



CHAPTER II

LITERATURE REVIEW

Lignocellulosic biomass comes in many different types, which may be grouped into four main categories: (1) wood residues (including saw mill and paper mill discards), (2) municipal paper waste, (3) agricultural residues (including corn stover and sugarcane bagasse), and (4) dedicated energy crops (which are mostly composed of fast growing tall, woody grasses).

2.1 The composition of lignocellulosic biomass

Lignocellulosic biomass mainly consists of three different types of polymers, namely cellulose (40–50 %), hemicellulose (25–35 %), and lignin (15–20 %), which are associated to each other. Composition of some lignocellulosics is given in Table 2.1.

Table 2.1 Composition of some agricultural lignocellulosic biomass (Saha, 2003)

	Composition (% , dry basis)		
	Cellulose	Hemicellulose	Lignin
Corn fiber ^a	15	35	8
Corn cob	45	35	15
Corn stover	40	25	17
Rice straw	35	25	12
Wheat straw	30	50	20
Sugarcane bagasse	40	24	25
Switchgrass	45	30	12
Coastal Bermuda grass	25	35	6

^a Contains 20 % starch

2.1.1 Cellulose

Cellulose exists of D-glucose subunits, linked by β -1,4 glycosidic bonds, as shown in Figure 2.1. The cellulose in a plant consists of parts with a crystalline (organized) structure, and parts with a, not well-organized, amorphous structure. Because of the β -1,4 linkage, cellulose is highly crystalline and compact, making it very resistant to biological attack. The cellulose strains are 'bundled' together and form so called cellulose fibrils or cellulose bundles. These cellulose fibrils are mostly independent and weakly bound through hydrogen bonding (Handriks *et al.*, 2009).

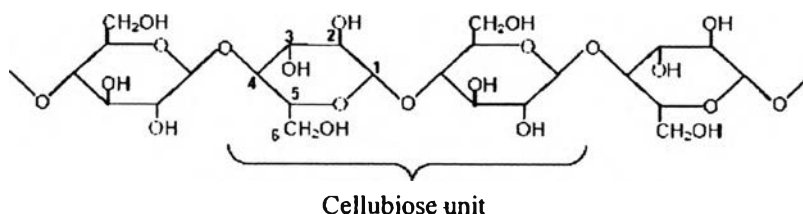


Figure 2.1 Schematic of the basic structure of cellulose (Kumar *et al.*, 2009).

2.1.2 Hemicellulose

Hemicellulose is a complex carbohydrate structure consisting of different polymers like pentoses (like xylose and arabinose), hexoses (like mannose, glucose, and galactose), and sugar acids, as shown in Figure 2.2. The dominant component of hemicellulose from hardwood and agricultural plants, like grasses and straw, is xylan, while from softwood is glucomannan. Xylans of many plant materials are heteropolysaccharides with homopolymeric backbone chains of 1,4-linked β -D-xylopyranose units.

Hemicellulose has lower molecular weight than cellulose, and branches with short lateral chains that consist of different sugars, which are easily hydrolyzable polymers. Hemicellulose serves as a connection between the lignin and the cellulose fibers, making the whole cellulose–hemicellulose–lignin network more rigid. The compactness and complexity of lignocellulose make it much more difficult than starch to enzymatically degrade to fermentable sugars.

The solubility of different hemicellulose compounds is in descending order: mannose, xylose, glucose, arabinose, and galactose, and the solubility increases with increasing temperature. The solubility of higher molecular polymers could not be predicted because of unknown melting points. The solubilization of lignocellulose components not only depends on temperature, but also on other aspects like moisture content and pH. The xylan of hemicellulose can be extracted quite well in an acid or alkaline environment, while glucomannan can hardly be extracted in an acid environment and needs a stronger alkaline environment. Xylan can be the most easily extracted. Of cellulose, hemicellulose, and lignin, the hemicelluloses are the most thermal-chemically sensitive. During thermal-chemical pretreatment, the side groups of hemicellulose will firstly react, followed by the hemicellulose backbone (Handriks *et al.*, 2009 and Saha., 2003).

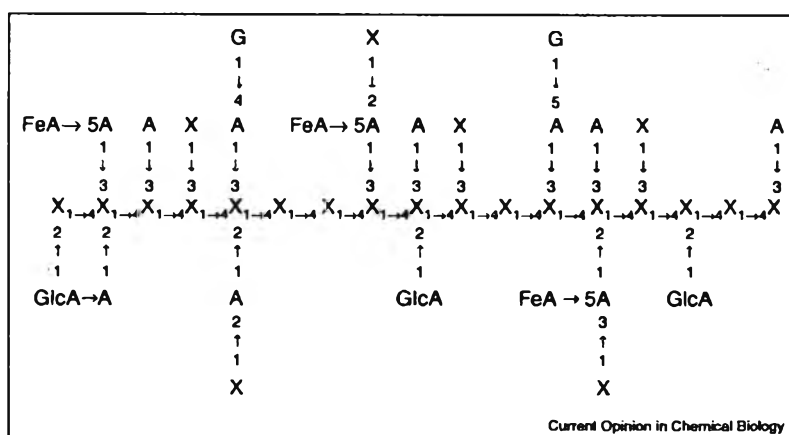


Figure 2.2 Schematic of the basic structure of hemicellulose. A, arabinose; FeA, ferulic acid; G, galactose; Glc, glucuronic acid; X, xylose (Gray *et al.*, 2006).

2.1.3 Lignin

Lignin is one of the most abundant polymers in nature, and is present in the cellular wall. It is an amorphous heteropolymer, consisting of three different phenylpropane units (p-coumaryl, coniferyl, and sinapyl alcohol) held together by different kind of linkages. The main purpose of having lignin is to give the plant structural support, impermeability, and resistance against microbial attack and oxidative stress. Lignin is also non-water soluble and optically inactive. All these

properties make the degradation of lignin very tough. The solubility of the lignin in acid, neutral, or alkaline environments depends, however, on the precursor (p-coumaryl, coniferyl, sinapyl alcohol or combinations of them) of the lignin (Hendriks *et al.*, 2009).

2.2 Pretreatment of lignocellulosic biomass

Pretreatment is required to alter the biomass macroscopic and microscopic size and structure as well as its submicroscopic chemical composition and structure so that hydrolysis of the carbohydrate fraction to monomeric sugars can be achieved more rapidly with greater yields (Mosier *et al.*, 2005). The goal is to break the lignin and hemicellulose, disrupt the crystalline structure of cellulose, and increase the porosity of the materials; as represented in the schematic diagram of Figure 2.3.

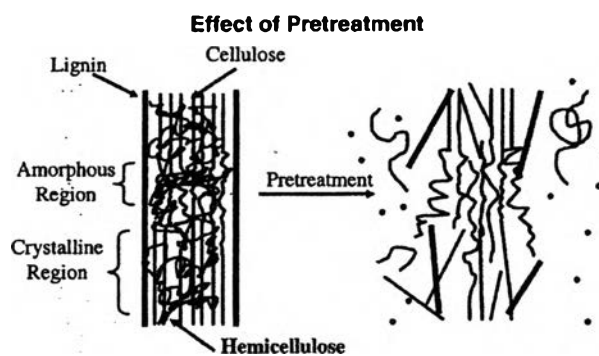


Figure 2.3 Schematic of goals of pretreatment on lignocellulosic material (Mosier *et al.*, 2005).

Pretreatment must meet the following requirements: (1) To improve the formation of sugars or the ability to subsequently form sugars by enzymatic hydrolysis; (2) To avoid the degradation or loss of carbohydrate; (3) To avoid the formation of byproducts inhibitory to the subsequent hydrolysis and fermentation processes; and (4) To be cost-effective. The process itself utilizes pretreatment additives and/or energy to form solids that are more reactive than native material, and/or generate soluble oligo- and monosaccharides (Figure 2.4).

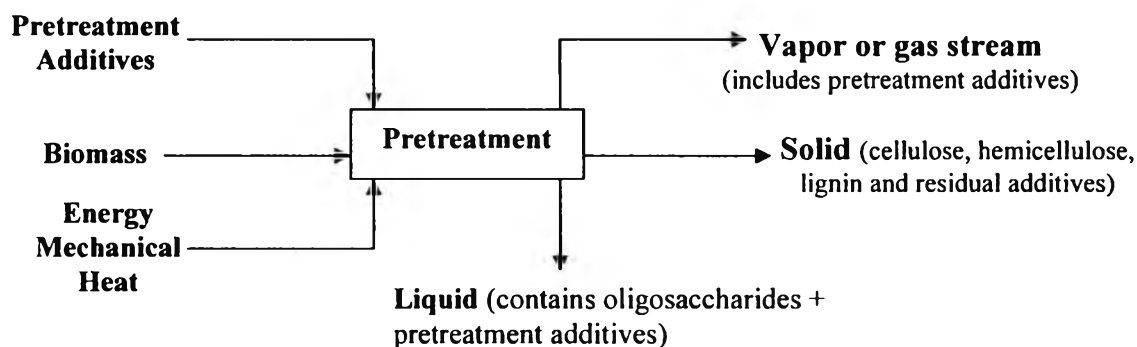


Figure 2.4 Schematic of pretreatment process (Mosier *et al.*, 2005).

A multitude of different pretreatment methods has been suggested during the past few decades. They can loosely be divided into different categories: physical (e.g. milling, grinding and irradiation), chemical (e.g. alkali, dilute acid, oxidizing agents and organic solvents), physicochemical (e.g. hydrothermolysis, wet oxidation, and steam pretreatment/autohydrolysis), and biological, or combinations of these (Galbe *et al.*, 2007). One of the most common methods used in this project is the chemical pretreatment.

2.2.1 Chemical pretreatment

The regular and cross-linked cellulose chains form a very efficient barrier against penetration of the enzymes into the fibers. Swelling of the pores can be achieved by alkaline pretreatment through soaking the material in an alkaline solution, such as *sodium hydroxide* (NaOH), followed by heating for a certain time. The swelling causes an increase in the internal surface area, and a decrease in the degree of polymerization and crystallinity. Usually, a major fraction of the lignin is solubilized together with some of the hemicellulose. A rather large fraction of the hemicellulose sugars are usually recovered as oligomers. Alkaline pretreatment breaks the bonds between lignin and carbohydrates, and disrupts the lignin structure, making the carbohydrates more accessible to enzymatic attack. As alkali pretreatment acts mainly by delignification, it is more effective on agricultural residues and herbaceous crops due to less lignin content than on wood materials generally containing a large amount of lignin. Pretreatment using *lime* instead of

NaOH is another alkaline method, especially suited for agricultural residues, e.g. corn stover, or hardwood materials, such as, poplar.

Dilute acid pretreatment is performed by soaking the material in dilute acid solution, followed by heating to temperatures between 140 and 200 °C for a certain time (from several minutes up to an hour). The main reaction occurring during the acid pretreatment is the hydrolysis of hemicellulose, especially, xylan, as glucomannan is relatively acid stable. The mechanism of the hydrolysis reaction includes (i) diffusion of protons through the wet lignocellulosic matrix; (ii) protonation of the oxygen of a heterocyclic ether bond between the sugar monomers; (iii) breaking of the ether bond; (iv) generation of a carbocation as intermediate; (v) solvation of the carbocation with water; (vi) regeneration of the proton with cogeneration of the sugar monomer, oligomer or polymer, depending on the position of the ether bond; (vii) diffusion of the reaction products in the liquid phase if it is permitted, depending on their forms and sizes; (viii) restarting the second step (Aguilar *et al.*, 2002). There are many types of the acid pretreatment, including use of sulfuric acid, hydrochloric acid, peracetic acid, nitric acid, or phosphoric acid. *Sulfuric acid*, at concentrations usually below 4 % (wt), has been of most interest in such studies since it is inexpensive and effective. The hemicellulose is hydrolysed and the main part is usually obtained as monomer sugars. It has been shown that materials that have been subjected to acid hydrolysis may be harder to ferment because of the presence of toxic substances (Balat *et al.*, 2008; Galbe *et al.*, 2007; and Sun *et al.*, 2002).

2.2.2 Inhibitors in fermentation

Dilute acid pretreatment methods generate significant microbial inhibitors, the undesirable lignocellulosic hydrolysate components. They can be divided into three groups on the basis of their origin:

- (1) *compounds released from the hemicellulose component* (e.g. acetic, ferulic, glucuronic, p-coumaric acids, etc.)
- (2) *lignin degradation products* (e.g. syringaldehyde, syringic acid, etc.)
- (3) *sugar degradation products* (e.g. furfural, hydroxymethyl furfural [HMF], and levulinic acid) (Figure 2.5)

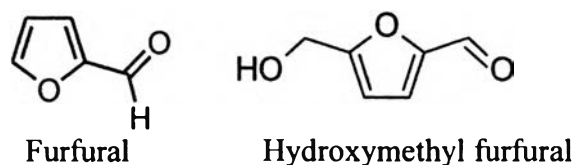


Figure 2.5 The chemical structures of furfural and hydroxymethyl furfural.

Therefore, to complete depolymerization of lignocellulosic biomass, it is difficult to totally avoid the generation of inhibitory compounds irrespective of the pretreatment and hydrolysis method utilized (Ezeji *et al.*, 2007).

2.2.3 Effects of chemical pretreatment conditions

2.2.3.1 *Dilute alkali pretreatment*

Zhao *et al.* (2009) studied effects of NaOH loading, temperature, treatment time, and liquid to solid ratio (LSR) on the sugarcane bagasse pretreatment. When the LSR was fixed, increasing NaOH loading caused higher delignification rate and enhanced the enzymatic digestibility. Temperature affected the kinetics of delignification and further affected the enzymatic digestibility. However, temperature had no significant effect on the yield of reducing sugar (YRS) at over 80 °C. They also found that treatment time had no significant effect on time profiles of enzymatic hydrolysis. The decrease of the degree of the delignification was probably due to the condensation of dissolved lignin when prolonging reaction time. Lastly, the results showed that a lower or higher liquid-to-solid ratio led to a decrease of conversion ratio of cellulose (CRC).

2.2.3.2 *Dilute acid pretreatment*

Silva *et al.* (2005) studied effects of sulfuric acid loading and residence time on the composition of sugarcane bagasse hydrolysates. They found that the increase in acid loading and residence time resulted in the increase in xylose concentration. However, these conditions also led to increasing concentrations of inhibiting byproducts, such as hydroxymethyl furfural and acetic acid. In 2002, Aguilar *et al.* found that acetic acid in hydrolysates was derived from the hydrolysis of the acetyl groups bound to the hemicellulosic monomers. The acid could be an inhibitor of microbial growth because it entered the cell membrane and decreased

intercellular pH, thus affecting the metabolism of the microorganisms in butanol fermentation process.

Laopaiboon *et al.* (2010) investigated the optimum conditions for hydrolysis of sugarcane bagasse. The results showed that xylose was the main product. At 90 °C to 110 °C, it was observed that the longer the reaction time, the higher the xylose concentration. However, at the highest temperature (120 °C), the maximum xylose concentration was obtained at the reaction time of 4 h. The highest xylose concentration (15.16 g/L) was obtained under 0.5 % HCl at 120 °C for 4 h. Under these conditions, glucose, 2.85; arabinose, 1.35; acetic acid, 0.04 and furfural, 0.66 (in g/L) were detected. In the case of the H₂SO₄ hydrolysis, the highest xylose concentration of 12.64 g/L was obtained when the bagasse was treated by 0.5 % H₂SO₄ at 110 °C for 4 h. Under these conditions, only glucose, 2.28; arabinose, 1.33 and acetic acid, 0.06 (in g/L) were detected.

Rodrigues *et al.* (2010) also studied morphological characteristics of sulfuric acid-pretreated sugarcane bagasse. Figure 2.6(a) shows a compact structure of “in natura” sugarcane bagasse. The acid hydrolysis interrupted the fibers of sugarcane bagasse after the partial removal of its hemicellulose fraction (Figure 2.6(b)).

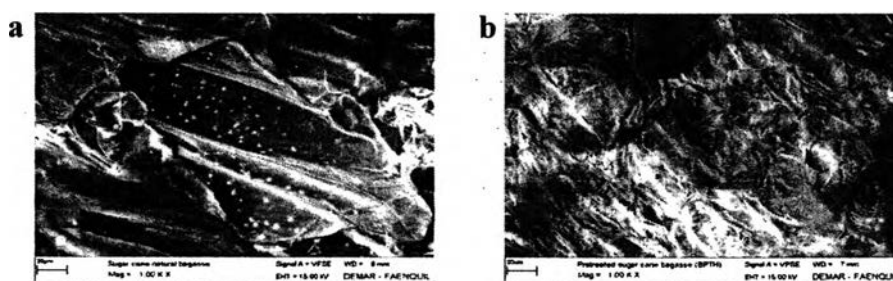


Figure 2.6 Scanning electron microscopy micrographs of sugarcane bagasse in the following forms: (a) “in natura”, and (b) after the acid hydrolysis in pilot reactor. Magnification of 1000x in secondary electrons mode at low-vacuum (Rodrigues *et al.*, 2010).

2.3 Microwave heating technique

In 1984, Ooshima *et al.* found that the microwave treatment for the cellulosic materials was a new effective technique for acceleration of their sacchafications, and explained the mechanism of microwave pretreatment. The cellulosic materials are heated internally, and water, cellulose, hemicellulose and the other low molecular compounds (such as the organic acid containing in the cellulosic materials) absorb the microwave radiation; the polar molecules and their neighboring clusters are then forced to orient to the specific direction. It, thus, appears that the microwave gives a directly serious shock to the polar molecules composing cellulosic materials.

Besides, Hu *et al.* (2008) reported that the beneficial effect of microwave pretreatment was due to volumetric and selective heating of the polar part of lignocelluloses. When microwave is used to treat lignocelluloses, it selectively heats the more polar part and creates a “hot spot” with the inhomogeneous materials. It is hypothesized that this unique heating feature results in an “explosion” effect among the particles, and improves the disruption of the recalcitrant structures of lignocellulose. In addition, the electromagnetic field used in microwave might create non-thermal effect that also accelerates the destruction of the crystal structures.

Zhu *et al.* (2005) compared microwave/alkali pretreatment with the alkali-alone pretreatment of rice straw, and found that microwave/alkali pretreatment could remove more lignin and hemicellulose from rice straw with shorter pretreatment time compared with the alkali-alone one. One year later, they investigated pretreatment of rice straw using different microwave/chemical pretreatment processes, namely, microwave/alkali, microwave/acid/alkali, and microwave/acid/alkali/H₂O₂. The results indicated that the rice straw pretreated by microwave/acid/alkali/H₂O₂ could remove the highest amount of lignin and hemicellulose, and also had the highest enzymatic hydrolysis rate.

Microwave-assisted alkali treatment of switchgrass was studied by Hu *et al.* (2008) who found that microwave heating increased the sugar yield in both pretreatment and enzymatic hydrolysis stages. When switchgrass was presoaked in alkali solution (0.1 g alkali/g biomass) and treated by microwave heating (190 °C, 50

g/L solid content for 30 min), a sugar yield from the combined treatment and hydrolysis was 99 % of maximum potential sugars.