CHAPTER II
LITERATURE REVIEW

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 which each other. The composition and percentages of these three different types of polymers vary from one plant species to another. Moreover, composition varies within a single plant (roots, stems, leaves), with age (heartwood versus sapwood), stage of growth (early wood versus late wood in annual rings) and with the conditions under which the plant grows. Table 2.1 gives the composition of some lignocellulosic biomasses.

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 is the main structural constituent in plant cell walls and is found in an organized fibrous structure. The structure of cellulose is shown in Figure 2.1.

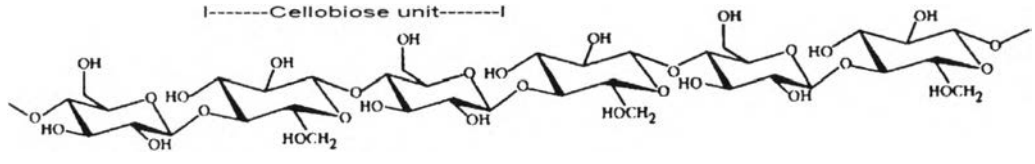


Figure 2.1 Illustration of a cellulose chain (Mousdale, 2008).

This high molecular weight unbranched linear polymer (glucan) consists of D-glucose subunits linked to each other by β-(1,4)-glycosidic bonds which can appear as a highly crystalline material. Cellobiose is the repeating unit established through this linkage, and it constitutes cellulose chains. The long-chain cellulose polymers are linked together by hydrogen and van der waals bonds, which cause the cellulose to be packed into microfibrils, as shown in Figure 2.2. Hemicellulose and lignin cover the microfibrils. Fermentable D-glucose can be produced from cellulose through the action of either acid or enzymes breaking the β-(1,4)-glycosidic linkages. Cellulose in biomass is present in both crystalline and amorphous forms (Stroeve *et al.*, 2009).

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.

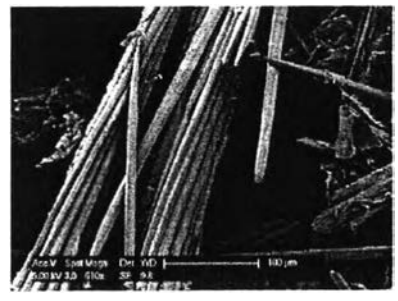


Figure 2.2 SEM micrograph of cellulose fibrils from *Miscanthus Sinensis* (Feng *et al.*, 2010).

Fukada *et al.* (2008) studied the effect of cellulose crystallinity on enzymatic hydrolysis of *Miscanthus Sinensis*. The yield of glucose from enzymatic hydrolysis (at 72 h of reaction) increased with the reduction in crystallinity, suggesting that the crystalline structure of *M. Sinensis* inhibits the enzymatic hydrolysis of cellulose. The initial rate of cellulose hydrolysis (at 3 h of reaction) also increased with decreasing crystallinity. This reflects the fact that cellulase hydrolyzes amorphous cellulose much more rapidly than crystalline cellulose. The quantity of amorphous cellulose can be increased by pretreatment.

2.1.2 Hemicellulose

Hemicellulose are heterogeneous polymers of pentoses (xylose, arabinose), hexoses (mannose, glucose, galactose), and sugar acid (glucuronic acid, methyl glucuronic acid), as shown in Figure 2.3. Unlike cellulose, hemicelluloses are not chemically homogeneous. Hemicellulose has a lower molecular weight than cellulose, and branches with short lateral chains that consist of different sugars, which are easily hydrolysable polymers. Hemicellulose serves as a connection between the lignin and the cellulose fibers and gives the whole cellulose-hemicellulose-lignin network more rigidity (Zeeman *et al.*, 2009).

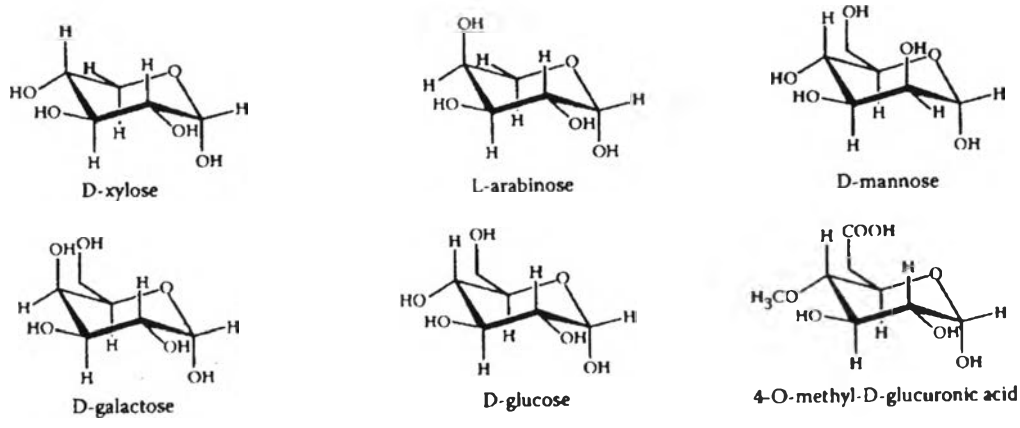


Figure 2.3 Chemical structures of sugar components of hemicelluloses (Mousdale, 2008).

Most plant species contain xylans (1,4-linked polymers of xylose). Besides xylose, xylans may contain arabinose, glucuronic acid, acetic acid, ferulic acid, and *p*-coumaric acid. Xylans can be categorized as linear homoxylan, arabinoxylan, glucuronoxylan, and glucuronoarabinoxylan. Xylans from different sources, such as grasses, cereals, softwood, and hardwood, differ in composition. Birch wood (Roth) xylan contains 89.3% xylose, 1% arabinose, 1,4% glucose, and 8.3% anhydrouronic acid. Rice bran neutral xylan contains 46% xylose, 44.9% arabinose, 6.1% galactose, 1.9% glucose, and 1.1% anhydrouronic acid. Corn fiber is one of the complex heteroxylans contains 48-54% xylose, 33-35% arabinose, 5-11% galactose, and 3-6% glucuronic acid. 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 shown in Figure 2.4.

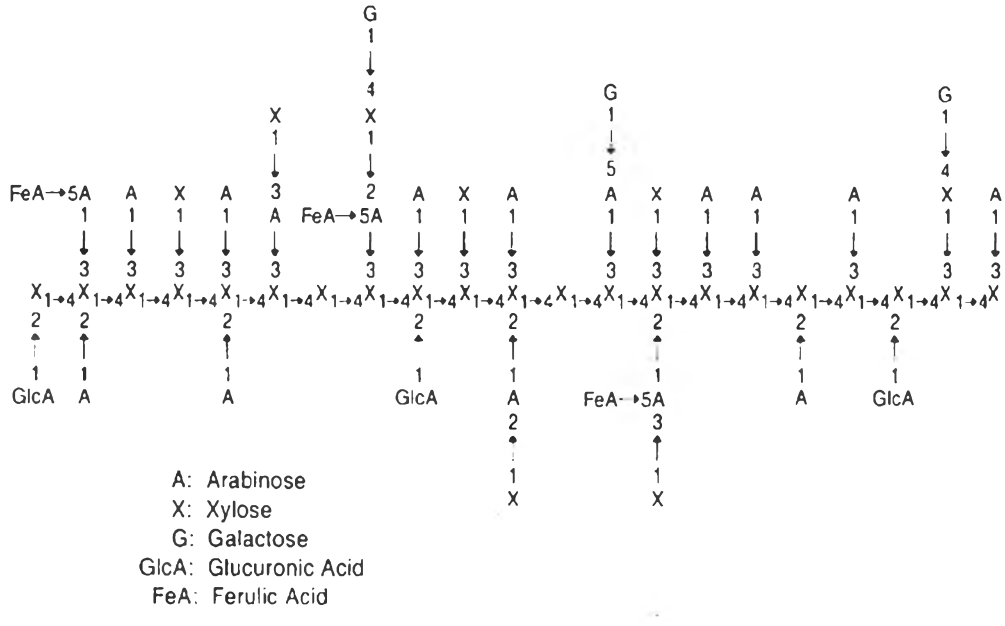


Figure 2.4 Schematic structure of corn fiber heteroxylan (Saha, 2003).

A model for the corn fiber cell wall is shown in Figure 2.5. The heteroxylans, which are highly cross-linked by diferulic bridges, constitute of a network in which the cellulose microfibrils may be imbedded. Structural wall proteins might be cross-linked together by isodityrosine bridges with feruloylated heteroxylans, thus forming an insoluble network.

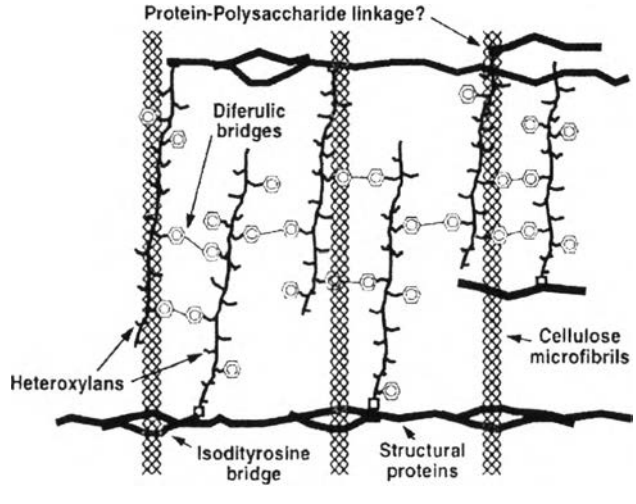
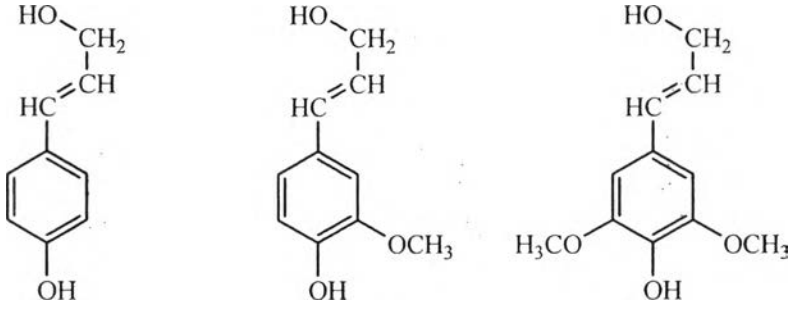


Figure 2.5 Model for corn fiber cell walls (Saha, 2003).

Yoon (1998) reported the effect of hemicellulose on enzymatic digestibility. Hemicellulose adsorbs cellulase enzyme and the adsorbed enzyme is unavailable for cellulose hydrolysis. Hemicellulose in a lignocellulosic substrate physically is also known to block the contact between cellulase enzyme and cellulose. It is, therefore, concluded that hemicellulose could be an important barrier to enzymatic hydrolysis of cellulose and its removal is a prerequisite for complete hydrolysis of cellulose in biomass.

2.1.3 Lignin

Lignin is an aromatic polymer with the substituents connected by both ether and carbon-carbon linkages. It is composed of three principal building blocks: *p*-coumaryl alcohol (p-hydroxyphenyl propanol), coniferyl alcohol (guaiacyl propanol), and sinapyl alcohol (syringyl propanol) (Figure 2.6) that are held together in a three-dimensional lignin structure (Figure 2.7).



p-Coumaryl alcohol Coniferyl alcohol Sinapyl alcohol

Figure 2.6 Lignin building blocks.

Generally, softwoods contain more lignin than hardwoods. In softwoods, coniferyl alcohol is the principal constituent; the lignin of hardwoods is composed of guaiacyl and syringyl units. Grass lignins contain guiacylsyringyl-, and *p*-hydroxyphenyl-units. Lignin inter-monomer linkages are similar in softwood, hardwood, and grass lignins. The main purpose of having lignin is to give the plant structural support, impermeability, and resistance against microbial attack and oxidative stress.

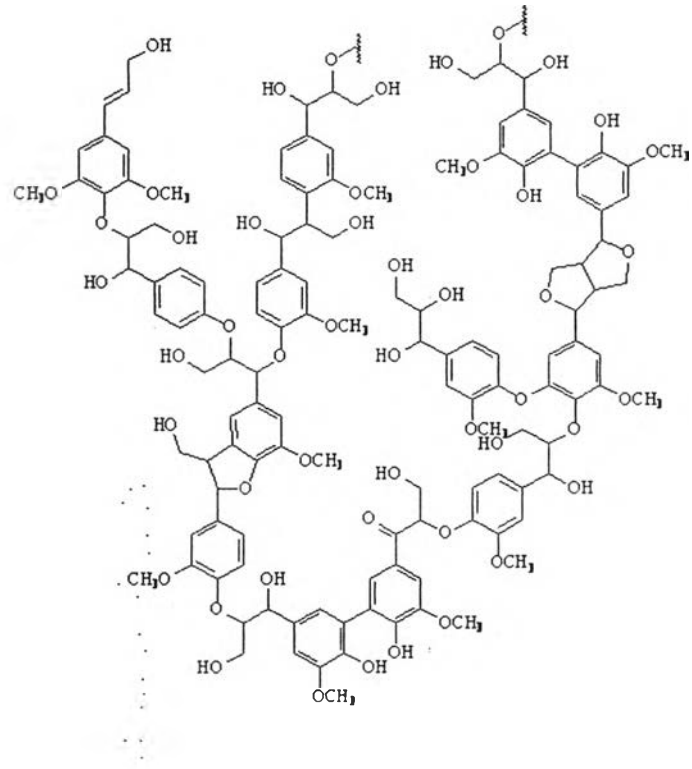


Figure 2.7 Chemical structure of lignin.

Jeffries *et al.* (1994) reported lignin-carbohydrate complexes (LCCs) are heterogeneous, poorly defined structures that are found in many plant species. Lignin is directly or indirectly bound covalently to carbohydrate, and the resulting complexes present a barrier to biological degradation. Relatively little attention has been given to enzymes capable of cleaving the chemical linkages between lignin and carbohydrate, the LC bonds. The proposed LC bonds include bonds to xylan, glucomannan, cellulose, various other hemicellulosic sugars, and pectin. They link the position of the phenyl propane lignin to either carboxyl or free hydroxyls of hemicellulose through ester or ether linkages, respectively. The most labile chemical bonds are ester linkages. The ester linkages occur between the free carboxyl group of uronic acids in hemicellulose and the benzyl groups in lignin. Many of these linkages are broken by alkali. Some ester bonds are present as acetyl side groups on hemicellulose, others are between uronic acids and lignin, and still others occur between hemicellulose chains. Unfortunately, lignin which contains no sugar encloses the cellulose and hemicellulose molecules, making them difficult to reach with fungi and bacteria for conversion to fuel.

2.2 Ethanol Conversion Process

Processing of lignocellulosics to ethanol consists of four major unit operations: pretreatment, hydrolysis, fermentation, and product separation/purification.

Pretreatment is required to alter the biomass macroscopic and microscopic sizes and structures 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 (Ladisch *et al.*, 2005).

Hydrolysis includes the processing steps that convert the carbohydrate polymers into monomeric sugars. The hydrolysis process currently used is either concentrated acid hydrolysis or enzymatic hydrolysis. Compared to acid hydrolysis, enzymatic hydrolysis is carried out by cellulase enzymes which are highly specific. Utility cost of enzymatic hydrolysis is low because it is usually conducted at mild condition and does not have a corrosion problem (lower equipment costs). Enzymatic hydrolysis leads to higher yields of monosacharide yields without sugar-degradation products. Enzymes are naturally occurring compounds which are biodegradable and therefore environmental friendly, but it requires pretreatment to improve the enzymatic digestibility. The pretreatment process can remove hemicellulose, reduce cellulose crystallinity, and increase the porosity of the materials. The structure of xylan is more complex than cellulose and requires several different enzymes with different specificities for complete hydrolysis.

2.3 Pretreatment of Lignocellulosic Biomass

Pretreatment is an important tool for practical cellulose conversion processes. Pretreatment 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, as represented in the schematic diagram of Figure 2.8.

Cheng *et al.* (2002) reported that the purpose of the pretreatment was to remove lignin and hemicellulose, reduce cellulose crystallinity, and increase the porosity of the materials. Pretreatment must meet the following requirements: (1) improve the formation of sugars or the ability to subsequently form sugars by enzymatic hy-

drolysis; (2) avoid the degradation or loss of carbohydrate; (3) avoid the formation of byproducts inhibitory to the subsequent hydrolysis and fermentation processes; and (4) be cost-effective.

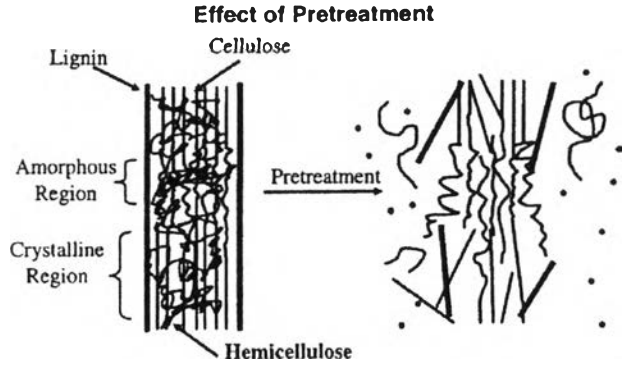


Figure 2.8 Schematic of goals of pretreatment on lignocellulosic material (La disch *et al.*, 2005).

Various pretreatment techniques change the physical and chemical structure of the lignocellulosic biomass and improve hydrolysis rates. 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) and biological, or combinations of these (Galbe *et al.*, 2007).

Stroeve *et al.* (2009) reported that the factors affecting the hydrolysis of cellulose included porosity (accessible surface area) of the biomass materials, cellulose fiber crystallinity, and content of both lignin and hemicellulose. The presence of lignin and hemicellulose makes the accessibility of cellulase enzymes and acids to cellulose more difficult, thus reducing the efficiency of the hydrolysis process. 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 and with greater yields. The hydrolysis process can be significantly improved by removal of lignin and hemicellulose, reduction of cellulose crystallinity, and increase of porosity through pretreatment processes.

2.3.1 Chemical Pretreatment

Chemical pretreatment methods have usually implied hydrolytic techniques using acids and alkalis, although oxidizing agents have also been considered.

- *Dilute acid pretreatment*

Among all the pretreatment methods, dilute acid pretreatment has been wildly studied because it is effective and inexpensive. The dilute sulfuric acid pretreatment can effectively solubilize hemicellulose into monomeric sugars (arabinose, galactose, glucose, mannose, and xylose) and soluble oligomers, thus improving cellulose conversion.

Cheng *et al.* (2005) studied the effect of sulfuric acid concentrations and pretreatment time on the rye straw and Bermuda grass. The increase severity of the pretreatment condition resulted in more solubilization of hemicellulose. For rye straw pretreatment, more than 50% of the hemicellulose was solubilized into monomeric sugars when pretreated with 1.2% sulfuric acid for 60 min or 0.9% sulfuric acid for 90 min. The monomeric glucose in the prehydrolyzate of rye straw was only 10% of glucan and kept constant under different pretreatment conditions. The total reducing sugar yield increased with the pretreatment severity and the non-pretreated control sample produced less total reducing sugars than the pretreated biomass. For Bermuda grass pretreatment, more than 60% of the xylan was hydrolyzed into monomeric xylose. Approximately 33% of glucan from Bermuda grass was converted into glucose when acid concentration and pretreatment time were 1.2% and 60 min respectively. Rye straw is harder to be hydrolyzed than Bermuda grass. It has been reported that the cell wall structure and components may be significantly different in plants, which may influence the biomass digestibility.

Feng *et al.* (2010) investigated the influence of the sulfuric acid pretreatment on the morphological and chemical changes of pretreated *Miscanthus* were elucidated by Scanning Electron Microscopy (SEM) and Fourier Transform Infrared Spectroscopy (FTIR). Figure 2.9 shows the SEM micrograph and FTIR spectrum of untreated *Miscanthus*. The assignments of the characteristic absorption bands of *Miscanthus* are listed in Table 2.2. From the SEM image, it can be seen that the *Miscanthus* sample exhibits a smooth and rigid structure.

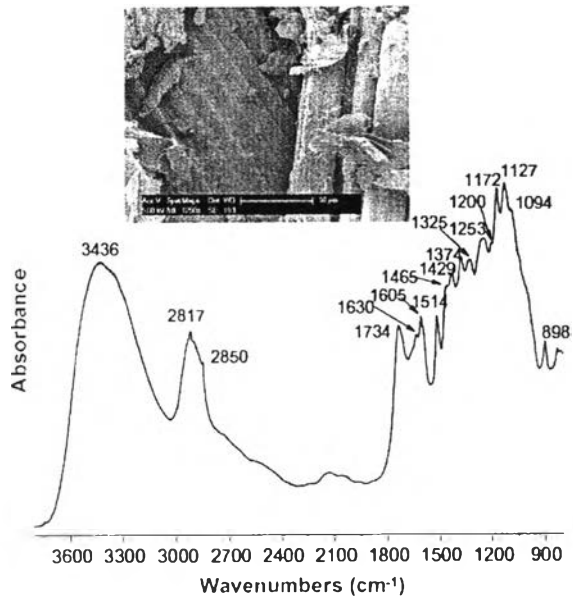


Figure 2.9 SEM micrograph and FTIR spectrum of raw *Miscanthus*.

Table 2.2 Assignment of characteristic absorption bands of *Miscanthus*

Wavenumbers (cm^{-1})	Assignment
898	C-O-C vibrations at β-glucosidic linkages in hemicellulose and cellulose[a]
1094	C-O, C-C stretching or C-OH bending in hemicellulose
1127	and cellulose[b]
1172	C-O-C stretching at β-glucosidic linkages in cellulose and
1200	hemicellulose[c]
1253	Aromatic C-O stretching out of lignin[d]
1325	C-C and C-O skeletal vibrations[e]
1374	Aliphatic C-H vibrations[f]
1429	Aromatic C=C stretching from aromatic ring[g]
1465	Aromatic C-H vibrations[h]
1514	Aromatic C=C stretching from aromatic ring of lignin [i]
1605	Aromatic skeletal vibrations[j]
1630	Bending of Absorbed residual water[k]
1734	C=O stretching of unconjugated ketone and carboxyl group[l]
2850	C-H stretching of methyl, methylene or methane group[m]
2917	
3436	O-H stretching[n]

The 1% H_2SO_4 pretreatment (200 °C, 8 min) more effectively broke down the rigid structure of *Miscanthus* shows in Figure 2.10. While this pretreatment may produce more reactive sites for enzymatic attack, part of the sample, shown in the circle in Figure 2.10A, remained unbroken. The FTIR spectra of the treated (Figure 2.10C) and the untreated *Miscanthus* (Figure 2.10B) reveal that the height of the peaks corresponding to the polysaccharides is dramatically lowered, indicating depolymerization of polysaccharides. The peaks at 1253, 1514 and 1734 cm^{-1} are, however, still visible with a lower intensity. These remaining lignin and ester linkages might hinder further hydrolysis.

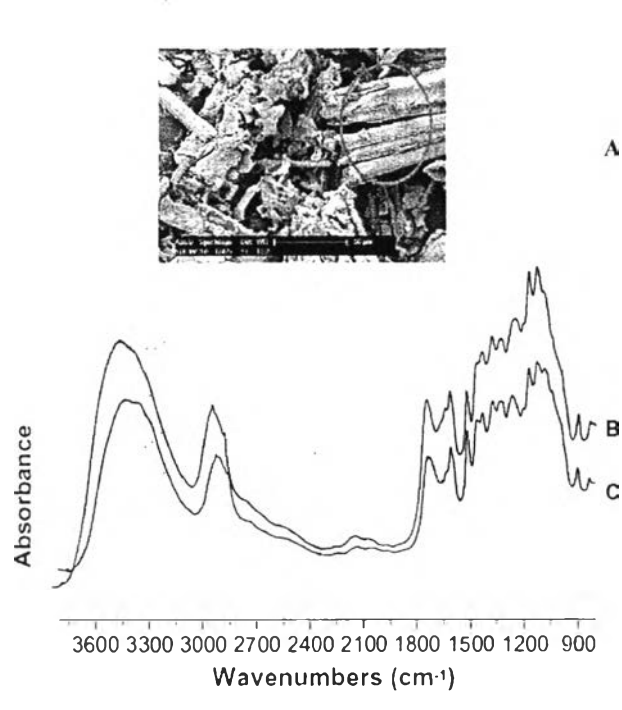


Figure 2.10 (A) SEM micrograph and (B) FTIR spectrum of raw *Miscanthus*, (C) FTIR spectrum of *Miscanthus* pretreated by 1% H_2SO_4 (200 °C, 8 min).

Jönsson *et al.* (2002) studied the effect of steam explosion by using different impregnating agents on sugar yields. Three parallel pretreatments were carried out, one without any impregnation, a second with sulfur dioxide, and a third with sulfuric acid as the impregnating agent. The pretreatment was performed at 205 °C for 10 min. The results showed the highest yields of xylose (16.2 g/100 g dry bagasse), arabinose (1.5 g/100 g), and total sugar (52.9 g/100 g) were obtained in the

hydrolysis of the SO_2-impregnated bagasse. The H_2SO_4- impregnated bagasse gave the highest glucose yield (35.9 g/100 g), but the lowest total sugar yield (42.3 g/100 g) among the three methods. The low total sugar yield from the H_2SO_4- impregnated bagasse was largely due to by-product formation, as the dehydration of xylose to furfural. The severe condition of H_2SO_4 pretreatment in addition caused a high degree of degradation of the released sugars.

- *Dilute alkali pretreatment*

Choi *et al.* (2009) studied the effectiveness of ammonia percolation pretreatment of wheat straw for ethanol production. The experiments were performed at treatment temperature of 50-170 °C and residence time of 10-150 min. The results showed that residual solid, delignification, cellulose, and hemicellulose were changed during the ammonia pretreatment of wheat straw. The solid remaining after the pretreatment varied from 81% (10 min, 50 °C) to 58.51% (150 min, 170 °C). The cellulose and hemicellulose content ranged from 34.94 to 36.49% and 10.14 to 12.69%, respectively. The delignification ranged from 21.65 to 67.39%. This means that the ammonia pretreatment is effective in removing lignin. Although delignification increased with increasing reaction time at low temperatures, there was little effect on delignification at high temperature. This suggests that temperature is a more important factor for removing lignin than the reaction time. In enzymatic digestibility, the pretreated biomass at 170 °C showed higher enzymatic digestibility (> 95%) while the untreated biomass was < 35%. The pretreatment temperature played a significant role in improving the enzymatic digestibility. The fermentability of the pretreated materials was evaluated using *S. cerevisie*. This study was carried out to confirm the inhibitor effect using YPD (10 g/L yeast extract and 20 g/L peptone) medium as a control. The initial glucose concentrations were 57.51 and 54.96 g/L for the control and wheat straw hydrolysate, respectively. The final ethanol concentrations for the control and wheat straw hydrolysate were 26.65 and 25.14 g/L, respectively, and the ethanol yields were 91.57 and 90.66% of the theoretical value, respectively. This means that the inhibitors were not created by ammonia percolation pretreatment.

Bazan *et al.* (2010) studied the effect of dilute ammonia pretreatment of sorghum. The treatment was carried out by mixing sorghum fibers, ammonia, and

water at a ratio of 1:0.14:8 at 160 °C for 1 h under 140–160 psi pressure. The results showed that approximately 44% of the initial lignin was removed during pretreatment. Inhibitors, such as furfural and HMF, were not formed during pretreatment and were not present during enzymatic hydrolysis or fermentation. Considerable amounts of hemicellulose (35%) and lignin (44%) were removed during the process. However, more than 90% of the cellulose was retained in the treated biomass. They believed that lignin removal increased the area and porosity of the biomass, thus enhancing enzymatic hydrolysis and fermentation.

2.3.2 Microwave Heating Pretreatment

Microwave irradiation has been widely used in many areas because of its high heating efficiency and easy operation. Some studies have shown that microwave irradiation could change the ultra-structure of cellulose, degrade lignin and hemicellulose in biomass and increase the enzymatic susceptibility of biomass. Fortunately, microwave irradiation could be easily combined with chemical reactions and, in some case, accelerate the chemical reaction rate.

Wen *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.

Wen *et al.* (2008) studied the effect of microwave heating on enhancing switch grass digestibility compared with conventional heating, used to pretreat switch grass, which was then hydrolyzed by cellulase enzymes. At the pretreatment stage, microwave pretreatment led to a much higher xylose yield than the conventional heating pretreatment. They showed that microwave heating enhanced the solubilization of hemicellulose (xylan) in the pretreatment stage, and high hemicellulose removal followed by a high cellulose digestibility in the enzymatic hydrolysis stage. A total sugar yield of 34.5 g/100 g biomass was obtained from combined microwave

pretreatment and hydrolysis, while the corresponding yield from the conventional heating pretreatment and hydrolysis was 22.6 g/100 g biomass.

Zhang *et al.* (2005) studied the combination pretreatment of rice straw using microwave and alkali and its enzymatic hydrolysis was investigated to compare with the alkali-alone pretreated process. Rice straw treated by microwave/alkali showed more weight loss, higher cellulose content and lower moisture, lignin and hemicellulose content than that treated by alkali alone. The weight loss came from the solubilisation of its components, such as lignin, hemicellulose. The reason that moisture decreases maybe arises from the enlarged pore size of rice straw by the treatment, which leads to diminishing its combined water. Higher microwave power with short pretreatment time and the low microwave power with long pretreatment time had almost the same effect on weight loss and chemical composition of rice straw when the irradiation power was set at 700, 500, 300 W and it was treated for 30, 42, and 70 min, respectively. Rice straw treated by microwave/alkali had a higher hydrolysis rate than that treated by alkali alone. This result justified the above conclusion that microwave irradiation could enhance the enzymatic hydrolysis of rice straw by removing more hemicellulose and lignin in its microwave/alkali pretreatment and increasing its accessibility to the enzymes.

2.4 Degradation of Lignocellulosic Biomass by Hydrolysis

Biomass provides a potential source of added-value chemicals, such as reducing sugars, furfural, ethanol and other products, by using enzyme- or acid-catalyzed hydrolysis. Hydrolysis procedures involve in treatment of lignocellulosic biomass at high temperature under acidic condition. The main degradation pathways are schematically presented in Figure 2.11.

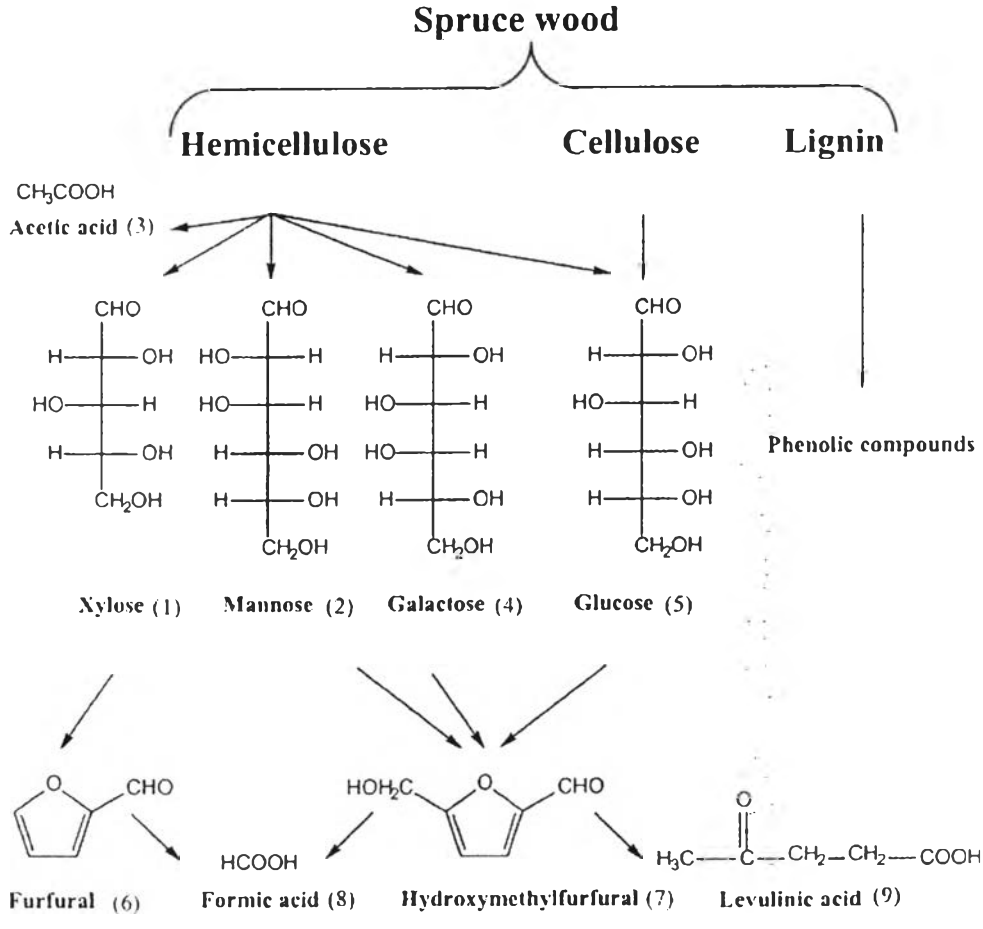


Figure 2.11 Main degradation products occurring during hydrolysis (Hahn-Hägerdal *et al.*, 2000).

When hemicellulose is degraded, xylose, mannose, acetic acid, galactose, and glucose (Figure 2.11, numbers. 1, 2, 3, 4, and 5, respectively) are liberated. Cellulose is hydrolyzed to glucose. At high temperature and pressure xylose is further degraded to furfural (Figure 2.11, no. 6). Similarly, 5-hydroxymethyl furfural (HMF) (Figure 2.11, no. 7) is formed from hexose degradation. Formic acid (Figure 2.11, no. 8) is formed when furfural and HMF are broken down. Levulinic acid (Figure 2.11, no. 9) is formed by HMF degradation. Phenolic compounds are generated from partial breakdown of lignin (Hahn-Hägerdal *et al.*, 2000). Since 5-carbon sugars degrade more rapidly than 6-carbon sugars, one way to decrease sugar degradation is to remove hemicellulose. Removal of hemicellulose has sometimes been considered to be

a desired characteristic of a biomass pretreatment because this reduces inhibitory compounds, such as furfural, generated from hemicellulose degradation via dilute acid treatment at high temperature and pressures (Anex *et al.*, 2008).

Byun *et al.* (2010) studied dilute sulfuric acid hydrolysis for the conversion of distillers' grains (DDG) and corn fiber to monomeric sugars. The influence of hydrolysis temperature on the formation of furfural and the effect of biomass loading on the solubility of monomeric sugars were investigated. The experimental results confirmed an increasing trend in the formation of monomeric sugars as a function of time. For the hydrolysis of DDG for which the hydrolysis temperature was 120 °C, the maximum yields were reached at the end of the reaction period (1 h). At the hydrolysis temperature of 140 °C the maximum amount of sugars was recorded during the first 20–30 min of the reaction and further increases in the reaction time resulted in decrease in the total amount of the formed sugars. The decreasing trend in the formation of monomeric sugars at 140 °C and acidic condition may be due to the thermal decomposition of monomeric sugars to organic compound such as furfural, hydroxymethylfurfural, acetic acid, levulinic acid and other organic compounds. In this study, the formation of furfural was monitored. The formation of furfural at 120 °C followed a similar behavior to that at 140 °C, but was much lower in concentration. The highest amount furfural was recorded at 3.0 mg/mL at 140 °C and 20 wt.% DDG loading. At 120 °C and otherwise identical conditions the furfural formation was measure at 0.15 mg/mL. Experimental results for the pretreatment of corn fiber reveal the formation of sugars followed a similar trend to the hydrolysis of DDG. The highest amount furfural was recorded at 3.8 mg/mL at 140 °C and 20% corn fiber loading. At 120 °C and otherwise identical condition the furfural formation was measured at 0.5 mg/mL. The solubilized material in the liquid fraction of the hydrolyzate contained monomeric and oligomeric carbohydrates, acid soluble lignin, oil, soluble proteins, minerals, and inhibitory compounds. The presence of non-carbohydrates in the liquid fraction may have a limiting effect on the solubility of monomeric sugars, resulting in the highest yields of monomeric sugars observed at the lower biomass loadings (5 and 10 wt.%). For example, at the hydrolysis temperature of 140 °C, when the DDG loading was increased from 5% to 10% the solubility was increased from 42.3 to 69.2 mg/mL of hydrolyzate (63% increase in solubility),

whereas, when the loading was increased from 15% to 20% the solubility was increased from 88.6 to 97.6 mg/mL of hydrolyzate (31% increase in solubility). High concentration of non-carbohydrates may be responsible in limiting the overall solubility of the substrate at larger loading levels.

Jönsson *et al.* (2002) reported thirteen different phenolic compounds in the hydrolyzates from lignin degradation of sugarcane bagasse, as shown in Figure 2.12.

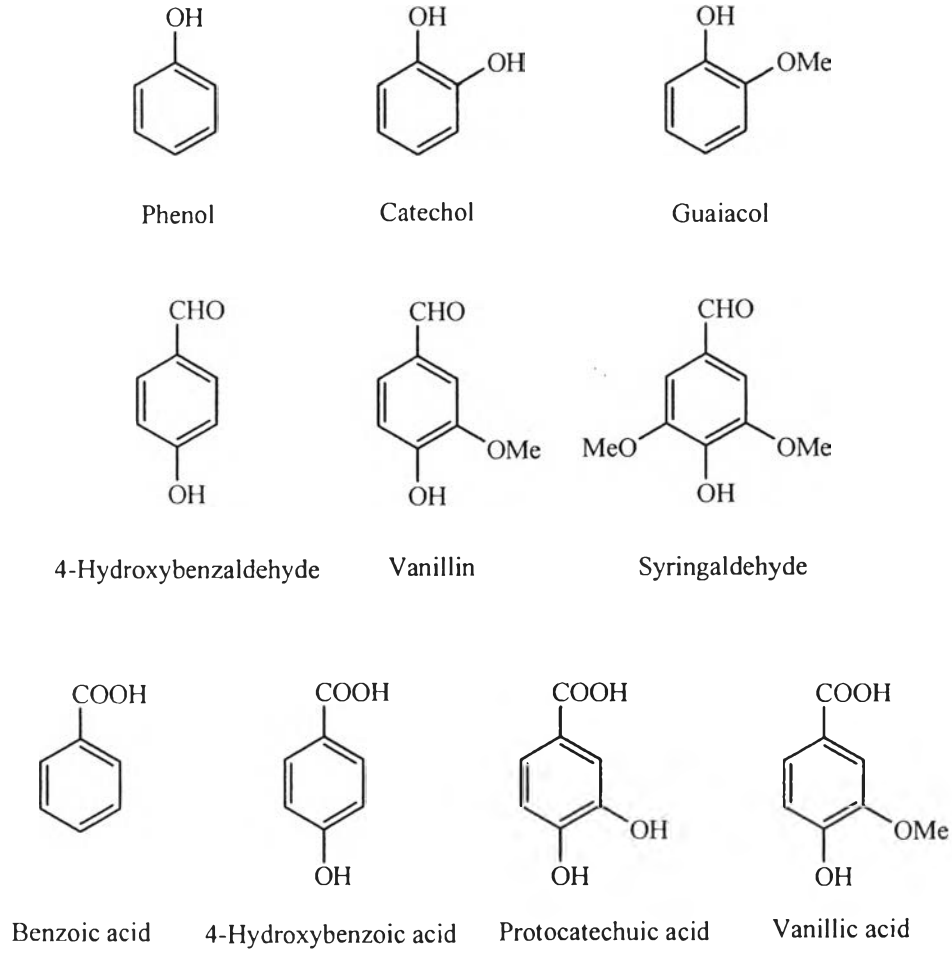


Figure 2.12 Thirteen different phenolic compounds from lignin degradation.