# CHAPTER II
LITERATURE REVIEW

2.1 The Composition of Lignocellulosic Biomass

Lignocellulose is the primary building block of plant cell walls. Plant biomass is mainly composed of cellulose, hemicellulose, and lignin, along with smaller amounts of protein, extractives (soluble nonstructural materials such as nonstructural sugars, nitrogenous material, chlorophyll, and waxes), and ash (Jorgensen *et al.*, 2007). The composition of these constituents can vary from one plant species to another. For example, hardwood has greater amounts of cellulose whereas wheat straw and leaves have more hemicelluloses, see Table 2.1 (Sun *et al.*, 2002). In addition, the ratios between various constituents within a single plant vary with age, stage of growth, and other conditions (Perez *et al.*, 2002). Basically cellulose forms skeleton which surrounded by other substances functioning as matrix (hemicelluloses) and excrusting (lignins) materials. Cellulose, hemicellulose, and lignin are closely associated and covalent-cross linked. They have been suggested to occur between lignin and polysaccharides (lignin-carbohydrate complex, LCC). The side groups of arabinose, lactose, and 4-O-methy-glucuronic acid are most frequently perceived as connecting link to lignin. It is generally agreed that the hemicelluloses molecular are oriented parallel to cellulose fibril, as shown in Figure 2.1 (Fengel *et al.*, 1983).
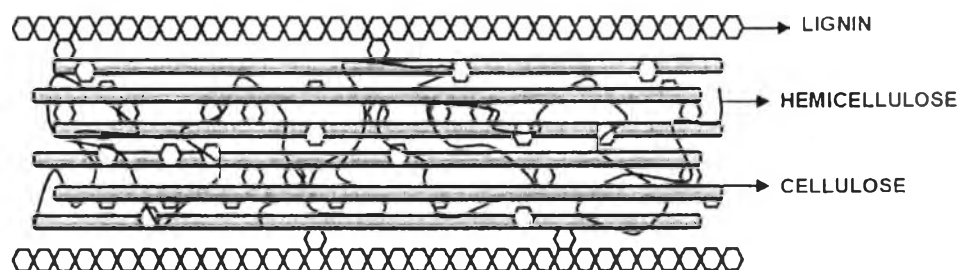
Figure 2.1 Representation of lignocellulose structure showing cellulose, hemicellulose and lignin fractions (Fengel *et al.*, 1983).

Table 2.1 Cellulose, hemicellulose, and lignin contents in common agricultural residues and wastes (Sun *et al.*, 2002)

Lignocellulosic material	Cellulose (%)	Hemicellulose (%)	Lignin (%)
hardwood 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
paper	85-99	0	0-15
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	60-70	10-20	5-10
solid cattle manure	1.6-4.7	1.4-3.3	2.7-5.7
coastal bermudagrass	25	35.7	6.4
Switchgrass	45	31.4	12
swine waste	6.0	28	na

2.1.1 Cellulose

Cellulose is main constituent of plant cell wall comprising about 50% of wood. Cellulose is closely associated with hemicelluloses and lignin, and isolation cellulose requires intensive chemical treatment. Cellulose consists of D-glucopyranose monomer unit bound by β-1-4 glycosidic linkages. The successive glucose residues are rotated by 180° relative to each other, and thus repeating unit of the cellulose chain is cellubiose unit, as shown in Figure 2.2. The degree of polymerization (DP) of cellulose varies between 7,000 and 15,000 glucose units, depending on source (Fengel *et al.*, 1983).
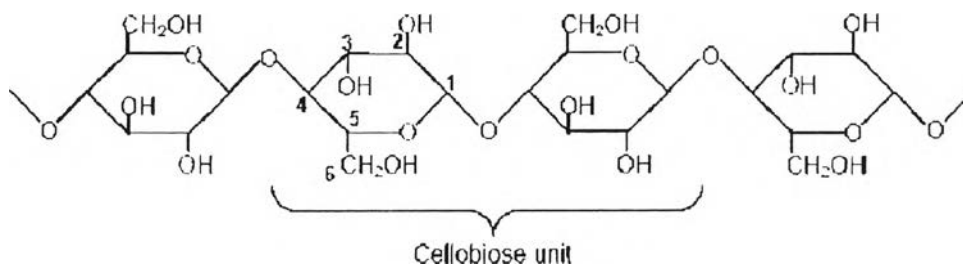
Figure 2.2   Illustration of a cellulose chain (Kumar *et al.*, 2009).

The functional groups in the cellulose chain are the hydroxyl group. These hydroxyl groups are able to interact with each other or with O-, N- and S- groups, forming hydrogen bond. Hydrogen bonds also exist between hydroxyl groups of cellulose and water molecule. These hydroxyl groups make the surface of cellulose largely hydrophilic. The cellulose chain has hydroxyl groups at both ends. The C_1 – end has reducing properties. The cellulose chain is stabilized by strong hydrogen bonds along the direction of the chain. In native cellulose found in plant source, cellulose chains are packed the individual cellulose chains are held together by hydrogen bond (O'Sullivan *et al.*, 1997). By forming these hydrogen bounds, the chains tend to arrange in parallel and form a structure. Therefore, cellulose microfibrils have both highly crystalline regions (around 2/3 of the total cellulose) and less-ordered amorphous regions. More ordered or crystalline cellulose is less soluble and less degradable (Zhang *et al.*, 2004, Taherzadeh *et al.*, 2008). The degree of cellulose crystallinity is a major factor affecting enzymatic hydrolysis of the substrate. It has been reported that a decrease in cellulose crystallinity especially influences the initial rate of cellulose hydrolysis. Physical or chemical pretreatment to disrupt the crystalline structure of cellulose is often used to promote the hydrolysis of biomass.

Christakopoulos *et al.* (2008) studied the effect of cellulose crystallinity on direct conversion of straw to ethanol by *Fusariumoxysporum*. The effect of ball milling on certain important physical characteristics of wheat straw is depicted. After 14 h of milling, the crystallinity index of cellulose was reduced to 23.6%. Ball milling, on the other hand, was not very efficient in increasing specific surface area of straw, due to particle agglomeration which was evident between 4 and 6 h of treatment. Pretreatment of wheat straw with ball milling markedly affected direct conversion of this material to ethanol by *F. oxysporum F3*. Of the main altered physical characteristics of straw, namely particle size, specific surface area, and crystallinity index, the latter showed a strong correlation with ethanol production. A highly correlated (r = 0.985) inverse linear relationship between wheat straw cellulose crystallinity index and ethanol production was established. This could be explained on the basis of enhanced action of cellulolytic

enzymes on noncrystalline (amorphous) cellulose, resulting in increased rates of saccharification and, consequently, ethanol production. Approximately 80% of straw carbohydrate was converted to ethanol when the cellulose crystallinity index was reduced to 23.6%.
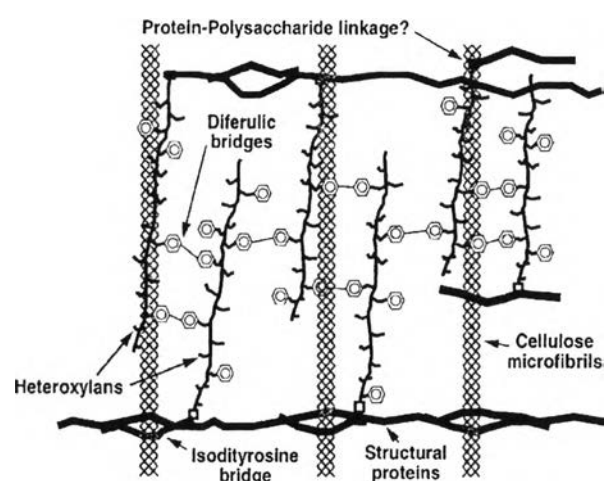
2.1.2 Hemicellulose

The main feature that differentiates hemicellulose from cellulose is that hemicellulose has branches with short lateral chains consisting of different sugars which are easy hydrolyzable polymers. These monosaccharides include pentoses (xylose, rhamnose, and arabinose), hexoses (glucose, mannose, and galactose), and uronic acids (e.g., 4-o methyl glucuronic, D-glucuronic, and D-galactouronic acids). Xylans of many plant materials are heteropolysaccharides with homopolymeric backbone chains of 1,4-linked β-D xylopyranose units. The backbone consists of 4-O-acetyl, α-L-arabinofuranosyl, α-1,2-linked glucuronic or 4-O-methyl glucuronic acid substituents. Xylans from different sources, such as grasses, cereals, softwood, and hardwood, differ in composition. About 80% of the xylan backbone is highly substituted with monomeric side-chains of arabinose or glucuronic acid linked to O-2 and/or O-3 of xylose residues, and also by oligomeric side chains containing arabinose, xylose, and sometimes galactose residues, as can be seen in Figure 2.3 (Saha *et al.*, 2003).

A: Arabinose
X: Xylose
G: Galactose
GlcA: Glucuronic Acid
FeA: Ferulic Acid
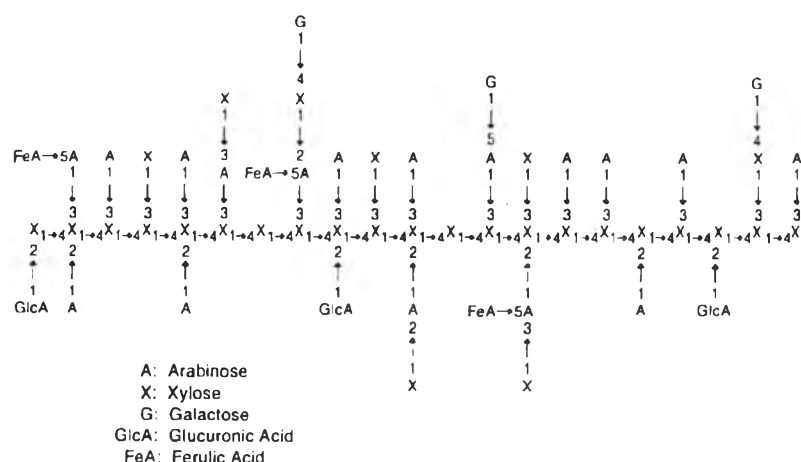
Figure 2.3   Schematic structure of corn fiber heteroxylan (Saha *et al.*, 2003).

A model for the corn fiber cell wall is shown in Figure 2.4. The heteroxylans, which are highly cross-linked by diferulic bridges, constitute a network in which the cellulose microfibrils may be imbedded. Structural wall proteins might be cross-linked together by isodityrosine bridges and with feruloylated heteroxylans, thus forming an insoluble network (Saha *et al.*, 2003). Hemicellulose serves as a connection between the lignin and the cellulose fibers and gives the whole cellulose-hemicellulose-lignin network more rigidity (Perez *et al.*, 2005).


Figure 2.4   Model for corn fiber cell walls (Saha *et al.*, 2003).

The solubility of the different hemicellulose compounds is in descending order: mannose, xylose, glucose, arabinose, and galactose. The solubility increases with increasing temperature. The solubilization of hemicellulose compounds into the water starts around 180 °C under neutral conditions (Bobleter *et al.,* 1994). Garrote *et al.* (1999) however mentioned that starting from 150 °C parts of the hemicellulose were solubilized. The solubilization of lignocelluloses components not only depends on temperature, but also on other aspects like moisture content and pH (Fengel *et al.*, 1983). 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 than xylan to be extracted. Xylan appears to be

the part that can be extracted the most easily. Of cellulose, hemicellulose and lignin the hemicelluloses are the most thermal-chemically sensitive. During thermal–chemical pretreatment firstly the side groups of hemicellulose react, followed by the hemicellulose backbone (Kumar *et al.*, 2009).

2.1.3 Lignin

Lignin is a complex, hydrophobic, cross-linked aromatic polymer. In nature, lignin is mostly found as an integral part of plant cell wall, embedded in a carbohydrate polymer matrix of cellulose and hemicelluloses. The main purpose of lignin is to give the plant structural support, impermeability, and resistance against microbial attack and oxidative stress. Lignins are polymers of phenyl propene unit: guaiacyl (G) unit from the precursor trans-conifery-alcohol, syringyl (S) units from trans-p-coumaryl alcohol, and p-hydroxy phenyl (H) unit from the precursor trans-p-coumaryl alcohol, as shown in Figure 2.5. The exact composition of lignin varies widely with species. In addition to classification as softwood, hardwood and grass lignin, lignins can be divided into two major groups: guaiacyl lignin and guaiacyl-syringyl lignins. Guaiacyl lignins are predominantly polymerization product of conifer alcohol while guaiacyl-syringyl lignins are composed of varying parts of the aromatic nuclei (Fengel *et al.*, 1983)
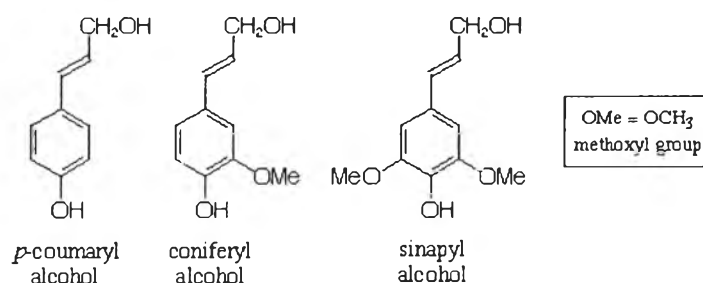
**Figure 2.5** Phenyl propene units (Fengel *et al.*, 1983).

Softwood contains mainly units while hard wood contains also syringyl extraction than hardwood. It has been suggested that the guaiacyl lignin restricted fibre swelling and thus the enzymatic accessibility more than syringyl lignin. It was observed that the residual substrate remained after extensive hydrolysis of the steam pretreatment aspen and eucalyptus was mainly composed of vessel elements. Vessel elements are known to have guaiacyl to syringyl ratio than other cells found in hardwood (Ramos *et al.*, 1992).

The major type of linkage in spruce lignins is ether linkage, of which aryl glycerol-β-aryl linkage is the most common. In addition, the phenyl propene units are linked by carbon-to-carbon linkage. The functional groups affecting the reactivity of lignin include free phenolic hydroxyl, methoxy, benzylic hydroxyl, benzyl alcohol, noncyclic benzyl ether, and carbonyl groups. Molecular dynamic simulation has suggested that the hydroxyl and methoxyl groups in lignin precursors and oligomers may interact with cellulose microfibrils despite the fact that lignin is hydrophobic in character. The structure scheme for softwood lignin constructed by Brunow *et al.* (1998) is present in Figure 2.6.

The chemical structure of native lignin is essentially changed under high temperature, such as the conditions during stream pretreatment. At reaction temperature higher than 200 °C, lignin has shown to be aggregated into smaller particle and separated from cellulose The studies on hardwood lignin have shown that β-O-4 aryl ether linkaged are cleaved in steam exploration causing a decrease in molecular weight and an increase in phenolic content (Marchessault *et al.*, 1981)

Lignin, just like hemicellulose, normally starts to dissolve into water around 180 °C under neutral conditions. The solubility of the lignin in acid, neutral, or alkaline environment depends however on the precursor (p-coumaryl, coniferyl, sinapyl alcohol or combinations of them) of the lignin (Bobleter *et al.*, 1994)
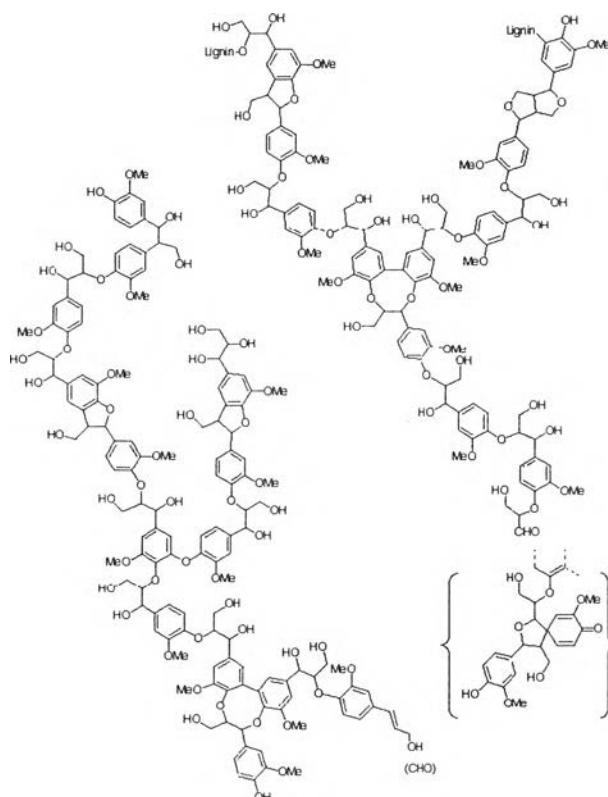
Figure 2.6   The structure of softwood lignin (Brunow *et al.*, 1998).

## 2.2 Ethanol Conversion Process

Processing of lignocellulosics to ethanol consists of four major unit operations: pretreatment, hydrolysis, fermentation, and product separation/ purification. The first step in bioconversion of lignocellosics to bioethanol is size reduction and pretreatment. Pretreatment is required to alter the size and structure of the biomass, as well as its chemical composition, so that the hydrolysis of the carbohydrate fraction to monomeric sugars can be achieved rapidly with greater yields.

As the pre-treatment is finished, the cellulose is prepared for hydrolysis, meaning the cleaving of a molecule by adding a water molecule (Balat *et al.*, 2008):

$$(C_6H_{10}O_5)_n + nH_2O \rightarrow nC_6H_{12}O_6. \qquad (2.1)$$

The hydrolysis process can be significantly improved by removal of lignin and hemicellulose, reduction of cellulose crystallinity and increase of porosity through pretreatment processes. In the hydrolysis process, the sugars are released by breaking down the carbohydrate chains, before they are fermented for alcohol production.

Chemical and enzymatic methods are the most common techniques for hydrolyzing cellulose. The chemical method, also known as concentrated acid hydrolysis, is conducted with mineral acids, such as H_2SO_4 or HCl (in the range of 10–30%), at temperatures of about 160 °C and pressures of about 10 atm. These harsh conditions (high temperature and acid concentration) are needed to liberate glucose from the tightly associated chains, because most cellulose is crystalline. In this process, acid concentration, temperature and time are crucial factors, and must be controlled to avoid the sugars and lignin degradation to by-products. Enzymatic hydrolysis has attracted increasing attention as an alternative to concentrated acid hydrolysis because the process is highly specific and can be performed under milder reaction conditions (pH around 5 and temperature less than 50 °C) with lower energy consumption and lower environmental impact. In addition, there are no corrosion problems, and it gives high yield of pure glucose with low formation of by-products that is favorable for the subsequent hydrolysate use in fermentation processes. Enzymatic hydrolysis of cellulose is a reaction carried out by cellulase enzymes, which correspond to a mixture of several enzymes, among which at least three major groups are involved in the hydrolysis of cellulose: (1) β-1-4-endoglucanase, which attacks regions of low crystallinity in the cellulose fiber creating free chain ends; (2) β-1-4-exoglucanase or cellobiohydrolase, which degrades the molecule further by removing cellobiose units from the free chain ends; (3) β-glucosidase, which hydrolyzes cellobiose to produce glucose. A schematic representation of the cellulase enzymes over the cellulose structure is shown in Figure 2.7 (Mussatto *et al.*, 2010).
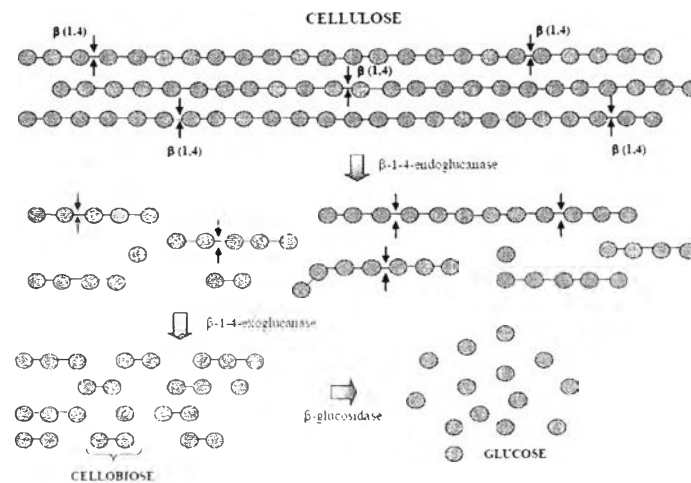
Figure 2.7 Schematic representations of the cellulase enzymes over the cellulose structure (Mussatto *et al.*, 2010).

2.3 Pretreament of Lignocellulosic Biomass

Pretreatment is a crucial process step for the biochemical conversion of lignocellulosic biomass into e.g. bioethanol. It is required to alter the structure of cellulosic biomass to make cellulose more accessible to the enzymes that convert the carbohydrate polymers into fermentable sugars (Mosier *et al.*, 2005).

Pretreatment involves the alteration of biomass so that enzymatic hydrolysis of cellulose and hemicellulose can be achieved more rapidly and with greater yields. Possible goals include the removal of lignin and disruption of the crystalline structure of cellulose (Figure 2.8). The following criteria lead to an improvement in enzymatic hydrolysis of lignocellulosic material:

- Increasing of the surface area and porosity

- Modification of lignin structure

- Removal of lignin

- (Partial) depolymerization of hemicellulose

- Removal of hemicellulose

- Reducing the crystallinity of cellulose

In an ideal case the pretreatment employed leads to a limited formation of degradation products that inhibit enzymatic hydrolysis and fermentation, and is also cost effective. However, these are actually the most important challenges of current pretreatment technologies
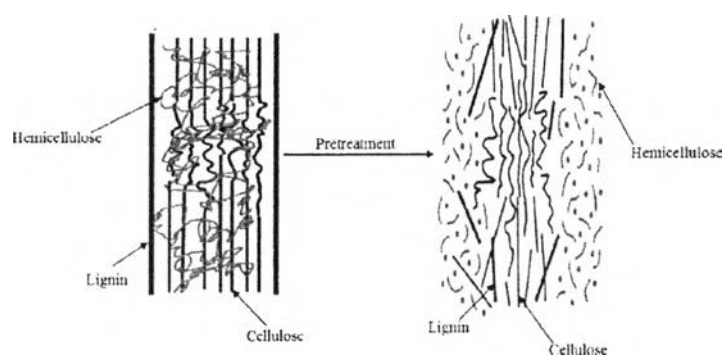
Figure 2.8 Schematic of the role of pretreatment in the conversion of biomass to fuel (Hsu *et al*, 1980).

Pretreatment methods can be roughly divided into different categories: physical (milling and grinding), physicochemical (steam pretreatment/auto-hydrolysis, hydrothermolysis, and wet oxidation), chemical (alkali, dilute acid, oxidizing agents, and organic solvents), biological, electrical, or a combination of these. Different pretreatment methods have been developed (Table 2.2).

Table 2.2 Comparison of advantages and disadvantages of different pretreatment options for lignocellulosic materials (Girio *et al.*, 2010)

Desirable features	Concentrated acid	Dilute acid	Steam explosion	Autohydrolysis	Organosolv	Solid Superacids	Alkaline	Ionic liquids	Supercritical fluids
High hemicellulose solubilisation	++	++	++	++	+	+	+	++	+
High hemicellulosic monosaccharides production	++	++	0	-	/0	+	/0	0/+	
Low hemicellulosic oligosaccharides production	+	+	0	-	/0	+	-/0	0/+	-
High cellulose recovery	++	++	++	++	-	++	+	+	++
High cellulose digestibility	++	++	+	+		+	++	+	0/+
High lignin quality	-	-	0/+	+	+	-/0	-	+	+
High chemicals recycling	-		0	n.r.	-	+	-/0/+	+	n.r./+
Low inhibitors formation	-	-	0	0	+	0	+	-	0
Low corrosion problems	-	-	0	0	-	0		/0	/0
Low need for chemicals	-	-	0	++	-	0/+	-/0	+	++
Low neutralisation requirements	-		0	n.r.	+	-/0	-/0	0	n.r.
Low investment costs	+	+	-	+	0	0	0/+	+	-
Low operational costs	-	0	++	+	-	0	-/0		
Low energy use	0	-	0	0	0	+	+	++	+

+, Advantage; -, disadvantage; 0, neutral; n.r., not relevant.

2.3.1 Physical Pretreatment.

Physical pretreatments operate based on the principle of particle size reduction by mechanical stress. Mechanical comminution of lignocellulosic materials through a combination of chipping, grinding, and/or milling can be applied to reduce cellulose crystallinity. The size of the materials is usually 10–30 mm after chipping and 0.2–2 mm after milling or grinding. This can be obtained by dry, wet vibratory, and compression based ball milling procedures, increasing the enzyme performance by improving the surface area to volume ratio and in some cases by reducing degree of polymerization and crystallinity of cellulose. Although physical pretreatments are not sufficient to dramatically increase sugar conversions, most other pretreatments require a minimal particle size reduction in order to be effective, especially to overcome mass and heat transport problems. It is also important to notice that beyond a certain particle size this type of pretreatment becomes economically unfeasible. The final particle size and biomass characteristics determine the power requirement for mechanical comminution of agricultural materials.

The energy consumption for size reduction of hardwoods and agricultural wastes as a function of final particle size and comminution ratio (size reduction) was quantified. It was proposed that, if the final particle size is held to the range of 3–6 mm, the energy input for comminution can be kept below 30 kWh per ton of biomass. The energy consumption is higher than the theoretical energy content available in the biomass in most cases (Kumar *et al.*, 2009).

2.3.2 Chemical Pretreatment

2.3.2.1 Dilute Acid Pretreatment

Dilute-acid processes have been viewed primarily as a means of pretreatment for the hydrolysis of hemicelluloses rendering the cellulose fraction more amenable for a further enzymatic treatment. Both cellulose and hemicellulose components can also be hydrolyzed using dilute-acid catalyzed processes. Compared to the concentrated acid pretreatment, one of the advantages of dilute acid pretreatment is the relatively low acid consumption, limited problem associated with equipment corrosion and less energy demanding for acid recovery. Under controlled conditions, the levels of the degradation compounds generated can also be low. Acid pretreatments normally aim for high yields of sugars from lignocellulosic biomass. There are many types of acid pretreatment including use of sulfuric acid, hydrochloric acid, peracetic acid, nitric acid, or phosphoric acid.

Martin *et al.* (2007) investigated the potential of dilute-acid pre-hydrolysis as a pretreatment method for sugarcane bagasse, rice hulls, peanut shells, and cassava stalks. The pre-hydrolysis was performed at 122 °C during 20, 40, or 60 min using 2% H_2SO_4 at a solid-to-liquid ratio of 1:10. Sugar formation increased with increasing reaction time. Xylose, glucose, arabinose, and galactose were detected in all of the pre-hydrolysates, whereas mannose was found only in the prehydrolysates of peanut shells and cassava stalks. The hemicelluloses of bagasse were hydrolyzed to a high-extent yielding concentrations of xylose and arabinose of 19.1 and 2.2 g/L, respectively, and a xylan conversion of more than 80%. High-glucose concentrations

(26–33.5 g/L) were found in the prehydrolysates of rice hulls, probably because of hydrolysis of starch of grain remains in the hulls. Peanut shells and cassava stalks rendered low amounts of sugars on pre-hydrolysis, indicating that the conditions were not severe enough to hydrolyze the hemicelluloses in these materials quantitatively.

Cara *et al.* (2007) studied dilute acid pretreatment of olive tree biomass. Pretreatment was performed at 0.2, 0.6, 1.0, and 1.4% (w/w) sulfuric acid concentrations while temperature was in the range 170–210 °C. Attention is paid to sugar recovery both in the liquid fraction issued from pretreatment (prehydrolysate) and that in the water-insoluble solid (WIS). As a maximum, 83% of hemicellulosic sugars in the raw material were recovered in the prehydrolysate obtained at 170 °C, 1% sulfuric acid concentration, but the enzyme accessibility of the corresponding pretreated solid was not very high. In turn, the maximum enzymatic hydrolysis yield (76.5%) was attained from a pretreated solid (at 210 °C, 1.4% acid concentration) in which cellulose solubilization was detected; moreover, sugar recovery in the prehydrolysate was the poorest one among all the experiments performed. The maximum value (36.3 g sugar/100 g raw material) was obtained when pretreating olive tree biomass at 180 °C and 1% sulfuric acid concentration, representing 75% of all sugars in the raw material.

Hsu *et al.* (2010) studied the operational conditions for the dilute acid pretreatment of rice straw. A maximal sugar yield of 83% was achieved when the rice straw was pretreated with 1% (w/w) sulfuric acid with a reaction time of 1–5 min at 160° or 180 °C. The FTIR spectra of raw rice straw and pretreated solid residues are shown in Figure 2.9. The broad band at 3350 cm^{-1} was associated with O–H stretching of the hydrogen bonds of cellulose. The absorption peak in the untreated rice straw was similar to that in the pretreated solid residues, implying that most of the crystalline cellulose in the rice straw was not disrupted by the acid-catalyzed reaction. In addition, the band at 2900 cm^{-1} was attributed to C–H stretching within the methylene of cellulose and this peak was slightly enhanced after pretreatment. Furthermore, the prominent bands at 1200–1000 cm^{-1} were typically related to the structural features of cellulose and hemicelluloses. The vibrations of these bands overlapped the C–O–H stretching of

primary and secondary alcohols at 1064 cm^{-1}, C–O–C glycosidic bond stretching at 1160 cm^{-1}, and C–O–C ring skeletal vibration at 1100 cm^{-1}. Nevertheless, the band at 910 cm^{-1} was dominated by the b-(1-4)-glycosidic bond (C–O–C); the adsorption peaks of these bands were enhanced. Furthermore, the ester linkage C=O with an absorption peak at 1720 cm^{-1} was usually defined as the acetyl group in hemicelluloses structure and/or the linkage between hemicelluloses and lignin that should reflect the presence of remaining ester linkage between lignin and hemicelluloses in pretreated solid residues. They also investigated the distribution of lignin-associated bands, the C=O groups in the alkyl groups of the lignin side chains was suggested to conjugate with the aromatic structure and then resulted in an adsorption peak at 1640 cm^{-1}. They found that the absorption peak for C=O group reduced in the pretreated solid residues since the acid hydrolysis reaction may cause partial lignin structure to release from raw rice straw. The degradation of the ester and ether linkages within the lignin by the acid-catalyzed reaction may also destroy the matrix structure and generate small lignin fragments. This redistribution of the lignin has been suggested to generate a trap effect that may hinder cellulase as it begins to attack the surface of the cellulose. The completely release of sugar (xylose and glucose) increased the pore volume of the pretreated solid residues resulted in an efficiency of 70% for the enzymatic hydrolysis. The extra pore volume was generated by the release of acid-soluble lignin and this resulted in the enzymatic hydrolysis being enhanced by nearly 10%.
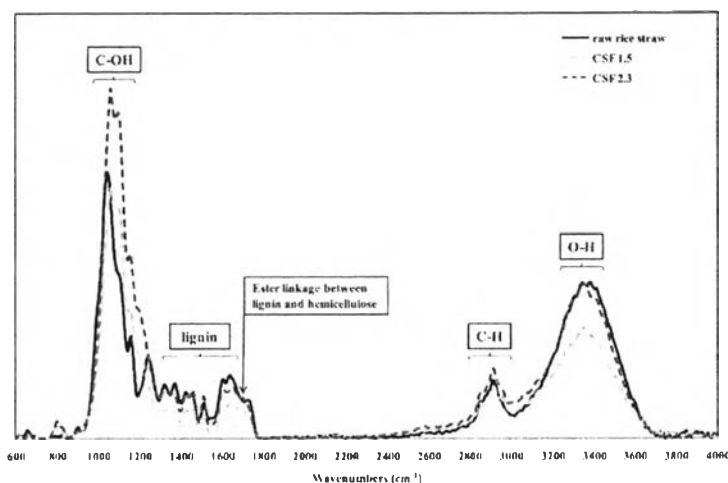
Figure 2.9 FTIR spectra of raw rice straw and pretreated solid residues under CSF 1.5 (180 °C/0.7% H_2SO_4/min) and 2.3 (180 °C/1.0% H_2SO_4/4 min) (Hsu et al., 2010).

2.3.2.2 Dilute Alkaline Pretreatment

The alkaline pretreatments can be divided into two major groups, depending on the catalyst used: pre-treatments that use alkaline/alkaline-earth metals based agents (typically sodium, potassium, or calcium) and those that use ammonia. Conversely to acid or hydrothermal processes, alkaline pretreatments are very effective for lignin solubilisation exhibiting only minor cellulose and slightly higher hemicellulose solubilisation.

Aita et al. (2010) studied high fiber sugarcane bagasse as feedstock for the production of cellulosic ethanol. Energy cane bagasse was pretreated with ammonium hydroxide (28% v/v solution), and water at a ratio of 1:0.5:8 at 160 °C for 1 h under 0.9–1.1 MPa. Approximately, 55% lignin, 30% hemicellulose, 9% cellulose, and 6% other (e.g., ash, proteins) were removed during the process. The maximum glucan conversion of dilute ammonia treated energy cane bagasse by cellulases was 87% with an ethanol yield (glucose only) of 23 g ethanol/100 g dry biomass. The enzymatic digestibility was related to the removal of lignin. Lignin dissolves at temperatures between 140° and 160 °C and the presence of ammonia lowers

its softening range. Furthermore, liquid ammonia changes the crystal structure of cellulose and causes cellulose swelling making sugars accessible to enzymatic attack. Significant morphological changes were observed in this study. Ammonia treated energy cane bagasse changed from compact and rigid to loose and swollen .It is speculated that the improvements in enzyme hydrolysis in the dilute ammonia treated samples are due to increased surface area and the presence of pores that can be accessed by enzymes.

Wang *et al.* (2009) studied sodium hydroxide pretreatment of coastal Bermuda grass. Coastal Bermuda grass was pretreated with NaOH at concentrations from 0.5% to 3% (w/v) for a residence time from 15 to 90 min at 121 °C. The pretreatments were evaluated based on total lignin removal and production of total reducing sugars, glucose and xylose from enzymatic hydrolysis of the pretreated biomass. Pretreatment time of 30 min was sufficient to achieve a significant amount of total lignin removal as long as the sodium hydroxide concentration was equal or over 1%. On the other hand, decreasing sodium hydroxide concentration from 1 to 0.5% significantly reduced total lignin removal, but there was no significant difference in lignin removal between 2 and 3% NaOH. Up to 86% lignin removal was observed. The optimal NaOH pretreatment conditions at 121 °C for total reducing sugars production as well as glucose and xylose yields were 15 min and 0.75% NaOH. Under these optimal pretreatment conditions, total reducing sugars yield was about 71% of the theoretical maximum, and the overall conversion efficiencies for glucan and xylan were 90.43% and 65.11%, respectively.

2.3.3 Microwave Pretreatment

Microwave irradiation has been widely used in many areas because of its high heating efficiency and easy operation. Some studies have shown microwave irradiation could be easily combined with chemical reaction and, in some case, accelerating the chemical reaction rate (Caddick *et al*, 1995).

Antonio *et al.* (2005) studied thermal effect of microwave irradiation. Microwave irradiation is rapid and volumetric, with the whole material heated simultaneously. In contrast, conventional heating was slow and introduced into the sample from the surface (Figure 2.10).
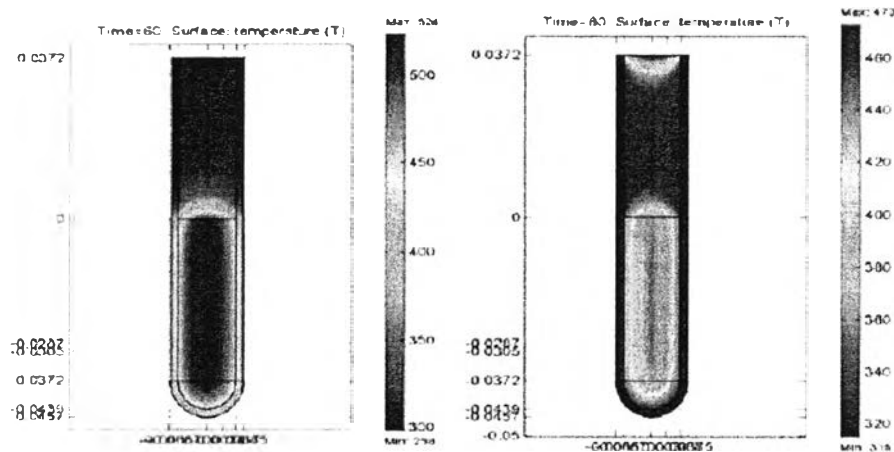
Figure 2.10   The temperature profile after 60 sec as affected by microwave irradiation (left) compared to treatment in oil bath (right).

Microwave irradiation raises the temperature of the whole reaction volume simultaneously, whereas in the oil heated tube, the reaction mixture in contact with the vessel wall is heated.

Hu *et al.* (2008) reported the benefit of microwave pretreatment. Microwave is an alternative method for conventional heating. Compared with conduction/ convection heating, which is based on superficial heat transfer; the microwave uses the ability of direct interaction between a heated object and an applied electromagnetic field to create heat. Therefore, the heating is volumetric and rapid. When microwave is used to treat lignocelluloses, it selectively heats the more polar (lossy) 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 effects that also accelerate the destruction of the crystal structures.

Hu *et al.* (2008) also studied microwave-based heating pretreated switchgrass, which was then hydrolyzed by cellulase enzymes. When switchgrass was soaked in water and treated by microwave, total sugar (xylose + glucose) yield from the combined treatment and hydrolysis was 34.5 g/100 g biomass, equivalent to 58.5% of the maximum potential sugars released. This yield was 53% higher than that obtained from conventional heating of switchgrass. To further improve the sugar yield, switchgrass was presoaked in different concentrations of alkali solutions and then treated by microwave or conventional heating. With alkali loading from 0.05 to 0.3 g alkali/g biomass, microwave pretreatment resulted in a higher sugar yield than from conventional heating, with the highest yield (90% of maximum potential sugars) being achieved at 0.1 g/g of alkali loading. Scanning electron microscope images revealed that the advantage of microwave over conventional heating was due to the disruption of recalcitrant structures. Finally, the effects of temperature, solid content, and treatment time on microwave pretreatment of switchgrass were investigated. At optimal conditions of 190 °C, 50 g/L solid content, and 30 min treatment time, the sugar yield from the combined pretreatment and hydrolysis was 58.7 g/100 g biomass, equivalent 99% of potential maximum sugars. The results demonstrate that microwave-assisted alkali treatment is an efficient way to improve the enzymatic digestibility of switchgrass.

Zhu *et al.* (2006) investigated microwave-assisted alkali pretreatment of wheat straw and its enzymatic hydrolysis and compared with the conventional alkali pre-treatment process. First, the effect of microwave power and pretreatment time on the weight loss and composition of wheat straw was examined. The results show that the higher microwave power with shorter pretreatment time and the lower microwave power with longer pre-treatment time had the same effect on the weight loss and composition at the same energy consumption. The comparison was then made between the effect of the microwave-assisted alkali pretreatment and the conventional alkali one on the weight loss and composition of wheat straw. The wheat straw had a weight loss of 48.4% and a

composition of cellulose 79.6%, lignin 5.7% and hemicellulose 7.8% after 25 min microwave assisted alkali pretreatment at 700 W, compared with a weight loss of 44.7% and a composition of cellulose 73.5%, lignin 7.2% and hemicellulose 11.2% after 60 min conventional alkali pre-treatment. The microwave assisted alkali pre-treatment removed more lignin and hemicellulose from wheat straw with shorter pretreatment time compared with the conventional alkali one. Finally, the enzymatic hydrolysis of pretreated wheat straw (substrate concentration 50 g l^{-1}, enzyme loading 20 mg g^{-1} substrate) was also investigated and the results indicated that the microwave-assisted alkali pre-treated wheat straw had higher hydrolysis rate, reducing sugar concentration and glucose content in the hydrolysate than the conventional alkali pretreated one. Microwave-assisted alkali pre-treatment is a potential alternative of wheat straw pre-treatment for it enzymatic hydrolysis.