A REVIEW ON BIOETHANOL PRODUCTION FROM AGRICULTURAL LIGNOCELLULOSIC BIOMASS

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Abstract: Bioethanol obtained from lignocellulosic biomass is the most promising biofuel resource to tackle problems like rapid depletion, rising global warming and crude oil prices related to the energy derived from conventional resources. There are three main processes required in the bioethanol production from agricultural lignocellulosic wastes viz. pretreatment, hydrolysis, and fermentation. Hydrolysis can be carried out either biologically (enzymatic) or chemically (acidic). The main factor limiting the bioethanol commercialization is in the development of the step called enzymatic hydrolysis. Challenges like high enzyme cost, phenolic compounds affecting the activity of enzymes and thermal inactivation of cellulase and hemicellulase enzymes make enzymatic saccharification a bottleneck of the overall bioethanol production. This paper reviews the steps involved in bioethanol production from lignocellulosics with emphasis on recent trends of enzymatic hydrolysis process for cost-effective bioethanol production.

Keywords **- Second Generation bioethanol, Pretreatment, Enzymatic hydrolysis, Cellulases**

I. INTRODUCTION

With unceasing development of country and progressive living standards, demand for energy is growing rapidly whereas fossil fuel resources are depleting which results into quest for alternative renewable energy resources (Hook and Tang, 2013) of which bioethanol is the dominating biofuel with an annual world production expanded over 7% during 2018, from 104 billion litres to 112 billion litres (REN21). This energy scarcity situation led to increased attention towards biofuel that can be produced from renewable feedstock such as agricultural lignocellulosic biomass (Rivarolo *et al*., 2016). Lignocellulosic biomass often referred to as cellulosic biomass, is the only known large scale sustainable raw material for biorefineries to fulfill the constantly growing energy demand (Lynd *et al*., 2008). Transformation of cellulosic biomass to value-added products like biofuels and other chemicals has enormous merits such as the reduction in greenhouse gas (GHG) emission, low dependence on non-renewable energy sources and improvement in energy security of the country (Wyman, 2007). Ethanol derived from lignocellulosic biomass (plant cell wall) is second-generation (2G) bioethanol whereas the one obtained from starchy resources like cane sugar, wheat, corn, etc. is termed as first-generation (1G) bioethanol (Jordan *et al*., 2012). Studies have shown that energy derived from lignocellulosic biomass is much higher with lower emission of GHG compared to starchy feedstock based bioenergy (Hsu *et al*., 2010). Moreover, unlike feedstock used for 1G biofuel, this lignocellulosic biomass does not have conflict over food vs fuel and thus is continuously receiving attention (Alvira *et al*. 2010). The intrinsic recalcitrance of lignocellulosic biomass, rigorous pre-treatment, costly enzymes are some of the factors contributing to the high cost of 2G ethanol (Lynd *et al*., 2008).

II. AGRICULTURAL LIGNOCELLULOSIC BIOMASS

2.1 Structure of lignocellulosic biomass

Lignocellulosics typically non-edible plant components, encompassing dedicated crops, and agricultural biomass which comprises of cellulose, hemicellulose, lignin, and other inorganic materials. The compositions of each differ with the origin of the lignocellulosic material (Saini *et al*., 2015) (Figure 1).

Typically, agricultural lignocellulosic biomass is comprised of cellulose (35-50%), hemicelluloses (15-30%), and lignin (12-35%). Cellulose and hemicelluloses, the chief components of agricultural biomass those can be potentially hydrolyzed to sugars which are further converted into ethanol while lignin can be converted to produce fuels and chemicals (Wyman and Ragauskas, 2015).

2.1.1 Cellulose

Cellulose, the major structural component in the plant cell wall, is a linear homopolysaccharide composed of a long chain of glucose monomers linked by β-1,4-glycosidic bonds. The extensive intra- and intermolecular hydrogen bonding network in its structure results in crystalline and strong matrix structure which increases the rigidity of cellulose (Ebringerova *et al*., 2005). Cellulose is the most prevailing organic macromolecule for the reason that approximately 50% of the organic carbon in the environment exists in the form of cellulose. Thus, its utilization for producing fuels and valuable chemicals has copious importance (Zhou *et al*., 2011; Taarning *et al*., 2011).

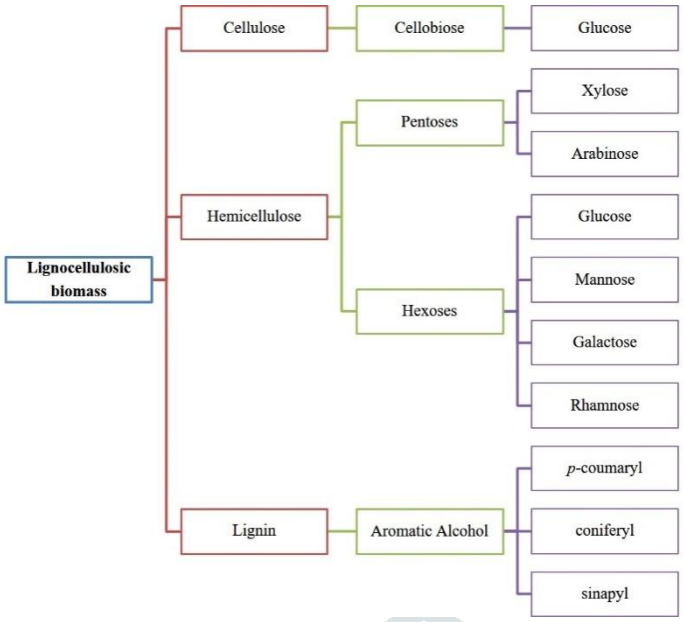


Figure 1 Diagrammatic representation of lignocellulosic biomass with its contents (Source: Sajith *et al.*, 2016)

2.1.2 Hemicellulose

Hemicellulose, the subsequent most prevalent polymer of plant biomass is a heterogeneous mixture of pentoses (including arabinose and xylose), hexoses (glucose, galactose, and mannose) and uronic acids (Howard *et al.*, 2004). Its composition is uneven which relies on the nature of plant sources. Generally, in softwoods mostly found from conifers and gymnosperm trees like spruce and pine, a hemicellulosic part is mainly made of mannan, especially glucomannan and galactoglucomannan, while hemicellulose in secondary walls of hardwood (e.g., angiosperm) and herbaceous plants consists of xylans (Girio *et al.*, 2010).

2.1.3 Lignin

Lignin is the most complex natural polymer consisting of a predominant building block of phenylpropanoid units like p-coumaryl alcohol, sinapyl alcohol, and coniferyl alcohol which are particularly difficult to biodegrade making lignin the most non-biodegradable component of the plant cell wall (Harmsen *et al.*, 2010).

The distribution of these three major polymers is not uniform in the cell walls of plants. Their structure and quantity vary with plant cell wall species, type of tissues, and maturity (Barakat *et al.*, 2013).

III. FROM BIOMASS TO BIOETHANOL

Conversion of lignocellulosics to ethanol includes the following steps: Pretreatment, Separation of lignin residues, Hydrolysis of cellulose and hemicellulose, fermentation of reducing sugars to bioethanol, and recovery and purification of ethanol (Figure 2) (Maurya *et al.*, 2015).

The appropriate blend of each of the above-mentioned steps is significant for accomplishing maximum bioethanol yield in an economical and practical approach (Saini *et al.*, 2015).

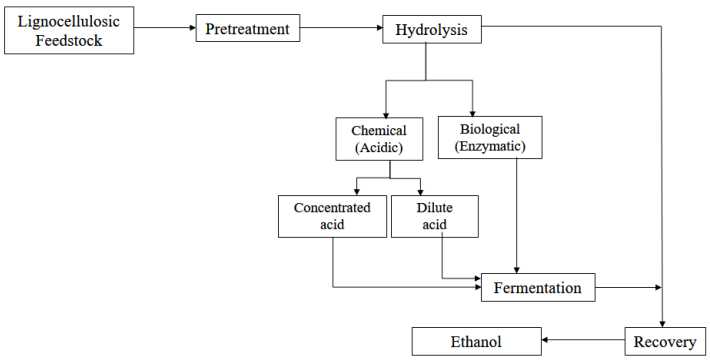


Figure 2 Flowchart of bioethanol production from lignocellulosic biomass (Source: Nicola *et al.*, 2011)

3.1 Pretreatment

It is a processing step to make lignocellulosic biomass more flexible to biological conversion rendering high yields that generally suffer from high processing expenses with low yield (Wyman *et al.*, 2013). A suitable process is required to accelerate effective enzyme access and hydrolysis of pretreated biomass to fermentable sugars (Maitan-Alfenas *et al.*, 2015).

Several pretreatment methods have been recognized during the last few years. Some of the most common pretreatment technologies used on lignocellulosic biomass are as shown in Table 1(Kumar and Sharma, 2017).

3.2 Hydrolysis

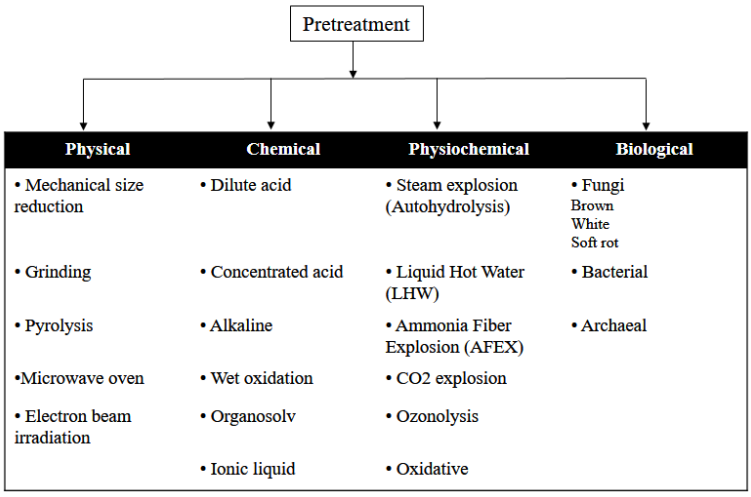
Hydrolysis of pretreated biomass is an important step to convert cellulosic and hemicellulosic part into fermentable sugar for biofuel production whose success depends on the effectiveness of the pretreatment operation used (Gamage *et al.*, 2010). Hydrolysis can be enacted in two ways either with the aid of acid, especially sulphuric acid or with enzymes **(**Nazhad *et al.*, 1995).

3.2.1 Acid Hydrolysis

Acidic reactions can be divided into dilute or concentrated acid hydrolysis. The main significance of this chemical hydrolysis is that acids can enter lignin without any preliminary pretreatment of biomass, thus hydrolyzing the cellulose and hemicellulose into individual sugar molecules. Various types of acids, diluted or concentrated can be used, such as sulphuric, phosphoric, hydrochloric, hydrofluoric, formic and nitric acid **(**Galbe and Zacchi, 2002). Among them, H_2SO_4 and HCl are the most frequently utilized acids for the hydrolysis of lignocellulosic biomass (Lenihan *et al.*, 2010).

Dilute hydrolysis (2-5%) is done at a high temperature of 200-240ºC to accomplish acceptable rates of cellulose conversion (Xiang *et al.*, 2003). This high temperature enhances the decomposition rates of hemicellulose sugars resulting into the formation of toxic compounds such as furfural and 5-hydroxymethyl-furfural (HMF). These toxic compounds are harmful to the effective fermentation stage that causes lower ethanol production. Additionally, they bring about the reduction of fermentable sugars and also increase the corrosion of equipment (Kootstra *et al*., 2009). The only advantage of this hydrolysis process is it requires a low amount of acid.

Table 1 Some of the most common pretreatment methods used on lignocellulosic biomass for production of 2G ethanol (Kumar and Sharma, 2017)



Nearly 10-30 % of the acid is used in the process of concentrated acid hydrolysis. This process takes place at low temperatures and produces high hydrolysis yields (Iranmahboob *et al*., 2002). However, large amounts of acids are required that cause corrosion problem to the equipment along with expensive acid recycling making it commercially less attractive (Hamelinck *et al*., 2005).

3.2.2 Enzymatic hydrolysis

In spite of the previously mentioned advantages of chemical hydrolysis, hydrolysis employing enzymes is preferred over it due to various advantages like high product yield, low generation of a toxic compound, lower chemical requirements, lower energy and mild environment conditions, and less formation of fermentation inhibitor products (Kuila *et al*., 2016). The production of sugar from cellulose and hemicelluloses obtained after the pretreatment of lignocellulosic biomass in the presence of enzyme results in effective production of monosaccharides (Maitan-Alfenas *et al*., 2015). Enzymatic hydrolysis of lignocellulose is complex because several structural characters contribute to its recalcitrant nature (Poovaiah *et al*., 2014).

i. Cellulose hydrolysis

Cellulases are glycoside hydrolases (GHs) that hydrolyse β-1,4-glycosidic bonds of cellulose into shorter chain polysaccharides such as cellodextrin, cellobiose, and glucose. Cellulase complex consists of endo-β-glucanase, exo-β-glucanase, and β-glucosidase. All these enzymes synergistically bring out the complete saccharification of cellulose (Bhat, 2000).

Mechanism: As shown in figure 4 Endoglucanases often termed as CMCase that degrades carboxymethyl cellulose (CMC) hydrolyze the β-1, 4-glycosidic bonds haphazardly within the cellulose chain to produce a soluble long-chain cellodextrin or fragments of insoluble cellulose (Zhang and Zhang, 2013). Exoglucohydrolases or Cellobiohydrolases (CBH) removes glucose units from the non-reducing ends of cyclodextrins. Finally, β-glucosidases bring hydrolysis of cellobiose into glucose. It is also responsible for the removal of glucose monomers from non-reducing ends of small cyclodextrins (Van den Brink and de Vries, 2011). They are vital for proficient cleavage of cellulose, as it hydrolyse cellobiose and small cello-oligosaccharides into glucose monomers and completes the saccharification (Lima *et al*., 2013).

Recently a novel class of oxidative enzymes called Lytic polysaccharide monooxygenases (LPMOs) has been discovered which oxidizes highly recalcitrant crystalline regions of cellulose, creating more reducing/non-reducing ends for cellulase components to attack thus, ultimately resulting into an increasing yield of product and reducing overall processing cost (Agger *et al*., 2014).

Sources:

Cellulolytic microorganisms mainly include fungi, bacteria, and some actinomycetes (Kim and Kim, 2012). Recently, fungi are the most studied cellulase producers considering their high protein secretion capabilities and multi-component, synergetic, cellulolytic, enzyme activity (Juturu and Wu, 2014). Some of the other notable cellulase producers encompasse the soft-rot, brown-rot and white-rot fungi (E.g. *Aspergillus*, *Penicillium*, and *Humicola*, bacteria (such as *Cellulomonas*, *Pseudomonas)* and actinomycetes (E.g. S*treptomyces)* (Sajith *et al.*, 2016)

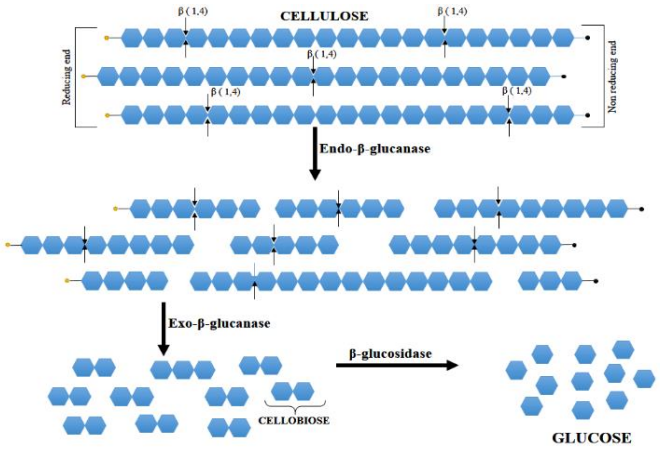


Figure 3 Diagrammatic representation of the cellulose hydrolysis by the synergistic action of cellulases (Sajith *et al*., 2016)

ii. Hemicellulose hydrolysis

Hemicellulases are responsible for the hydrolysis of hemicellulose polymer. Xylan, the major component of hemicellulose is hydrolysed by endo-ß-1,4-xylanase, ß-xylosidase, and many accessory enzymes including α-L-arabinofuranosidase, α-glucuronidase, α-galactosidase, acetyl xylan esterase, and ferulic acid esterase. The internal bond of xylan is randomly broken down by endo-ß-1,4-xylanase to release xylo-oligosaccharides while ß-xylosidase hydrolyses the non-reducing ends of xylose chains to release xylose (Kumar and Murthy, 2013).

iii. Lignin hydrolysis

Ligninases (Lignases) are the enzymes that hydrolyze lignin into low molecular weight compounds that are absorbed by other microorganisms. Generally, two types of ligninases are found namely laccases and peroxidases (lignin peroxidase and manganese peroxidase). Laccases are copper-containing glycoproteins responsible for the degradation of lignin to form phenoxy radicals and quinines (Kunamneni *et al*., 2007). Microorganisms that can produce laccase include *Aspergillus nidulans, Pleurotus pulmonarius, Phellinus ribis, and Phanerochaete chrysosporium* (Arora and Sharma, 2010).

Peroxidases cause depolymerization of lignin utilizing H_2O_2. Lignin peroxidase is a protein containing heme that possesses high redox potential with a low optimum pH of nearly 3 (Arora and Sharma, 2010) whereas Manganese peroxidases utilise manganese as electron donor and cause oxidisation of phenolic structures to phenoxyl radicals (Chinedu *et al*., 2005).

3.3 Fermentation

Once the lignocellulolytic enzymes hydrolyze biomass into fermentable sugars, the fermentation process is carried by several microorganisms to produce bioethanol. Figure 4 depicts various saccharification and fermentation bioprocess integrations reported for bioethanol production (Sarkar *et al*., 2012). Each process has its merits as well as limitations.

Saccharomyces cerevisiae, Zymomonas mobilis, Escherichia coli, Pachysolen tannophilus, Candida shehatae, Mucor indicus etc. are few microorganisms employed in the fermentation process (Sukumaran *et al*., 2010; Girio *et al*., 2010). *S. cerevisiae* and *Z. mobilis* are most resilient among them to be employed in ethanol production (Talebnia *et al*., 2010). However, *S. cerevisiae* is found to be inefficient in utilizing pentose sugar, xylose for producing ethanol. Therefore, to increase ethanol yield and reduce its cost, efforts are continuously made not only to make mesophilic/thermophilic microorganisms efficient to ferment both pentoses as well as hexoses but also to make microorganisms break down cellobiose and higher cellodextrins promptly to ethanol and other valuable products (Kumar *et al*., 2016).

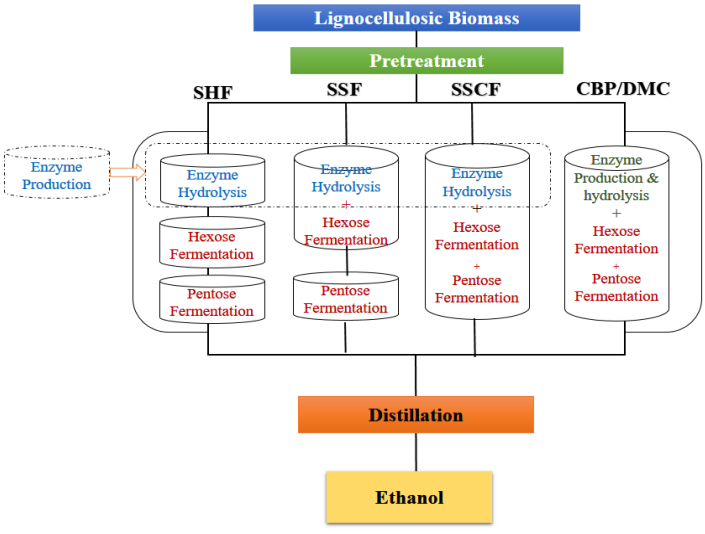


Figure 4 Diagrammatic representation of the cellulose hydrolysis by the synergistic action of cellulases (Source: Ali *et al*., 2016)

[SHF: Separate (or sequential) Hydrolysis and Fermentation; SSF: Simultaneous Saccharification and Fermentation; SSCF: Simultaneous Saccharification and Co-Fermentation; CBP: Consolidated BioProcessing (CBP)/DMC:Direct Microbial Conversion]

IV. CONCLUDING REMARKS

Second-generation ethanol derived from agricultural residues and other lignocellulosic biomass is an excellent substitute for conventional energy resources. Painstaking efforts of researchers towards improvising the efficacy of biomass conversion methods are making it more economically acceptable. Recent progress in enzyme sector, pretreatment and fermentation process in lignocellulosic derived bioethanol will surely make it a promising technology to accomplish energy demand and security in nearby future.

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