (Konovalova et al. 2018).

Mediators may be synthesized by the biocatalysts themselves or exogenous (artificial) mediators may be added to the BFC. Species such as *Pseudomonas sp., S. oneidensis,* and *E. coli* have been shown to synthesize pyocyanin, riboflavin, and quinones for extracellular electron transport. Examples of artificial mediators include methylene blue, riboflavin, neutral red, thionine, and humic acid. Adding such exogenous mediators is not a sustainable option—they are toxic both to the microorganisms and to the environment. The cytotoxicity of the mediators also causes the reduction of power output over time. From the practical point of view, periodic addition of mediators is not economically or technologically feasible either (Cao et al. 2019).

9.3.2 Extra Electron Transfer Pathways: At the Molecular Level

While the apertures and mechanisms of electron transfer remain the same, at molecular level, different species use different compounds and pathways to achieve the same. This section describes the extra electron transfer (EET) pathways of the model electroactive organisms *Shewanella oneidensis* and *G. sulfurreducens.*

9.3.2.1 Mtr Pathway: *S. oneidensis*

Shewanella oneidensis, a Gram-negative facultative anaerobe, has been supposed to contain 42 c-cytochromes: 14 containing four or more hemes and 80% of the known found on the outer membrane. It utilizes Mtr pathway for EET, one of the most extensively studied EET mechanisms till date (Fig. 9.4). Initially, quinols are oxidized by TorC and CymA, both of which are tetraheme cytochromes. This allows electron transport in between NADH-dehydrogenase and inner membrane cytochromes, leading to creation of a proton gradient and ultimately ATP synthesis. TorC passes electrons to the periplasmic reductase TorA, which passes it on to the terminal electron acceptor trimethylamine N-oxide, an outer membrane permeable compound, reducing it to trimethoxyamphetamine. CymA, on the other hand, reacts with different redox carriers in the periplasms, thus creating pathways with a number of terminal electron acceptors. For this reason, *S. oneidensis* is capable of utilizing a large assortment of substrates. Some of the redox carriers include octaheme SirA that

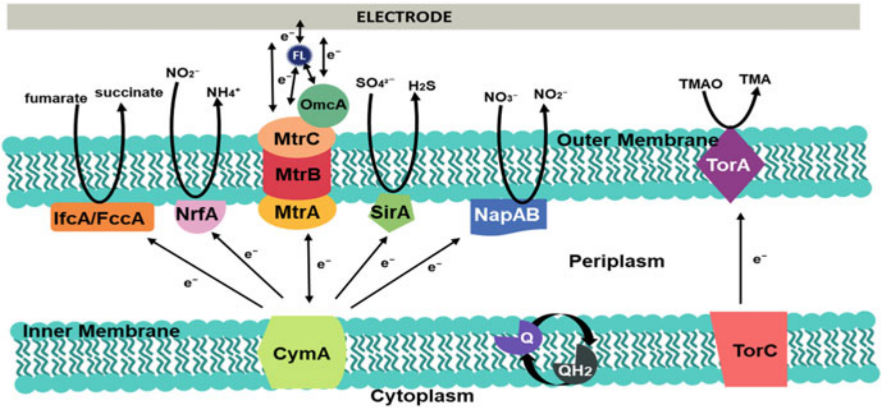

Fig. 9.4 Extra electron transfer in *S. oneidensis*

reduces sulfite; nitrite by pentaheme cytochrome NrfA; NapAB reductase to reduce nitrate; FccA and IfcA reductase for formate reduction; and decaheme cytochrome MtrA which passes electrons on to decaheme cytochrome MtrC and finally to the electrode. MtrA and MtrC are stabilized by a porin molecule MtrB, which together form the MtrCAB complex.

Other dissimilatory metal-reducing and -oxidizing bacteria like *Thermincola spp., Albidiferax,* and *Deferribacter* that use electrodes as terminal electron acceptors have been shown to consist of homologous genes to that of the Mtr pathway.

S. oneidensis can also perform indirect electron transfer through secretion of flavin molecules (FL) extracellularly. These small diffusible shuttles facilitate electron transport through OMCs to the electrode or bound to OMCs as cofactors (Kracke et al. 2015; Kumar et al. 2017a, b).

9.3.2.2 Branched OMC System: *G. sulfurreducens*

G. sulfurreducens is a Gram-negative, obligate anaerobic model exoelectrogen organism. It has been found that as many as 111 genes code for c-cytochromes, out of which 73 contain two or more hemes that support extra electron transfer. *G. sulfurreducens* transfers electrons by direct transfer through biofilm formation and nanowires, the former showing more effective performance and higher power densities. The biofilm thickness is directly proportional to the current generation, up to a particular thickness. A proposed model of the electron transfer to the electrode from the inner cell membrane is via the branched outer membrane cytochrome (OMC) system (Fig. 9.5). This starts with the metal reduction-associated cytochrome (MacA), a diheme cytochrome c peroxidase upon the inner cell membrane that facilitates electron transport to the periplasmic c-type cytochrome (PpcA), a triheme c-type cytochrome in the periplasm. The electrons are further transferred to the outer membrane cytochromes (OMCs) by PpcA. OMCs relocate the electrons

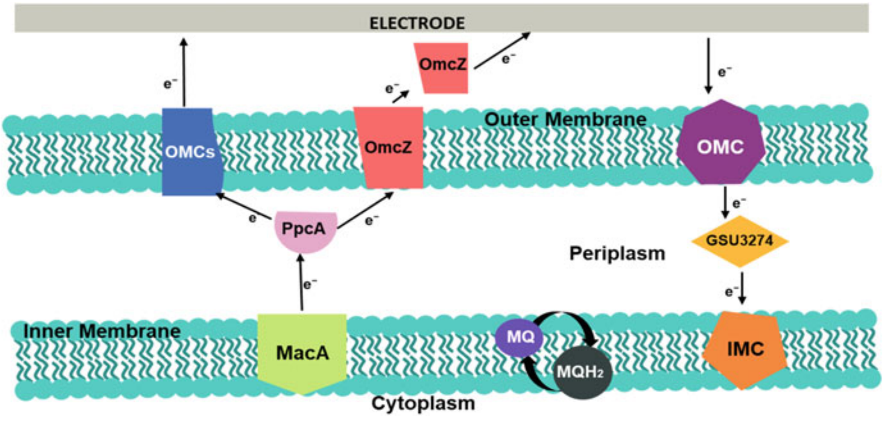

Fig. 9.5 Extra electron transfer in *G. sulfurreducens*

extracellularly to the electron acceptor. The exact mechanisms of extra electron transfer through these OMCs have not been elucidated clearly, but several studies have helped draw conclusions about the involvement of particular OMCs. Recent genetic knockout studies have shown dodecaheme cytochrome OmcB and hexaheme cytochrome OmcS transport electrons from the periplasm, passing them on other OMCs; OmcZ, an octaheme cytochrome, is vital in extra electron transmission, wherein the deficiency of the same leads to a 90% decrease in current output.

The electron transfer mechanisms from cathode into the cells are yet to be elucidated. However, studies have proved that GSU3274 gene is essential for electron uptake into the cell.

Another mechanism involved in electron transfer is through e-pili or microbial nanowires which are connected to the inner membrane of the cell, transporting the electrons through physical contact to electrode. The exact mechanisms are still being debated. One model is built on aromatic amino acid residues in the nanowires, owing to π-stacking, where electron delocalization takes place, similar to metallike conductivity. The supercharge or incoherent multistep hopping model proposes that electrons "hop" from redox-active proteins, by a cascade of redox reactions, finally to reach the terminal electron acceptor (Kracke et al. 2015; Kumar et al. 2017a, b).

9.3.3 Resistances

While the electrons are transferred through the external circuit, an equal number of protons are transported to the cathode chamber through diffusion. This diffusion is a slow process, and also a limiting factor of the reaction rate, causing internal resistance of BFCs. The internal resistance of a cell is defined as the opposing resistance to the current flow of the operational system. The internal resistances found in BFCs have been divided into different types, by different authors. Fan and Li have divided them into three major types—ohmic, charge transfer/active resistance, and diffusion/concentration resistance. Ohmic resistance is caused through electrolyte and proton-exchange membrane, while active resistance and diffusion resistance occur on the surface of the electrode in contact with the electrolyte.

Ohmic resistance is usually the major cause for internal resistance in BFCs, caused by resistance due to ion movement in the electrolyte, electron transport within the cell, and contact resistance. However, unlike the typical fuel cells, BFCs are complex systems that are influenced by environmental factors affecting the output and internal resistance in variable ways. The overall potential difference of the cell is formed when the protons diffuse across the membrane from the anode, initiating the flow of electrons through the external circuit to the cathode. The potential difference is caused by two forces—proton motive forces due to diffusion of protons to the cathode and electron motive force passing electrons to the cathode through the circuit (Fan and Li 2016).

9.4 Some Major BFC-Coupled Biocatalysis Pathways

9.4.1 Glucose Pathway and Energy Calculations in Saccharomyces cerevisiae

When glucose is used as the substrate and *Saccharomyces cerevisiae* is the biocatalyst, the following reactions take place at the electrodes:

$$\text{Anode (oxidation)} : C_6H_{12}O_6 + 6H_2O \rightarrow 6CO_2 + 24H^+ + 24\ e^-$$

$$\text{Cathode (reduction)} : 6O_2 + 24H^+ + 24\ e^- \rightarrow 12H_2O$$

$$\text{Overall reaction} : C_6H_{12}O_6 + 6O_2 + 6H_2O \rightarrow 6CO_2 + 12\ H_2O$$

Glucose is oxidized to pyruvate via the glycolysis pathway in the cytoplasm. The pyruvate is further completely oxidized to carbon dioxide and water. The electrons released during this process proceed to the electron transport chain in the mitochondria, where they are carried to the electrode through a series of redox mediators which include quinones and cytochrome proteins. ATP is synthesized with the help of proton concentration gradient, using ATP synthase, created due to the flow of electrons. Figure 9.6 shows a diagrammatic representation of the metabolism of glucose in *Saccharomyces cerevisiae*. Overall, an entirely oxidized glucose molecule generates 24 electrons; assuming that in 1 h, one glucose molecule is completely oxidized, the quantity of electricity (ampere-hour) obtained from 1 kg of glucose can be calculated using the Faraday constant (96,485 C/mol) as shown below:

$$\frac{(24 \times 96,485 \times 1000)}{(3600 \times 180)} = 3574\ \text{ampere} - \text{hour}$$

The redox potentials of the reactions taking place at the anode and cathode are −0.42 V and 0.82 V, respectively; hence the theoretical maximum potential generated by complete oxidation of glucose is 1.24 V, irrespective of the volume of the

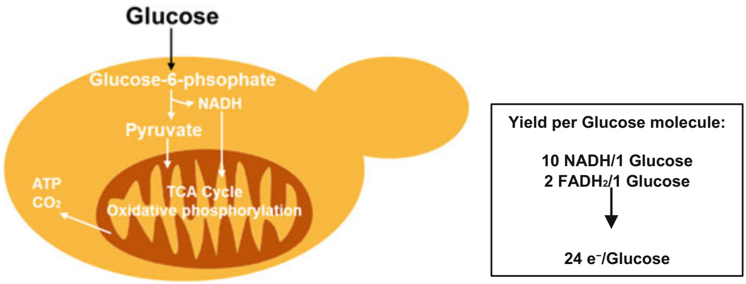

Fig. 9.6 Metabolism of glucose in *Saccharomyces cerevisiae*

substrate. As a consequence, the electrical energy of 4.431 kilowatt-hours can be accomplished if the potential is 1.24 V.

9.4.2 Plant–Microbe Symbiotic Association P-MFCs

In plant-based microbial fuel cells (P-MFCs), soil microbial flora forms the anodic biofilms, degrades root exudates, and transfers the released electrons to the anode (Fig. 9.7). Root exudates contain carbohydrates, carboxylic acids, and amino acids. P-MFCs are comparatively more sustainable systems as the root exudates provide a continuous supply of fuel for the microbes. In general, aquatic plants are preferred for use in P-MFCs, as the water serves as an electrolyte. Tuberous and leguminous plants have also been shown to produce high power densities, owing to the higher amount of root exudates released (Sophia and Sreeja 2017). Factors that contribute towards the efficient functioning of P-MFCs include solar irradiation, photosynthetic efficiency, photosynthesized carbohydrates, rhizodeposits, inoculum used, species forming anodic biofilm, MFC energy recovery, plant growth season, and improvement of leaf area configuration of the P-MFC (De Schamphelaire et al. 2010; Mathuriya et al. 2018).

Rice is the most preferred plant for use in P-MFCs. Bacterial communities which form biofilms on the anode of rice plant-based microbial fuel cells are *Desulfobulbus*

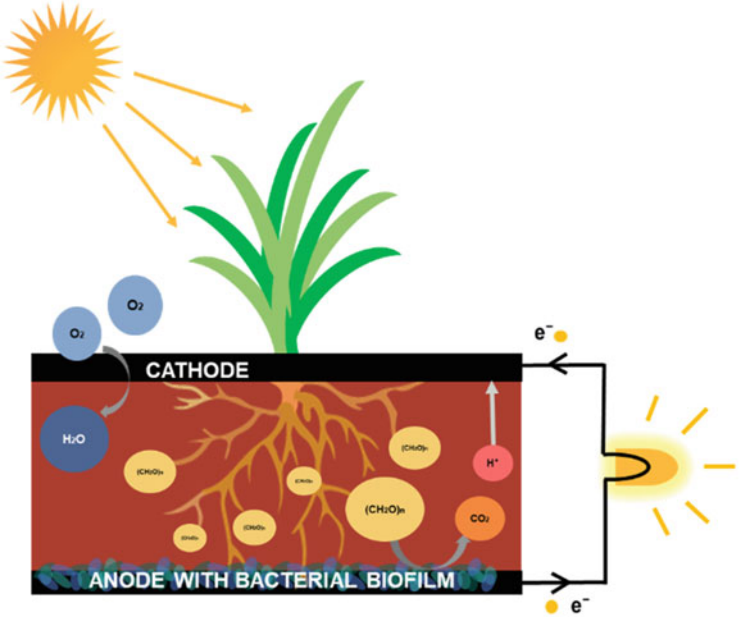

Fig. 9.7 Schematic representation of a plant-based microbial fuel cell (P-MFC)

sp., *Geobacteraceae* family, *Deltaproteobacteria*, *Chloroflexi*, *Anaeromyxobacter*, *Anaerolineae*, *Betaproteobacteria*, Alphaproteobacteria, and Firmicutes. Hydrogenotrophic methanogens, important archaea found in indigenous rice paddy soil as well, are the dominant archaea found on the anodic chamber (De Schamphelaire et al. 2010; Cabezas et al. 2015).

9.4.3 Wastewater Treatment and Recalcitrant Pollutant Degradation

The most cited application of BFCs is signified in the treatment of wastewater expending biocatalysts along with simultaneous production of electricity. Depending on the source, wastewater contains a wide range of components like organic carbon compounds, ammonium, dyes, phosphates, organochlorides, sulfides, nitrates, and nitrites. A complex interspecific coordination occurs between exoelectrogens, pollutant-degrading bacteria, and some other non-exoelectrogenic bacteria. While the pollutant-degrading bacteria initially break down the pollutants, exoelectrogens and other minority of bacteria may be involved in bioprocesses such as electron transfer, uptake of nutrients, nitrification, denitrification, biomass synthesis, and degradation. It has also been observed that the non-exoelectrogens maintain an anaerobic condition, facilitating optimum condition for higher power generation. For these reasons, mixed microbial consortiums and communities show better performances than a single pure culture. Table 9.3 depicts some reactions that take place in the electrodes (Ilamathi and Jayapriya 2018; Guo et al. 2020).

9.4.4 Metal Recovery

Metal-containing wastewaters are released from many human activities and industries such as mining, burning fossil fuels, manufacturing batteries, plastics, paints, and leather tanning. These metals become environmental hazards, as they gradually leach into the soil and contaminate the environment, accumulating in living organisms as well. A number of physical, chemical, physicochemical, and biological treatments have been established to eradicate such metals from the wastewaters. Microorganisms have been known to remove metals by biosorption, bioaccumulation, bioreduction, and biomineralization.

Recently, MFCs have been used for metal recovery and simultaneously to produce bioelectricity. The substrate oxidized at the anode releases electrons, which flow and reach the cathode via the circuit. At the cathode, the metal or metalloid ions act as the terminal electron acceptors, and are reduced by the electrons, hence accumulating in the cathodic chamber as deposits on the cathode (Table 9.4). However, this is not the case for all metals—redox potential of the

Table 9.3 Biocatalysts in wastewater treatment

Biocatalysis reaction	Pollutant degraded/ removed	Species involved
Anodic reactions		
$NH4^{+} + HO^{-} \rightarrow NO_2/NO_3^{-} + H^{+} + e^{-}$	Ammonia	Aridibacter, *B. thuringiensis*, Nitrosomonas sp., Nitrospira, Pirellula
Organic pollutants $\rightarrow CO_2 + H^{+} + e^{-}$	Polycyclic aromatic hydrocarbons (PAHs)	Desulfovibrio, *Geobacter metallireducens*, Lactococcus, Paludibacter, Thauera
	Phenols	Acinetobacter, Aeromonas, Arcobacter, *B. subtilis*, Cloacibacterium, *E. cancerogenus* BYm30, Geobacter sp., *Pseudomonas fluorescens*, Rhodococcus, Shewanella
	Polychlorinated biphenyls (PCBs)	Acetoanaerobium, Actinobacteria, Alcanivorax, Chloroflexi, Clostridium, Comamonas, Dehalogenimonas, Gordonia, Hydrogenophaga, Longilinea, Mycobacterium, Parvibaculum, Sedimentibacter
Cathodic reactions		
$- N{=}N- + 2e^{-} + 2H^{+} \rightarrow - NH - NH-$ $- N{=}N- + 4e^{-} + 4H^{+} \rightarrow -NH_2 - NH_2-$	Azo dyes	*Betaproteobacteria, G. sulfurreducens, Proteus hauseri ZMd44, Pseudomonas aeruginosa*
$NH_4^{+} + PO_4^{3-} + CO_2 + H_2O \rightarrow$ microbial biomass	Phosphate	*Microalgae, Cyanobacteria, Desulfomicrobium*
$NO_2^{-}/NO_3^{-} + H^{+} + e^{-} \rightarrow N_2\uparrow + H_2O$	Nitrogen	*Acidovorax sp., Bdellovibrio, Bosea, Brevundimonas, Chlorella biomass with Azospirillum and Rhizobium (photosynthetic organism consortium), Exiguobacterium sp., Flavobacterium, Geobacter, Hydrogenophaga, Mesorhizobium, Nitratireductor sp. Nitratireductor sp., Pseudomonas stutzeri, Rhodobacter, Thauera, Thiobacillus, Zoogloea*

Table 9.4 Direct metal recovery using MFCs

Metal (electron acceptor)	Cathodic half-cell reaction(s)	Redox potential
Cobalt(III)	$LiCoO_2 + 4H^+ + e^- = Co^{2+} + Li^+ + 2H_2O$	+1.61 V
Chromium(VI)	$Cr_2O_7^{2-} + 8H^+ + 6e^- = Cr_2O_3 + 4H_2O$ $Cr_2O_3 + 6H^+ = 2Cr^{3+} + 3H_2O$ $Cr_2O_7^{2-} + 14H^+ + 6e^- = 2Cr^{3+} + 7H_2O$	+1.33 V
Gold(III)	$AuCl_4^-(aq) + 3e^- \rightarrow au(s) + 4Cl-$	+1.002 V
Vanadium(V)	$VO_2^+(aq) + 2H^+ + e^- = VO_2^+(aq) + H_2O$	+0.991 V
Mercury(II)	$2Hg^{2+} + 2e^- = Hg2\ 2+$ $Hg_2^{2+} + 2e^- = 2Hg$ $Hg^{2+} + 2e^- = Hg$ $Hg_2^{2+} + 2Cl^- = Hg_2Cl_2$ $Hg_2Cl_2 + 2e^- = 2Hg + 2Cl-$	+0.911 +0.796 +0.851 + 0.268
Silver(I)	$Ag^+ + e^- = ag$	+0.799 V
	$Ag(NH_3)^{2+} + e^- = ag + 2NH_3$	+0.373 V
	$[AgS_2O_3]^- + e^- = ag + S_2O_3^{2-}$	+0.250 V
Iron(III)	$Fe^{3+} + e^- = Fe^{2+}$ $Fe^{2+} = FeO/Fe(OH)_2$	+0.77 V
Selenium(IV)	$Se^{+4} + 4e^- = se$	+0.41 V
Copper(II)	$Cu^{2+} + 2e^- = Cu$	+0.286 V

cathodic half-cell should be greater than the anode potential of the cell. Silver(I), gold(III), chromium(VI), cobalt(III), copper(II), mercury(II), selenium(IV), and vanadium(V) are examples of metals that have positive redox potentials, and have been precipitated using MFCs, simultaneously removing organic wastes as well (Nancharaiah et al. 2015).

In the case of metals and metalloids like Cd(II), Co(II), Ni(II), Zn(II), and Pb (II) that have a lesser or close redox potential to the anodic potential, an additional potential should be supplied so the electron flow is directed towards the cathode (Table 9.5). These bioelectrochemical systems that require an external power source for their functioning are known as microbial electrolysis cells. MFCs are usually coupled to the MECs so that the power generated in the MFCs can be utilized as the external source of power towards metal reduction in MECs (Wang and Ren 2014).

Table 9.5 Recovery of metals by MFCs requiring external power source

Metal (electron acceptor)	Cathodic half-cell reaction(s)	External voltage applied (V)	Redox potential
Lead(II)	Pb2+ + 2e- = Pb	0.34	−0.13 V
Cobalt(III)	Co2+ + 2e- = co	0.3–0.5	−0.232 V
Nickel(II)	Ni2+ + 2e- = Ni	0.5–1.1	−0.25 V
Cadmium(II)	Cd2+ + 2e- = cd	1.7	−0.403 V
Zinc(II)	Zn2+ + 2e- = Zn	1.0	−0.764 V

9.5 Recent Developments and Prospective Paths

9.5.1 Genetic Modification and Applying Synthetic Biology

Synthetic biology is an interdisciplinary field that integrates techniques of biology and engineering used to design organisms and construct new organisms that can perform specific functions. Synthetic biology can be used to improve the performance of existing biocatalysts or create new biocatalysts. Existing biocatalysts can be modified such that they can utilize more range of substrates, have an increased substrate uptake and/or electron flux, produce electron mediator molecules, or improve biofilm formation. *S. oneidensis*, a model exoelectrogen, has been used in several studies to expand its substrate utilization range and altering electron transfer rates. *S. oneidensis* possessing Mtr pathway genes has been successfully introduced and expressed in *E. coli*; phenazine-1-carboxylic acid synthesis pathway, expressing an electron shuttle from *P. aeruginosa*, has also been inserted in a different study—both resulting in higher current production than the wild strains (Logan et al. 2019). In *G. sulfurreducens*, another model exoelectrogen, a gene encoding proteins with a PilZ domain, gene GSU1240, was deleted. A biofilm six times more conductive, producing 70% more power densities than the wild type, was the result. The biofilms could be peeled off without damaging the electrodes, and used as novel conductive biomaterials (Leang et al. 2013). Through biofilm engineering, living conductive biomaterials that can self-heal and self-replicate can be created (Glaven 2019).

9.5.2 Chemical Treatment

Due to their outer lipopolysaccharide (LPS) layer, most Gram-negative bacteria are nonconductive. In a study by Liu et al., it was shown that treating *P. aeruginosa* biofilm with polyethyleneimine (PEI) augmented the power density twice in comparison to the control. This is due to the formation of channels and large pores formed in the treated cells, allowing free movement of the redox mediators pyorubin and pyocyanin produced by the bacteria itself (Liu et al. 2012). The thick cell wall of

Gram-positive bacteria hinders the electrons to be transferred to the electrode. Lysosome treatment of *Kocuria rhizophila*, an exoelectrogen that is used predominantly in direct electron transfer mechanisms, had shown an increased power density by 1.75 times (Azuma and Ojima 2019).

9.6 Conclusion

In BFCs, biocatalysts are the initiators of bioelectricity generation. The integration of biology and electrochemical and architectural systems, along with their optimum performances, is influenced by a number of biological, chemical, and physical factors, which make BFCs even more sophisticated systems. While the work in electrochemical and architectural aspects is making significant progress, there are still many unanswered questions and unclear concepts with respect to the biological aspects. Only when we dive deeper into the cores of the biocatalysts, following the very paths of electrons, can we find answers, and BFCs can perform to their best.

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Chapter 10
Bioelectric Fuel Cells: Recent Trends to Manage the Crisis on Resources for Conventional Energy

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Abstract Bioelectric fuels are substantial alternatives to electrical energy derived from the chemical energy of copious organic biomass. The battle on managing the organic matter-rich biomass needs a live accelerator. The industrial revolution has led to huge dumping of non-hazardous solid waste. Land filling was the major practice followed by many industrial sectors due to ease. Practices like implementing the bioelectric fuel system in every industry can bring up a change in non-hazardous waste management that requires the knowledge on utilizing the biomass by specific biosystems for the purpose to act as bioelectric system. Such biological matter which utilizes this biomass or even any organic matter that gets converted to electrical energy is the prime emphasis of this chapter.

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10.1 Bioelectric Fuel Cell

Electrochemical devices use the biological catalyst to generate electricity from any of the substrates (organic or inorganic). These devices facilitate the oxidation of substrate by the protons and electrons through any biological mediators at the site of the anode or capable of acting as an anode; such biotransformation of energy is driven by chemical to electrical form of energy in the form of bioelectricity production. Microorganisms serves as the biocatalyst and these are fuel cells that can derive energy from biomass, plant and light, through the anaerobic digestion technology; some forms of bioelectric fuel cells are unique in their way of design, environment and working. The classification of the bioelectric fuel cell is widely based on many factors including substrates, mediators, enzymes, sediments, cytochromes, design and methods.

10.1.1 Introduction

The rapid increase in population is becoming an uncontrollable factor in the world. The growing population needs more resources for their comfortable living needs. Energy needs are exponentially increasing whereas the fossil resources for energy production are declining with a threat to biodiversity and living needs. All the countries' energy policies focus on alternative energy resources and invest in the same for the prospect of safeguarding the earth from the calamities like global warming and climate change. Sustainable energy that does not deplete the natural resources can be the game changer. Innovation, improvement, investment in free energy and alternative energy that can be sustainable are the prime focus of research in the twenty-first century. This has led to the development of sustainable energies like the bioelectric fuel cell.

10.1.2 Working

The working principle of this bioelectric fuel cell can be explained with the common glucose as the source of energy. The substrates [simple to complex] are oxidized and the oxidant is reduced to produce electrons and protons as given below:

$$C_6H_{12}O_2 + 6H_2O \rightarrow 6CO_2 + 24H^+ + 24e^-$$

$$24H^+ + 24e^- \overset{6O_2}{\rightarrow} 12H_2O$$

$$E_O = 1.23\ \text{V}.$$

This process is mediated by a biocatalyst that participates in election transfer indirectly through the metabolic process or directly through the transfer of electron chain from the substrate to the anode.

10.1.2.1 Acetate Oxidation

$$2HCO3^- + 9H^+ + 8e^- \overset{6O_2}{\rightarrow} CH_3COO^- + 4H_2O$$

$$CH_3COO^- + 2O_2 \overset{6O_2}{\rightarrow} 2HCO3^- + H^+$$

On oxido-reduction the MFC potential is $E_O = 1.042$ V.

Similarly, any simple carbon sources to complex can be produced by the fermentation process mediated by biological stems that are electrochemically active to oxidize the substrates and its by-products to fuels (Fig. 10.1).

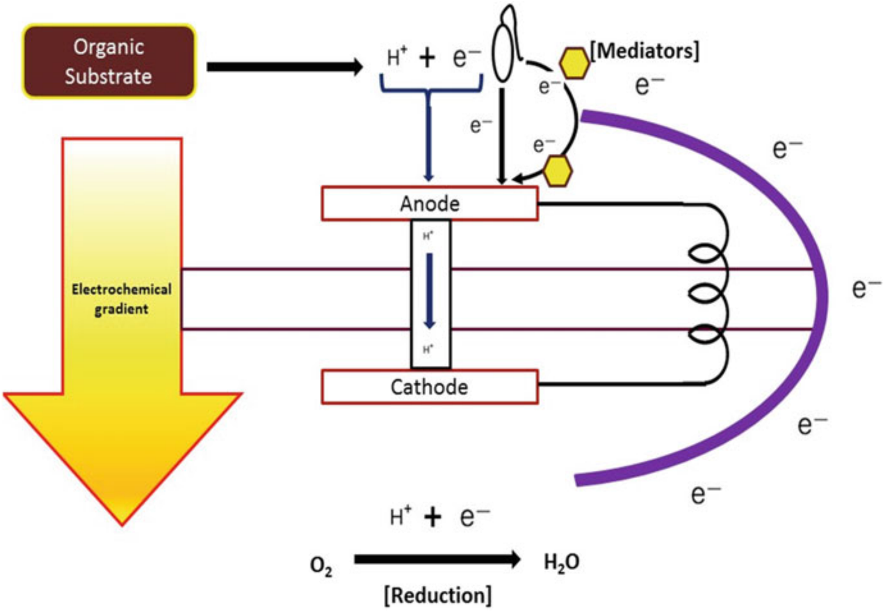

Fig. 10.1 MFC working mechanism

10.1.3 Chamber Mechanism

The first basic reaction proposed in the MFC has a two-chamber model with the anodic and cathodic elements in turn separated by membranes that facilitates exchange of protons.

Key Points Enrolled in MFC Working and Construction

1. Organic substrate is digested to protons and electrons by bioelectrochemical means through a specialized structure called microbes which are living things.
2. The electrons produced by the substrate reduction are transported to the positive electrode through chemical or microbial mediators facilitated by means of direct transfer.
3. The electron deposited over anode is transferred to the negative electrode chamber facilitated by means of an external circuit; meanwhile the protons (H^+) are transferred to negative electrode-containing chamber from its origin through the proton- or cation-exchange membrane creating a proton and electron chemical gradients.

10.1.4 Classification of Microbial Fuel Cell

The classification of MFC can be considered before designing any MFC. Depending on the selection of biological system there can be some modification imposed to improve the efficiency of the bioelectricity generation. Classification of the MFC is generally based on the parameters like configuration, light requirement, temperature, nutrition, and mediator (Fig. 10.2); concerning biological system there can be an elaborative classification as given in Sect. 10.3.

10.1.5 Requirements

10.1.5.1 Anode Chamber

Anode chamber is the hotline for the start of MFC and any bioelectrical fuel cell. The anode chamber has to provide sufficient space for microbial growth and pumping out the electrons to the positive electrode. An important part of any positive electrode-containing chamber is the electrode material. It has to provide low resistance, high chemical stability, physical stability, and conductance. These features of anode material are more important than any other chamber parameters like compatibility, stability, and configurational arrangement.

The potential anode material used as reported by many other studies is the carbonaceous material. This is the site where microbes undergo oxidation reaction

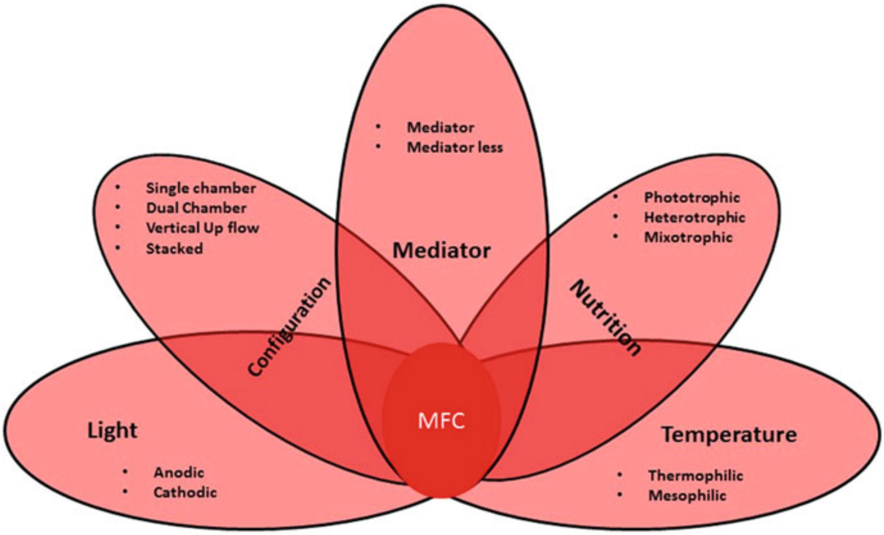

Fig. 10.2 Classification of MFC

and hence the material chosen should support the biofilm and electron transport. The important factor considered for biofilm formation is surface chemistry, which can provide the basic amenities for the microbes to get accommodated.

MFC technology for the feasibility and properties of the anode varieties is the prime factor for material selection. The range of anode materials studied includes carbon cloth (Guerrini et al. 2014; Santoro et al. 2013), carbon brush (Liao et al. 2015), carbon rod (Jiang and Li 2009; Liu et al. 2004), carbon paper (Santoro et al. 2014; Srikanth et al. 2008), carbon mesh (Wu et al. 2017; Wang et al. 2009), carbon felt (Seviour et al. 2015; Roy et al. 2014), granular activated carbon (Yasri and Nakhla 2017; Zhao et al. 2016; Jiang et al. 2011), granular graphite, carbonized cardboard (Kretzschmar et al. 2017), graphite plate (Heijne et al. 2008), reticulated vitreous carbon (Lepage et al. 2012), electrospun carbon fibres (He et al. 2011), activated carbon nanofibres (Karra et al. 2013), carbonized plant materials (Karthikeyan et al. 2015), metallic anode materials (Guo et al. 2016) and composite material (Mustakeem 2015; Sharma et al. 2008). Due to the importance developed for surface chemistry, the surface modifications proposed at the site of anode materials are favouring the growth of fuel generator. Chemical treatments, surface coating and thermal treatments were already being imposed (Lowy et al. 2006).

10.1.5.2 Cathode Chamber

This chamber is the site of reduction of oxygen and is called an oxygen reduction reaction site (ORR). ORR is considered as one of the rate-limiting processes for bioelectricity generation. It has been evident from many studies of MFC designs that different materials at the electrode chamber have an impact on bioelectricity generation rate. And in a way, the studies also suggest selecting the metals as electrode/ cathode from the list as that of from anode. Poor catalytic activity is indicated in using carbonaceous material as the cathode. The cathode materials can be classified as

1. Biocathode
2. Metal-free catalyst
3. Pt-based catalyst
4. Non-Pt-based catalyst

These classifications are based on the properties that facilitate the oxygen reduction reaction like mechanical strength, catalytic property, electrons and conduction of ions (Santoro et al. 2017; Mustakeem 2015). The materials studied so far include carbon Pt, double-layer polydimethylsiloxane (PDMS), iron phthalocyanine, cobalt tetra methoxy phenylporphyrin (CoTMPP), lead dioxide carbon, palladium, lead oxide, activated carbon and N_2-doped graphene nanosheets.

10.1.5.3 Membrane System

The membrane system is the set-up that supports the proton movement and electron movement from its origin site of the positive electrode-containing chamber to the negative electrode-containing chamber. The material selection properties include high permeability of protons, less gas penetrability, better heat stability and resistance (Liu and Logan 2004; Zhao et al. 2009; Li et al. 2011; Hernandez-Flores et al. 2015). Based on the function of membrane they can be classified as

1. PEM: Membrane that facilitates proton exchange
2. CEM: Membrane that facilitates cation exchange
3. AEM: Membrane that facilitates anion exchange
4. BPM: Membrane that can facilitate exchange of both ions
5. PCPM: Membrane made of polymer composite that facilitates proton

The material selection of the membrane system is still haunting due to certain drawbacks for large-scale implementation of this system for commercial purpose. The materials studied so far are enlisted as below:

S. no	Membrane	Authors
1.	Sulphonated polyether ether ketone (SPEEK) membrane	Literature reports
2.	Polysulphone-based anion-exchange membrane	Literature reports
3.	Sulphonated polybenzimidazole (PEM) Sulphonated poly (ether amide) with fluorenyl and trifluoromethyl quartz	Singha et al. (2016), Kumar et al. (2016)
4.	Sand chamber	Literature reports
5.	Glass wool	Literature reports
6.	Ceramic wall (unglazed)	Khalili et al. (2017)
7.	Ceramic floor (unglazed)	Khalili et al. (2017)
8.	Electrospun microtube array	Literature reports
9.	Salt bridge	Min et al. (2005)
10.	Glass fibre membrane	Zhang et al. (2011)

10.1.6 Design and Construction

10.1.6.1 Designs

The design of MFCs is important for generating enough amount of current density to implement into the large-scale application. The existing viable MFC reactor designs include single/dual chamber, up-flow cylindrical/up-flow tubular type, flat bed, stacked (Pandey et al. 2016) and sediment type of designs. Applications of each design are detailed in Table 10.1.

10.1.6.2 Single-Chamber MFC

This is a design with a single chamber, hence derived its name. This type exhibits a membrane-less character. Simple one chamber is integrated with both the anode and the cathode (Park and Zeikus 2003). This type of MFC has a potential implementation in commercial sectors due to its less expensive implementation cost, simple design and more efficiency in producing the power density by simple modification. The modification that was reported to have competitive power generation includes adjusting the spacing between the electrodes and integrating catholyte, electrode material, etc. (Ringeisen et al. 2006).

10.1.6.3 Double-Chamber Designs

Double- or dual-chamber MFC is the first simple and durable type of reactor when compared to all MFCs (Kumar et al. 2016). As indicated by the name the design will

Table 10.1 Application of various designs at MFC

S. no.	Application	Design	Output and references
Single chamber			
1	Brewery wastewater as a fuel source	Carbon cloth as both electrodes, coated with the platinum catalyst	Power density up to 205 mW/m at 30 °C with 37% of COD clearance efficacy Literature reports
2	Wastewater treatment	Granular activated carbon (GAC) chamber containing graphite rod as anode and carbon cloth coated with platinum as air cathode	200 mg/L of COD and pH 7.2 that can be used as source of the fuel (Jiang and Li 2009)
3	Treatment of activated sludge	The capacity of 1.29 L consists of 30 wt% air cathode which is a wet-proofed carbon cloth and Pt/C-coated carbon (20%) cloth as anode	Reduced power density due to internal resistance; COD removal increased from 88.5% to 94% (Jeon et al. 2013)
Double chamber			
4	Dairy wastewater	The chambers are filled with granular graphite material and connected with electrodes made of graphitic rods	80–90% COD removal efficiency with 60% coulombic efficiency. Power density up to 27 Wm^{-3} Literature reports
Vertical upward flow design			
7	Beer brewery waste sludge	The MFCs consist of 0.5 mg/m^2 coated membrane as air cathode and the anode is carbon veil, and it was operated for more than 7 months	Independently connected MFC modules showed maximum power density up to 6% and 36% at 0.8 and 0.08 g/L of sucrose (Kim et al. 2011)
8	Industrial wastewater	1.7 L capacity reactor built with single chamber and 0.5 mg/cm platinum-coated air-cathode electrode, and the combination of graphite bar and carbon granules as positive electrode with ionic liquid-type membrane	Continuous electricity production (Salar-García et al. 2016)
Stacked design			
9	Treatment of ethanolamine containing synthetic wastewater at the pilot-scale level	Anode is the carbon cloth and wet-proof carbon cloth (30%) is used as air cathode	Power density = 0.86 W/m^2 clearance of carbon and ammonia efficiency up to 95.30 and 95.70% Literature reports
10	Brewery wastewater	Activated carbon-coated PTFE as cathode and carbon brush weaved with titanium wire as anode	Power density = 0.027 kWh/m^3 reduced the suspended solids (SS) up to 86.3% Literature reports

be with two chambers, where one serves as the positive electrode (anode) and the next chamber serves as the negative electrode (cathode). These two chambers will be disconnected by the PEM or salt bridge. They are ‘H’-shaped designs with specific anode and cathodes that can act as substrates to produce current density. The dual-chamber design factors like chamber material, electrode material, membrane efficiency and viability affect the output potential. This design is a batch-mode operative framework and hence has its disadvantages in maintenance.

10.1.6.4 Vertical or Up-Flow Chamber MFCs

Vertical up-flow is a new design favouring wastewater and composite substrate treatment rather than the scope of electrical potential efficiency considerations. This is a single-chamber design with anode at the bottom serving as the inlet of wastewater or composite material that needs treatment. The inlet is pumped up to cathode that is not physically separated but dispensed with glass medium. The gradient developed within the electrode aids in developing electric fuel. The design is not reported with any use of specific substrates to act as anolyte or catholyte. The internal resistance developed within the reactor continues to diminish the electric potential of the design. The upward flow of the design facilitates the proton transfer efficiencies (Zhou et al. 2013; Venkata Mohan et al. 2014).

10.1.6.5 Stacked Designs

Stacked designs have their advantage in power density production. The stacked MFC is intervened to improve the power density by combining the sets of single-chamber units or dual-chamber units or the hybrid one with the single and dual chamber to have high stable power density. In addition to the benefit mentioned by the stacked designs, they also have been reported for high COD removal (Kumar et al. 2017).

10.1.7 Drawbacks of Each Design

Single-chamber MFC is reported to have the drawback of microbial backflow and so reverse transport of oxygen from negative electrode to positive electrode. Single-chamber designs are simpler and economic designs. This type of MFC has an anodic chamber with no requirement of air in a cathodic chamber (Rabaey et al. 2004, 2005). Size of the reactor in large-scale applications is a constraint. The power density yield is also less compared to other designs. Dual chamber is a feasible design with better power density yield and compatible model for many applications. The potential of the design lies in the efficiency of the membrane. Vertical up-flow designs favour in best of the wastewater treatment whereas power efficiencies are negligible. The power consumption or the up-flow of the waste is also highly incurring.

10.2 Non-hazardous Solid Waste

Non-hazardous solid wastes are described in 27 CCR 20220 (California Code of Regulation) which states all putrescible and non-putrescible solid, semi-solid and liquid wastes, including garbage, trash, refuse, paper, rubbish, ashes, industrial wastes, demolition and construction wastes, abandoned vehicles and parts thereof, discarded home and industrial appliances, manure, vegetable or animal solid and semi-solid wastes and other discarded waste (whether of solid or semi-solid consistency), provided that such wastes do not contain wastes which must be managed as hazardous wastes, or wastes which contain soluble pollutants in concentrations which exceed appropriate water quality objectives, or could cause deprivation of waters of the state (i.e., designated waste).

Some of the examples of non-hazardous wastes as defined by Tudor et al. (2008) include domestic wastes, food waste, hygiene waste, packaging waste, recyclable waste (e.g. paper, aluminium and glass), wooden waste furniture, construction waste and ground waste produced.

10.2.1 Categories and Sources

Non-hazardous solid wastes can be originated from industries or household. Depending on the type of industry, the sources and categories can be defined as per the flow chart given in Fig. 10.3.

According to Fig. 10.3, non-hazardous solid wastes originate from industrial activities or household utilities as two major sources. Some of the wastes generated from the paper and pulp industry, iron and steel manufacturing and petroleum refineries are given below.

10.2.2 Technical Disposal

Non-hazardous solid wastes are treated usually to extract usable materials before disposal. Like any waste treatment process which follows 4Rs such as reduce, reuse, recycle and recover, non-hazardous solid wastes are managed through the traditional methods used for all solid wastes. Some of the most common waste management procedures are described in the flow chart in Fig. 10.4.

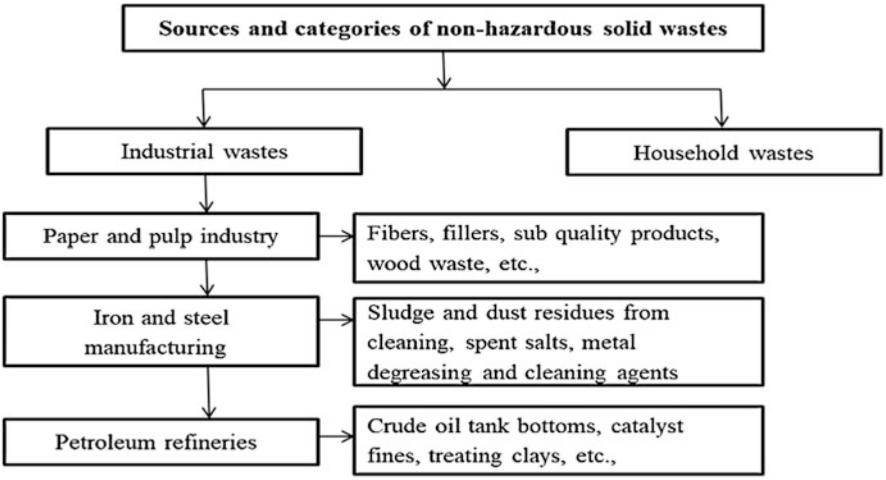

Fig. 10.3 Sources, categories and some examples for each type listed (Jonathan Seth Krones 2016)

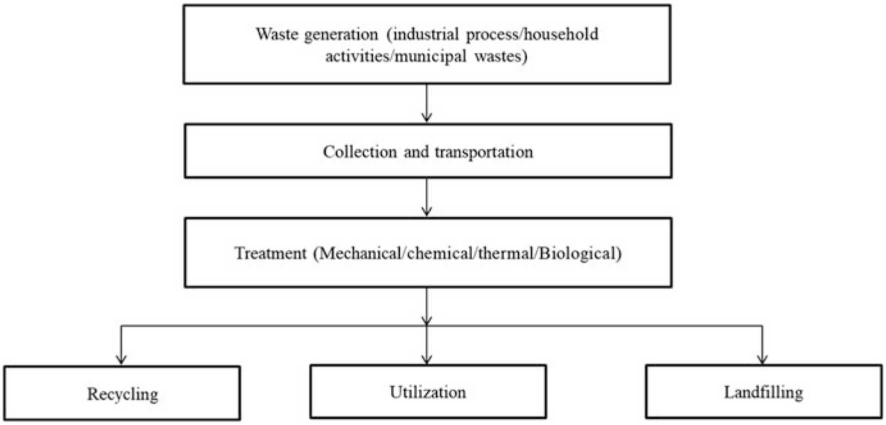

Fig. 10.4 Waste management process of solid waste (Thomas H. Christensen 2010)

Based on the data published on the treatment of non-hazardous waste (as reported in Statista for European Union in the year 2016), a pie chart representation is provided in Fig. 10.5.

It is noted that a majority of non-hazardous waste (836 million tons of a total of 2.23 billion tons) is landfilled. It was followed by recycling (~38%) and release into water bodies (~7%). Only about 0.8% of non-hazardous waste is incinerated.

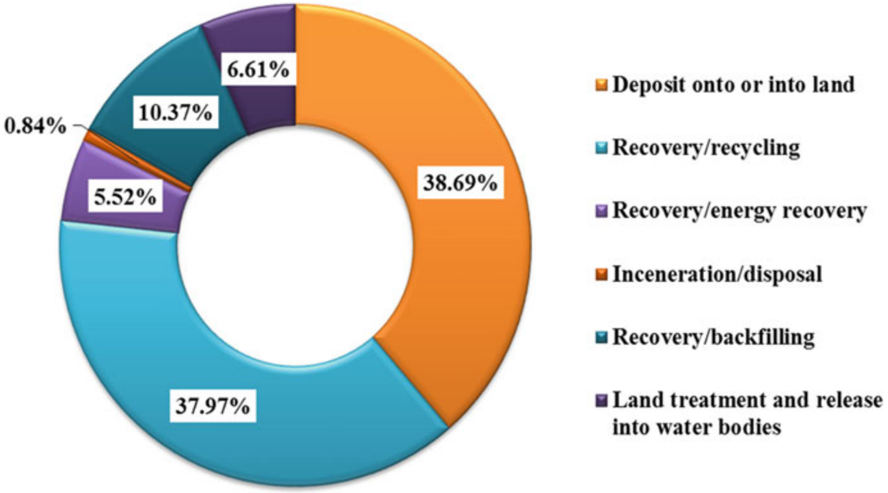

Fig. 10.5 Treatment of non-hazardous waste in the European Union in the year 2016

Table 10.2 Technical disposal of non-hazardous solid waste, and its advantage and disadvantage

S. no.	Method of disposal	Advantage	Disadvantage
1	Landfills	Relatively cheap Landfill gas recovery Can deal with a large volume of waste	Creates water and soil pollution Deforestation Ecological imbalance
2	Incineration/combustion	Can destroy all matter effectively Requires small area for disposal	Requires high capital cost Harmful gas emission is unavoidable
3	Recovery/recycling	Energy-efficient method	Additional costs incurred for recycling
5	Composting	Enhances soil quality Eco-friendly	Requires initial investment Unpleasant odour
6	Waste to energy (energy recovery)	Environmental friendly	Requires initial cost of operation

10.2.3 Advantages and Disadvantages

Each type of waste disposal had an advantage and disadvantage. Table 10.2 gives a brief description of the type of disposal, and its advantage and disadvantage.

10.3 Biological Systems

10.3.1 Classification of Biological Systems

The MFC classification based on the biological system can be categorized as microbial MFC, algal MFC and plant MFC. Microbes used in MFC are always embedded to any MFC; the substrate or the other roles of the biological system in MFC enable the classification of the biological system as in the hierarchy given below.

10.3.2 Microbes

10.3.2.1 Bacteria

Many bacteria have the potential to be the active MFC, being the largest in the microbial family. The microorganisms especially bacteria were identified with any of the characteristics as anode respiration (respiration happening in the anode of MFC utilized to conserve energy for electron acceptance); anode reducing (bacteria donates electrons to anode); exoelectrogen (bacteria donate electrons to the anode by either mediator or mediator less); electrotroph (bacteria pull an electron from cathode); cathodophile (oxidizing bacteria that attract electrons from cathode); and electrochemically active bacteria (donate/accept electrons with or without the help of mediators).

Although daunting results are there for the microbes studied to assess the microbial fuel cells in the past two decades, there are some potential microbes reported in the commercial scale of MFC due to the good yield of bioelectricity. Top-notch of the list is deserved by the *Geobacter* species and *Shewanella sp.* due to oxidization of organic materials to CO_2 with more electron transport (Nevin et al. 2008).

Some of the potential species under this genus are *Geobacter sulfurreducens* (Holmes et al. 2006), *Desulfuromonas acetoxidans* and *Geobacter* . *Clostridium sp.* are good as bioelectricity generator, viz., *Clostridium acetobutylicum* (Singh and Sharma 2010). Some of the other species in this genus include *Desulfovibrio, Aeromonas and Tetrathiobacter* (Kumar et al. 2017b). Some of the *Proteobacteria, Aeromonas* and *Enterobacter sp.* were reported as electricigen. *Enterobacter cloacae* are the wild species reported one decade ago (Mohan et al. 2008) with mediators. In concern with the by-products, *A. hydrophila* is marked with high power production with the degradation of chitin.

Other potential electrogens in the bacterial community are *A. butzleri*, *Klebsiella pneumoniae* L17 (Zhang et al. 2008), *Klebsiella oxytoca* ADR13 (Kingsly et al. 2017), *Corynebacterium humireducens sp.* (Wu et al. 2011), *Citrobacter sp.* (Xu and Liu 2011), *Cupriavidus basilensis*, *Shewanella oneidensis* (Ringeisen et al. 2006) and *Ochrobactrum anthropi* (Zuo et al. 2008). Park and Zeikus (2003) were the first to report on co-culture and mixed bacterial culture of five different strains with the highest yield of 500 mV/m^2.

10.3.2.2 Fungi

Fungal cells have the potential to produce many exo-enzymes which are widely used for various domestic applications; one such is the fungal fuel cells where the fungal cells are used as bioanode or biocathode to produce bioelectric fuel system (Fig. 10.6). The fungal enzymes have the potential to break complex polysaccharide and contaminants into simple carbohydrates (van Leeuwen et al. 2013). Many fungi reported to be an effective agent in bioremediation were studied for MFC under various processing efficiency. Fungal enzymes as exudates in the media can be used as a suitable biological alternative to perform as the anode. Similarly, the whole fungal cells can also be used as bioanode and in biofilm development. Various applications of the fungal MFC studied are presented in Table 10.3.

10.3.2.3 Yeast

Yeast is a filamentous organism with some morphological features of fungi. These type of microbes are enormously hosted in the soil and moisture-rich conditions. *S. cerevisiae* is a yeast variety that grows in the presence and absence of oxygen. This is the yeast widely studied in MFC and they were reported to be used as

Fig. 10.6 Types of fungal MFC

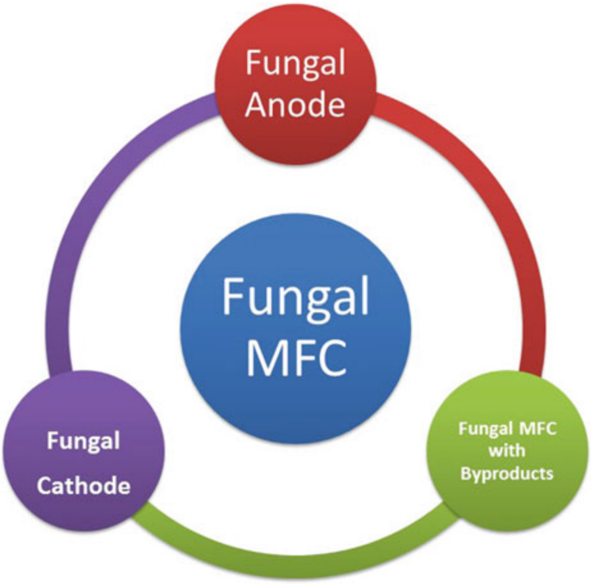

Table 10.3 Application of fungus in MFC

S. no.	Fungus	Type	Application	Reference
1.	*Scedosporium dehoogii*	Bioanode	Biofilms	Mbokou et al. (2016)
2.	*Aspergillus awamori*	Biodegradation	Two-step process: fungal fermentation to by-products	Ray et al. (2017)
3.	*Aspergillus sp.*	Biodegradation	Dual chamber: Solid reduction and by-products like chitosan, organic acids	Rabaey et al. (2004), Maghsoodi et al. (2009)
4.	*Rhizopus sp., Aspergillus sp., Penicillium sp.*	Air cathode	Biocatalyst for the reduction of oxygen in MFC (oxidase enzymes) laccase enzyme	Morant et al. (2014)
5.	*Trametes versicolor*	Enzyme based	Laccase enzyme	Kipf et al. (2013)
6.	*Coriolus versicolor*	Biofilm as biocathode	Fungal cells are immobilized in the graphite as biocathode	Wu et al. (2012)
7.	*Trametes versicolor* with *Shewanella oneidensis*	Fungi-bacteria-assisted MFC	Dye decolourization electron	Literature reports
8.	*Gloeophyllum* and *Rhizopus*	Liquid fungal cultures as analyte and catholyte	Remote sensor devices Laccase enzyme production	Literature reports
9.	*Pleurotus ostreatus*	Microbial anode Laccase enzyme-based cathode	Textile wastewater dye treatment Decolourization	Sankaran et al. (2010)
10.	*Trichoderma viride*	Derived peptaibiotics	Sewage and sludge	Ray et al. (2017)
11.	*Trichoderma atroviride*	Trichotoxin, alamethicin, citreoviridin to exhibit methanogens	Sewage and sludge	Ray et al. (2017)

assistive culture mode along with algal MFC. The result of many studies on this yeast indicates the considerable bioelectricity generation only in the presence of mediators. The electron transport from yeast is also justified due to the presence of metal reductase that favours transfer of electrons directly (Hubenova and Mitov 2015). The characteristic features of metal reductase enzyme in yeast are also used in desalination plants coupled with MFC (Mardiana et al. 2016). *Candida melibiose* has been studied for MFC by Hubenova and Mitov (2010) with detailed metabolism structures. Similarly, another yeast (*Arxula adeninivorans*) was also positively reported for bioelectricity generation.

10.3.3 Algae

Algae are a special group of micro- and macro-organisms that support MFC by fixing the atmospheric CO_2 as the carbon along with sunlight to generate energy (ATP).

They can also do favour in generating bioelectric potential without sunlight as given in Fig. 10.7.

Some algae are heterotrophic and grow without sunlight and are called photobioreactors. These phototrophic and heterotrophic algae are subjected to the single and dual chamber by many researchers who are studying the following algal species. Figure 10.8 illustrates the working of algal MFC in a dual chamber.

The studied species include *Chlamydomonas reinhardtii* (Nishio et al. 2013), *Chlorella vulgaris* (Velasquez-Orta et al. 2009), *Cyanobacteria* (Yuan et al. 2011 and Zhou et al. 2012), *Ulva lactuca* (Velasquez-Orta et al. 2009), *Microcystis aeruginosa* (Wang et al. 2012), *Chlorella vulgaris* (Wang et al. 2012), *Arthrospira maxima* (Inglesby et al. 2012), *Scenedesmus obtusus* (Rashid et al. 2013), *Laminaria saccharina*, *Scenedesmus obliquus* (Kondaveeti et al. 2014; Hur et al. 2014), *Chlorella vulgaris* (Lakaniemi et al. 2012), *Dunaliella tertiolecta* (Lakaniemi et al. 2012) and mixed algae (Strik et al. 2008; Wang et al. 2012).

Some of the testing was also done on the mixed cultures of algae for generating electrical energy. Algae MFC can replace the aeration system when grown on the cathode chamber. Figure 10.9 depicts the working mechanism of algae as catholyte.

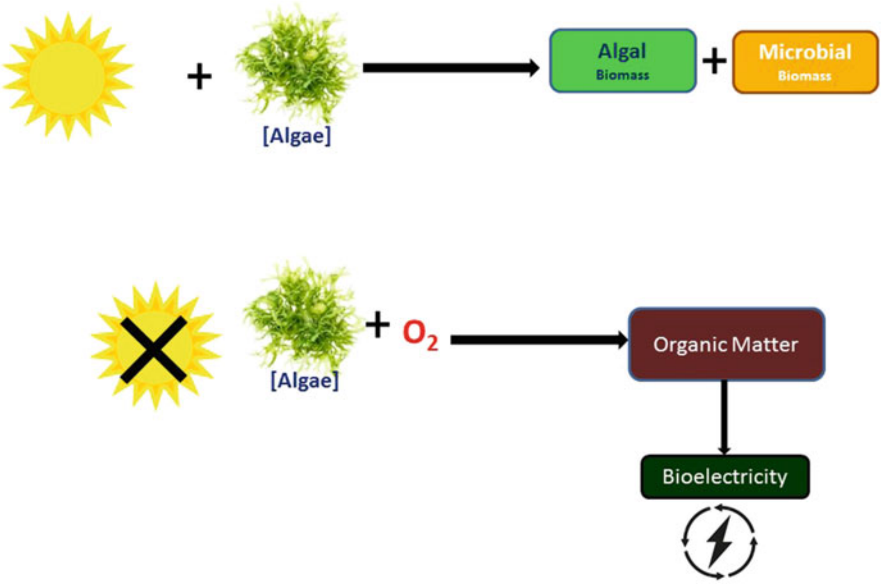

Fig. 10.7 Involvement of algae in bioelectricity generation

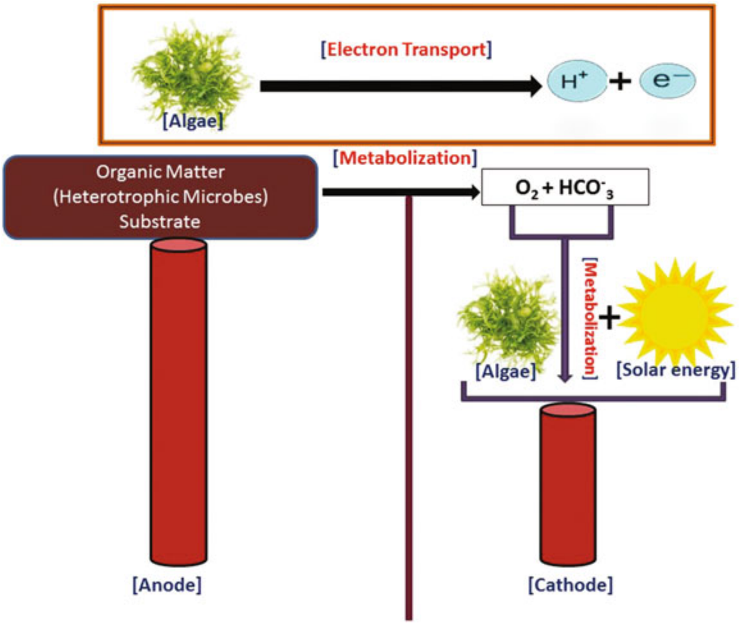

Fig. 10.8 Working of algal MFC in dual chamber

The anode-assisting algae are utilized for the electrochemical catalysis and as substrates for electricity generation, whereas the cathode-assisting algae can aid in oxygen production, carbon dioxide production, biomass, treatment of wastewater and illumination effect in bioelectricity generation. Some of the algae studied for their potential in different types of MFC set-up are enlisted in Table 10.4.

10.3.4 Plants

Microbial fuel cells use organic sources for chemical energy and convert them to electricity. This was made possible by the sediment MFC, widely used in the wastewater purification system. The new and slight modification of this SMFC gives rise to the plant MFC, wherein the organic material is derived by photosynthesis instead of chemical energy. This uses the free energy (solar) for organic source and the microbes will utilize them to produce bioelectric power. Figure 10.10 illustrates how the plant MFC fixes the carbon and converts it to bioelectric power.

The major anodes reported in PMFC are graphite, carbon fibre, activated carbon, granular carbon, carbon brush, glassy carbon, carbon felt, etc., in which among the so far reported anode materials graphite is the commonly studied applied to plant MFC. The studies have indicated the plant and bacterial preferential for the anode material and it is also reflected in the various advantages in current density, stability

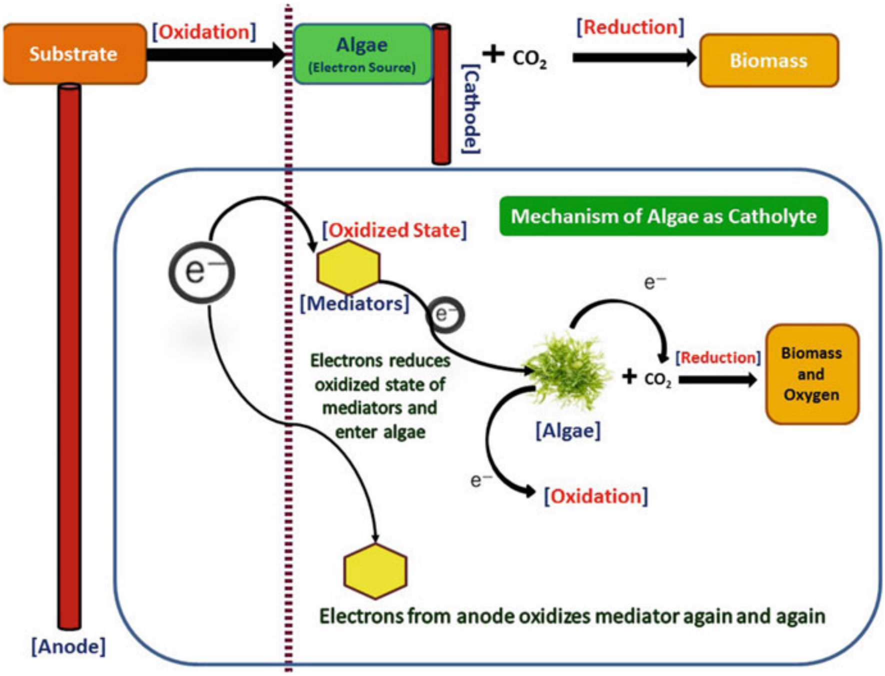

Fig. 10.9 The working mechanism of algae as catholyte

and conductivity. The privilege of granular carbon as anode material in the establishment of new plant electricity connections was supported in an earlier study.

Similarly, for cathode materials, carbon and graphite are widely used along with O_2 as the electron acceptor for reduction reaction (Nitisoravut and Regmi 2017). So many plants were tested so far based on the purpose of research (Table 10.5). The maximum electrical energy derived from PFMC so far is recorded as 679 mW/m^{-2} by *S. anglica*. The electrical energy derived from this type of PMFC is low and has nevertheless improved a lot by using solar energy through photosynthesis and converting their derivatives to electrical energy.

Some of the factors influencing the PFMC include weather, organic matter, pH, electrical conductivity, plant type and microbial community.

10.4 Biomass

10.4.1 Classification

Any residue originated from a living thing is termed biomass. The source of biomass includes plants, animals and microbial biomass (Fig. 10.11). Biomass exploitation capability in terms of utilization and synthesizing energy can be a suitable alternative

Table 10.4 Different types of algal MFC configuration

S. no.	Type	Algae	References
1.	Coupled up-flow algal MFC	*Chlorella vulgaris*	Powell et al. (2009)
2.	Single chambered	*Spirula platensis*	Lin et al. (2013)
3.1.	Dual chamber	*Chlorella vulgaris*	Rodrigo et al. (2009)
		Chlorella salina *Tetraselmis gracilis* *Isochrysis sp.* *Nannochloropsis sp.* *Dicrateria sp.* *Chaetoceros calcitrans* *Pavlova sp.* *Synechocystis sp.* *Dunaliella sp.*	Ramanathan et al. (2011)
1.1.1.1.			
		Dunaliella tertiolecta	Lakaniemi et al. (2012)
		Laminaria saccharina	Literature reports
		Scenedesmus obliquus	Kondaveeti et al. (2014)
		Chlorella pyrenoidosa	Xu et al. (2015)
4.	Sediment algal fuel cell	*Chlorella vulgaris*	Jeon et al. (2012)
5.	Twelve reactors algal fuel cell Four closed-circuit systems Four open-circuit systems Four anaerobic reactors	*Chlorella vulgaris* *Ulva lactuca*	Cheng and Liu (2006)
6.	Nine cascades algal fuel cell	*Synechococcus leopoliensis*	Winfield et al. (2013)
7.1.	Anode assistance with phototrophic microorganisms	*Rhodobacter sphaeroides*	Literature reports
		Chlamydomonas reinhardtii	Nishio et al. (2013)
8.	Cathode assistance algal MFC	*Chlorella vulgaris* *Desmodesmus sp.* A8 *Microcystis aeruginosa*	Kokabian and Gude (2013), Wu et al. (2014)

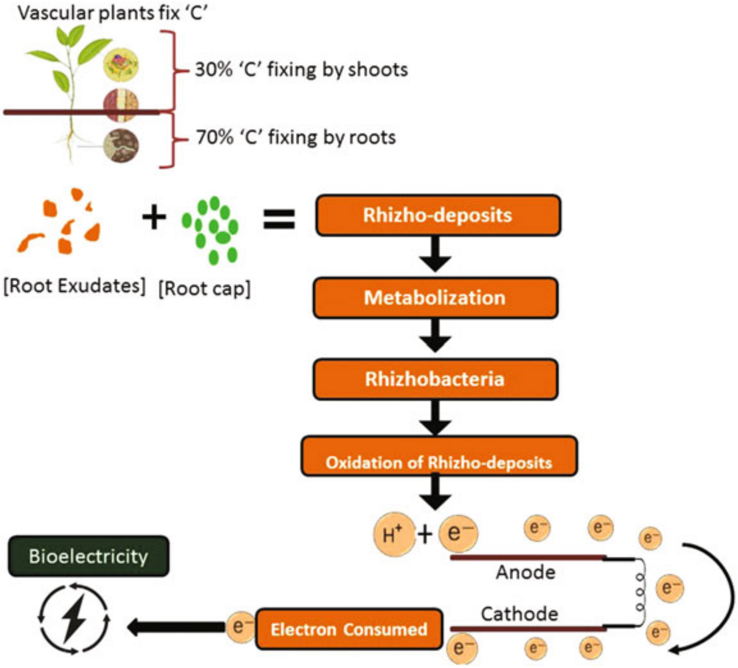

Fig. 10.10 Mechanism of plant MFC in generating bioelectricity

to all growing needs of the population. The biomass and other applications of microbes in MFC were detailed in the previous section of this chapter. The residues arise due to the non-hazardous solid waste from industries.

The other biomass and their potential to be converted into the existing MFC can be discussed further. The bioenergy value of biomass utilization is least addressed by the countries. The growing population needs for energy can be satisfied with the help of biomass as the source to generate fuel for all needs as an alternative to non-renewable sources like fossil fuel.

These classified sources of biomass are rich in carbon content terms either in simple form or in complex form. Some of the forms of carbon in these residues include lignocellulose, cellulose, hemicellulose and lignin.

The biomass, especially plant biomass and microbial biomass, was explored well in the biofuel production like biodiesel, biogas and biohydrogen. Due to the known composition of the biomass content, it was also made available in commercial production.

Animal biomass, municipal waste and processed crop residue can be utilized effectively for carbon sources to enable microbial growth. The suitable modern applications of animal waste and municipal waste would be anaerobic digestion and utilizing them for bioelectricity. Unlike other fuel production, this does not require any changes in the device for utilizing this energy.

Table 10.5 Different types of plants tested for MFC and their power density (Sivasankar et al. 2018)

S. no	Plant type	Power density
1.	*Arundinella anomala*	22
2.	*Canna indica*	18
3.	*Eichhornia crassipes*	224.93
4.	*Echinochloa glabrescens*	115
5.	*Glyceria maxima*	67
6.	*Glyceria maxima*	80
7.	*Glyceria maxima*	12 (MA)
8.	*Ipomoea aquatica*	12.42
9.	*Lolium perenne*	55
10.	*Oryza sativa*	6
11.	*Oryza sativa*	19 ± 3.2
12.	*Oryza sativa*	126.3
13.	*Oryza sativa*	72
14.	*Oryza sativa*	14.44
15.	*Oryza sativa*	33
16.	*Oryza sativa*	80
17.	*Oryza sativa*	23
18.	*Pennisetum setaceum*	163
19.	*Spartina anglica*	222
20.	*Spartina anglica*	110
21.	*Spartina anglica*	679 (PGAc)
22.	*Spartina anglica*	240
23.	*Typha latifolia*	6.12
24.	*Sedum album*	0.0024
25.	*Sedum hybridum*	0.092
26.	*Sedum kamtschaticum*	>0.001
27.	*Sedum reflexum*	>0.001
28.	*Sedum rupestre*	0.0155
29.	*Sedum sexangulare*	0.0084
30.	*Sedum spurium*	>0.001
31.	*Oryza sativa*	33
32.	*Phragmites australis*	22
33.	*Spartina anglica*	82
34.	*B. juncea*	69.32
35.	*Trigonella foenum-graecum*	80.26
36.	*Canna stuttgart*	222.54
37.	*Oryza sativa*	1.3

(continued)

Table 10.5 (continued)

S. no	Plant type	Power density
38.	*Hydroponic plants (rooted plants grown on the water surface; Bryophyllum pinnatum, Solanum lycopersicum (tomato), Oryza (rice) sativa, Lycopodium and Adiantum (ferns)), submerged plants (Hydrilla verticillata), Myriophyllum and self-grown algae*	110
39.	*Ipomoea aquatica*	11.2
40.	*Myrtillocactus*	90
41.	*Spartina anglica*	211
42.	*Spartina anglica*	440
43.	*Lemna minuta*	380
44.	*Lemna valdiviana*	140
45.	*Glyceria maxima*	390
46.	*Phragmites australis*	43
47.	*Canna indica*	15.73
48.	*Phragmites australis*	9.4

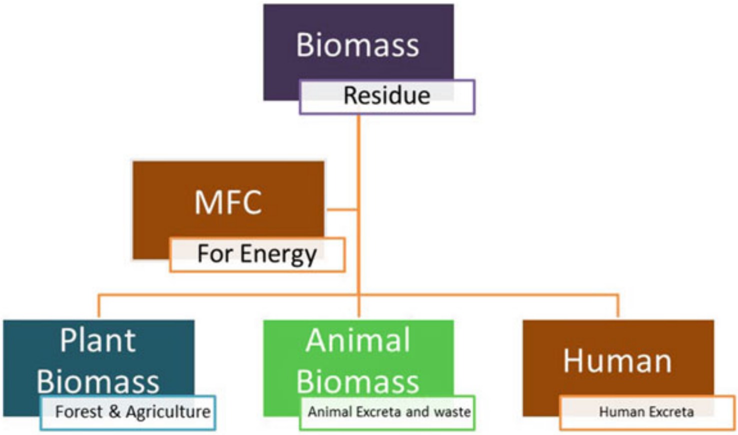

Fig. 10.11 Classification of biomass for energy production

10.4.2 Energy Values of Biomass

10.4.2.1 Agricultural Biomass

The crop residue left in the soil is collected after harvesting the crop account for the agricultural biomass. Every year tons of agricultural products are produced from every country to meet the food demand. The demand also constantly increases in proportionate to the population of the country besides the season and region variation.

The crops including field crops for staple food and vegetable crops are continuously cultivated to align with the demands of nutritional supplement to the healthy population.

Agricultural biomass is mostly homogenous in composition besides seasonal variations. The energy values range from 11,550 to 18,600 KJ/kg with an average of 16,300 KJ/kg nearly three decades above. Now in the year 2020, there is no proper statistics on the plant biomass generated but the agricultural countries in Asia lead the list in both production and population. The decade from 2010 had an incline in all agricultural produce, in turn generating more residues. For example, some European countries have predicted statistical data for 2020 which is topped by France followed by Germany at 84 and 58 million tons (MT) in 2013, 90 and 62 MT in 2020 and 96 and 66 MT may be in 2030 for 12 crops like barley, maize, oats, olives, rapeseed, rice paddy, soybeans, sugar beet, rye, triticale, sunflower seed and wheat. These crops will in turn, account for 0.4 MT and 0.15 MT biopower production by France and Germany, respectively.

10.4.2.2 Forest Biomass

In plant biomass, another huge source is the forest. The wood and its industrial products account for the forest biomass. The wood is the raw material for the forest biomass. Only less than 5–10% of the wood is used for energy as raw materials. The residue from the forest and industrial processing is forest residue or forest waste.

Forest waste is accounted from dead trees, litterfall and wastes generated during logging. It is roughly estimated that logging residues account more when compared to any other wastes from forest. In a study on biomass utilization, it is found that Russian Federation tops with 5718 MT (dry weight)/year forest residues followed by Indonesia, the USA, Brazil and China with the residue level as 2221, 2078, 1613 and 807 MT dry weight/year (Tripathi et al. 2019).

These forestry residues are either cellulose or lignocellulose with carbon, oxygen and hydrogen as major components. The energy value of logging residue is 18,610 KJ/kg (dry weight).

10.4.2.3 Animal Residues (or) Biomass

Global animal rearing is topped by East and Southeast Asia followed by the USA and European countries. It is estimated to touch 10,000 million tons in 2100 in the world due to the growing population's nutritional requirements. The animal waste accounting per unit area is very less when calculated overall due to the unconfirmed rearing of animals, whereas the same when calculated in the industrial rearing sectors is very high and the management of animal biomass is complex because of the high moisture concentration.

The energy value of animal wastes is 17,450 KJ/kg dry matter due to the complex composition of animal waste based on their varieties. Their management in terms of collection transportation and energy conversion is also feasible for large farming areas. The animal waste is rich in nitrogen, phosphorus and potassium. If they are not managed properly in the confirmed zone, it poses threat to the land resources and water resources during run-off.

They are managed mainly by converting them to the organic manners and dispersing to the agricultural areas; unlike the municipal solid waste this animal biomass (mainly volatile solids) and waste are used for biogasification for methane production.

10.4.2.4 Human Waste

Municipal sewage waste includes wastewater and the sludge arising out of it. The sewage sludge is categorized into four types from the wastewater treatment of municipal sewage waste. It includes primary, secondary, tertiary and digested sludge. Primary sludge is the settled sludge and secondary sludge arises due to activation or trickling of filter humus. Tertiary sludge arises due to tertiary treatment. The digested sludge is solid that arises due to anaerobic digestion of the waste.

All the sludge, except the primary sludge, is rich in organic matter: It is estimated by the USA that an average of one million people can produce 98 tons of dry solids each day which can be huge or less according to the population statistics of each country. It is high in moisture content. The organic matter accounts for nearly 50% in dry digested sludge. They do have high components of nitrogen, phosphorus and potassium with trace elements of heavy metals. The energy content of raw municipal waste accounts for 16,284 KJ/kg of dry solids. Apart from the difficulty in converting the sludge to the energy sources, the impact of sludge on the environment is not favourable due to the trace of heavy metals. Hence their segregation and process of converting them to fuel or electricity should be focused much better.

10.5 Future Perspective

Though the invention of MFC and its application originated 20 years ago, there is still a huge space that needs to be addressed for MFC commercialization and large-scale application. The MFC design, classification and types are growing day by day. There is more number of new designs that are getting developed based on the feasibility and the scope of research areas.

The co-culture and mixed-culture studies are at a nascent stage. The perfect consortium can bring up high potential in bioelectricity. The consortia can be considered to make up as many as other microorganisms in combination with other biological materials to build up green energy. Microbes that are capable of utilizing the remnants of biomass can be used to convert the sludge into an organic matter-rich material. This in turn, can be converted into energy and stabilizing that with artificial intelligence and control can reduce the labour, improve the world fuel necessity and help for sustainable energy production.

This chapter in turn has addressed the need for studies in non-hazardous solid waste management. This is the waste that accounts for a huge share. Non-hazardous dumping and combustion will cause only added environmental problems. Non-hazardous soil waste has huge scope in utilizing the MFC applications. MFC and its variation can make use of the sludge and these non-hazardous waste as the potential substrate to meet out the energy needs.

Biomass for bioelectricity is not well envisaged by the researchers despite the known energy value and organic matter content. The suitable system for the biomass can yield high energy values.

The in silico analysis of the mechanism of identifying the microbial genome responsible for or favouring the electron production and transfer can be brought to spotlight. Such type of genes can be aligned to organisms. Metagenomic studies on biomass utilization and non-hazardous waste utilization can be enhanced for huge productivity. In silico consortial engineering can be done through metabolic flux, dynamic flux analysis and metabolic pathway analysis which has a wide scope for using as much as residue and connecting them into bioelectric energy.

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Chapter 11
Bioethanol: Substrates, Current Status, and Challenges

Anita Saini, Deepansh Sharma, and Swati Sharma

Abstract Bioethanol, an oxygenated fuel, is a potent alternative to fossil fuels in the transport sector. Its efficiency in internal combustion engines and cleaner combustion makes it an attractive fuel to either replace or blend with gasoline in different proportions. Bioethanol can be produced from a wide variety of substrates rich in different carbohydrates. Currently, bioethanol is being produced at commercial levels from starch- and sugar-containing substrates. The bioethanol made from food crops is known as "*first-generation bioethanol*." The cellulose and hemicellulose components of the lignocellulosic biomass from agriculture, industrial, or forest origin can also be utilized as a source of fermentable sugars which are converted to ethanol. The bioethanol generated from lignocellulosic substrates is named as "*second-generation bioethanol*." Also, bioethanol can also be produced using algal biomass, rich in starch and/or cellulose, as a substrate, and this is called as "*third-generation bioethanol*." The technology is well developed for first-generation ethanol production. However, the environmental concerns and limited availability of feedstock for first-generation ethanol production require a shift to the technology for higher generations of bioethanol production. Several economic and technological constraints, in the way of second- and third-generation bioethanol production, need to be overcome. The cost of the biomass pretreatment, cellulase enzymes, and logistics during feedstock procurement pose major challenges in the commercialization of second-generation bioethanol. In the case of algal-based bioethanol, biomass yield, biomass pretreatment, and fermentation efficiency are the primary areas that require developments.

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11.1 Introduction

Bioethanol, a renewable fuel, is known across the world for its importance in the transport sector (Balat 2009). This biofuel has gained attention for its potential in replacing fossil fuels, the primary global energy source at present. The exponential rise in fossil fuel demands, along with the concerns of their limited availability and longer generation times, has necessitated a switch to alternative sources such as biofuels. Bioethanol, having chemical similarity with the fossil-derived ethanol, is produced from natural green biomass. The biological origin of bioethanol feedstock is an advantage to the producers. This is because the photosynthetic ability of the green plants and algae ensures the inexhaustibility of the feedstock. Bioethanol not only is seen as a potent solution to the rising demands of liquid transport fuels but also offers an answer to environmental issues due to fossil fuels. Being an oxygenated fuel, its combustion emits lesser amounts of environmental pollutants (Balat et al. 2008).

Ethanol has many advantages as a transport fuel. It has high octane number (an indicator of gasoline quality), high anti-knock value (for early ignition), lower cetane number, broader flammability range, higher laminar flame velocity, and higher latent heat of vaporization than that of gasoline. All these characteristics account for the high efficiency of internal combustion engines (Balat et al. 2008). Also, the use of ethanol eliminates the requirement of toxic methyl tertiary butyl ether (MTBE) blending in gasoline for raising the octane number (Almodares and Hadi 2009). Thus, ethanol is a promising fuel for spark ignition internal combustion engines and can be used in ethanol-dedicated engines and flexible fuel vehicles (FFV) either directly or as a gasoline blend in different ratios. The first internal combustion engine prototype run on ethanol was constructed in 1826 by Samuel Morey. Later on, in 1908, the first model "T" car was launched by Ford Motor Company, runnable on alcohol-gasoline fuel. After the revelation of advantages of ethanol blends in gasoline and establishment of Energy Policy Act (1992) aiming at cutting down the use of petroleum by 30%, the ethanol bending programs were initiated by different nations. Examples include the USA (E10, for FFV E85), Canada (E10, for FFV E85), Sweden (E5, for FFV E85), Brazil (E20, for FFV E25), Australia (E10), Thailand (E10), Peru (E10), Paraguay (E7), China (E10), and India (E5) (Balat 2011). Amendments are done in these programs from time to time.

Bioethanol can be produced from a wide range of feedstock, all essentially having carbohydrates in their biomass. These substrates may include sugar-rich food crops, starch-containing materials, lignocellulosic biomass, and algae. Depending on the feedstock used as the starting source of fermentable sugars, several generations of bioethanol are known such as first-, second-, third-, and fourth-generation ethanol (Robak and Balcerek 2020). Currently, bioethanol is produced commercially from sugar- and starch-based biomass using a well-established technology. However, the climatic concerns and debate of “food vs. fuel” have driven the researchers towards ethanol production from non-edible materials such as lignocellulosic wastes from the industrial, agricultural, forest, and municipal origin. The cellulosic and hemicellulosic components of lignocellulose account for a significant amount of fermentable sugars in lignocellulosic wastes, which can be converted to ethanol. However, the primary challenge in the bioconversion of lignocellulose to ethanol is the efficient and cost-effective hydrolysis of carbohydrate polymers of lignocellulose to their respective constituent sugars. Efforts are underway to overcome the techno-economic impediments in the way of commercialization of technology for second-generation ethanol production from the lignocellulose, mainly through the reduction of cost of pretreatment and hydrolytic enzymes required for the saccharification of sugar polymers. Additionally macroalgal and microalgal biomass rich in starch and other carbohydrates may also be employed as a substrate for bioethanol production.

11.2 Bioethanol Generations

Since its establishment, bioethanol production technology has undergone changes resulting in several generations of ethanol. The advanced generation is mostly focused on minimizing the limitations associated with the lower generation technology. Currently, this distinction is primarily based on differences in the feedstock used for bioethanol production. The change in feedstock also accounts for the change in the process technology as well as the challenges pertaining to different production processes. Ethanol is preferably produced from C6 sugar, primarily glucose, through fermentation by an ethanologenic microorganism. *Saccharomyces cerevisiae* yeast is the most commonly employed microorganism for ethanol fermentation processes. The glucose is found in a variety of plant carbohydrates such as cellulose, starch, and several disaccharides. Therefore, theoretically, all of the glucose-rich carbohydrates qualify as a feedstock for their bioconversion into ethanol. The bioethanol generated from sugar- and starch-rich food materials is referred to as the “*first-generation ethanol*.” The ethanol produced from the (hemi) celluloses in the lignocellulosic biomass is known as “*second-generation ethanol*” or “*cellulosic ethanol*” (Robak and Balcerek 2018). Algae have gained attention as a feedstock for ethanol and the ethanol generated from them is called as “*third-generation ethanol*” (Dutta et al. 2014). More recently, genetically modified crops and algae have become a subject of research around the globe. The ethanol produced from them is generally classified as “*fourth-generation bioethanol*” (Ziolkowska 2020).

11.2.1 First-Generation Ethanol

11.2.1.1 Feedstock and Production Technology

First-generation ethanol feedstock can broadly be classified into two types: (1) sugar-rich feedstock and (2) starch-rich feedstock (Table 11.1).

Sugarcane

Sugarcane (*Saccharum officinarum*), a tropical C4 plant (perennial) from the Poaceae family, contains high amounts of sucrose and is, therefore, widely used for sugar production at commercial levels. Sucrose may account for nearly 50% of the sugarcane culm dry mass (de Souza et al. 2014). Only 10% of total sugars (wet weight) are constituted by glucose or fructose (Bertrand et al. 2016). The abundance of sugars in sugarcane also makes it the second most used feedstock for bioethanol production, accounting for two-thirds of the global sugar production. Brazil, one of the major sugarcane-cultivating countries (with 632 billion tons of production) and one of the major bioethanol producers of the world, accounts for nearly 21 million m^3 of the total sugarcane-based ethanol (Bertrand et al. 2016).

The ethanol can be produced from either the cane juice or the molasses generated as a by-product during the process of sugar formation (Fig. 11.1). In Brazil, the ethanol facilities associated with sugar mills use molasses or juice-molasses mixture, whereas dedicated distilleries prefer cane juice for ethanol production (Lopes et al. 2016). The production process starts with the extraction of juice from the clean sugarcane. The juice is clarified and separated from the fibrous residue called bagasse. The clarified juice is concentrated, sterilized, and transferred to the fermentation tank, where ethanologenic culture is added. The alcoholic fermentation is carried out by maintaining the required conditions. In the initial stage of the fermentation process, the yeast's endogenous invertase enzyme hydrolyzes the sucrose into glucose and fructose (fermentable sugars), and later on glucose is fermented to ethanol (Zabed et al. 2014). The ethanol produced is recovered by distillation or filtration methods and dehydration may be done to obtain anhydrous ethanol (Dias et al. 2011). The bagasse is commonly used for generating heat or electricity in the plant. However, nowadays it is considered an attractive feedstock for cellulosic ethanol especially in the distilleries based on the integration of first-generation with second-generation ethanol production technology. In the industries

Table 11.1 Primary feedstock for first-generation ethanol

Feedstock	Plant part	Examples
Sugar-rich crop	Roots	Sugar beets
	Stalks	Sugarcane, sweet sorghum
Starch-rich crop	Cereal	Wheat, corn, barley, rye, sorghum, etc.
	Roots (tubers)	Potato, cassava

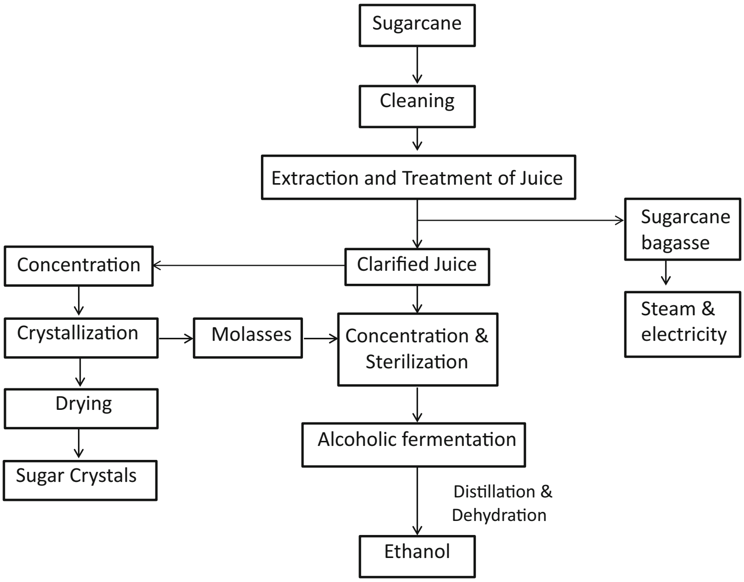

Fig. 11.1 Scheme for production of ethanol and sugar from sugarcane

where ethanol production unit is associated with a sugar mill, the clarified juice is concentrated and crystallized followed by drying to obtain sugar crystals. The molasses generated as a by-product is separated, concentrated, sterilized, and subjected to the fermentation process. The fermentation can be executed in different modes, i.e., batch, fed-batch, or continuous fermentation. In batch mode, the biomass of the ethanologenic strain, which is most commonly *Saccharomyces cerevisiae*, is separated from the fermentation tank by centrifugation for reuse in the next batch. In Brazil, the fed-batch mode is preferred for bioethanol production. The method can result in high product yields relative to batch mode due to addition of fresh substrate at a specific flow rate which eliminates the problem due to inhibitory effect of the high substrate or product levels (Sanchez and Cardona 2008). Around 17% of ethanol distilleries in Brazil employ continuous fermentation processes, while 83% prefer fed-batch processes (Lopes et al. 2016).

Owing to the ethanol production potential of sugarcane various research groups across the world are focused on improving sugarcane productivity, by working on different parameters including tolerance to drought and cold, improving fiber yield, early flowering and fast growth, fixing of culm height to diameter, ability to outcompete weeds, etc. (de Souza et al. 2014).

Sugar Beet

Sugar beet (*B. vulgaris* L.) is another widely used crop for the production of sugar (Bertrand et al. 2016). The crop is predominantly cultivated in temperate climatic

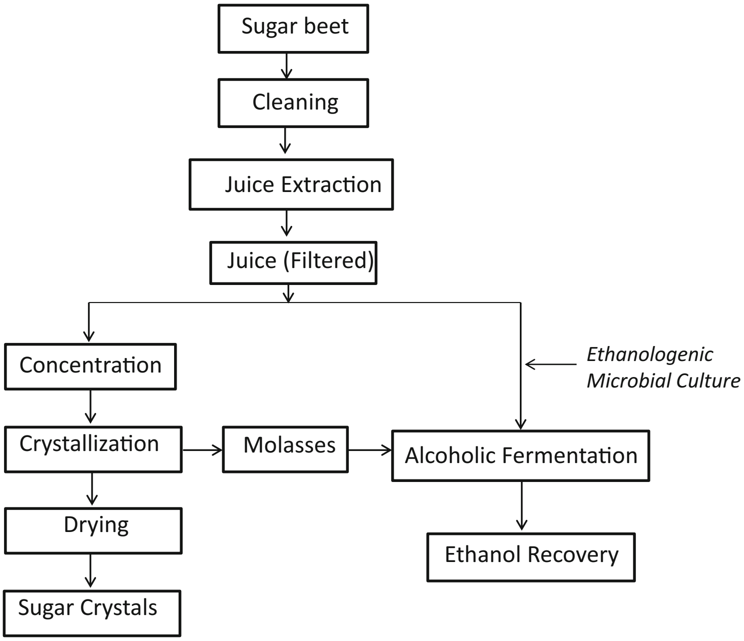

Fig. 11.2 Scheme of ethanol and sugar production from sugar beet

conditions in countries such as Russia, Germany, France, and the USA. Europe alone accounts for 80% of the world's sugar beet cultivation (Marzo et al. 2019) for sugar and ethanol production. Sugar beet tubers contain 75% of water while the remaining 25% is dry matter, of which 75% is made up of sugars (mainly sucrose). The sugar content, however, may vary from 12% to 20% depending on the variety of crop, breeding, and location of cultivation (Bertrand et al. 2016; Marzo et al. 2019). The short cycle of the crop, higher yield, and tolerance towards a wide range of climatic conditions, drought, and lower fertilizer demands make sugar beet a promising feedstock for bioethanol production at commercial levels (Balat et al. 2008). Bioethanol can be produced from raw juice or sugar-processing intermediates (thick juice and molasses) (Bušić et al. 2018) (Fig. 11.2). Beet molasses, the main by-product generated during sugar production, is the most common substrate in annexed distilleries.

Sweet Sorghum

Sweet sorghum (*Sorghum bicolor* L.) is a potential energy crop (C4 plant) rich in fermentable sugars (Bertrand et al. 2016). The crop shows a short cycle (4–5 months), high photosynthetic efficiency, and higher carbon assimilation rate (50 g/m^2 per day) (Zabed et al. 2014). Besides, other unique characteristics such as drought resistance, tolerance to cold temperatures, salinity and alkalinity,

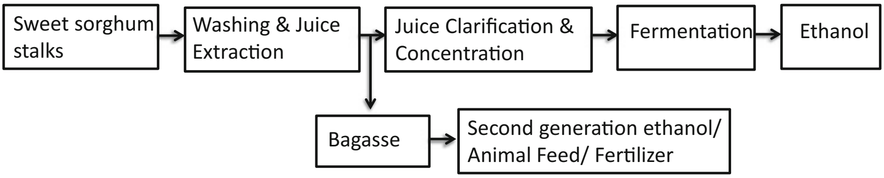

Fig. 11.3 Scheme of ethanol production from sweet sorghum

resistance to pest and disease, lower input demands, and wider acclimatization enable the plant to grow in a range of temperate and tropical climatic niches in irrigated as well as arid lands (Balat et al. 2008; Zabed et al. 2014). The stalks of the plant consist of extractable sugars such as glucose (9%), fructose (6%), and sucrose (85%) (Bertrand et al. 2016). The processing of raw juice starts with the step of liming which is performed to precipitate out the impurities removable by filtration technique. The thin juice (clarified) obtained from filtration is concentrated in an evaporator which yields thick juice, which is transferred to the fermentation tank for ethanol production. The pulp generated in the multistage process is dried and utilized for its pharmaceutical value or as animal feed. The bagasse generated after juice extraction can be used as animal feed or fertilizer, or even as a substrate for cellulosic ethanol (Holou and Stevens 2012; Ray et al. 2019). Additionally, starch-containing grain part of sweet sorghum can also be converted into ethanol (Di Nicola et al. 2011; Ray et al. 2019). Fig. 11.3 depicts the simplified scheme for production of ethanol using sweet sorghum as a feedstock.

Corn

Corn is a starch-rich crop. Starch is a homopolymer of glucose sugar units linked together by glycosidic linkages. The starch may consist of amylose in which glucose molecules are linked together by α-1,4 bonds and amylopectin which is a highly branched polymer having α-1,6 linkage between glucose molecules. In plants, starch is accumulated as granules in the amyloplast organelles in plant cells. The bioconversion of starch to ethanol involves hydrolysis of starch carbohydrate chains into glucose monomers, which can subsequently be fermented to ethanol. The liquefaction of starch is carried out enzymatically using amylase enzymes. α-Amylase, obtained from bacteria, is an endo-amylase that breaks down the α-1,4 bonds randomly in the polymer chain and yields dextrins of variable lengths along with small amounts of glucose. The liquefaction is generally carried out at high temperatures near 90–110 °C. In the next step, saccharification is done at relatively lower temperatures of 60–70 °C, using glucoamylase which is obtained from fungi such as *Aspergillus* or *Rhizopus*. The glucoamylase is an exo-amylase which removes glucose molecules from the ends of the polymers of dextrin (Fig. 11.4) (Di Nicola et al. 2011). During the bioconversion of corn to ethanol, dry milling produces dried

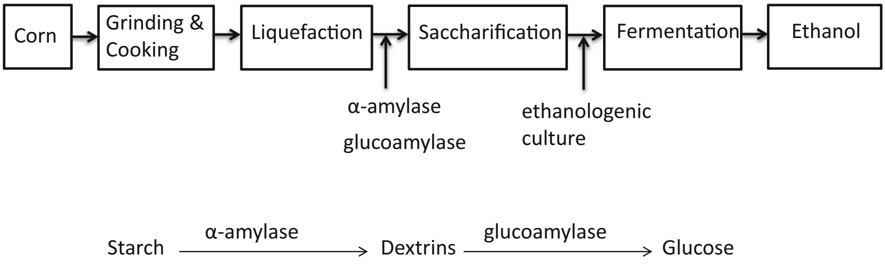

Fig. 11.4 Scheme of ethanol production from corn

distiller's grain with solubles (DDGS) as a co-product, which is primarily used as animal feed (Sanchez and Cardona 2008).

Wheat

Wheat is produced in around 120 countries. European Union, China, and India are among the largest producers of wheat (Patni et al. 2013; Mohanty and Swain 2019). Temperate regions are most suitable for growth of this crop (Di Nicola et al. 2011). The global wheat production in the year 2017 has been reported to be 737.83 million metric tons (Mohanty and Swain 2019). Though it is primarily used as a food crop, in several countries, mismanagement in the storage houses leads to the destruction of significant amounts of wheat, which could have been used as a feedstock for ethanol production (Patni et al. 2013).

The scheme of bioethanol production from wheat is shown in Fig. 11.5a. The wheat grains are milled in the first step followed by liquefaction of the meal obtained from milling at a higher temperature. Mixing is ensured and the starch-rich mash is produced, which is saccharified enzymatically in the next step of hydrolysis. The hydrolyzed mash is subjected to fermentation using appropriate ethanologenic microbial strain and the ethanol produced is recovered at the end mostly by using a distillation method (Kahr et al. 2012).

In France, wheat is also used for ethanol production other than beet molasses (Patni et al. 2013). However, the wheat has not been used as a preferred starchy feedstock for ethanol production compared to corn because of lesser productivity of wheat per hectare of land.

Cassava

Cassava, belonging to the family Euphorbiaceae and native of South America, is a rich source of starch found in tropical and subtropical countries. Approximately 70–90% of the total dry matter of cassava is made up of starch. It is a tuberous plant, which is known among the five most common starch crops in the world (Marx

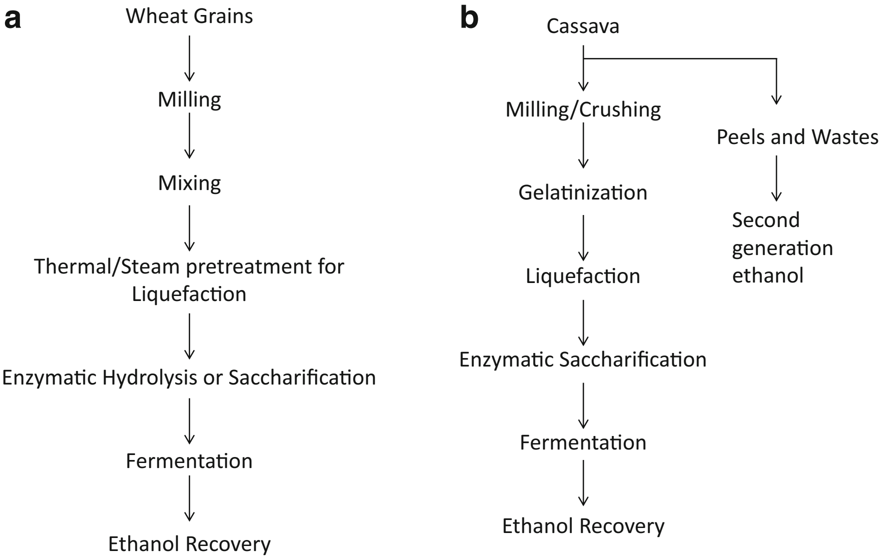

Fig. 11.5 Scheme of bioethanol production from (**a**) wheat and (**b**) cassava

2019). The plant is tolerant to drought, demands lesser inputs, and can be cultivated on marginal lands. The global cassava production is estimated to be approximately 281 million tons a year (Bertrand et al. 2016). Africa only accounts for more than 50% of the global cassava production (Bertrand et al. 2016). In 2014, Nigeria produced larger than 52 million tons of cassava (Marx 2019). Asia contributes to approximately one-third of the world's cassava production, with Thailand and Indonesia being the main producers (Bertrand et al. 2016). A large number of products such as chips, food, feed, flakes, and flour are produced from cassava (Marx 2019). It is also used for the production of glucose syrups (Sanchez and Cardona 2008). The ethanol production from cassava is similar to ethanol production from other starchy materials such as corn. However, compared to cornstarch, cassava starch requires lower gelatinization temperature and solubilizes amylases effectively (Sanchez and Cardona 2008). The higher moisture content causes cassava to deteriorate rapidly; hence, it is often converted into sun-dried cassava chips for further use. The process for ethanol production from cassava is outlined in Fig. 11.5b. The process begins with the grinding of fresh roots or chips and milling with water followed by liquefaction or cooking (usually performed at temperatures above 50 °C using α-amylase enzymes), saccharification (with glucoamylase enzymes), and then fermentation to ethanol (Sanchez and Cardona 2008; Marx 2019).

Ethanol from Other Starchy Materials

Besides corn, cassava, and wheat, ethanol can also be produced from other starchy materials such as barley, oat, sorghum, and rye (Chiaramonti 2007; Sanchez and Cardona 2008). Barley shows slower starch and ethanol recovery compared to corn (Gibreel et al. 2009). This is because it generates viscous β-glucans due to the presence of mixed glucan in its endosperm, which are not hydrolyzed easily and its hydrolysis sugars are not fermented by *S. cerevisiae* (Nghiem et al. 2010). However, barley hull can be used for the production of cellulosic ethanol and other contents can be used for the production of different nutraceutical and functional food products, which may offer the benefits of biorefinery (Gibreel et al. 2009). Recently new starch-rich varieties of barley are being developed to obtain higher yields of ethanol from them.

The production processes from starchy feedstock involve similar steps of milling, liquefaction, saccharification, and fermentation. The cereal grains can be subjected to either *wet milling* or *dry milling*. The wet milling involves direct milling of grains without pretreatment, followed by addition of water and heating for liquefaction. In wet milling, grains are steeped in water (at 50 °C for 1–2 days) for their softening, and then softened grains are milled (Chiaramonti 2007). In all substrates, optimization is generally required to ensure maximum yield at every step during the multistep process of ethanol production.

11.2.1.2 Current Status and Challenges

The bioethanol came in use as an alternative fuel after the oil crisis of the 1970s. Programs were made by different countries to develop fuels from the different feedstock. After another oil crisis of 1979, the production of ethanol was started at a commercial scale as an alternative fuel for the transport sector (Balat 2009). The global bioethanol production reached 88.69 billion liters (~23.42 billion gallons) in 2013 from 13.5 billion gallons in 2006 (Balat et al. 2008; Gupta and Verma 2015). In the USA, more than 130 ethanol plants were established till 2007 (with nearly 26 billion liters of total capacity on an annual basis). Brazil also increased its capacity for ethanol production by 40% between 2005 and 2009 (Naqvi and Yan 2015).

Currently, most of the global ethanol supply relies on first-generation feedstock. This is because production technology for bioethanol from starch and sugar crops is well developed. Bioethanol is mostly produced from corn (USA and China), sugarcane (Brazil), sugar beet and wheat (EU), and sugarcane molasses (India). Sugarcane-based ethanol is also being promoted in sub-Saharan Africa and Latin America. Less commonly used feedstock involves cassava (Southeast Asia and China), sweet potato, and sweet sorghum (China) (Gasparatos et al. 2013). Among different countries, the USA and Brazil account for more than 85% of the world's total ethanol production (Lopes et al. 2016). The USA and Brazil produce approximately 15.25 billion gal and 7.3 billion gal of bioethanol annually, respectively

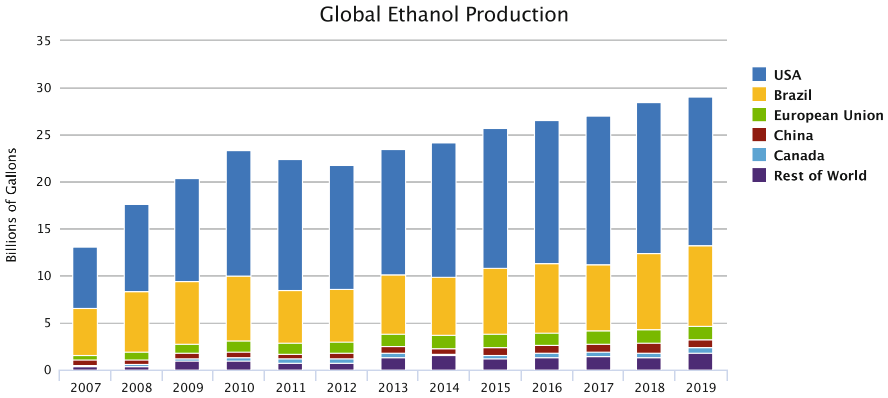

Fig. 11.6 Ethanol production by different countries from 2007 to 2019 (source: AFDC 2020)

Table 11.2 First-generation ethanol production in different countries

Country	Feedstock	Annual ethanol production (billion liters)	Costs (US $/L)
USA	Corn (maize)	50.3	0.25–0.40
Brazil	Sugarcane	25.5	0.16–0.22
EU	Cereal and sugar beet	4.5	–
Canada	Wheat/cereal	1.8	–
Thailand	Cassava	1.0	0.18
France	Sugar beet	1.0	0.60–0.68
China	Molasses, sweet sorghum	–	0.32, 0.29
Argentina	Sugarcane	0.5	–
Belgium	Wheat	0.4	–
Spain	Barley, wheat	0.4	–
Australia	Sugarcane	0.3	–
Poland	Rye	0.2	0.55–0.65
Sweden	Wheat	–	0.40–0.45

Source: Bertrand et al. (2016).

(Mohanty and Swain 2019). The ethanol produced in the USA is estimated to replace around 500 million barrels of petroleum every year (Ramos et al. 2016). Around 10% of the bioethanol produced in the USA is exported to different countries including Brazil and Canada, and a major part of ethanol produced in Brazil is used in the domestic market only. Brazil imported around 1200 million L of ethanol from the USA in 2019 (Susmozas et al. 2020).

The global ethanol production by different countries from 2007 to 2019 has been given in Fig. 11.6. Table 11.2 enlists different countries producing first-generation ethanol from the various feedstock. The USA and Brazil do not only differ in their bioethanol feedstock, but these countries also rely on different technologies for

Table 11.3 World's current annual ethanol fuel production (billion gallons)

Country	2018	2019	% of world ethanol production
USA	16.091	15.778	54%
Brazil	7.990	8.590	30%
European Union	1.450	1.370	5%
China	0.770	1.000	3%
Canada	0.460	0.520	2%
India	0.430	0.510	2%
Thailand	0.390	0.430	1%
Argentina	0.290	0.280	1%
Rest of the world	0.529	0.522	2%
Total	28.400	29.000	

Source: RFA analysis of public and private data sources, https://ethanolrfa.org/statistics/annual-ethanol-production/

ethanol production. The distilleries in the USA distil the fermented medium along with yeast cells because of high solid concentrations, and therefore, yeast cells are not recycled. As a result, fermentation yield is relatively lower (Lopes et al. 2016). In Brazil, autonomous industries ferment juice only, while those attached to sugar industries use molasses or a mix of juices for fermentation (Lopes et al. 2016).

According to estimates of the year 2016, around 95% of the total bioethanol produced in the USA was contributed by corn, while only 3% was produced from wheat (Mohanty and Swain 2019). The ethanol production in the USA has increased not only due to high productivity of corn, but the higher oil prices and increased subsidies have also been among major contributors to this increase (Naqvi and Yan 2015). Corn-based ethanol could not be increased much in many countries because of higher costs. Similarly, sugarcane-based ethanol could not be expanded in Africa and Latin America owing to lower crop yield compared to Brazil (Naqvi and Yan 2015). Among different cereals, wheat is most widely cultivated in different countries, yet it is not preferred over corn as the primary feedstock for producing ethanol due to its lower productivity compared to corn (Mohanty and Swain 2019). Table 11.3 gives an overview of current ethanol production in different countries from 2018 till 2019.

Other than Brazil and the USA, many other countries have also emerged as bioethanol producers. China and Canada produce around 1000 million gal and 436 million gal of first-generation ethanol, respectively, using different starchy crops such as corn, cassava, rice, and wheat (Mohanty and Swain 2019). The ethanol production has also been done by countries like India (1 billion L), France (1 billion L), Germany (750 million L), and Australia (500 million L) from crops such as sugarcane, sugar beet, molasses, and wheat (Mohanty and Swain 2019). The world's third largest bioethanol industry is in China (Zhao et al. 2015). China contributes to 21% of the world's corn production but it accounts for only 3% to the total ethanol production in the world (Mohanty and Swain 2019). Owing to the advanced fermentation technology and corn cultivation techniques, the price for bioethanol production in China is 1.5–2.0 times higher compared to the production cost in the USA (Wang et al. 2011). China is likely to implement the E10 mandate after 2020, for which it has planned for more bioethanol plants in the country. China is also expanding its technology in countries like Nigeria (Susmozas et al. 2020).

By 2008, sugar beet, wheat, and other cereal grains were the primary feedstock for bioethanol production in the EU. After 2012, corn-based ethanol was expanded. By 2013, 70 plants were set up in the EU which could produce approximately 2.5 billion gallons of ethanol annually (Ramos et al. 2016). In 2014, the EU produced 40% of it from corn, 26% from wheat, and 20% from the sugar beet. However, in 2018, Europe imported 0.618 billion L ethanol from different countries such as Pakistan, the USA, and Brazil (Susmozas et al. 2020). The corn-based ethanol is likely to increase in future in the EU (Takács-György et al. 2020).

The biggest challenge in the expansion of first-generation bioethanol is its sustainability. The sustainability of biofuel is assessed in terms of not only its technical feasibility but also its economical affordability, environment, and social viability (Gomiero 2015).

- First-generation bioethanol is generated from food crops, and they are already not sufficient to meet the growing demands of growing populations across different nations (IEA 2008). Therefore, the production of fuel from edible crops contributes to higher food prices. It has been reported that only 2% of the world's farmland is used for cultivation of food crops and this is likely to contribute to the increase in prices of food as well as animal feeds (Bertrand et al. 2016). There are many studies which have also debated over *food* vs. *fuel* issue. The associated problems of burden on agricultural land, change in land use, and excessive fertilizer input lead to other issues (Naqvi and Yan 2015).
- The net cost of bioethanol is too high to ensure energy security to future generations when the cost is assessed by excluding government grants and subsidies (IEA 2008). One alternative for cost reduction is the use of biorefinery. Takács-György et al. (2020) have documented that selling of co-products generated from ethanol-producing plants in the EU can also reduce the deleterious effect of biofuel on land use and the environment. Furthermore, though many studies have claimed the GHG mitigation potential of first-generation biofuels, recent life cycle assessments have concluded limited potential especially when all factors in its production are considered (IEA 2008; Naqvi and Yan 2015). Moreover, the calculation for carbon debts and carbon payback time is based on several assumptions so that the final conclusions are not certain (Gasparatos et al. 2013). Also, the claimed environmental benefits are not going to remain the same with the changing scale. The biomass characteristics are subject to variation with changing conditions of the environment and soil (IEA 2008). Also, different studies have presented different reports. For some pollutants, the life-cycle emissions of pollutants from sugarcane ethanol have been found to be higher than those from the conventional transport fuels (Gasparatos et al. 2013).

- Deforestation for biofuel production is one serious threat to biodiversity and is detrimental to nature (IEA 2008). The associated problems of soil erosion are significant.
- Additionally, a sustainable water supply is another constraint. Varying water requirements for biofuel production from different feedstock in different geographical locations may contribute to the added burden on water resources of a country (Gasparatos et al. 2013). The biofuel-related water pollution can also be a serious concern in the future.

One important point is that the social perspectives are also going to decide the future of first-generation biofuels. The expansion in first-generation biofuels has raised conflict in developing countries regarding land-use change, land tenure issues, as well as other concerns such as regulation required for biofuel production. However, considering the benefits, the rural development through the creation of employment and income can be a driving force for first-generation ethanol expansion in several regions (Gasparatos et al. 2013; Naqvi and Yan 2015).

11.2.2 Second-Generation Ethanol

11.2.2.1 Production Technology

The second-generation ethanol came as an alternative to the limitations associated with the first-generation ethanol. The use of second-generation ethanol has the potential to eliminate problems of sustainability of first-generation ethanol. The second-generation ethanol or cellulosic ethanol relies on carbohydrates locked in the cell walls of waste lignocellulosic biomass, which can vary in its origin from natural to agro-industrial residues. The primary constituents of lignocelluloses in all plants include cellulose, hemicellulose, and lignin (Isikgor and Becer 2015). Small quantities of pectin, proteins, extractives, and inorganic chemicals are also present (Baruah et al. 2018). The biomass composition varies among plants belonging to different species or geographical locations with varying climatic conditions. Other factors such as plants' age and variety also play a significant role in the final composition.

Cellulose is a homopolysaccharide (a macromolecule) made up of D-glucose, a fermentable sugar, in which monomeric units are linked together in linear chains through β-(l-4)-glycosidic bonds (Robak and Balcerek 2020). However, the degree of polymerization differs in celluloses from different sources. The chains of cellulose are held together by multiple intra-chain and interchain hydrogen bonds and other hydrophobic interactions and make macromolecular structures called as *macrofibrils*, made up of smaller bundles of *microfibrils*. The macrofibrils are aggregated together to form cellulose fibers, which are tough structures requiring specific treatment for the hydrolysis (Bajpai 2016). The supramolecular structure of cellulose may have crystalline (high packing density) and amorphous regions (less

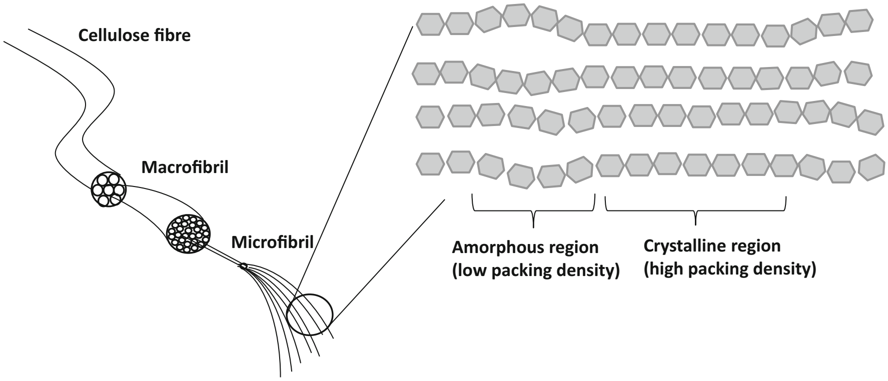

Fig. 11.7 Structure of cellulose

ordered) (Chen 2014). The crystallinity of cellulose also varies among different sources, and the crystalline form is relatively more difficult to hydrolyze during chemical and enzymatic hydrolytic processes (Fig. 11.7). Hemicellulose is a branched complex heteropolymer made up of different C5 sugars (D-xylose and L-arabinose), C6 sugars (D-glucose, D-galactose, and D-mannose), and uronic acids (D-glucuronic acid, D-galacturonic acid) (Bajpai 2016) (Fig. 11.8) mostly linked together by β-1,4-glycosidic bonds. The hemicellulose interacts with other components of lignocellulose and confers physical strength to the cell wall. The acetylation occurs in the hemicellulose at several points, which may pose steric hindrance against binding of hydrolytic enzymes, therefore making enzymatic hydrolysis of hemicellulose difficult. Xylan, a polymer of xylose, is the most abundant polymer of hemicellulose. Other types of hemicelluloses may include xyloglucans, glucuronoxylans, mannans, glucans, arabinoxylans, glucomannans, galactomannans, and galactoglucomannans (Chen 2014; Bajpai 2016). The hemicellulose is amorphous in nature as side groups and branching hinder the formation of highly ordered structure and, therefore, hemicellulose is easy to hydrolyze compared to cellulose.

The cellulosic and hemicellulosic sugars can be fermented to yield ethanol. These polymers are present as a matrix embedded in tough cementing component, i.e., lignin. Lignin is a branched heteropolymer, which is chiefly composed of three phenylpropanoid macromolecules (monolignols), i.e., p-coumaryl, coniferyl, and sinapyl alcohol (Hatfield and Vermerris 2001). Various alkyl- or aryl-ether interunit linkages give a highly branched and amorphous structure to the lignin (Bajpai 2016). Different types of plants vary in their composition of lignin; for example, coniferyl alcohol is found abundantly in softwoods, whereas hardwoods are rich in coniferyl and sinapyl alcohols (Fig. 11.8). Lignin is known to play important roles in the plant cell walls. It confers rigidity and resists chemical and microbial attack making cell wall an impervious and resistant structure (Bajpai 2016). Lignin is present within the cell wall matrix and as a cover over sugar polymers. It also adsorbs hydrolytic enzymes competitively resulting in their inhibition, thereby

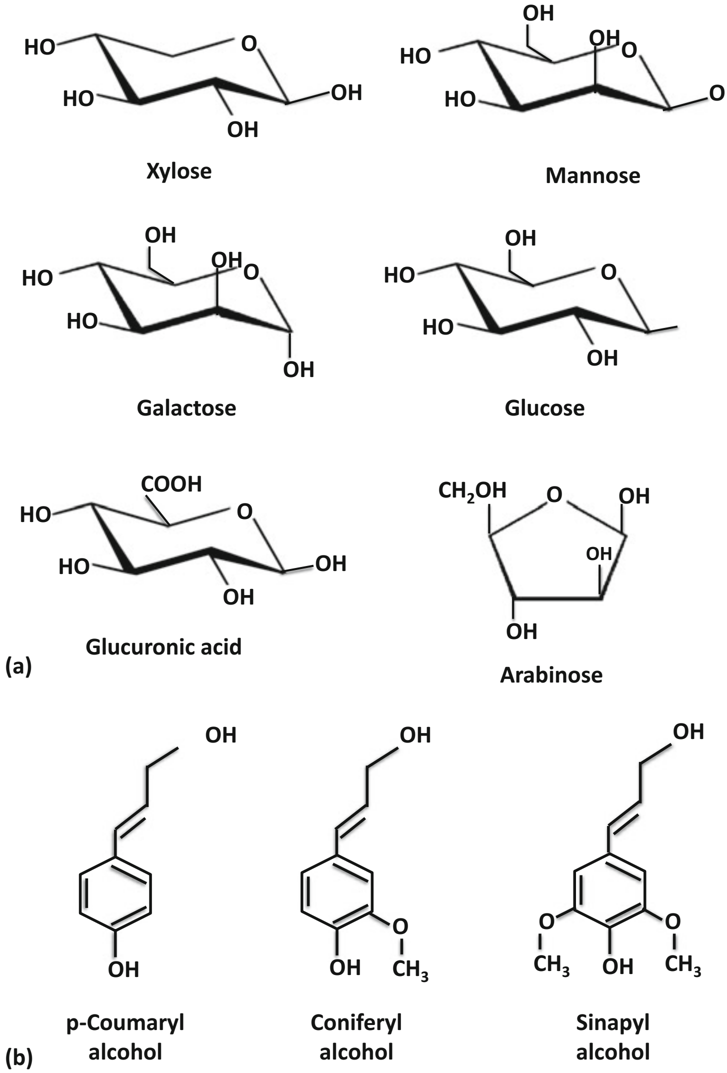

Fig. 11.8 Primary constituents of (**a**) hemicellulose and (**b**) lignin

reducing the digestibility of the lignocellulosic biomass (Li et al. 2016). The removal of lignin through appropriate pretreatment is often considered essential during the bioconversion of lignocellulose to ethanol.

The lignocellulosic bioconversion to ethanol can be carried out either thermochemically or through biochemical route (Fig. 11.9). In the thermochemical pathway, syngas is produced from biomass by subjecting it to high temperatures (750–120 °C) in the presence of limited amounts of oxygen (Robak and Balcerek 2020). The syngas is a mixture of gases such as carbon monoxide (CO), carbon

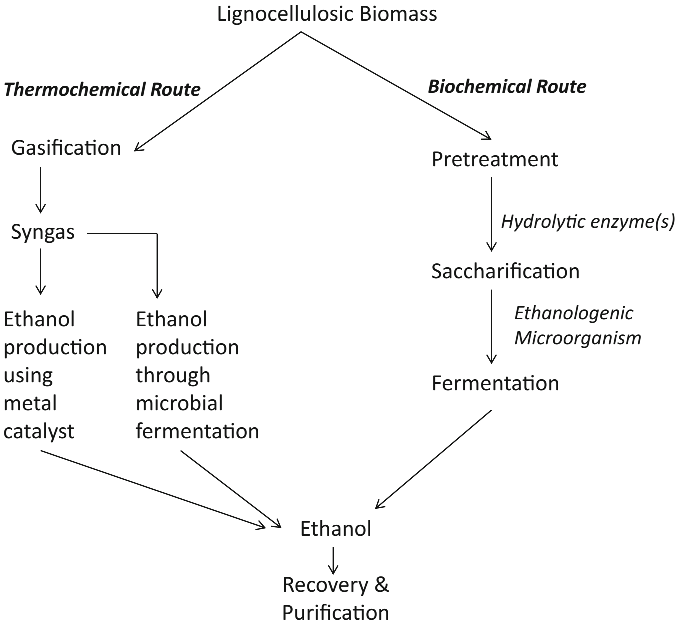

Fig. 11.9 Second-generation ethanol production scheme: thermochemical and biochemical route

dioxide (CO_2), and hydrogen (H_2), with small amounts of methane (CH_4), nitrogen (N_2), tar, short-chain alkanes, etc. The clean syngas can be converted to ethanol using a metal catalyst or through microbial fermentation using anaerobic acetogenic bacteria, which ferment the syngas mixture into acetic acid, ethanol, and other co-products (Devarapalli and Atiyeh 2015). The advantage of thermochemical pathway is that all biomass components including lignin are utilized for bioethanol production (Vohra et al. 2014).

The biochemical conversion of lignocellulose to ethanol starts with the step of "*pretreatment*" in which lignin and/or hemicellulose are removed. Often removal of lignin is essential as it decreases the digestibility of biomass. The hemicellulose is removed if only cellulose is to be converted to ethanol. Though hydrolysis of both hemicellulose and cellulose can generate fermentable sugars, different ethanologenic microbes are used in carrying out fermentation of hexoses and pentoses effectively. Simultaneous fermentation of hexoses and pentoses requires genetically modified strain or mixed culture (will be discussed under different fermentation strategies for ethanol production). The pretreatment removes the lignin and exposes the underlying sugar polysaccharides for their saccharification in the next step. Thus, pretreatment deconstructs the cell walls making them susceptible to degradation in the subsequent step. The financial viability of second-generation biofuel production depends on the efficiency of the pretreatment. The pretreatment may be carried out using different physical, chemical, physiochemical, and biological methods (Kumar et al. 2009). In the following step of "saccharification," the biomass is subjected to

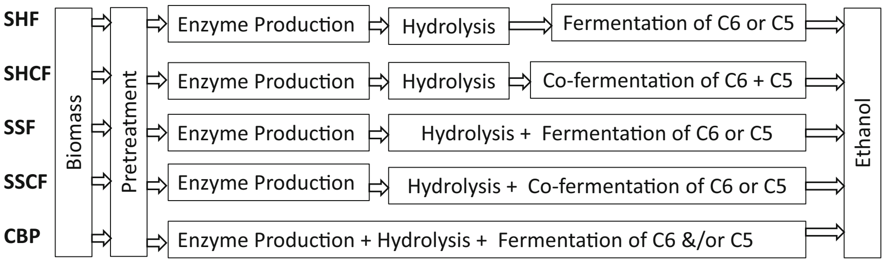

Fig. 11.10 Fermentation strategies for bioethanol production processes

acid or enzymatic hydrolysis. The dilute acid hydrolyzes the hemicellulose and relatively higher concentrations of acid are required to hydrolyze cellulose. However, enzymatic hydrolysis is preferred over chemical method as acid hydrolysis generates inhibitory compounds which may interfere in the subsequent step of fermentation. The enzymatic saccharification involves the use of cellulolytic and hemicellulolytic enzymes for the hydrolysis of cellulose and hemicellulose, respectively (Robak and Balcerek 2020). The hydrolysate obtained after saccharification process contains glucose as a result of cellulose hydrolysis and primarily C5 sugars from the hydrolysis of hemicellulose. Finally in the last step, fermentation of C6 or C5 sugars is carried out using appropriate wild or genetically modified ethanologenic strain, and the ethanol is recovered at the end of the process.

The ethanol production from lignocellulose may be carried out using different fermentation strategies such as separate hydrolysis and fermentation (SHF), separate hydrolysis and co-fermentation (SHCF), simultaneous saccharification and fermentation (SSF), simultaneous saccharification and co-fermentation (SSCF), and consolidated bioprocessing (CBP) (Zabed et al. 2016) (Fig. 11.10). In the SHF method, after the pretreatment step, hydrolysis and fermentation steps are carried out in separate fermentation vessels. Although it allows separate regulation and optimal operation of saccharification and fermentation steps (Putro et al. 2016), the saccharification yield is often low owing to the inhibitory effect of glucose and cellobiose accumulated in the hydrolysate as end products of cellulose saccharification. Also, the use of separate bioreactors makes it a costly method. In the SHCF approach, the fermentation of hexoses (C6) and pentoses (C5) is integrated in one step, whereas saccharification and pretreatment are carried out separately (Zabed et al. 2016). The method is advantageous as it utilizes both hexoses and pentoses released from biomass hydrolysis for ethanol production. The method may involve the use of either genetically modified microbial strains capable of fermentation of both hexoses and pentoses or co-culture of pentose- and hexose-fermenting microbes. In the SSF method, both hydrolysis and fermentation of the biomass (appropriately pretreated) are integrated in a single vessel. The glucose and cellobiose sugars released from the hydrolysis are utilized simultaneously in the process without their accumulation in high concentrations so that the problem of their inhibitory effect or low yield of reducing sugars is eliminated. However, as optimal conditions for fermentation and hydrolysis are different, a compromise is made between their optimal temperatures

(Olofsson et al. 2008). Studies have revealed significant enhancement in final ethanol yields in SSF methods compared to SHF strategy (Putro et al. 2016). In SSCF, the pretreated biomass is subjected to saccharification and fermentation of both pentoses and hexoses in a single fermenter (Putro et al. 2016). This strategy is considered advantageous because the cost of the process is reduced due to decrease in the number of vessels, and the problem of feedback inhibition is solved. The method results in higher ethanol production compared to SSF. The most integrated approach of CBP involves production of hydrolytic enzyme, saccharification, and fermentation of the pretreated biomass in the same fermentation vessel. The method is also referred to as "direct microbial conversion (DMC)." Theoretically, CBP approach is the most cost effective among all of the above-discussed configurations (Putro et al. 2016).

11.2.2.2 Feedstock

Second-generation ethanol can be produced from a wide variety of feedstock such as wastes from agricultural, industrial, forest, and municipal origin; dedicated energy crops; and weed biomass (Robak and Balcerek 2020).

Every year globally large amounts of agricultural wastes, the leftover residues after crop harvesting, are generated in the form of straws, husks, stalks, leaves, roots, peels, cobs, shells, and nuts. Examples include rice straw, wheat straw, corn cob, sugarcane bagasse, sorghum straw, coconut husk, cotton stalk, vegetables' and fruits' peels, bamboo wastes, etc. Management of this waste is usually a problem for several countries, especially when the waste is bulky or undergoes slow degradation in the soil. This lignocellulose-rich biomass has huge potential for second-generation ethanol production. The availability of different types of agricultural wastes varies among different geographical and climatic regions in various countries. The choice of the feedstock, thus, depends on these factors. Among different agricultural wastes, residues from major cereal crops and vegetables have gained attention worldwide. However, the spectrum of feedstock for cellulosic ethanol is being broadened in order to ensure uninterrupted supply of substrate for ethanol production.

Rice, the third most widely cultivated crop worldwide, generates huge quantities of waste as rice straw. Only a small portion of it could be utilized or managed. The bulkiness of these residues, the presence of high mineral content, and the problem of pathogen development in slowly degrading straw make the management of this waste a concern. The Western, European, and many Asian countries have banned the open-field burning of rice straw on account of the resulting air pollution. Therefore, researchers have started utilizing rice straw for producing cellulosic ethanol. Wheat straw generated from wheat, the second largest cereal crop all over the world, is also a potential agricultural waste for cellulosic ethanol production. Despite different uses, the disposal of wheat straw generated in huge amounts after every crop is a concern for the farmers and environmentalists. Its utilization for bioconversion into ethanol may offer an alternative strategy for the management of this waste.

Sugarcane is grown in most countries (tropical and subtropical) primarily for sugar production. The estimates have shown 1900 million metric tons of sugarcane production worldwide in the year 2013 (Bezerra and Ragauskas 2016). The bagasse is a lignocellulosic, fibrous by-product from the sugarcane industry, having no nutritive value. Each year around 279 million metric tons of sugarcane bagasse is generated globally and its disposal is a major concern (Jugwanth et al. 2020). The easy availability and lignocellulosic composition of sugarcane bagasse make it an attractive feedstock for bioethanol production. Corncob, a globally abundant by-product from the corn industry, is mostly used either as fodder or for humus production. The corncobs are rich in holocellulose (cellulose + hemicellulose), which makes them a potent candidate for cellulosic ethanol production (Arumugam et al. 2020). In countries such as the USA where first-generation ethanol is produced from corn, the integration of second-generation ethanol from corncob can prove an interesting and profitable biorefinery approach for enhanced ethanol production. Similarly, the potential of other agricultural residues is shown in Table 11.4.

Lignocellulosic wastes produced by different industries including pulp and paper industries, cardboard industry, textile industries, food processing industries, etc. have also proved to be potential candidates for bioethanol production. Brewer's and distiller's spent grains can also be utilized for second-generation ethanol production. Liguori et al. (2015) have documented production of 0.53 g/L ethanol from alkaline-acid pretreated brewers' spent grain saccharified by commercial cellulases and then fermented to ethanol using *Saccharomyces cerevisiae* NRRL YB 2293. Megala et al. (2020) have reported production of 0.58 g/l, 0.543 ml/g, and 0.334 mL/g of bioethanol from textile cotton waste after its acid, ultrasonic, and solvent pretreatment, respectively. M'barek et al. (2020) have exploited olive mill waste for ethanol production using consolidated bioprocessing method. They used *F. oxysporum* as CBP microorganism and achieved production of 2.47 g/L ethanol with 0.84 g/g yield.

Forest residues such as litter, wood chips, shavings, forest thinning, hardwood, and softwood have also been explored for their potential as bioethanol feedstock. For example, 22.90 g/l of ethanol has been produced from palm wood after its hydrothermal pretreatment using *Trichoderma reesei* MTCC 4876 for saccharification and *Kluyveromyces marxianus* MTCC 1389 for fermentation to ethanol (Sathendra et al. 2019). Similarly, ethanol production from other forest-based lignocellulosic biomass is shown in Table 11.4.

Municipal solid wastes, such as food waste, waste papers and boards, packaging papers, waste clothes, and other woody refuse, are also rich in lignocelluloses. It has been estimated that by 2050, the world's annual municipal waste production is likely to reach 3.4 billion tons (Dornau et al. 2020). Management of such huge amounts seems difficult and valorization of this waste to the valuable product like bioethanol is a promising approach. Dornau et al. (2020) pretreated organic portion of municipal solid waste by autoclaving followed by enzymatic saccharification and fermentation using different microbial strains. They could achieve 69%, 70%, and 72% of highest theoretical fermentation yield using *Z. mobilis*, *S. cerevisiae*, and *Rhodococcus opacus*, respectively. Prasoulas et al. (2020) have also documented

Table 11.4 Bioethanol production from various lignocellulosic feedstock

Feedstock	Bioethanol production strategy	Ethanol production achieved	Reference
Rice straw	Simultaneous saccharification and co-fermentation of biomass subjected to combined pretreatment using alkaline peroxide and mixture of ionic liquid-water, by employing cellulase enzyme and SHY 07–1 yeast	91.9 g/L Ethanol	Hong et al. (2019)
Rice straw	SHF of NaOH-pretreated biomass using cellulase and hemicellulase from *Aspergillus fumigatus* (200 FPU/mL crude enzyme) and ethanologenic yeast of *Saccharomyces tanninophilus*	9.45 g/l (83.5% yield)	Jin et al. (2020)
Wheat straw	Substrate pretreated by combined microwave-NaOH treatment followed by saccharification using *Bacillus* sp. BMP01 (termite gut origin) and then fermentation of hexoses and pentoses by *S. cerevisiae* MTCC 174 and *Zymomonas mobilis* subsp. *mobilis* MTCC 91, respectively	68.2% Ethanol yield	Tsegaye et al. (2019)
Sugarcane bagasse	NaOH-pretreated biomass subjected to semicontinuous SSF using 10 FPU/g Cellic® CTec2 and fermented by *S. cerevisiae* strain (CLQCA-INT-005)	8.36–10.79% (v/v) ethanol level	Portero Barahona et al. (2020)
Sugarcane bagasse	SSF of steam-assisted salt-alkali-pretreated biomass using Celluclast 1.5 L enzyme and *S. cerevisiae* as ethanologen	4.88 g/l Ethanol	Jugwanth et al. (2020)
Corncob	SSF involving prehydrolysis using commercial Cellic CTec 2 cellulase (160 FPU/ml) and microaerophilic fermentation by *S. cerevisiae* strain BY4743	42.24 g/l Ethanol	Sewsynker-Sukai and Kana (2018)
Sunflower stalk	NaOH- and *Trichoderma reesei*-pretreated biomass saccharified by enzyme cocktail of commercial cellulase and β-glucosidase, followed by fermentation using *S. cerevisiae* TISTR5020	12.562 g/L Ethanol with 71.6% fermentation efficiency	Manmai et al. (2020)
Mixture of rice hull and orange peel	Biomass pretreated and hydrolyzed by sulfuric acid, and then fermented to ethanol using *S. cerevisiae*	22.77 g/l Ethanol (or 0.295 l ethanol/kg dry matter)	Taghizadeh-Alisaraei et al. (2019)

(continued)

Table 11.4 (continued)

Feedstock	Bioethanol production strategy	Ethanol production achieved	Reference
Eucalyptus grandis biomass	SSF of steam-exploded biomass	299 kg Ethanol per metric ton of dry biomass	McIntosh et al. (2017)
Beech wood	Biomass pretreated by acetone-water oxidation method, saccharified by commercial enzyme (Cellic® CTec2) and fermented using *S. cerevisiae*	75.9 g/l Ethanol	Katsimpouras et al. (2017)
Miscanthus	Trifluoroacetic acid-delignified biomass (with high-purity cellulose) subjected to ethanol production using a consortium of *S. cerevisiae* MY4242, *Pachysolen tannophilus* Y3269, and *Scheffersomyces stipitis* Y3264	3.1–3.4% Bioethanol yield	Kriger et al. (2020)
Napier grass	Simultaneous saccharification and fermentation of alkali-pretreated biomass	86.6% Ethanol yield	Tsai et al. (2018)
Reed	Biomass pretreated by combined liquid hot water (LHW) and sodium carbonate with oxygen subjected to SSF for bioethanol production	66.5 g/l Ethanol (13.3 g/100 g reed)	Lu et al. (2020)
Yellow poplar	Biomass pretreated by deacetylation and oxalic acid treatment followed by ethanol production	0.34–0.47 g/g Ethanol yield 27.21 g/l Ethanol produced through SSF	Kundu et al. (2015)
Water hyacinth	Biomass treatment with *A. terreus* F-98 and then acid hydrolysis (H_2SO_4), followed by fermentation using *C. tropicalis* Y-26	14 g/l bioethanol	Madian et al. (2019)
Parthenium hysterophorus	NaOH-delignified biomass, hydrolyzed by combination of 1% sulfuric acid and 80 mg cellulase/g biomass, fermented using three strains of yeast *Torulaspora delbrueckii* R3DFM2, *S. cerevisiae* R3DIM4, and *S. pombe* R3DOM3	0.24, 0.27, and 0.27 g ethanol/g dry biomass using three strains, respectively	Tavva et al. (2016)

bioethanol production from dilute acid-pretreated household food wastes using in site-produced enzyme (from *Fusarium oxysporum* F3) and *S. cerevisiae* as ethanologen. The researchers showed production of 20.6 g/L ethanol using mixed culture, and the yield was enhanced further to 30.3 g/L when glucoamylase enzyme was supplemented in the mixed culture.

Several crops, known as "*energy crops*," are cultivated with the sole purpose of obtaining bioenergy from the biomass. These crops include many nonfood herbs and short-term wood crops as well (Gent et al. 2017). Examples include switchgrass, *Miscanthus*, reeds, willow and *Populus*, napier grass, etc. (Hattori and Morita 2010). These crops are low-maintenance crops with high solar energy conversion efficiency. They demand lower energy inputs and lesser chemicals are required for their cultivation. Zhang et al. (2017) studied the bioethanol productivity from switchgrass and also assessed the impact of its cultivation in marginal land soil on the environment during its complete life cycle. The authors found that switchgrass grown over 59 million hectares of China's marginal land could give rise to 22 million tons of ethanol. They reported that the entire marginal land of China could potentially produce a net energy of 1.75×10^6 million MJ. A lowered potential of global warming was also observed during the study. Bioethanol production from several other dedicated energy crops is shown in Table 11.4.

Recently, weed biomass from different plants has been considered an unconventional feedstock for ethanol production. The weed biomass can be obtained from both aquatic and terrestrial habitats. Water hyacinth (*Eichhornia crassipes*) is a perennial, aquatic weed plant that is known as a destructive invasive species in Africa and Asia. It is primarily found floating over the surface of water bodies. It blocks rivers and channels, exerts harmful influence on other aquatic plants, and restricts the light and air rendering it unavailable for the underwater animals and plants. The water hyacinth biomass consists of 7–26% lignin, 18–31% cellulose, and 18–43% hemicellulose (Zhang et al. 2016). The utilization of water hyacinth biomass can not only provide a feedstock for bioethanol but also offer an alternative for the management of this weed. Similarly, *Parthenium hysterophorus* is a terrestrial weed plant showing various ill effects on animals, other plants, as well as human beings. Several reports have even documented the negative impact of this weed on the natural biodiversity of different areas. Different physical, chemical, and biological methods used for the control of this weed have been unsuccessful in eradicating this weed or minimizing it to manageable levels. Therefore, weed biomass utilization for bioethanol production has been suggested as an alternative approach. Similarly, Borah et al. (2019) have evaluated the ethanol production potential of a composite of eight invasive weeds, i.e., *A. donax, Chromolaena odorata, Mikania micrantha, Lantana camara, Eichhornia crassipes, Ipomea carnea, Parthenium hysterophorus,* and *Saccharum spontaneum*. The group achieved a net 20 g bioethanol yield per kg of raw biomass.

11.2.2.3 Current Status and Challenges

Second-generation biofuels have evolved independently from first-generation biofuel production technology. They offer many benefits such as greater energy output compared to fossil fuels, production from a wide spectrum of lignocellulosic feedstock, alternative to land management issue, and environment-benign fuel. Owing to the advantages of cellulosic ethanol over first-generation ethanol, its production is being encouraged around the globe through various programs and legislation.

In the USA, total 115 facilities have been identified for all types of advanced biofuels, of which 39 represent the facilities for cellulosic ethanol (including proposed, existing, and under-construction plants) with approximately 1.37 billion liters of capacity (UNCTAD, 2016). Majority of the production is done in the Midwest, where corn stover serves as the primary feedstock. The second-generation ethanol production was first commercialized in 2013 by INEOS Bio from vegetative, agricultural, and municipal solid waste. By 2015, INEOS Bio, POET, Abengoa, and Quad County Corn Processors became the major cellulosic ethanol producers in the USA (UNCTAD 2016).

Renewable Energy Directive (RED) and Fuel Quality Directive (FQD) in the European Union aimed at using 10% renewable energy in the transport sector by 2020, which could not be met using food crops without having a negative effect on the environment. As a result, a shift to the lignocellulosic feedstock (agricultural, forest biomass, and energy crops) has been suggested as one alternative (Padella et al. 2019). In 2014, cellulosic ethanol accounted for 2.9% of total ethanol production; however, no increase is expected in the near future (Takács-György et al. 2020).

Brazil, which is known among the major producers of first-generation ethanol, has started to think about developing R&D for cellulosic ethanol production after experiencing a threshold in the agricultural and industrial yields of first-generation ethanol. The estimates have shown production potential of 6.75 billion liters of cellulosic ethanol using new mills, expansion of existing mills, and integrating of second- and first-generation ethanol production (UNCTAD 2016). Two commercial scale plants are operational in Brazil, which include Bioflex 1 plant, GranBio (around 65 kt per year of production capacity), and Raízen’s Costa Pinto Unit (36 kt of annual ethanol production capacity using bagasse) (Padella et al. 2019).

Other than developed countries having well-established first-generation biofuel production capacity, developing countries including Brazil, China, India, and Thailand have also made growth in the last few years first in the first-generation biofuel sector. Since 2015 Thailand and India have started making progress towards cellulosic ethanol production also. Some of the commercial cellulosic ethanol plants operational around the globe have been listed in Table 11.5.

Currently, wide-scale commercialization of second-generation ethanol is not possible around the globe because of various techno-economic challenges in the production process (Fig. 11.11). At the outset, availability of the feedstock in sufficient amounts may pose a problem. The feedstock may affect around one-third of the ethanol production cost (Dien et al. 2003). The bioethanol production technology needs to be optimized according to diverse feedstock types available in different geographical locations as setting up the facility in any country is dependent on the feedstock availability indigenously. The distantly located industries may face problems of logistics, i.e., transport and storage of biomass, which

Table 11.5 List of some global commercial-level second-generation ethanol plants

Company (status)	Country	Capacity (ktons)
Abengoa Bioenergy Biomass of Kansas, Hugoton, (idle)	USA	75
Aemetis (planned)	USA	35
Beta Renewables (on hold)	USA	60
DuPont (idle)	USA	83
INEOS Bio (idle)	USA	24
POET-DSM Advanced Biofuels (operational)	USA	75
Beta Renewables (acquired by Versalis) (on hold)	China	90
COFCO Zhaodong Co. (planned)	China	50
Longlive Bio-technology Company Limited (idle)	China	60
Borregaard Industries AS (operational)	Norway	16
GranBio (operational)	Brazil	65
Raízen Energia (operational)	Brazil	36
Beta Renewables (acquired by Versalis) (on hold)	EU (Slovakia)	55
Beta Renewables (acquired by Versalis) (idle)	EU (Italy)	40
North European Bio Tech Oy (NEB) (planned)	EU (Finland)	40

Source: Padella et al. (2019).

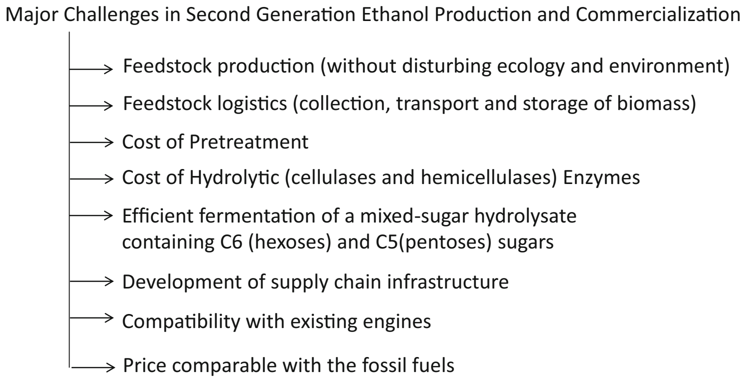

Fig. 11.11 Challenges in cellulosic ethanol production

may further increase the cost of the final marketed product. Different approaches are being adopted by various nations to address this issue. The cultivation of energy crops in marginal lands, with lesser energy and chemical inputs, is being encouraged. The diversification of the feedstock including exploration of bioethanol production potential from unconventional biomass is also seen as a potent alternative. The agricultural residues generated in surplus amounts and the waste by-products generated from industrial settings are attractive options. The integration of first-generation ethanol production plans with the second-generation ethanol production facility will offer the advantage of utilization of cellulosic waste from the first-generation ethanol plant. Various studies have already validated the potential of this biorefinery concept. Dias et al. (2012) have also shown in their study that integration of first-generation ethanol production with cellulosic ethanol production using sugarcane was more economic compared to the individual plant, especially when involving advanced saccharification methods and C5 fermentation in the production process.

Major challenges in the production process include (1) overcoming the recalcitrance of lignocellulosic biomass, which often requires harsh and expensive pretreatment physicochemical methods; (2) cost of removal of fermentation inhibitors generated during pretreatment processes; (3) production of efficient and cost-effective hydrolases to saccharify cellulose and hemicellulose polymers in the biomass (Marriott et al. 2016); (4) simultaneous utilization of both C6 and C5 sugars from the hemicellulose and cellulose carbohydrates of the lignocellulose; and (5) developing stress-tolerant ethanologenic strains for higher ethanol yields.

The cost of the pretreatment can be reduced effectively by employing greener methods of biomass deconstruction including use of ionic liquids and biological methods for biomass pretreatment. The use of harsh chemicals and treatment conditions is being avoided. Lowering of fermentation inhibitors is the prime focus of newer pretreatment technologies. However, efficient removal systems for inhibitors are also designed where pretreatment methods' efficiency is considerably high. The nonproductive binding of cellulases to lignin can be reduced by using surfactants (Robak and Balcerek 2020). For enhancing the production of hydrolytic enzymes, several strategies may be employed which include (1) exploration of potent cellulase producers, (2) enhanced cellulase synthesis by optimization studies, (3) use of low-cost substrates for the production of hemicellulolytic enzymes, (4) genetic engineering of microbes for enhanced cellulase synthesis, (5) protein engineering and random mutagenesis for improved cellulases, (6) recycling of enzymes, etc. (Saini et al. 2018). The utilization of both hexoses and pentoses can be carried out using fermentation strategies of separate hydrolysis and co-fermentation, and simultaneous saccharification and co-fermentation. For this, either genetically modified microorganisms are developed which are capable of fermenting both hexoses and pentoses present in the mixed-sugar hydrolysate or a consortium of C6 and C5 fermenters may be used. Also, CBP, integrating different steps in the same fermenter, may be employed for cost reduction during cellulosic ethanol production. To develop CBP-enabled microorganism, different approaches can be used. The cellulolytic microbes may be made ethanologenic by genetic modification. Certain microbes having both cellulolytic and ethanol production potential may be improved genetically for obtaining higher yields of ethanol. On the contrary, the ethanologenic strains can be engineered genetically to make them cellulolytic (Olson et al. 2012). The ethanol-producing microorganisms may also be engineered to make them tolerant to high ethanol levels and other stresses of temperature and presence of salts, etc. Furthermore, many microbes can be immobilized to different supports in order to enhance the fermentation yield under various stress factors including high temperature, pH fluctuations, and high substrate levels (Robak and Balcerek 2020).

In addition to the production process, commercialization also requires distribution facilities for the cellulosic ethanol having compatibility with the existing infrastructure. In general, the local distribution of ethanol minimizes the transportation cost; however, distribution is essential to distantly located stations. According to the U.S. Department of Agriculture, most of the ethanol produced in Midwest is transported to East and West regions by train (carrying capacity approximately 30,000 gallons) or truck (carrying capacity 8000–10,000 gallons). Though pipelines are more efficient alternative, ethanol's affinity for water requires dedicated pipeline system (US department of Energy 2018). Only if the production and distribution cost of cellulosic ethanol is reduced, its price can become comparable to petroleum-based fuels. The initiatives by government, subsidy programs, and collaborative efforts of R&D are required to make commercialization of second-generation ethanol successful across the globe. The biorefinery is an ongoing concept which has the potential to reduce the production cost of ethanol significantly. The estimated cost of ethanol production can be as low as $1.00–$1.20 per gallon in a small lignocellulosic biorefinery (100 tons per day capacity) generating three million gallons of ethanol along with various co-products (Chandel et al. 2007). Biorefinery aims at utilization of all components of the biomass for the production of different value-added products, other than just ethanol, to maximize the benefit of biomass utilization. The co-products can be purified and marketed commercially, thus contributing to cost reduction of individual product formation. Some of the chemicals which can be produced alongside cellulosic ethanol production include 1,2-butanediol, 1,3-propanediol, 1,4-butanediol, 2,3-butanediol, acetaldehyde, acetic acid, acetic anhydride, acetone–butanol–ethanol, ethyl acetate, ethyl lactate, ethyl tert-butyl ether, ethylene, ethylene glycol, ethylene-propylene-diene monomer, furfural, furfuryl alcohol, glutamic acid, isobutanol, itaconic acid, lactic acid, lactide, lysine, microfibrillated cellulose, xylitol, terpenes, succinic acid, glycerol, sorbitol, etc. (Rosales-Calderon and Arantes 2019).

11.2.3 Third-Generation Ethanol

11.2.3.1 Feedstock and Production Technology

The fuels which are derived from algal biomass are called as third-generation biofuels. Algal bioethanol offers many benefits which are not achievable with first- and second-generation ethanol. One of the biggest advantages is the higher growth rate and, hence, a shorter harvesting cycle of algae (Chowdhury et al. 2019). The algae contain a high level of carbohydrate and the biomass can be produced in a wide range of aquatic habitats. The cultivation in aquatic systems eliminates the problem of land management for growing biomass to obtain bioethanol. The use of algae as bioethanol feedstock offers the additional advantage of the reduction of GHGs as algae remove CO_2 from the atmosphere. The photosynthetic efficiency of algae may reach up to 5% which is larger than that of the terrestrial biomass with efficiency varying from 1.8 to 2.2%. The low lignin and hemicellulose content in algae further enhances its value for its bioconversion to bioethanol. Some algae accumulate higher

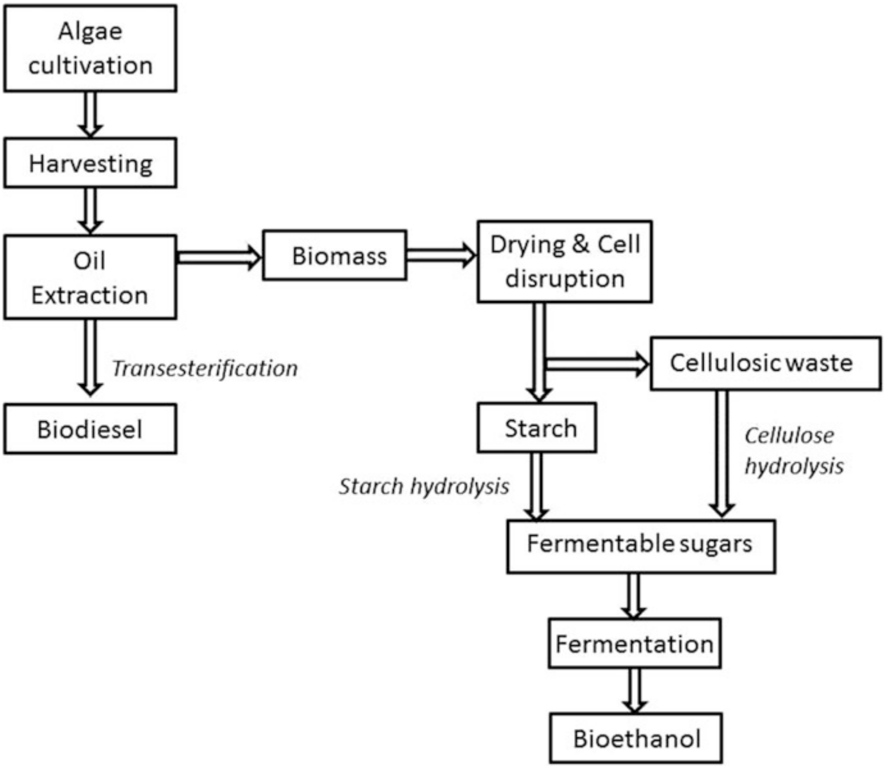

Fig. 11.12 Scheme of bioethanol production from algal biomass

starch levels intracellularly which is a rich source of fermentable sugars. Furthermore, the chemical composition of algal biomass confers it high potential for producing a vast range of biorefinery products, which holds significance for economic production of value-added products from the biomass while maximizing the benefits obtained from biomass utilization.

The general outline of the scheme of ethanol production from algae has been shown in Fig. 11.12. The algae are cultivated in natural (ponds) or anthropogenic systems such as photobioreactor. Natural systems are economically more sustainable, especially at a higher scale. However, the advantages of photobioreactors cannot be overlooked as these systems allow greater control over the process with minimized chances of contamination and result in high productivity. The harvesting procedures may involve flocculation (using inorganic or organic flocculants) followed by techniques of filtration, centrifugation, sedimentation or flotation, and then ultrasonication. An appropriate method can be chosen depending on the size of the algae. After the extraction of oil for biodiesel production, the leftover biomass can be subjected to the thermochemical route for conversion into bioethanol.

The bioethanol can be produced from both microalgae and macroalgae, inhabiting a wide range of habitats including freshwater, marine water, wastewater,

Table 11.6 Various microalgae varying in their carbohydrate content

Microalgal species	Carbohydrate content (%)	Microalgal species	Carbohydrate content (%)
A. cylindrica	25–30	*Nannochloropsis oceanica*	22.70
Chlamydomonas reinhardtii	17	*Porphyridium aerugineum*	45.8
Chlorella pyrenoidosa	26	*Porphyridium cruentum*	40–57
Chlorella sorokiniana	35.67	*Scenedesmus dimorphus*	21–52
Chlorella vulgaris	12–17	*Scenedesmus obliquus*	10–17
Chlorococcum humicola	32.50	*Spirogyra sp.*	33–64
Dunaliella bioculata	4	*Spirulina maxima*	13–16
Dunaliella salina	32	*Spirulina platensis*	8–14
Euglena gracilis	14–18	*Tetraselmis maculata*	15

Source: Velazquez-Lucio et al. (2018), Chowdhury et al. (2019).

and extreme aquatic habitats. The microalgae are microscopic (2–200 μm) algae with thalloid structure, involving Cyanophyta (blue-green algae), Pyrrophyta (Dinoflagellates), Chrysophyta (diatoms and golden brown algae), and Chlorophyta (microscopic green algae). They are mostly unicellular and planktonic. The microalgae show high adaptability to extreme environmental conditions, such as saline, drought, anaerobic, high-temperature conditions; photooxidation; osmotic pressure; and ultraviolet radiations (Abdullah et al. 2019). Table 11.6 illustrates some microalgal strains which can serve as potential feedstock for biomass conversion into bioethanol. The chemical composition of biomass varies among different microalgae. The selection of the strain is crucial for producing ethanol from algae. A strain with high carbohydrate content is most valuable for this process. Bioethanol can be produced from the cellulose, hemicellulose, pectin, starch, and other carbohydrates present in the algal biomass. The potential of microalgal components is useful not only for bioethanol production, but also for the production of value-added products useful in the food, pharmaceutical, cosmetics, and fertilizer industries.

The macroalgae or seaweeds are known popularly for their economic importance in a broad range of applications including their utilization as food supplements. They represent marine biomass which is available throughout the year. The important members belong to *Chlorophyceae*, *Rhodophyceae*, and *Phaeophyceae* groups. The content of carbohydrate varies among different microalgae relative to their protein and lipid content. The estimates have shown that 730 tons of macroalgal biomass can be generated per hectare per year having carbohydrate content of 40.140 kg per hectare per year, which can potentially produce 23,400 liters of ethanol per hectare per year (Adams et al. 2009). The algae from *Phaeophyceae* consist of the highest carbohydrate levels among all seaweeds. However, the carbohydrate composition is

Table 11.7 Carbohydrate composition of different macroalgae

Macroalgae	Polysaccharide component
Green algae (Chlorophyceae)	Cellulose, starch, mannan, ulvan, etc.
Red algae (Rhodophyceae)	Carrageenan, agarose, cellulose, starch
Brown algae (Phaeophyceae)	Laminarin, mannitol, alginate, fucoidan, cellulose

Source: Roesijadi et al. (2010).

not the same for different seaweeds. Table 11.7 shows the types of carbohydrate polysaccharides found in different macroalgae. The production of bioethanol can be achieved from most of these polysaccharides and storage carbohydrates.

The steps involved in the production of bioethanol from algae are (1) biomass pretreatment and enzymatic saccharification: The biomass is first of all washed with water to remove any salt, sand, epiphytes, chemicals, or contaminants adhering on to them. Size reduction, which is required for increasing the surface area for subsequent treatment, is then carried out by drying of the biomass and subsequent powdering or slurry preparation. This is followed by various physical, chemical, and biological pretreatments of algal biomass aiming at increased availability of sugar monomers for fermentation. Hot water and alkali or acid hydrolysis are typical methods followed to extract polysaccharides such as alginates, fucans, laminarin, agarans, carrageenans and ulvans. The brown, red, and green macroalgae respond well to acid hydrolysis by dilute sulfuric acid at high temperature. The acid breaks down the glycosidic linkages that connect chains of polysaccharides. The conversion into simple monomers (and uronic acids) can also be carried out by enzymatic hydrolysis in which treatment with lytic enzymes is carried out. The hydrolysis of starch, β-glucans (such as laminarin, chrysolaminarin, and cellulose), and galactans (agarose and carrageenan) yields different fermentable sugars, which can be fermented into ethanol. On the other hand, bioconversion of alginate requires engineering metabolic pathways and ethanol is produced from the simple sugars released from the conversion steps as a process by-product (Al Abdallah et al. 2016). Enzymatic hydrolysis is not only environment friendly in comparison to chemical methods; the porosity of algal based feedstock also enhances the accessibility of enzyme to substrate, thus making it an efficient process. Additionally, extraction can be done using supercritical fluids (Bharathiraja et al. 2015). (2) Fermentation: For ethanol fermentation, microorganisms such as bacteria, yeast, and fungi have been employed. Characteristics such as high selectivity, lower by-product formation, high yields of ethanol, and faster rate of fermentation make *Saccharomyces cerevisiae* and genetically engineered *Escherichia coli* favorable strains to carry out the bioethanol fermentation. Advanced bioethanol fermentation processes such as SHF or SSF are being progressively used to increase the economic feasibility of bioethanol production (Dahnum et al. 2015). Different wild and genetically modified microorganisms are used to ferment different sugars and uronic acids (Al Abdallah et al. 2016). (3) Purification: Techniques like rectification, distillation, and dehydration are used to carry out the purification step. Among these, distillation columns and vacuum evaporation are widely used for bioethanol production in labs (Borines et al. 2013).

11.2.3.2 Current Status and Challenges

Presently, bioethanol production technology from algal biomass is not economically viable at a commercial level. The estimates have shown that they are economical only for crude petroleum with more than $100 price per barrel. Currently, commercial production of bioethanol from algae is being carried out by companies like Algenol, Seambiotic, and Sapphire Energy reaching a production of 1 billion gallons of ethanol per year with a cost of around 85 cents/l (Khan et al. 2018). The R&D for microalgal biofuel is still not mature. The feasibility of microalgal bioethanol can be increased by maximizing the biomass production and reducing the operation and maintenance cost during the process (Khan et al. 2018). Therefore, most of the studies and production plants are focused on enhancing biomass.

Macroalgae diversity varies worldwide; however, their production facilities are more common in Asian countries including China, Indonesia, the Philippines, Japan, Korea, and Malaysia, thus making them potential sites for algal bioethanol development. China leads in the production of microalgae followed by Korea and Japan. Further two states of Malaysia, Sabah and Sarawak, are actively involved in seaweed production. With a number of cultivation techniques being used, the seaweed industry in Malaysia was targeted to generate around 150,000 metric tons of macroalgae by the end of 2020. This amount of algal production is crucial for biofuel development, specifically bioethanol in Malaysia (Jambo et al. 2016). A number of countries at the border of the Mediterranean Sea including Morocco, Algeria, Tunisia, and Egypt have huge algal production potential because of high temperature and a large expanse of unutilized land of the desert area. Libya, Cyprus, and Turkey too have marginal land that can be used to harvest algae (Singh and Gu 2010). Although a number of Asian and Mediterranean countries have favorable conditions for abundant algal growth, most of the companies related with algae production and harvesting are based in America (Edward 2009). Table 11.8 enlists various companies across the world involved in the production of algae at large scale.

In spite of a number of advantages associated with algal biomass such as plant like characteristics in terms of carbohydrate content, high growth rate and productivity, bioremediation, etc., there are a few challenges in the way of algae-based bioethanol production. The first and foremost challenge is the identification of the algae among different strains growing under diverse conditions, which is also well suited to the site selected for its growth. Further, the media and culture conditions and cultivation method have to be optimized according to the selected strain. Another challenge associated with algal bioethanol is that the production requires a lot of water, nitrogen, phosphorus, and CO_2. Although utilization of excess CO_2 is advantageous in bio-fixation, the residual product formed after the algae have absorbed various heavy metals such as lead, mercury, arsenic, and cadmium that are present in several

Table 11.8 List of some algae-producing companies around the world

Company	Cultivation method	Region	Comments
PetroSun, Scottsdale, Arizona	Open ponds	USA	Has 1100 acres of saltwater algal ponds with a potential to make 110 million pounds of algal biomass annually
Neste Oil, Helsinki		Europe	Aims to produce renewable fuel with majority of its feedstock from nonfood-chain materials
Ingrepo, Netherlands		Europe	Efficient algal production at industrial level
Seambiotic, Ashkelon, Israel		Mediterranean	Algal cultivation for various applications including biofuels
Neptune Industries, Boca Raton	Natural settings	USA	Creates sustainable, eco-friendly aquaculture. Fish waste is utilized in Aqua-Sphere system (patented) for cultivation of algae for producing biofuels and methane gas
Blue Marble Energy, Seattle		USA	Has developed a business model for simultaneous cleaning of polluted water and production of algal biomass
Aquaflow Bionomic, New Zealand		New Zealand	Harvests algae directly from polluted water systems and other nutrient-rich water (open-air environments)
Biofuel Systems, Spain		Europe	Aiming at energy production from marine algae
A2BE Carbon Capture, Boulder, Colorado	Closed systems	USA	Developed carbon capture and recycle (CCR) systems to recycle industrial CO_2 through algal cultivation
GreenFuel Technologies, Cambridge, Massachusetts		USA	Manufactures algal growth systems based on recycled CO_2
Solazyme, Inc. San Francisco		USA	Uses sugar-fed large tanks for growing algae in the dark (1000 times higher productivity)
Algenol Biofuels, Fort Meyers, Florida		USA	Aims of developing industrial scale algal cultivation systems (based on seawater and CO_2) on desert land for bioethanol production
Inventure Chemical Technology, Seattle		USA	Has used algae from facilities in Israel, Arizona, and Australia to produce alga-based fuel
Solena, Washington State		USA	Gasification of algae and other organic materials using its plasma technology
Solix Biofuels, Fort Collins, Colorado		USA	Set up with the aim to cultivate microalgae using CO_2 generated from a closely located brewery for producing biofuel

(continued)

Table 11.8 (continued)

Company	Cultivation method	Region	Comments
XL Renewables, Phoenix, Arizona		USA	Low-cost alga production using local agricultural and irrigation systems
Bionavitas, Snoqualmie, Washington		USA	Developed higher alga production technology using biofactories

Source: Edward (2009), Singh and Gu (2010).

industrial sources of CO_2 is unfit to be utilized as animal fodder (Saad et al. 2019). Freshwater, saltwater, or wastewater may be used to meet the water requirement. Among these, using wastewater is sustainable and it provides an added advantage since some amount of phosphorus and nitrogen is usually present in it. However, factors such as the presence of algal pathogens and toxic contaminants and distance between algal production sites and wastewater collection/treatment plants make it a costly affair. Conversely, coupling a wastewater treatment plant with the production and utilization of algae for biofuel can provide a cost-effective and sustainable solution (Cabanelas et al. 2013). In addition, processes such as harvesting and extraction of compounds from the feedstock followed by conversion to specific biofuel require labor, machinery, and operating and maintenance costs. Nevertheless, algae-based bioethanol production definitely scores on being environmentally friendly and in the utility of by-products. The cost-effectiveness can further be improved by engineered algae with greater substrate production and faster growing rates, which can be used to purify wastewater as well as to produce biofuels (Saad et al. 2019).

11.2.4 *Fourth-Generation Ethanol*

Recently scientists have also started taking genetically modified feedstock into consideration for enhancing ethanol production. As a result of advancement in plant biotechnology and genetic engineering, the modifications of plants and algae have made it possible to incorporate desirable characteristics in such biomass types.

Genetically modified algal biomass can be designed with high photosynthetic efficiency and higher carbohydrate content. Additionally, some of the strategies documented by Abdullah et al. (2019) include an increase in penetration of light and reduction in photoinhibition. The penetration of light may be increased in algal biomass through the reduction in the size of the antenna of chlorophyll and modifications in light-absorbing pigments. The range of absorbing spectrum of microalgae may also be widened to improve the efficiency of photosynthesis. All forms of algae, i.e., microalgae, macroalgae, and cyanobacteria, can be modified genetically to serve as a feedstock for fourth-generation bioethanol. The cultivation of modified algae can be carried out both under highly controlled conditions (contained systems) and in ponds (uncontained systems). The choice of the cultivation system depends on the economic considerations, scale of the process, and several other factors.

Other than providing feedstock for bioethanol production, the use of genetically modified algae may offer other advantages including CO_2 assimilation, mitigation of GHGs, and heavy metal bioremediation useful for wastewater treatment. The fourth-generation biofuel production has in fact been considered carbon negative. However, several researchers have also highlighted the disadvantages associated with the use of genetically modified algae such as allergies, toxicity, disposal problems, negative impact on native species and biodiversity, and horizontal gene transfer. More studies are required to reach a conclusion if genetically modified algae can be produced at large scale or not.

The modifications in the lignocellulosic biomass from the plants can also be used as a potent strategy for improved ethanol yield from biomass. When using lignocellulosic biomass for second-generation ethanol production, the cost of pretreatment contributes significantly to the overall ethanol production cost, which seems to be economically unaffordable to meet future energy demands on the planet. From the lignocellulose, the recalcitrant and non-sugar phenolic component of lignin is removed during pretreatment. This is essential to enhance the digestibility of the biomass. The physicochemical methods are costly and are not environmentally benign. The biological methods, on the other hand, are very slow. If genetic modifications in plants are made to aim at enhancing the digestibility of the biomass, then the problem of pretreatment cost can be solved considerably. The genetic modification can be done to reduce the lignin content or modify lignin, but other approaches may also be used for enhancing ethanol production from lignocellulose. These approaches may include an overall increase in biomass, increase in (hemi)-cellulose content, lowered crystallinity of cellulose, and synthesis of cell wall-hydrolyzing enzymes by feedstock plants themselves (Sticklen 2008; Wang and Zhu 2010). To introduce modifications in plants, gene gun method and *A. tumefaciens*-mediated gene transfer have been used successfully (Sticklen 2008). Research studies by various researchers have documented the use of transgenic plants showing higher enzymatic hydrolysis and ethanol yields. In a study by Fan et al. (2020) poplar was modified genetically to overproduce brassinosteroid phytohormone resulting in a reduction in biomass crystalline index as well as degree of polymerization in cellulose. In the hemicellulosic portion, xylose/arabinose ratio was lowered. As a consequence, the porosity and digestibility of the biomass were increased, which improved the enzymatic hydrolysis, and 15.68% bioethanol yield was achieved from the biomass pretreated using Na_2S + Na_2CO_3. However, fourth-generation biofuels are still in the stage of experiments and development.

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Chapter 12
Progress and Perspectives of Nanomaterials for Bioenergy Production

Alka Pareek and S. Venkata Mohan

Abstract Bioenergy that comprises biodiesel, biogasoline, bioethanol, biobutanol, hydrogen, etc. is one of the emerging renewable energies capable of tackling climate change and promising long-term durability. There is an upsurge in the interest in scientific field to enhance the output of the biofuel industry that seeks intervention of nanotechnology to overcome the limitations. Nanotechnology is a tremendously growing field merging and effecting a wide range of technological, biological and pharmacological applications but still its usage for bioenergy production from biomass is at a budding stage. Employing nanomaterials in the production of bioenergy increases efficiency and reduces process cost. Nanosized materials enhance the reaction kinetics of catalysis process by providing more catalytic sites and considerably large surface area for interaction. Wide range of nanomaterials are synthesized with distinct properties and surface features to accommodate the demand of cost-effective and process-efficient biofuel industry. The promising role of nanotechnology in the biofuel industry can be realized from studies like increase in biodiesel production rate by nano-catalyst-based microbial enzymes, use of nanomaterial additives to enhance the biogas yield and improvement of anaerobic digestion process using magnetic nanoparticles. This chapter focuses on the role of bionanomaterials in biofuel production and highlights the impact of nanotechnology-based bioenergy generation through comprehensive literature study.

Keywords Bioenergy production · Nanomaterials · Biodiesel · Biogas · Microbial fuel cells · Carbon-based materials · Nanostructured materials

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12.1 Introduction

Ever-growing and huge energy demands are becoming the main challenge with respect to future energy requirements. It is well known that 90% of global energy is obtained from depleting carbon-based fossil fuels, which has been contributing to global warming. Moreover, surge in prices of crude oil and its refined products indicates continued depletion of fossil fuels (Waqas et al. 2018). Apart from incessant consumption of fossil fuels, there is also threat to environment causing serious global devastation. To accommodate these issues, bioenergy can be a lucrative alternative as bioresources are self-prevalent, ubiquitous, inexhaustible and extremely eco-friendly (Hoel and Kverndokk 1996; Lin and Huber 2009). Biofuels produced from biomass include biogas, bioethanol, biodiesel, biohydrogen and biomethane. Bioethanol production includes the following processes: pretreatment, hydrolysis, saccharification and fermentation using sugarcane, corn, wheat, potatoes, lignocellulose biomass, etc. (Kim Keon Hee 2018). On the other hand, biodiesel is synthesized by transesterification process in which reaction of triglycerides and an alcohol produces acid alkyl esters. Biodiesel exhibits less CO_2 emission, high combustion efficiency, flash point, lubricant efficiency and cetane number (Abbaszaadeh et al. 2012). Regardless of these advantages, bioenergy is still underutilized due to lack of reliable techniques that can harvest biomass in an efficient manner (Zebda et al. 2018). Therefore, presently highly specific and focused modification methods are required to untap the unused potential of biomass. To enhance the production of biodiesel and biogas numerous approaches are explored recently. One such emerging technique that can contribute progressively towards the biofuel industry is nanotechnology that provides promising economical and productive modification tools to enhance biofuel generation. Nanotechnology is a branch of science that deals with materials of dimension or surface features in the size range of approximately 1–100 nm (Rahman et al. 2016). Nanomaterials are advantageous in biofuel systems due to unique properties like high surface areas, degree of crystallinity, adsorption power, catalytic activity, stability, longevity and storage capacity that can cumulatively optimize and make the entire process efficient (Donaldson and Poland 2013) (shown in Fig. 12.2). Moreover, it also provides a system having higher probability for recyclability, reusability and recovery. Nanoparticles thus used as co-catalysts in a system could bring a biotransformation of microbial species that maximizes the bioproduct production and hence promotes bioenergy generation (Ingle et al. 2019). The tool of nanotechnology in the biofuel industry participates in numerous applications like hydrogenation, transesterification, pyrolysis, anaerobic digestion and gasification (Zhang et al. 2013b). Some of the applications of nanotechnology in the bioindustry are shown in Fig. 12.1 (Srivastava et al. 2017). The choice of nanoparticle depends on the type of bioprocess and required yield of biofuels. This chapter summarizes the impact of nanotechnology on bioenergy production and parametrial dependency of each other through a comprehensive literature review.

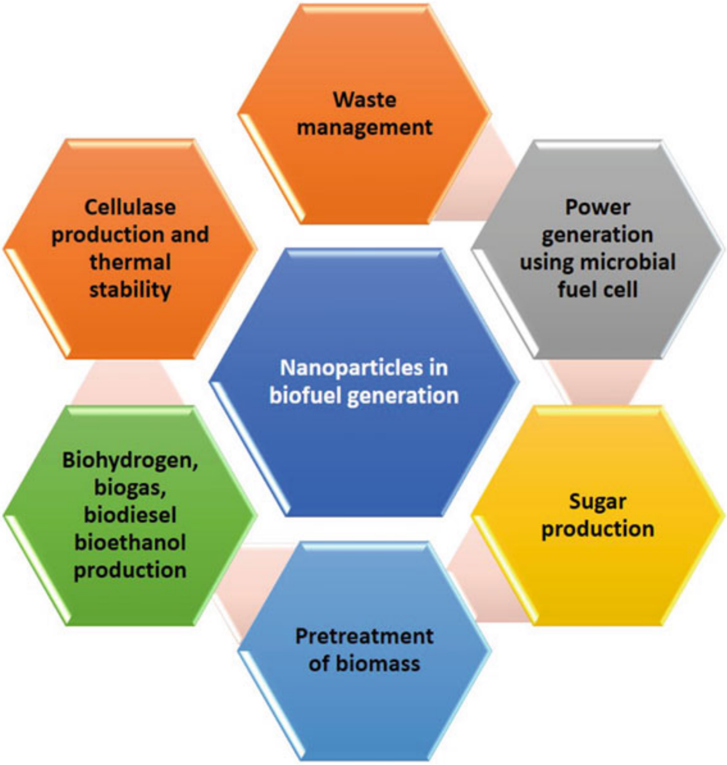


Fig. 12.1 Application of nanoparticles in the biofuel industry

12.2 Characteristics and Properties of Nanoparticles

Tremendous efforts are devoted to designing nanoparticles that can act as a functionalized catalyst for modifying biomass and making biosystems more efficient. These specially designed nanoparticles possess the potential for creating an economic, efficient, stable and durable biosystems capable of achieving higher bioproduct quality and yields. Therefore, it is imperative to study important characteristics and properties of nanoparticles relevant to bioenergy generation.

12.2.1 Characteristics of Nanoparticles

Recently, huge interest has risen in the organization of nanoscale structures into predefined superstructures due to their excellent physicochemical, optical, electrical and photoelectrochemical properties (Chandrasekharan et al. 2000). The small features in nanomaterials provide more functionality and accessible area in a defined

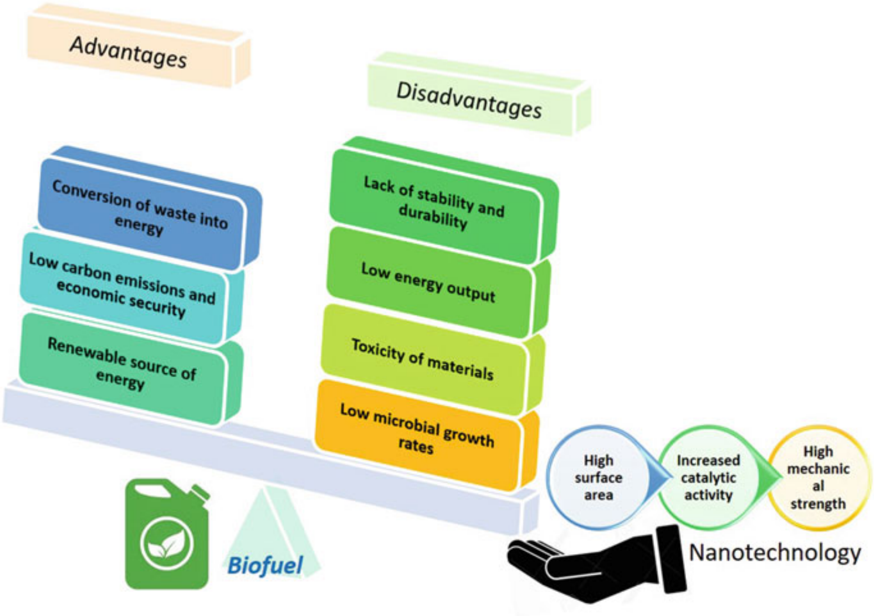


Fig. 12.2 Role of nanotechnology in the biofuel industry

space. Nanotechnology is not merely miniaturization from micro range to nanoscale but physical characteristics of nanomaterials distinctively vary from their bulk counterpart. Nanomaterials have low melting point, reduced lattice constant and enhanced catalytic properties as compared to bulk counterpart. For example, bulk aluminium is stable but highly combustible in nanoform; similarly macroscale metals like gold, silver and platinum are inert but their nanoparticles are highly reactive and possess catalytic properties. Even crystal structures of bulk materials that are stable only at high temperatures can be stabilized at a considerably low temperature in nanoform. These changes in properties are observed at nanoscale due to the fact that the population of surface atoms or ions significantly increases as compared to the total number of atoms and hence surface energy increases that controls the physical properties of nanomaterials. Nanoparticles are beneficial for various processes on account of their distinctive properties like enhanced Rayleigh scattering, surface plasmon resonance (SPR), Raman scattering in metal nanoparticles, confinement or quantization effects in semiconductor nanoparticles and superparamagnetic properties in magnetic materials (Schmid 2004). Nanotechnology plays an increasingly crucial role in many key technologies of the era like optoelectronics, catalysis, solar cells, water treatment, biomedical, electromagnetic, energy and nano-remediation (Guo et al. 2013). Owing to its versatility and important role in numerous applications, engineering of nanoparticles with different morphologies and surface properties is explored at an enormous scale. Researchers are working relentlessly towards either improving existing techniques or exploring new techniques to produce various shapes of nanoparticles like rectangular, triangular, flower, tubes, rods, wires and thin films. With nanotechnology being a vast interdisciplinary subject, various methods have been adopted to synthesize nanoparticles using physical, vapor, chemical, biological and hybrid techniques.

12.2.2 Nanoparticles for Bioprocesses

Nanoparticles along with liquid biomass exhibit an important part in water purification due to its antibacterial properties (Stoimenov et al. 2002). Utilization of nanomaterials has been proved to be the most beneficial technique for water treatment due to its high surface area and enhanced catalytic properties (Qu and Alvarez 2013). Recently, nanotechnology has also shown potential for applications like adsorption, photocatalysis, membrane processes, microbial control, disinfection, sensing and monitoring. Moreover, they are also employed in processes involving detection and subsequently removing chemical and biological substances like nutrients (phosphate, ammonia, nitrate), metals (Cd, Cu, Zn), cyanides, organic substances, algae species (cyanobacterial toxins), parasites, viruses and bacteria. Though some properties are useful for bioenergy production some may show adverse effects like toxicity and concentration of nanoparticles (Lazar 2011). Microorganisms have the tendency to respond to various nanoparticles that can significantly affect the efficacy of biological process. Antimicrobial properties of nanoparticles may impose potential adverse effects on microorganisms and harm cell membranes that leads to change in structures and causes more permeability in bacteria (Lazar 2011). This effect is dependent on various factors like size, shape and concentration of nanoparticles. Antibacterial property of nanoparticles is dependent on two parameters: firstly, physicochemical properties of nanoparticle and secondly, bacteria type.

In one of the studies, it is claimed that the Ag nanoparticle-treated coliform bacteria irradiated with ultrasonic waves enhanced the antibacterial activity. Even sometimes nanoparticles in close vicinity with a microbial community reduce the efficiency of anaerobic digestion process causing unsuccessful treatment process and release of contaminated effluent (Hoffmann and Christofi 2001). For similar reasons, silver nanoparticles are utilized in various medical processes like dental treatment, tubes including catheters and curing burn wounds (Klasen 2000). Adverse effects of silver nanoparticles were studied using *E. coli* and observed to be reliant on the concentration of nanoparticles and exposure time. Harmful effects of nanoparticles on bacteria are associated with the leakage of reducing sugars and proteins, cell disruption, enzyme inhibition and scattered vesicles that inhibits cellular respiration and hence cell growth. It is observed that the toxicity of nanoparticles can be largely controlled when nanoparticles are exposed to sludge. The effect of toxicity on bioenergy yield is dependent on the concentration, nanoparticle size, exposure time and microorganism type. Moreover, behaviour of nanoparticles and its interaction with biomass are also dependent on environmental factors like pH, light, ionic strength and natural organic matter (Klaine et al. 2008). Both size and shape of nanoparticles play a crucial role in bioprocesses; for example it is reported that nanoparticles of size less than 30 nm are cytotoxic towards *E. coli* and *S. aureus* (Martinez-Gutierrez et al. 2010) as compared to nanoparticles of size 80–90 nm (Martínez-Castañón et al. 2008). Similarly shape of nanoparticles is also important; for instance, the triangular shaped AgO nanoparticles exhibit the highest bactericidal effect on *E. coli* in comparison to spherical or rod-shaped AgO in both agar plate and broth cultures.

12.3 Role of Nanoparticles in Bioenergy Generation

12.3.1 Biodiesel Production

Biodiesel is an eco-friendly fuel that behaves like fossil diesel which is synthesized domestically using vegetable, animal and waste cooking oil (shown in Fig. 12.3) (Marchetti et al. 2007). The process of conversion of these oils to biodiesel is known as transesterification (Vasudevan and Briggs 2008). The transesterification process is the mechanism in which fat/oil (triglyceride) reacts with an alcohol to form esters and glycerol. This fuel possesses properties like density, air/fuel ratio and heat of vaporization that are comparable to mineral diesel. Moreover, it is beneficial for the environment as it reduces carbon monoxide, sulphur oxide and smoke emissions. Nanomaterials have been widely studied for the optimization of yield and quality of biodiesel. Magnetic nanoparticles are used as catalysts for industrial scale biodiesel production owing to their ease of separation from the final product, reusability and economic nature (Gardy et al. 2018, 2019). Similarly, metal oxides like TiO_2 (Gardy et al. 2017), CaO (Liu et al. 2008), MgO (Verziu et al. 2008) and SrO (Liu et al. 2007) nanoparticles show great catalytic activity for efficient biodiesel production. Enhanced biodiesel generation has been reported using carbonaceous materials like

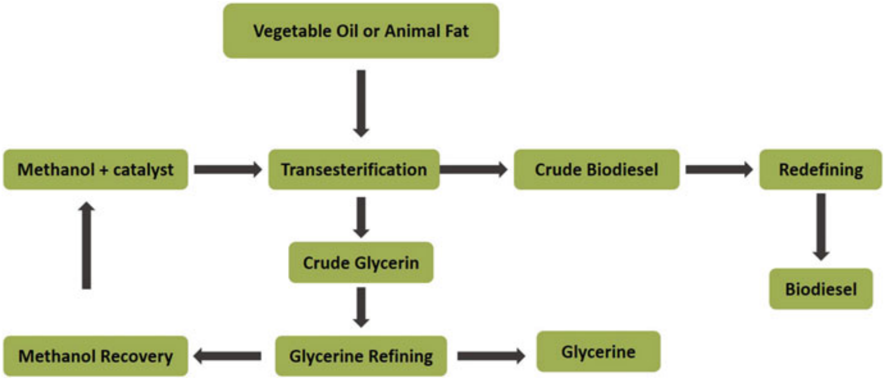


Fig. 12.3 Schematic showing the process of biodiesel production

graphene oxide (Mahto et al. 2016), carbon nanotubes (Guan et al. 2017), carbon nanofibres (Stellwagen et al. 2013) and biochar (Dehkhoda et al. 2010). Few mesoporous nanomaterials are also studied with excellent structural properties that exhibit improved catalytic activities for biodiesel production. Tangy et al. used microwave irradiation and studied SrO nanoparticle-decorated SiO_2 beads for generation of biodiesel from waste cooking oil (Peralta-Yahya and Keasling 2010). High conversion values as large as 99.4 wt % (in 10-s irradiation time) were obtained using composite nano-catalyst, leading to the development of economical biodiesel in a very short time. Jayanthi et al. studied the efficiency and emission characteristics of DI diesel engine filled with biodiesel using copper nanoparticle additives. Reports suggested that brake thermal efficiency was enhanced to B20+ 80 PPM CuO and also specific fuel consumption was reduced at full load conditions (Jayanthi and Rao 2016). There are some reports that studied improvement in biodiesel generation by employing alumina (Al_2O_3) and cerium oxide (CeO_2) nanoparticles. In one such study, Al_2O_3 and CeO_2 nanoparticles of each 30 ppm were used in DI diesel engine that improves the brake thermal efficiency by 12%, followed by reduction of 30%, 60%, 44% and 38% in NO, CO, hydrocarbon and smoke emission, respectively (Prabu 2017). Similarly, Ramesh et al. studied performance, combustion and emission characteristics of diesel engine by employing alumina nanoparticles as additive with poultry litter (Ramesh et al. 2018). Chaichan et al. reported the effect of alumina nano-fluid (aqueous) on diesel engine's performance and emission characteristics (Tariq et al. 2017). The group studied the impact of nano-Al_2O_3 (51 nm diameter) with varying weight fractions of 1%, 3%, 5%, 7% and 10% that resulted into improved brake thermal efficiency by 5.5% and decreased the relative fuel consumption by 3.94%. Kim et al. reported the design of nanoparticles (magnetic and non-magnetic), carbon nanotubes and carbon nanofibres as nano-immobilized biocatalysts for biodiesel generation (Kim Keon Hee 2018). Recently, Ajala et al. synthesized nano-catalysts using waste iron filling for biodiesel production (Ajala et al. 2020). Numerous groups have reported nanotechnological advancements towards efficient biodiesel production using nano-catalysts (Dantas et al. 2020; Xie and Wang 2020; Mofijur et al. 2020).

12.3.2 Biogas Production

Biogas is a kind of biofuel that is generated naturally from the decomposition or breakdown of organic waste such as food scraps and animal waste in an environment absent of oxygen (anaerobic environment). Decomposition of waste in anaerobic conditions releases a blend of gases, primarily methane and carbon dioxide, and the above process is also known as anaerobic digestion. Common sources of biogas generation constitute sewage treatment plants, landfills, organic industrial waste and mesophilic and thermophilic digestion of organic wastes (Ganzoury and Allam 2015). The anaerobic digestion process contains mainly four steps, namely, hydrolysis, acidogenesis, acetogenesis and methanogenesis. To improve the efficacy of

Table 12.1 Biogas production using different nanoparticles

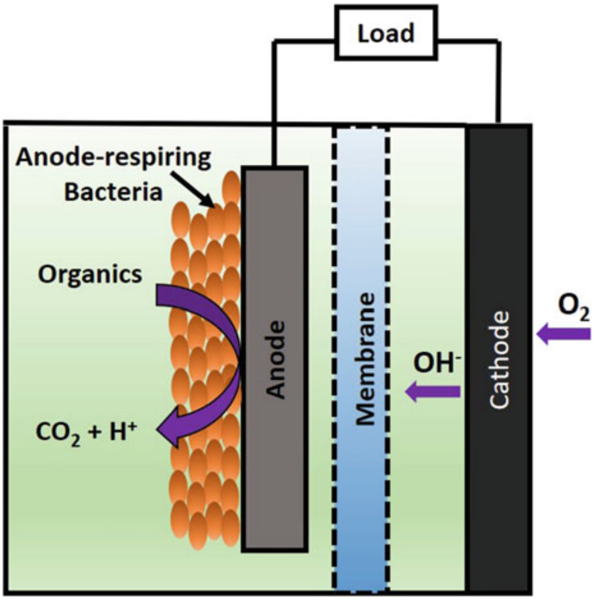
Type of material	Nanoform	Effect of nanomaterial on biogas
Transition metal oxides/zero-valent metals	Titania, ceria, nano-zero-valence iron (NZVI)	Outcome is dependent on the nanomaterial concentration and process digestion time
Metal oxides	ZnO, CuO, MnO_2, Al_2O_3	Reduction in the rate of biogas production
Zero-valent metals	Zero-valent iron nanoparticles	Leads to enhanced methane generation
Transition metal oxides	Metal oxide nanoparticles encapsulated in porous SiO_2	Considerable enhancement in methane
Nanoform ash and carbon nanostructures	Ag/Au nanoparticle	Biogas production depends on the concentration
	Micro/nano fly ash or bottom ash	Enhanced biogas
	C60 (fullerene) and SiO_2 nanoparticles, SWCNTs	Unaltered biogas

this process, numerous nanomaterials were utilized as additives to improve the biogas quality and yield. During anaerobic digestion process, employing of various nanoparticles like iron oxide, fly ash, zero-valence iron, bottom ash and metal oxides has promisingly increased methane production (Table 12.1). Though Mohamed et al. have extensively discussed a variety of nanoparticles employed till now for biogas production in the form of review paper, few studies are presented here (Ganzoury and Allam 2015). Abdelsalam et al. carried out biogas production using laser irradiation and Ni nanoparticles from anaerobic digestion. The combination of laser irradiation (irradiation time ~2 h) and 2 mg L^{-1} Ni nanoparticles obtained maximum specific biogas and methane generation of 679.5 mL and 453.3 mL, respectively (Abdelsalam et al. 2018). Unsar et al. studied anaerobic digestion using CuO, Ag and CeO_2 nanoparticles and elaborated their long- and short-term impacts using municipal waste-activated sludge (Ünşar et al. 2016). Casals et al. studied programming related to iron oxide nanoparticles disintegrated in anaerobic digesters that boosted biogas generation (Casals et al. 2014). Ambuchi et al. have shown enhancement in biogas production using Fe_2O_3 nanoparticles and multiwall carbon nanotubes (MWCNT) (Ambuchi et al. 2016). The study demonstrated that 0.75 g/L and 1.5 g/L concentration of Fe_2O_3 nanoparticles and MWCNT, respectively, caused faster substrate consumption and higher biogas generation. Abdelsalam et al. studied the effect of cobalt and nickel nanoparticles on methane and biogas production (Abdelsalam et al. 2017). The study revealed that the maximum biogas and methane generation was observed with 2 mg/L nickel nanoparticles showing 0.61 l biogas and 0.36 l methane, respectively. Duc et al. synthesized CeO_2 nanoparticles of size 192 nm and 10 mg/l concentration that increased the biogas production from UASB sludge by 11% (Nguyen et al. 2015). Similarly, 7.5 nm size TiO_2 nanoparticles of concentration 1120 mg/l by Garcia et al. enhanced the wastewater treatment sludge by 10% (García et al. 2012). In another report, Fe_3O_4 nanoparticles of size 7 nm were synthesized that increased the biogas yield by 180% and methane by 234% from wastewater sludge (Casals et al. 2014). Lo et al. reported the use of micro/nano fly and micro/nano bottom ash in anaerobic digestion that increased the biogas production by 2.9 times and 3.5 times, respectively (Lo et al. 2012). Al-Ahmad et al. reported the synthesis of nickel (Ni)-, cobalt (Co)-, iron (Fe)- and platinum (Pt)-encapsulated porous SiO_2 structures and their effect on anaerobic digestion process. It was found that methane production was increased in the range of 70%, 48%, 7% and 6% with Ni, Co, Fe and Pt nanoparticles, respectively (Al-Ahmad et al. 2014).

12.3.3 Bioelectrochemical Systems

Bioelectrochemical system (BES) is a promising technology for converting chemical energy of waste into electrical energy and other valuable products employing the technologies like microbial fuel cells (MFC) and microbial electrolysis cells (MEC). MFC is the most studied technology in which bacteria carry out a chain of redox reactions to transform organic mass into electric current (Butti et al. 2016). Generally, MFCs generally consist of two important parts: anode compartment and a cathode compartment divided by a proton-exchange membrane (PEM), as depicted in Fig. 12.4. The chemical mixture contained in anode gets oxidized using microbial metabolism following anaerobic conditions, which generates electrons and protons. The electrons migrate to anode and travel via external circuit producing electrical

Fig. 12.4 Schematic showing MFC cell set-up



current; on the other hand, protons are transferred to cathode via PEM. MFCs are categorized into two groups: mediator-assisted MFC (where mediators are introduced to the system) and mediator-less MFC. In mediator-less MFCs, bacteria consist of conductive pili or electrochemically active membrane-associated cytochromes secrete redox-mediating molecules that facilitates the flow of electron. MFC has matured as an emerging technology for biological treatment of municipal or industrial wastewater (He et al. 2005; Logan et al. 2006). Still, this technology is not apt for commercialization owing to its poor power outcome. To improve the output power of MFC, research is carried out in two broad aspects: (1) the bacteria that work efficiently in mediator-less MFC (2) and the material used to make electrode. Electrode material is a vital aspect as physical and chemical characteristics of various electrode materials influence microbial attachment, electron transfer, electrode resistance and rate of electrode surface reaction. Nanostructuring of electrodes is an easy and cost-effective way of improving MFC performance (Choudhury et al. 2017). Carbon-based materials are most dominantly followed by anode and cathode materials in history and present era of MFC technology owing to outstanding chemical, electrochemical and biological stability. Carbon-based materials are studied in different shapes and sizes like graphite rod, graphite fibre (Chaudhuri and Lovley 2003), brush, carbon cloth, carbon paper, carbon felt (Chen et al. 2012) and reticulated vitreous carbon (RVC) (Wei et al. 2011). But there are some shortcomings associated with this class of materials like limited electrical conductivity. In the case of graphite, electrical conductivity is 2–3 times lesser than metals. Metal electrodes were also explored as anode; though they offer good electrical conductivity they offer poor microbial adhesion or biofilm formation, electrochemical corrosion and metal ion-related water pollution. Few of them like Cu and Ag are even known to show antimicrobial properties, which restricts their candidature as anode material in MFC (Yamashita and Yokoyama 2018). To overcome these problems, nanostructured materials like CNT and graphene are studied that offer higher surface area for bacterial colonization, biocompatibility, great conductivity and extraordinary mechanical strength due to their distinct features and morphologies (Pareek and Mohan 2018, 2019). Moreover, they also offer excellent charge-storing capacity that further enhances output power density of MFC and provides possibility of constructing hybrid MFC. But synthesis of CNT (Mohanakrishna et al. 2012; Zhang et al. 2013a) and graphene (Singh et al. 2011; Gautam et al. 2016; Pareek et al. 2019a, b, c) includes complicated methods that increase the cost associated with MFC operation. Moreover, stacking of graphene sheets owing to strong van der Waals forces reduces their surface area as a result of negatively charged bacteria that experience electrostatic repulsions from graphene. Metal oxide nanomaterial (Mehdinia et al. 2014; Winfield et al. 2016) is another class of material, which can replace carbonaceous electrodes owing to its greater mechanical strength, morphological and electrochemical stability, biocompatibility, redox activity, electrochemical stability, low cost and eco-friendly nature. Still their performance in MFC is delimited due to relatively low conductivity, porosity and surface area. In view of this, reticulated vitreous or 3D sponge-like carbon nanomaterials were fabricated that provide hierarchically micro-, meso- or macrospores, establishing outstanding power density (Yang et al. 2016; ElMekawy et al. 2017). These hierarchical porous structures enhance the bacterium attachment due to the micro- and mesoporous pores, provide superior bacteria and electrode interaction enhancing the charge transfer process and form good-quality stable biofilm for long-term application. However, there is clogging of mesopores with time due to rapid bacterial growth, which prevents further electrolyte diffusion and limits further bacterial colonization of anode surface.

12.4 Conclusions

Nanomaterials exhibit a crucial role in enhancing the efficiency of bioenergy production by interconnecting biological processes with nanomaterials. It has impacted various bioprocesses like fermentation, enzyme hydrolysis, biomass pretreatment, product separation and microbial fuel cells. This chapter briefly discusses reports highlighting different nanomaterials that have been utilized to improve the efficiency of biofuels like biodiesel, biogas and bioelectrochemical systems. Wide range of nanomaterials are extensively explored as catalysts in biodiesel production and as additives in biogas production. A high surface area-to-volume ratio of nanosized particles provides greater reactivity and hence catalytic activity to them. On the other hand, the antibacterial activity of nanoparticles poses adverse effect or inhibits the bioprocesses, which is dependent on nanoparticle size, concentration of nanoparticles and time of exposure. There is enormous possibility to explore the effect of nanomaterials on biofuel generation using other biomasses, like agricultural waste and municipal solid waste (MSW). It is required to study optimum concentration and exposure time to reduce the toxicity of nanoparticles and also bioactive nanometal oxides.

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Chapter 13
Nanofarming: Nanotechnology in Biofuel Production

Shivani R. Pandya, Aaeen Alchi, Parth Lakhlani, and Arushi Chawla

Abstract The world is currently facing various problems, among which one of the severe concerns is energy crises which include restricted span of fossil fuels and few alternate energy options. To make the world a better place to live amidst the complicated environmental and economic situation, renewable energy alternatives like wind, solar, and other bioenergy resources exist recognized as excellent clean energy sources as they produce zero-carbon residue. This series presents the newest research, various approaches, and many perspectives on clean energy production, which exist in industrial and other emerging parts of the world under one roof and curated and also well developed by the long-established institutions and subject experts' global leadership associated with the theme. There has been an immediate need to find alternative energy sources as there is limited fossil fuel availability. Hence, the potential applications of an emerging field, "nanotechnology," for the assembly of viable bioenergy and biosensors, have fascinated researchers' consideration in recent years to analyze the innovative nanoscaffolds. It can contribute to the construction of stout nanobiocatalytic systems. Various nanomaterials have stayed lately developed as per the world's emerging need for the application purpose. Many types of metal nanomaterials remain to be established to have their application in biofuel production. This application could be both directly and indirectly.

Keywords Nonconventional energy source · Biofuels · Nanofarming · Biofuel production

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13.1 Introduction

Inside the twenty-first century, the human populace has extended at the very most satisfactory stage. Due to population increase, worldwide global economic challenges are faced by many sectors like agricultural, industries, manufacturing, and energy production. The energy zone is one of the most demanding sectors nowadays. Production of energy using fuel has many applications like generating energy in vehicles, aeroplanes, ships, industrial production, and electricity for house and buildings. In the current period, petroleum fuels are used widely around the world. Day by day resources of petroleum are decreasing, increasing the demand in the automobile and industrial sectors. Worldwide there are many alternatives available like biofuel, nuclear fuel, and renewable solar energy. Biofuel is one of the best options due to its easy availability as well as sustainability.

13.1.1 What Is Biofuel?

Biofuel is a combustible fuel created from biomass like plant materials and animal waste. Biofuel is the product of hydrocarbon present in biomass, so the essential constituent of biofuel is carbon. Biofuel refers to the user to generate energy that exists as labeled as bioenergy. The main advantage of biofuel is that its production is from bioorganic wastage, but the other side of chemical composition of biomass is not consistent. It generally contains carbon, water, and other organic matters in a different ratio.

The production of biofuel from biomass is one of the most emerging topics in the field of chemistry. Technological advancement requires more and more research and development. With the development of new technology, there are new challenges in converting biomass into biofuel. The practical use of biofuel requires sustainable techniques for converting biomass into biofuel like bioethanol, biomethanol, bioether, and other biological fuels (Fig. 13.1).

13.1.2 Classification of Biofuel

The crude materials stay assembled in biofuel. These biofuels are broadly classified into two groups which include primary and secondary. The primary biofuel is a natural biofuel derived from animal wastes or plants, while the secondary biofuel is derived from microalgae and other microbial species, which is again categorized under the category of an advanced biofuel. Various researchers have been making constant attempt to bring about a genetic modification in the biofuel production and this genetically modified biofuel production is known as the fourth-generation biofuel. The amount of biofuel production largely depends on the type of

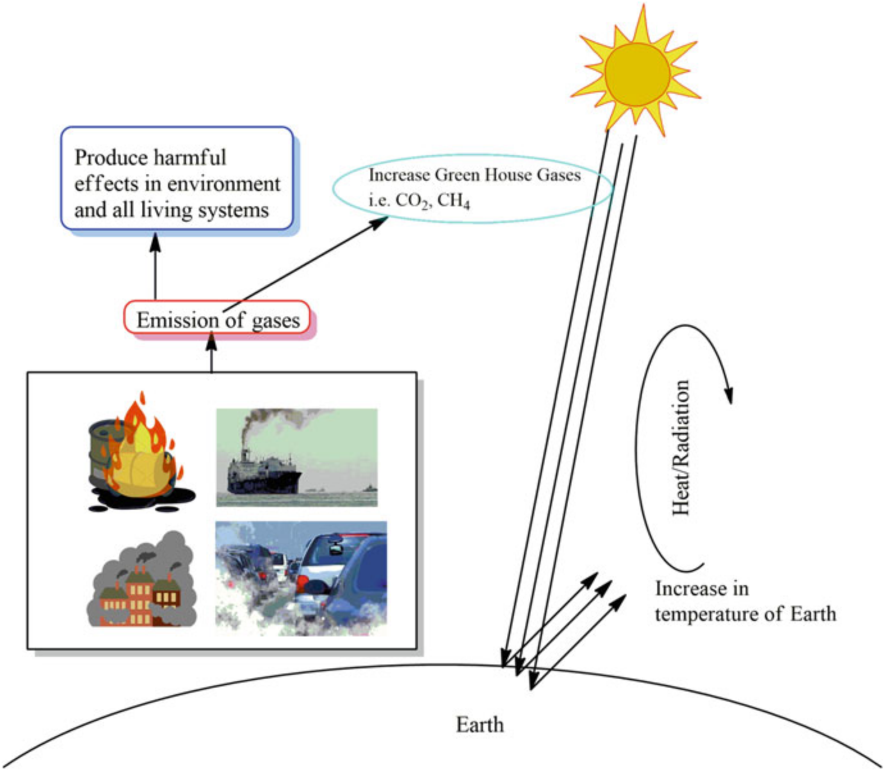

Fig. 13.1 Pictorial representation of greenhouse gas effect on the burning of fossil fuels

microorganism selected and the favorable conditions which are required for its growth. The composition of the media also primarily affects the production and amount of biofuel produced.

13.1.3 Production of Biofuel

One of the most suitable biofuel production sources is biomass like the plant, algae, microbial biomass, and various other waste materials (Swain 2014). The first biodiesel plant, i.e., Pacific Biodiesel, existed custom-made in the United States in the year 1996. This plant mainly focused on biodiesel production, which was derived from cooking oils (Wong 2014). There was a sudden surge in biodiesel production after 2001 as the prices of petroleum products hiked. This surge was mainly due to biodiesel's various advantages, which included emission of less toxic waste, cost sufficiency, and being different from other fossil fuels (Chanakya et al. 2013). There

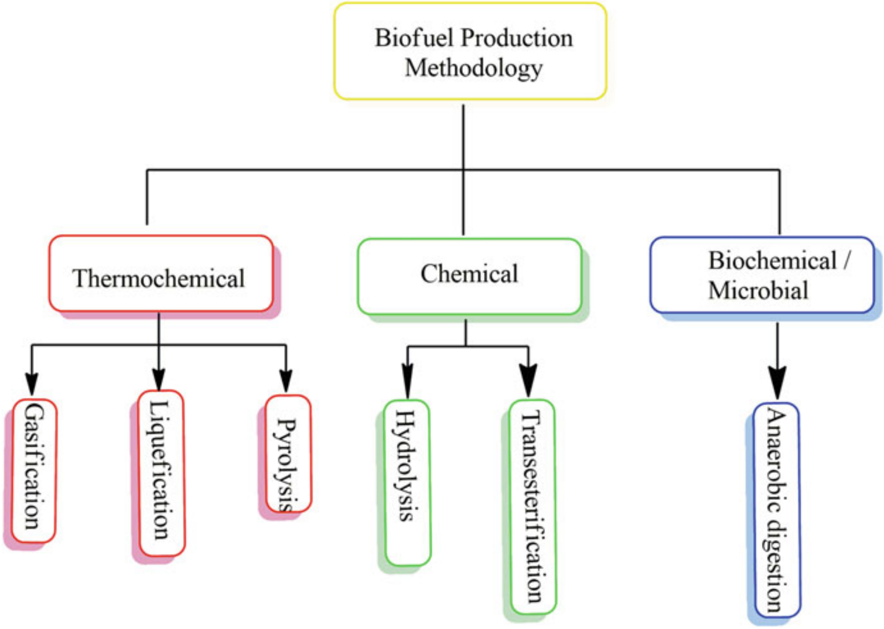

Fig. 13.2 Methods used for biofuel production

are various techniques and multiple-unit operations involved in biofuel production (Fig. 13.2).

13.1.3.1 Manufacture Techniques for Biofuel

The production of biofuels depends on various methods used to produce reactor types, reactor volumes, biofuel, other reactor parameters including pH and temperature, mixing, many other biological materials, and various production methods for the production of biofuel processes.

The manufacturing technologies of biofuel may be primarily divided into three organizations, which are:

- First-era biofuel generation for biodiesel (Dash and Lingfa 2017) and bioethanol production (Dias et al. 2012)
- Second generation of biofuel generation for lignocellulosic biomass conversion (Davis et al. 2013)
- Third-technology biofuel for algae processing (Leite et al. 2013)
- Fourth-technology biofuel era for genetically modified algae

The production technology followed manufacturing of the first-technology biofuel and the transesterification system to manufacture biodiesel and bioethanol. All the more impressive complex lignocellulosic biomass-processing technology

Table 13.1 Biofuel production from biomass as an alternative to conventional energy sources

Biofuel generation	Raw material	Method	References
First generation	Food crops	Transesterification, or yeast fermentation	Alalwan et al. (2019)
Second generation	Lignocellulosic or woody biomass, or agricultural residues/ waste (maize, wheat, rice, and sugarcane)	Anaerobic digestion and alcoholic fermentation	Chandra et al. (2012), Sims et al., (2010)
Third generation	Aquatic cultivated feedstock mostly algae including macroalgae and microalgae	Biochemical transformation, thermochemical change, complex reactions, and direct burning are vital for biomass treatment to deliver high-esteem synthetic substances as bioethanol	Neto et al. (2019)
Fourth generation	Hereditarily adjusted microorganisms, for example, microalgae, yeast, organisms, and cyanobacteria, are used as sources	Alleviation practices, pyrolysis, gasification, redesigning, and also sun-powered fuel pathways	Abdullah et al. (2017), Alalwan et al. (2019)

inclusive of thermochemical and biological translation strategies is hired to produce the second-period biofuels. Nearly 33% of the 1/3-innovation biofuel creation from green growth incorporates the oil extraction and transesterification methods. The generation of integrating algae oil processing and the second-era biofuel manufacturing is actively studied. The fourth-generation biofuel now uses genetically modified (GM) algae to enhance biofuel manufacturing. GM algae biofuel is well known for fossil fuel production. An assessment of these issues and conceiving reasonable relief methods to manage them are critical for a successful popularized fourth-innovation biofuel creation. Table 13.1 indicates the assessment of the biofuel production system. As shown, the conversion technology changes in line with the bodily characteristics of the feedstock, which appear within the form of sugar, starch, and vegetable oil-based substances (first technology); lignocellulosic biomass (second generation); algae (third generation); and genetically changed microorganisms (fourth generation).

13.1.3.2 Historical Perspective

Plant biomass is a bountiful, environmentally friendly power source containing different carbon, hydrogen, oxygen, and minerals. The availability of plant biomass is considered to be more reliable than any other sources of energy which include solar energy, wind energy, and hydropower, as it can be quickly produced in variable and favorable environmental conditions. Alongside, as the density of biomass is less it makes it more feasible for storing and transportation purposes. In developing countries like India, plant biomass is actually prepared for household purposes, underestimating its value as a potential biofuel. The boiling of petroleum derivatives and plant biomass is the principal supporter of the expansion in CO_2 level and straightforwardly impacts an unnatural weather change. GHG discharges and an unnatural weather change open up the entryway for the novel cycles of biofuel age. The lignocellulosic biomass is a highly complex biopolymer of cellulose (40–60%), hemicellulose (10–40%), and lignin (15–30%) as the primary components which gives support to plant, and it is resistant to various microbial degradations as well as biochemical conversion (Demartini et al. 2013; Himmel et al. 2007; Schutyser et al. 2018). Cellulose and hemicellulose consist of monomeric sugar unit. And after pretreatment, the lignocellulosic biomass sugar component through further saccharification is transformed into suitable biofuel (Chen and Chen 2014).

The lignin segment is the phenylpropane unit, which is more obstinate as compared to cellulose and hemicellulose. During the process of kraft pulping, lignin is used for the co-power generation, which is then separated and suitably valorized into useful precursors for the production of biofuel. However, the hydrogenation process is a suitable method for converting lignin into a valuable product and converting raw material into biofuel (Bawadi Abdullah et al. 2017; Chen and Chen 2014). Lignin valorization strategy utilizes the complete conversion of lignocellulosic biomass into biofuel generation.

Many different methodologies have been adapted from time to time in order to treat the plant biomass for the route of biorefinery and the methods that are applied have many advantages as well as limitations. At the same time, paper and pulp productions are deliberated as the optimal samples for lignocellulosic biomass biorefinery and are presently developing as fermentative manufacture means of bioethanol (Hahn-Hägerdal et al. 2006; Limayem and Ricke 2012; Sugawara and Nikaido 2014). Many existing conventional techniques have resulted into a low yield of primary products which are extracted from the lignocellulosic biomass (Davis et al. 2013). Further, the optimization process remains achieved by understanding the process. Biofuel production directly impacts the carbon sink; therefore, biofuels produced by oil-based fuels are always considered a better choice than GHG emissions into the environment as per the critics of biofuels, which depends on the route of production. Biofuel's choice depends on food versus fuel; land utilized for feedstock development consistently contends with food interest and land utilization for creations. Hence the governments from different countries take initiatives for the continuous growth of the biofuel industry, which also includes assistance in providing funds for research and development along with mandating laws of the Environmental Protection Agency (EPA) for the blending of biofuel with conventional fuel. Inexhaustible fuel standard (IFS) tackels the influence of biofuels and foundation, and high production of biofuels that should be delivered and broadly accessible for clients with cost effectiveness (Richards 2013).

These fuels are derived from lucrative and renewable causes like vegetable oils, which remain imitative from diverse biological causes like algal biomass, microbial biomass, and plant biomass. Nanomaterials have incipient biofuel production applications within the current scenario, mainly thanks to their exclusive structural comportment, just like the nanomaterials' small size (nanoscale size). These distinctive nanomaterials now augment biofuel production by taming the biosynthesis conduits. As mentioned formerly, fuels occur quarried from lucrative and renewable sources. These nanomaterials augment biofuel fabrication by refining the biosynthesis conduits. These nanomaterials upgrade biofuel foundation by improving the biosynthesis conduits in differing types of gases, including CO_2, sulfur dioxide, and other diverse varieties.

During the current times, the availability of fossil fuels is limited and may be exhausted after a particular time. If the situation worsens further, biofuel will be reflected as a potential candidate for the source of energy. Hence it has been stated that biofuel production is useful in the future as an alternative energy source (Palaniappan 2017). Biofuel is also referred to as an energy source generated from different biomass types, and these biomasses come from living organisms like algae, bacteria, plants, and agricultural waste. These biofuels can quickly terminate and overturn the demand for fossil fuels in the future and minimize the effect of fossil fuel combustion, which creates many environmental differences (Demirbas 2009). A recent approach for biofuel production remains grounded on various types of microorganisms like cyanobacteria and microalgae. These microalgae contain a high amount of lipid used as a raw material for biofuel production.

In the past few decades, we have also witnessed the production of biofuels from plant materials, but recently the focus is on the production of biofuel from microalgae. Also, plant and algal biomass are described as natural sources for biofuel production (Demirbas 2009). Compared to other conventional methods, nanostructures have better efficiency and faster adsorption, and hence nanoparticles play an important role in biofuel production and enhanced production of biohydrogen methane, biodiesel (Savage and Diallo 2005).

Algal Biodiesel

The production of biodiesel from microalgae is very expensive as there is a tremendous requirement of energy source and maintenance of the condition of growth to cultivate algal species. Algal biofuel production is a very complicated process. A variety of mechanisms are involved in the production process (Fig. 13.3). Many researchers are working to reduce production cost and enhance biofuel production (Chanakya et al. 2013).

Biohydrogen

In the present times, about 99% of hydrogen exists spawned from fossil fuels (Shaishav et al. 2013). The conventional techniques have a few disadvantages like existence of high temperature, i.e., more than 840 °C, and processes which are not favorable to the environment (Shaishav et al. 2013). This process is possible whenever the electricity is inexpensive because electricity covers the significant

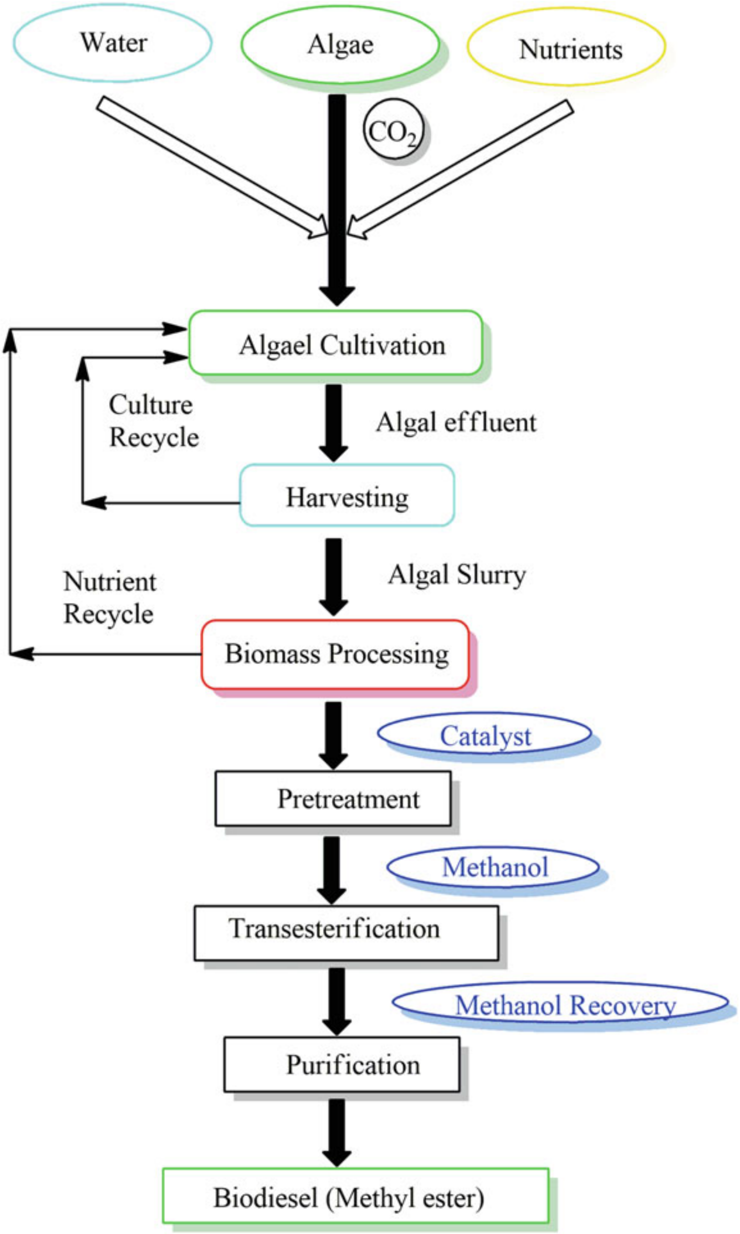

Fig. 13.3 Schematic representation of algal biodiesel production

cost which is around 80% of the production of hydrogen (Karthic et al. 2012). This chapter focuses on the inexpensive techniques which are environmentally safe for the production of biohydrogen. Hence, biohydrogen stays spawned during the

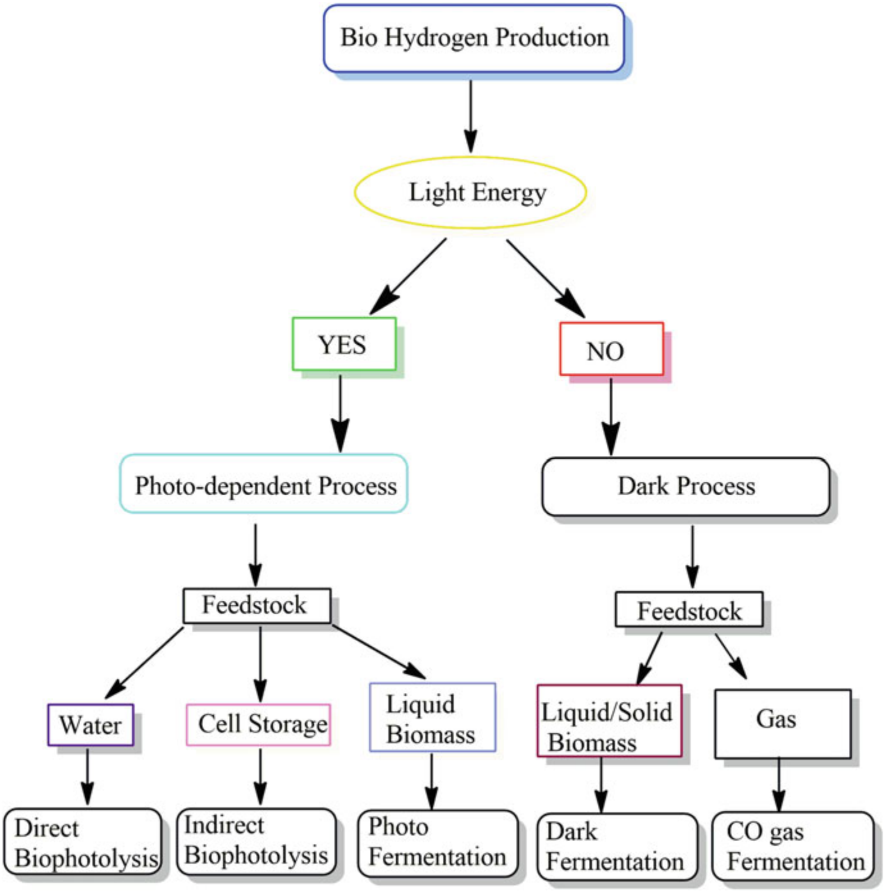

Fig. 13.4 The flowchart represents biohydrogen production methods from various raw materials

photolytic reaction in photosynthetic microorganisms such as microalgae and plants (Fig. 13.4). Various types of microorganisms have remained deliberate for biohydrogen production, such as *Chlamydomonas moewusii, Scenedesmus obliquus, Rhodobacter sphaeroides* (photosynthetic bacteria), and *Enterobacter aerogenes* (fermentative bacteria) (Fabiano and Perego 2002).

13.2 Nanotechnology in Biofuel Production

13.2.1 Synthesis and Properties of Nanomaterials

As we discuss the use of nanomaterials in biofuel and the waste management processes, it is important to know about the synthesis mechanism and the basic properties of nanoparticles. A wide range of nanomaterials are available. Small-size particles come together and form nanomaterials (Biswas et al. 2012).

Other methods also remain a charity for the synthesis of nanomaterials. In the process of coprecipitation, nanomaterials which exist are created by precipitation in the presence of gravitational forces and magnetic field (Kalantari et al. 2012). The technique of arc discharge is considered to be one of the best methods for synthesizing good-quality nanotubes which would require warmth and carbon hotspot for nanotubes' blend (Saifuddin et al. 2013). Argon gas is required later, for better interaction (Kishore et al. 2012). After the blend of nanomaterials, another essential cycle is surface functionalization. Surface functionalization is a process that enhances the property of nanomaterials. It also enhances nanomaterials' binding capacity with various types of enzymes (Pavlidis et al. 2012). A variety of synthetic polymeric materials are currently being used as functionalization materials (Requicha 2003). As we add the functional groups with the nanomaterials which are responsible for enhancing the properties of nanomaterials, it leads to different surface charges, which in turn provides a link between functional groups of more than one nanomaterial. This minimizes the size of the pore entrance, which is applicable for enzyme immobilization (Lee et al. 2010). The advanced properties of these nanomaterials are responsible for the application of nanomaterials in various fields like drug delivery system, environmental engineering, and production of bioenergy (Sekoai et al. 2016).

13.2.2 Nanotechnology and Biofuel Production

During the process of the production of biofuel, nanoparticles enhance the process by increasing the catalytic reaction. This section of the chapter mainly focuses on applying several types of nanoparticles and their role in biodiesel, biohydrogen, bioethanol, biogas, and bioethanol (Fig. 13.5).

13.2.2.1 Biohydrogen Production

As there are many routes of metabolism, biohydrogen production is highly dependent on several parameters which include pH, temperature, retention time, and composition of media (Łukajtis et al. 2018). At the same time we know that nanoparticles can enhance the production of biohydrogen in the microbial system by increasing the electron transfer rate in the microbial cell (Serrano et al. 2009). Dark fermentative production of biohydrogen is known to be an inexpensive and an extremely eco-friendly process. Here, biohydrogen production depends on various renewable feedstock and microbial species (Nagarajan et al. 2013). Different types of nanomaterials have different applications in the field of biohydrogen production. Gold nanoparticles enhance the production as they have a small size and

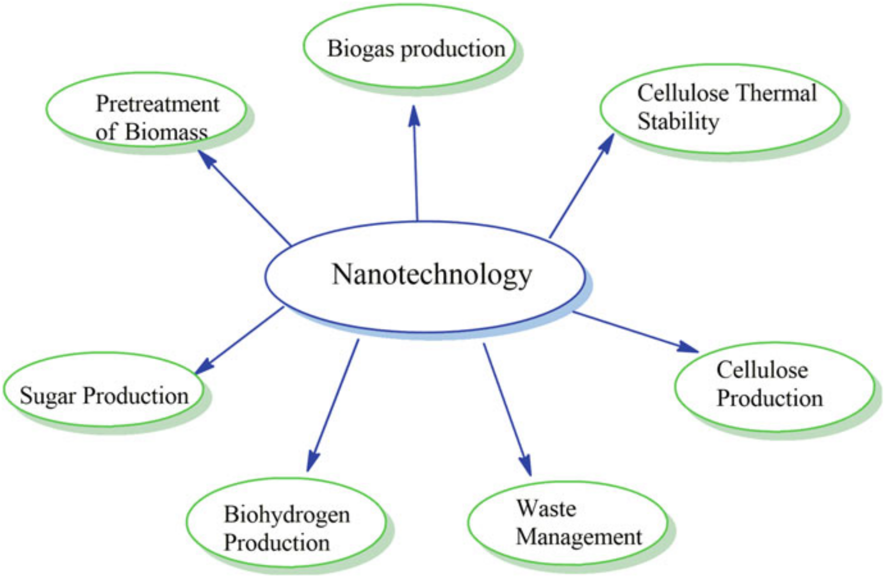

Fig. 13.5 Potential application of nanotechnology in the production industries

comparatively larger surface area which are responsible for the binding of microbial cells to the active sites.

Gold nanoparticles can also enhance the activity of biohydrogen synthesis machinery, which is also responsible for the biohydrogen production. The nanoparticles are also beneficial to the microorganisms when used in optimum concentration. Higher concentration of silver nanoparticles can restrict the growth of microorganisms and hence decrease the production of biohydrogen. The addition of silver nanoparticles can enhance the substrate utilization up to 62%, which is responsible for the enhancement of biohydrogen production up to 2.48 mol H_2/mole substrate. The main pathway for biohydrogen production is the acetic reaction (Yang and Wang 2018; Zhao et al. 2013). In the current times, silica nanoparticles have a unique emerging application in the process of dark fermentation for the production of biohydrogen.

Nanoparticles are used for the enhancement of the production of biohydrogen in photosynthetic microorganisms. These photosynthetic microorganisms like microalgae and photosynthetic bacteria are used for the production of biohydrogen. The enhancement of growth, physiological process, photosynthetic efficiency, protein synthesis, lipid synthesis, and nitrogen metabolism in microbial species occurs as the addition of nanoparticles takes place (Eroglu et al. 2013). These nanoparticles enhance the activity of various enzymes like glutamate dehydrogenase and glutamine synthetase production. The accurate concentration of gold and silver nanoparticles is known to enhance the photosynthetic activity of *Chlorella vulgaris* (Eroglu et al. 2013) which can increase the biohydrogen production by the transfer of electrons from zerovalent particles.

Biogas is produced through various methods in which digestion of organic material occurs due to a variety of microorganisms (Romero-Güiza et al. 2016). The four crucial steps are hydrolysis (breakdown process), acidogenesis (alcohol production), acetogenesis (acetic acid production), and methanogenesis (production of methane gas) (Mao et al. 2003). Acetogenesis pathways of biogas synthesis produce acetic acid and other ingredients. The concluding step of these ways engenders methane gas by various methanogens. Khan et al. (2019) and Su et al. (2013) reported that adding the zerovalent iron nanoparticles ensures to improve the production of biogas from waste materials.

13.2.2.2 Biodiesel Production

Biodiesel has numerous promising applications later on because of fewer contaminations, being eco-accommodating, and being delivered from eatable and nonedible oils (Araújo et al. 2014). Nonedible oils considered are microbial oil and plant oil changed over into biodiesel through different systems. This cycle stays replicated and valuable because these nonedible oils exist frivolous and available in nature's colossal sum (Rathore and Madras 2007; Alchi and Solanki 2017; Alchi et al. 2020). Nanoparticles upgrade the synergist reaction during transesterification, consequently improving the creation of biodiesel. Guan et al. (2017) have revealed that the biodiesel creation yield progressed within the sight of Fe_3O_4/ZnMg(Al)O nanoparticles. This interaction improves biodiesel creation up to 85% from soybean cooking oils (Dantas et al. 2017). Immobilization improves the effectiveness of biofuel creation and decreases the creation cost. For example, microbial compounds, such as lipase from *Pseudomonas cepacia*, are immobilized on the outside of nanoparticles and upgrade biofuel creation because of improved transesterification response (Goh et al. 2012). In this cycle, different kinds of cooking and algal oils have stood operational. Algal oils have a high return of creation within the sight of these particle silica nanocomposites (Chiang et al. 2015).

13.2.2.3 Bioethanol Production

Bioethanol has different preferences like high dissipation enthalpy and high octane number (Saini et al. 2015). Hereditary designing in microorganisms is likewise applied to improve bioethanol creation (Balan 2014; Kuhad and Singh 1993). Different sorts of nanomaterials have arising applications in the creation of bioethanol. Cherian et al. (2015) examined that MnO_2 nanoparticles increment the bioethanol creation by utilizing sugarcane leaves. MnO_2 nanoparticles give the expansive surface zone responsible for limiting chemicals on their dynamic locales that causes an improvement in ethanol creation (Cherian et al. 2015). Bioethanol creation utilizing immobilized microbial cells on superficial level nanoparticles has remained accounted for in numerous works of writing that have examined that yeast cells delivered extra ethanol when immobilized outside of attractive nanoparticles

(Ivanova et al. 2011). Yeast cells are responsible for more ethanol creation. These immobilized cells upgraded bioethanol creation yield up to 100%, yet the creation yield of suspended cells was 88%. Its application with nanotechnology can give better outcomes at business-level bioethanol creation (Galazzo and Bailey 1990).

Nanomaterials have favorable solicitation in biofuel creation because of their small size, enormous surface zone-to-volume proportion, and excellent reactant properties liable for improving the creation of different biofuels like biohydrogen, biodiesel, and bioethanol. From a state-of-the-art concentrate in nanotechnology, the creators infer that these methodologies can be utilized in biofuel ventures to upgrade biofuel creation and limit biofuels' expense because of nanomaterials' remarkable underlying properties.

Astoundingly, the need for superseding chattels like oil and coal with environmentally pleasant supremacy and biofuel bases has incited copious experts to appear for distinctive, pioneering methodologies. As an example, the prominence of trading petroleum yields in instruments for energy got from vast sources is suitably inveterate discerning that the vehicle area is often steadfast by 60% of the gauged oil interest for 2030, around 116 million casks every day. Viable power integrates a couple of replacements, like sun oriented, wind, geothermal, and biomass. Accordingly, new and advanced approaches are indispensable to undertake achievable cycles and a broader and quicker variance within the worldwide vigor framework. Among the new turns of events, nanotechnology has been a prolific field of inspection, retention, and auspicious prospects for various solicitation zones of social and mechanical awareness. This improvement is often exploited in innumerable fields like hardware, factual turn of procedures, preparation, and life disciplines, among others, and has, together with other amalgamation progresses (biotechnology, data innovation, and psychological sciences), unusual potential to enhance human existence (Demetzos 2016; Wolf and Medikonda 2012). Nanotechnology has great potential in innumerable biofuels and bioenergy fields, like modification in feedstocks and more dynamic impetuses. For example, compounds have stood essentially to hydrolyze biomass to make biofuels like ethanol and biogas or catalyze biodiesel design from oils and fats (Michalska et al. 2015). During this specific situation, nanostructures can supersede the catalysts or immobilize them, leading to better catalysis or preferring biocatalysts' convalescence from the medium. Additionally, this innovation reminisces choices that attractive properties remain added to immobilized frameworks (Verma and Barrow 2016). This part presents an overview of nanotechnology's prospects as an important device to take care of some dedicated issues in bioenergy and biofuel creation. At present, world energy supply is done transcendently through coal, oil, gaseous petrol, atomic, hydro, and sustainable sources (Brazilian Energy Balance 2015). The production of conventional energy sources takes decades to form fuel, which triggers scientists to work on the production of nonconventional energy sources to fulfill the current requirement worldwide, energy critical creation has remained lengthened, and therefore the current market drivers are (Mtoe) China: 2555, the USA: 1989, Russia: 1334, Saudi Arabia: 630, India: 571, Indonesia: 457, Canada: 452, Australia: 357, Iran: 308, and Brazil: 26 (Yearbook Enerdata 2015a). Brooding about various causes, the planet's energy resource uses certain chief sources, tailed by petroleum gas and coal. As is often noticed, the planet's remaining parts are unequivocally reliant on petroleum derivatives. But sustainable power has been representing almost 10% of the whole world energy source from around 40 years (Brazilian Energy Balance 2015). Likewise, the utilization of wind and sun for power creation exists exclusively in Portugal: 24.5, Spain: 23.9, New Zealand: 21.7, Italy: 16.7, Germany: 15.2, the UK: 10.3, Belgium: 10.0, Romania: 9.6, Sweden: 7.6, and the Netherlands: 6.6 (Yearbook Enerdata 2015b). Biofuels have moreover assimilated specific features and have existed in various nations, with expanded creation. For example, around 25 million gallons of bioethanol is produced in the world annually. The more protuberant bioethanol makers include the USA, with around 14,000 gallons annually by utilizing corn as a crude material, followed by Brazil, with around 7000 gallons annually, utilizing sugarcane juice as a carbon source (Renewable Fuels Association 2015). As per Goldemberg (2006), if just ethanol delivered from sugarcane juice could supplant 10% of absolute burned-through gas universally, fuel by-products might be decreased to 66 million tons annually. Quite possibly, the foremost basic expected material for biofuel creation is the vegetal biomass, chiefly considering lignocellulosic intensifiers that are the foremost plentiful inexhaustible carbon source on the earth. These materials have an expected production of 10–50 billion tons annually, with plenty of essential biomass conceivably accessible for reuse (Zhao et al. 2013). Consequently, they will remain utilized in bioprocesses for delivering biofuels or other value-added compounds. During this situation, green innovation and nanotechnology can supply distinctive mechanical areas to appeal for items of required origin for the manageable advancement measure.

13.2.3 Nanotechnology in Bioenergy Production

Distinctive nanomaterials, like carbon nanotubes and attractive and metal oxide nanoparticles, are favorable to act as a fundamental piece in bioenergy creation (Rai et al. 2016). Nanotechnology can improve bioenergy creation by utilizing various sorts of nanoparticles. They will build energy creation and fill in as a solution, which may handle energy creation. Water, sun-based, and biogas energies are various sorts of sustainable sources, and their creation can stay progressed by applying nanotechnology (Hussein 2015). While using nanotechnology in biogas production, biogas remains generated from anaerobic assimilation of natural waste like the plant, agribusiness, and animal and human waste. Biological waste is wealthy in carbon and nitrogen sources, and therefore the production of energy from the anaerobic cycle relies upon the C:N proportion (Feng et al. 2015).

There is proof that expansion of explicit metal particles increments the action of methanogenic microbes, and consequently acts as an impetus for the creation of energy. Since methanogenic microbes require a modest quantity of iron, cobalt, and nickel for anaerobic assimilation, scientists exhibited that nanomaterials are advantageous rather than utilizing nuclear or cumbersome materials (Feng et al. 2015).

Magnetic nanoparticles have a potent paramagnetic property and high coercivity, and subsequently are often utilized during the methanogenesis (Yang et al. 2015). Methanogenesis process requires very less quantity of metal, i.e., iron, nickel, and cobalt to catalyze the reaction under the anaerobic condition that enables the use of nanomaterials instead of the bulk. It has been also reported that metal oxide nanomaterials are more advantageous than the metal nanomaterials. In a later report, Abdelsalam et al. (2017) detailed that the impact of various nanoparticles like Fe, Fe3O4, nickel (Ni), and cobalt (Co) yields the foremost imperative biogas and methane from anaerobic processing of dairy cow compost. Likewise, Casals et al. (2014) detailed that when Fe3O4 nanoparticles were functional in the natural waste within the anaerobic digester, improvement of the movement of degradation and therefore increase in the yield of methane biogas were noted. Nanoparticles have vital uses in biofuel creation thanks to their outstanding physicochemical properties. Numerous nanomaterials like TiO2, Fe3O4, SnO2, ZnO, carbon, graphene, and fullerene, with select properties, are applied for biofuel creation. Also, attractive nanoparticles have wide biofuel creation applications thanks to their high surface-to-volume proportion, quantum properties, and immobilizing property due to their tiny size (Patumsawad 2011).

13.2.3.1 Nano Facilitators in Biodiesel Fabrication

Biodiesel is a blend of esters customarily created by transesterification of vegetable oils or animal fats with short-chain alcohols (methanol or ethanol) that fulfils explicit guidelines to remain shortly disbursed as fuel diesel motors. When juxtaposed through petroleum harvests, biodiesel presents numerous focal points, like biodegradable and predominant grease properties, without creating destructive emanations, just as the chance of being delivered by sustainable assets (Feyzi et al. 2016).

There are a few unique prospects for the use of nanocatalysts in this field. Blended corrosive functionalized attractive nanoparticles are utilized as heterogeneous nanocatalysts for biodiesel creation. Biodiesel creation was done from cooking oils, utilizing CaO and MgO nanoparticles incorporated by sol-gel and sol-gel self-ignition strategies, respectively. CaO nanoparticles showed a critical expansion in the biodiesel yield compared to MgO nanoparticles. Another intriguing methodology is utilizing attractive nanocatalysts that can remain proximately recuperated and reused, preferring the cycle's monetary practicality. In this examination, writers utilized iron/cadmium and iron/tin oxide nanoparticles with attractive properties arranged by coprecipitation strategy as nanocatalysts for biodiesel creation. Between the two utilized nanocatalysts, iron/tin oxide nanoparticles showed the most extreme viability by creating about 84% biodiesel.

Further, the inventors fathomed biodiesel manufacture by developing the amalgamated nanocatalysts for the transesterification of soybean oil and methanol in different molar amounts. Various factors such as nanocatalyst concentration, reaction time, and reaction temperature are also very important parameters studied by scientists (Qiu et al. 2011). The outcomes exhibited that the retort combination comprising methanol and oil within the fraction of 16:1 having 6% nanocatalyst at 60 °C for 2 h caused the foremost substantial biodiesel yield of about 98%. Wen et al. (2010) have reported the production of biodiesel using 30–100 nm particle sized KF/Cao nanocatalyst by impregnation method from Chinese tallow seed oil. Within the molar proportion of 5:1 and 4:1, respectively, these nanocatalysts showed to be latent for the manufacture of biodiesel, bringing about 94% of interaction yield at 60 °C with continuous stirring for 100 min. Another method exhibited the MgO nanocatalyst unification in nano-sheet structure by aerogel procedure and proposed its utilization to make biodiesel from rapeseed and sunflower-seed oil with 97% yield. Inventors likewise reconnoitered the adequacy of the mutual nanocatalyst toward biodiesel manufacture from nonedible crude oils like Jatropha oil (Verziu et al. 2008). Limit of 98.54% biodiesel yield was accounted for at states of methanol-to-grease fraction in 515:1 molar proportion, 130:1-min reaction time at 0.05:1 (w/w) nanocatalyst fraction.

13.2.3.2 Nano Reagents in Bioethanol Fabrication

Accordingly, the development of cutting-edge procedures, which could offer the recuperation and reusing of compounds, can decrease creation cost. Nanotechnology offers immobilization of various chemicals, for instance, cellulases and hemicellulases, related to the bioethanol creation through various nanomaterials. For example, immobilization of the catalyst in attractive nanomaterials may be a promising strategy that provides straightforward recuperation of the protein by applying a beautiful field that allows chemical recuperation and reuses for a couple of cycles (Alftrén and Hobley 2012). Existing studies show that utilization of attractive nanoparticles accounts for immobilization of chemicals involved in bioethanol creation. By and large, chemical immobilization in nanoparticles stands accomplished by covalent restricting or actual adsorption. Notwithstanding, the covalent restricting technique is more appropriate because it lessens protein desorption due to the event of covalent connections among catalyst and nanoparticles (Abraham et al. 2014). For stable immobilization of the protein in nanomaterials, these mixtures should be adjusted or covered with a synthetically dynamic polymer to offer the sensible gathering to the chemical's linkage. Lee et al. (2010) exhibited the immobilization of the β-glucosidase protein on polymer attractive nanofibers by ensnarement strategy for cellulosic ethanol creation. β-Glucosidase is a catalyst for changing cellobiose into glucose, which microorganisms can use to make bioethanol-appraised β-glucosidase, secluded from an organism immobilization in attractive nanoparticles, utilized as nanocatalyst for bioethanol creation. Additionally, we tried reusing proteins within the hydrolysis of microcrystalline cellulose utilizing carbodiimide as the connecting polymer for chemical immobilization in Fe3O4 nanoparticles. Due to the reactive nature of nanoparticles, the catalyst could be recuperated rapidly and reused multiple times. Another investigation (Goh et al. 2012) showed that the bioethanol creation compound existed immobilized in single-walled carbon nanotubes, which remained consolidated by attractive iron oxide nanoparticles that give attractive properties. In this examination, the immobilized protein execution could exist constrained by adjusting iron oxide nanoparticles' grouping in nanotubes. In this manner, the immobilized protein can be put away in acetic acid derivation support at 4 °C for its more extended stockpiling. Diverse nanomaterials have been used for immobilization of catalysts. For instance, Xie et al. (2012) exhibited lipase's immobilization in attractive chitosan microspheres combined by compound coprecipitation technique.

In this work, glutaraldehyde remained utilized as connecting particles for the covalent restriction between lipase protein and attractive chitosan microspheres. It showed that the immobilization of cellulase recuperated from *Aspergillus fumigatus* in manganese dioxide nanoparticles by covalent restriction. Creators confirmed that immobilized catalysts showed the potential to improve their thermostability property contrasted with free proteins, introducing strength up to 70 °C. After reuse for around five cycles, the immobilized chemical showed 60% of its movement. Aside from attractive nanoparticles, other nanomaterials can remain utilized in nanotechnology measures, like silica and TiO_2, polymeric nanoparticles, and carbon materials like fullerene, graphene, carbon nanotubes, and others. These materials have remained effective for immobilization of various chemicals concerning bioethanol creation (Huang et al. 2011).

In another examination, Lupoi and Smith (2011) contemplated the immobilization of cellulase in silica nanoparticles, exhibiting the adequacy of immobilized and free chemicals in the hydrolysis of cellulose into glucose. Creators saw that immobilized cellulase chemicals showed expanded glucose yield contrasted with free proteins, confirming that immobilized catalysts can stay utilized in concurrent saccharification and ageing. Microbial cells can likewise be immobilized in nanoparticles and applied to the ageing advance of ethanol creation. Subsequently, the examinations performed either on the immobilization of chemicals or on the entire microbial cells in distinctive nanomaterials prove that such methodologies are advantageous for the protected and prudent creation of bioethanol from least expensive lignocellulosic materials.

13.3 Conclusion

Lamentably, a couple of studies have stayed complete on the well-being appraisal of nanoparticles utilized for biofuel and bioenergy creation. During their amalgamation and application, nanoparticles can exist in the climate, posing a danger to humans and the climate (Gupta et al. 2012). The simple section is conceivable employing the cycle of ingestion, inward breath, and infiltration through flawless and broken dermis layers (Abdullah et al. 2017; Gupta et al. 2012; Tang et al. 2009). Because nanoparticles have a more modest size, they can, without much of a stretch, enter the human and animal cells. They can raise a ruckus to the ordinary working of the cell. As metal nanocatalysts (Asharani et al. 2011) such as carbon nanotube and carbon nanofiber (Erdely et al. 2013; Simon-Deckers et al. 2008) and zirconia-based nanoparticles have been accounted for to initiate the poisonousness.

Taking everything into account, their outflow from the vehicles and industry can cause destructive impacts. Platinum nanoparticles have likewise stayed investigated for their potential impacts on the early improvement stage. Writings have revealed that, contingent upon their focus, they bring down the pulse, postpone the incubating cycle, and influence the touch reaction, pivot ebb and flow (Asharani et al. 2011). Even though the top-to-bottom component of toxicity is until now not comprehended, the harmfulness is reliant on the size (Mostafalou et al. 2013), shape, portion (Foldbjerg et al. 2011), creation, surface covering, and construction (Gupta et al. 2012). Whenever reacted with organic molecules, the nanoparticle causes interference, consequently leading to cell wall perforation, followed by the potential harm in DNA molecules for instigation of nanoparticle cooperation (Guan et al. 2017; Kim et al. 2018).

The worldwide ecological issues, for example, the nursery impact created by various synthetic compounds, including petrol and coal, have required looking for elective environmentally friendly power and biofuel sources. The interest in elective sources is likewise because of the abrupt consumption of existing oil resources. Among the new options, nanotechnology is acquiring significance to handle bioenergy and biofuels by various applications through utilization of employable impetuses and alterations for feedstock. Reassuringly, different nanomaterials, like carbon nanotubes and attractive metal oxide nanoparticles, having one-of-a-kind properties, are utilized for biofuel creation. Among all the nanoparticles tried for biofuel creation, certain nanoparticles are being frequently utilized because they can exist proximately recuperated because of their attractive properties.

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Chapter 14
Potential of Extremophiles in Bioelectrochemical Systems and Biohydrogen Production

Tejas Oza, Saklein Malek, Prinsa Siddhapura, Swati Rani, Vrinda S. Thaker, Kairavi J. Desai, and Gaurav Sanghvi

Abstract Microorganisms are omnipresent and diverse entities present in the nature. Among the diverse categories, the extremophiles hold key significance in regard to the evolution of microbes. The features like survival and adaptation in extreme conditions ranging from extreme cold to high temperatures make them ideal candidates for exploration in diverse biotechnological applications. Apart from the huge applications of extremozymes in biotechnology industries, the extremophiles also hold potential in bioenergy production. The electrocatalytic properties of extremophiles are used in various bioelectrochemical applications. Using the economical substrates like carbohydrates, agro-residues and waste water, bioelectricity is generated using the electrocatalytic activity of extremophiles using microbial fuel cells. Apart from bioelectricity, biohydrogen is seen to be a next-generation fuel with the highest energy yield among the other fuels. Due to its easy transportation, biohydrogen is considered as the most environmentally safe fuel. Extremophiles can become the substitute of the physical and chemical processes practiced for the production of biofuel. This chapter thoroughly discusses on the prospective role of extremophiles in bioelectricity generation and biohydrogen generation.

Keywords Extremophiles · Electrocatalytic activity · Biohydrogen · Extremozymes

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14.1 Introduction

The main challenges for the globally developed world economies are increasing oil consumption and global warming. Approximately 86% of the world's energy is currently derived from fossil fuels. The use of non-renewable resources like fossil fuel, however, has increased the impact of greenhouse gases (GHGs) that raise the global mean temperature with possible adverse effects on the atmosphere, humans and other living forms present on earth. This has encouraged researchers to pursue new sources of renewable energy to supplement fossil fuels (Watson et al. 2015). In the United States, primary energy usage in 2016 was almost 96 quadrillion BTUs (U.S. Energy Information Administration, Monthly Energy Review, April 2017). Most of the developed nations heavily depend on the non-renewable sources of energy for the economic as well as societal development. Major burdens on nuclear plants have incurred negative effects on environmental niche as well as on human life (Kyne et al. 2016). Energy-generating plants running on the non-renewable sources like coal and petroleum products are found to be responsible for many cardiovascular and adverse disorders (Pandit et al. 2011). The non-renewable sources are posing great challenges with respect to its rapid exhaustion, health risks and global warming (Navanietha Krishnaraj et al. 2015; Yu et al. 2017).

Microbial electrolysis cell (MEC) is a young-generation bioenergy technology that has a huge potential for the generation of hydrogen and other value-added chemicals (methane, formic acid and hydrogen peroxide) along with the treatment of waste water, aside from the traditional approach of discovering green energy sources (Escapa et al. 2016; Zhang et al. 2019a, b).

Bioelectrochemical systems (BES) offer a novel and attractive option for electricity production using biological entities (Pant et al. 2012). BES has become an attractive tool utilising the power of the microbes to catalyse the reaction and mediate the process of biological electrocatalysis (Fig. 14.1).

The microorganisms or enzymes work as catalysts for electricity generation and are called the microbial and enzyme electrocatalysts. Apart from the bioelectricity production, the electrocatalytic property of microbes is used in the biofuel processing like generation of biohydrogen, alcohols and its derivatives, biodiesel production, wastewater treatment and production of value-added compounds by BES (Sleutels et al. 2012).

Moreover, in comparison to the non-renewable energy sources the BES offers advantages like low cost, cheap raw materials and efficient operational systems. Many reports show the efficient usage of the bioelectrochemical cells as biological fuel cells, bioelectrocatalytic cells, bioelectrosynthetic cells and biological sensors (Logan et al. 2006a, b; Navanietha Krishnaraj et al. 2015) (Fig. 14.2).

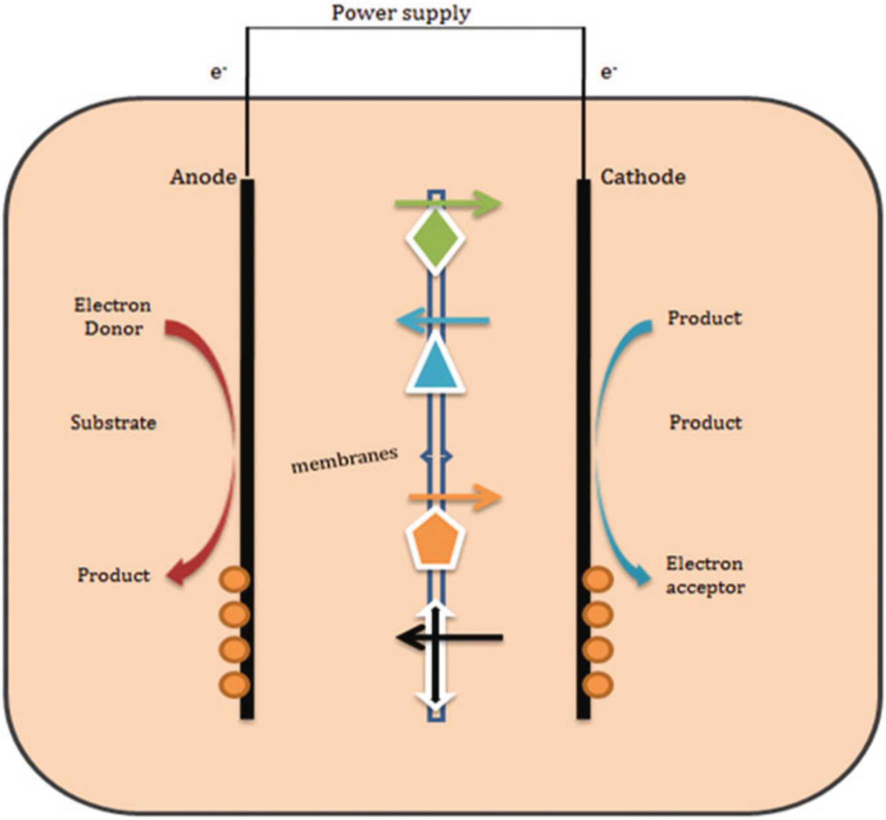

Fig. 14.1 The schematic view of bioelectrochemical systems (BES)

14.2 Microbial Electrolytic Cells (MECs)

Microbial electrolytic cells (MECs) are electrochemical devices operating with the major application as catalysis in microbial fuel cells (MFCs). The major difference with respect to the microbial fuel cell is the way it generates and converts electrical energy to chemical energy. The movement of the electrons and its operating principle are different with objectives of energy generation. The reverse flow of electrons has led to the generation of carbon dioxide and also biohydrogen. With the help of the external voltage series, electricity is generated due to microbial and enzymatic capacity of the microbes. Substrate is oxidised at the anode compartment through the electrigens (electroactive microbes) leading to the release of electrons and protons (H^+ ions). The electrons are initially arrested at anodes with further extension joining to the cathode via an external circuit. With the presence of mediator electrolytes, the protons produced at anode get passed through the cathode. This sequential reaction leads to hydrogen production by a combination of H+ ions.

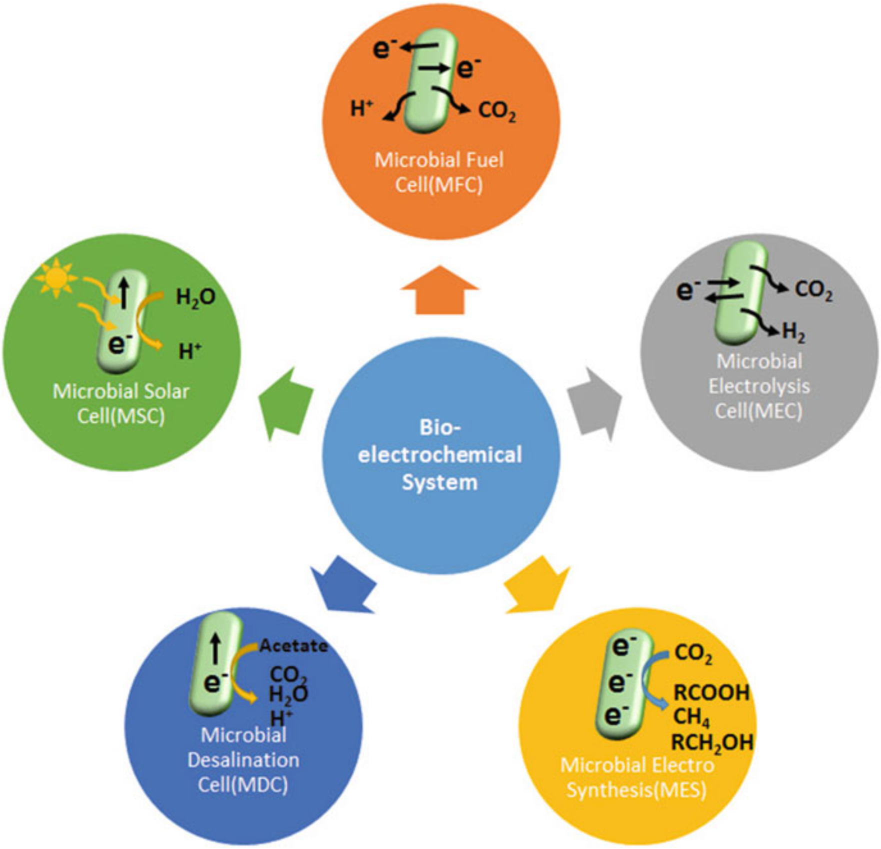

Fig. 14.2 Applications of BES

The electrochemical potential of the catalytic reaction is usually found to be insufficient for the production of hydrogen via oxidation reaction at anode. The reaction requires additional power supply for significant electricity generation. The voltage usually in the range of 0.2–1.0 V is required for hydrogen production (Logan et al. 2008). However to the merits, MECs required very less voltage supply from the external source when compared with the classical approach in the water electrolysis process. The MECs and MFCs offer many advantages in terms of substrate usage and product formation (Fig. 14.3).

The important parameter for designing the process is about the selection of the microorganism. The strain and the parameters tend to be very specific and also work under the limited set of operating conditions. The microbes are very sensitive to the external environmental factors like temperature, pH, and pressure. The selective and specific binding of the enzyme at the substrate site is also playing a crucial role in designing operational parameters for the microbial catalysis. In this case, the microbes surviving at extreme conditions are very useful for the generation of

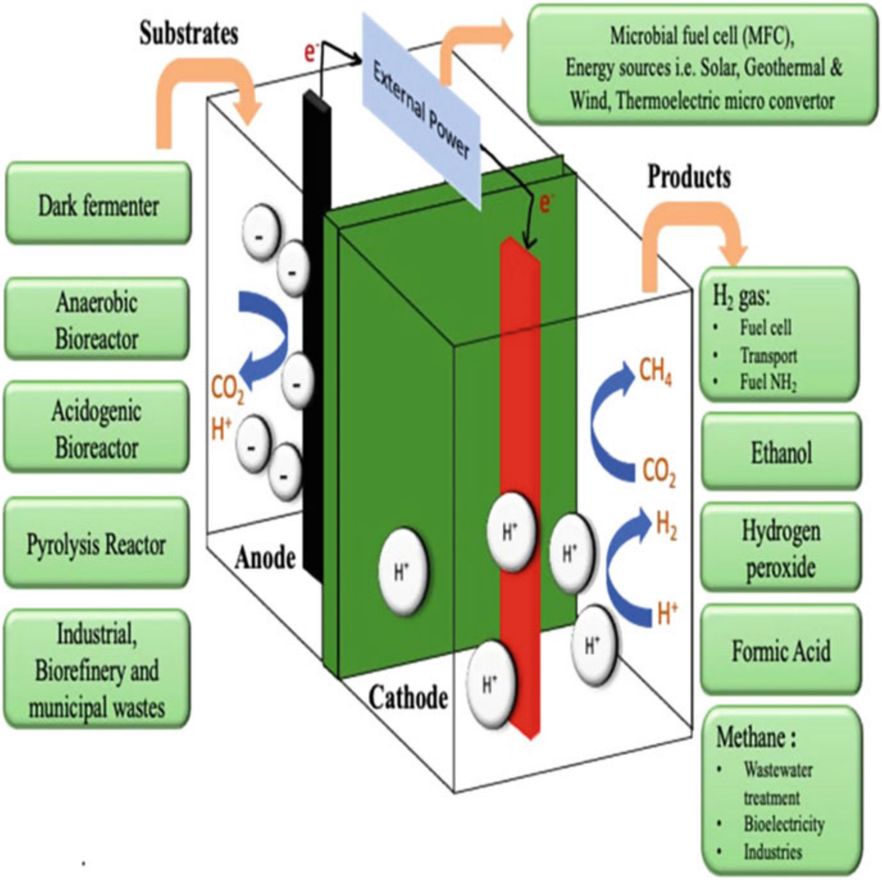

Fig. 14.3 Applications of MFCs and MECs

bioelectricity as its natural environment is unusual. The extremozymes can also restore the limitation of the mesophilic microbes. To add on, extremophiles can easily grow on the many substrates and lignocellulose biomass (Turner et al. 2007; Bhalla et al. 2014a, b). The chapter explains the use of extremophiles in MECs and in renewable energy production.

14.3 MECs General Concepts

Depending on the substrate-binding efficiency and the type of substrate and enzyme, the substrate is typically oxidised by a chemical or microbial catalysed reaction in order to form one or more items. In MECs, the derived electrons are further passed either directly or indirectly to the conductors (anode). To form the desired product, electrons will be combined at the other end (cathode). This reaction can occur at the anode through microbes or metals that act as catalysts.

In MECs, at the anode site, microorganisms which serve as catalysts are known as electricigens. The microbes dwelling on this anode site are also known as ‘electrogens’ or ‘anophiles’. The extremophiles from many phyla are known to work as electricigens; however in particular phyla representing members of the *Proteobacteria* and *Firmicutes* are most favourable for the process. Among the group members of this family, *Geobacter sulfurreducens* and *Shewanella oneidensis* are reported to be the most studied and used for the biocatalysis process (Caccavo et al. 1994; Venkateswaran et al. 1999). Under ideal conditions, the anode usually does not use any of the external alternative electron sources and also cell transmission mechanisms. The direct mechanism involves the transfer of electrons anaerobically via electron transport chain using the insoluble electron acceptor (the anode). The transfer of electrons is processed by the c-type of the cytochromes and proteins loaded with iron sulphur to be found on the cell surface (Liu et al. 2014).

Recently, as reported by Reguera et al. (2005), the electrons can be transferred directly through electricigens using nanowires. The study on the ways and pathways used by these electricigens to transfer electrons from the anode site using the mediators like cytochromes in composition of nanowires is going on (Lovley and Malvankar 2015; Malvankar et al. 2015). In the mechanisms of indirect electron transfer to the anode, the mediators like redox shuttles are extensively used. Mediators can be either organic like humic acid or organic like SHS shuttle. In the indirect mechanism, the movement of electrons can also mediate the use of the planktonic cells which can form biofilms on the surface of the anode to facilitate the electron transfer and movement to the other site. The other type of the diversion of electrons was observed in the methanogenesis process using methanogens in the system.

14.4 Cathode’s Reactions

Electrons move from the anode to cathode site to complete the reduction reaction for generation of bioelectricity. The final electron acceptor differs as per the system types and also about its operations. For example, oxygen was used for electricity generation as the electron acceptor due to its wide and abundant presence and also working with water as the reducing element (Logan et al. 2006a, b; Clauwaert et al. 2007). On the contrary, MECs work on the requirement of additional external energy to complete the power supply for production of valuable resources like cathode hydrogen. The main advantage of biohydrogen production through the MECs is that higher production can be achieved compared to the conventional classical method of water electrolysis for energy consumption.

14.5 Separators

As indicated in many reports, electrons are separated by the membranes preferably by the ion-exchange one for the separation of anolytes and catholytes having varied compositions. The ion-exchange membrane also aids in stopping the phenomena of crossing over of the substrate and product. This step helps in creation of the pure product facilitating the transport of the unique ionic loads and refusal of the opposed ionic loads. This ion-exchange membrane (anion exchange) allows the flow and transfer of the negative ions with refusal of the positive ones. On the contrary, the cation-exchange membrane allows the positive ion movement with refusal of the negative ions. These applications and selective ion-exchange movement help in optimising conditions for maximum production. For example, just a simple optimisation in the bipolar movement aids in maintaining the pH gradient between the anode and the cathode (Ter Heijne et al. 2006; Harnisch et al. 2008; Harnisch and Schroder 2009).

14.6 Couplings of MFC-MEC

In theory, an applied voltage of 0.14 V is necessary for driving hydrogen power in MECs. In practice, a tension of 0.6 V or more for a high-efficiency hydrogen production is required because of the overpotential (Call and Logan 2008). A typical MFC open-circuit voltage can in particular reach up to 0.8 V (Min et al. 2008) and can thus be done by the use of an MFC to power a MEC, providing a combined MFC-MEC device, to achieve the high-efficiency processing of hydrogen. This method can harvest hydrogen from substrates and does not need an external power supply. Min et al., who combined a single-chamber MFC with an air cathode and a dual-chamber MEC, reported the first demonstration of an MFC-MEI connector. As an electron donor for both the MFC and MEC, the device's hydrogen output rate approaches 2.2 0.2 mL $L^{-1}d^{-1}$ with acetate (0.1 g L^{-1}). Cathode hydrogen recovery and Columbia's systemic overall performance were, respectively, 88–96% and 28–33%. The systemic hydrogen production was 1.21 mol-H/mol-acetate in average (Min et al. 2008). The performance of the coupling system was tested in different configurations: the results showed that the hydrogen output values of 2.9 ± 0.2–0.2 ± 0.0 ml $L^{-1}d^{-1}$ were variable when the resistor shifted from 10X to 100X. The hydrogen production rate grew dramatically when the MFCs were connected in one line, while when connected in parallel, it slowed slightly (Sun et al. 2009).

14.7 Microbial Photo-Coupled Device

The direct use of renewables such as solar is a visible, but still difficult, solution to hydrogen production in an environmentally friendly way. A MEC (dye-sensitised solar cell (DSSC)) system where an external solar cell replaces the electrical partition was combined with the MEC device to provide the additional power required (Ajayi et al. 2010; Chae et al. 2009; Jeon and Kim 2016) as reported in the literature. Furthermore a solar MEC device combining the microbe anode and the photocathode of a semiconductor has been shown to produce effective hydrogen. The material preparation and development costs of a DSSC-powered MEC can be reduced.

14.8 MECs and Fermentation

Due to thermodynamical limitations, many organic compounds produced by dark fermentation cannot further degrade to hydrogen via fermentation (Call and Logan 2008). A MEC can be combined with fermentation to further degrade these dead-end products. In an ethanol-type fermentation reactor, for example, Lu et al. (2009) fed effluent into a single-chamber MEC. The MEC has achieved a hydrogen production rate of 1.41 ± 0.08 m3 $L^{-3}d^{-1}$ with a voltage of 0.6 V significantly higher than that of the fermenting reactor (0.70 $m3L^{-3}d^{-1}$) (Lu et al. 2009). The recalcitrant substrate fermentation effluents, including lignocellulose and cellobiose, have also been degraded with MECs. Lalaurette et al. (2009) achieved the hydrogen output rate for the lignocellulose and cellobiose fermentation effluent at MECs of 0.96 ± 0.16 L (cellobiose) and 1.00 ± 0.19 $L^{-1}d^{-1}$ (lignocellulose) (Lalaurette et al. 2009). Yan et al. (2015) fed MFCs fermentation effluent of xylose and corncob hydrolysate. When a current was formed, MFCs were used for hydrogen production. For xylose and corncob hydrolysate effluent the production rates for hydrogen were 41.7 and 23.3 mmol per mol acetate, respectively. Fermentation effluents were also employed in MECs in the form of cellulose (Wang et al. 2011) and glycerol (Selembo et al. 2009).

14.9 Wastewater-Processing MECs

7.6 kJ L^{-1} energy from household waste water has been reported by Heidrich et al. (2011), indicating the abundance of energy in waste water. Often used for the recovery of energy from waste water are the MFCs and MECs. MECs have many advantages over MFCs from the economic and environmental points of view (Sleutels et al. 2012; Zhang and Angelidaki 2014). Some MEC reactors have been designed for the treatment of waste water. Ditzig et al. (2007) developed the first MEC to use domestic waste water as a substratum.

The domestic waste water in the anode chamber was treated with a double-chamber reactor with applied voltages of 0.2–0.6 V. The MEC was run in fed-batch mode, with COD removed almost completely (87–100%). The hydraulic yield (approximately 10% theoretical value) was poor because of low substrate conversion and loss of hydrogen. Laboratory results on the pilot scale must be used in order to assess the functional application of MECs and to evaluate the durability of their critical components, such as electrodes and membranes. Cusick et al. (2011) designed the first pilot MEC to handle real waste water from a winery. The MEC was a 1000 L volume single-chamber reactor using graphite fibre brushes as anode and SS mesh as cathode. The MEC achieved a 0.2 $L^{-1}d^{-1}$ hydrogen output rate and a mean soluble COD removal of 62%. The emissions of the gas were, however, mainly CH_4 (86%) and CO, with trace quantities of hydrogen, as methanogens converted hydrogen to CH_2 further. A 120^{-L} volume MEC device consists of six separate modules of MEC for domestic wastewater treatment using a stainless steel cathode and low-cost microporous membrane was developed by Heidrich et al. (2011). The MEC system produces mainly pure hydrogen gas ($100 \pm 6.4\%$) for more than 3 months with an average efficiency of 34% with a hydrogen output rate of 0.015 L d^{-1}.

The use of electron acceptors in cathodes can also minimise the recalcitrant pollutants (such as nitrobenzene and 4-chlorophenol) because the possible cathode of MECs can be regulated by electricity. Compared to the conventional electrochemical reduction, removal of these pollutants in MECs needs much less energy. In addition, the excess potential of electrochemical reactions can significantly be decreased by electroactive microorganisms in the anode or cathode, thus achieving improved effectiveness and removal rate.

14.10 Electrochemical Constraints

14.10.1 Electrodes

Electrodes are the main sources for generation of electrons and are also the active sites for biochemical reactions. Electrodes also support the formation of biofilms for electrogenic/electroactive interface between the surface of the electrodes and microbial species. Many carbon-based and metal-based electrodes have been used for the production of the novel material for achieving more significant production. The use of different materials will lead to increase in energy production with cheap raw sources and also better chemical treatment costs. The use of low-conductivity material needs to be avoided as it might cause problems in large-scale energy production. The porous nature of electrodes provides higher and better prospects in energy generation and also in long-term industrial economical applications. In comparison of the porous electrodes, the anodes made of carbon- and metal-dependent anodes face major issues due to the poor strength and corrosive nature (Butti et al. 2016). The use of energy production with combination of microbes and heavy materials like brass and aluminium can result in significant production. The inputs to increase durability, reliability and material composition for the anode production are in progress (Navaneeth et al. 2015). With inputs on the anode material, it is important that the biocompatibility of the material with the microbes is well studied. The more the biocompatibility, the more chances of biofilm production which in turn increases energy production.

14.10.2 Design

Design of the electrodes and the vessel makes it important for initialising the pilot-level studies. The raw material choice, composition, construction and efficiency play crucial roles in designing single cell or stacked cells (Oliveira et al. 2013). Apart from raw materials, the cost of the cells is driven by the reactor volume. The identical cathode system was found to be insufficient for power supply without the membrane separators. The other factors found to lower the efficiency include electrical configuration and also distance between the electrodes. The less distance between electrodes proved better for system performance (Liu et al. 2008). Electrolyte strength in the membrane-free reactors was reported to have lower flow of ions and can also result in the increase in internal resistance to 38–60% due to resistance (Ohm) (Fan et al. 2008). Advancement in the designing and type of materials for surface electrodes makes it an ideal candidate for better output of microbial fuel cells. The reports published by Hsu et al. (2013) show that the intensity of power is not always found to be linear and it can change exponentially. The research by Cheng and Logan (2011) reports that increasing the size of the cathode leads to significantly more than 60% of electricity. On the contrary, the output in power decreases to 12% when the process is initiated to the anode. This shows that changes in the anode site of cells will not make a process efficient for the energy production and also a factor to be taken care of is greater performance of the cathode electrode which can make cathode site a rate-limiting factor for energy generation.

14.10.3 Connectivity

The arrangement of individual MFC units often explicates electric communication in scalable systems. These scalable units are linked mainly by potential means of a serial or analogue combination of both current and voltage necessities (Liu et al. 2008). The power system usually calculates the total end product and a potential reduction during the process of ion crossover (Kim et al. 2012; Zhuang and Zhou 2009). The reports by Galvez et al. (2009) indicate that at the sites where leaching is prominent, the multiple-series MFCs were found to produce significant energy output. In the study by Ewing et al. (2014), the relation between single MFC and multiple MFCs was found to be significant and upscaling in the process has led to greater energy generation. The combination has delivered more than 3% of the output compared to the single microbial fuel cells. Similar studies conducted by Zhuang et al. (2012b) suggest that the output generation of a combination of multiple MFCs yields better energy generation. Based on all these reports, it clearly points out that the connected MFCs can achieve high electrical power in comparison to the analogue system for energy production.

14.10.4 Operational Restrictions

Effective functioning of the catalyst acts as the main factor in the designing and performance of the catalyst. The role of catalyst is the most crucial when MECs are used in the large-volume wastewater treatment facility. However, it can become a rate-limiting factor when it is quantified with the starting line-up of energy production. The value can vary in between hundreds and thousands depending on its operation conditions as well as set-up (Feng et al. 2010; Liu et al. 2008). The type of substrate and inoculum concentration affect the operation of the MFC (Aelterman et al. 2006). Additionally the selection of the type of microbial strain also makes the system more viable and operationally substantial. The pH load also affects biological film creation and hence its commencement (Patil et al. 2011). Although this is feasible for better start-ups, large volumes (>1 mm3) of MFCs are anticipated to function on an industrial scale with the enriched substrate/culture (~10–20 m3 reactor volume), thereby restricting its usefulness. Fe (III) or fumarate was reported to be the fastest for effective functional MFC before the inoculation with culture of *Geobacter sulfurreducens* (Torres et al. 2009). Connectivity at the anode site also regulates the intensity of the current and the formation and configuration of biofilm at the anode site of the cell. The preservation of desired conductivity levels can thus inhibit non-exoelectrogen growth and thus promote start-ups. The regulation of potential at the anode site can also offer a promising solution in designing efficient operational parameters (Wang et al. 2010).

14.10.5 Loading of Substrates

The loading rate (OLR) and load rate of sludge (SLR) are important for bulk-scale systems during the initialisation process. Those parameters calculate the strength of the reactor per volume per unit and the number of microbes in organic substrates (Oliveira et al. 2013). Numerous studies have reported about the overall influence of OLR and SLR on productivity of microbial fuel cells, and clinched that these factors are directly proportionate to biomass yield and degradation and reversely to efficiency of the cell and internal and external resistance (Martin et al. 2010).

The use of waste water was not beneficial because the system was improved with regard to cathode surface, and increased power loss was observed with significantly lower charge rate (Cheng and Logan 2011). It was reported that the OLR can cause a direct effect on the anode-site oxidation and also an increase in substrate depletion (Martin et al. 2010). On the contrary, the decreased or lower OLR can increase cell efficiency with lower methane activity with significant rise in resistance in the system that lowers the OLR. The balanced approach of recovering the electron at optimum levels at both sites is a prerequisite for scale-up industrial operations. In this context, the MFC needs to be operational at optimum level at both SLR- and OLR-level values for better biomass generation and also efficient power production.

14.10.6 Economics

The MEC proficiency can guarantee an independent biological process for simultaneous waste depletion; however the overall usefulness is largely restricted by fiscal dynamics. Overall, the laboratory installation cost with a planned 10-year life cycle leads to the investment cost of around US$ 3 to 1 k (Fan et al. 2008). When considering large-scale operations, the economic importance of this aspect will vary. A qualitative analysis of traditional treatment schemes may also include a techno-economic viewpoint. An MFC shows an advantage over conventional sludge therapy where additional benefit is expanded (Liu and Cheng 2014). MECs also offer other economic advantages as compared to the other classical as well as advanced treatment technologies. The main advantages include (1) minor biomass and no requirement for the aeration and very minor temperature regulation requirements and (2) high convertible energy values (Liu and Cheng 2014).

Several other published reports showed encouraging consequences in the development of economically feasible MEC applications. But on the other hand, long-term consumer acceptance is not feasible. The material and production costs of the MFC are a significant impediment to overcoming (Zhuang et al. 2012a, b). The major costs are associated with pretreatment by chemical electrodes, the use of valuable and costly metallic elements, and the composition and specificity of electrodes as current producer and collector (Seelam et al. 2015). Electrode constituents, accumulators, promoters (catalyst) and membrane exchangers/separators are too costly and also contribute to the overall economy of the system development. Although the price of anode products has fallen, as an important site for energy generation the different types of cathodes are still overpriced. To reduce the price, the utilisation of anodes made up of graphite material would be preferable to the economical one (Feng et al. 2010). Usually, cathodes account for 75% of the overall price of cells (Rozendal et al. 2008). Reduced cathode content costs alone will reduce capital costs and also make the system more economic and viable (Fan et al. 2012). During modification and designing of cathodes, when the base material is of iron and nickel the power generation and intensity were found to be around 23–36 W/m^3 (Aelterman et al. 2006). To prevent any damage and to increase shelf life of cathode in the cell, the base material like stainless steel can be used. Stainless steel is a cost-efficient, non-corrosive and efficient binder. The different separators also add to the cost of the cells. In order to reduce the cost, the distance between electrodes and also separators is narrowed down to make sure that efficient power generation is possible with less volume in the reactor (Liu and Cheng 2014).

Although the use of individual-compartment MECs will require a smaller amount of investment than double/multi-compartment arrangement because there is no separator, the efficiency of bioelectricity is normally not affected (Butti et al. 2016). It can be beneficial to have separators because they prevent short circuits and have more potential for gaps in electrode arrangement (anode and cathode). This type of arrangement boosts electrical efficiency on a measurable quantity. Inexpensive substitute separators are under evaluation, although reliability and quality of their long-term operation have yet to be assessed (Butti et al. 2016). Biofeuling and scaling adversely impact MECs in the operating front, ultimately impacting its shelf life, strength and power competence (Liu and Cheng 2014). During continuous process, membrane cleansing may be vital in a two/multi-chambered facility. Published reports have recommended the importance of bio-based cathodes in a MEC set-up. However, to set up bio-based cathodes can also be a pricey concern. As the biological entity is bound to cathode, aeration plays a crucial role in the designing of a biological cathode and in the supplying and transferring of oxygen (Cheng and Logan 2011). The use of activated carbon in combination with a wire mesh collector can be a potential sturdy substitute as a catalyst for oxygen reduction. Another encouraging solution is to use air cathodes. The single-chamber MECs with air cathode and fabricated electrode assembly can be created for attainment of more efficiency. Such designs can provide comparatively fine return since it is cheaper, and easy to manufacture, control and produce elevated power. For ecological considerations as well as fiscal viability, MECs necessitate lower power operational needs. Waste water usually works as precious feedstock and supplies extra financial gain to the MEC skeleton. Fornero et al. (2010) found poor electrode competence using waste water and reported that this technology is not viable for the production of electricity. Wang and Ren (2013) reported that MEC technology continues to be an expensive variant in waste flows as the cost of the cell-grade electrode material is also too high. The MEC is a silent budding technology, but these financial limitations signify that expenditure matter remains oversized. However, it can be assumed that technological drift in science advancement can most likely solve this barrier for its use in industrial application.

14.10.7 Extremophiles

Electroactive microorganisms are cells that can display electrocatalytic action. Electrons can be produced/consumed and the electrons pass through the electrode-electrolyte interfaces after oxidation/reduction from the electrode donor and electron acceptor. They are the key actors and can serve as electrocatalysts in electrochemical response in any bioelectrochemical process. Electron transfer becomes troublesome when microbial electrocatalysis happens deeper within the cell. Electron transfer is difficult. However the redox positions of the enzymes/microorganisms cannot be transferred to an electrode surface. For example, the Gram-positive bacteria have a layer of peptidoglycan and a periplasmic intermembrane distance. However, this trouble can be evaded by cautiously amassing electrically active microbes. Additionally, the microbes possess excellent oxidation-reduction potential and surface-reactive proteins on the cell wall, making the system better for electron transmission characteristics to the electrodes. Further to the merits, activity over broad environmental parameters like pH, temperature, metal tolerance and toxin resistance is also useful for energy generation. Microorganisms can carry electrons either via direct transport of electrons or through electron shuttle compounds. The direct transmission of electrons is conceded by microbes via mediators like different cytochromes and pilis or using extracellular proteins. Reguera et al. (2005) reported the mechanism underlying the electron transfer in *Geobacter sulfurreducens* through pili.

Recent studies have reported about the use similar to the metal conductance of these microbial nanowires. This microbial assembly will facilitate the guided electron transmission across syntonic species, in addition to being able to pass electrons among acceptors and donors of electrons (Malvankar and Lovley 2012). For example, the species *Shewanella oneidensis* can directly transfer electrons via mediators like c-type cytochromes which are found to be entrenched in periplasmic membranes (Schuetz 2009). Apart from the mediators certain intracellular and extracellular proteins were found to have compounds which can transfer/mediate the electron transmission at electrode interfaces. Few microbes were found to generate mediators or shuttles like compounds of class quinine which can mediate electron transfer (Schuetz 2009; Rabaey 2004). Analysis at gene level indicates that proteins and gene expressions are regulated and usually produced at the surface of microbes during its interaction/attachment on the electrode surface (Holmes 2006). The morphology and conductivity of certain electroactive microorganisms in pili nanowires are also stated in a report by Malvankar et al. (2015). Vargas (2013) has revealed that *Geobacter sulfurreducens* shows good conductivity through pili due to the presence of essential aromatic amino acids.

Due to the presence of catalytic activity and adaptivity at harsh environments, the use of extremophiles will benefit for better performance in bioelectrochemical system designing. Extremophiles represent species which can tolerate extreme salt, pH, temperature, pressure, saline, metals, etc. Additionally, this group of microbes are found to be good mediators of the oxidation-reduction potential and also possess proteins having good electron transfer abilities. As substrate, extremophiles can use many recalcitrant, xenobiotic as well as lignocellulosic biomass for energy generation. The additional benefit is that extremophiles can be used for the development of a safe, highly advantageous system for the efficient commercialisation of any microbial/enzymatic technology. For example, numerous thermophiles were isolated and reported due to the presence of its cellulose/hemicellulose-degrading ability (Rastogi et al. 2010). The sequencing results of the isolated culture demonstrated that the sequences were related to the species of *Actinobacteria, Bacteroidetes, Chloroflexi* and *Deinococcus-Thermus*. Several isolates belonging to the family *Bacillus and Cohnella* showed prospective for depletion of cellulose and also raw sawdust. Among many important species, *Geobacillus* sp. is one of the species which were widely studied by many researchers. Among that the strain WSUCF1 was studied due to its high cellulose-degrading efficiency. The optimal pH and temperature were 5.0 and 70 °C. The genome of whole-*Geobacillus* strains was sequenced. The annotated results indicate that the polysaccharide degradation was correlated with 70 open reading frames, 3 cellulose degradation ORFs and 13 ORFs among the 865 carbohydrate metabolism enzymes that were annotated as xylan-degrading ones. The strains found to degrade polysaccharides were also found to be significant producers of endoglucanase, xylanase and beta xylosidase (Bhalla et al. 2014a; Bhalla et al. 2015). Recent research has revealed that the WSUCF1 thermophile strain can be used in the carbon-free electrode and can conduct direct transmission reactions. The results indicate the impact of species for creation of new MEC systems utilising lignocellulosic substrates. Any bioelectrochemical approach will considerably reduce running costs and ensure that these wastes obtained in large quantities from different environmental sources are disposed of in an inexpensive and abundant lignocellulosic biomass.

Most studies reported attributes that extremophiles are paving way for improvement in the electrocatalytic process and are also known to boost for forming electrocatalytic films in the sense of severe conditions (e.g. high heat). *Thermincola ferriacetica*, for instance, has had an exceptionally high current concentration in comparison to mesophile species. Due to operating high temperature adaptability and surface characteristics these thermopiles are working well in electrical operation. However, it cannot be predicted that whether the particular biological engineering further leads to enhanced catalytic activity in thermophiles.

Extremophiles' electrocatalytic behaviour has been studied in depth with the intention of mediating electrooxidation/mediate/dons/electron acceptors and electron transmission property (Hawkins et al. 2011; Sokolovskaya et al. 2015). Extremophile catalytic action is used in many bioelectrochemical applications (Dopson et al. 2016). Bioelectricity from various substances, such as glucose, xyloses, cellulose, acetate, lignocelluloses, waste water and heavy metals, has been produced using the extremophiles for bioelectricity processing in anode or cathode compartments (Wrighton et al. 2008; Jong et al. 2006; Lusk et al. 2015). A thermophile possessing similar cellulose-degrading ability and acetate production ability was reported to be closely related to *Thermincola carboxydophila* (Mathis et al. 2008).

Microbes residing in deep-sea sediment barophile microorganisms were isolated and used as electron donors for microbial fuel cells. Extremophile electrochemical activity is also reported in the literature for the development of biohydrogen in microbial electrolysis cells (MECs) (Lu et al. 2011). Lusk et al. (2007) reported about the designing of microbial electrolytic cell using a combination of two thermophilic species, i.e. *Thermoanaerobacter* and *Thermincola*, member of phylum Firmicutes. Using cellulose as substrate, the developed MECs work in the anodic compartment at 60 °C and have provided a current intensity of 6.5A m^2 and a coulombic (CE) effectiveness of 84% without CH production (Lusk et al. 2007). Further, the other thermophilic species *Moorella thermoautotrophica* growing at 60 °C was reported for bioelectrosynthesis and the simultaneous reduction in formate and acetate (Yu et al. 2017).

14.11 Extremophiles and Its Types

14.11.1 Acidophiles

In conditions with low pH (<7.0) the microbes which survive are known as acidophiles. The extreme acidophiles (*Acidithiobacillus* sp.) grow well under extremely low pH conditions (<3.0). In a pH range of 3–5, mild *Bacillus acidoterrestris* sp. live (Gerday and Glansdorff 2007). All three realms, viz. Archaea, Bacteria and Eukarya, are representative of some acid-tolerant bacteria. The mesophilic microbes grow in a range of 20–40 °C, moderate microbes live at 40–60 °C and thermophilic/extreme microbes thrive at >60 °C (Gerday and Glansdorff 2007). Acidophiles can thrive in acidic conditions with notable examples of *Metallosphaera* sp. and *Acidithiobacillus ferrooxidans*. The acidophiles are usually aerobic heterotrophs which can oxidise iron and sulphur and also species like Acidianus sp. that minimise iron concentration. Acidophiles can handle acidic waste (3–5 pH) due to the presence of biological processes (e.g. nitrification and fermentation), from volcanic areas and from the mining industries of geothermal and coastal coal and steel (Johnson et al. 1992). *A. cryptum* was the first acidophile used in MEC experiments (Borole et al. 2008). Acidophiles are used in a number of further subsequent experiments for the treatment of mine sulphide waste (Ni et al. 2016), leachate food waste (Li et al. 2013) and waste distillery (Kim et al. 2014). Usage of *Ferroplasma sp.*, *Desulfovibrio sp.* and *Acidithiobacillus sp.* has also shown its application in MECs (Rojas et al. 2017; Sulonen et al. 2015; Sulonen et al. 2016).

In acid conditions, acidophiles used in the MES anode act and function as follows: (1) inhibit methanogenic activity; (2) increase the pH gradient between the anode and the cathode body; and (3) promote proton transfer through the cation-exchange membranes. In addition, acidophiles are more commonly treated with sulphides, minerals and acidic wastewater by-products. More anodic overpotential is nevertheless sustained, which offsets the metabolic losses needed to maintain neutral conditions of cytoplasm (Dopson et al. 2016).

14.11.2 Challenges in Design

Electrode and membrane materials are vulnerable to substrate degradation issues, including corrosion, drowning and oxidation when exposed to acidic circumstances (Martínez-Huerta and Lázaro 2017; Yi et al. 2017). Under acidic conditions, glassy carbon electrodes are highly vulnerable to oxidation, especially with higher electrode potential (more positive potential), and glassy carbon electrodes are affected by problems linked to agglomeration, reduced porosity and increased mass transport limitations (Yi et al. 2017). The lower pH reduces proton-exchange durability for normal membranes. Acidic conditions also promote deposition of electrode products on the membrane surface, resulting in increased resistance to diffusion and a decrease in MES's electrochemical efficiency. It is necessary to choose oxidation and corrodible materials but without sacrificing biocompatibility, conductivity and large specific surface area desirable features.

14.11.3 Alkaliphiles

The alkali-tolerant bacteria (pH 8–9) or alkali-resistant bacteria (pH $>$10) are known as alkaliphiles (pH 8–9), e.g. *Bacillus alcalophilus*. Alkaliphiles are divided into subclasses depending on the tolerance towards pH. Usually, depending on the pH range, they are categorised into moderate pH of around 9 and extreme alkaliphiles with pH range of 12–13 (Horikoshi 1999). All three cellular life classes comprise alkaliphiles, such as prokaryotes, eukaryotes and archaea. Alkaliphiles can use a diverse source of energy and can be found in different ecological niches such as aerobic as well as anaerobic conditions. The alkalophilic group of organisms can include phototrophic, fermentative, sulphur-oxidising (e.g. Thioalkalimicrobium), acetogenic and methanogenic (e.g. Methylomicrobium) groups.

In the MES operations alkaliphiles are advantageous. They allow anodic reactions, increase the anode potential to more negative values first under higher pH conditions and improve overall electrochemical conversion efficiency. Secondly, the formation of alkaline environments reduces accidental emissions of methanogens. Finally, alkaline situations produce a greater number of electroactive moieties in anodic biofilms. In a recent study by (Zhang et al. in 2016), the benefits from alkaline conditions (pH 1/4 10) in MFCs were seen in the mixed microbial population from a brewing waste treatment plant-activated sludge. At the end of the study, high-performance sequencing studies found that most group of microbes include *Firmicutes* (88.14%), *Alkalibacter* (5.14%), *Bacillus* (2.14%), *Alkaliflexus* (2.107%), *Anoxyna tronum* (0.48%) and *Alkaliphilus* (0.09%). Among different microbe groups, *Corynebacterium sp.* were the dominant species of alkaline-enriched biofilm. The methods used in the processing of paper and cement from livestock can be used to process alkaline waste streams from the electroplating industry and to treat potato lye.

Enzymes and useful by-products for their processing capability (starch degradation enzymes, cellulases, lipases, xylanases, pectinases, chitinases) can be further explored (2-phenylamine, carotenoids, siderophores, and cholic acid derivatives).

14.11.4 Design and Challenges

Graphite components are inert under alkaline conditions (Iken et al. 2007). However, metal compounds (e.g. stainless steel) are susceptible to consistent corrosion and stress corrosion cracking at high levels of caustic soda and potash used in experiments with MEC to protect under alkaline conditions (Iken et al. 2007). Due to stress, corrosion cracking problems can be worsened by the increased impact of MECs under thermophilic and high pressures. One way to overcome these challenges is the use of compatible materials such as nickel alloys and stainless steel withstanding hot (>95 °C) and strong 50% alkaline conditions (Jones 1992). To avoid stress corrosion, it is better to minimise copper electrode sensitivity towards MEC ammonia-based alkalis (Fontana 2005).

14.11.5 Thermophiles

The operation of MES is activated at 45 °C for most thermophiles. The literature suggests that extremophiles isolated from the coal waste piles tolerating temperature of about 65 °C can be employed for the method of MESs (Beffa et al. 1996). The use of such thermophiles can be used in compost and silage piles for household and industrial applications (Singh 2012). Archaea species tolerate an exceptional temperature of 100 °C between prokaryotes (such as methanogenesis, decreased sulphate, sulphur oxidation, nitrate reduction and hydrogen oxidation with little effect on their metabolic chemolithotrophic activities) (Gerday and Glansdorff 2007). Thermophilic conditions speed up the kinetics of waste oxidation in MECs, through enhanced substrate solubility, mass transfer characteristics and microbial behaviour.

They can also kill pathogens and reduce other infection hazards. Recent studies have demonstrated the use in thermophilic MECs, viz. *Thermincola ferriacetica* and *Thermoanaerobacter pseudethanolicus* (Dopson et al. 2016; Lusk et al. 2015). Thermophiles were also used for the processing of energy supplies in multiple electrolytic microbial cell (MEC) systems, such as hydrogen (Dopson et al. 2016; Dai et al. 2017; Fu et al. 2015).

14.11.6 Design and Challenges

Thermophilic MECs require thermally jacketed cells or advanced autoclave systems in order to withstand redox reactions at desired temperatures. It is a daunting task to enforce thermal conditions in the MECs (Wildgoose et al. 2004). In system, the MEC reactors and their components (electrodes and membranes) are built to resist the high temperatures and pressures faced. Specifically, the metallic electrodes are susceptible to corrosion and high temperature. High temperatures minimise the solubility of standard electrolyte gases (e.g. CO_2). Higher temperatures (>45 °C) may lead to problems with degradation of electrode materials, membrane surface agglomeration and a resulting loss of resilience (Chandan et al. 2013). For instance, Nafion membrane seems to be degraded at temperatures above 100 °C (Rahimnejad et al. 2015). The literature shows that sulphonated hydrocarbon polymers, composite membranes (e.g. doped polymers with graphene oxide) and solid acid membranes are ideal for thermophilic conditions (Park et al. 2011).

14.11.7 Halophiles

Halophiles are a group of microbes that include a group of oxygenic and anoxygenic phototrophs, anaerobic heterotrophs, fermenting and denitrifying agents, sulphate reducers and methanogens, as well (Crowley 2017; Oren 2002). Mixed cultures and pure halophile cultures have been studied in MFCs, MCDCs and MECs.

14.11.8 Design and Challenges

Fouling and corrosion problems can arise during exposure of the MES components to saline waste streams. Fouling is an inherently difficult issue with the typical elements of electrodes and membrane that are exposed to salt water primarily due to alkalisation, evaporation, sorption and crystallisation (Jiang et al. 2011; Zhuang et al. 2012a; Thomas 2003). Corrosion processes result in skewed electrochemical signal measurements, improved impedance to acceptable faradaic processes and lowered MES performance.

14.11.9 Biohydrogen Production

Hydrogen (H_2) is a desirable alternative fuel for the future, due to its non-polluting and non-exhaustible nature. Global research supports growth in processing biological hydrogen (biohydrogen), in order to mitigate the stresses produced by the emissions of carbon dioxide and the lack of fossil fuel. Biohydrogen will replace current hydrogen-processing technologies that are heavily dependent upon fossil fuels with electricity generation.

The use of non-renewable fossil fuels leads to the emission of greenhouse gases. On the other hand, biohydrogen ($BioH_2$) production is environmentally friendly (non-polluting in nature) and renewable, since it can be extracted from biomass, and no GHGs are generated. H_2 microbial generation would also meet the requirements for a workable prospect for biofuels and provide a renewable alternative to traditional production practises which is environmentally free and energy saving. Several options for the biological production of H_2 are well studied, such as microalgae and cyanobacteria water biophotolysis, use of photo-fermentation photosynthetic bacteria for organic substances and dark fermentation by anaerobic organisms using organic substances. The final solution is typically preferred, i.e. dark fermentation, as the provision of light sources does not depend on this.

The major benefit in the fermentation (dark) process includes (1) ease of fermentor (reactor) unit, (2) known biochemical pathways and biochemical activity, (3) broader substrate range and (4) elevated H_2 production compared to classical methods (Kumar et al. 2015; Saripan and Reungsang 2013).

14.11.10 Hydrogen Production Pathway

The Embden-Meyerhof (EMP) pathway is followed by the formation of BioH_2 through the decarboxylation leading to formation of acetyl-CoA. During this pathway, the protons are reduced to hydrogen using the protein ferredoxin (Verhaart et al. 2010). Routinely, in aerobic conditions, the pyruvate generated at the end of the glycolysis pathway is further reduced to the form lactate by enzyme lactate dehydrogenase. In anaerobic conditions, the enzyme pyruvate oxidoreductase reacts with pyruvate and acetyl-CoA. The product acetic acid is formed depending on the environmental conditions. For formation/generation of biohydrogen, in strict anaerobes two pathways are followed; pathway follows the use of nicotinamide adenine dinucleotide phosphate, glyceraldehyde 3-phosphate dehydrogenase and pyruvate ferredoxin-oxidoreductase (PFOR) (Akhtar and Jones 2008). The reaction for production of the biohydrogen in anaerobic condition was found to be thermodynamically unfavourable (Akhtar and Jones 2008; Hallenbeck and Ghosh 2009). However, among the classes of extremophiles, methanogens were found to be better suited for biohydrogen production. Methanogens have the ability to reduce sulphur and in turn lower the partial pressure for biohydrogen formation. The resultant lower biohydrogen pressure favours the acetate formation. In case of methanogens, it became evident that the partial pressure and high temperature will not affect the hydrogen generation. Using the methanogens and optimum conditions, biohydrogen up to 4 moles can be generated along with side production of 2 moles of acetate.

Hydrogenase is the main BioH_2 synthesis enzyme with two forms depending on the metal content. During the fermentative EMP route, H_2 is generated when pyruvates are transformed into acetyl-CoA.

The thermophilic bacteria can generate elevated H_2 quantity compared to mesophiles (Van Niel et al. 2003; Pradhan et al. 2015). The fermentation process of hyperthermophilic bacteria at high temperatures has several advantages. For example, other microorganisms (Pradhan et al. 2015; Mohan 2010) would be at lower risk for infection and improvement of pathogens (Sahlstrom 2003), due to higher temperature, during the procedure. Furthermore the process will benefit from a higher degree of reaction, lower viscosity and improved mixing. Compared to mesophilic reactions, reaction susceptibility is less selectively at high H_2 partial pressure (Van Niel et al. 2003). In the presence of different feedstocks, bacterial growth in combination with the metabolism of production of H_2 can vary, apart from the benefits above (Pradhan et al. 2016). Thermotogales were seen as the preferred alternative to biofuel production and for industrial applications with a form of hyperthermophilic bacteria because they have a capacity to produce high H_2 yields (1.5–3.85 mol H_2/1 hexoses) from various cellulosic waste materials (Cappelletti et al. 2012). The total potential gain of intensive thermophilic bacteria (4 mol hydrogen/mol glucose) yields 83–100%. Bear in mind that H_2 also depends on many variables, including substrate concentration, immobilised cells, anaerobic conditions, etc. Various factors producing H_2, such as glycerol levels, medium-growth composition, pH and temperature, are studied in a wide range (Lo et al. 2013).

14.11.11 Thermophilic Species

A wide variety of sugar compounds can be used and fermented into acetate, CO_2 and H_2 by Thermotogales roots to intense hyperthermophiles with the potential to expand at a temperature of 80 °C. Thermotoga species are Gram-negative bacteria within Thermotogales, which are mainly fermentative anaerobes with optimum rising temperatures in the 75–80 °C range and found primarily in geothermally heated sediment regions (Huber and Stetter 1992).

Many complex sugars can be reduced to acetate, carbon dioxide and hydrogen belonging to the group of Thermotogales hyperthermophilic microbes which can resist temperature up to 80 °C. Thermotoga species belongs to a group of Gram-negative bacteria. The species belonging to the class of Thermotogales are facultatively fermentative anaerobes with optimum temperature of about 75–80 °C. The habitat for the Thermotoga species is usually the geothermal sediments or hot springs.

14.11.12 Thermotoga Species

Thermotoga species represents the microorganisms which have large classes of hydrolysis enzymes which are suitable for biomass fermentation but majorly lack the exoglucanase enzyme which plays a vital role in the degradation of cellulose (Han et al. 2014). Over the last few years, *Thermotoga maritima* was the sort of strain that researchers have been most interested in (optimum growth at 80 °C). Due to its enormous ability, *T. maritima* has been chosen to produce hydrogen from simple and complex carbohydrates. The genome of the species shows that 7% of the coding sequences were functioning for monosaccharide and polysaccharide metabolism (Nelson et al. 1999; Chhabra et al. 2003; Conners et al. 2005). Other species of *T. maritima* catabolise sugar and polymer results during anaerobic cellular respiration generating hydrogen and carbon dioxide. These species can be predicted as future sources for replacement of non-renewable sources. These can also be reported to utilise cellulose and xylan. This species also represents anaerobic respiration by decreasing Fe (III) metabolites.

Another species in the same community that researchers primarily use for biohydrogen production is *T. neapolitana*. *Thermotoga neapolitana* is also a hyper-thermophilic organism that grows on a large variety of substrates, such as glycerol, glucose, xylose and starch (Pradhan et al. 2016). These microorganisms can display fast kinetic growth (Pradhan et al. 2016), oxygen tolerance (Pradhan et al. 2015) and low contamination risk (Nguyen et al. 2010). It was claimed by D'Ippolito et al. (2010) that anaerobic growth of species is highly influenced by parameters like pH buffering, culture/headspace volume ratio, stirring and N2 sparging for hydrogen production. During their analysis, the maximum hydrogen yield was 3.85 mol H_2/mol glucose and the development rate was 51 ml L^{-1}h.

14.12 Conclusion

The use of extremophiles in MESs has presently commenced and the oxidising potential of the proteins along with the ability to form biofilms will reveal some novel uses for energy generation and remediation of polluted/degraded sites. The bioremediation of waste water, which reflects a combination of many recalcitrant factors, proffers substantial potential for the crucial role of microbes in designing MESs. In order to treat the desired waste water, the identification of extreme microbes will be a crucial research requirement which involves sampling of different extreme conditions and optimising of the concentration of microorganisms used in MESs. However other unresolved issues still remain, including the pollutants used as electron donors, as well as the energy requirement that is available at the same location to both donors and acceptors. In all cases, electron acceptors are needed to provide a sufficient power gain when coupled with those donors.

MESs that have advantages for electricity generation can also be used to treat high-pH waste water. Alkaliphiles are very less reported in MESs and very limited numbers have been studied in pure and mixed cultures. New electricigens are required for better on-site performance in different extreme conditions like high-pH conditions. Additional research on cathodes, anodes, economic considerations and membranes, and especially their selectivity, may also determine the potential large-scale application of MES. Biohydrogen generation utilising the agricultural substrates is however at its initial phase because of the unanswered problems at some stage in the fermentation process.

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