# Biomass, Bioenergy, and Biofuels

## 16

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**Abstract**

The greatest challenge for humanity is the continuous provision of a sustainable source of energy while considering the environmental concerns of global climate change. These factors, along with the rising prices of fossil fuels, require research into various sources for the production of environmentally friendly renewable energy. Biomass has emerged as a key source in the contribution of renewable energy to meet future energy needs in the form of biofuels. It is a potential candidate for the production of electricity, heat, and transport fuels. The proper management of bioenergy will ensure energy security in the future and reduction of environmental pollution and realize the potential of organic waste, for economic and social development. The chapter gives an insight into the potential of biomass and technologies used for its conversion into bioenergy. The newly found use of algae and microbial cells as fuel has also been discussed.

**Keywords**

Biomass · Bioethanol · Biofuels · Bioenergy · Microalgae · Fuel cells

## Abbreviations

| | |
|---|---|
| ABE | Acetone-butanol-ethanol |
| AD | Anaerobic digestion |
| CHP | Combined heat and power |
| DMC | Direct microbial conversion |

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| | |
|---|---|
| EU | European Union |
| MSW | Municipal solid wastes |
| PUFA | Polyunsaturated fatty acid |
| SSF | Solid state fermentation |

## 16.1 Introduction

The use of biomass for various applications from food to feed as well as the generation of biofuels and biorefinery products has been under discussion since the last century (Garba 2020; Kumar and Verma 2021a). The energy crisis faced by the world since the 1970s has led several countries to shift their focus on the use of biomass for generating biofuels. Although various technological advances to reduce fossil fuel prices slowed down the development of biomass-to-biofuel conversion approaches for quite a long time, however, the ever-increasing fuel demand, fluctuating prices, limited supply of fossil-based fuels, and the emission of greenhouse gases and other harmful gases leading to global warming and environmental pollution have remotivated research into the development of biomass-based bioenergy(Lee et al. 2019; Kumar et al. 2020a; Kumar and Verma 2021b).

Biomass consists of solar energy along with $CO_2$ stored in the form of chemical energy (carbohydrate) via the process of photosynthesis. The trapped carbon can be released along with energy generation that makes biomass a potential renewable energy source (Sansaniwal et al. 2017; Chaturvedi and Verma 2013). The energy generated from biomass by using fuels is termed bioenergy. It is a renewable form of energy and can be harvested to meet global energy demand. Biomass can be used directly via combustion for the generation of thermal energy or can be converted through various technological innovations to generate different types of biofuels that can be used for various applications (Kumar et al. 2020b). Since the production of bioenergy from biomass has the advantage of providing sustainable energy while having additional benefits to the environment, the study of biomass potential and various aspects that have influenced its implication along with different technologies for biomass conversion is necessary (Long et al. 2013).

The energy contribution of biomass is already more than 90% in the rural areas of developing nations and is expected to be the leading energy source in the near future with a 10%–15% share globally (Bhavanam and Sastry 2011; Pathak et al. 2013). The chapter describes the types of biomass sources, key factors, technologies, and commonly used industrial biofuels.

## 16.2 Biomass

Biomass being a renewable source of energy has been considered a prospective source to be used as a feedstock for the generation of a sustainable form of energy to meet the present energy demand while providing security to future energy requirements. The application of biomass for energy generation dates back to the traditional use of firewood to generate thermal energy (Lee et al. 2019). For industrial applications, different range of feedstock can be used for biofuel production (Fig. 16.1).

Biofuels produced from edible food crops such as corn, sugarcane, sunflower, etc. are termed the first-generation biofuels, while lignocellulosic biomass such as switchgrass, straw, jatropha, etc. are used for the production of the second generation of biofuels (Naik et al. 2010; Sims et al. 2010). Recently algae are also being explored for the fabrication of the third generation of biofuels (Fig. 16.2) (Chowdhury et al. 2019).

### 16.2.1 Factors Affecting Resource Potentials

The biomass, due to its complex nature, makes it very obscure to estimate its potential as bioenergy with different literature showing zero technical to 1500 EJ of theoretical potential of the biomass for energy generation (Edenhofer et al. 2011). These differences depend on the assumptions of different scenarios such as plant type, yield, available area, and the methodologies used. Though different studies have been conducted for the estimation of the biomass to bioenergy potential, none of these studies include all the factors that could have an impact on the biomass potential. The biomass diversity, availability, and demand, water requirements, type and availability of land areas, superfluousness of food, competition with the other sectors for the resources in use, etc. are some factors that influence the potential of biomass for its use for generating bioenergy (Fig. 16.3) (Dornburg et al. 2010).

The type of crop, agricultural administration, history of a previous application for land under consideration, etc. directly affect the diversity of crops, which also affects

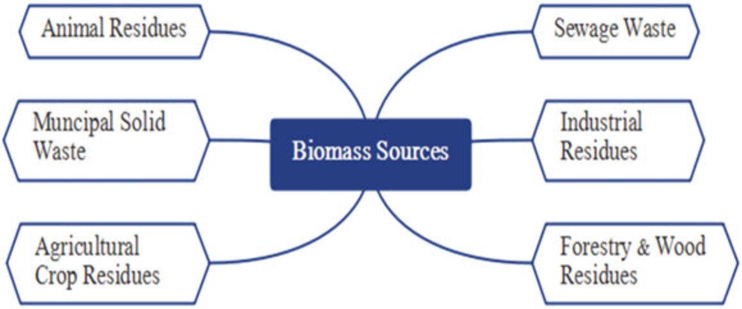

**Fig. 16.1** The potential sources of biomass available for energy generation

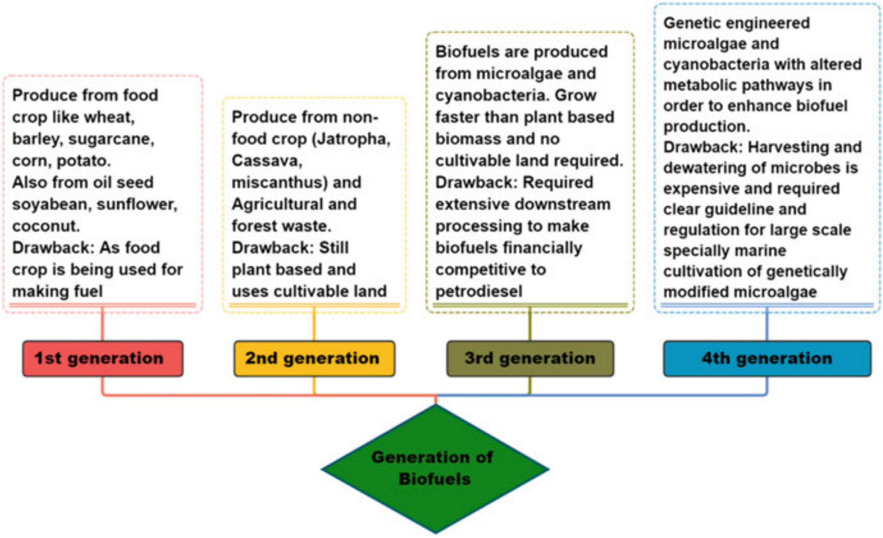

**Fig. 16.2** Classification of biofuels based on biomass source

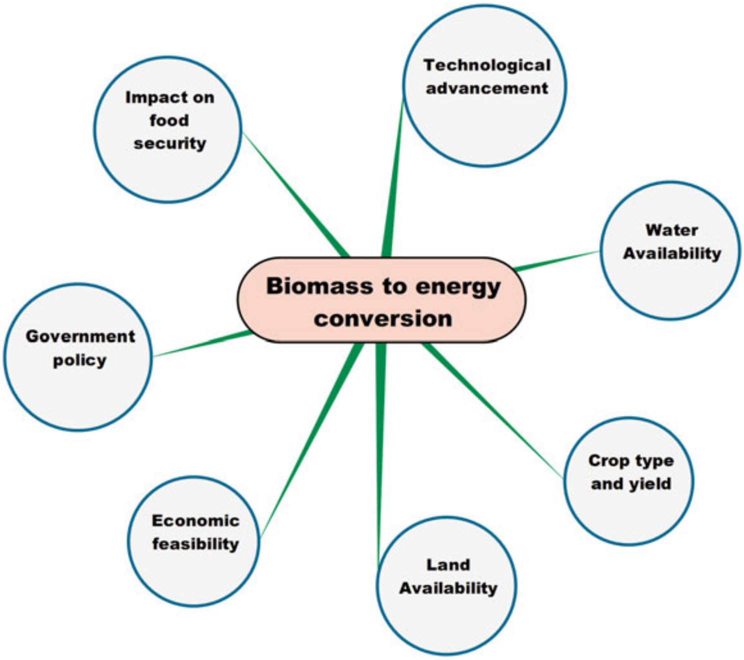

**Fig. 16.3** Different factors that have an influence on biomass potential as a bioenergy source

the biomass potential. Furthermore, lower biomass demand for power generation due to its competition with other forms of renewable energy is another important factor. The demand for biomass is also dependent on the development of technologies to generate these alternate energy sources. Although various bio-based chemicals and biomaterials such as wood and fiber may have increasing biomass demand, they are not considered for biomass potential. To add to this, increasing food demand and the rising price of agricultural commodities have created a huge competition between the growing agricultural market and the use of bioenergy (Dornburg et al. 2010). It is estimated that if laws are enforced for the blending of fossil fuels and biofuels for transportation purposes, the price of the biofuel will upsurge by 10% using the first-generation biofuel crops (Banse et al. 2011). The use of cereals, sugarcane, oilseeds, etc. in biofuel production collides with food supply, while the use of pasture and grasses builds competition for limited resources such as land and water. The growing population shows a trend of increasing water demand for domestic and agricultural practices from 60 to 220% by 2050. Changing pattern of rainfall and increased rate of evapotranspiration due to climatic change all add up the scarcity of water for its use on bioenergy crop production. However, proper planning for the efficient use of water can provide favorable opportunities for biomass production for bioenergy production. Finally, an important factor to consider for biomass potential is its yield. The development of an efficient agricultural system with the application of modern technologies other than crop type can have a positive impact on biomass yield, yet successful implementation of these technologies in developing countries is an overwhelming task. However, the data regarding biomass potential are based on the use of perennial crops that usually have yields higher than the annual crops. Therefore, there are doubts about the reliability of these data while using annual biomass crops (Dornburg et al. 2010).

### 16.2.2 Feedstock Conversion Technologies

For the utilization of biomass potential, numerous conversion technologies are employed for generating energy in different forms. The type of conversion technology depends on biomass type, its characteristics, and quantity available, project specificity, end-use requirements or energy form required, economic and environmental policies, etc. with the most decision based on the form of energy required and pathway for its generation (McKendry 2002).

There are three main types of conversion technologies used for the generation of bioenergy from biomass to bioenergy: biochemical, thermochemical, and physiochemical with each type further divided into various processes for generating different energy types (Adams et al. 2018). Anaerobic digestion (AD) and fermentation are grouped under the biochemical technology of feedstock conversion, where AD is carried out by microorganisms for the conversion of organic waste to produce gaseous biofuel, i.e., biogas, whereas fermentation requires pretreatment and saccharification for the release of simple sugar molecules which are then fermented to produce liquid biofuel (ethanol). AD is more economical than fermentation as the alcohol produced at the end of fermentation is diluted and thus requires an additional step of distillation (Deublein and Steinhauser 2011). Photobiological reactions are also being explored for the generation of biogas (biohydrogen) via conversion of biomass using the phototrophic organisms (Lee et al. 2019). Organic compounds are decomposed using high-temperature treatment to bring about their chemical conversion into biochar or to produce a liquid or gaseous biofuel in the thermochemical technology (Goyal et al. 2008). Combustion, pyrolysis, liquefication, and gasification are the options available under this technology. Combustion is the most common process used for the generation of heat and electricity (Kataki et al. 2015). In all thermochemical processes, pyrolysis is the initial stage as it brings about a chemical reaction in the absence of oxygen to produce all three forms of energy, i.e., solid, liquid, and gas (Patel et al. 2016). Physiochemical technology is used for the extraction of oil from the seed of different biomass such as linseed, which is also known as mechanical extraction. The oil is further esterified to generate biodiesel, which is used as a transportation fuel (Adams et al. 2018).

Faster reaction time, the ability to bring about the decomposition of even the recalcitrant biomass, and higher efficiency of the thermochemical process compared to other technology make them a popular option as feedstock conversion technology. Moreover, the energy released can be released in any form for various applications (Adams et al. 2018). Moreover, the readily available infrastructure, low water requirement, and the ability to use plastic waste for energy generation make thermal technology a widely used method (Uzoejinwa et al. 2018).

## 16.3 Bioenergy Production

The biomass can be used for the production of different biofuels, i.e., solid, liquid, as well as gaseous biofuels. Gaseous biofuel is biogas, whereas solid biofuels are sawdust, briquettes, straw, etc., while bioethanol, biodiesel, and biomethanol are liquid biofuels. Of these, liquid biofuels have higher demand as transportation fuels with biodiesel and bioethanol being the only biofuel used in the European nations (76 and 20%) (Brodziński et al. 2014).

### 16.3.1 Bioethanol

Ethanol generated using the renewable plant and microbial biomass as its substrate is termed bioethanol or good ethanol. It is an environmentally friendly and renewable source of energy (Johnston 2008). The production and usage of bioethanol reduces the energy dependency on fossil-based fuels and reduced the emission of greenhouse gases. Bioethanol is the most extensively used among liquid biofuels and is produced via the fermentation of sugar molecules from different sources. Different crops and agro-wastes can be used as feedstock for bioethanol production, such as sugarcane and corn, rice straw, switchgrass, pulpwood, as well as food waste and municipal solid wastes (MSW) (Demirbas and Demirbas 2010).

The use of cellulosic material for ethanol production requires pretreatment steps followed by saccharification, fermentation, and finally distillation for ethanol separation (Nigam and Singh 2011). Pretreatment is a necessary step for making cellulose molecules trapped in the hemicellulose and lignin components of the biomass accessible. Further saccharification of the cellulose and hemicellulose polysaccharides will convert them into simple fermentable sugars (Demirbas and Demirbas 2010).

Different technological advancement in ethanol production has been investigated to improve each step with *S. cerevisiae* as the most common organism for the fermentation step. Further technologies with combined saccharification and fermentation steps are being developed for ethanol production using lignocellulose. This SSF technology has the advantage of a high hydrolysis rate resulting in a higher yield at a shorter duration. Similarly, direct microbial conversion technology, i.e., DMC, involves single-step production, hydrolysis, and fermentation of cellulose that makes the overall process economical (Vasić et al. 2021).

Bioethanol finds its application in different industries such as cosmetic and alcoholic beverage production. However, the main studied application of bioethanol in the current scenario is bioenergy production. Bioethanol is mostly used as a transportation fuel with cars run entirely on pure ethanol or “gasohol,” i.e., a blend of gasoline with ethanol (Bielski et al. 2015). The ethanol used in blending needs to be anhydrous with no engine modification required for its use as a blend. It enhances the octane rating of the fuel and at the same time reduces the amount of pollutants generated in unleaded gasoline (Demirbas and Demirbas 2010). The oxygen content of bioethanol is approximately 35%, while a low level of nitrous oxide is released in the environment. In the European nations, 5% blend of ethanol can be used with petrol without engine modification according to the EU quality standard EN 228 along with the vehicle warranty, while engine modification is required for using higher bioethanol percentage (Brodziński et al. 2014). Other countries such as the USA, China, Brazil, and Canada are also engaged in bioethanol production and use, with the highest production being shown by the USA which contributes more than 50% to the total global ethanol production followed by Brazil with 27% (Zabed et al. 2017; Vasić et al. 2021).

Though new technologies have greatly increased ethanol production, there are opportunities to increase the efficiency of the pretreatment processes of the cheap lignocellulosic biomass substrate, optimization of different components required for increased production, and enhancing tolerance and stability of the organisms used to make the overall process economical for wide-scale commercial applications (Nigam and Singh 2011).

### 16.3.2 Biobutanol

Butanol is a four-carbon alcohol that belongs to the same category as ethanol and methanol which are used as biofuel. Four isomers of butanol are present, namely, *n*-butanol, 2-butanol, iso-butanol, and tert-butanol, based on the orientation of the carbon atoms which either form a straight chain or branched structure and the corresponding position of –OH (Zheng et al. 2015). Though these isomers have different properties, the energy generation and application in gasoline blending and combustion are identical. However, the production method of these isomers varies (Jin et al. 2011; Ramey 2004). *n*-Butanol is the commonly generated end product of the fermentation process with the fermentation technology being referred to as acetone-butanol-ethanol (ABE) fermentation as these three compounds are generated as the end product in a 3:6:1 ratio. The petrochemical-based product, tert-butanol, cannot be synthesized using biological methods, while 2-butanol production involves a two-step process, the first step being the bacterial fermentation of sugar to obtain an intermediate product, followed by chemical modification of the intermediate to 2-butanol directly in the fermentation medium. Iso-butanol is produced in a small amount by the yeast during the process of winemaking; however, large-scale production of iso-butanol is slowly being considered with different companies showing an increased interest in its production considering the issues with the production of *n*-butanol. Though commercial production of *n*-butanol goes back a long way, the complexity of the production process, toxic nature, and difficulty in recovery and purification make the overall process costly (Nigam and Singh 2011).

The fluctuation in the price of crude oil and achievement in bioethanol production has led to increased interest in the use of alcohol as a biofuel with the development in biobutanol production also being considered (Kumar et al. 2012). The same feedstock used for bioethanol generation can also be applied for butanol production, providing opportunities for the farmers as well as expanding the biofuel market since it is used alone or in synergy with ethanol for the preparation of gasoline blend. The higher hydrogen and carbon content of butanol makes blending with gasoline easier and has higher energy compared to ethanol. Butanol with a value of 85% can be used in gasoline blend to be used as fuel without modification in the car engines. The lower evaporation rate compared to gasoline and ethanol and emission of less volatile organic compounds make it an easy and safe alternative as biofuel. The lower corrosive nature makes transport and distribution of butanol easier using the system (Nigam and Singh 2011). Butanol also finds its application as a solvent and industrial cleaners other than acting as a fuel additive (Jin et al. 2011). However, the success of butanol production depends on technological advancement in the ABE fermentation technology. High pretreatment and recovery cost, the high toxicity of butanol to the fermenting microorganisms and end-product inhibition, and development of efficient strain for the fermentation process are the challenges to be addressed in this regard (Veza et al. 2021).

### 16.3.3 Biohydrogen

Hydrogen is another source of renewable energy with the potential for use as an alternative to conventional energy sources. It has numerous applications from use in fuel cells for generating electricity, fertilizer, and methanol production; in oil refineries for removal of impurities; as a reducing agent, hydrogenating agent, or rocket engine fuel; and in cryogenics, pharmaceuticals, etc. Also, it is carbon-neutral energy with an energy yield 2.75 times higher than fossil fuels (Singh and Mahapatra 2019).

Though there are different methods for hydrogen production such as the electrolysis of water, fossil fuels, and natural gas, the commonly used method of hydrocarbon reformation generates carbon monoxide gas as a byproduct, which has environmental consequences. The thermochemical technologies used for biomass conversion to hydrogen also produce different toxic substances; thus the biological process of biomass conversion to biohydrogen is gaining attention (Mona et al. 2020). Biohydrogen production using photosynthetic organisms with zero pollution is one of the most efficient approaches with sunlight, water, and minimal nutrient requirements, and further technological advancement can provide a platform for commercial production of biohydrogen as renewable energy sources (Rodionova et al. 2017). The different species of bacteria, cyanobacteria, green algae, and plants produce biohydrogen along with oxygen via photosynthesis (Stevens 2001). Dark fermentation is another biological process where anaerobic fermentative bacteria are used for the conversion of sugar substrate to release hydrogen along with organic acid byproducts (Sen et al. 2008). This method of hydrogen production though has a higher yield and low concentration of hydrogen; however, it makes it uneconomical as a purification needs to be added for its use in fuel cells. Furthermore, the hydrogen yield depends on the fermentation pathway with 4 mol or 2 mol of $H_2$/mol of glucose being generated depending on whether acetate or butyrate is produced along with it (Wang et al. 2003). Photo-fermentation is another biological process in which photosynthetic organisms such as *Rhodopseudomonas palustris*, *R. capsulata*, *R. sphaeroides*, and *Rhodospirillum rubrum* use the energy from light for the anaerobic conversion of organic molecules to release hydrogen along with carbon dioxide (Basak and Das 2007).

Though biohydrogen can be used as a valuable and renewable source of energy, for economic viability and eco-friendly production, the use of cheap biomass for fermentation by the microorganism is preferred. Thus, studies focused on technological advancement in this area can enable the industrial production of biohydrogen using biomass and microorganisms (Saratale et al. 2019). The problems of storage and transportation are some other challenges that need to be exploited to make its commercial application feasible (Srivastava et al. 2020).

### 16.3.4 Biogas

Biogas is a gaseous alternate biofuel that is produced via the process of AD by microorganisms using different types of organic matter. The composition of biogas mainly consists of two gases, i.e., methane ($CH_4$) and $CO_2$ at 60% and 40%, respectively, with the presence of traces of other substances such as $H_2S$, siloxanes, $NH_3$, and water (Chaemchuen et al. 2016; IEA Bioenergy Task 37 2018). Various organic wastes such as agricultural residues, food scraps, municipal solid waste, and industrial waste rich in organic compounds, etc. can be used for biogas production (Hanifzadeh et al. 2017). The use of these organic compounds helps to meet environmental guidelines while managing waste and providing cheaper or in some cases negative cost of substrate for generating renewable energy. The energy crops can also be used for generating energy with reduced emission of greenhouse gases (Zhu et al. 2019). Biogas can be produced in sewage and wastewater treatment plants, agricultural waste digestion plants, landfills, etc. through the action of mesophilic and thermophilic microorganisms (Chaemchuen et al. 2016). Furthermore, the residue generation at the end of AD can be used as fertilizer, thus making biogas a promising alternative biofuel generated using biomass (Palop et al. 2010).

Biogas can be used for the sole purpose of generation of heat as well as in combined heat and power (CHP) plants, where it can be used for generating electricity along with heat. The high content of $CO_2$ is an issue in the industrial utilization of biogas (Palop et al. 2010). The separation of $CO_2$ from biogas is essential to improve its calorific value. In addition, when biogas is used as a fuel, the purification of biogas is crucial as the contaminants present in the biogas can cause damage to the device and cause emission of undesirable compounds (Chaemchuen et al. 2016). The removal of $CO_2$ and other substances via purification generates an upgraded version of biogas known as biomethane, which has properties similar to fossil fuels and can be used directly with the existing transportation and distribution facilities (Zhu et al. 2019). Biomethane has received significantly increasing attention in the recent past in the context of renewable energy, with the number of biomethane plants rising from 187 to 465 within a span of 4 years (2011–2015) in Europe alone with a market significance of 90% globally (Cucchiella et al. 2017).

The development of technologies for the purification and cleaning of biogas is the key bottleneck for the exploitation of biogas to its maximum potential. Since the composition of contaminants depends upon the substrate source, with the contamination of $H_2S$ being of major environmental concern, the separation technologies need to be established accordingly. The materials used in the purification steps should have high stability and nonreactivity to these contaminants. Therefore, biotechnological advancement in the evolution of suitable technologies with economic feasibility, maximum efficiency, and low energy are future expectations for large-scale commercial application of biogas as biofuel (Chaemchuen et al. 2016).

## 16.4 Algal Biomass as Fuel Cells

While the use of various food crops as feedstock for biofuel generation has all the environmental benefits of reduced carbon-dioxide emission and continuous supply of energy for the growing population, their use, however, can pose a major challenge in meeting the food demand and ensuring food security globally. Further limited land and water resources, exploitation of agricultural land for maximum production of these feedstock crops via the use of chemicals and fertilizers, soil erosion, loss of crop biodiversity, etc. can have a negative impact on soil health. A further limitation

**Table 16.1** Application of algal biomass for the production of different biofuels

| Biofuel | Technique | Algal species | Reference |
|---|---|---|---|
| Bioethanol | Fermentation | *Tribonema* sp., *Chlorella*, *Dunaliella*, *Chlamydomonas*, *Scenedesmus* | Wang et al. (2014), Özçimen et al. (2015) |
| Biodiesel | Transesterification | *Oedogonium*, *Spirogyra*, *Scenedesmus* sp. | Hossain et al. (2008), Chen et al. (2012) |
| Biogas | Anaerobic digestion | *Botryococcus braunii*, *Nannochloropsis oculate*, *Macrocystis pyrifera*, *Euglena gracilis* | Vergara-Fernández et al. (2008), Mussgnug et al. (2010), Frigon et al. (2013), Buxy (2014), Ciudad et al. (2014) |
| Biohydrogen | Biophotolysis | *Chlamydomonas reinhardtii*, *Synechococcus elongatus*, *Anabaena variabilis* | Happe et al. (1994), Markov et al. (1997), Mathews and Wang (2009) |
| | Fermentation | *Ulva* sp., *Chlorella vulgaris*, *Dunaliella tertiolecta*, *Chlorococcum littorale* | Ueno et al. (1998), Carver et al. (2011), Margareta et al. (2020) |
| Bio-oil | Pyrolysis | *Chlorella protothecoides*, *Nannochloropsis* sp., *Microcystis aeruginosa* | Miao et al. (2004), Miao and Wu (2004), Pan et al. (2010) |
| | Hydrothermal liquefaction | *Chlorella pyrenoidosa*, *Scenedesmus*, *Spirulina* | Yu et al. (2011), Vardon et al. (2011, 2012) |

of current technological advancement leading to the high cost of pretreatment methods and low yields makes the use of the lignocellulose uneconomical (John et al. 2011). To address these issues, the third generation of biofuels based on algal biomass is being introduced, which can serve as the best alternative for addressing the urgent demand for biofuels without compromising food security or the limitation of agricultural land (Subhadra and Edwards 2010) (Table 16.1).

Microalgal biofuels show characteristics similar to fossil fuels and have gained considerable attention in the past decade. Different species of algal are being explored for their capability to produce biofuels, especially bioethanol with increased investment in the research and development in this area by the different fuels-based companies (Kiran et al. 2014). The higher content of oil in their biomass makes the microalgae a potential substitute for crude oils (Mehariya et al. 2021). Other advantages of the algae over other feedstock crops include higher $CO_2$ sequestration (1.83 kg $CO_2$/kg of algal biomass); bioremediation of industrial, agricultural, and municipal wastewater via removal of chemicals; and heavy metals $NH_4^+$, $NO_3$, $PO_4^{3-}$, etc. The algae can be cultivated throughout the year and have the ability to thrive and grow under a low nutritional environment with no meddling with food security (Goswami et al. 2021). Their water requirements are lower than the other feedstock crops, and they can be cultivated in nonarable land with no requirement of fertilizer or pesticides. The fast growth rate and accumulation of different neutral lipids for a higher yield of biodiesel are favorable for algal biomass-based biofuels (Goswami et al. 2021). Other important compounds such as PUFA, pigments, dyes, proteins, etc. can also be obtained from algal biomass (Kiran et al. 2014).

The algal biomass can be used for biofuel production via the conversion of algal metabolite into simple sugars. The algae assimilate a large amount of starch in their cell or, in some cases, other carbohydrates such as cellulose or laminarin, which can then be harvested and converted into simple sugars to be used for fermentation using efficient ethanol-producing organisms, similar to most biofuel generation technologies (Bhardwaj et al. 2020a; Agrawal et al. 2020). In the dark, the algae generate energy through the breakdown of stored carbohydrates, i.e., starch and glycogen via oxidative reaction. If the anaerobic condition is maintained during this time, the incomplete degradation of starch can lead to the generation of ethanol, $H_2$ gas, organic acids, etc. in varying proportions depending on the algal species. This process can be modified for these algae to operate in the form of a mini-factory for ethanol production via dark fermentation. Further attempts at the development of microalgae genetically engineered for direct ethanol production were also done (John et al. 2011; Chaturvedi et al. 2021).

## 16.5 Improving the Capabilities of Microbial Strains for Bioenergy Production

Improved conversion of plant biomass into various bioenergy sources could be achieved by implementing genetic engineering to construct relevant microbial strains with enhanced lignocellulosic degradation capabilities (Singhvi et al. 2014). Various genetic engineering methods can help to improve biochemical reaction rates to achieve maximum end-product production. Various methods such as heterologous expression of genes encoding plant biomass hydrolytic enzymes, expression of transporter proteins and carbon uptake pathways, expression of $CO_2$ fixation pathways, etc. in relevant microorganisms could be used to achieve improved bioenergy production. Some important approaches are discussed.

### 16.5.1 Consolidated Bioprocessing (CBP)

An innovative approach to effective bioenergy production is consolidated bioprocessing (CBP). In this approach, all three steps of biofuel production, enzyme production, and saccharification and fermentation are combined in the same reactor, where the pretreated plant biomass is efficiently converted into the desired product by the microbial consortium without the addition of saccharified enzymes (Kumar and Verma 2020a, b).

There are two strategies for CBP: native strategy and recombinant strategy. Native strategies include naturally hydrolytic enzymes producing microbial strains and improving biofuel production by using different approaches such as isolating new strains for CBP and using different substrates (Salehi Jouzani and Taherzadeh 2015). Microbial candidates suitable for this strategy include hydrolytic enzyme-secreting bacteria and fungi and cellulosome-forming bacteria. Liu et al. investigated the ability of the native cellulolytic bacterium, *Clostridium thermocellum* DSM 1237, to produce bioethanol from lignocellulosic biomass. The industrial applicability of this bacterium was concluded by high rates of cellulose degradation and the ability to survive and grow at higher temperatures of 50–60 °C, both properties that are advantageous for industrial processes (Lamed and Bayer 1988). In this study, the strain *C. thermocellum* DSM 1237 was cultured in 3-L fermenter and anaerobic flasks, and the growth of the strain was evaluated under different cellular growth conditions such as different temperatures, different carbon sources (glucose, cellobiose, and xylose), and substrates and its ability to produce ethanol. The different fermentation substrates used for the evaluation included rice straw, corn straw, sugarcane bagasse (SCB), peroxide fortified alkali-treated SCB, and SCB treated with alkali. With 0.5% (weight/volume) cellobiose and an optimum temperature of 60 °C, the strain produced ethanol with a yield of 0.60 g/L at 0.80 g/g cell biomass. Utilization of alkali-treated sugar bagasse showed an increased yield of ethanol to about 0.68 g/L. The addition of enzymes such as xylanases and cellulases in the 3-L fermenter showed further improved yield of ethanol, i.e., 0.86 g/L, which is 83.3% of the theoretical yield. Thus, the integrated one-step hydrolysis and fermentation process and the on-site addition of lignocellulolytic enzymes, i.e., CBP, proved to be an effective approach to increase bioethanol production (Liu et al. 2020; Bhardwaj et al. 2020b). Second, the recombinant strategy involves engineering the ability to secrete hydrolytic enzymes in non-cellulolytic microorganisms and implementing these modified strains for enhanced production of bioenergy such as biofuels. Some commonly used microbial hosts for the recombinant strategy are bacteria such as *Escherichia coli*, *Lactobacillus lactis*, *Zymomonas mobilis*, *Bacillus subtilis*, etc. and yeasts such as *Saccharomyces cerevisiae*. *E. coli* FBR strains were constructed using ldh (lactate dehydrogenase) and pfl (pyruvate formate lyase) strains. The strains were transformed with pLOI297 plasmids containing the pet operon system. These recombinant strains of *E. coli* were able to produce ethanol from various substrates such as xylose, arabinose, and glucose. Strain FBR5 showed maximum ethanol production with 0.46–0.51 g/L yield (Dien et al. 2000). Xylose fermentation ability was introduced into *Z. mobilis* by insertion of four *E. coli*-derived xylose fermentation genes: *xylA* (encodes xylose isomerization), *xylB* (encodes xylulose kinase), *tktA* (encodes transketolase), and *tktB* (encodes transaldolase). The genes were introduced into the bacterium under the influence of two strong constitutive promoters: Glyceraldehyde 3-phosphate dehydrogenase and enolase promoters. The transformed strain thus produced, *Z. mobilis* CP4 (pZB5), showed efficient conversion of xylose to produce a high yield of ethanol of about 86% (Parker et al. 1995). Another important approach to increase bioenergy production under CBP is the overexpression of hydrolytic enzymes. Such overexpression of lignocellulolytic enzymes can be achieved by various techniques, such as increasing the copy number of genes, engineering the specific promoters, protease enzyme deficiency, etc. Yamada et al. showed increased production of ethanol from *S. cerevisiae* by the technique of increasing the copy number of genes. When grown on substrates such as pretreated rice straw and cellulose treated with phosphoric acid, the transformed yeast showed ethanol production with yields of 7.7 g/L and 7.6 g/L, respectively (Yamada et al. 2010).

In addition to the advantages of the CBP approach, both strategies have their limitations. The native biomass-degrading microorganisms are usually wild type and are poorly characterized. Such organisms isolated with the desired CBP potential perform well below satisfactory levels of bioenergy production. In the recombinant strategy, setbacks include the undesirable effects arising from the co-expression of multiple genes, improper protein folding, variations in the corresponding expression levels of the different genes, inadequate fermentation, etc. These limitations could be efficiently addressed to make CBP an efficient approach for future biotechnological fields.

### 16.5.2 Clustered Regularly Interspaced Short Palindromic Repeat (CRISPR)/CRISPR-Associated Proteins (Cas9)

Recent advances in the fields of genetic engineering have provided a plethora of techniques and tools to modify the physiological behavior of a relevant microorganism in the desired form and consolidate its applicability in the industrial fields. In the genetic engineering approach, the desired gene can be inserted, deleted, or regulated at a specific site of the chromosome of the host microorganism. There are two major types of genetic engineering: MEM engineering and REM engineering. MEM genetic engineering includes three methods zinc finger nucleases (ZFNs) (Miller et al. 2007), transcription activator-like effector nucleases (TALENs) (Joung and Sander 2013), and meganuclease system (Silva et al. 2011). REM genetic engineering includes CRISPR/Cas9 technology.

CRISPR/Cas9 technology was originally derived from the adaptive immune systems in bacteria and archaea. In the bacterial genome, this system contributes 40% of the endogenous adaptive immune defense and approximately 70% in members of archaea (Burstein et al. 2016). CRISPR/Cas9 provides defense in three stages: adaptation, expression, and interference against invading exogenous DNA. The invading DNA is fragmented into multiple fragments by Cas genes to produce protospacers. Such protospacer fragments are introduced into the CRISPR locus tandem array. In the second phase of defense, expression, the locus with the integrated spacer is transcribed to produce long precursor CRISPR RNA (pre-crRNA), which forms a complex with transactivating CRISPR RNA (tracrRNA) and Cas9 protein. The RNA hybrid is recognized by an RNA exonuclease enzyme, which converts the complex into a mature form. The mature form of the RNA hybrid combines with the Cas9 protein, which cuts the DNA to create double-strand breaks at the desired locations. Replacing the dual RNA hybrid with a specifically designed guide RNA, called sgRNA, forms the basis of CRISPR/Cas9-mediated gene manipulation to incorporate desired traits into host cells. The sgRNA attachment requires a protospacer adjacent motif (PAM) present immediately downstream of the target DNA site. Generated DSBs are repaired via two repair pathways: homologous repair (HR) pathway and the nonhomologous end-joining repair (NHEJ) pathway. NHEJ can lead to gene insertion and deletion, while the HR pathway uses an exogenous DNA donor to recombine at the desired sites. Gene knockout and gene knocking introduced via DNA repair can be used for the addition or elimination of desirable traits within microorganisms.

CRISPR/Cas9 gene-editing technology has been applied to a variety of industrial microorganisms, including bacteria, yeasts, and fungi, to enhance their capabilities in the production of bioenergy, such as biofuels. This approach of genetic manipulation has been studied to install a number of desirable traits in the producing strains, such as:

1. Building increased tolerance to biofuels: different microorganisms exhibit differences in their biofuel tolerance. Several functional, genomic, and transcript profiling analyses of *S. cerevisiae* revealed the presence of genes controlling tolerance to ethanol (Lewis et al. 2010), suggesting an increase or decrease in ethanol tolerance following site-specific mutations at such genes. In *Z. mobilis*, ethanol tolerance was increased by a frameshift mutation in the gene encoding the enzyme NADH dehydrogenase (Ulaganathan et al. 2017).
2. Increased tolerance to various inhibitors: tolerance to inhibitors is important to prevent low yields due to the production of toxic compounds during pretreatment processes of lignocellulosic biomass. Ramos et al. developed increased tolerance to acetate in *S. cerevisiae*. The removal of a single amino acid from four different genes, GLS4, ADH3, SKS1, and ASG1, which were present at different sites, showed increased tolerance of the yeast cell to acetic acid (González-Ramos et al. 2016).
3. Enhancing the tolerance to temperatures: one amino acid alteration in NADH dehydrogenase enzyme and pyruvate kinase showed enhanced ethanol production and more thermotolerance in the bacterium *Z. mobilis* (Benjaphokee et al. 2012).

However, although equipped with several advantages, the recent technology of CRISPR/Cas9 provides certain limitations which include off-target effects, low efficiency of gene manipulation by HR, absolute dependence on the PAM site, as well as the challenges of the generation and the delivery of sgRNA. Such limitations need to be addressed in a more efficient way to make this technology applicable on industrial grounds (Table 16.2).

## 16.6 Conclusion and Prospects

Biomass and bioenergy have become a major global issue, with a remarkable increase in research and development and an expanding market for bioenergy. Biomass is a continuously available renewable energy resource, and its full potential for use as a bioenergy source has yet to be explored. It has the potential to contribute

**Table 16.2** Strategies for improving the ability of microbial strains for bioenergy production

| Consolidated bioprocessing (CBP) | | | | | |
|---|---|---|---|---|---|
| **Native strategies** | **Microorganisms** | **Method** | **The objective of the method** | **Yield** | **References** |
| | *Thermoanaerobacterium* sp. | Deletion of *ldhA* gene encoding lactate dehydrogenase enzyme | Improvement in hydrogen production | Hydrogen production increased by twofold | Shaw et al. (2008) |
| | *Clostridium saccharoperbutylaceticum* strain $N_{1–4}$ | Hydrogenase gene (*hup*CBA) cluster downregulation using antisense RNA technology | Improvement in hydrogen production | Improvement in hydrogen evolving activity by 3.1-fold | Nakayama et al. (2008) |
| | *Clostridium thermocellum* CT2 | Anaerobic single-step conversion of banana crop waste by co-culture fermentation of CT2 with *C. thermosaccharolyticum* and *Thermoanaerobacter ethanolicus* ATCC 31937 strains | Enhanced bioethanol production | A maximum ethanol yield of 22 g/L obtained on the substrate of 100 g/L concentration | Harish et al. (2010) |
| | *Clostridium acetobutylicum* | Inactivation of *hbd* gene essential in butyrate synthesis | Improvement in ethanol production | High ethanol yield of 0.38 g/g of glucose with a productivity of 0.5 g/L/h | Lehmann and Lütke-Eversloh (2011) |
| **Recombinant strategies** | *Clostridium cellulolyticum* | Heterologous expression of two genes: *Pdc* and *Adh* encoding pyruvate decarboxylase and alcohol dehydrogenase enzymes, respectively, and isolated from *Zymomonas mobilis* | Improvement in ethanol production | Improvement in ethanol production by 53% | Guedon et al. (2002) |
| | *Escherichia coli* | Heterologous expression of different genes: *thl*, *bcd*, *crt*, *etfA*, *etfB*, and *adhE* involved in butanol pathway and isolated from *C. acetobutylicum* | Production of biobutanol | Highest butanol titer by BUT2 strains reported was 1184 mg/L | Inui et al. (2008) |
| | *Saccharomyces cerevisiae* | | | | |

| | | | | | |
|---|---|---|---|---|---|
| | | Co-expression of endoglucanase enzyme from *Trichoderma reesei* and β-glucosidase from *Saccharomycopsis fibuligera* | Enhanced production of ethanol | Higher yield of ethanol with 1 g/L | Den Haan et al. (2007) |
| **CRISPR/Cas9** | | | | | |
| | *Myceliophthora thermophila* | Multiplex genome editing using CRISPR/Cas9 method. Efficient mutation of the imported gene amdS in the genome of the fungi | Production of strain with hyper-cellulase activity | Increase in lignocellulosic activities by 5- to 13-fold | Qian Liu et al. (2017) |
| | *Chlamydomonas* sp. | CRISPR/Cas9-mediated knockout of gene involved in lipid degradation | Improvement in lipid accumulation | 6% increase of lipid accumulation in the mutant cell | Nguyen et al. (2020) |
| | *Escherichia coli* ASA02 | CRISPR/Cas9-mediated integration of butanol synthetic cassettes after the elimination of gene involved in ethanol production | Production of *n*-butanol | Butanol production by 4.32 g/L | Abdelaal et al. (2019) |

greatly to the global energy supply in the coming years. Although its commercial realization depends on its success in competing with fossil fuels and on environmental and agricultural policies, its share in the global energy market is steadily increasing. Various factors and available technologies for biofuel production are the most important considerations in selecting from the wide range of available biomass sources. The study of all these factors and technological advancements will further improve the biomass and bioenergy market.

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## References

Abdelaal AS, Jawed K, Yazdani SS (2019) CRISPR/Cas9-mediated engineering of *Escherichia coli* for n-butanol production from xylose in defined medium. J Ind Microbiol Biotechnol 46(7):965–975

Adams P, Bridgwater T, Lea-Langton A, Ross A, Watson I (2018) Biomass conversion technologies. In: Greenhouse gas balances of bioenergy systems. Elsevier, pp 107–139

Agrawal K, Bhatt A, Bhardwaj N, Kumar B, Verma P (2020) Algal biomass: potential renewable feedstock for biofuels production–part I. In: Biofuel production technologies: critical analysis for sustainability. Springer, Singapore, pp 203–237

Banse M, Van Meijl H, Tabeau A, Woltjer G, Hellmann F, Verburg PH (2011) Impact of EU biofuel policies on world agricultural production and land use. Biomass Bioenergy 35(6):2385–2390

Basak N, Das D (2007) The prospect of purple non-sulfur (PNS) photosynthetic bacteria for hydrogen production: the present state of the art. World J Microbiol Biotechnol 23(1):31–42

Benjaphokee S, Koedrith P, Auesukaree C, Asvarak T, Sugiyama M, Kaneko Y, Boonchird C, Harashima S (2012) CDC19 encoding pyruvate kinase is important for high-temperature tolerance in *Saccharomyces cerevisiae*. New Biotechnol 29(2):166–176

Bhardwaj N, Agrawal K, Verma P (2020a) Algal biofuels: an economic and effective alternative of fossil fuels. In: Microbial strategies for techno-economic biofuel production. Springer, pp 59–83

Bhardwaj N, Kumar B, Agrawal K, Verma P (2020b) Bioconversion of rice straw by synergistic effect of in-house produced ligno-hemicellulolytic enzymes for enhanced bioethanol production. Bioresour Technol Rep 10:100352

Bhavanam A, Sastry RC (2011) Biomass gasification processes in downdraft fixed bed reactors: a review. Int J Chem Eng Appl 2(6):425

Bielski S, Dubis B, Jankowski K (2015) The energy efficiency of production and conversion of winter triticale biomass into biofuels. Przem Chem 94(10):1798–1801

Brodziński Z, Kryszk H, Kurowska K (2014) Market of producers and processors of agricultural biomass for energy purposes. Pol J Environ Stud 23(2):619–627

Burstein D, Sun CL, Brown CT, Sharon I, Anantharaman K, Probst AJ, Thomas BC, Banfield JF (2016) Major bacterial lineages are essentially devoid of CRISPR-Cas viral defence systems. Nat Commun 7(1):1–8

Buxy S (2014) Techniques to enhance methane production from terrestrial (sugarbeet) and algal (*Nannochloropsis oculata*) biomass. University of Florida

Carver SM, Hulatt CJ, Thomas DN, Tuovinen O (2011) Thermophilic, anaerobic co-digestion of microalgal biomass and cellulose for $H_2$ production. Biodegradation 22(4):805–814

Chaemchuen S, Zhou K, Verpoort F (2016) From biogas to biofuel: materials used for biogas cleaning to biomethane. ChemBioEng 3(6):250–265

Chaturvedi V, Verma P (2013) An overview of key pretreatment processes employed for bioconversion of lignocellulosic biomass into biofuels and value added products. 3 Biotech 3(5):415–431

Chaturvedi V, Goswami RK, Verma P (2021) Genetic engineering for enhancement of biofuel production in microalgae. In: Biorefineries: a step towards renewable and clean energy. Springer, pp 539–559

Chen L, Liu T, Zhang W, Chen X, Wang J (2012) Biodiesel production from algae oil high in free fatty acids by two-step catalytic conversion. Bioresour Technol 111:208–214

Chowdhury H, Loganathan B, Mustary I, Alam F, Mobin SM (2019) Algae for biofuels: the third generation of feedstock. Elsevier, Second and third generation of feedstocks, pp 323–344

Ciudad G, Rubilar O, Azócar L, Toro C, Cea M, Torres Á, Ribera A, Navia R (2014) Performance of an enzymatic extract in *Botryococcus braunii* cell wall disruption. J Biosci Bioeng 117(1):75–80

Cucchiella F, D'Adamo I, Gastaldi M (2017) Biomethane: a renewable resource as vehicle fuel. Resources 6(4):58

Demirbas T, Demirbas A (2010) Bioenergy, green energy. Biomass and biofuels. Energy Sources A Recovery Util Environ Eff 32(12):1067–1075

Den Haan R, Rose SH, Lynd LR, van Zyl WH (2007) Hydrolysis and fermentation of amorphous cellulose by recombinant Saccharomyces cerevisiae. Metab Eng 9(1):87–94

Deublein D, Steinhauser A (2011) Biogas from waste and renewable resources: an introduction. Wiley

Dien BS, Nichols NN, O'bryan PJ, Bothast RJ (2000) Development of new ethanologenic *Escherichia coli* strains for fermentation of lignocellulosic biomass. Appl Biochem Biotechnol 84(1):181–196

Dornburg V, van Vuuren D, van de Ven G, Langeveld H, Meeusen M, Banse M, van Oorschot M, Ros J, van den Born GJ, Aiking H (2010) Bioenergy revisited: key factors in global potentials of bioenergy. Energ Environ Sci **3**(3):258–267

Edenhofer O, Pichs-Madruga R, Sokona Y, Seyboth K, Kadner S, Zwickel T, Eickemeier P, Hansen G, Schlömer S, von Stechow C (2011) Renewable energy sources and climate change mitigation: special report of the intergovernmental panel on climate change. Cambridge University Press

Frigon J-C, Matteau-Lebrun F, Abdou RH, McGinn PJ, O'Leary SJ, Guiot S (2013) Screening microalgae strains for their productivity in methane following anaerobic digestion. Appl Energy 108:100–107

Garba A (2020) Biomass conversion technologies for bioenergy generation: an introduction. Biomass, IntechOpen

González-Ramos D, De Vries AR, Grijseels SS, Van Berkum MC, Swinnen S, Van Den Broek M, Nevoigt E, Daran JM, Pronk JT, Van Maris AJ (2016) A new laboratory evolution approach to select for constitutive acetic acid tolerance in *Saccharomyces cerevisiae* and identification of causal mutations. Biotechnol Biofuels 9(1):1–8

Goswami RK, Mehariya S, Verma P, Lavecchia R, Zuorro A (2021) Microalgae-based biorefineries for sustainable resource recovery from wastewater. J Water Process Eng 40:101747. https://doi.org/10.1016/j.jwpe.2020.101747

Goyal H, Seal D, Saxena R (2008) Bio-fuels from thermochemical conversion of renewable resources: a review. Renew Sustain Energy Rev 12(2):504–517

Guedon E, Desvaux M, Petitdemange H (2002) Improvement of cellulolytic properties of *Clostridium cellulolyticum* by metabolic engineering. Appl Environ Microbiol 68(1):53–58

Hanifzadeh M, Nabati Z, Longka P, Malakul P, Apul D, Kim D-S (2017) Life cycle assessment of superheated steam drying technology as a novel cow manure management method. J Environ Manage 199:83–90

Happe T, Mosler B, Naber JD (1994) Induction, localization and metal content of hydrogenase in the green alga *Chlamydomonas reinhardtii*. Eur J Biochem 222(3):769–774

Harish KR, Srijana M, Madhusudhan RD, Gopal R (2010) Coculture fermentation of banana agro-waste to ethanol by cellulolytic thermophilic *Clostridium thermocellum* CT2. Afr J Biotechnol 9(13):1926–1934

Hossain AS, Salleh A, Boyce AN, Chowdhury P, Naqiuddin M (2008) Biodiesel fuel production from algae as renewable energy. Am J Biochem Biotechnol 4(3):250–254

IEA Bioenergy Task 37 (2018) Green gas-facilitating a future green gas grid through the production of renewable gas

Inui M, Suda M, Kimura S, Yasuda K, Suzuki H, Toda H, Yamamoto S, Okino S, Suzuki N, Yukawa H (2008) Expression of *Clostridium acetobutylicum* butanol synthetic genes in *Escherichia coli*. Appl Microbiol Biotechnol 77(6):1305–1316

Jin C, Yao M, Liu H, Chia-fon FL, Ji J (2011) Progress in the production and application of n-butanol as a biofuel. Renew Sustain Energy Rev **15**(8):4080–4106

John RP, Anisha G, Nampoothiri KM, Pandey A (2011) Micro and macroalgal biomass: a renewable source for bioethanol. Bioresour Technol 102(1):186–193

Johnston J (2008) New world for biofuels. Energy Law 86:10–14

Joung JK, Sander JD (2013) TALENs: a widely applicable technology for targeted genome editing. Nat Rev Mol Cell Biol 14(1):49–55

Kataki R, Chutia RS, Mishra M, Bordoloi N, Saikia R, Bhaskar T (2015) Feedstock suitability for thermochemical processes. Elsevier, Recent advances in thermo-chemical conversion of biomass, pp 31–74

Kiran B, Kumar R, Deshmukh D (2014) Perspectives of microalgal biofuels as a renewable source of energy. Energ Convers Manage 88:1228–1244

Kumar B, Verma P (2020a) Enzyme mediated multi-product process: a concept of bio-based refinery. Ind Crops Prod 154:112607. https://doi.org/10.1016/j.indcrop.2020.112607

Kumar B, Verma P (2020b) Application of hydrolytic enzymes in biorefinery and its future prospects. In: Microbial strategies for techno-economic biofuel production. Springer, pp 59–83

Kumar B, Verma P (2021a) Biomass-based biorefineries: an important architype towards a circular economy. Fuel:119622

Kumar B, Verma P (2021b) Techno-economic assessment of biomass-based integrated biorefinery for energy and value-added product. In: Biorefineries: a step towards renewable and clean energy. Springer, pp 581–616

Kumar M, Goyal Y, Sarkar A, Gayen K (2012) Comparative economic assessment of ABE fermentation based on cellulosic and non-cellulosic feedstocks. Appl Energy 93:193–204

Kumar B, Bhardwaj N, Agrawal K, Chaturvedi V, Verma P (2020a) Current perspective on pretreatment technologies using lignocellulosic biomass: an emerging biorefinery concept. Fuel Process Technol 199:106244

Kumar B, Bhardwaj N, Agrawal K, Verma P (2020b) Bioethanol production: generation-based comparative status measurements. In: Biofuel production technologies: critical analysis for sustainability. Springer, Singapore, pp 155–201

Lamed R, Bayer EA (1988) The cellulosome of Clostridium thermocellum. Adv Appl Microbiol 33: 1–46

Lee SY, Sankaran R, Chew KW, Tan CH, Krishnamoorthy R, Chu D-T, Show P-L (2019) Waste to bioenergy: a review on the recent conversion technologies. BMC Energy 1(1):1–22

Lehmann D, Lütke-Eversloh T (2011) Switching *Clostridium acetobutylicum* to an ethanol producer by disruption of the butyrate/butanol fermentative pathway. Metab Eng 13(5):464–473

Lewis JA, Elkon IM, McGee MA, Higbee AJ, Gasch AP (2010) Exploiting natural variation in *Saccharomyces cerevisiae* to identify genes for increased ethanol resistance. Genetics 186(4):1197–1205

Liu Q, Gao R, Li J, Lin L, Zhao J, Sun W, Tian C (2017) Development of a genome-editing CRISPR/Cas9 system in thermophilic fungal *Myceliophthora* species and its application to hyper-cellulase production strain engineering. Biotechnol Biofuels 10(1):1–4

Liu Y, Xie X, Liu W, Xu H, Cao Y (2020) Consolidated bioprocess for bioethanol production from lignocellulosic biomass using *Clostridium thermocellum* DSM 1237. Bioresources 15(4):8355–8368

Long H, Li X, Wang H, Jia J (2013) Biomass resources and their bioenergy potential estimation: a review. Renew Sustain Energy Rev 26:344–352

Margareta W, Nagarajan D, Chang J-S, Lee D-J (2020) Dark fermentative hydrogen production using macroalgae (*Ulva* sp.) as the renewable feedstock. Appl Energy 262:114574

Markov S, Thomas A, Bazin M, Hall DJ (1997) Photoproduction of hydrogen by cyanobacteria under partial vacuum in batch culture or in a photobioreactor. Int J Hydrog Energy 22(5):521–524

Mathews J, Wang G (2009) Metabolic pathway engineering for enhanced biohydrogen production. Int J Hydrog Energy 34(17):7404–7416

McKendry PJB (2002) Energy production from biomass (part 2): conversion technologies. Bioresour Technol 83(1):47–54

Mehariya S, Goswami R, Verma P, Lavecchia R, Zuorro A (2021) Integrated approach for wastewater treatment and biofuel production in microalgae biorefineries. Energies 14(8):2282. https://doi.org/10.3390/en14082282

Miao X, Wu Q (2004) High yield bio-oil production from fast pyrolysis by metabolic controlling of *Chlorella protothecoides*. J Biotechnol 110(1):85–93

Miao X, Wu Q, Yang C (2004) Fast pyrolysis of microalgae to produce renewable fuels. J Anal Appl Pyrolysis 71(2):855–863

Miller JC, Holmes MC, Wang J, Guschin DY, Lee YL, Rupniewski I, Beausejour CM, Waite AJ, Wang NS, Kim KA, Gregory PD (2007) An improved zinc-finger nuclease architecture for highly specific genome editing. Nat Biotechnol 25(7):778–785

Mona S, Kumar SS, Kumar V, Parveen K, Saini N, Deepak B, Pugazhendhi A (2020) Green technology for sustainable biohydrogen production (waste to energy): a review. Sci Total Environ 728:138481

Mussgnug JH, Klassen V, Schlüter A, Kruse O (2010) Microalgae as substrates for fermentative biogas production in a combined biorefinery concept. J Biotechnol 150(1):51–56

Naik SN, Goud VV, Rout PK, Dalai A (2010) Production of first and second generation biofuels: a comprehensive review. Renew Sustain Energy Rev 14(2):578–597

Nakayama SI, Kosaka T, Hirakawa H, Matsuura K, Yoshino S, Furukawa K (2008) Metabolic engineering for solvent productivity by downregulation of the hydrogenase gene cluster hupCBA in *Clostridium saccharoperbutylacetonicum* strain N1-4. Appl Microbiol Biotechnol 78(3):483–493

Nguyen TH, Park S, Jeong J, Shin YS, Sim SJ, Jin E (2020) Enhancing lipid productivity by modulating lipid catabolism using the CRISPR-Cas9 system in *Chlamydomonas*. J Appl Phycol 32(5):2829–2840

Nigam PS, Singh A (2011) Production of liquid biofuels from renewable resources. Prog Energy Combust Sci 37(1):52–68

Özçimen D, İnan B, Biernat K (2015) An overview of bioethanol production from algae. In: Biernat K (ed) Biofuels—status and perspective. InTech, Croatia, pp 141–162

Palop JJ, Mucke L, Roberson ED (2010) Quantifying biomarkers of cognitive dysfunction and neuronal network hyperexcitability in mouse models of Alzheimer's disease: depletion of calcium-dependent proteins and inhibitory hippocampal remodeling. In: Alzheimer's disease and frontotemporal dementia. Springer, pp 245–262

Pan P, Hu C, Yang W, Li Y, Dong L, Zhu L, Tong D, Qing R, Fan Y (2010) The direct pyrolysis and catalytic pyrolysis of *Nannochloropsis* sp. residue for renewable bio-oils. Bioresour Technol 101(12):4593–4599

Parker C, Barnell WO, Snoep JL, Ingram LO, Conway T (1995) Characterization of the Zymomonas mobilis glucose facilitator gene product (glf) in recombinant *Escherichia coli*: examination of transport mechanism, kinetics and the role of glucokinase in glucose transport. Mol Microbiol 15(5):795–802

Patel M, Zhang X, Kumar A (2016) Techno-economic and life cycle assessment on lignocellulosic biomass thermochemical conversion technologies: a review. Renew Sustain Energy Rev 53: 1486–1499

Pathak B, Chaudhari S, Fulekar M (2013) Biomass-resource for sustainable development. Int J Adv Res Technol **2**(6):271–287

Ramey D (2004) Butanol advances in biofuels. The Light Party

Rodionova MV, Poudyal RS, Tiwari I, Voloshin RA, Zharmukhamedov SK, Nam HG, Zayadan BK, Bruce BD, Hou H, Allakhverdiev SI (2017) Biofuel production: challenges and opportunities. Int J Hydrog Enenrgy 42(12):8450–8461

Salehi Jouzani G, Taherzadeh MJ (2015) Advances in consolidated bioprocessing systems for bioethanol and butanol production from biomass: a comprehensive review. Biofuel Res J 2(1):152–195

Sansaniwal S, Pal K, Rosen M, Tyagi S (2017) Recent advances in the development of biomass gasification technology: a comprehensive review. Renew Sustain Energy Rev 72:363–384

Saratale GD, Saratale RG, Banu JR, Chang J-S (2019) Biohydrogen production from renewable biomass resources. In: Biohydrogen. Elsevier, pp 247–277

Sen U, Shakdwipee M, Banerjee R (2008) Status of biological hydrogen production. J Sci Ind Res 67:980–993

Shaw AJ, Podkaminer KK, Desai SG, Bardsley JS, Rogers SR, Thorne PG, Hogsett DA, Lynd LR (2008) Metabolic engineering of a thermophilic bacterium to produce ethanol at high yield. Proc Natl Acad Sci U S A 105(37):13769–13774

Silva G, Poirot L, Galetto R, Smith J, Montoya G, Duchateau P, Pâques F (2011) Meganucleases and other tools for targeted genome engineering: perspectives and challenges for gene therapy. Curr Gene Ther 11(1):11–27

Sims RE, Mabee W, Saddler JN, Taylor M (2010) An overview of second generation biofuel technologies. Bioresour Technol 101(6):1570–1580

Singh L, Mahapatra DM (2019) Waste to sustainable energy: MFCs–prospects through prognosis. CRC Press

Singhvi MS, Chaudhari S, Gokhale DV (2014) Lignocellulose processing: a current challenge. RSC Adv 4(16):8271–8277

Srivastava RK, Shetti NP, Reddy KR, Aminabhavi T (2020) Biofuels, biodiesel and biohydrogen production using bioprocesses. A review. Environ Chem Lett 18(4):1049–1072

Stevens D (2001) Hot gas conditioning: recent progress with larger-scale biomass gasification systems; update and summary of recent progress

Subhadra B, Edwards M (2010) An integrated renewable energy park approach for algal biofuel production in United States. Energy Policy 38(9):4897–4902

Ueno Y, Kurano N, Miyachi S (1998) Ethanol production by dark fermentation in the marine green alga, *Chlorococcum littorale*. J Ferment Bioeng 86(1):38–43

Ulaganathan K, Goud S, Reddy M, Kayalvili U (2017) Genome engineering for breaking barriers in lignocellulosic bioethanol production. Renew Sust Energ Rev 74:1080–1107

Uzoejinwa BB, He X, Wang S, Abomohra AE-F, Hu Y, Wang Q (2018) Co-pyrolysis of biomass and waste plastics as a thermochemical conversion technology for high-grade biofuel production: recent progress and future directions elsewhere worldwide. Energy Convers Manage 163: 468–492

Vardon DR, Sharma B, Scott J, Yu G, Wang Z, Schideman L, Zhang Y, Strathmann T (2011) Chemical properties of biocrude oil from the hydrothermal liquefaction of *Spirulina* algae, swine manure, and digested anaerobic sludge. Bioresour Technol 102(17):8295–8303

Vardon DR, Sharma BK, Blazina GV, Rajagopalan K, Strathmann T (2012) Thermochemical conversion of raw and defatted algal biomass via hydrothermal liquefaction and slow pyrolysis. Bioresour Technol 109:178–187

Vasić K, Knez Ž, Leitgeb M (2021) Bioethanol production by enzymatic hydrolysis from different lignocellulosic sources. Molecules 26(3):753

Vergara-Fernández A, Vargas G, Alarcón N, Velasco A (2008) Evaluation of marine algae as a source of biogas in a two-stage anaerobic reactor system. Front Environ Sci Eng 32(4):338–344

Veza I, Said MFM, Latiff Z (2021) Recent advances in butanol production by acetone-butanol-ethanol (ABE) fermentation. Biomass Bioenergy 144:105919

Wang C, Chang C, Chu C, Lee D, Chang B-V, Liao C, Tay J (2003) Using filtrate of waste biosolids to effectively produce bio-hydrogen by anaerobic fermentation. Water Res 37(11):2789–2793

Wang H, Ji C, Bi S, Zhou P, Chen L, Liu T (2014) Joint production of biodiesel and bioethanol from filamentous oleaginous microalgae *Tribonema* sp. Bioresour Technol 172:169–173

Yamada R, Tanaka T, Ogino C, Kondo A (2010) Gene copy number and polyploidy on products formation in yeast. Appl Microbiol Biotechnol 88(4):849–857

Yu G, Zhang Y, Schideman L, Funk T, Wang Z (2011) Hydrothermal liquefaction of low lipid content microalgae into bio-crude oil. Trans ASABE 54(1):239–246

Zabed H, Sahu J, Suely A, Boyce A, Faruq G (2017) Bioethanol production from renewable sources: current perspectives and technological progress. Renew Sustain Energy Rev 71:475–501

Zheng Z, Li C, Liu H, Zhang Y, Zhong X, Yao M (2015) Experimental study on diesel conventional and low temperature combustion by fueling four isomers of butanol. Fuel 141:109–119

Zhu T, Curtis J, Clancy M (2019) Promoting agricultural biogas and biomethane production: lessons from cross-country studies. Renew Sustain Energy Rev 114:109332