Chapter 9
Biofuels Production from Diverse Bioresources: Global Scenario and Future Challenges

I. Abernaebenezer Selvakumari, J. Jayamuthunagai, K. Senthilkumar, and B. Bharathiraja

Abstract Roadmaps toward bioeconomy strategy included biofuel production from sustainable biomass. This is due to the worldwide increasing environmental concerns, fast fossil fuel depletion, and the need for energy security. Although complete replacement of petroleum-derived fuels is not possible, the marginal substitution of diesel with biofuel could prolong the depletion of oil resources. The biofuel produced as an alternate energy source is currently a top priority in many nations' research and development sectors. Biofuels are produced by the fermentation process using various starch or sugar-containing feedstocks by microorganisms. Lignocellulosic biomass sources like oilseeds, oils, agricultural residues, forest wastes, paper industrial wastes, municipal solid wastes, and microalgae were potential abundant feedstocks widely used for biofuel production at low cost. This chapter mainly focuses on the diverse significant bioresources used for biofuel production, global scenario in biofuel development, biofuel policies, challenges, and future perspectives in biofuel production across the world. The first segment explains the need for biofuels, the next segment presents a detail presentation on different potential substrates used for biofuel generation and the last section deals with the current biofuel policies and concerns of biofuel.

9.1 Introduction

The concern for the world's dwindling petroleum demand and price volatility has been increasing globally. The ultimate aim of the petroleum market is to expand nominally from 50% by the next 10 years to 118 million barrels per day (mbd),

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with a lead consumption rate of 28, 16, and 15 mbd by United States, Europe, and China, respectively. In India since 1990, fuel production has raised from roughly 650 thousand barrels per day (tbd) to around 1 mbd. In the meantime, utilization has expanded from 1.2 mbd from 1990 to about 3 mbd in 2008 (Pathak et al. 2012). In a global context, various nations are mindful of their energy surveillance because of the substantial depletion of crude oil reserves and the controls of global petroleum reserves are declining continuously. Consequently, the ever-growing populace and rapid industrial growth expand the gap between the production and consumption of petroleum resources in the past few years. In India, the demand for oil is increasing annually and so the import has lifted from US$ 6 to 15 billion during 1990–2000 and to US$77 billion in 2008. Under this circumstance, different nations have stepped in suitable actions principally intended in improving energy security by endowing in renewable resources and developing policies for exploiting alternative energy sources. Thus, these uncertainties in future energy supply, unfeasible patterns of energy consumption, and the price of expanding as certain fossil fuel reserves have for various energy analysts and researchers around the world to explore alternative and renewable sources of energy such as biofuel (Dufey 2006).

Biofuels are obtained from biological components, primarily from microorganisms, plants, animals, and wastes. Every type of biofuels possesses similar basic as well as sustainable origin. Biofuels offer numerous priorities, including renewability, availability, lower CO_2 emissions, provide energy security, regional development with social structure. Biofuels have the potential to manage two major issues. At first sight, they are known to be carbon–neutral (the carbon emitted by biofuels is neutralized by the atmospheric plants by absorbing from the atmosphere while growing), renewable (surplus supplies can be grown), and suitable for being cultivated in different environments (Dutta et al. 2014). The full picture, yet, is further complex as different biofuels impose different economic, social, and environmental impacts. Due to extensive available opportunities for biomass resources, fossil-fuel-based technology could be possibly replaced by bio-based fuel technology.

9.2 Biofuel and Types

Biofuels are attributed to renewable kind of fuels integrated from biomass originated from organic matters that has been processed to play a valuable role in providing a viable energy source. Distinctive biofuels bring about a different variety of fuel types (liquid, gaseous, and solid forms) for generating energy seems as a signifying alternative energy source with related properties to petroleum fuel. The prevalent types of biofuel include biodiesel, bioethanol, and biohydrogen (Kumar et al. 2019; Rastegari et al. 2019).

Biodiesel is obtained as the result of transesterification of vegetable oils or animal fat with alcohol (methanol/ethanol) in the existence of catalytic agents that can be utilized in pure as well as blended forms with vehicle diesel. The second most common one is bioethanol, the product of fermented sugars/starch biomass is utilized in its purest form in the specially designed vehicles as well as blended form mixed with gasoline in a specific ratio and the other fuel requirements are performed corresponding to regulations. Biohydrogen is a third kind of biofuel produced by living microorganisms as the source of energy via fermentation and photolysis process in a specialized container or a bioreactor and known to be an advanced biofuel (Patni et al. 2011).

9.3 Biofuels Feedstocks

The biomass feedstock utilized in the production of biofuel can be sorted into the following groupings. The first-generation biofuels were produced from feedstocks grown for starch, sugar, and oil such as corn, barley, wheat, soybean, cassava, rye, sugar beet, sugarcane, or sweet sorghum using conventional technologies such as fermentation, distillation, and transesterification (Msangi et al. 2007). Bioethanol, an additive to gasoline are primarily produced through fermentation of starch and sugar substrates with by-products of butanol and propanol. The major advantage of bioethanol is that it burns cleaner with zero carbon emission and hence produces negligible greenhouse gases. Another important biofuel called biodiesel, produced from plant oil or animal fat through a process called transesterification in which the oil exposes with an alcohol (methanol/ethanol) in the existence of a catalyst (acid/alkali). This was followed by the distillation process in which the biodiesel separation from other by-products takes place. Biodiesel can be used as an alternative fuel in many diesel engines in a proportionate mixture of petroleum diesel and biodiesel. These first-generation biofuels symbolize a step toward energy independence and promote rural communities and agricultural industries through increased demand for crops. The first-generation biofuel production has also counter effect in contributing global price increase for food and animal feeds and have a possible negative impact on biodiversity and competition for water in several regions (Singh and Singh 2010). Additionally, they provide only a minimal advantage over fossil fuels in regards to greenhouse gases since anyhow they require a large degree of energy for feedstock cultivation, collection, and processing. Prevailing production practices employ fossil fuels for power generation in the production process of first-generation biofuels. Thus, they are a more expensive choice than gasoline, concluding it economically unfeasible.

Researchers are then aimed at promoting second-generation technologies in the production of biofuels from nonedible dedicated energy crops such as agricultural waste, forest residue, organic residue, food waste, and industrial waste (Sims et al. 2010). These feedstocks need to undergo thermochemical or biochemical pretreatment steps to unlock the sugars embedded in the plant fibers. Forest residues such as straw have to encounter thermochemical pretreatment in order to generate syngas (a mixture of carbon monoxide + hydrogen + methane). The hydrogen formed in this manner is the biohydrogen, employed as a biofuel. The biochemical pretreatment route converts the various polymeric sugars (cellulose and hemicellulose) present in the crop feedstock to sugar monomers, fermented by microorganisms to biofuels. No compete between fuels and food crops has been noted in the second-generation biofuels as they were derived from independent biomass. Additionally, they endorse the usage of poor quality land where food crops fail to grow. Recent estimates show that second-generation biofuels production costs are double the times to petroleum fuels on the basis of energy equivalence as they requires more energy and materials.

To cut down the biofuel formulation cost, the third generation feedstocks are based on distinctively engineered crops specifically algae as the energy source. The oil extracted from algal species is converted into biodiesel through transesterification process, or it can be enriched into other fuels as petroleum alternatives. This field is presently under far-reaching research toward enhancing the production as well as the separation of bio-oil from nonfuel elements and to further reduce the manufacturing costs. Algae are highly beneficial in the following manner that they can be cultivated as cheapest, immense-energy, and absolutely renewable source of energy. This can grow in municipal as well as industrial wastewater, saltwater, such as oceans or salt lakes, and can deliver the dual purpose of biofuel production along with phytoremediation. The ability of these microorganisms to develop under both oxygen consuming and anaerobic conditions has made them less demanding to move inside various cultivating modes to start biohydrogen generation (Suali and Sarbatly 2012). In this manner, the development of wastewater microalgae affords the numerous focal points in the treatment of wastewater, production of algal biomass, and greenhouse gas mitigation all the while. However, further research still needs for further extraction process in order to make it economically competitive to petroleum-based fuels. The various feedstocks used for biofuel production are tabulated in Table 9.1.

Table 9.1 Various bioresources for biofuel production

Biofuel	Country	Bioresource	References
Biodiesel Bioethanol	Australia	Sugarcane, Molasses, Wheat, Palm oil, Cotton oil	Araújo et al. (2017)
Biodiesel Bioethanol Biohydrogen	Brazil	Sugarcane, Soybean, Palm oil, Wheat straw, Vinasse wastewater	Bajpai and Tyagi, (2006), Kaparaju et al. (2009)
Bioethanol	Canada	Corn, Wheat	Araújo et al. (2017), Demirbas (2009)
Biodiesel	Malaysia	Palm oil, Waste cooking oil	Dufey (2006), Elbehri et al. (2013)
Bioethanol	Thailand	Cassava, Molasses, Sugarcane	Balat et al. (2008)
Bioethanol	Indonesia	Sugarcane, Cassava	Balat et al. (2008)
Biodiesel Bioethanol	China	Corn, Cassava, Sweet potato, Rice, Jatropha	Bajpai and Tyagi (2006), Demirbas (2009)

(continued)

Table 9.1 (continued)

Biofuel	Country	Bioresource	References
Biodiesel Bioethanol Biohydrogen	EU	Rapeseed, Sunflower, Wheat Sugar beet, Barley, Sewage manure, Food wastes,	Araújo et al. (2017), Bajpai and Tyagi (2006), Elbehri et al. (2013)
Biodiesel Bioethanol	India	Molasses, Sugarcane, Jatropha	Elbehri et al. (2013), Ghosh and Ghose (2003)
Biodiesel Bioethanol	USA	Corn, Switchgrass, Soybean, Sunflower	Demirbas (2009). Dufey (2006)

9.4 Biodiesel

Biodiesel also known as monoalkyl esters of long-chain fatty acids, supposed as a viable equivalent of conventional petroleum diesel could be derived from various renewable feedstocks, such as vegetable oil, animal fats, microbial oils, etc. These fatty acid methyl/ethyl esters are generally attained from triglycerides by the process of transesterification with respective alcohol (methanol/ethanol). In the beginning, diglycerides and alkyl esters were produced from the triglycerides, followed by the production of monoglycerides, and later biodiesel (alkyl esters) and glycerol were formed. Various catalysts were investigated for the transesterification process that includes acids, bases, both in heterogeneous and liquid forms using free as well as immobilized enzymes as catalysts (Haas et al. 2003).

9.4.1 Substrates for Biodiesel

9.4.1.1 Biodiesel from Vegetable Oil

Several plants are highly effective in transforming solar energy toward reduced form of hydrocarbons or oils. Therefore, vegetable oils have come in advance for biodiesel production due to their feasibility. The association between the composition of vegetable oils and petroleum-derived diesel fuel made the vegetable oils as a suitable substrate for biodiesel conversion (Demirbas 2009; Tiwari et al. 2007). They are made up of one glycerol to three fatty acids, so that commonly referred to as triglycerides. The vegetable oils comprise edible oils, nonedible oils, and waste/used edible oil. The selection of vegetable oil for biodiesel production depends on availability and locality.

9.4.1.2 Biodiesel from Tree Born Oils

These are nonedible oil including jatropha (*Jatrophacurcas*), castor (*Ricinus communis* L.), rubber seed (*Hevea brasiliensis*), Paradise Tree (*Simarouba glauca*), sea mango (*Cerbera manghas*), and Indian Beech Tree (*Pongamia pinnata*). One of the major limitations in converting this nonedible oil into biodiesel associates with their abundant free fatty acid (FFA) content. India is one of the leading jatropha cultivators and set aside around 1.72 million hectares of land for cultivation of jatropha and few pilot plants of jatropha biodiesel are being handed over to oil companies belonging to public sector (Bajpai and Tyagi 2006).

9.4.1.3 Biodiesel from Animal Fats

Animal fats acquired from poultry, beef, and pork and are the common substrates used for biodiesel production (Sharma et al. 2008). Researchers have also attempted to produce biodiesel from fish oil like salmon oil and animal fat residue. As it might not be cost-effective to nurture fish and different animals merely for fat, the utilization of by-products of fat residues from cattle, poultry, and hogs increase the profit of the livestock industries (Reyes et al. 2006).

9.4.1.4 Biodiesel from Microbial Oils

Microbial oils of micro- and macroalgae, bacteria, and fungi have been examined for the production of biodiesel by many researchers (Kour et al. 2019; Schenk et al. 2008; Raju et al. 2009). Microalgae are regarded as a promising candidate for biodiesel production as they are highly rich in oils (over 80% of their dry weight) (Chisti 2008; Manzanera 2011). Moreover, microalgal cultures demand minimal maintenance and could even cultivate in non-potable water, waste effluents, and water sources regarded as unfit for agriculture, and also in the seawater (Mata et al. 2010). This microalgal biodiesel production could also be connected with the greenhouse gas removal from power stations or the synthesis of several value-added products (Harun et al. 2010; Banerjee et al. 2002). Various investigations have exhibited hat the oil composition of algae obtained per hectare is 200 times higher than the fertile land crops. Thus, it is a hopeful eminence for new generation biofuels, devoid of perplexing the food supply as microalgae could be grown on nonagricultural lands. Additionally, diverse prokaryotes and eukaryotes can also incorporate an increased amount of lipids in terms of TAGs. The most important prokaryote included *Mycobacterium* sp., *Rhodococcus* sp., *Nocardia* sp., *Dietzia* sp., *Micromonospora* sp., and *Gordonia* sp., accompanying streptomycetes that incorporate TAGs in their cells as well as mycelia. Within eukaryotes, apart from microalgae, yeasts of the genera Candida (Waltermann and Steinbüchel 2010), Saccharomyces (Maity et al. 2014), and Rhodotorula (Benson et al.2014) are also the most significant candidates for the production of biodiesel. Global biodiesel production is depicted in Fig. 9.1.

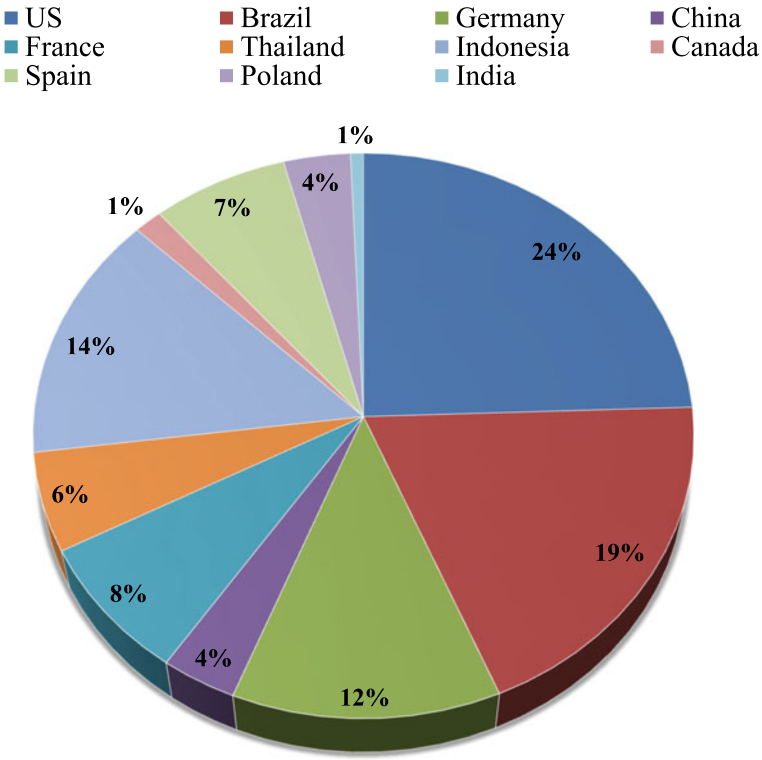

Fig. 9.1 Global status of biodiesel production till 2018

9.5 Bioethanol

Ethanol produced from renewable substrates is known as bioethanol. It is considered as eco friendly and renewable and considered to be one of the excellent substitutes for petroleum-based fossil fuels. The bioethanol producing substrates include sugar-loaded crops such as sugarcane, sugar beet, starch-loaded crops (corn and cassava), lignocellulosic residual biomass, and microbial consortia. The choice of feedstock relies on the countries' agricultural policies. Presently, around 60% of bioethanol is produced from sugar-based crops and the remaining is starch-based.

9.5.1 Substrates for Bioethanol Production

The well-known commercial technology for bioethanol production is crop based, making use of molasses, corn starch, sugarcane juice, and beet juice. As the expense of these raw feedstocks accounts for above 40% of the bioethanol production cost (Balat et al. 2008), researchers started focused on employing lignocelluloses substrates since the late 90s. This naturally abundant cheap polymer is endowed as an agricultural residue (wheat and rice straw, sugarcane bagasse, soybean residues, corn stalks), industrial wastes (paper and pulp industry), forestry residues, municipal solid wastes, etc. (Wyman 1999).

9.5.1.1 Bioethanol from Sugars

In general, the sugarcane juice and cane molasses are the chief substrates for the production of bioethanol. Brazil accounts for 79% of bioethanol production from fresh juice of sugarcane and the rest from cane molasses (Seelke and Yacobucci 2007). In India, sugarcane molasses is the major raw source for bioethanol production (Ghosh and Ghose 2003); Molasses as well as sugar beet juices are the alternate sources of fermentable substrates for bioethanol fermentation in Europe. In the large-scale industries, bioethanol is produced by*saccharomyces cerevisiae*, as it hydrolyzes cane sucrose into easily assimilable glucose and fructose. In the midst of various bacteria, the highly significant one is *Zymomonas mobilis*, yielding bioethanol around 97% of theoretical maximum with a narrow range of fermentable sugars like glucose, sucrose, and fructose. The bioethanol yield could be increased by supplementing additional growth factors that include ergosterol, soy flour, oleic acid, chitin, fatty acids, vegetable oils, and skimmed milk powder (Patil and Patil 1989; Wilkie et al. 2000; Shigechi et al. 2004; Pimentel and Patzek 2005).

9.5.1.2 Bioethanol from Starch

Starch is the best yielding feedstock for bioethanol production, but the only limitation is that the yeast *S. cereviciae* is unable to exploit it directly. Prior hydrolysis is necessary to synthesis bioethanol from starch through fermentation. Earlier, starch hydrolysis was done by acids, but later, due to the enzyme specificity at milder reaction conditions skipping the secondary reactions has made use of the amylases as catalysts for the bioethanol fermentation process. Amylase hydrolysis includes two steps, namely, liquefaction and saccharification. In the first step, the starch suspensions are subjected to high temperatures of 90–110 °C for collapsing the starch kernels. At the end of the liquefaction process, the resultant liquid contains dextrines and fewer quantity of glucose. In the next step, the melted starch is subjected to saccharification at moderate temperatures in the range of 60–70 °C through glucoamylase obtained from *Aspergillus niger* or *Rhizopus species* (Pandey et al. 2000; Kaparaju et al. 2009).

9.5.1.3 Bioethanol from Corn

In the US, bioethanol is produced solely from corn substrate. Corn is grounded for starch extraction, and also enzymatically hydrolyzed for collecting glucose syrup that was further fermented to bioethanol. Corn milling can be done by both wet and dry methods in industries. During the process of wet milling, corn grain is detached allowing the starch to convert into bioethanol and other fermented co-products. In the course of dry-milling, grains are not evenly fragmented and their source of nourishments is condensed as a distillation co-product employed as animal feed (Dried Distiller's Grains Soluble (DDGS)) (Gulati et al. 1996).

9.5.1.4 Bioethanol from Wheat

The most common method of bioethanol production in Europe is from beet molasses whereas, in France, wheat is used as a primary substrate. In order to increase the productivity and yield of bioethanol, attempts have been done for improving the fermentation process conditions. Wang et al. (1999) have optimized the temperature as well as specific gravity for the fermentation of the wheat mash and Soni et al. (2003) have determined the optimal process parameters using α- amylase and glucoamylase for starch hydrolysis of wheat bran in solid-state fermentation.

9.5.1.5 Bioethanol from Cassava

Cassava, a substitute source of starch widely preferred for bioethanol and glucose syrup production. Cassava is the tuber that grabs keen attention by various researchers as it is available abundantly in tropical countries and ranked to be one among the top ten significant tropical crops. Bioethanol could be produced using either the whole cassava tuber or the extracted starch. Starch extraction could be attained by the Alfa Laval extraction method (FAO 2004) in the industrial-scale process or through the conventional process in small- and mid-scale industrial plants.

9.5.1.6 Bioethanol from Other Feedstocks

Apart from corn and wheat, bioethanol can also be synthesized from sorghum (Prasad et al. 2007), barley, rye, triticale (Wang et al. 1997) with pretreatments. Abd-Aziz (2002) recommended the employment of sago palm for bioethanol production. Bioethanol from bananas and their peels have been investigated by Hammond et al. (1996) with commercial α- amylase and glucoamylase. The malt processing of starch-containing food wastes has been patented in 2002 (Chung and Nam 2002). Other highly assuring widely used crops for the production of bioethanol are sweet sorghum, which produces seed granules (high starch), shaft (high sucrose), leaves, and bagasse (high lignocellulosic).

9.5.1.7 Bioethanol from Lignocellulosic Biomass

Various lignocellulosic feed stocks have been approved for the synthesis of bioethanol. Generally, lignocellulosic substrates can be classified into six major groups that are involved in bioethanol production: crop residues (wheat straw, barley and rice straw, corn stover, rice hulls, pulps, olive stones, and sweet sorghum bagasse), hardwood (poplar and aspen), softwood (pine and spruce), herbaceous biomass (switchgrass, alfalfa hay, coastal Bermuda grass, reed canary grass, timothy grass), cellulose wastes (newsprint, recycled paper sludge, waste office paper,), and municipal solid wastes (MSW). However, a large extent of complexity implicit in feedstock processing is the only major limiting factor. This is associated with the nature and their distribution of lignocellulosic biomass and so the fermentation process using these substrates is quite complicated and energy-consuming.

9.5.1.8 Bioethanol from Algal Biomass

First studies as algal biofuels are concentrated on biodiesel production. However, the carbohydrates in the structure of algae made to consider as potential substrate utilized for bioethanol production after hydrolysis. Marine algae can exhibit a large amount of carbohydrates every year. Also, it is expected that algal species could meet the future biofuel demand by harvesting at short time and regarded more highly reproducible than other raw materials. Microalgae with a high amount of starch in the cell walls include *Dunaliella, Chlorella, Chlamydomonas, Scenedesmus* are widely used in bioethanol production. Like microalgae, macroalgae could also serve as potential renewable feedstock for bioethanol production. The absence or less amount of complex lignin molecules in their structure, simplifies the hydrolysis treatment process (Araújo et al. 2017). The fermentation of algal polysaccharides such as starch, sugar, and cellulose yields bioethanol and the carbohydrate content (mostly starch) of microalgae can be enhanced up to 70% under specific conditions. Global bioethanol production is depicted in Fig. 9.2.

9.6 Biobutanol

Butanol ($C_4H_{10}O$) contains a higher number of hydrogen as well as carbon related to ethanol (Ramey 2004). In the early twentieth century, Chaim Weizman identified a bacterial strain able to produce promising amounts of acetone as well as butanol and was labeled as *Clostridium acetobutylicum.* Biobutanol could readily intermix with gasoline and other hydrocarbons, and also possess extended heat energy, not as great corrosive as ethanol, and can be conveyed across functioning pipelines and fueling stations. 85% butanol–gasoline-blended mixture could be employed directly in automotive engines and is regarded as minimal evaporative compared to both gasoline and ethanol. Thus butanol is highly safer to utilize and generates mild

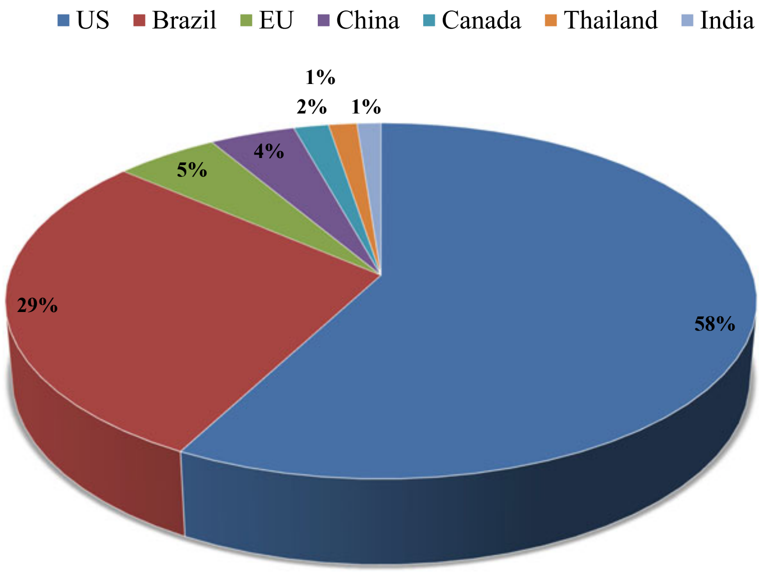

Fig. 9.2 Global status of bioethanol production till 2018

volatile organic compound (VOC) emissions (Qureshi et al. 2005). It is composed of 22% oxygen that originating it as an environmentally friendly fuel that burns cleanly and produces only carbon dioxide.

9.6.1 Substrates for Biobutanol Production

Biobutanol productive microorganisms can make use of an extensive variety of carbohydrates containing glucose, lactose, fructose, arabinose, mannose, sucrose, starch, xylose, dextrin, and inulin obtained from raw fermentable substrates, namely, whey refine, sugar beet, maize, wheat, millet, oats, rye, paper industry residues such as Jerusalem artichoke, and sulfite waste liquor. The ability of microbes to ferment all these kinds of carbohydrates as well as cellulosic substrates (pentose sugars) including xylose and arabinose makes it feasible to utilize variety of agricultural feedstocks such as agricultural residues, woody biomass, and energy crops.

9.6.1.1 Biobutanol from Cane Molasses and Whey Permeate

Just as feedstock, sugarcane molasses posses' higher superiority than maize together with effortless handling and contains simple sucrose molecules that could be easily broken down by solventogenic Acetone–Butanol–Ethanol (ABE) clostridia in accordance with the conversion of sugars into biobutanol. Whey permeate is another valuable feedstock approximately with 45–50 g/l lactose. Butanol synthesizing microbial cultures can easily hydrolyze lactose into assimilable sugar without any additional enzymes (Maddox et al.1993). Soya molasses could also use as a potential substrate for the fermentation of butanol. It contains relatively 745 g carbohydrates per kg of which 58% (434 g/kg) are easily fermentable into galactose, glucose, fructose, and sucrose (Jesse et al. 2002). Sugars like verbascose, raffinose, pinitol, stachyose, and melibiose are incapable of fermentation by *Clostridia,* and therefore require prior enzyme or acid hydrolysis. In addition, fruit industry wastes and contaminated maize also have been illustrated as a useful substrate for biobutanol fermentation (Qureshi et al. 2001).

9.6.1.2 Biobutanol from Starch

In the consideration of *Clostridia,* it could efficiently hydrolyze starch in the range of 45–48 g/l, potatoes and their wastes have been examined for biobutanol production. To investigate the effect of hydrolysis prior to fermentation, each of two hydrolyzed and unhydrolyzed potatoes were subjected to fermentation. The starch content of unhydrolyzed potato yields 12 g/l ABE, much as hydrolyzed potato produced 10–11.4 g/l ABE indicating that hydrolysis is not strictly required for ABE fermentation. In addition, maize starch also could be easily bioconverted to ABE.

9.6.1.3 Biobutanol from Lignocellulose

Besides all the previous traditional fermentable substrates, there exist few promising lignocellulosic feedstocks including switchgrass, maize fiber, maize stover, rice straw, wheat straw, barley straw, corn cobs, hemp waste, DDGE, and sunflower husks usable for ABE generation. Maize fiber, a maize residual obtained during the wet milling process contains 60–70% carbohydrates and produces around 25% of butanol by the fermentation process. Ezeji and Blaschek (2008) employed hydrolyzed DDGS for the biosynthesis of biobutanol using *C. beijerinckii* P260*, C. beijerinckii* BA101*, C. sacchrobutylicum* P262*, C. acetobutylicum* 824*, and C. butylicum* 592*.* Pretreated wheat straw in dilute (1%, v/v) sulfuric acid followed by enzyme hydrolysis is used as a potential substrate for the bioconversion of ABE using *C. beijerinckii* P260. Switchgrass is another energy crop that can also be applied for the generation of biofuels including ABE. In order to minimize the utilization of food and feed-grade fermentable substrates such as rye flour and molasses, efforts were made by researchers to employ agricultural residues such as corn cobs, and sunflower shells. They are rich in fermentable pentose and reduced hexose sugars and are easily hydrolyzed by dilute sulfuric acid at reaction temperatures extending from 115 to 125 °C. The substantial corn cob hydrolysis and fermentation for the synthesis of biobutanol using *C. acetobutylicum* was demonstrated by Marchal et al. (1992).

The Jerusalem Artichoke juice was also used by several researchers for biobutanol production.

9.6.1.4 Microbial Production of Biobutanol

Biobutanol can be produced by anaerobically by solventogenic Clostridia; the rod-shaped, spore-forming Gram-positive bacteria which can ferment diverse feedstocks from monosaccharides made up of pentoses and hexoses sugars (Jones and Woods 1986). Besides various solventogenic Clostridia, *Clostridia beijerinckii, Clostridia acetobutylicum, Clostridia saccharobutylicum,* and *Clostridia saccharoperbutyl acetonicum* are the dominant solvent producers. On the other hand, the biobutanol yield is certainly minimal as two moles of CO_2 is derived from per mole of glucose with the formation of various by-products, namely ethanol, acetic acid, acetone, butyric acid, and gaseous hydrogen with solvent toxicity. Considering the yield enhancement and solvent tolerance, genetically engineered recombinant microbial strains would be established (Chen et al. 2013). However, many strains displayed no makeable increase in biobutanol yield except in few hyper-butanol producing strains, such as *Clostridium beijerinckii* P260 and *C.beijerinckii* BA101. (Ezeji and Blaschek 2008). *Clostridia strain* TU-103and *Clostridium cellulolyticum* are capable of direct fermenting cellulose to biobutanol by the anaerobic process without any pretreatment (Qureshi and Blaschke 2005). Some genetically engineered non-Clostridial stains such as *Saccharomyces cerevisiae* BY4742*, Ralstoniaeutropha* H16, *Escherichia coli, Bacillus subtilis* KS438*, Pseudomonas putida* S12 can convert various substrates to biobutanol with superior solvent tolerance (Schenk et al. 2008). For instance, between the non-Clostridial strains*, P. putida* S12 can manage solvent concentration up to 6% (v/v). Microalgal species, such *as Dunaliella, Chlorella, Spirulina, Chlamydomonas,* and *Scenedesmus* are known to possess high amount (>50% of the dry weight) of starch, cellulose, and glycogen that could be used as potential raw material for biobutanol production (Surriya et al. 2015).

9.7 Biohydrogen

Hydrogen is one among the most assuring alternative forms of energy carriers. Similar to electricity, hydrogen is not regarded as a primary form of energy but considered as a secondary source of energy produced from natural as well as bioresources. Hydrogen is witnessed to be a clean fuel with zero toxic emissions as well as been regarded as the future energy source practiced in the fuel cells for the electric current generation. Utilization of hydrogen in the automotive sector either as fuel in combustion engines or fuel cell in electrical energy has earned benign consideration in an energy policy issue (Sorda et al. 2010). Hydrogen utilization is highly eco friendly as it is devoid of noxious gas as well as CO_2 emission whereas the only co-product formed is water vapor. Thus vehicles running by hydrogen energy remarkably decrease the dependency on fossil fuel in the near future.

9.7.1 Substrates for Biohydrogen Production

9.7.1.1 Biohydrogen from Biomass

Wood substrates are the earliest scheme of energy employed by humankind. Wood biomass, agricultural crops and their residues, animal and municipal solid waste (MSW), food industrial wastes, aquatic plants, and algal species are the common potential biomass sources used for biohydrogen production. Biological as well as thermochemical approaches are the major processes in biohydrogen production. Hydrogen can be produced by thermochemical processes via gasification (supercritical water gasification (SCWG) and steam gasification), steam reforming of bio-oils and pyrolysis. The advantage of the thermochemical process is highly economic and highly efficient (up to 52%) (Zhou and Thomson 2009). Indeed, dark fermentation, photo-fermentation, biophotolysis of water by the aid of algal species, and developing hybrid reactor systems are the common biological hydrogen production processes. To establish biomass-based fuelling processes, the chemical, as well as organic composition of biomass employed in the fermentation process, should be scrutinized. Cellulose (40–50%), hemicelluloses (25–30%), lignin (15–20%), and extractives are the four primary components of all lignocellulose biomass and the estimated molecular weights of first three substrates are relatively high, whereas the last one is limited with minimal quantity (Mofijur et al. 2015).

9.7.1.2 Biohydrogen from MicroOrganisms

Investigations on biohydrogen producing anaerobic bacteria initiated in the 1980s and have been expanded due to its environmentally friendly characteristics. The widely known hydrogen producers are cyano-bacteria, anaerobic bacteria, and fermentative bacteria.Hydrogen generating microalgae include *Chlamydomonas reinhardtii, Chlorella fusca, Platymonas subcordiformis, Chlorococcum littorale,* and *Scenedesmus obliquus* were reported under direct biophotolysis method of biohydrogen production (Philipps et al. 2012; Mussgnug et al. 2010). So far, numerous studies were reported on the biological synthesis of hydrogen by the dark fermentation process using facultative (e.g., *Escherichia coli, Enterobacter cloacae, Enterobacter aerogenes,* and *Citrobacter intermedius*) and obligate anaerobic bacteria (e.g., *Ruminococcus albus, Clostridium beijerinckii,* and *C. paraputrificum*). There exists a substantial consent in employing mixed microbial consortia as a biocatalyst and feasible choice for scale-up of biohydrogen production chiefly with wastewater as the carbon energy source substrate (Sambusiti et al. 2015). This technique is widely approved because of the simple operation, stability, security, distinct biochemical

Table 9.2 Microbial strains used in biofuel production

Biofuel type	Microbial strain	Yield (g/g consumed feedstock)	References
Biodiesel	*Acinetobacter calcoaceticus*	0.69	Tiwari et al. (2007)
Bioethanol	*S. cerevisiae*	0.38	Shigechi et al. (2004)
Bioethanol	*E. coli*	3.87	Seelke and Yacobucci, (2007)
Biodiesel	*Zymomonas mobilis*	1.33	Waltermann and Steinbüchel (2010)
Biodiesel	*Clostridium thermocellum*	0.84	Surriya et al. (2015)
Biobutanol	*Clostridium acetobutylicum*	9.2	Solomon and Bailis (2014)
Bioethanol	*Clostridium beijerinckii*	1.8	Qureshi et al. (2001)
Bioethanol	*Bacillus coagulans*	0.33	Pathak et al. (2012)
Bioethanol	*Thermoanaerobacter mathranii*	3.48	Shigechi et al. (2004)
Bioethanol	*Coriolusversicolor*	2.96	Patni et al. (2011)
Biodiesel	*Mucor circinelloides*	6.45	Singh and Singh (2010)
Biobutanol	*Synechococcus elongatus*	0.45	Ramey (2004)
Biodiesel	*Botryococcus braunii*	1.2	Qureshi et al. (2001)
Bioethanol	*Chlamydomonas reinhardtii*	1.94	Philipps et al. (2012)
Biodiesel	*Scenedesmus dimorphus*	1.53	Prasad et al. (2007)
Biohydrogen	*Carboxydothermus hydrogenoformans*	1.32 ml H_2/L/h	Araújo et al. (2017)
Biohydrogen	*Nannochloropsis*	0.6 ml H_2/L/h	Schenk et al. (2008)
Biohydrogen	*Rhodopseudomonas faecalis*	2.76 ml H_2/L/h	Raju et al. (2009

functions, and the possibility of adopting an extensive range of substrates serving the dual purpose of biohydrogen generation as well as wastewater treatment. Various microbial strains involved in biofuel production are tabulated in Table 9.2.

9.8 Worldwide Biofuel Scenario

At present, Asia's best biofuel producers are Malaysia, Philippines, Indonesia, China, Thailand, and India. Malaysia produces biodiesel majorly from palm oil, after all, several researchers have been focusing on Jatropha for large-scale production. Malaysia and Thailand have established their first commercial plantation in the

1960s. Malaysia accounts for 0.5 million tons of waste cooking oil production annually and a mild refining and conversion process of this oil can simply be converted into high-value biodiesel. Thailand established around eight hundred gas stations marketing B-5 biodiesel in 2007. This even progress of Indonesia and Thailand were chiefly as long as the opportunity to utilize a different variety of feedstock. For bioethanol production in Thailand, the major feedstocks are cassava, molasses, and sugarcane and in Indonesia, sugarcane and cassava. Conversely, Malaysia focused on palm oil for biofuel production which made them more liable to the price fluctuations of petroleum as well as palm oil.

Poland is the only country favorable for cultivating oilseed rape among the newer producers due to the plentiful availability of agricultural lands and suitable climatic conditions. It is a net biofuel merchant (Kondili and Kaldellis 2007). In Lithuania, only two pilot-scale plants are in working in which one for the production of biodiesel and the other for the production of bioethanol and restricted their biofuel production for domestic purposes. Romania is recognized as a net sponsor of bioethanol by exploring excellent research in fuel processing as well as biofuel production using various feedstocks (Kondili and Kaldellis 2007).

Due to increased oil cost, Brazil started to develop sugarcane-based bioethanol and has become the most likely example of profitable utilization of biomass for bioenergy production. A great deal of experience in bioethanol production from sugarcane has driven Brazil the most leading producer worldwide. In 2001, South American countries like Peru and Colombia have enforced new law in order to promote the production and consumption of bioethanol derived from sugarcane which declared that the composition of gasoline should comprise 10% ethanol by 2009, with a progressive increment up to 25% in the next 20 years (IPS 2006). They are presently producing about 1,050 million liters of bioethanol per day and investigating diverse alternate substitute sources includes cassava and sugar beets for production of bioethanol. Their interest is not only focused on accomplishing the nation's demand for biofuel but also in the attainment of chances for biofuel export (Dufey 2006). Australia is performing a powerful position for bioethanol utilization within their transporting system (Dufey 2006). Colombia encouraged significant investment since 2005 in the biodiesel production by announcing an imperative demand of 5B biodiesel in their automotive fuel. In the United States of America, soybeans-based biodiesel production elevated from 284 million liters to 950 million liters in 2005–2006 (UNCTD 2008). In April 2006, Argentina endorsed the "Biofuels Act", which demands a 5% demand of biodiesel in petroleum by-products from January 2010 that require 60,000 tons of biodiesel annually for the indigenous market (IPS 2006).

The report of International Energy Agency "World Energy Outlook 2007" suggests that the global energy requirement would be 50% greater by the next 10 years than today. In this scenario, India and China were exclusively supposed for 45% of the increment in fuel demand. The Indian Ministry of Petroleum and Natural Gas has initiated the first stage of the Ethanol Blended Petrol (EBP) Program on 2003 that authorized 5% blending of ethanol in gasoline for 9 states out of 29 and 4 union territories out of 6 (Su et al. 2015; Khanna et al. 2013). As for India, the production of biodiesel was chiefly concentrated on nonedible crop oils such as Jatropha, Neem, Karanja, and Mahua. In China, currently, 80% fuel class ethanol was produced from corn, and remaining from wheat. They use the inferior quality corn for fuel-grade ethanol production to prevent the food stock. Sweet sorghum and cassava are used on an experimental basis and the fuel class ethanol production and marketing is reserved by state resident companies (Mofijur et al. 2015). There are six promising biofuel feedstock, i.e., corn-derived ethanol; sweet sorghum-derived ethanol; cassava-derived ethanol; Jatropha-derived biodiesel; soybean-derived biodiesel; and used cooking-oil-derived biodiesel. Chinese method of biodiesel production is marginal compared to the production of ethanol and their biodiesel production estimated to roughly 300,000 metric tons annually based on waste vegetable oils or animal fat (Elbehri et al. 2013). Various biofuel policies across the world are shown in Table 9.3.

Table 9.3 Biofuel policies across the world

Country	Timeline	Action	Economic measures	Impact
China	November 2018	10% blending mandates in some regions of the country	Tax exemption for biodiesel from animal or vegetable oil and Used cooking oil	Launched the world's first coal-to-ethanol production facility and signed a $100 million agreement of intent to jointly construct about 100 municipal solid waste-to-bioethanol plants by 2035
Japan	January 2017	Upper limits for blending are 3% (ethanol) and 5% (biodiesel)	Subsidies for bioethanol production and tax exemptions	Aim for 10,000–20,000 L of bio-jet fuel production in 2020
Indonesia	August 2016	Target for 30% blending in the transport fuel supply in 2025	Providing biofuels subsidies to producers and also support the domestic agricultural economy to mitigate climate change	The blending mandate B20 program was established domestically
Philippines	July 2006	Diesel: 1% coconut blend; 2% by 2009 Ethanol: 5% by 2008; 10% by 2010	Tax exemptions and priority in financing for biodiesel and bioethanol producers	Stop the sale of biofuels and biofuel-blended gasoline and diesel that are not in conformity with the specifications

(continued)

Table 9.3 (continued)

Country	Timeline	Action	Economic measures	Impact
Thailand	December 2018	Target to increase the current blend from 7% to 10 or 20%	No import tariff for biodiesel greater than B30 and up to and including B100	The government has raised the second large biodiesel plant in 2018, adding 210 million liters per annum to its current 450 million liters production capacity
India	December 2009	Blending 5% ethanol in gasoline in designated states in 2008, to increase to 20% by 2017	Ethanol and diesel: set minimum support prices for purchase by marketing companies	Conversion of surplus grains and agricultural biomass helps in price stabilization
Malaysia	December 2018	7% blending mandates	Plans to subsidize prices for 7% blended diesel	The use of palm oil would be subsequently reduced to zero by 2030

Sources Mofijur et al. (2015), Solomon and Bailis (2014), Pathak et al. (2012)

9.9 Challenges

The main challenges with first- and second-generation feedstocks include (i) threatening the food security, (ii) excess land requirement as well as farming inputs, (iii) high capital investment (Patni et al. 2011), (iv) little net energy benefits, (v) superior allegations over gaseous emission reductions (Solomon and Bailis 2014). The challenges regarding the land allotment for the cultivation of nonedible oil crops intend to be done on “wastelands” in the forest and nonforest areas but the definition of “wasteland” is not clear till now. However, according to few nations policy-makers and rulers, the term ‘wasteland’ means ‘the uncultivated land that did not offer revenue to the government’, i.e., semi-jungle lands, drylands, and wetlands (Zhou and Thomson 2009). In India, there is no agreement of mutual understanding among policy-makers regarding the vacancy of sufficient wasteland for the cultivation of biofuel crops to satisfy the future demand for driving fuel. The existing preferable crop was Jatropha. In the favor of reaching the aspiring target of B-10 biodiesel mandate, Indian government had assured to plant Jatropha on 11.2–13.4 million hectares area by 2012. Prominently, recommending suitable land allotments for Jatropha cultivation is one of the prime concerns in Indian biofuel production (Goswami and Choudhury 2015). Khanna et al. (2013) stated that no order available for the division of wasteland suitable for Jatropha cultivation for biofuel production in India. Further, it is concluded that policy-makers failed to consider farmers while framing decisions. Widely, Indian farmers cultivated Jatropha as a fence crop and certain farmers were objected for Jatropha plantation as a monoculture. Meanwhile, few farmers who grow Jatropha were extremely upset because of reduced productivity and profits.

Another major limitation includes the diverse tax structures, i.e., dissimilar state tax policies that vary from state to state. Raju et al. (2012) reported that though each state admits its own custom tax, biodiesel is excluded from 4% central excise duty as a marketing incentive. Researchers so far identified more than 400 species of nonedible oil seeds for biofuel production, but the feasible experiments affirmed a limited feedstock source. Various research communities are experimenting in the developing genetically improved eminent yielding nonedible plants and microbial species, but of limited success rate (Koçar and Civaş. 2013). Many National Policies on Biofuels did not establish their laws within the stipulated period likewise; very few voluntary institutions have been scheduled to accomplish the importing profits to farmers for gaining the carbon credits. Consequently, it is certain to focus the consequences through the country's traditional or novel mechanisms.

9.10 Conclusion and Future Prospects

In the future, biofuel will be the only possible option that plays a promising role in meeting the energy requirements of the world. To meet this large energy demand, the abundant raw material source is the typical need. Each generation of biofuel has its own pros and cons. Therefore, if a country has to evolve with satisfactory biofuel production, the dominant indigenous biofuel crops are essential to be planted within the country aside from influencing the food supply. Several standardization and promotional actions should be employed for the replacement of conventional fuels. It has been well approved globally that biofuel would serve as an energy source to meet the nation's energy security and it is solely a matter of time before they are added on the market than petroleum fuels. The expansion and application of biofuels still need progressive technological development, to extend its utility by upgrading the energy balance, lessening the noxious emissions, and manufacturing cost, so that the purpose of biofuels' future scheme as true alternatives will be accomplished.

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