



LIGNOCELLULOSIC MATERIAL FOR PRODUCTION OF ETHANOL: A REVIEW

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ABSTRACT

At present buildings with floating column is a typical feature in the modern Lignocellulosic biomass can be utilized to produce ethanol, a promising alternative energy source for the limited crude oil and climate change. The Present world energy scenario is focused at nonconventional sources. The biomass has emerged as one of the dependable nontraditional feed stocks for the production ethanol. Biomass derived from corn has become one of the primary feedstocks for bioethanol production for the past several years however, the argument of whether to use food as biofuel has led to a search for alternative non-food sources. Consequently, industrial research efforts have become more focused on low-cost large-scale processes for lignocellulosic feedstocks originating mainly from agricultural and forest residues along with herbaceous materials and municipal wastes. Although cellulosic-derived biofuel is a promising technology, there are some obstacles that interfere with bioconversion processes reaching optimal performance associated with minimal capital investment. This review summarizes current approaches on lignocellulosic-derived biofuel bioconversion and provides an overview on the major steps involved in cellulosic-based bioethanol processes.

Key words: Lignocellulosic; Pretreatment; Hydrolysis; Fermentation; Ethanol

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1. INTRODUCTION

Global depletion of fossil fuels, rising fuel prices, environmental concerns, and pressures for oil independence are creating a strong market for bio-fuels [USDA, 2016]. Bio-fuels have the potential to be domestically and globally available for energy security, with most being carbon neutral (introducing no additional carbon to the global carbon cycle) or potentially carbon negative (if coupled with carbon sequestration) and supportable within the current agricultural infrastructure [DOE, 2015a]. Presently, one of the most promising alternatives for petro-fuels is bio-ethanol. Ethanol is a simple alkyl alcohol that can

be used as a transport fuel in spark ignition engines. It has high octane levels and can be either blended into petrol or used in unmodified vehicles, or run as pure ethanol in a converted engine [Rudkin, E., 2002].

Ethanol can be produced from a variety of feed stocks, including sugar substances, such as sugarcane juice and molasses, as well as starch-based materials such as wheat and corn [Jones, A.M. *et.al*, 1994], where the corn starch based ethanol production is dominant in the US bio-ethanol industry[Korves, R, 2016]. However, this technology may not be practical in the long run due to the fact that wide planting of corn for ethanol

production will compete for the finite arable land and thus threaten the national food security [Giampietro, M., *et al*, 1994]. Lignocellulosic biomass has been suggested as the most promising alternative for the traditional starch feedstock. Lignocellulosic feedstocks have the best well-to-wheel assessment, considering its abundance, low cost and high polysaccharides (cellulose and hemicellulose) content [Fujii, T *et al*, 2009].

Lignocellulosic biomass represents the major fraction of most plant matter. Common examples of lignocellulosic biomass include agricultural and forestry residues, the paper and much of the remaining organic fraction of municipal solid waste (MSW), industrial processing residues such as wastes in the paper and pulp industry, and herbaceous and woody plants grown as feedstocks for the production of fuels. The major fraction of such materials, typically of the order of 35-50%, is a polymer of glucose known as cellulose. The next largest fraction, of the order of 20-35%, is hemicellulose. Hemicellulose is also a polymer of sugars, but the types and distributions of these sugars vary depending upon the particular biomass source. For many types of lignocellulosic biomass, the five carbon sugar xylose represents the predominant fraction of the hemicellulose component. The third largest fraction, of the order of 15 to about 25%, is typically lignin, a phenyl-propene polymer of complex composition that cannot be broken down to form sugar molecules. A number of other compounds such as plant oils, proteins, and ash make up the remaining fraction of the lignocellulosic biomass structure [Li, Xuan, 2010].

Biomass conversion involves three basic steps: (1) Pretreatment of raw feedstock to increase the accessibility of enzymes to the polysaccharides (cellulose and hemicellulose); (2) enzymatic hydrolysis to break down the lignocellulose constituents (polysaccharides) into a mixture of fermentable sugars; and (3) microbial fermentation, mediated by bacteria or yeast, to convert these sugars to ethanol [DOE, 2005b]

Ministry of Water and Energy (MoWE) of Ethiopia has developed bio-fuel strategy recently. The objective of the strategy is enhancing energy

security and access to transport fuels. The program will also promote an agriculture based industry for increased agricultural and industrial outputs, employment and exports. The main goals of bio-fuel program in Ethiopia are (i) coordinating the production to gain 194.9 Million Liter at the end of strategic period, (ii) expanding bio-fuel blending facility through oil distribution companies, to have 8 benzene-ethanol blending stations at the end of the strategic period, (iii) enhancing benzene-ethanol blending level to reach 25% and to use 64.4 million liter of bioethanol for blending, (iv) rising awareness and attract private investors on bio fuel development by hosting international forums and conferences, and (v) addition of 25 project designs on already existing 3 projects and coordinating bio-fuel project designs to reduce GHG emission and make the country beneficiary from CDM Program [Asrat G. W *et al*, 2015].

2. LIGNOCELLULOSIC FEEDSTOCK

2.1. Lignocellulosic Biomass Composition

Lignocellulosic material can generally be divided into three main components: cellulose (30-50%), hemicellulose (15-35%) and lignin (10-20%) [Pettersen RC, 1984]. Cellulose and hemicelluloses make up approximately 70% of the entire biomass and are tightly linked to the lignin component through covalent and hydrogenic bonds that make the structure highly robust and resistant to any treatment [Mielenz JR, 2001]. Potential lignocellulosic feedstocks and their composition are summarized in Table 1.

2.1.1. Cellulose

Cellulose is a structural linear component of a plant's cell wall consisting of a long-chain of glucose monomers linked by (1/4)- glycosidic bonds that can reach several thousand glucose units in length. The extensive hydrogen linkages among molecules lead to a crystalline and strong matrix structure [Ebringerova A *et al*, 2005]. This cross-linkage of numerous hydroxyl groups constitutes the microfibrils which give the molecule more strength and compactness. Although starchy materials require temperatures of only 60-70 °C to be converted from crystalline to amorphous texture, cellulose requires 320 °C as well as a pressure of 25 MPa to shift from a rigid crystalline

structure to an amorphous structure in water [Deguchi S, Et al, 2006]. Cellulose is the most prevalent organic polymer and is approximately 30% of the plant composition [U.S., 2016]. Cotton, flax and chemical pulps represent the purest sources of cellulose (80-95% and 60-80%, respectively) while soft and hardwoods contain approximately 45% cellulose [Demirbas A, 2005].

2.1.2. Hemicellulose

Hemicellulose is an amorphous and variable structure formed of heteropolymers including hexoses (D-glucose, D-galactose and D-mannose) as well as pentose (D-xylose and L-arabinose) and may contain sugar acids (uronic acids) namely, D-glucuronic, D-galacturonic and methylgalacturonic acids [69]. Its backbone chain is primarily composed of xylan b (1/4)-linkages that include D-xylose (nearly 90%) and L-arabinose (approximately 10%) [Girio FM et al, 2010]. Branch frequencies vary depending on the nature and the source of feedstocks. The hemicelluloses of softwood are typically glucomannans while hardwood hemicellulose is more frequently composed of xylans [McMillan JD. 1993]

Although the most abundant component in hemicellulose, xylan composition still varies in each feedstock [Aspinall GO 19 80].

2.1.3. Lignin

Lignin is an aromatic and rigid biopolymer with a molecular weight of 10,000 Da bonded via covalent bonds to xylans (hemicelluloses portion) conferring rigidity and high level of compactness to the plant cell wall [Mielenz JR, 2001]. Lignin is composed of three phenolic monomers of phenyl propionic alcohol namely, coumaryl, coniferyl and sinapyl alcohol. Forest woody biomass is primarily composed of cellulose and lignin polymers. Softwood barks have the highest level of lignin (30-60%) followed by the hardwood barks (30-55%) while grasses and agricultural residues contain the lowest level of lignin (10-30% and 3-15%, respectively) [16]. Conversely, crop residues such as corn stover, rice and wheat straws are comprised mostly of a hemicellulosic heteropolymer that includes a large number of 5-carbon pentose sugars of primarily xylose [20]. Previously, little interest has been given to lignin chemistry potential on

hydrolysis. However, lignin components are gaining importance because of their dilution effect on the process once solids are added to a fed batch hydrolytic or fermentation bioreactor in addition to their structure and concentration effects that would affect potential hydrolysis [21]. For instance, the adsorption of lignin to cellulases requires a higher enzyme loading because this binding generates a non-productive enzyme attachment and limits the accessibility of cellulose to cellulase [22]. Furthermore, phenolic groups are formed from the degradation of lignin. These components substantially deactivate cellulolytic enzymes and hence influence enzymatic hydrolysis. Chen et al. (2006) demonstrated that lignin modification via genetically engineering practices targeting its biosynthetic pathways could considerably reduce lignin formation and improve ethanol yield. However, this could be somewhat problematic as lignin components serve as the major plant defense system to pathogen and insects and its modification could disrupt the plants' natural protection[23].

PATHWAYS OF BIO-ETHANOL PRODUCTION

Lignocellulosic biomass can be transformed into bioethanol via two different approaches, (i.e. biochemical or thermochemical conversion) [24]. Both routes involve degradation of the recalcitrant cell wall structure of lignocellulose into fragments of lignin, hemicellulose and cellulose. Each polysaccharide is hydrolyzed into sugars that are converted into bioethanol subsequent followed by a purification process [25]. However, these conversion routes do not fundamentally follow similar techniques or pathways. The thermochemical process includes gasification of raw material at a high temperature of 800°C followed by a catalytic reaction. Application of high levels of heat converts raw material into synthesis gas (syngas) such as hydrogen, carbon monoxide and CO₂. In the presence of catalysts, the resulting syngas can be utilized by the microorganism *Clostridium ljungdahlii* to form ethanol and water can be further separated by distillation [26].

Unlike the thermochemical route, biochemical conversion involves physical (i.e. size reduction) or/and thermo-chemical with possible

biological pretreatment. Biochemical pretreatment is mainly used to overcome recalcitrant material and increase surface area to optimize cellulose accessibility to cellulases [27]. The upstream operation is followed by enzymatic or acidic hydrolysis of cellulosic materials (cellulolysis) and conversion of hemicellulose into monomeric free sugars (saccharification) subsequent to biological fermentation where sugars are fermented into ethanol and then purified via distillation [25,27]. Concurrently, lignin, the most recalcitrant material of cell walls is combusted and converted into electricity and heat [28]. Overall, biochemical approaches include four unit-operations namely, pretreatment, hydrolysis, fermentation and distillation [29]. Currently the biochemical route is the most commonly used process [30].

3.1. Pretreatment Overview

Effective pretreatment is fundamental for optimal successful hydrolysis and downstream operations (See Fig.1) [31]. Pretreatment upstream operations include mainly physical, (i.e., biomass size reduction) and thermo chemical processes that involve the disruption of the recalcitrant material of the biomass. This upstream operation increases substrate porosity with lignin redistribution. Therefore, it enables maximal exposure of cellulases to cellulose surface area to reach an effective hydrolysis with minimal energy consumption and a maximal sugar recovery [27].

Zhu and Pan [32] concluded that the pretreatment process of woody biomass differs substantially from the agricultural biomass due to differences in their chemical composition and physical properties. Unlike woody biomass, agricultural residues pretreatment does not require as much energy as recalcitrant woody material to reach size reduction for further enzymatic saccharification. Emphasis on the importance of the energy consumption from the mechanical operation (size reduction) primarily based on the estimation of woody biomass pretreatment energy efficiency (Pretreatment $\frac{1}{4}$ Total sugar recovery (kg)/Total energy consumption (MJ)). In addition to sugar recovery and ethanol yield, this energy efficiency ratio and mass balance was deemed crucial for the complete estimation of pretreatment

efficiency [32, 33]. Toxic inhibitory level estimation has also been considered important for evaluating pretreatment cost effectiveness primarily when dilute acid is added. Costly detoxification steps could be a major hindrance to reach high-performance pretreatment [34]. Overall, the ratio including energy consumption versus sugar yield with regard to feed stock versatility [32, 33] as well as toxic inhibitors formed per level of sugars recovered are of prime consideration on the estimation of the pretreatment efficiency and cost effectiveness of the operation in an effort to reach optimal conditions. Several pretreatment methods, namely, mechanical, chemical or microbiological have been used to remove the recalcitrant cell wall material of lignocellulosic biomass depending on the raw material being extracted [35]. More recently, there has been considerable advancement in development of pretreatment processes [36]. Table 2 illustrates some of the pretreatment methods that have been examined over the years. Although most of these treatments can liberate hemicellulose and cellulose from the cell wall, some of them remain economically unfeasible due to key technical issues. Furthermore, they are not all able to overcome the recalcitrant material found mainly in wood-based feedstocks. Typically, few treatments are endowed with ability to overcome feedstock versatility [37]. Unlike agriculture residues, forest and wood materials are high in lignin (approximately 29%) and cellulose (approximately 44%) [16] which renders them more recalcitrant. Agricultural residues such as corn stover, rice and wheat straws are mostly composed of hemicellulose (32%) and low levels of lignin (3-13%) conferring to them a less resistant texture but a higher level of pentose sugars rendering them less practical than woody recalcitrant material.

The most prevalent treatments include acid hydrolysis, hot water, dilute acid pretreatment and lime [92,93]. However, the conventional methods using acidic treatments (usually dilute sulfuric acid with concentrations below 4 wt% and temperatures greater than 160°C) [109] are always accompanied by formation of toxic inhibitors such as furfural from xylose and hydroxymethyl furfural (HMF) from glucose in addition to phenolics and

acetic acid [93]. Acetic acid resulting from dilute acid pretreatment of agricultural residues as well as herbaceous and hardwoods is pH dependent and can reach a high concentration of approximately 10 g/L [36] that is more difficult to separate and detoxify than HMF and furfural. Unlike dilute acid pretreatment, ammonia fiber explosion (AFEX) treatments are sufficient to hydrolyze primarily agricultural residues such as corn stover and have not been associated with the formation of toxic products including HMF [97]. Given that woody feedstock is gaining increasing attention for its attractive attributes over low-lignin materials, organosolv along with steam explosion [38] and sulfite pretreatment to overcome recalcitrance (SPORL) [39] have become of prime interest for their ability to degrade high-lignin forest materials [32,39]. A recent study reported that steam explosion consumed the highest level of energy yielding the lowest pretreatment energy efficiency ratio of 0.26 kg sugar/MJ when compared to organosolv (0.31-0.40 kg sugar/MJ) and SPORL (0.35-0.43 kg sugar/MJ) [32]. While the organosolv treatments degrade high-lignin woody biomass including both softwood and hardwood, they produce considerable quantities of inhibitors namely furfural and HMF, yield a low hemicellulosic sugar concentration and are also associated with a high capital investment [40]. Consequently, SPORL remains the most attractive candidate for its flexibility and ability to overcome both hardwood and softwood recalcitrance with the highest sugar recovery and lowest energy consumption [32]

3.2. Hydrolysis

The success of the hydrolysis step is essential to the effectiveness of a pretreatment operation [28]. During this reaction, the released polymer sugars, cellulose and hemicellulose are hydrolyzed into free monomer molecules readily available for fermentation conversion to bioethanol [25]. There are two different types of hydrolysis processes that involve either acidic (sulfuric acid) or enzymatic reactions [41]. The acidic reaction can be divided into dilute or concentrated acid hydrolysis. Dilute hydrolysis (1-4%) requires a high temperature of 200-240 °C to disrupt cellulose crystals [42]. It is followed by hexose and pentose

degradation and formation of high concentrations of toxic compounds including HMF and phenolics detrimental to an effective saccharification [43].

To optimize alcohol yield and reduce inhibitory and toxic byproducts the process uses sulfuric acid H₂SO₄ (0.5 wt%) that flows continuously to the biomass at a high temperature of 150-180 °C in a short period of time allowing for a greater sugar recovery [44]. Concentrated acid hydrolysis, the more prevalent method, has been considered to be the most practical approach [45]. Unlike dilute acid hydrolysis, concentrated acid hydrolysis is not followed by high concentrations of inhibitors and produces a high yield of free sugars (90%); however, it requires large quantities of acid as well as costly acid recycling, which makes it commercially less attractive [46]. While acid pretreatment results in a formation of reactive substrates when acid is used as a catalyst, acid hydrolysis causes significant chemical dehydration of the mono saccharides formed such that aldehydes and other types of degradation products are generated [43]. This particular issue has driven development of research to improve cellulolytic-enzymes and enzymatic hydrolysis. Effective pretreatment is fundamental to a successful enzymatic hydrolysis [47].

During the pretreatment process, the lignocellulosic substrate enzymatic digestibility is improved with the increased porosity of the substrate and cellulose accessibility to cellulases. *Trichoderma reesei* is one of the most efficient and productive fungi used to produce industrial grade cellulolytic enzymes. The most common cellulase groups produced by *T. reesei* that cleave the β/1,4 glycosidic bonds are β-glucosidase, endoglucanases and exoglucanases [40]. However, cellulase enzymes exposed to lignin and phenolic-derived lignin are subjected to adverse effects [48,49] and have demonstrated that phenolic derived lignin have the most inhibitory effects on cellulases. A study reported that a ratio of 4 mg to 1 mg peptides, reduced by half the concentration of cellulases (i.e. β-glucosidases) from *T. reesei*. This strain was also shown to be 10 to 10 fold more sensitive to phenolics than *Aspergillus niger*. In addition to phenolic components effect on

cellulases, lignin has also an adverse effect on cellulases. As mentioned previously, the lignin adverse effect has two aspects including non-productive adsorption and the limitation of the accessibility of cellulose to cellulase. Although considerable genetic modifications (GMs) have been deployed to transform lignin effects, lignin has been shown to be a potential source of self-sustaining-energy and added-value components. Consequently, several research studies have determined practical approaches in eliminating inhibition of cellulases without involving GM approaches. Lui et al. [50] have demonstrated that the application of metal components namely, Ca(II) and Mg(II) via lignin-metal complexation substantially enhanced enzymatic hydrolysis. Additionally, Erickson et al. [51] have reported the importance of additives namely, surfactants and bovine serum albumin (BSA) in blocking lignin interaction with cellulases. Sewalt et al. [49] have reported that the adverse effect of lignin on cellulases can be surmounted by ammoniation and various N compounds. Moreover, the enzymatic treatment can be accomplished simultaneously with the engineered co-fermentation microbial process known as simultaneous saccharification and fermentation (SSF) [52]. This process has been of interest since the late 1970s for its effectiveness to minimize cellulolytic product inhibition and subsequently increase alcohol production [52]. Typically, separate hydrolysis and fermentation (SHF) processes involve the inhibition of the hydrolytic enzymes (cellulases) by saccharide products such as glucose and cellobiose. Unlike SHF, the SSF process combines hydrolysis and fermentation activities simultaneously and hence keeps the concentration of saccharides too low to cause any considerable cellulase inhibition [53].

3.3. Fermentation

Pretreatment and hydrolysis processes are designed to optimize the fermentation process. This natural, biological pathway depending on the conditions and raw material used requires the presence of microorganisms to ferment sugar into alcohol, lactic acid or other end products [25]. Moreover, industrial yeasts such as *S. cerevisiae* have been used in alcohol production mostly in the

brewery and wine industries for thousands of years. *S. cerevisiae* has also been utilized for corn-based and sugar-based biofuel industries as the primary fermentative strain. Once becoming accessible for enzymatic or acidic hydrolysis, the pretreated cellulosic slurry is subsequently converted into fermentable free sugars. The sugars are mixed with water to form a broth. Typically, during batch fermentation *S. cerevisiae* ferments hexose sugars, mainly glucose, into ethanol in a large tank via the Embden Meyerh of pathway under anaerobic conditions and controlled temperature. Yeast-based fermentation is always accompanied by formation of CO₂ by-products and supplemented by nitrogen to enhance the reaction. This conventional strain is optimal at a temperature of approximately 30°C and resists a high osmotic pressure in addition to its tolerance to low pH levels of 4.0 as well as inhibitory products [54]. *S. cerevisiae* can generate a high yield of ethanol (12.0-17.0% w/v; 90% of the theoretical) from hexose sugars [55].

Traditionally, separate hydrolysis and fermentation (SHF) sequential steps are used in bioethanol production. However, there is particular interest in targeting bioethanol production that can be derived from lignocellulosic biomass materials where both hexose and pentose sugars are available from the hemicellulose fraction. Despite its broad tolerance to stressful bioethanol process conditions, *S. cerevisiae* is not able to ferment sugars other than hexose. Unfortunately, lignocellulosic material includes a large proportion of hemicellulosic biomass that contains mainly pentose sugars such as D-xylose [56]. Moreover, an optimal fermentative microorganism should be tolerant to a high ethanol concentration and to chemical inhibitors formed during pretreatment and hydrolysis process. In response to this inability of *S. cerevisiae* to ferment pentose sugars, extensive efforts have been employed to develop genetically engineered microorganisms that are capable of fermenting pentose and hexose sugars simultaneously. An optimal fermentative microorganism should be able to utilize both hexose and pentose simultaneously with minimal toxic end-products formation. Different techniques including SSF and consolidated bioprocessing (CBP)

have been developed to ensure the combination of hydrolysis (step 3) and fermentation (step 4) in one single reactor and thus, reduce product inhibition and operation costs. In addition to continuing downstream steps, CBP processing integrates both fermentation and cellulase formation in one fermentative/ cellulolytic microorganism [21]. However, despite the extensive range of prokaryotic and eukaryotic microorganisms that have been shown to be able to produce ethanol from sugars, most of them remain limited in terms of sugars co-fermentation, ethanol yield and tolerance to chemical inhibitors, high temperature and ethanol. In an effort to summarize relevant advantages and major limitations of microbial fermentative species.

3.4. Distillation

Bioethanol obtained from a fermentation conversion requires further separation and

purification of ethanol from water through a distillation process. Fractional distillation is a process implemented to separate ethanol from water based on their different volatilities. This process consists simply of boiling the ethanol water mixture. Because the boiling point of water (100 °C) is higher than the ethanol-boiling point (78.3 °C), ethanol will be converted to steam before water. Thus, water can be separated via a condensation procedure and ethanol distillate recaptured at a concentration of 95% [23]. Typically, most largescale industries and biorefineries use a continuous distillation column system with multiple effects [57]. Liquid mixtures are heated and allowed to flow continuously all along the column. At the top of the column, volatiles are separated as a distillate and residue is recovered at the bottom of the column. Totally the general procedures in Fig. 2.

Table 1. The contents of cellulose, hemicellulose, and lignin in Lignocellulosic materials^a

Lignocellulosic materials	Cellulose (%)	Hemicellulose (%)	Lignin (%)
Hardwoods stems	40–55	24–40	18–25
Softwood stems	45–50	25–35	25–35
Nut shells	25–30	25–30	30–40
Corn cobs	45	35	15
Grasses	25–40	35–50	10–30
Wheat straw	30	50	15
Sorted refuse	60	20	20
Leaves	15–20	80–85	0
Cotton seed hairs	80–95	5–20	0
Newspaper	40–55	25–40	18–30
Waste papers from chemical pulps	60–70	10–20	5–10
Primary wastewater solids	8–15	NA ^b	24–29
Swine waste	6.0	28	NA ^b
Solid cattle manure	1.6–4.7	1.4–3.3	2.7–5.7
Coastal Bermuda grass	25	35.7	6.4
Switch grass	45	31.4	12.0

^a Source: Reshamwala et al. (1995), Cheung and Anderson (1997) and Boopathy (1998)

^bNA – not available.

Table 2. Pre-treatment methods and key characteristics

Pretreatments	Key characteristics
Dilute acid (H ₂ SO ₄ , HCL (0.5-5%))	<ul style="list-style-type: none"> ✚ Practical and simple technique. Does not require thermal energy. ✚ Effective hydrolyze of hemicelluloses with high sugar yield, Generates toxic inhibitors & Requires recovery steps
Hot water	<ul style="list-style-type: none"> ✚ The majority of hemicelluloses can be dissolved. ✚ No chemicals and toxic inhibitors. ✚ Average solid load & Not successful with softwood.
Lime	<ul style="list-style-type: none"> ✚ High total sugar yield including pentose and hexose sugars. ✚ Effective against hardwood and agricultural residues. ✚ High pressure and temperature hinder chemical operation. ✚ Commercial scalability problem
Ammonia fiber expansion (AFEX)	<ul style="list-style-type: none"> ✚ Effective against agricultural residues mainly corn stover without formation of toxic end-products. ✚ Not suitable for high-lignin materials. ✚ Ammonia recovery ✚ No wastewaters
Ammonia recycle percolation (ARP)	<ul style="list-style-type: none"> ✚ High redistribution of lignin (85%) ✚ Recycling ammonia ✚ Theoretical yield is attained
Steam explosion with catalyst	<ul style="list-style-type: none"> ✚ Effective against agricultural residues and hardwood. ✚ High hemicelluloses fractions removal ✚ Not really effective with softwood
Organosolv	<ul style="list-style-type: none"> ✚ High yield is enhanced by acid combination. ✚ Effective against both hardwood and softwood. ✚ Low hemicellulosic sugar concentration ✚ Formation of toxic inhibitors, Organic solvent requires recycling & High capital investment
Sulfite pretreatment to overcome recalcitrance (SPORL)	<ul style="list-style-type: none"> ✚ Effective against high-lignin materials, both softwood and hardwood. ✚ Highest pretreatment energy efficiency ✚ Minimum of inhibitors formation &-- Accommodate feedstocks versatility. ✚ Steam explosion combined to SPORL in presence of catalyst becomes effective against softwood materials ✚ Cost-effective.
Ozone	<ul style="list-style-type: none"> ✚ Effectively remove lignin from a wide range of cellulosic material without generating inhibitors. ✚ Expensive
Alkaline wet oxidation	<ul style="list-style-type: none"> ✚ The combination of oxygen, water, high temperature and alkali reduce toxic inhibitors. ✚ High delignification and solubilization of cellulosic material ✚ Low hydrolysis of oligomers
Fungal bioconversion	<ul style="list-style-type: none"> ✚ Environmentally friendly ✚ Low use of energy and chemical ✚ Slow bioconversion

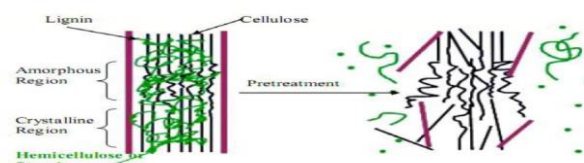


Figure 1. Schematic Representation on Biomass Pre-Treatment (Mosier et al, 2004)

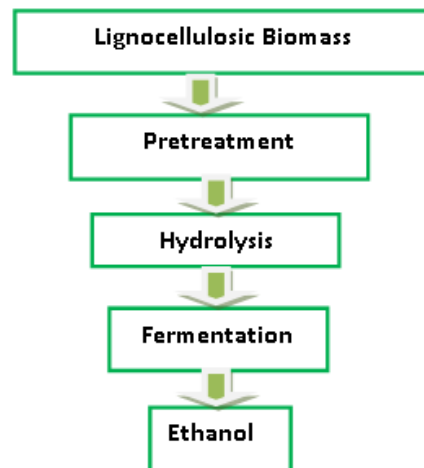


Figure 2. Schematic diagram of cellulosic ethanol production

CONCLUSIONS

Ethanol is comes into traditional fuel for transportation in last decades and it is less polluting and clean burning fuel. Cellulosic-based ethanol is a potential alternative over food derived ethanol originating mainly from corn starch and sugarcane provided. This review highlights cellulosic biomass with different pretreatment, hydrolysis and fermentation methods to produce ethanol. The review assessed the potential of biomass for producing energy could increase into huge amount. Pretreatment, the most costly step is of particular concern due to the high recalcitrance of lignocellulosic raw materials. Given that lignocellulosic feedstock is a versatile material and bioethanol is a commodity product, it has been deemed imperative to design a general pretreatment combination that would be effective against a wide range of cellulosic material and hence deal with feedstock variability. For instance, researchers have shown that pretreatments involving steam explosion with either catalyst or lime are potential candidates to agricultural residues, herbaceous materials and hardwoods. The inability of steam explosion combined with catalyst to degrade softwood materials can be compensated by the low-cost and the energy efficient pretreatment approach. Genetic engineering also works to improve the efficiency of microorganisms for increase yield as well as minimum cost of production. Commonly *Pichia stipites* and *saccharomyces cerevisiae* is used by

different researchers. Enzymatic hydrolysis may be the most potent alternative process for saccharification of complex polymer contains feed. Forest, industrial wastes typically unused and available in large amount. Agricultural waste is renewable, less costly and available in nature. Agriculture waste do not demand separate land, water and energy requirement. They do not have food value as well. Most of the municipal solid waste contains starch, lignocellulos, hemicelluloses and sugar that are sufficient for fermentation and very good raw materials compared to the other. While technological accomplishments and multiple research coalition efforts are still progressing, an efficient combination of the most advanced systems analysis and economical techniques designed to cope with feedstock versatility and commodity should emerge as the option of choice in an attempt to achieve optimal second-generation biofuel performance. In recent years it has been investigated that, instead of traditional feed stocks (starch crops), cellulosic biomass, including forest and industrial residues, agriculture waste and municipal waste, could be used as an ideally inexpensive and sufficient amount of sugar for production of ethanol by fermentation. Researchers mainly concern about cheapest and unused available resources to produce ethanol.

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