

Chapter 8

Microbial Biofuel and Their Impact on Environment and Agriculture



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Abstract It is a paramount concern to make certain the proper and impervious disposal of organic matter due to piling of organic wastes, changes in the climate, energy security which result in the pollution prevailing in the environment that leads to emerging issues such as epidemics, diseases, obnoxious odors, ammonia release, etc. The emergence of such issues has grabbed the attention of researchers to perform investigations regarding the applications of organic wastes with respect to the concepts of the biotechnology. Researches in these particular domains propose a wide range of advantages both economically and ecologically, which include restrained utilization of fossil fuel, reductions in the emissions of greenhouse gases (GHGs), generation of economical raw materials, substrate development required for numerous microbes. Energy obtained from the biofuels (end-product) is an appealing elucidation for legitimate dumping of feedstocks. This chapter puts spotlights on the production of biofuel from numerous microorganisms and their impact on the agriculture and environment. This also gives insights on the certain examples, which describe the biofuel generation from different microbes or agricultural residues via different mechanisms and concepts. This chapter also describes the commercialization of the biofuels and associated concepts.

8.1 Introduction

The global deadlock of energy has put the universe into erratic and agitated situations because of the increasing demands and rapid reduction of resources on daily basis and it has been observed that soon these available resources will vanish out completely. In situations like these, much attention is required for the concept of the exploitation of renewable resources for the production of energy. With global consumption of fossil fuels, the demerit of fossil fuels is that they are not unsustainable in nature

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since they elevate the levels of carbon dioxide and hence results in the accumulation of greenhouse gases (GHGs) and thus leading to a noxious climate. In order to keep certain concepts on point like a clean and pure environment, sustainable development, etc., it is a necessity to develop and produce eco- friendly fuels, namely biofuels (Schenk et al. 2008). These eco-friendly biofuels are produced from numerous types of biomass obtained from distinctive products of agriculture and forest domain and biodegradable wastes of various industries (Dufey 2006; Yadav et al. 2017, 2019). Examples of biofuels like biodiesel (Shay 1993), butanol (Dürre 2007), *Jatropha curcas* (Becker and Makkar 2008) and algae (Sheehan et al. 1998). It has been estimated that the production of biofuels will be 35 billion liters, approximately. Brazil, the United States (US), and the European nations are considered as the world's biggest producers of biofuels (precisely biodiesel) (Khan et al. 2017).

An alternative source of energy (biofuels) is being considered at a large extent since (a) there is no requirement to modify the engine, (b) helps in the reduction of emissions of greenhouse gases (GHGs), (c) endows security of energy, (d) assure sustainable environment, (e) push the development of rural areas due to switching on the power obtained from the agricultural industries rather than petroleum industries. The exploitation of biofuels is comparatively much more compliant and alluring with respect to the present energy (Hassan and Kalam 2013). The goal of the investigations and studies associated with the development and production of the biofuels is to generate products from numerous sources of biological origin which produces energy like alcohols (ethanol, propanol, butanol, propanediol, and butanediol), biodiesel, biohydrogen, biogas, etc. (Elshahed 2010).

From the burning of the biofuels, there has been a reduction in the emission of greenhouse gases such as carbon dioxide, methane, carbon monoxide, etc., as compared to the fossil fuels and thus they are perceived as an eco-friendly approach to produce energy rather than from the fossil fuels. Additionally, the initial raw materials required for the production the biofuels are present in ample amount in the United States (US) and other nations with developed industries and economies. Hence, from the political, sustainable development, and environment point of view, it is pretty much appropriate to raise the concerns in each and every society regarding the research on the development and production of the biofuels and thus eliminating the reliance on foreign nations for oil (Elshahed 2010; Rastegari et al. 2020).

In accordance with the renewable energy policy network (REN 21), in the year of 2011, it has been estimated that around 78% of energy is consumed from fossil fuels all over the globe, 3% of the energy was consumed from nuclear energy and the rest 19% of the consumption was from renewable sources such as wind energy, solar energy, geothermal energy, hydrothermal energy, and biomass from agricultural sector or industries. It has been acknowledged that approximately 13% of the renewable source of energy is exploited from materials that were rich in carbon and was feasible on earth either by direct burning of the biomass or by converting biomass into heat and power via a thermochemical process (Balan 2014; Mohr and Raman 2013).

Presently, approximately 10% of the demand for energy all over the globe has been transformed by biomass. With the increase in the prices of crude oil, exhaustion of the resources, environmental instability, and biomass has the ability to meet the demands of energy prevailing all over the globe. Biomass obtained from plants is present in ample amount and is the renewable energy source which is rich in carbohydrates and thus effective for conversion into biofuels via microbes. Till date bioethanol is one such commercialized product in the industries but not yet exploited for transportation purposes. This chapter gives insights into biofuels produced from numerous types of microbes and their impact on the agriculture and environment, respectively. Additionally, this chapter deals with certain examples of microbial biofuels to gain a more and clear knowledge of different aspects of microbial biofuels (Antoni et al. 2007).

8.2 Microbial Setup for Biofuels

By consuming organic substrates with the aid of microorganisms and also their exploitation in the processes dealing with the metabolic help in the generation of the favorable products, which will be the source of energy production (Kumar and Kumar 2017). Figure 8.1 gives an insight into the generation of microbial biofuel from numerous microorganisms and pathways.

There are certain factors such as (a) choice of microorganisms, (b) substrates to be utilized, (c) process required to produce biofuels are essential with respect to the synthesis of biofuels. For example, the production of ethanol from corn (substrate) requires more intake of energy from fossil fuels in comparison to the production of ethanol when sugarcane is being used as a substrate (Goldemberg et al. 2008). Furthermore, it is required to have a positive balance of energy (on an average) with a viewpoint of commercialization. The other important concern is the selection of an efficient substrate for microbes (Chang et al. 2013). Lignocellulosic biomass can be exploited and transformed into biofuels by dismantling sugars. This approach usually initiates first with the step of pretreatment of the lignocelluloses following hydrolysis by employing enzymes or by centralized bioprocessing approaches (Kumar et al. 2009; Mosier et al. 2005). The biomass will further be hydrolyzed either by simple cocktails of enzyme cellulose or by a cellulolytic microbe (Lynd et al. 2002).

When compared to carbon dioxide, there is less emission of methane, a component of natural gas, but it is more persuasive in nature (Yvon-Durocher et al. 2014). The production of methane is from landfills or from anaerobic digestion of numerous wastes with organic content. There has been a dramatic flow of methane in the recent past and thus grabs the attention of the researchers to look for an effective source of carbon. By employing methanotrophs which will feed on methane (from landfills and natural gas) directly and produce or another approach is to transform methane into to methanol via methanotrophs and hence the biofuel production (Liao et al. 2016). The oxidation of methane via methanotrophs was done by first reducing the oxygen atoms of hydrogen peroxide followed by the conversion of methane to

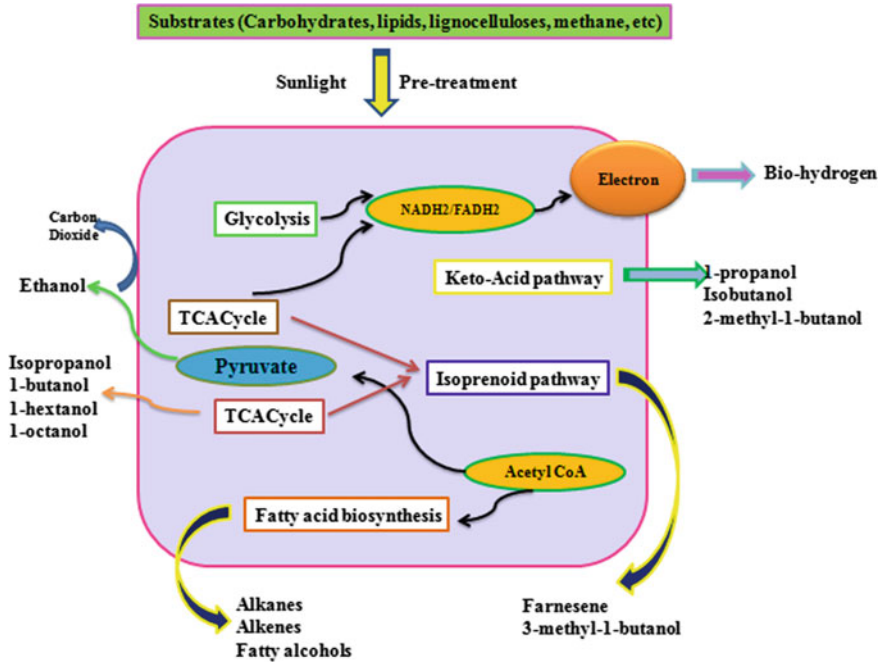


Fig. 8.1 Synopsis of biofuels from different pathways (Kumar and Kumar 2017)

methanol by utilizing methane monoxygenases (Fuerst 2013). There are two types of methane monoxygenases: (a) soluble methane monoxygenases and (b) particulate methane monoxygenases. It has been noticed that the cells which consist of particulate methane monoxygenases have high abilities to grow along with the high affinity for methane than cells which consist of soluble methane monoxygenases (Kumar and Kumar 2017).

8.3 Biofuels from Microbes

The two basic categories of biofuels are primary biofuels and secondary biofuels. The primary biofuels are basically the unprocessed form of fuel used chiefly during heating, cooking, generation of electricity like fuelwood, wood chips, etc., whereas production of secondary biofuels are from the processing of biomass, for example, ethanol, biodiesel, etc., which can later be exploited in the transportation sector and for numerous processes of industries (Rana et al. 2019; Rastegari et al. 2019a). The secondary biofuels are also categorized, on the grounds of raw material and the technology to employ to produce biofuels, into first-generation biofuels, second-generation biofuels, and third-generation biofuels, respectively. There has been

Table 8.1 Various microorganisms and their significance in the production of biofuels

Microorganism's name	Importance in terms of biofuel production
<i>Anaebaena variabilis</i>	It is a cyanobacteria which produces biohydrogen
<i>Clostridium acetobutylicum</i>	It is considered as one of the significant microorganism which produces butanol
<i>Micrococcus luteus</i>	This source generates alkenes of long chains
<i>Zymomonas mobilis</i>	It has a role in the fermentation process of ethanol with a high level of ethanol tolerance
<i>Saccharomyces cerevisiae</i>	Significant source for the production of ethanol
<i>Rhodospseudomonas palustris</i>	This microorganism which belongs to the phototroph family and produces producing hydrogen gas
<i>Saccharophagus degradans</i>	This microorganism helps in the degradation of the various biopolymers

Source Wackett (2008)

extensive research done in the recent past regarding the production of biofuels from numerous microorganisms (Table 8.1) (Nigam and Singh 2011).

Present-day technologies which include biotechnology are doing huge exercises to convert biomass into substances with a potential to utilize them as a fuel at a particular cost, which can go up against in the contest of the high prices of the crude oil. There will be a release of carbon dioxide into the atmosphere when these substances of the biomass are burned that were fixed by the process of photosynthesis and thus resolving the issue of global warming (Fig. 8.2). Additionally, the costs and yield associated with the production of biofuels from biomass is a matter of concern. The favorable outcome of biofuels depends on the economic process of the conversion of biomass into biofuel having physicochemical features which function as a substitute for fuels generated from fossil fuels (Nigam and Singh 2011).

While burning fossil fuels, there is a release of carbon dioxide into the atmosphere resulting in an increase in the concentration of carbon dioxide and thus contribution to global warming. Plant or certain photosynthetic microorganisms help in fixing some portion of the carbon dioxide prevailing in the atmosphere. There are certain microorganisms that either employ carbon dioxide (photosynthetic microorganisms) or biomass as a source of carbon or produce various carbon substances which are later utilized as fuels (Fig. 8.2). The biggest obstacle for the biotechnology is to generate such substances in an economical, sustainable, and appropriate way. One can derive biomass by cultivating dedicated crops that generate energy either by reaping residues of forests and plants or from the wastes of biomass (Gullison et al. 2007). There are numerous feedstocks of biomass such as crops of sucrose and starch (sugarcane and corn), lignocellulosic materials (rice straw and switchgrass), which can be utilized for the production of biofuels but the cost associated with the hydrolysis of the lignocellulosic materials is a matter which one must consider (Sharma and Arya 2017).

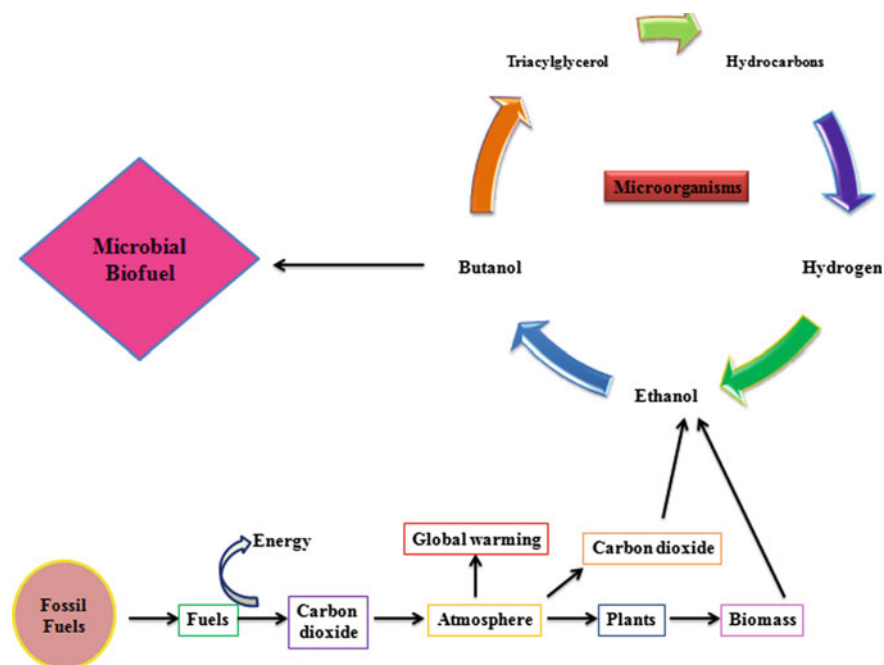


Fig. 8.2 Generation of biofuels from numerous microorganisms (Rojo 2008)

8.3.1 Generation of Biofuels from Microbes

In the recent past, the advancements have depicted that there are certain species of microbes like yeast, fungi, microalgae that have the potential to utilize them for the production of biofuels like biodiesel, biohydrogen, bioethanol, etc., since it is possible to synthesize biologically along with the storage of huge content of fatty acids in the biomass (Kour et al. 2019a; Yadav et al. 2020; Xiong et al. 2008). It has been reported by the team of researchers back in the year 2009 (Huang et al. 2009) about the production of microbial oil from the wastes of rice straw. The microbial oil can also be generated from hydrolysate of the rice straw, which was treated with sulfuric acid (H_2SO_4) by cultivating *Trichosporon fermentans*. It has been observed that the fermentation of rice straw which was treated with sulfuric acid (H_2SO_4) when detoxification was not done results in low yields of lipid, that is approximately 0.17% w/v (1.7 g l^{-1}). Group of researchers (Huang et al. 2009) exercised to enhance the yield of the process. The pretreatment stages (detoxification) consist of (a) overliming, (b) concentration, and (c) adsorption via Amberlite XAD-4 have enhanced the fermentation capability of rice straw, which was treated with sulfuric acid (H_2SO_4) in a significant manner.

The concept of pretreatment has assisted in augmenting the yield of lipids via removal of the inhibitors present in the rice straw treated with sulfuric acid. It has been

recognized that the biomass of the microbe in total was 28.6 g l^{-1} after a fermentation process for 8 days. The lipid content of the rice straw which was treated with sulphuric acid (H_2SO_4) observed was 40.1% which corresponds to the yield of lipid to around 11.5 g l^{-1} after cultivating with *Trichosporon fermentans*. Furthermore, apart from rice straw which was treated with sulphuric acid (H_2SO_4), *Trichosporon fermentans* has the ability to metabolize more sugars like mannose, galactose, cellobiose, etc., which are present in the hydrolysates of additional lignocellulosic materials of natural origin and can be utilized as a source of carbon. *Trichosporon fermentans* has the ability to evolve and employ hydrolysate of the rice straw in order to increase the content of lipid within the biomass of the cell and thus resulted in increased yields (10.4 g l^{-1}). Hence, this particular microorganism can be utilized as a potential candidate for the production of microbial biofuels (Nigam and Singh 2011).

Another group of researchers have performed experiments (Zhu et al. 2008) and produced biofuel microbial from the wastes of molasses. It has been published that the lipids generated in the biomass of the microbe can later be utilized for producing biodiesel. There has been optimization of the constituents of the growth medium in order to cultivate the culture of interest and after that studies have been done to check the consequence of conditions which are required by the culture on microbial biomass and generation of the lipid via *Trichosporon fermentans*. The favorable source of nitrogen and carbon and the molar ratio of carbon to nitrogen (C: N) with respect to the yield of the lipids was peptone, glucose, respectively. Also, the favorable pH and temperature required for the growth medium for cultivation was 6.5 and $25 \text{ }^\circ\text{C}$, respectively. Within such a favorable environment, there has been a cultivation of the culture for seven days with an outcome of the 28.1 g l^{-1} biomass yield from the microbial strain which consists of an amount of lipids to approximately 62.4% which was found to be more when compared to the original data, that is 19.4 g l^{-1} and 50.8%, respectively (Xiong et al. 2008; Zhu et al. 2008). It is also possible to cultivate the strain *Trichosporon fermentans* in a medium which consist of wastes of molasses collected from the sugar industries. From the already published reports, the yield of the lipids was approximately 12.8 g l^{-1} and the total concentration of the sugar (in terms of w/v) was 15% when converted biologically from the wastes of the at a pH value of 6.0 (Zhu et al. 2008).

It is possible to improve the assembly of lipids inside the cells of the microbes by adding numerous sugars into the molasses (pretreated) (Chen et al. 1992; Fakas et al. 2007). It has also been noticed that the amount of the lipid was augmented to 50% of the mass of the cells. The lipid present in the microbes major includes palmitic acid, stearic acid, oleic acid, and linoleic acid along with the unsaturated fatty acids approximately 64% of the total content of the fatty acids (Zhu et al. 2008). The reports have suggested that yeast has the capability to evolve and cultivate pretty well over the lignocellulosic biomass (which was already pretreated). This evolution has resulted in an increase in the accumulation of the lipids in an effective way and thus a potential candidate to produce microbial biofuel from residues of the agriculture sector in an economic and eco-friendly way (Nigam and Singh 2011).

8.3.2 Generation of Biofuels from Algae

Algae being the oldest form of life are existent in all the ecosystems surrounding the earth, and thus represent a huge diversity of species active in a broad range of conditions of the environmental (Mata et al. 2010). They are basically called the primitive plants named thallophyte that is no roots, no stems, and no leaves and even does not have a sterile cell covering surrounding the reproductive cells. The primary pigment of photosynthesis of algae is chlorophyll a (Farrell et al. 1998). When growth conditions are innate, the sunlight was absorbed by phototrophic algae and thus the assimilation of carbon dioxide (CO₂) from the air and obtains nutrients from the aquatic biosphere. It has also been observed that there can be a production of lipids, protein carbohydrates in bulk amounts from microalgal species in a very brief time which can later be exploited for the production of the biofuels and other worthy related products (Brennan and Owende 2010). But the production of biofuels from lipids, proteins is a limited affair because of the restrained availability of the sunlight and hence results in the commercialization of such biofuels only in those regions where sunlight is available in bulk amount (Pulz and Scheibenbogen 2007). The capability of microalgal species for carbon dioxide fixation has been considered as an alternative to diminish the emissions of greenhouse gases (GHGs). Furthermore, algal species are highly rich in the oil and thus can be utilized for the production of the biodiesel (Gislerød et al. 2008).

Researchers have observed that (Widjaja et al. 2009) when a particular or appropriate nitrogen source was absent there has been the production of oil, in the majority, while when the sunlight was present there has been the production of sugars, proteins, etc., from carbon dioxide (CO₂). One of the species of microalgae, namely, *Chlorella protothecoides* accumulates lipids when grown in the presence of autotrophic and heterotrophic environment later which can be utilized for the production of biodiesel. In order to enhance the accumulation of the lipids via microalgal species, the best approach is to limit the source of nitrogen. Apart from the accumulated lipids, there has been a progressive change in the arrangement of the lipids from free fatty acids into triacylglycerols (TAGs) which are comparatively better for the production of biofuels (Meng et al. 2009; Tsukahara and Sawayama 2005).

There are primarily two approaches to convert microalgal species for their utilization and they are (a) thermochemical conversion approach and (b) biochemical conversion approach. The former approach of conversion deals with the decomposition of the organic constituents into the fuel thermally like direct combustion, gasification, thermochemical liquefaction, pyrolysis, etc. (Energy 2002). The later one deals with the conversion of energy obtained from the biomass into certain other fuels via processes like anaerobic digestion, fermentation by employing alcohols, production of hydrogen from photobiological approach, etc. (Grant 2009).

Presently, there are only certain multinational companies (MNCs) owned privately and certain research groups (funded publicly) which are engaged onto the cultivation of algal species and on the condition to lower down the cost of production of oil from the microalgal species at a modest range. A Colorado-based company, Solix

Biofuels, have manufactured a closed-tank bioreactor which employs the generated carbon dioxide (waste) and produces beer. A company of New Zealand named Aqua flow Bionomics is engaged in the biofuels generation through harvesting wild algal species obtained from the foul waterways (Nikolić et al. 2009). Researchers have reported that only the algal species are not capable enough to produce an acceptable amount of fuels so later they shifted their focus on the heterotrophic species of algae and exploited the substances which employ carbon instead of carbon dioxide fixation into the environment. All the algal strains have the ability to use up everything from the glycerol wastes and the wastes of the sugar cane and convert it into the pulps of sugar beets and molasses, respectively (Nikolić et al. 2009).

8.4 Upscaling the Production of Biofuels via Metabolic Engineering

For producing biofuels from microbes, a particular metabolic pathway and various groups of catalytic enzymes are required. For instance, it has been observed that there has been a direct decarboxylation of pyruvate in *Saccharomyces cerevisiae* (baker's yeast), which results in the generation of the ethanol whereas, in *Escherichia coli*, there has been an activation of the acyl group by coenzyme A (CoA) in the course of pyruvate decarboxylation and ultimately leads to ethanol. Hence, the concept of metabolic engineering of aforementioned pathways can be considered as a worthy opportunity to enhance the production of biofuels and be exploited in numerous ways in order to improve the production of biofuels by utilizing microbes. One approach is to produce ethanol from two numerous pathways (mentioned above) in *Saccharomyces cerevisiae* (baker's yeast) and in *Escherichia coli* (*E. coli*), respectively. It has been reported that the best and effective way to produce ethanol is when coenzyme A (CoA) is absent (Liao et al. 2016). For this reason, it is possible to express such pathways in certain microbes via approaches which make use of genetic engineering for producing ethanol. Likewise, the microbes in which the metabolic routes are absent for producing specific biofuel, an injection of imperative genes, enzymes (extracted from particular microbes with an efficiency to produce biofuel) can be given which will help in converting the microbes which do not produce biofuel into microbes which will produce biofuels (Rastegari et al. 2019c). This very particular approach is advantageous to utilize numerous substrates for producing biofuels in the near future.

The second approach can be thought of the competing pathways which will drain either the products, that are biofuels or the precursors like pyruvate, acetyl-Coenzyme A. Additionally, certain enzymes create hindrances in the synthesis process of the biofuel that can be knocked out by exploiting metabolic engineering approaches. For instance, in *Escherichia coli*, an acyl carrier protein (ACP) impedes the route of synthesis of fatty acids (Davis and Cronan 2001). In order to overcome this inhibition, the over-expression of thioesterase enzymes proves beneficial and thus allows the

synthesis of fatty acids in free form, which eventually leads to the production of a precursor, that is acyl-Coenzyme A (for the synthesis of fatty alcohol).

Furthermore, to improve the catalytic activity of the enzymes that are specific to a particular substrate and to improve the turnover number, maneuvering the genetic material of an enzyme by utilizing progressive design tools and certain experimental methods. Also, proteins manipulated via computational tools can also be exploited to support amino acids of unnatural origin in order to fabricate and imitate enzymes with all the desired features and properties which later can be exploited for producing biofuels. Although, performing such manipulation is a challenging task which requires a high level of tools with an efficiency to control the proteins at a particular stage of mRNA levels in order to perform very well in an artificial route or environment (Kumar and Kumar 2017).

8.5 Examples of Microbial Biofuels

8.5.1 *Producing 1,3-Propanediol from Microbe Klebsiella Pneumoniae via Utilization of Crude Glycerol*

Biodiesel a derivative of triacylglycerols (TAGs) through a transesterification reaction which utilizes alcohols (short-chains) have gained a lot of attention in the recent past due to certain properties such as renewability, biodegradability, non-toxicity, etc. (Andrade and Vasconcelos 2003; Xu et al. 2003). There has been a generation of byproducts called glycerol during the biodiesel synthesis, which is regarded as a 10% (w/w) of an ester which can be utilized further to enhance the desirability of the whole process (Mu et al. 2006).

The process of conversion of glycerol into 1,3-Propanediol via microbe is an alluring approach and grabbing huge attention of the researchers since it is a comparatively easy process with no generation of lethal byproducts. There are various applications of 1,3-Propanediol such as in polymers, cosmetics section, foods, lubricants, medicines, etc. Production of 1,3-propanediol industrially is considered as an important approach for the synthesis of an advanced class of polyester, namely, polytrimethylene terephthalate (PTT) (Zeng and Biebl 2002). One of the significant constraints for producing 1,3-propanediol via microbe at an industrial scale is the increased costs of the raw materials. It is advised to employ crude glycerol with no purification beforehand with respect to the economic point of view. More research has been going on about the study of the bacterial growth on glycerol of low grade apart from the study of using them as a substrate (Papanikolaou et al. 2004).

In this very particular example, the work has been done on performing shake-flask fermentations and fed-batch fermentations by *Klebsiella pneumoniae* for the production of 1,3-Propanediol either by utilizing the pure form of glycerol or crude form of glycerol acquired during methanolysis of soybean oil. There are no reports till date on the production of 1,3-propanediol by *Klebsiella pneumoniae* from crude

glycerol. The concentration of 1,3-propanediol from crude glycerol obtained during the methanolysis of soybean oil using alkali catalyst was 51.3 g/l^{-1} whereas the concentration of 1,3 propanediol was from crude oil when lipase catalyst was utilized 53 g/l^{-1} . The yield when crude oil was employed was $1.7 \text{ g l}^{-1} \text{ h}^{-1}$ whereas when pure glycerol was employed the yield was $2 \text{ g l}^{-1} \text{ h}^{-1}$. Thus, in conclusion, crude oil has the ability to directly transform into 1,3-Propanediol with no requirement of purification beforehand. Also, this work also suggests that the fermentation cost is less which is one of the important factors while employing byproducts and transforms it into certain significant substances (Mu et al. 2006).

8.5.2 *Microbial Biofuels from Fatty Acids and Chemicals Obtained from Biomass of Plants*

With the increase in the costs of the crude oil and associated concerns of environment, much effort has been put by the researchers for the production of fuels that obey the concept of sustainability (Fortman et al. 2008). The significant efforts have been made on the production of biofuels from various microbes from an economic viewpoint (Lynd et al. 2005). Fatty acids are derivatives of long-chain alkyl and are considered as a primary metabolite being utilized by cells for certain functions. They are the energy-rich compounds being separated from the oils obtained from plants and animals and are considered as a broad group with a range of fuels to oleochemicals. For this particular class of compounds, another pathway to produce an economical biofuel is from the utilization of microbes and which will help in the transformation of feedstocks (renewable) into fuels (Steen et al. 2010).

This example demonstrates the engineering of *Escherichia coli* for the production of artificial fatty esters (called biodiesel), fatty alcohols, waxes, etc., from simple sugars by a direct route. Additionally, this example also provides information regarding the engineering of the cells that generate biodiesel in order to assert hemicelluloses—a significant constituent of biomass which is obtained from plants. Oils obtained from plants and animals are considered as raw materials for the production of biofuels like biodiesel, surfactants, solvents, lubricants, etc. (Hill et al. 2006). A substitute for sustainable development is the production of such oils with a direct route to produce these products directly from sufficient and economical sources which are renewable in nature via the fermentation process. *Escherichia coli* is one such microbe of an industry which constitutes about 9.7% lipid and generates metabolites of fatty acids with productivity at a commercial level of $0.2 \text{ g l}^{-1} \text{ h}^{-1}$ per gram of mass of the cell in order to grow and accomplish the yield of mass to around 30–35% (Rude and Schirmer 2009). Another merit is that it is possible to manipulate the genetic make-up of the microbe in a flexible way. This particular line of work provides strength to such products enabling them to excel at commercial levels. Also, extensive research has been going on regarding the enhancements of

strain and development of advanced process via scale-up bioprocesses keeping in mind the point of view of commercialization (Tsuruta et al. 2009).

8.5.3 *Ionic Liquid for the Production of Microbial Biofuel*

It has been well reported that biomass of lignocelluloses is present in ample amount and can be readily used for producing biofuels in a sustainable with high commercial values. In recent days, microbial engineering (Liu and Khosla 2010; Wen et al. 2013) is grabbing a lot of attention as it has a potential to produce biofuel along with the utilization of a broad range of feedstocks such as biomass from woods, residues of native grass, agricultural products like corn stover, etc. (Bokinsky et al. 2011; de Jong et al. 2012). As it is a prerequisite that the pretreatment approach is required in order to take care of the recalcitrant biomass and leads to free polysaccharides free from lignin either through enzymatic hydrolysis or through the use of chemicals for fermenting sugars. There are some ionic liquids of hydrophilic nature, which can be exploited for solubilizing lignocellulosic biomass. These ionic liquids are very efficient and eco-friendly candidate to utilize in the pretreatment process and result in the generation of inhibitors obtained from biomass in very less amount in comparison to low numerous traditional methods of pretreatment (Liu et al. 2012; Mora-Pale et al. 2011). A common ionic liquid named imidazolium has certain demerits like the generation of which leads to impairment in the growth of hosts (*Escherichia coli* and *Saccharomyces cerevisiae*), which will produce biofuel inherent toxicity of microbe thus inhibition in the efficiency of the production of the biofuel (Ouellet et al. 2011). Furthermore, another issue is the severe reduction of the product yield at the end of the process of the production of biofuel (Park et al. 2012).

In this example, a mechanism has been developed that will help in resisting the ionic liquids. This mechanism includes two adjoining genes from the strain *Enterobacter lignolyticus* (soil bacteria), which can tolerate ionic liquids having imidazolium. Such genes have the ability to hold their complete functional property during their transform into *Escherichia coli* which will ultimately produce biofuel with resistant ionic liquid which have been established by a transporter present in the inner membrane which is further regulated by an ionic liquid inducible repressor. The transporter is adjusted in such a way so that the expression will be directly through ionic liquids, which will enable the growth and production of biofuel at a particular stage of ionic liquids which is lethal for indigenous strains. Such original autoregulatory mechanisms (by EilR repressor) are efficient for converting lignocellulosic biomass into biofuels. The researchers have chosen the targeted functional screening method for identification of important genetic elements which are subjected to Cl tolerance and to uncover that such genes have efflux pump and regulator. Researchers have transferred such genes into an *Escherichia coli* (engineered host) and demonstrated the improved production of biofuel based on terpenes (secondary metabolites) in the presence of ionic liquids (Ruegg et al. 2014).

This mechanism of efflux has enhanced the production of biofuel when ionic liquid is present in a low amount. We anticipate that engineering IL-tolerant biofuel pathway enzymes and production strains with tolerance to inhibitors originating from biomass breakdown (Klinke et al. 2004) are needed to further increase yields. Furthermore, the tenacity of the strain *Escherichia coli* has been strengthened via an autoregulatory mechanism of efflux of ionic liquids via repressor EilR. Apart from the strain improvement, the production of biofuels has also been efficiently improved for fermentation processes at the industry level in which the levels of ionic liquid fumbles within the batches of the biomass. Additionally, such mechanism forestalls the requirement of expensive molecules for an induction process. It renders the fermentation process cost-effective with the aseptic environment by inhibiting the growth of the contaminants of the microbes (Ruegg et al. 2014).

8.6 Influence of Microbial Biofuel on the Agricultural Sector

With an increase in the requirement of energy and fuel, the global economy is increasing at a fast pace in order to scrutinize the strength of advanced stage biofuels (Kour et al. 2019b; Yang et al. 2009). These forms of bioenergy will help nations to curb the import of petroleum reserves from foreign countries rendering to provide an elucidation regarding dual obstacles which are the issue of security of energy and changes in the climate. However, the major matter is the escalation of emissions of greenhouse gases (GHGs) coming from biofuels produced from the crops with expanded lands (Gibbs et al. 2008). The production of biofuel all over the world is increasing and has stretched to the remarkable levels in the recent past (Gerber et al. 2008). According to the reports, from the year 2001 to the year 2007, the global production of ethanol gets world ethanol production has been intensified, that is, from 20 to 50 billion liters and that of the biodiesel increased from 0.8 to 4 billion liters (Banse and Meijl 2008).

It has been a tough task to make an estimate of numerous calculations of the generation of biofuels on the prices of food commodities and agricultural commodities, respectively (Gerber et al. 2008). Furthermore, compared to the countries with well-developed industries, the developing nations will be more competitive for producing biofuels because of the reduced production and the opportunity of availing the reasonable agricultural land for cultivating feedstocks for the generation of biofuels. With necessary global trades and investments for developing nations, there has been a prediction of numerous challenges. Also, increased prices already exist in the markets of feedstocks (sugar, rapeseed, soybeans, jatropha) for the production of biofuel (Ottinger 2007).

In opposition, poor belonging to urban and rural areas of the nation which imports food to other countries have to pay increased prices of simple and important food with less availability of grains to feed the humanity (Cassman 2007). It has been

recorded that trading of certain food products like wheat was high in terms of pricing in the year 2006–2008. There are numerous nations which have adopted certain strict policies regarding the promotion of biofuels as an alternative of gasoline in the transport section. For example, around 10% of the use of gasoline for the United States is generated from corn ethanol which will grow to 30% in the year 2022 (Charles 2012). Such hikes will create disturbances in the weak and poor sections of society by spending crooked shares of their monthly salary at a high rate on food commodities in order to meet the cliché requirements of nutrition (Charles 2012). It has been investigated globally that the production of biofuels from first-generation feedstocks along with the use of agricultural land for the generation of foods will have an adverse and serious implication on the supplies of agriculture and food (Ottinger 2007). Universally, the land is the scarce source and thus more pressure on the effective allocation of land with innovative agricultural practices (Lambin and Meyfroidt 2011).

The significant difference in food crops and energy crops is the relationship between yield and the input. In case of food crops, the major concern is the yield and to clinch the same, there should be a willingness to people have been willing to boost the supply of inputs like water, fertilizer, labor, machinery, etc. (Sang 2011). There are certain crops that produce biofuels which are in need of bulk amount of water required to cultivate the crops and this a major concern specifically to the areas where water supplies are scarce. Additionally, reduction of supply of water and contamination of resources of water has an acute effect on the health of humans and animals (Ottinger 2007).

From the study of World Bank, it has been found out that in the year 2006–2008 there has been an increase in the prices of the food commodities for the production of biofuel by 70–75% and thus more worldwide focus regarding the relation between biofuels and prices of the commodities of the food. Since the already utilized crops are not sufficient enough to meet the aims and requirements of the production of energy in a sustainable way, so the extensive research is going on the contemporary crops that will provide energy. In order to overcome the aforementioned issue, it is required to cultivate and grow such contemporary crops on marginal lands which are not appropriate for the production of food. These marginal lands must have increase yield of biomass which requires a very little requirement of irrigation facilities. There should be a minimization of certain inputs which requires energy such as tilling, planting, harvesting, storage, transportation, etc. Such crops are known as second-generation energy crops (Heaton et al. 2008; Karp and Shield 2008; Oliver et al. 2009). The most promising advantage of biofuels is their positive effect on the employment rates in agricultural lands and practices and enhancement in the livelihoods of poor farmers in rural sectors. Apart from this potential merit, the serious implications of biofuels are on the global market of agriculture. There is strength in the production of biofuels to generate employment opportunities in rural sectors but the majority of shares of employment are for those agricultural workers that are not extremely skilled or knowledgeable and such workers are precisely more in jeopardy (Kumar et al. 2019; Rastegari et al. 2019b).

There are numerous risks associated with the health of the farmers while working in the agricultural fields or lands majorly because of exploiting the agrochemicals by improper means like no proper and full information regarding their use and no means of safety equipment to them while working. A better environment for sound work should be considered while mentioning the constituents of the standard protocol for the production of biofuels along with their trading (Rosegrant 2008). The production of biofuels from second-generation feedstocks such as residues of animals, crops, timber, food, etc., provide an edge to overcome the existing competition with respect to the food for human consumption but such residues are a vital source of nutrients with respect to the growth and development of plants. With the burning of such residues of crops results in the decrease in the amount of organic matter from the soils and increased utilization of fertilizers such as ammonia. All these practices are exploited under high-energy usage (Bisth et al. 2015).

Considering all the aspects, merits, demerits of biofuel production from second-generation feedstocks, currently biofuel production from third-generation feedstocks are grabbing a lot of attention of researchers. There has been the production of biofuels such as biodiesel from microorganisms like cyanobacteria, microalgae, etc. Microorganisms are pretty much better alluring feedstock for the production of biofuel as compared to the traditional feedstocks such as oil from crops since they have high efficiency of photosynthesis and a high amount of lipids. Photosynthetic microorganisms such as algae and cyanobacteria are the major and significant producer in aquatic animals and cover approximately 71% earth space (Andersen 2005).

These photosynthetic microorganisms have the ability to transform carbon dioxide into various hydrocarbons like lipids. It has also been published that these algal lipids can be considered as an alternative of fossil fuels in the near future because of the building up of them in the cells present at the end stage of growth (Abdeshahian et al. 2010; Kenthorai et al. 2011). Another advantage of these microorganisms are that (a) they have the capability to grow in the nutrient medium with minimum supplements like water, photon, carbon dioxide, (b) the simple mass cultivation, (c) simple process of extraction and purification and thus appropriate for the production of biofuel in bulk (Hu et al. 2008). Since the impact of agriculture is both on the climate and production of food. Thus, the adverse impacts will be more (a) on the nations which are highly vulnerable to the changes in the climate (b) on the farmers with low-income source and thus high chances of poverty. The developing nations have a positive impact of biofuels, that is poor farmers of the rural sector may get employment for producing biofuels from microbes and thus helps in improving the development of the area and their livelihoods. Thus, on the whole, biofuels, when compared to the traditional fuels, provide many advantages such as security of energy in the near future, less serious implications on the environment, savings during the foreign exchange, and prevention of certain socioeconomic problems (Mohammady 2007).

8.7 Influence of the Microbial Biofuel on the Environment

From the period of an automobile long ago, oil was considered as an exclusive source of energy of the transport sector. In the year 2007, 95% of the energy for transportation worldwide was from petroleum resources (Hill et al. 2009). Extensive research regarding the search of alternative source of energy is going at full pace. The forces behind such extensive research and urgency are the elusive prices of oil, the global increase in the demand of the fuel and so the energy, more dependence on the imports of fuels from unsettled regions, awareness regarding harmful implications of greenhouse gases and pollution in the air, etc. (Maclean and Lave 2003). Numerous nations are trying to enhance the security of energy along with the economic aspects to diminish the emissions of the harmful greenhouse gases to lessen the effect on the changes in the climate worldwide. Research has been done to develop biofuels from biomass for transportation. Such biofuels will help in replacing the fuels generated from the petroleum reserves, which consist of atmospheric carbon instead of fossil carbon, hence addresses the concerns related to the emissions of greenhouse gases (Frank et al. 2012).

Recent policies associated with energy labeling the problems of the environment such as the development of eco-friendly technologies for increasing the supplies of energy and to support clean and effective utilization of energy which considers certain issues such as air pollution, global warming, and changes in the climate (Demirbas 2009). Traditional fuels are answerable toward certain problems associated with the presence of the pollutants and greenhouse gases in the atmosphere which are contributing to global warming day by day. The ramification of greenhouse gases is a natural event where a portion of the infrared radiations are kept by the atmosphere of the earth because the greenhouse gases get reflected back. Back in the 1990s, The International Panel on Climate Change (IPCC) classified three significant preferences in order to mitigate the concentration of carbon dioxide in the atmosphere via agricultural domain and they are (a) reducing the emissions associated with the agriculture, (b) fabrication of carbon sinks in the soil, (c) generation of biofuels in order to take the place of fossil fuels (Bisth et al. 2015).

There has been a firm conviction that biofuels can be considered as an alternative to mollify the current changes in the climate. This faith has forced the government to think of biofuel by promoting the ethanol production, biodiesel production via certain policies that will ensure the market and grants incentives to producers and consumers, respectively (Sexton and Zilberman 2008). One of the significant reasons that forced to shift the gears on the production of biofuels as a substitute of energy is with respect to the perks being provided to the climate like sequestration of harmful gases and thus called as greenhouse gas neutral. This particular thought of clean and green energy has led one to include biofuel in numerous nations, where industrialization is significant like Unites States of America (USA), European Union (EU), etc. (German and Schoneveld 2011). A significant notion regarding biofuel is the limit up to which biofuel will help in reducing the emissions of carbon dioxide in case of deforestation. Other questions like damage to local worthy goods and services obtained from the

forests. While using cultivated land, there are chances that there will be a loss of production of food along with the reduction of the security of food. There are certain potential risks while producing biofuel that might alter the conventional patterns of land, social alliances, favorable circumstances of livelihood, precisely in case of production at a large scale instead of small scale (Lima and Skutsch 2011). All over the globe, the problem of soil erosion is prevailing at a big platform. Biofuel is helpful in accelerating the aspect of geology if the productivity of the cultivated lands is increased (Bisth et al. 2015).

Keeping in mind such issues, much effort has been made on the production of biofuels and cut down the exploitation and consumption of petroleum reserves, increasing the exploitation of the land use, reduction in the emissions of the greenhouse gases (GHGs). With respect to this microalgal species are gaining interest since they have the potential to produce large yields when compared to the grass, grains, trees, etc. Also, they can also generate oils that can be transformed into products like diesel, gasoline, etc. It is mandatory to quantify the products obtained from algal biofuel ad fuels from reserves of petroleum as it requires enough energy to produce fuel (Frank et al. 2012). There is a requirement of cost-effective feedstocks for the production of biofuels but resources such as land, water, nutrients required to produce biofuel from the crops should be readily available. The production of biofuel from woods has serious implications in the form of deforestation or the impact of the emissions of greenhouse gases. It has been reported that productivity of biofuel from the photosynthetic microorganism such as microalgae, cyanobacteria, etc., was large when the comparison was made with terrestrial plants which consist of cellulose such as grass, grains, trees, etc. Such quality for agricultural practices suggests a high yield of biomass per acre. Furthermore, energy can be stored in the lipid of algae which later can be transformed into products like diesel and gasoline with the help of advanced technologies which will play a significant role to recycle carbon (Frank et al. 2012).

8.8 Conclusion and Future Prospects

Presently, the universe is dealing with three crucial issues such as increased prices of fuels, changes in the climate and environmental pollution. Recently, issues like the high demand of energy, rapid depletion of nonrenewable sources of energy, elevated levels of pollution in the environment, etc. have to be resolved as soon as possible. To this, biofuels come to the rescue which provides the security of energy worldwide, make environment amiable, and sustainable (reduction in the emissions of the greenhouse gases), huge savings during the foreign exchange, development of rural areas, etc. For many decades, biofuels are the part of many debates of various nations but in the past years, there has been a shift in the debate and considers the increase in the prices of crude oil. Not because of the prices, there are several reasons (mentioned above) why the government has a keen interest in the development of

biofuels even though the subsidies are required for their commercialization. It is difficult to calculate the impact of the biofuels on agricultural commodities with respect to the midterm projections. Biofuels generated from the microbes have enough potential in the domain dealing with the research on the development and generation of the biofuels as an alternative energy source. Much research is required in the biology domain in order to enhance the production of biofuels via breeding energy plants, hydrolysis by using enzymes, strains to treat the wastes and to exploit during the fermentation process.

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