Chapter 8
Fungal Bioengineering in Biodiesel Production

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8.1 Biodiesel and Its Advantage

The exploitation of fossil fuel and their impact on the environment have led to seek an alternate and sustainable source of energy. Biofuels may emerge as a new source of renewable energy. Biodiesel is an alternative fuel made from biological sources. It is an attractive energy source due to its environmental benefits. Biodiesel is produced by the process of transesterification. It can be extracted from algae, bacteria, and fungi. Fungi have great capacity to accumulate the lipids intracellularly. Due to its environmental benefits and renewable resources, biodiesel has become more lucrative in recent years. Microbial oils may serve as a potential feedstock for the production of biodiesel which need further research. Biodiesel, a liquid fuel, can be obtained from biological materials such as vegetable oil and animal fats. These contain free fatty acids, phospholipids, sterols, water and other impurities. Many vegetable oils have been explored for the production of biodiesel such as palm oil, soybean oil, sunflower oil, coconut oil, and rapeseed oil (Shay 1993). The advantage of biodiesel over fossil fuel is of its low toxicity, renewability, and rapid degradation more in comparison with diesel fuel. To use biodiesel, there is no need for engine modifications (Romano and Sorichetti 2011). Biodiesel fuel has drawn attention globally as a blending part of fuel diesel in vehicles (Demirbas 2009).

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8.2 The Raw Material for Biodiesel Production

There are various raw materials which are used for biodiesel production. Two types of plant oils, i.e., edible and nonedible, have been used for the biodiesel production.

8.3 Edible Plant Oils

Currently, biodiesel has been predominantly produced from edible vegetable oils and considered as the first generation of biofuel. Biodiesel is chiefly produced from soybean in the United States, rapeseed in Canada, sunflower in Europe, palm in Southeast Asia, and jatropha in India. Due to competition with edible oils, the large-scale production of biodiesel has been of great concern (Refaat 2010). The use of edible oil for production is mainly carried out by biodiesel producers which are the European Union, the United States, Brazil, and Indonesia. The worldwide largest biodiesel producers are the United States and Brazil; they produced 6 and 4.3 billion liters of biodiesel in 2017, respectively (Fig. 8.1) (https://www.statista.com/statistics/271472/biodiesel-production-in-selected-countries). In addition to overcome edible oil, researchers have sought some other renewable resources for biodiesel production (Ahmia et al. 2014).

8.4 Nonedible Plant Oils

As nonedible plantoils are not consumed by human due to its toxic components, these plants can be cultivated on wasteland, which provides a better choice for biodiesel production (Ahmad et al. 2011). Jatropha, karanja, tobacco, mahua, neem, and castor are important nonedible oil plants.

8.5 Microalgae

Microalgae are also used as raw material for biodiesel production and are called third-generation biofuel resource. Due to rapid biomass production and a good source of high oil content, the microalgae have been recognized as good sources for biodiesel production (Rengel 2008).

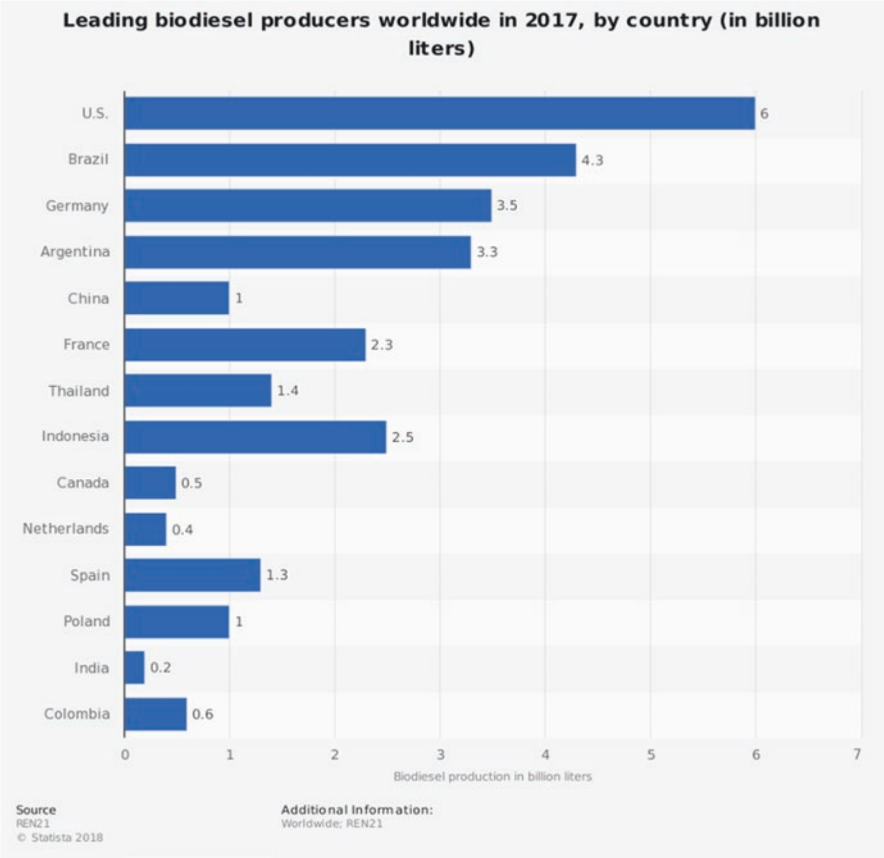

Fig. 8.1 Leading biodiesel production worldwide in 2017 (www.statista.com)

8.6 Animal Fat

The tallow, choice of white grease or lard, fish fat (in Japan), and chicken fat are an example of animal fats, which are used to produce biodiesel. In comparison with plant crops, these fats frequently offer a commercial benefit.

8.7 Biodiesel Production

Due to free fatty acids, phospholipids, sterols, water, odorants, and other impurities, the oil cannot be used as fuel directly. To overcome some chemical modification like pyrolysis, microemulsion, dilution, and transesterification have been done. Biodiesel is the mono-alkyl esters of long-chain fatty acids derived from renewable feedstocks. It is composed of fatty acid methyl esters which are formed by the triglycerides in vegetable oil by transesterification process.

8.8 Transesterification

It is also known as alcoholysis, in which the displacement of alcohol takes place (Srivastava and Prasad 2000). This process has been used for the reduction of high viscosity of triglycerides. The process is called as methanolysis if methane is used in this process. Transesterification is a reversible reaction which is continued by mixing the reactants in the presence of a catalyst. The function of catalyst is to accelerate the reaction. It may be a strong acid or base.

The molar ratio of alcohol to oil, catalyst concentration reaction temperature, and reaction time are the main factors that affect the process of transesterification. Transesterification consists of a number of reversible and consecutive reactions. The triglyceride molecule is removed in the form of glycerin during the process. The triglycerides are broken stepwise into diglycerides and monoglyceride and finally converted into methyl esters and glycerol (Fig. 8.2).

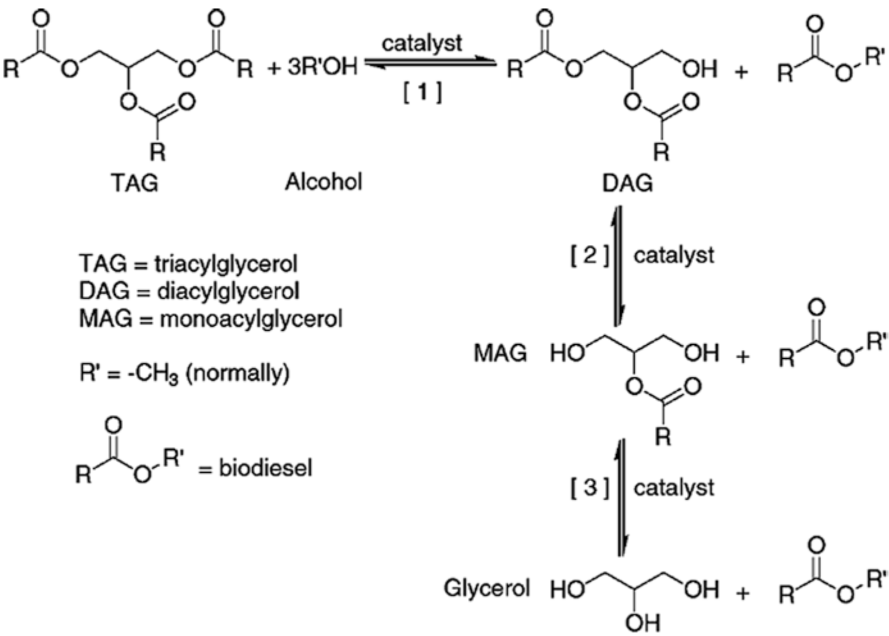

Fig. 8.2 Process of transesterification for biodiesel production (Gasahw et al. 2015)

8.9 Biodiesel Production by Lignocellulosic Biomass

Lignocellulosic biomass is the most attractive and largest biomass resource in the world that can be used as a raw material for the economic production of microbial oils. The production of ethanol and butanol has been done successfully. The major lignocellulosic biomass resources come from the residues generated by agricultural, forest, and industrial sources (Yousuf 2012). Lignocellulosic biomass can be categories into two groups (Fig. 8.3).

The use of agro-industrial residues has attracted the attention of researchers worldwide to overcome the limitations of traditional feedstock. This residual substrate is served as the nutritional source for microorganisms that accumulate lipids intracellularly. Lignocellulosic biomass is the cheapest and promising raw material to produce microbial oil from agrowaste (Huang et al. 2009; Dai et al. 2007). Lignocelluloses are complex in nature; they need pretreatment to release simple sugar. Various pretreatment techniques, physical, chemical, and biological methods, are used, depending on the properties of the substrate, pretreatment may be. Physical pretreatment involves the milling, grinding, steam explosion, sonication, etc. Chemical pretreatment can be done by acid or base hydrolysis (Lenihan et al. 2010; Taherzadeh and Karimi 2008). Enzymes produced by a variety of microorganisms are capable of breaking down the lignocellulosic materials to sugars, and it is considered as biological pretreatment.

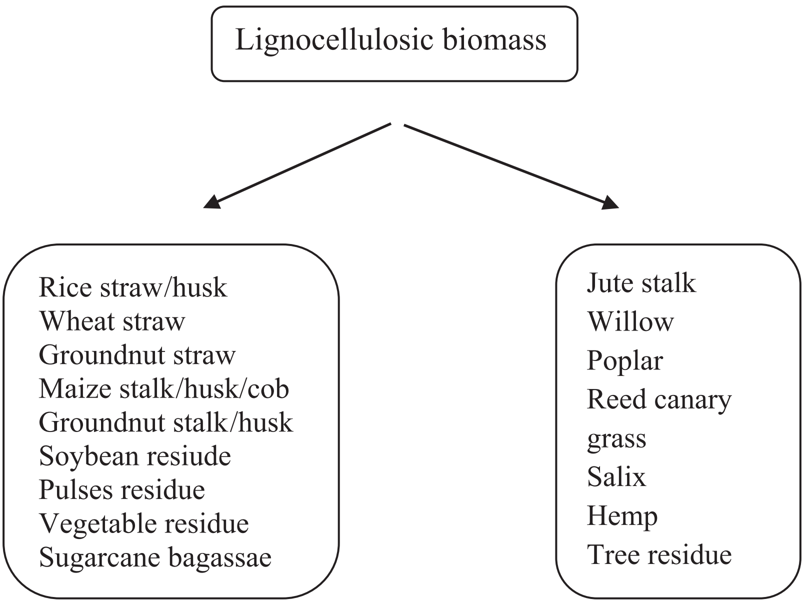

Fig. 8.3 Showing different lignocelluloses biomass for biodiesel production

8.10 Role of Fungi in Biodiesel Production

To overcome the negative impact of the biodiesel production from plants or animal resources, the use of microbes which can be easily grown can yield large amounts of lipids, like triglyceride; they have been considered as the most promising solutions (Tao et al. 2006). An oleaginous microorganism contains more than 20% of lipids of its dry weight, and it produces intracellular lipid (Mutanda et al. 2011; Meng et al. 2009). The production of microbial oil has many advantages over vegetable oils and animal fats due to fast growth of microbes, and oil requires less effort (Li et al. 2008). Several oleaginous yeasts and microalgae had been reported to accumulate as single cell oil (Aggelis and Sourdis 1997). Some lipid-accumulating yeasts are *Rhodotorula graminis* (Guerzoni et al. 1985), *Lipomyces starkeyi* (Holdsworth and Ratledge 1988), *Rhodosporidium toruloides*, *Cryptococcus albidus*, *Trichosporon pullulan*, and *Yarrowia lipolytica* (Liu et al. 2000; Liang et al. 2006). The oil accumulation in fungi depends on the carbon, nitrogen ratio, temperature, pH, oxygen, and concentration of trace elements and inorganic salt.

8.11 Advantage of Fungal Oil for Biodiesel

In microbial oil production, its cost depends mainly on the species of fungus chosen for cultivation and lipid concentration within cells. The biomass and oil productivity of oleaginous microorganisms are higher than plant oil productivity. The lipid content may be less due to slight change in the amount of sunlight. The use of low-cost and effective alternative feedstocks such as wastewater, municipal, and lignocellulosic wastes can improve the economics of single-cell oil production. In terms of a fast growth rate and productivity, oleaginous microbes are the choice for biodiesel production, as edible and nonedible oil plant farming needs lots of space and maintenance. The independence of weather provides an advantage for the microbe cultivation (Thevenieau and Marc-Nicaud 2013).

8.12 Fungal Bioengineering for Biodiesel Production

Fungal bioengineering is a part of genetic engineering that aims to either increase or decrease any metabolic product or any change in the microorganism for the production of a specific enzyme to enhance the production of the desired product. By this technique, the modification is done at the genetic level in the metabolic pathway. The desired gene can be inserted or knocked out according to the need. There are two approaches to increase the production of the biodiesel or desired product. The first way is changing the expression of specific genes to overcome specific rate-limiting steps in the target pathway that result in decreased catabolism of target compounds combined with inhibition of competitive pathways. The second way is to change the expression of regulatory genes that controls structural genes downstream of multiple biosynthesis genes. In the context of biodiesel or biofuel production, genetically modified organisms are in focus for the production of green fuel (Verpoorte and Memelink 2002). In the transformation of microbes into the desired cell factories with high efficiency of biodiesel production, genetic engineering plays a key role. In gene cloning, RNAi technology is one of the best techniques used for bioengineering of biodiesel-producing fungi and also the genetic manipulation of lignocellulosic-degrading enzymes for the production of lipid. Bioengineering stability of engineered strains and methods are an important issue. The modification of lipid degree of fatty acid unsaturation or chain length of fatty acid is the main challenge, which all are regulated by different enzymes. Fungal bioengineering involves the cloning of the gene of an enzyme, their transgenic expression, and modification of genes to achieve high microbial recombination (Kalscheuer and Steinbuchel 2003).

A number of genetic tools have been developed to transform plasmids, knock out genes, and develop both episomal and integrative expression cassettes to enable metabolic engineering approaches for the bioengineering of fungi. For biodiesel production, microbial fatty acid metabolism is of great interest for the production of biodiesel. The microorganism has been modified in various aspects by overexpression of key fatty acid and TAG biosynthesis enzymes for high-level fatty acid production (Beopoulos et al. 2011). The overexpression of three fatty acid biosynthesis genes, namely, ACC1, FAS1, and FAS2, could increase the lipid accumulation by 17%. It was reported that the deletion of glycerol 3 phosphate dehydrogenase gene (GUT2) in yeast *Y. lipolytica* can increase the lipid synthesis manyfold (Runguphan and Keasling 2014). Due to deletion of GUT2, the formation of dihydrogen acetone phosphate (DHAP) is prevented from glycerol-3-phosphate which is the cause of the overexpression of glycerol-3-phosphate dehydrogenase1 (GPD1) and synthesis of more lipids. For the improvement of biodiesel feedstock, some other lipid biosynthetic pathway genes have been explored. They are acetyl-CoA carboxylase (ACC), diacylglycerol acyltransferase (DGA), D9-desaturase (D9), and ATP citrate lyase (ACL) genes regulating various steps in the lipid synthetic pathway. Presently, the oleaginous fungus *Mortierella isabellina* has also been reported for intracellular lipid production (Ruan et al. 2013, 2014). The successful bioengineering of the fungi to produce biodiesel requires more research and a better understanding of the strain improvements.

8.13 Conclusion

There is a need to focus more on alternative substitute of the fossil fuel and green source of energy. For biodiesel production, microbial oil might become one of the potential feedstocks due to renewability, low cost, and fast growth rate. Biodiesel obtained from fungal strain by employing cost-effective experimental approach can serve as one of the alternative platforms to compensate the rising energy crisis.

Genetic modifications through bioengineering can open new doors for the performance improvement of microorganisms producing oils. Biodiesel can provide an alternative solution to the environmental pollution caused by fossil fuel combustion and meet the increasing world energy demand.

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