Conversion of Lignocellulosic Biomass to Bioethanol: An Overview with a Focus on Pretreatment

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Abstract. The present review article aims to highlight various pretreatment technologies involved in the biochemical conversion of biomass to bioethanol from lignocellulosic biomass without the process modification. Pretreatment technologies are aimed to increase the enzyme susceptibility to the biomass for high yield of ethanol production through microbial fermentation. Broadly, pretreatment methods are divided into four categories including physical, chemical, physico-chemical and biological. This paper comprehensively reviewed on the lignocellulosic biomass to bioethanol process with focuses on pretreatment methods, their mechanisms, combination of different pretreatment technologies, the addition of external chemical agents, advantages, and disadvantages. It also discussed the ethanol productions from biomass in details without disturbing the process integrity.

1. Introduction

The world is facing serious energy problem due to depletion of fossil fuels. At the same time, the demand and need for energy in the transport sector have risen vigorously. One viable solution to this problem is making used out of alternate and renewable resources. Many countries have implemented policies for bioethanol production in other to replace the petrol-based engines. In this review, an attempt is made to understand the overview of research activities in the area of bioethanol production from lignocellulosic biomass. The review focused on the different pretreatment technologies that are involved in the synthesis of bioethanol.

According to the International Energy Agency (IEA), the global demand for biofuel would rise significantly by 2050. The increasing global warming and its impact on the environment due to CO₂ emission can be minimized by using the biofuel and bioenergy [1]. One of the most promising biofuels that we have is ethanol produced from lignocellulosic biomass by microbial fermentation. Lignocellulosic biomass (LCB) such as crop residues, grasses, sawdust, wood chip [180], oil palm, fruit bunch, trunk etc. are the potential feedstock for production of bioethanol [2, 178]. These raw materials are easily available in local areas throughout the world. It is rather to say that ethanol production would be a more rural-friendly occupation.

Bioethanol energy or ‘Bioenergy’ is one of the most challenging renewable energy in modern fuels. The challenge is to ensure that biomass energy is produced in large scale and compensate the increased price of food. In other words, bioenergy should do a better job than fossil fuels in terms of its production, efficiency, and availability. Modern bioenergy resources include liquid biofuels, biomass-fired electricity, or methane from animal wastes. It also includes cellulosic ethanol and Fischer-Tropsch fuels [3].

Bioethanol is a clean and renewable fuel [4, 179]. It is one of the most important products of industrial biotechnology. The main raw materials used for the production of ethanol in industry scale are sugar-based such as sugarcane, molasses, and starch-based materials such as wheat and corn. Based on such raw material, bioethanol may be divided into first, second and third-generation [5]. The second generation of biofuel is more promising in terms of efficiency and low cost of raw material as compared to the first and third generation of biofuel. The second generation of biofuel is mainly depended on the lignocellulosic biomass. It seems that those
countries, which have a significant agronomic-based economy or agricultural work for their livelihood, have greater advantages to incorporate currently available technology for fuel ethanol production. Bioethanol production will not be signed without proper improvement in the process as well as lowering energy consumption. During the last two decades, technology for bioethanol production from different feedstock has been developing in much higher and large-scale production became reality.

This paper comprehensively reviewed on the lignocellulosic biomass to bioethanol process with focuses on pretreatment methods, their mechanisms, combination of different pretreatment technologies, the addition of external chemical agents, advantages, and disadvantages. It also discussed the ethanol productions from biomass in details without disturbing the process integrity. It is very essential to highlight the different pretreatment methods as all the methods are equally important and it is not possible to have common methods for different types of biomass. It is because, biomass possess different types of composition and varies according to the type of the biomass.

2. Composition of lignocellulosic biomass (LCB)

Lignocellulose is the most abundant renewable biomass with a worldwide annual production of 1x1010 MT [6]. The term “Lignocellulosic biomass” is used when referring to higher plants such as hardwood or softwood. The main components of LCB are cellulose, hemicelluloses, lignin and inorganic materials [7]. Cellulose is the main component in lignocellulosic material followed by hemicelluloses then lignin. Cellulose and hemicelluloses are polysaccharides which are tightly bound to lignin by covalent cross-linkages or non-covalent forces [8] whereas, lignin is an aromatic polymer made up of phenylpropanoid precursors. Apart from the three basic chemical compounds (cellulose, hemicellulose, and lignin), lignocellulosic biomass content water, proteins, minerals and other compounds.

The organic component of biomass plays a major role in processing and producing biofuels. Cellulose is a major structural component of cell walls. It provides mechanical strength and contributes major fuel. The solar energy is absorbed through the process of photosynthesis and stored the energy as cellulose or hemicellulose [9]. It has been estimated that around 7.5 X 10^{10} tonnes of cellulose are consumed and regenerated every year [10]. This is the main reasons why cellulose is considered as the most abundant organic compound on the Earth. The composition and proportion of these compounds vary from species to species depending upon plant cell wall structure [11-12]. Table 1 summarized the composition of the lignocellulosic biomass of different sources.
<table>
<thead>
<tr>
<th>Lignocellulosic materials</th>
<th>Cellulose (%)</th>
<th>Hemicellulose (%)</th>
<th>Lignin (%)</th>
<th>References</th>
</tr>
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<tr>
<td>Hardwoods stems</td>
<td>40–55</td>
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<td>35–50</td>
<td>10–30</td>
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<tr>
<td>Paper</td>
<td>85–99</td>
<td>0</td>
<td>0–15</td>
<td>[13]</td>
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<tr>
<td>Wheat straw</td>
<td>30</td>
<td>50</td>
<td>15</td>
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<td>Sorted refuse</td>
<td>60</td>
<td>20</td>
<td>20</td>
<td></td>
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<tr>
<td>Leaves</td>
<td>15–20</td>
<td>80–85</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Cotton seed hairs</td>
<td>80–95</td>
<td>5–20</td>
<td>0</td>
<td>[13]</td>
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<tr>
<td>Newspaper</td>
<td>40–55</td>
<td>25–40</td>
<td>18–30</td>
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<tr>
<td>Waste papers from chemical pulps</td>
<td>60–70</td>
<td>10–20</td>
<td>5–10</td>
<td></td>
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<tr>
<td>Primary wastewater solids</td>
<td>8–15</td>
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<td>24–29</td>
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<tr>
<td>Swine waste</td>
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<td>1.4–3.3</td>
<td>2.7–5.7</td>
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<tr>
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<td>35.7</td>
<td>6.4</td>
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<td>31.4</td>
<td>12.0</td>
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<tr>
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<td>44</td>
<td>27</td>
<td>7</td>
<td>[16]</td>
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<tr>
<td>Rice straw</td>
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<td>26</td>
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<td>16</td>
<td>11</td>
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<tr>
<td>Cotton straw</td>
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<td>12</td>
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<td>19.7</td>
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<tr>
<td>Barley bran</td>
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<td>32.7</td>
<td>21</td>
<td>[19]</td>
</tr>
<tr>
<td>Pine tree</td>
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<td>26</td>
<td>29</td>
<td>[14]</td>
</tr>
<tr>
<td>Card board</td>
<td>47</td>
<td>25</td>
<td>12</td>
<td>[14]</td>
</tr>
<tr>
<td>Sweet sorghum</td>
<td>45</td>
<td>27</td>
<td>21</td>
<td>[20]</td>
</tr>
</tbody>
</table>

2.1. Cellulose

Cellulose \((\text{C}_6\text{H}_{10}\text{O}_5)_x\) is made up of a linear chain of D-glucose linkages by \(\beta\)-(1,4)-glycosidic bonds. This linear chain together makes the cellulose fibre. Due to the intra and intermolecular hydrogen bonds [21] linked between the cellulose fibers made it insoluble in water and other organic solvents [22]. The single molecule structure of cellulose is given in Fig. 1.

![Figure 1. Structure of single cellulose molecule (Adapted from [23])](image)
2.2. Hemicellulose

Hemicelluloses \((C_5H_8O_4)_m\) are heterogeneously branched biopolymers (Fig. 2) with different pentoses (\(\beta\)-D-xylose, \(\alpha\)-L-arabinose), hexoses (\(\beta\)-D-mannose, \(\beta\)-D-glucose, \(\alpha\)-D-galactose) and some uronic acids (\(\alpha\)-D-glucuronic, \(\alpha\)-D-4-0-methyle-galacturonic) [24]. As compare to cellulose fibers, they are easy to hydrolyse because of their branched structure, amorphous. In the fermentation process, hemicelluloses are more relatively sensible to temperature, retention time and hence must be controlled to avoid the formation of furfurals and hydroxymethyl furfurals, which inhibits fermentation [25].

![Figure 2. A schematic representation of the hemicellulose in LCB (Adapted from [10])](image)

2.3. Lignin

Lignin \((C_{31}H_{34}O_{11})_n\) is the stuff that makes the biomass woody in nature. It is a giant polymer molecule with both aliphatic and aromatic portions synthesized from phenylpropanoid precursors (Fig. 3). In general, lignin is made up of three basic building blocks such as P-coumaryl, coniferyl and sinapyl alcohol.

![Figure 3. P-coumaryl-, coniferyl- and sinapyl alcohol: dominant building blocks of the three-dimensional polymer lignin (Adapted from [10])](image)

3. Overall biochemical conversion process

Ethanol is currently produced in great amounts from corn-derived starch in the United States and sugar cane-derived sucrose in Brazil. However, these dependent raw materials (first generation) could not supply enough bioethanol for transportation in lower middle-income countries. The ultimate significant raw material with high potential of sustainable production of ethanol is LCB. Moreover, ethanol production from LCB becomes major outlook. Many steps are involved in the production of ethanol from LCB, however, some of the main steps that can be considered are; Conversion of cellulose and hemicellulose to soluble sugars by depolymerization process, Sugar fermentation as well as fermentation of inhibitory compounds and cost effective use of lignin where lignin content can be minimized.
The polysaccharides obtained from LCB could be hydrolyzed into simple monomer sugar by acid or enzyme treatment. It has been reported that enzymatic hydrolysis are generally more preferred than acid hydrolysis in terms of its environmentally friendly. The monomeric sugars obtained from hydrolysis is used in fermentation to produce ethanol by using microbes like bacteria, yeast, filamentous fungi or genetically modified microorganisms [26]. The final product ethanol is purified by the distillation process. The remaining valuable co-products have many industrial applications and new technologies are under development to convert it into higher value products [27]. The brief schematic diagram of bioethanol conversion from biomass is shown in Fig. 4.

**Figure 4. Schematic representation of LCB conversion to bioethanol**

### 3.1. Pretreatment methods for LCB

One of the most common and important steps in biochemical conversion of biomass into ethanol is pretreatment technology. The aim of the pretreatment technology is to loosen the strong bond held between the cellulose fibers. The pretreatment induces breaking of the bonds that bind with lignin, cellulose, and hemicelluloses. Pretreatment process is necessary to achieve higher enzymatic degradation in the production of ethanol from LCB. It is essential because: (1) It allows to attack by enzymes (2) avoiding the formation of inhibitory compounds (3) hemicellulose and cellulose recovery (4) size reduction and reducing the cost of materials for construction of fermentation reactors (5) Increases pore size Fig. 5.
There are several methods for pretreatment technology of LCB prior to enzymatic hydrolysis. These methods could be classified into “Physical pretreatment”, “Physico-chemical pretreatment”, “Chemical pretreatment”, and “Biological pretreatment” [29-32]. Schematic outline of pretreatment technologies is given in Fig. 6. The enzyme-based pretreatment is more advantageous than chemical treatment due to higher feasible efficient conversion, no substrate loss, lower reaction temperatures, neutral pH, and use of biodegradable and non-toxic reagents.

3.1.1. Physical pretreatment

Physical pretreatment is the process of applying mechanical force such as ball milling, two-roll milling, hammer milling, colloid milling, vibrato energy milling [33], chipping, grinding [13] irradiation by gamma rays, electron beam or microwaves etc. to the LCB to reduce its size. This process provides more surface areas, decrease degree of polymerization of cellulose molecules and most importantly decrystallisation.
3.1.1. Milling

There are several types of milling pretreatment method used in bioethanol production. Ball milling, two-roll milling, hammer milling, colloid milling and disk milling are commonly used pretreatment methods. This method enforces to reduce the size of the biomass by applying physical forces. The size of the particle obtained from milling pretreatment depends on the methods enforced. However, 10-30 mm and 0.2-2 mm of particle size are achieved in chipping [13]. The milling pretreatment has got some drawbacks which include high energy consumption and high cost of investment. To overcome such drawbacks, wet disk millings are highly adopted. Another disadvantage of milling is its inability to remove lignin from the LCB thereby restricting the enzyme activity [30].

3.1.1.2. Irradiation

Irradiation is another physical pretreatment technology where a ray of light is allowed to impose on LCB. The cellulose component is degraded by irradiation due to dissociation of glycosidic bonds of the cellulose molecules. A high beam irradiation, above 100 MR, can disintegrate glucose ring structure [34]. It includes gamma rays, electron beam and microwaves irradiation techniques. By using this technique, it was achieved that the glucose yield of pretreated bagasse became double as compared to untreated one prior to its enzymatic hydrolysis [34]. It helps in improving enzymatic hydrolysis of LCB in the conversion of cellulose to glucose [35-36]. However, some of the irradiation techniques are quite expensive and not applicable for industrial application.

3.1.1.3. Microwave mediated pretreatment

Microwave mediated pretreatment is a combination of the thermal effect of microwave and chemical reagents. Mostly, the chemical used in this process are acids and alkali. The thermal effect in microwave is due to the dielectric heating of the system. This process is rapid and volumetric. The microwave mediated pretreatment have been significantly using in the production of ethanol from LCB. These effects could be used efficiently in improving the ethanol production. It is one of the efficient and modified ways for easily treating the sample in domestic level [37]. In this method, the parameters of microwave conditions are very important in dissociating the cellulose fiber from lignin. In pretreatment of rice straw by this process increases the saccharification by removing lignin and hemicellulose in large amount. Maximum reducing sugar obtained from the process was found to be 1334.79 µg/mL [37].

3.1.1.4. Extrusion

It is a promising physical pretreatment technology for biomass where LCB were subjected to heating, mixing and shearing through the extruder. During this technique, physical and chemical modification of cellulose fibers took place thereby causing defibrillation, fibrillation and shortening of the fibers [38-39]. The technique is highly acceptable due to its high shear, rapid mixing, no furfural formation, easy scale up and no washing and conditioning [38]. Pretreatment of soybean hulls by this technique with screw speed 350 rpm, barrel temperature of 80 °C, barrel moisture 40% could achieve 94.8% glucose conversion and glucose yield of 0.37 g/g biomass [40].

3.1.2. Chemical pretreatment of LCB

3.1.2.1. Acid hydrolysis

In this method, dried powdered biomass is treated with the acidic solution under specific temperature for a period of time. The treated biomass was subjected for filtration to separate out the liquid from the unhydrolyzed debris. The hydrolysis reaction constant is directly correlated with hydrogen ion concentration thus the more negative pKa value of the acid; more is the effective hydrolysis process [41]. There are different acids which can be used in the pretreatment of LCB. For example, sulphuric acid and phosphoric acid are broadly used for treating LCB because of its efficient in hydrolyzing celluloses. Similarly, hydrochloric acid and nitric acid are used and have
better cellulose to sugar conversion rate than sulphuric acid [42]. However, both acids are more expensive than sulphuric acid. When LCB is treated with acids, hemicellulose portion is degraded faster than cellulose molecules due to a structural configuration [43-44].

**Figure 7. Hydrolysis of cellulose in acidic media [45]**

### 3.1.2.2. Dilute acid pretreatment (DAP)

Industrially made acids can be diluted and used in the pretreatment of biomass for ethanol production. These acids include H₂SO₄, HNO₃, and HCL. Different concentration of acids could be used however, 0.2-2.5% w/w is used for treating the LCB in high temperature at 120-210 °C and pressures [46]. DAP is effective in terms of low acid consumption and process severity. Another advantage is that low acid concentration releases essential nutrients such as sulphur and phosphorus that enhance downstream fermentation of sugars to ethanol [47]. Sometimes two stages processes could be used where both dilute and concentrated acid treatment have to perform. In such case, most of the hemicelluloses solubilized in dilute acid and celluloses hydrolysed in concentrated acid [48-49].

The rate of cellulose conversion to glucose depends on the severity of the acid concentration. Pretreatment of grass, newspaper, potato peeling with the acids such as H₂SO₄, HNO₃ or HCL found that glucose conversion was high when the acid concentration was high [50]. In pretreatment of rye straw with different acids, dilute HNO₃ pretreatment was found to be more effective in the conversion of glucose than dilute H₂SO₄ [42]. Nevertheless, the main disadvantage of using dilute HNO₃ is the by-product formation which is difficult to remove from the system.

Dilute H₃PO₄ acid can be used in treating potato peels where 82.5% of sugar yield was achieved [51] and in corn stover 85% of glucose, 91.4% of xylose was yielded [52]. Not only this, some people had tried to combine acids to improve process performance. HCL and H₂SO₄ were mixed in pretreating of sweet sorghum bagasse, but the performance was not effective [53]. However, a combination of H₂SO₄ and H₃PO₄ used in pretreating of oil palm empty fruit recorded high yield of xylose [47] as compared to single acid treatment.

Beside these mineral acids, organic acids such as maleic, fumaric [54] and oxalic [55-56] have been also using in pretreating the biomass of corn stover [57], maple wood [58] etc. It was found that oxalic and maleic acids can degrade more hemicellulose than sulphuric acid [54]. On treatment of corn stover with oxalic acid the best result was obtained at 160 °C for 10 minutes at a concentration of 200Mm [57], whereas on maple wood, a total of glucose (87.7%), xylose (86.9%) and sugar (87.4%) was achieved.

### 3.1.2.3. Concentrated acid pretreatment

The efficiency of the pretreatment of LCB depends on process adapted and affected by acid/biomass ratio, process temperature, time and concentration of the acid. Acid pretreatment can be effectively used in two ways with respect to temperature. High temperature and low acid concentration (dilute acid pre-treatment) or Low temperature and high acid concentration...
(Concentrated acid pretreatment). Enzymatic hydrolysis of LCB could be improved with treatment of acids at high temperature. Among the acids, sulphuric acid is most widely used while other acids such as HCL, HNO₃ [59] and H₃PO₄ were also used in pretreating the biomass. Concentrated acids such as sulphuric (65-86% w/v), hydrochloric (41%) and phosphoric (85% w/w) were generally used in pretreating the dried LCB (5-10% moisture) at low temperature (30-60 °C).

3.1.2.4. Chemical additives and acids pretreatment

Even though, acids treatment have good efficiency and viable ethanol conversion, using of combined treatment with some other additives like chemical agents gives a better product. P-cresol, a phenol derivative with acid enabled complete separation of lignin and cellulose in oil palm empty fruit bunch [60]. Another additive ZnCl₂ solution was used with dilute acids [61]. COSLIF (cellulose solvent and organic solvent based lignocelluloses fractionation) is another way of treating biomass with acids where biomass is fractionated by adding concentrated H₃PO₄ under condition of 50 °C, 1 atm, and 60 min followed by addition of solvents such as ethanol (95% v/v) under room temperature for 10 min [62]. COSLIF allows the high enzyme accessibility to cellulose and hence more sugar yield. In a combination of COSLIF and ionic liquids give better digestibility of glucans in a low dose of an enzyme [62].

3.1.2.5. Alkali pretreatment

In alkali pretreatment, LCB are mixed with alkali solutions such as NaOH, Ca(OH)₂, Potassium hydroxide, ammonia [63] and sodium bicarbonate (Na₂CO₃) [64] at specific temperature and pressure to remove lignin and to degrade ester and glycosidic side chains [65] (Fig. 8). Alkali treatment helps in extraction of a hemicellulose part from the polysaccharide and efficiently increases the accessibility of enzyme to the cellulose. In most of the treatment process, a high concentration of base is required with relatively low temperature and longer time. In contrast, low alkali concentration (4% w/w) at high temperature and pressure could be used with high retention times [66]. After the treatment, two formed of by-products are obtained, a wet solid fraction (Cellulose) and a liquid fraction (Hemicellulose, lignin, inorganic chemicals). The solids portions could be separated by simple filtration and washed with warm water. Washing helps in removing enzyme inhibitors and other chemicals. So in many cases, the bases- NaOH and Ca(OH)₂ have been extensively used in many industries and research field for pretreatment of LCB.

![Figure 8. Cleavage of ether bond of lignin in alkaline solution Adapted from [67]](image)

3.1.2.6. Sodium hydroxide (NaOH) pretreatment

Sodium hydroxide is used widely as an agent for treating the biomass of agricultural residues and wood. As compared with acid or oxidative reagents, alkali pretreatment appears to be the most effective technique for breaking the esters bonds in between lignin, hemicellulose, and cellulose.
Dilute NaOH treatment loosens the biomass structure, increases the internal surface area, decreases the degree of polymerization and crystallinity, and disrupts the lignin structure [69]. The treatment of biomass with NaOH is totally depended on the temperature, concentration, and pH of the solution. Increasing pH from 10 to 13 increased the removal of lignin from 40 to 80% w/w in the wheat straw at 140 °C [70]. It is suggested that alkali treatment has more capability to delignify than acid hence NaOH pretreatment appears to improve enzymatic hydrolysis, sugar yield and biomass utilisation rate [71]. For example, pretreatment for enzymatic conversion of cotton stalk using various agents like sulphuric acid, sodium hydroxide, hydrogen peroxide and ozone treatment, the highest level of delignification (65% with 2% NaOH, 90 min, 121 °C) was achieved by using sodium hydroxide with 60.8% cellulose conversion rate [72].

3.1.2.7. Alkaline peroxide (H$_2$O$_2$) pretreatment

It is an effective method for pretreatment of LCB such as wheat straw [73], water hyacinth [74] grass stover [75], rice hull [76] and corn stover [77]. In this technique, LCB was soaked with H$_2$O$_2$ at room temperature, pH of 11-12 using NaOH, for a period of 6-24 hr. Pretreatment of wheat straw with alkaline peroxide showed excellent sugar yield (97%) by enzymatic saccharification [73]. A good result was also got in rice hull with 96% conversion of sugar at (7.5% H$_2$O$_2$ v/v, pH 11.5, 35 °C, 24 hr) [76]. One of the major advantages of this treatment is that there is no formation of furfural and hydroxymethylfurfural (HMF). In another report, two stages process could be done based on steam explosion followed by alkaline treatment on wheat straw [78]. Firstly, wheat straw was steam at 200-220 °C, 15-22 bar. Then it was delignified with 2% H$_2$O$_2$ at 50 °C, pH 11.5, 5hr. In the first segment of steam explosion pretreatment, there is a significant loss of hemicellulose and 11-12% lignin removal. In the second segment of post-treatment, 81-88% lignin was able to remove.

3.1.2.8. Lime pretreatment

Lime (quick lime and slaked lime) has been extensively used as pretreatment agent due to its low cost, easily available, safety in handling, and easy recovery. Lime pretreatment has been considerably applied in many feedstocks with encouraging results. This pretreatment process is usually applied to biomass having low lignin content such as grasses [79], sugarcane bagasse [80] and soft wood. Lime pretreatment improves hydrolysis rates of biomass promoting cellulose accessibility by enzymes [81]. In general, LCB is pretreated with lime water at 130 °C temperature in the presence of oxygen to enhance delignification [46, 82]. For example, Corn stover was pretreated in non-oxidative condition with an excess supply of calcium hydroxide at 55 °C for 4 weeks with aeration. A total of 91.3% glucose and 51.8% xylose was yielded [83]. Similarly, rice hulls were pretreated with different concentration of lime mixed with water for ethanol production [83]. A total yield of sugar (12.6%) was achieved at 100 mg lime/g rice hulls in 1 hr. It was also found that lime pretreatment enhancing enzymatic saccharification without generating furfural and HMF in the hydrolysate. Despite the merits of lime pretreatment, it has got many drawbacks such as longer reaction time as compared to NaOH treatment, lesser dissolving rate of water thus requires more volume of water to hold the process.

3.1.2.9. Potassium hydroxide (KOH) pretreatment

Potassium hydroxide (KOH) can be used as an agent for pretreatment of various feedstocks. In pretreating the Switch grass with KOH, the maximum sugar retention was achieved with 99.26% and sugar yield of 58.2% [85]. This pretreatment process needs to be extensively investigated for comprehensive cost analysis and may be required to use some other chemical agents in addition to existing protocol.

3.1.2.10. Organosolv pretreatment

This pretreatment process involved extraction of lignin from plant biomass by addition of organic solvent mixture in presence of water with /without a catalyst – such as an acid (HCl, H$_2$SO$_4$), base (NaOH), or salt (MgCl$_2$, Fe$_2$(SO$_4$) under certain conditions like temperature and
The commonly used solvent are ethanol, methanol, acetone, and ethylene glycol. These solvents hydrolysed the internal bond between lignin and hemicellulose and glycosidic bonds of hemicellulose and cellulose. The maximum temperatures used in the process is 200 °C and lower temperature is depends on the type of biomass as well as the type of catalyst used. The technique involved pretreatment of biomass and separation of pretreated residues by filtration and washing with distilled water. From such pretreatment processes, usually, three main fractions could be produced—high purity lignin, a hemicellulose syrup, and relatively pure cellulose. However, variation in the reaction time, temperature, concentration of solvent and dose of the catalyst affects the physical characteristics (crystallinity, degree of polymerisation and length of fibre) of the pretreated residues.

Using of sulphuric acid as catalyst in organosolv pretreatment process has been extensively showing a good yield of sugar despite its corrosive nature. This catalyst degraded the monosaccharide into furfural and 5-HMF. It was found that on the treatment of pine with various catalyst, the most efficient catalyst was with acid treatment which produced maximum ethanol yield. Pretreatment of wheat straw using glycerol based autocatalytic organosolv showed 98% cellulose retention. Likewise, organosolv pretreatment was also seen in using horticultural waste (HW) for bioethanol production. A modified approached of organosolv method was introduced in HW and Japanese cypress (Chamaecyparis obtusa) where, used of ethanol under mild condition followed by H2O2 post-treatment. It was achieved that a total of 11.69g/L ethanol was produced from HW hydrolysate after 8 hr of enzymatic hydrolysis and fermentation using Saccharomyces cerevisiae.

3.1.2.11. Wet oxidation (WO)

This technique is one of the common platforms for both ethanol and biogas production. In this technique, biomass is allowed to undergo oxidation in the presence of aqueous solutions with conditions like acid, neutral, and alkali provided with oxygen at an elevated temperature of 125-315 °C, 0.5-5 MPa. The process is exothermic, and therefore, it is self-supporting with respect to heat once the reaction starts. The pretreatment process involved the formation of acids from a hydrolytic process and the oxidative reaction. Due to this, cellulose, hemicellulose, and lignin undergo cleavage. Interestingly, hemicelluloses were cleaved to monomeric sugars; whereas lignins undergo oxidation and cellulose degraded to sugars. The pretreated suspension was filtered to separate out the cellulose and hemicellulose substrate. The solid component was washed with de-ionized water before going through enzymatic hydrolysis. Hemicellulose portion was oxidised into intermediates such as carboxylic acids and then cleavage into phenolic compounds, acetaldehydes, and alcohol and then finally degraded into CO2 and H2O. One of the main problems of WO pretreatment is about the heat generation, which is very rapid and high because of the fast rate of reaction, hence difficult to control the reactor temperature.

Alkaline WO pretreatment helps in reducing the inhibitors of enzymes such as furfural and HMF as compared to acidic and neutral conditions. However, the presence of high concentration of phenolic compounds in the biomass is much more toxic than HMF and furfural. It is because phenolic compounds causing partition and loss of integrity of the cell membrane of the fermenting microorganism. Feedstock such as spruce, wheat straw, rape straw, and rice husk have been successfully treated with WO and achieved good hydrolysis rate and fermentation yield.

3.1.2.12. Sulphite pretreatment

This pretreatment process is usually used in pulp and paper industry. In this method, ground biomass was mixed with sulphite (Na2SO3, NaHSO3, etc.) solution 1-10% w/w in acidic, basic, or neutral condition at temperature 80-200 °C and reaction time of 30-180 min. The pretreated solids were separated by filtration and washed with distilled water to remove unwanted inhibitors. This dried pretreated solid further proceed for enzymatic saccharification. In the entire process, the biomass is allowed to degrade partially and lignin tied up with sulphite to form sulphonates lignin.
This technique helps in enhancing glucose yields. Later on sulphite forms a sulphonlic acid that helps in improving the hydrophilicity of the pretreated substrates [104]. Sometimes, formaldehyde is added in this process to enhance removal of lignin by a formation of sulpho-methyl groups in lignin and high sugar yield was achieved [105]. While treating with corn stover in alkaline NaHSO₃ at 140 °C, a total of 78.2% of sugar was yielded with 92% lignin removal after enzymatic hydrolysis which was higher than other alkali based methods in similar conditions [106].

Sulphite pretreatment technique is a new emerging promising technique. This technique can be applied in many feedstocks with good results. For example, on corn cob pretreatment, at 156 °C, 1.4h, 7.1% charge and solid loading of 1:7.6 w/w, 79.3% of total glucan could be converted into glucose and cellobiose with 72.2% theoretical ethanol yield [107]. However, the residue of corn cob in treating with sulphite can produce highest glucose of 81.2% [104]. In a proprietary process, known as Sulfite pretreatment to overcome recalcitrance of lignocelluloses (SPORL) is an emerging method for pretreatment technology.

3.1.2.13. Sulfite pretreatment to overcome recalcitrance of lignocelluloses (SPORL)

In this pretreatment process, biomass is pretreated in a dilute solution of sulphuric acid and sodium bisulphite (NaHSO₃). The residual solids are separated out from hydrolysate. The lignin portion is removed by sulphonating. SPORL (Sulfite pretreatment to overcome recalcitrance of lignocellulose) pretreatment is a good technology for removal of the recalcitrance of sulfate lignin by 32% in spruce wood [108] and this technique can be applied to biomass having high lignin content [109-110]. Using SPORL pretreatment technology, it can reduce inhibitors of enzymes up to 65% and increases in sugar yield from 57% to 88% in softwood [109-110, 108]. In lodgepole pine pretreatment (180 °C, 25 min, liquor/wood = 3:1/vw), 276 L/ton ethanol was yield by SPORL process [110]. In switch grass, treatment with SPORL can digest the raw material more vigorously than other dilute acid and alkali treatment processes [58].

On pretreatment of agave stalk with SPORL, high yield of sugar and lower inhibitors were found as compared to dilute acid and NaOH treatment [111]. Moreover, this process was also found to be more efficient in sugar recovery than organosolv and steam explosion pretreatments [110]. The process has advantages such as high sugar yield, lignin removal, and biomass recovery. The drawbacks include sugar degradation, a large volume of water required in washing the pretreatment solids, a high cost of recovering pretreatment chemicals etc.

3.1.2.14. Glycerol

Glycerol is a by-product of diesel production. It can be used in the pretreatment of many LCB for ethanol production through fermentation [112]. This pretreatment causes an efficient removal of lignin from biomass. Usually, glycerol and water mixed in the same ratio could be utilised for pretreatment of biomass. In pretreatment of wheat straw (230 °C, 4 h) and water hyacinth (230 °C, 1 h) with glycerol, a total of 423 mg/g of reducing sugar was produced for wheat straw and 705 mg/g was produced for water hyacinth through enzymatic hydrolysis [113]. Glycerol pretreatment with biomass such as poplar, acacia, oak and fir recorded higher cellulose conversion rates as compared to dilute acid treatment [114]. However, a combination of acid and glycerol pretreatment yielded more sugar level than glycerol alone. Pretreatment of sugarcane bagasse at 90 °C, 30 min, with acidified glycerol carbonate could produce 80 % glucose and 90% glucan digestibility [58].

3.1.2. Physico-chemical pretreatment

Physico-chemical pretreatment methods are two stage processes where both chemical and physical treatment are combined together to enable the ethanol production [115]. This method comprises a large group of various processes. In this review, we mentioned the important processes which are useful for the LCB conversion.

3.1.2.1. Steam explosion (autohydrolysis)

This pretreatment method is substantially used in both ethanol and biogas production. It is one of the most commonly used methods for LCB pretreatment [116]. This pretreatment method allows
to open the biomass fibers, and makes the biomass polymers to easily accessible by enzymes in hydrolysis, fermentation, or densification processes. It is also a common pretreatment process for the production of solid biofuel pellets to increase the calorific value. In most of the cases, biomass is treated with high-pressure saturated steam, and then sudden released of pressure makes the materials to undergo an explosive decompression. This allows to degrading hemicelluloses into sugars and lignin transformation due to high temperature, thereby increasing the potential of cellulose hydrolysis in later subsequent steps. In the process, the temperature is set in the range of around 160-260 °C, pressure 0.69-4.83 MPa, 30 sec to 20 min before materials are exposed to atmospheric pressure [13]. The steam mixture is held for a certain period to hydrolyze the hemicellulose into sugars, and the process is terminated by an explosive decompression.

In poplar chips pretreated by the steam explosion, a total of 90% efficiency of enzymatic hydrolysis was achieved in 24 hr as compared to untreated chip, which produces near about 15% hydrolysis efficiency [117]. Steam explosion method was also applied in sunflower stalk at temperature 180-230 °C before enzymatic hydrolysis [118]. The highest glucose yield was obtained at 220 °C and hemicellulose recovery at 210 °C. In another report, poplar and eucalyptus chips were pretreated at 210 °C for 4 min, wheat straw at 190 °C for 8 min, Brassica carinata residue at 210 °C at 8 min and sweet sorghum bagasses at 210 °C for 2 min [119]. In all cases, hemicellulose sugars were extensively solubilised and decreased 75-90% of xylose content. This technique seems to be cost effective and satisfied most of the requirement in pretreatment process. Therefore, many researchers have tested this method on lab and pilot scale related to industrial scale production. For example, Masonite plants were set up for large scale production [88].

3.1.2.2. Catalyzed steam-explosion

A steam explosion can be discussed in many ways with the addition of various chemical agents. The chemical agents such as SO2, H2SO4, CO2, oxalic acid, etc. are used as a catalytic agent to impregnate the biomass. Use of such catalytic agents prior to steam-explosion has a greater impact on complete hemicellulose removal with better enzymatic digestion and less formation of inhibitory compounds [120]. For example, SO2 impregnated was used in pretreating agricultural residues [110]. Moreover SO2 impregnated biomass is relatively much easy to hydrolyze and ferment the hydrolysate than that of dilute acid impregnated because the later produces inhibitory compounds [121].

In steam explosion treatment of willow biomass with addition of SO2, the maximum glucose yield was found to be 95% at 1% SO2, 200 °C [122]. However, the yield of xylose was as low as compared to other pretreatment technologies.

3.1.2.3. Ammonium fiber/freeze explosion (AFEX)

It is one of the alkaline physico-chemical pretreatment processes. In this treatment, biomass is exposed to hot liquid ammonia at 90- 100 °C for 30 min under high-pressure and then the sudden release of pressure disrupts the structure of LCB leading to increasing digestibility and simultaneously delignifying it [28]. This process can modify or effectively reduce lignin content in biomass without disturbing hemicellulose and cellulose fractions. The optimum condition for pretreatment of LCB by AFEX process varies according to nature of the material. For example, in switch grass optimum conditions of pretreatment were 100 °C, ammonia loading of 1:1 kg of ammonia per kg of dry matter and 5 min retention time [123]. It was also observed that AFEX pretreatment is very much effective in fiber digestion and produces lower a level of inhibitors than acid pretreatment [124-125], and it is a highly effective method for pretreatment of grasses. Further studies on AFEX pretreatment technology found that compounds like 4-hydroxybenzaldehyde, lactate and acetate inhibits fermentation of E. coli KO11 strain [126]. That is why this technology is difficult to have one general method for a different type of biomass pretreatment. But it was observed that for agricultural residues AFEX, wet oxidation and liquid hot water (LHW) pretreatment technologies were more successful [127-129].
One of the major advantages of AFEX process is that there is no formation of inhibitory by-products such as furans [130]. There are certain disadvantages for this process. AFEX pretreatment does not significantly solubilize hemicellulose like other pretreatment processes does. The ammonia present in the process must be recycled after pretreatment to reduce cost and mitigation of environment [131, 13].

3.1.2.4. Ionic liquids (Green solvents) pretreatment

Ionic liquids (ILs) are salts having a melting point below 100 °C with large cations (mostly organic) and small anions (mostly inorganic). It is nonflammable [132], liquid at room temperature [113] with low volatility. ILs could be used in the pretreatment of specific biomass under optimal condition [133]. In IL pretreatment, biomass powder is mixed with solvents in the presence of water and acid. The solution is incubated at a temperature ranging from 80 to 160 °C for 10 min to 24 hr. Separately, an antisolvent chemical is also added to precipitate the cellulose fraction. The pretreated solids were retained via filtration or centrifugation and washed with distilled water to remove inhibitory. The washed cellulose is lyophilized and later saccharified. The added antisolvent was removed from the IL by the flash distillation process. The remaining IL could be further reuse.

The effectiveness of the pretreatment process in IL could be predicted using Kamlet-Taft hydrogen bond acceptor ability. Higher the β value, high is the lignin removal and vice versa [134]. One of the important factor in IL pretreatment is temperature. It influences sugar released, saccharification kinetics and sugar yields of the substrate [135]. Some of the selected biomass which has extensively pretreated with IL are shown in Table 2.

### Table 2. ILs pretreatment of some biomass

<table>
<thead>
<tr>
<th>Biomass</th>
<th>Solvent</th>
<th>Temperature (°C), time (min)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eucalyptus</td>
<td>Emim-AC</td>
<td>150, 3</td>
<td>[136]</td>
</tr>
<tr>
<td>Poplar</td>
<td>Emim-AC</td>
<td>120, 1</td>
<td>[137]</td>
</tr>
<tr>
<td>Pine</td>
<td>Amim-Cl</td>
<td>120, 5</td>
<td>[138]</td>
</tr>
<tr>
<td>Spruce</td>
<td>Amim-Cl</td>
<td>120, 5</td>
<td>[138]</td>
</tr>
<tr>
<td>Energy can bagasse</td>
<td>Emim-AC</td>
<td>120, 0.5</td>
<td>[139]</td>
</tr>
<tr>
<td>Switchgrass</td>
<td>Emim-AC</td>
<td>120-160, 6</td>
<td>[140]</td>
</tr>
<tr>
<td>Bamboo</td>
<td>Emim-Gly</td>
<td>120, 8</td>
<td>[141]</td>
</tr>
<tr>
<td>Wheat straw</td>
<td>Bmim-AC</td>
<td>100-150, 2-6</td>
<td>[142]</td>
</tr>
<tr>
<td>Rice straw</td>
<td>Ch-Aa</td>
<td>90, 2</td>
<td>[143]</td>
</tr>
<tr>
<td>Water hyacinth</td>
<td>Bmim-AC</td>
<td>100-150, 0.17-1</td>
<td>[113]</td>
</tr>
<tr>
<td>Kenaf powder</td>
<td>Ch-AC</td>
<td>110, 16h</td>
<td>[144]</td>
</tr>
<tr>
<td>Catalpa</td>
<td>Amim-cl</td>
<td>100, 3h</td>
<td>[145]</td>
</tr>
<tr>
<td>Maple wood flour</td>
<td>Bmim-AC</td>
<td>90, 6h</td>
<td>[134]</td>
</tr>
<tr>
<td>Willow</td>
<td>Bmim-AC</td>
<td>120, 22h</td>
<td>[146]</td>
</tr>
<tr>
<td>Red Oak</td>
<td>Emim-AC</td>
<td>110, 16 h</td>
<td>[147]</td>
</tr>
<tr>
<td>Rubber wood</td>
<td>Mmim-ME</td>
<td>25, 0.5h</td>
<td>[148]</td>
</tr>
<tr>
<td>Rice hulls</td>
<td>Amim-Cl</td>
<td>90, 4h</td>
<td>[149]</td>
</tr>
<tr>
<td>Corn stover</td>
<td>Emim-AC</td>
<td>140, 3h</td>
<td>[150]</td>
</tr>
</tbody>
</table>

Dmim-SO₄: 1,3-Dimethylimidazolium methyl sulphate, Emim-DePO₄: 1-Ethyl-3 methylimidazolium diethyl phosphate, Ch-Aa: Cholinium amino acid, Ch-AC: Cholinium acetate,

3.1.2.5. Ozonolysis

The word seems to be a very negative impact, as we know about the ozone depletion in today’s scenario. However, ozone could be used as a good agent for pretreatment process. In this process, ozone is sparged into a mixture of biomass and water at specific room temperature in a particular time to solubilize the lignin and hemicellulose [23]. The entire process depends on the type of biomass, the moisture content of the sample, concentration of ozone and ozone flow rate [151]. Ozone pretreatment is not suitable for all biomass. It gives low sugar yield in wheat and rye.
straw [151]. In the treatment of cotton stalk with ozone, the lignin content was reduced 11.97-16.6%. It was observed that the combination of ozone and ethanosolv in Sweetgum, Miscanthus, and loblolly pine gives high delignification and low carbohydrate lost [152]. In addition, combined ozonolysis and autohydrolysis was reported to give high ethanol yield, low waste products, low use of other chemicals and high hemicellulose solubility [152].

Ozonolysis is not so common process as it cannot be applicable to the majority of the biomass. The process is relatively expensive due to a large amount of ozone consumption. But, it has got some advantages; effective removal of lignin, low inhibitory productions, high dry matter concentration (45-60%) and reactions can be performed in atmospheric conditions [153-154].

3.1.2.6. Hydrothermal pretreatment

It is a new pretreatment technology. It seems to be a very promising process, as organic solvents were not used. This process also lowers the operational cost, which was high in other methods. In this treatment, hemicellulose is removed effectively by subjecting elevated temperature under varied pressure leaving cellulosic biomass susceptible to enzymatic degradation and thereby producing ethanol [155].

Another method called sonification pretreatment is used where biomass is treated with sound waves which help in removing lignin from lignocellulosic [156]. The high frequency of sound wave disrupts the lignin coating and thereby releasing the cellulose and hemicellulose portion.

3.1.2.7. Inorganic Salts

Some of the inorganic salts such as iron (III) chloride [157] and calcium chloride [158] could be used in pretreatment process of some biomass effectively. The salt forms a strong acid solution while dissolved in water. This makes rapid removal of hemicellulose from the biomass. For example, pretreatment of olive tree residue with salt concentration 0.26 M, 152.6 °C, 30 min got 100 % hemicellulose removal with 36.6 g glucose/100 g biomass yield [157]. In another report, CaCl₂ pretreatment in combination with microwave treatment on corn stover (162.1 °C, 12 min) gives much more glucose recovery than that of steam explosion [158].

3.1.3. Biological pretreatment

These pretreatment methods employed wood degrading microorganism such as white, brown, soft-rot fungi and bacteria for the production of bioethanol from biomass. The biological pretreatment by white rot fungi is a co-oxidative process where a carbon source is required. The required carbon is supplied from cellulose and hemicelluloses present in the biomass. The employed microorganism modifies the chemical composition of LCB so that enzyme can easily digest the biomass [159, 54, 160]. Different types of fungi can be employed according to the target substrate requirement. In general, brown and soft rots are used to attack the cellulose component of biomass while white rot fungi usually degraded lignin [161]. Among the fungi used in the biological pretreatment of biomass, White rot fungi are the most promising one [162]. In one of the treatment, using four different types of white rot fungi on wood chips for 30 days it was found that Trametes versicolor MrP1 could hydrolyze maximum substrate up to 45% and glucose conversion rate of 35% was achieved [163]. In another treatment of Japanese red pine Pinus densiflora with 3 species of white rot fungi such as Ceriporia lacerata, Stereum hirsutum, and Polyporus brumalis, the most effective in lignin degradation was observed in Stereum hirsutum [54].

Selection of the type of biomass and choosing the species to be used for treatment is an important aspect in biological pretreatment. The pretreatment of biomass like corn stover with Cyathus stercoreus found that there is an improvement of 3 to 5 fold in enzymatic digestibility [164]. While in treating of bamboo with different 35 isolates species of white-rot fungi, lignin degradation was more active in 3 species namely Echinodontium taxodii 2538, Trametes versicolor G20 and Coriolus versicolor B1 out of 35 [165].
Biological pretreatment has potential advantages over physical/chemical pretreatments. It is promising technique and has many advantages over chemical treatment method likes, low energy input, mild environment conditions, environment-friendly etc. [13]. However, the characters like slow rate, a large amount of space required, consumption of hemicellulose and cellulose by microorganism shows disadvantages of using biological pretreatment. Therefore, bio-pretreatment faces techno-economic challenges which led to less attractive in commercialization [54].

3.1.4. Other developing pretreatments methods

Some of the new methods which were developed to enhance production of bioethanol from LCB by pretreatment technology are used of supercritical CO₂, used of weaker acids such as phosphoric acid etc. [166-167]. The Mineral acids, which are used in pretreatment process, have several drawbacks in terms of rusting and toxicity. It requires a reactor that is made up of exotic metals. The weaker acids can use the reactor made up of stainless steel. This helps in lowering the toxic by-products level [166].

The hydrolysate obtained from phosphoric acid treatment were usually resisted by ethanologenic bacteria such as Escherichia coli strains and this bacteria can ferment both the sugars obtained from hemicellulose and cellulose derivatives in a single vessel, this process is termed as simultaneous saccharification and co-fermentation (SScF). SScF shows increased in ethanol yield up to 0.27g ethanol/g bagasse (dry weight) [168] as compared to concentrated sulphuric acid pretreatment method.

The overall year wise publication data of pretreatment technologies since 1996 to 2015 (march) is given in Fig. 9. The maximum number of publication came from chemical treatment even though it has many side effects on environmental issues. A complete tough comparison could be obtained in between biological and physical treatment. Physico-chemical treatment is just enhancing parameters in other pretreatment methods, so a number of publications are less. From this, one can conclude about the research work that is going on in a particular field with irrespectiveness of its side effects. However, in days to come more biological pretreatment methods should be adopted in other to produce more ethanol efficiently.

Figure 9. Comparision of pretreatment methods year wise publication from 1996 to 2015 (March).
Source: Scopus.

4. Simultaneous Saccharification and Co-Fermentation

Fermentation is the final cease in biomass conversion process for bioethanol production. It is usually carried out in different ways like separate hydrolysis and fermentation (SHF), simultaneous saccharification and fermentation (SSF) and simultaneous saccharification and co-fermentation (SScF). Firstly, SSF method was introduced by Gulf Oil Company, the US and
the University of Arkanas [169-170]. Subsequently, the process has become more elaborate and more improvised to yield efficient bioethanol from biomass. In SSF, hydrolysis of cellulose and fermentation process are performed in a single unit with microorganism *S. cerevisiae*. Various reports on bioethanol production had mentioned that SSF is a better process than SHF [171]. Nevertheless, current trends adapted more in the production of bioethanol with less process complexity, solid-liquid separation, neutralization of hydrolysate toxins (SHF) and co-fermentation of hexose and pentose (SScF). Hydrolysate resistant biocatalysts such as *E.coli* MM160 [168, 172] and *S.cerevisiae* 424A [173- 177] has been developed. Lignin+ SScF and SScF processes combine both C-6 and C-5 sugar fermentation in a single and more modified unit process simplification has been developed. These gave more yield of bioethanol as compared to SHF.

5. Conclusions and Future Perspective

The commercial production of bioethanol has been started around at 1975, during energy crisis at Brazil. In other countries after 35 years of research and development, commercial plants for the production of bioethanol have become reality. Therefore, during next decade, bioethanol technology is likely to embark the bio-refineries all over the world for transportation and domestic use. Extensive research is going on to develop the economically feasible bioethanol production that can meet the ethanol demand of the world. The challenges led to the production of around 30 billion gallons of bioethanol by 2020. Many advanced pretreatment technologies have been developed but dilute acid and steam explosion are commercially feasible. A major improvement has been made on genetic engineering of biomass or enzymes even production of cellulase inside plant biomass. However, the cost of the cellulase enzymes is also a main concerned. Approaches have been made to minimize enzyme cost providing more robust cellulase production. Genetic engineering is one such major area where it may play a potential role for the future development of biofuel production as well as in agriculture sector. Multiple transgenic strategies need to be developed to tackle the lack of metabolic pathways. Apparently, production of different chemicals, using of lignin by-products, and integration of ethanol production with power and heat plant are anticipated to take place, owing to improved financial returns and cost effective in the market. However, the existing pretreatment technologies also need to improve and possible process simplifications are the major targets in near days to come.

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Conflict of interest

Authors declared there is no conflict of interest

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