Chapter 10
Production of Bioethanol from Mixed Lignocellulosic Biomass: Future Prospects and Challenges

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Abstract The demand for alternative fuels like biofuels, which are produced from lignocellulosic biomass, is increasing day by day due to the depletion of natural resources continuously and rapidly, which is a major cause of global warming. So, cheaper and more sustainable biofuel production, especially bioethanol, which can be used as a substitute, forest materials, energy crops, agricultural residues, and municipal solid waste are used as raw materials for bioethanol production. While biochemical conversion of raw material into bioethanol involves the process of pretreatment followed by hydrolysis and fermentation, most of the work has been done on the second generation of bioethanol, and this chapter also elaborates on lignocellulosic biomass as having a key role in producing second-generation bioethanol (second-generation bioethanol). In the future, research is being conducted to produce third-and fourth-generation bioethanol from algae and genetically engineered plants, respectively. This chapter demonstrates and highlights the key role of lignocellulosic material in producing ethanol and its importance in the future.

10.1 Introduction

Natural fossil fuels hold the world's economy. But recently, diesel and petrol-like energy sources are going to be depleted due to an increase in population, technological devices, and transportation. This could lead to an increase in fossil fuel demand and its price (Haq et al. 2016). Thus, there is a dire need for non-conventional alternate bioresources to meet the energy demand and reduce the depletion of fossil fuels and global warming (Demain et al. 2005; Hill et al. 2006; Lin and Tanaka 2006; Ragauskas et al. 2006). Therefore, fuel produced from other sources, like plant biomass, is termed "biofuel" (Chiaramonti 2007). Bioethanol, bio-methanol, bio-hydrogen, biodiesel, and biogas come under biofuels (Balat 2008). Bioethanol is the most sustainable, renewable, and easily producible fuel

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among all of these, which is considered a substitute for gasoline (Tamburini et al. 2011).

10.2 Generations of Biofuel

There are three types of raw materials that are used to produce bioethanol. These include sugar/starch, lignocellulose-based materials, and algae. Based on raw materials, bioethanol is classified. Bioethanol is referred to as "first generation" if sugar and starch are used as raw materials for fermentation. "Second-generation" bioethanol is referred to as the one that is produced from lignocellulose-based material. "Third-generation" bioethanol is produced using corn cobs as a raw material. It is an emerging one (Nigam and Singh 2011; Sahoo 2016). Biofuel has been produced by various technologies or methods like thermal processes, biochemical and chemical processes of the fourth, third, second, and first generations.

10.2.1 First-Generation Biofuels

The bioconversion of sucrose and vegetable oils into bioethanol and biodiesel and crop plants containing energy-rich molecules like sugars, oils, and cellulose is called the first-generation biofuel (McAloon et al. 2000). It has a bad impact on food and fuel yields. Besides this, there was a need to introduce non-food feedstock to overcome all types of crises; including food, cost, and energy quality for the transport sector as well (Chiong et al. 2018).

10.2.2 Second Generation Biofuels

Second-generation biofuels are being produced from lignocellulosic material and residuals, and thermal processes like hydrotreating are being used for synthetic fuel production. The product of second-generation biofuel is under development and varies with the first-generation fuel product. It requires pretreatment as well as hydrolysis steps (Maeda et al. 2013*). The study has shown that developing the efficient hydrolysis process and the efficient enzymes for this purpose is the key step for second-generation bioethanol (Scordia et al. 2010*).

10.2.3 Third-Generation Biofuels

The third generation of biofuels is the production of fuel from algal biomass (Mielenz 2001). Currently, researchers are focused on the improvement of the metabolic production (Brennan and Owende 2010) of fuels and the process of separation in bio-oil production to remove non-fuel components and minimize the costs (Sun and Cheng 2002).

10.2.4 Fourth-Generation Biofuels

The fourth generation of biofuels is based on photobiological solar fuels and electrofuels, which are expected to cause changes in biofuels. Through emerging technology, this solar biofuel could be a wonder (Scaife et al. 2015) by which solar energy is directly converted into biofuel using cheap, inexhaustible, and easily available raw materials. For such advanced technology, synthetic biology is considered to be the best (Cameron et al. 2014*). Solar biofuel production from cyanobacteria has also been possible (Scaife et al. 2015).

Sugar and starch-based raw materials-based bioethanol increases food scarcity and food prices. So, the renewable nature of lignocellulosic biomass is widely used for the production of bioethanol (Asgher et al. 2013; Asgher et al. 2011). Globally, 10–50 billion tonnes of lignocellulosic biomass are produced each year (Srivastava et al. 2015). Pretreatment, hydrolysis, fermentation, and distillation are the four steps in the production of bioethanol from lignocellulosic material (Srivastava et al. 2015; Xiao et al. 2012). The quality of bioethanol produced depends upon the type of sugar source and also the method of pretreatment.

Similarly, acid pretreatment yields more glucose as compared to xylose (Demirbaş 2005; Mood et al. 2013).

10.3 History

The production of bioethanol has exponentially increased from 200 million gallons (1982) to 2.9 billion gallons (2003). Then, in 2009, production was extended to 11 billion gallons. The US became the world's leader in biofuel production in 2010, by producing approximately 13.5 billion gallons (Renewable Fuels Association 2010). In 2014, 24.5 billion gallons of bioethanol were produced globally, and this amount increased from the 23.4 billion gallons of bioethanol produced in 2013 (Demirbas and Balat 2006).

10.3.1 Lignocellulosic Biomass

Lignocellulosic biomass available for bioethanol production is categorized into three types:

- Primary sources (crops or key products)
- Secondary sources (residues from production processes)
- Tertiary sources (municipal solid waste)

10.3.2 Energy Crops

It includes crops such as perennial grasses and other delicate crops or key products like short-rotation energy plantations and sugarcane.

10.3.3 Forest Materials

It includes forest products like hard and softwood, bark thinning residues, pruning, and sawdust. Softwood originates from pines, firs, and spruces.

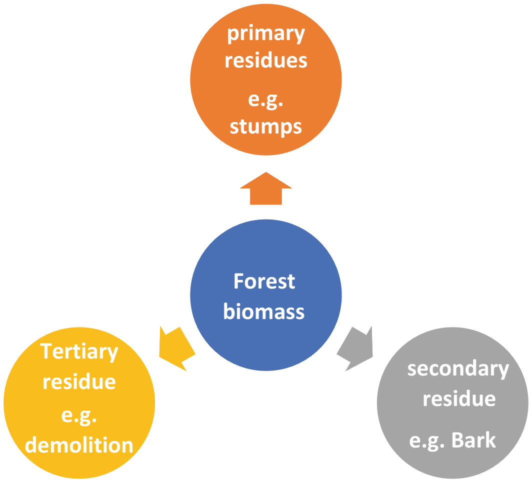
They are gymnosperms, while hardwoods originate from maples, oaks, and birches, and they are angiosperms (Bond 2002). A large amount of bioethanol is produced from forest materials. Approximately 370 million tonnes of woody biofuel are mass-produced in the USA annually (Perlack 2005). Sawdust, branches, wood chips, bark, and stumps are used for ethanol production (Amarasekara 2014).

10.3.4 Agricultural Residues

It is the most common way of producing bioethanol in many countries. It includes agricultural residues such as corn stover, rice husk, straw, wheat straws, and sugarcane bagasse [24]. Approximately 25–35% more hemicellulose content is available in agro-waste as compared to crop biomass (Demirbaş 2005).

10.3.5 Organic Portion of Municipal Solid Wastes

Bioethanol can be produced from industrial and municipal waste. It is an effective and inexpensive method of bioethanol production (Shi et al. 2009). On the other hand, household by-products and organic waste can be easily utilized before disposal in the environment (Khanna 2011).



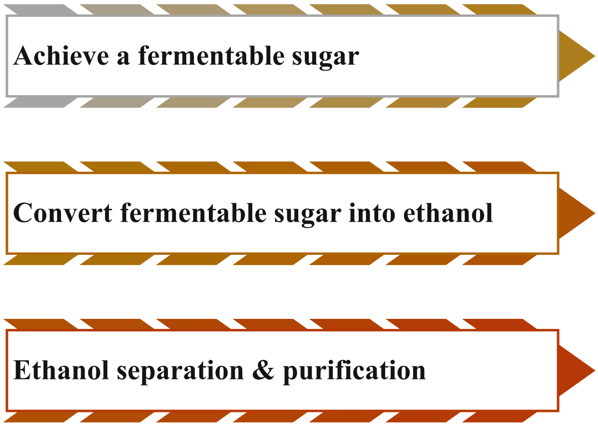
10.3.6 Marine Algae

It is used to produce third-generation bioethanol. Refinery expansion also helped to produce biofuels, mostly bioethanol. It is an effective and suitable raw material [28]. According to a study, it is estimated that approximately ten times more ethanol is produced per corn growing area (Ferrell and Sarisky-Reed 2010).

10.4 Lignocellulosic Molecular Component

Lignocellulosic biomass is composed of cellulose, hemicellulose, and lignin. In addition to these, lignocellulosic biomass consists of water, protein, and lipids (Edye and Doherty 2008).

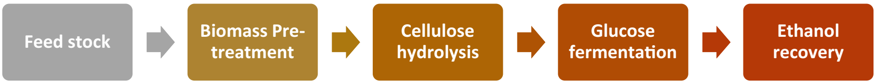
About 70% of the whole biomass is composed of cellulose and hemicellulose. They are resistant to chemical or physical treatment due to their connection with lignin through covalent bonds (Balat et al. 2008; Edye and Doherty 2008).



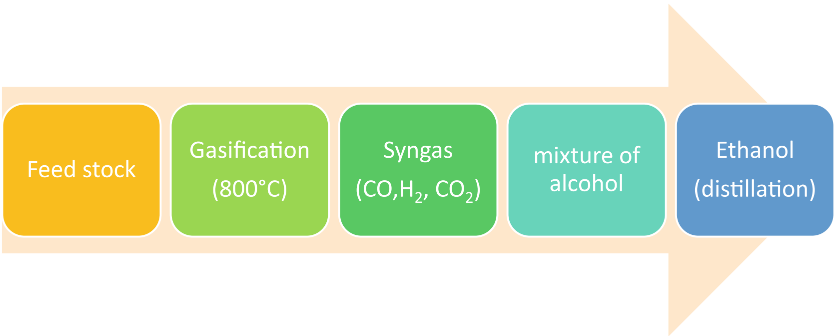
Steps involved in ethanol production from biomass

10.4.1 Routes of Bioethanol Production

Bioethanol can be produced from lignocellulosic biomass in two ways. Thermo-chemical conversion and biochemical conversion (Demirbas 2007).



Biochemical conversion



Thermochemical conversion

Biochemical route is most widely used today for ethanol production.

10.5 Pretreatment

Pretreatment is the first step in the conversion of lignocellulose-based materials to ethanol. This step helps to remove the hemicellulose and lignin so that the cellulose content is available for enzymatic hydrolysis. Pretreatment steps help to increase the yields of fermentable sugar. Pretreatment is furthermore categorized into different broad groups:

- Physical method
- Chemical method
- Biological method

10.5.1 Physical Method

The physical method involves pyrolysis, mechanical methods, thermolysis, milling, and irradiation. Generally, all physical methods work by decreasing the degree of polymerization of cellulose by increasing the surface area and pore volume of biomass (Szczodrak and Fiedurek 1996).

- **Extrusion Method**

  It is a physical method that involves the subsequent heating, mixing, and shearing of biomass when it passes through the extruder. To increase the availability of carbohydrates to enzyme action, barrel temperature and screw speed help to disrupt the structure of biomass and result in short fibers (Kang et al. 2013).
- **Freeze Pretreatment**

  It is a unique physical approach that helps improve the enzyme digestibility of feedstocks like rice husk. The advantage of this method is that it has less impact on the environment. But this technique is not widely used due to its cost.
- **Microwave Pretreatment**

  It is an easy to implement and effective physical method. This method helps to change the structure of the feedstock and degrade hemicellulose and lignin so that cellulose is available for further processing (Chang et al. 2011).

10.5.2 Chemical Methods

Chemical pretreatment involves various methods like acid pretreatment, alkaline pretreatment, organosolv pretreatment, and ozonolysis.

- **Acid Pretreatment**

  The conditions under which acid pretreatment is used depend upon the type of substrate (Dagnino et al. 2013). This method is used to remove lignin and solubilize hemicellulose content in lignocellulosic biomass. For this, various acids like sulfuric acid (H_2SO4), hydrochloric acid (HCl), nitric acid (HNO_3), and phosphoric acid (H_3PO_4) were used in varying concentrations. They can be used either in concentrated or in diluted form, but the diluted form is preferable.

10.5.2.1 Dilute Acid Hydrolysis

Hydrolysis by dilute acid is an old technique, and it is done at high temperatures and pressures. Temperature ranges exist from 200 to 400 °C. Sulfuric acid is mostly used for this purpose. 0.73% sulfuric acid is used for this purpose. This method has an advantage over concentrated acid hydrolysis, as it does not corrode equipment as acid hydrolysis does (Gírio et al. 2010). However, there is a disadvantage to using this method in that it produces cell wall inhibitors.

10.5.2.2 Concentrated Acid Hydrolysis

It is an effective method of hydrolysis because it produces 80% of free sugar. And it also produces a lower number of cell wall inhibitors. This method was performed at moderate temperatures (Zhang et al. 2007). But despite the major advantage of this method, there is a drawback that a large amount of acid is required to perform this method, so it is not cost-effective (Hamelinck et al. 2005).

- **Alkaline Pretreatment**

  The most widely used alkalis are sodium hydroxide (NaOH), potassium hydroxide (KOH), calcium hydroxide ($Ca(OH_2)$), and ammonium hydroxide (NH_4OH). This method of chemical pretreatment takes place at room temperature and ranges from a few seconds to days. To improve yield for the next step, calcium and sodium hydroxide are mostly preferable.
- **Organosolv Pretreatment**

  Simultaneous delignification and pre-hydrolysis are involved in this type of chemical pretreatment. This method uses a mixture of organic solvents, for example, ethanol, methanol, ethylene glycol, and acetone. Some catalysts like H_2SO_4, HCl, and salicylic acid are also used along with these solvents (Sahoo 2016).
- **Ozonolysis**

  In this method, ozone gas is used to remove the lignin and hemicellulose to improve the digestibility of the cellulose. The advantage of this method is that it can be performed at room temperature without the formation of toxic compounds (Travaini et al. 2013). However, there is a disadvantage to acquiring a large amount of ozone.

10.5.2.3 Physico-chemical Pretreatment

Physico-chemicalpretreatment method involves various methods like steam explosion, CO_2 explosion, wet explosion, ammonium fiber explosion (AFEX), and the liquid hot water method.

- **Steam Explosion Method**

  It is a thermo-mechanical-chemical process that helps to break down the lignocellulosic biomass by steam heating and shearing force (Jacquet et al. 2010). It is a widely used physico-chemical pretreatment method for the breakdown of lignocellulosic biomass and is highly cost-effective (Yang et al. 2017; Zhang and Chen 2012).
- **Ammonia Fiber Explosion (AFEX) Pretreatment**

  In this method, concentrated high-temperature and high-pressure ammonium gas is exposed to the lignocellulosic biomass, then the pressure is reduced abruptly. The main advantage of this method is that biomass is digested to a higher extent (Liu et al. 2013), and this could lead to higher production of bioethanol. It significantly improves the hydrolysis and fermentation processes by removing the lignin, resins, and latex (Chundawat et al. 2012). The cost of this process mainly depends on the ammonium price and the consumption of energy.

  Therefore, to make this process more economical, we have to recover the ammonia.
- **CO_2 Explosion Pretreatment**

  CO_2 is used as an effective solvent to digest the lignocellulosic biomass because it provides many advantages, such as being low cost, non-flammable, non-toxic, environmentally friendly, and easy to recover. Along these lines, the biggest advantage is that the process is carried out at low temperatures.

10.5.2.4 Biological Pretreatment

This method is used to digest the lignocellulosic biomass with the help of microorganisms.Among the microorganisms, white-rot fungi, soft-rot fungi, and brown-rot fungi are commonly used for this purpose, while white-rot fungi are widely used (Sarkar et al. 2012). Lignin and hemicellulose are targeted by white-rot and soft-rot fungi, while brown-rot fungi attack cellulose. For the bioconversion of lignocellulosic biomass, this method is preferable. but it has a serious disadvantage, i.e., it provides lower hydrolysis rates as compared to the other pretreatment methods. This method requires more incubation time.

10.6 Hydrolysis

- Pretreatment of lignocellulosics is followed by hydrolysis. Cellulose and hemicellulose are broken down, for further analysis, into their monomeric soluble compounds.

10.6.1 Enzymatic Hydrolysis

The enzymes used in this step can break down the glycosidic bond between cellulose and hemicellulose. After the enzymatic reaction, biomass is converted into glucose and xylose. This reaction takes place at an optimum temperature of 45–55 °C and an optimum pH of 4–5. The advantage of this method is that it produces fewer cell wall inhibitors.

Cellulase and hemicellulase enzymes are used for enzymatic hydrolysis. **Cellulase** is composed of **endoglucanase, exoglucanase,** and **cellobiohydrolase**. While hemicellulase is a complex enzyme composed of **exo-xylanase, endo-xylanase, β-xylosidase**, **ferulic acid esterase, acetyl xylan esterase**, **α–arabinofuranosidase,** and **α–glucoronisidase**. These ligninolytic enzymes are produced by microorganisms like bacteria and fungi. Bacteria like Bacillus and Clostridium and fungi like Penicillin, Aspergillus, and Trichoderma produce these types of enzymes.

10.6.2 Fermentation of Sugar

Hydrolysis is followed by the fermentation process to produce ethanol from biomass. This process needs microorganisms that produce alcohol from sugar. For this conversion of sugar, mostly bacteria and fungi are used in the absence of oxygen (anaerobically) to convert the monosaccharide and disaccharide into ethanol and by-products like CO_2 (Sarris and Papanikolaou 2016). Bacteria like ***Zymomonas mobilis*** are used to ferment sugar. Mostly, the microorganism used for fermentation is **yeast**, which is known as ***S. cerevisiae***. This yeast ferments the sugar in the absence of oxygen at 30 °C and converts it into alcohol. This is known as **separate hydrolysis and fermentation (SHF)**. Because of this process, hydrolysis and fermentation take place in different steps separately.

But ***S. cerevisiae*** is not able to ferment all sugar. They can only ferment hexoses. Therefore, we need other methods that can ferment all types of sugar. According to the latest research, **simultaneous saccharification and fermentation (SSF)** is used for hydrolysis and fermentation takes place simultaneously.

One of the various advantages of using this method is the low cost of the process and avoiding the production of various inhibitory compounds. Fermentation can be done in one of its three modes:

- Batch fermentation
- Fed batch fermentation
- Continuous fermentation (Oliveira et al. 2016)

Which mode of fermentation is selected depends upon the type of microorganism used for fermentation and the type of substrate.

10.7 Distillation

After fermentation, the resulting product is a mixture of alcohol and water. To separate these compounds, **distillation** processes were used. Mostly, fractional distillation has been performed for the separation of ethanol from water depending upon the different volatilities. For this purpose, a column is used, known as a **distillation column**, which is heated, and then the mixture can be poured onto the top of the column. Ethanol is collected first at the lower boiling temperature of 78.3 °C. While water is separated at its boiling temperature of 100 °C, 92% of ethanol can be recovered in this process. To obtain 99% ethanol, further, dehydration is required (Cardona and Sánchez 2007). Some other techniques can be used for the recovery of ethanol. It includes gas stripping, pervaporation, gas stripping, liquid-liquid extraction, and steam extraction.

10.8 Factors Affecting the Bioethanol Production

- Temperature
- pH
- Incubation time
- Initial substrate concentration
- Microbial load
- Accumulation of by-products

10.9 Conclusion: Future Perspective

For energy, bioethanol is one of the most promising renewable sources, which can be produced from several sources. Currently, on a commercial scale, a large amount of bioethanol is produced from sugarcane and corn biomass. But the energy requirements are very high, so we need other sources to produce bioethanol. For this purpose, lignocellulosic biomass is used. There are several different lignocellulosic biomasses available to produce bioethanol. Therefore, much effort has been put into using lignocellulosic biomass to produce bioethanol. But still, some challenges are faced by scientists. The major problem is the energy consumption in the pretreatment step, and sugar degradation is the main concern, which increases the cost of the product. It is trying to improve the technologies and optimize the different factors used in the processing of bioethanol from lignocellulosic biomass. Efforts have been made to produce bioethanol that is low in cost. For these new enzymes, improved techniques and the production of new systems are required. Therefore, according to current trends, genetic engineering, biotechnology is being directed to improve processes and products. Therefore, research is being directed toward third- and fourth-generation bioethanol.

In the third generation, algae can be used as raw material, while in the fourth generation of bioethanol, there is a concept that we can use specially engineered plants to produce hydrocarbons. This could help us to preserve our natural resources and also fulfill the demand for bioenergy. The concept of fourth-generation bioethanol in the future could lead to significant changes (Niphadkar et al. 2018).

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