# CHAPTER II

THEORETICAL BACKGROUND AND LITERATURE REVIEW

2.1 Characteristics of Lignocellulosic Biomass

Lignocellulosics, the most abundant biomass available on earth, have attracted considerable attention as an alternative feedstock for the production of various value added products due to their renewable nature and low cost availability (Gupta *et al.*, 2011). The main components of the lignocellulosic materials are cellulose, hemicelluloses, and lignin, along with smaller amounts of pectin, protein, extractives (soluble nonstructural materials such as nonstructural sugars, nitrogenous material, chlorophyll, and waxes), and ash (Harmsen *et al.*, 2010).
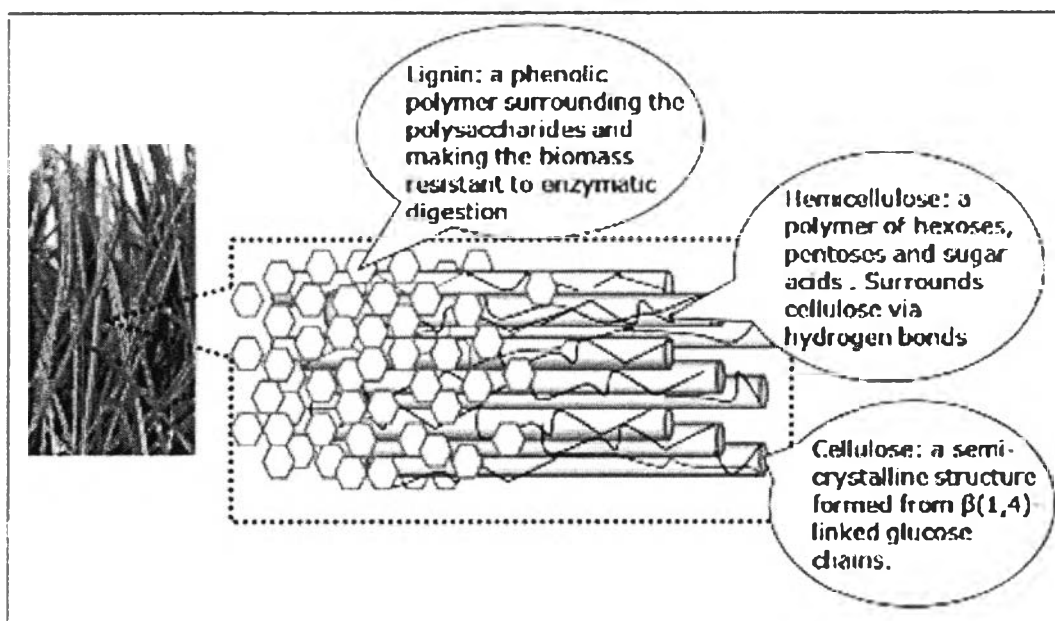
Figure 2.1 Lignocellulose structure
(http://www.napier.ac.uk/randkt/rktcentres/bfrc/Pages/MicrobialBiofuels.aspx).

The composition of lignocellulose highly depends on its source. Table 2.1 shows the composition of lignocellulose encountered in the most common sources of biomass.

Table 2.1 Composition of lignocellulose in several sources on dry basis (Harmsen *et al.*, 2010)

Lignocellulosic materials	Cellulose (%)	Hemicellulose (%)	Lignin (%)
Hardwoods stems	40–55	24–40	18–25
Softwood stems	45–50	25–35	25–35
Nut shells	25–35	25–30	30–40
Corn cobs	45	35	15
Grasses	25-40	35–50	10–30
Wheat straw	30	50	15
Leaves	15–20	80–85	0
Coastal Bermuda grass	25	35.7	6.4
Switchgrass	45	31.4	12

2.1.1 Cellulose

Cellulose is the main structural constituent in plant cell walls and is found in an organized fibrous structure. It consists of β-glucose subunits linked to each other by β-(1,4)-glycosidic bonds. Cellobiose is the repeat unit established through this linkage, and it constitutes cellulose chains (Kumar *et al.*, 2009). The structure of cellulose is shown in Figure 2.2.

Cellulose in biomass is present in both crystalline and amorphous forms. Crystalline cellulose is a major proportion of cellulose while a small proportion is amorphous cellulose. The long-chain cellulose polymers are packed into microfibrils by hydrogen and van der waals bonds. The microfibrils are covered by hemicelluloses and lignin. In this way cellulose can obtain a crystalline structure

highly resisting to attack by enzymes (limited accessibility of cellulose chains) which is the major proportion of cellulose.
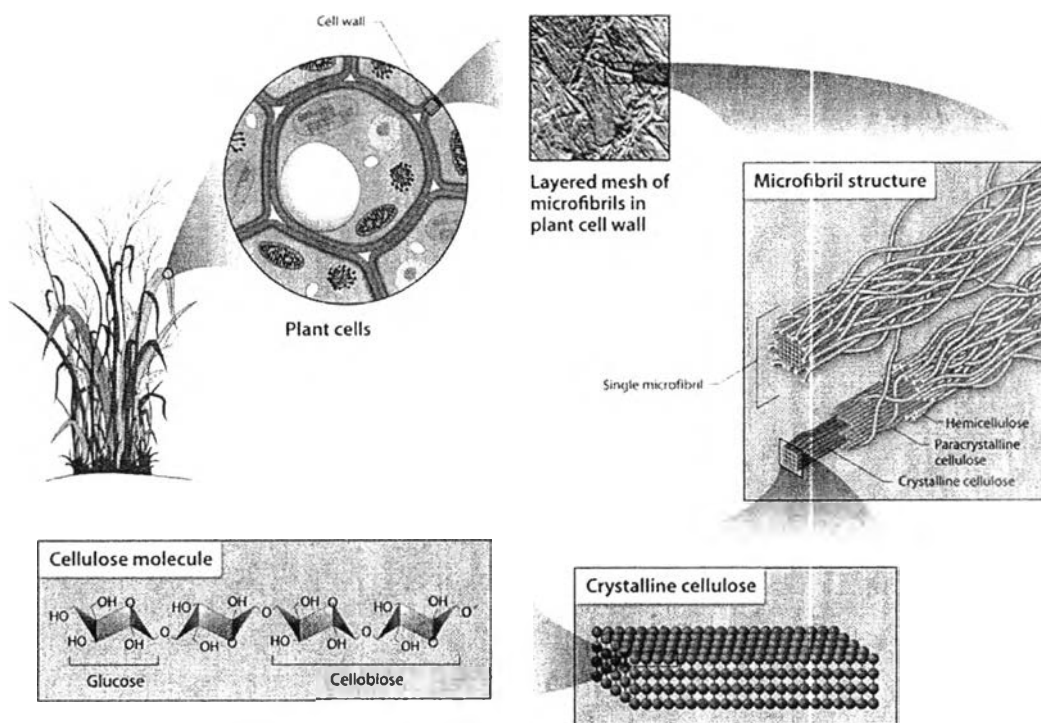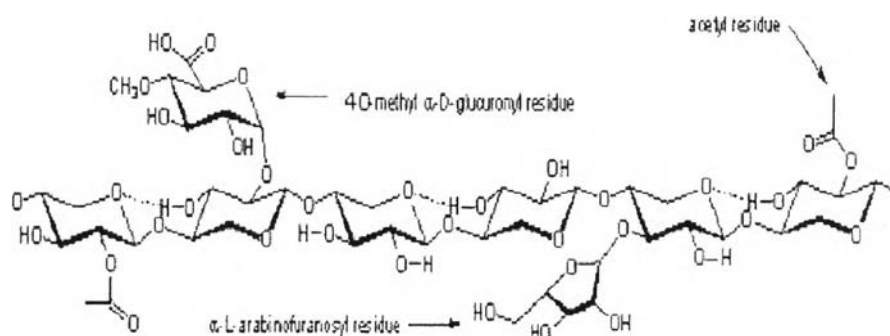

Figure 2.2 The structure of cellulose
(http://www.plantoils.in/portal/ce/blog/2008_12_01_archive.html).

2.1.2 Hemicellulose

Hemicellulose has a lower molecular weight than cellulose and is composed of mainly pentoses (like xylose and arabinose) and hexoses (like mannose, glucose, and galactose) (Brodeur *et al.,* 2011). The structure of hemicellulose is the lack of crystalline structure due to the highly branched structure and amorphous forms that are easy to hydrolyze. The composition is either a homopolymer or a heteropolymer with short branches linked and the presence of acetyl groups connected to the polymer chain. Figure 2.3 shows the structure of hemicellulose.

**Figure 2.3** The structure of hemicellulose (Harmsen *et al.*, 2010).

2.1.3 Lignin

Lignin is a complex, large molecular structure containing cross-linked polymers of phenolic monomers. It is an amorphous three-dimensional polymer with phenyl propane units as the predominant building blocks which are particularly difficult to hydrolysis. More specifically, p-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol basis are the ones most commonly encountered (Harmsen *et al.*, 2010). Lignin presents in the primary cell wall, imparting structural support, protecting cell wall polysaccharides from microbial degradation and impermeability.
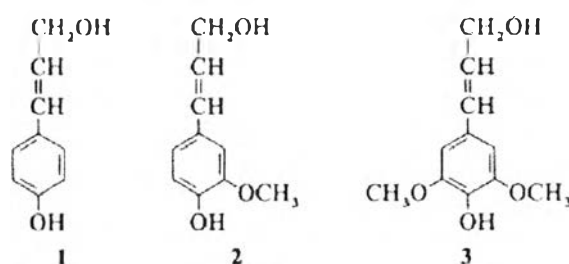
Figure 2.4 P-coumaryl- , coniferyl- and sinapyl alcohol: dominant building blocks of the three-dimensional polymer lignin (Harmsen *et al.*, 2010).

2.2 Effects of Biomass Compositional and Structural Features on Lignocelluloses
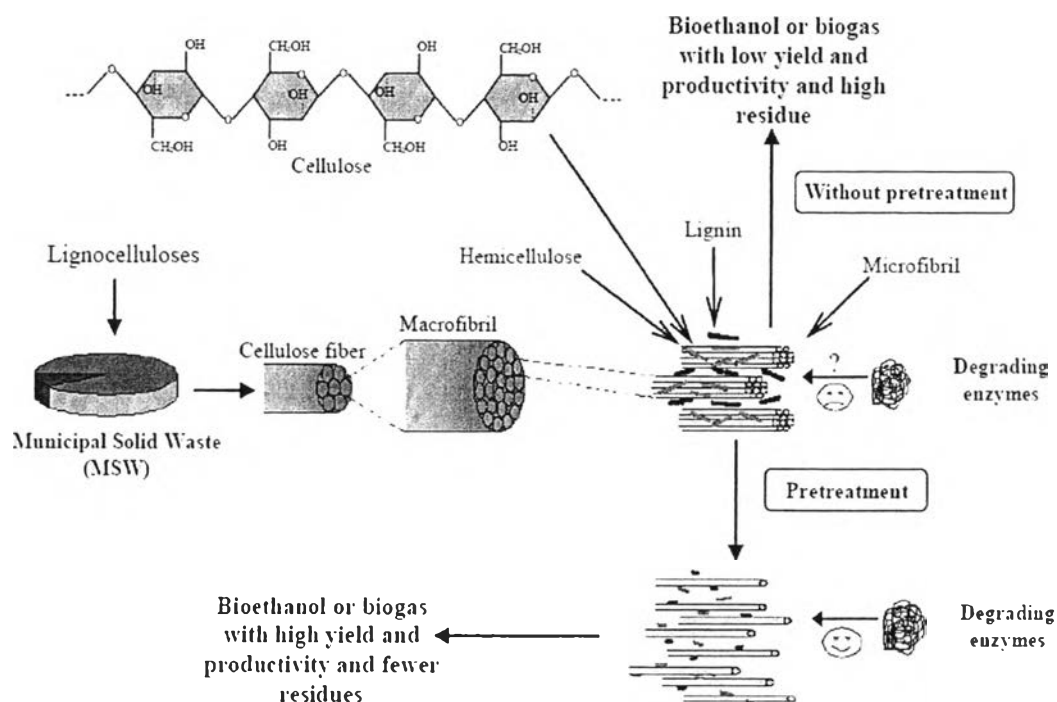
Figure 2.5 Effect of pretreatment on accessibility of degrading enzymes (Taherzadeh, and Karimi, 2008).

2.2.1 Cellulose Crystallinity

The major part of cellulose (around 2/3 of the total cellulose) is in the crystalline form. As the amorphous portion of cellulose is more accessible by cellulase hydrolysis, the enzymatic hydrolysis is resisted by crystalline portion. However, some research showed that the crystallinity information has no relationship to the rate of hydrolysis. The degree of crystallinity increased slightly with delignification (from 43% to 60%) because amorphous components such as hemicellulose and lignin were removed as reported by Kim and Holtzapple, 2005. Although the crystallinity is an important factor in digestibility of lignocelluloses, it is not the only factor in effective enzymatic hydrolysis.

2.2.2 Specific Surface Area of Cellulose

Lignocellulosic materials have two different types of surface area: external and internal. Lignocelluloses are swelled with water and polar solvents make a very large internal surface. The main reason for improvement in enzymatic hydrolysis by removing lignin and hemicellulose is related to the cellulose accessible surface area (Taherzadeh, and Karimi, 2008).

2.2.3 Lignin Barrier

Lignin, a biologically resistant, reticular polymer surrounding cellulose and hemicellulose correlates inversely with enzymatic digestibility. Lignin removal increases enzyme effectiveness by eliminating nonproductive adsorption sites and by increasing access to cellulose and hemicellulose.

2.2.4 Hemicellulose Content

Hemicellulose is a physical barrier which surrounds the cellulose fibers and can protect the cellulose from enzymatic attack. Many pretreatment methods were shown to be able to remove hemicelluloses and consequently improve the enzymatic hydrolysis (Taherzadeh, and Karimi, 2008). Alkali remove acetyl groups from hemicellulose (mainly xylan), that reduce the steric hindrance of hydrolytic enzymes and improve carbohydrate digestibility.

2.3 Fundamental Of Microwave-based Process

Microwaves are between infrared and radio-frequency radiation in the electromagnetic (EM) spectrum, as shown in figure 2.6. The frequency range is 300MHz to 30 GHz. To prevent interference with radar and telecommunications, most industrial microwave systems operate at either 900 MHz or 2.45 GHz.

Generally, the microwave systems is a device called the magnetron which is responsible for generating microwave radiation. Figure 2.7, a cathode filament is heated by using direct current to release electrons that start to navigate toward a circular anode that surrounds the cathode. Under normal conditions, these electrons would navigate in a straight line directly to the anode. Nevertheless, the magnetron

generates a permanent magnetic field that runs parallel to the cathode filament. Hence, the electrons experience a Lorentz force and accelerate towards the anode in a spiral path (Keshwani, 2009) to generate electricity from the power outlet and converts it into microwaves.
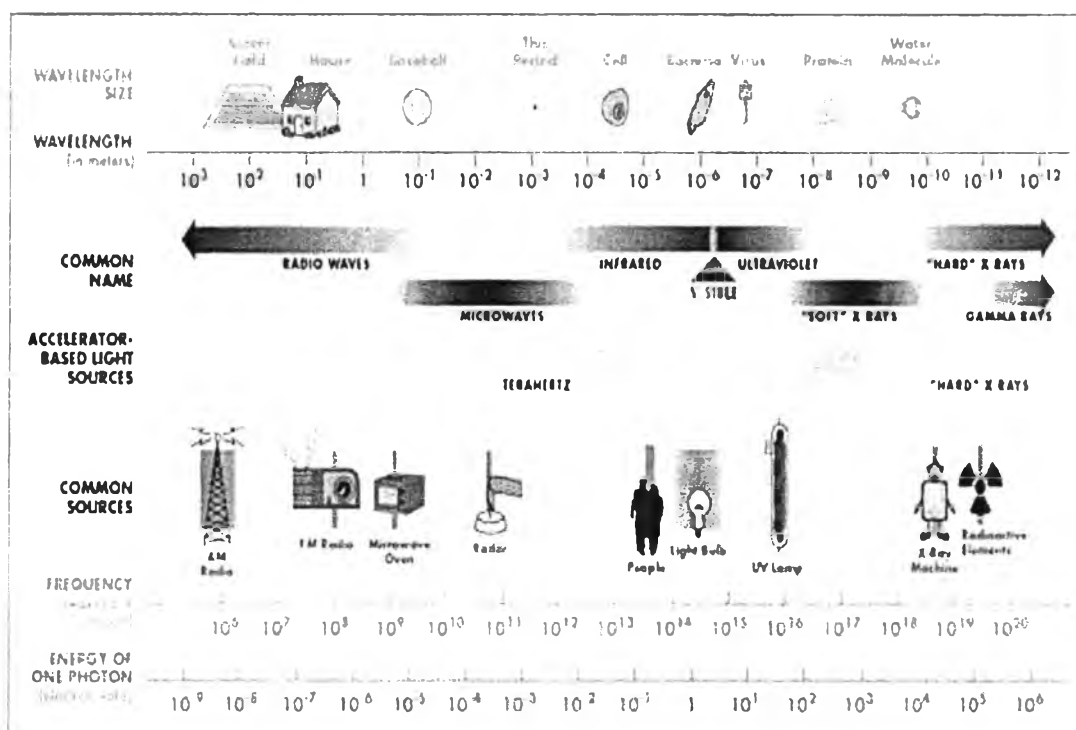

Figure 2.6 The electromagnetic spectrum with their applications at various frequencies (http://www.unc.edu/depts/mtcgroup/litmeetings/microwaves.pdf).

The electric component causes microwave heating by two main mechanisms

- Dipolar polarization: microwave radiation supplies internal heat to the biomass resulting from the vibrations of polar bonds in the material and surrounding aqueous medium. The radiation generates a continuously changing magnetic field causing the polar bonds to vibrate (Keshwani *et al.*, 2007). The polar bonds are disrupted contributing to accelerate chemical processes. This mechanism is called the dipolar polarization mechanism.

- Ionic conduction: the dissolved ionic oscillate back and forth with the changing direction under the electric field. The ions collide with each other that create heat in the system.
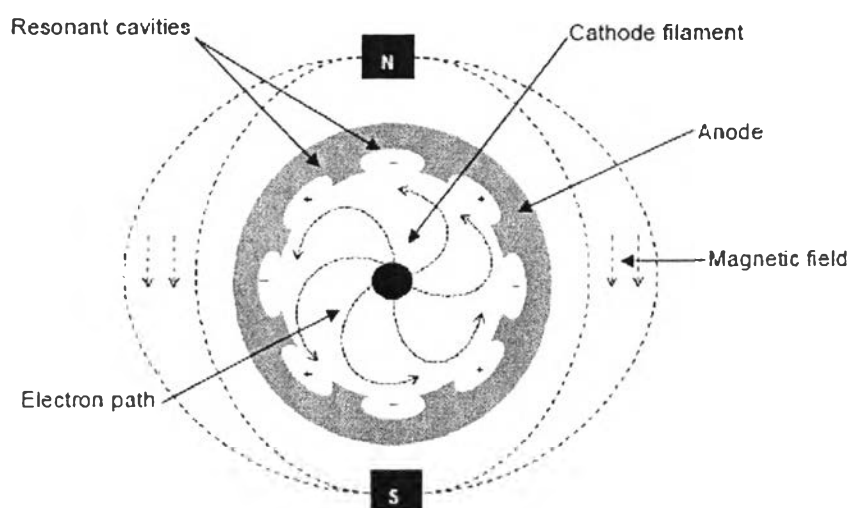
Figure 2.7 Schematic of a magnetron in microwave systems based on information (Keshwani, 2009).

In addition, the radiation can accelerate biological and physical processes of material which contains polar molecules and ions. Besides, microwave technology has other benefits and challenges, as shown in Table 2.2.

Table 2.2 Benefits and challenges of microwave processing (Clark *et al.*, 2004)

| Benefits	Cost savings (time and energy, reduced floor space)
	Rapid heating of thermal insulators (most ceramics and polymers)
	Precise and controlled heating (instantaneous on/off heating)
	Selective heating
	Volumetric and uniform heating (due to deep energy penetration)
	Short processing times
	Improved quality and properties
	Synthesis of new materials

Table 2.2 (Cont.) Benefits and challenges of microwave processing (Clark *et al.,* 2004)

Benefits	Processing not possible with conventional means
	Reduction of hazardous emissions
	Increased product yields
	Environmentally friendly (clean and quiet)
	Self-limiting heating in some materials
	Power supply can be remote
	Clean power and process conditions
Challenges	Heating low-loss poorly absorbing materials
	Controlling accelerated heating (thermal runaway)
	Exploiting inverted temperature profiles
	Eliminating arcing and controlling plasmas
	Efficient transfer of microwave energy to work piece
	Compatibility of the microwave process with the rest of the process line
	Reluctance to abandon proven technologies
	Timing
	Economics

2.3 Pretreatment

The overall purpose of the pretreatment is to remove lignin, hemicellulose, and increases the porosity of the material. Pretreatment must meet the following requirements: (1) improve the formation of sugars or the ability to subsequently form sugars by hydrolysis, (2) avoid the degradation or loss of carbohydrate, (3) avoid the formation of byproducts that are inhibitory to the subsequent hydrolysis and fermentation processes, and (4) be cost-effective (Kumar *et al.,* 2009).
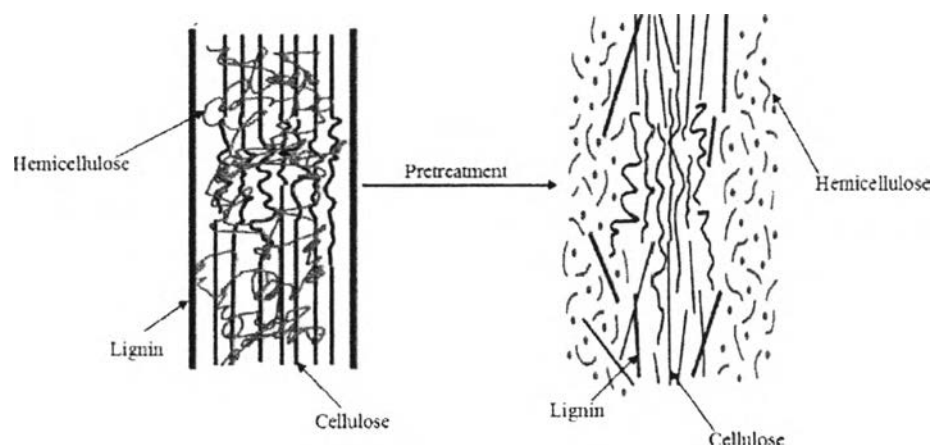
Figure 2.8 Schematic of pretreatment effect on lignocellulosic biomass (Kumar *et al.*, 2009).

Pretreatment techniques have generally classified into four categories, including physical, chemical, physic-chemical, and biological pretreatment. However, none of those can be the best pretreatment because each pretreatment has advantages and disadvantages.

2.3.1 Physical Pretreatment

2.3.1.1 Mechanical Comminution

Generally, the particle size of biomass, cellulose crystallinity, degree of polymerization and reduced. On the other hand the specific surface area of biomass an increased by breaking down the biomass into smaller particles, rendering the substrate more amenable to subsequent enzymatic hydrolysis. The mechanical comminuted by acombination of chipping, grinding, and/or milling. The size of the materials isusually 10–30 mm after chipping and 0.2–2 mm after milling or grinding (Kumar *et al.*, 2009). However, the mechanical comminution is time-consuming, energy-intensive and expensive; furthermore, mechanical comminution is much less effective than chemical pretreatments since it does not result in lignin removal, which has been proved to significantly restrict accessibility of cellulose and inhibit cellulases (Zheng *et al.*, 2009).

2.3.1.2 High Energy Radiation

Digestibility of cellulosic biomass has been enhanced by the use of high energy radiation methods, including γ-ray, ultrasound, electron beam, pulsed electrical field, UV, and microwave heating (Zheng *et al.,* 2009). These methods can increase specific surface area of cellulosic biomass, decrease the degrees of polymerization and crystallinity of cellulose, hydrolysis of hemicellulose, and partial depolymerization of lignin. However, they are usually slow, expensive, and some composition of lignocellulosic material might be decomposition at high temperature.

2.3.2 Chemical Pretreatment

2.3.2.1 Acid Pretreatment

The concentrated acid hydrolysis such as sulfuric acid and hydrochloric acid had been an important technology for hydrolyzing lignocellulosic biomass for fermentable sugar production. Even if they are effective agent for cellulose hydrolysis, concentrated acid is toxic, corrosive and hazardous so require the construction materials for reactor to resistant corrosive that make the process is expensive, the concentrated acid must be recovered after hydrolysis to make the process is economical. Advantages of concentrated acid hydrolysis are the flexibility in terms of feedstock choice, high monomeric sugar yield as well as mild temperature conditions that are needed (Harmsen *et al.,* 2010).

2.3.2.2 Alkaline Pretreatment

Alkaline pretreatment applies various bases, including sodium hydroxide, calcium hydroxide (lime), potassium hydroxide, aqueous ammonia, ammonia hydroxide, and sodium hydroxide incombination with hydrogen peroxide or other. The use of an alkali causes the degradation of ester and glycosidic side chains resulting in structural alteration of lignin, cellulose swelling, partial decrystallization of cellulose, and partial solvation of hemicellulose (Brodeur, *et al.,* 2011). The mechanism of alkaline pretreatment is believed to be saponification of intermolecular ester bonds crosslinking hemicellulose and other components. Moreover, alkali pretreatments remove acetyl, and the various uronic acid substitutions on hemicellulose that lower the accessibility of the enzyme to the

hemicellulose and cellulose surface that reported by Chang and Holtzapple, (2000). Pretreating of lignocellulosic material causes swelling, increased internal surface area, disruption of the lignin structure, and separation of structural linkages between lignin and carbohydrates.

In type of alkaline pretreatment, sodium hydroxide is used the most. It is more effective on hardwood, herbaceous crops, and agricultural residues with low lignin content than on softwood with high lignin content. Some authors observed that the digestibility of NaOH-treated hardwood increased from 14% to 55% with the decrease of lignin content from 24%–55% to 20%. In contrast, effect of dilute alkaline was found for softwoods with lignin content greater than 26%. It can be performed at ambient conditions, but longer pretreatment times are required at higher temperatures. Calcium hydroxide (lime) is shown to be an effective pretreatment agent and is the cheapest compared to sodium hydroxide and potassium hydroxide. Lime pretreated corn stover obtained maximum lignin removal of 87.5% at 55°C for four weeks with aeration studied by Kim and Holtzapple, 2005. Neutralizing step to remove lignin and inhibitors (salts, phenolic acids, furfural and aldehydes) is required before enzymatic hydrolysis. In neutralization of lime pretreatment by neutrallizing with carbon dioxide eliminates the solid-liquid separation step before hydrolysis, resulting in 89 wt% glucose recoveries from rice straw.

2.3.2.3 Organosolv Process

In the process, an organic or aqueous organic solvent mixture with inorganic acid catalysts such as H_2SO_4 or HCl is used to break the internal lignin and hemicellulose bonds. Normally, the presence of catalyst can increase the solubilization of hemicellulose and the digestibility of substrate is also more enhanced.

Benefit of organosolv pretreatment includes the production of a high-quality lignin, which might facilitate higher-value applications of lignin such as production of (platform) chemicals. The enzyme costs are low by separation of lignin before the enzymatic hydrolysis of the cellulose fraction. In addition to improved accessibility of the cellulose fibres, absorption of cellulase enzymes to lignin is minimized by actual removal of lignin beforehand (Harmsen *et al.,* 2010).

2.3.2.4 Ionic Liquids (ILs) or Green Solvent Pretreatment

Ionic liquids are salts, typically composed of a small anion and a large organic cation, which exist as liquids at room temperature and have very low vapor pressure (Brodeur *et al.*, 2011). They can dissolve large amounts of cellulose at considerable mild conditions and feasibility of recovering nearly 100% of the used ILs to their initial purity makes them attractive (Zheng *et al.*, 2009). The low vapor pressure of IL and similar solvents make them more than 99% recoverable in a number of operations that they are termed green solvents so reducing costs of solvent usage. ILs owns many advantages over regular volatile organic solvents of biodegradability, low toxicity, low hydrophobicity, low viscosity, enhanced electrochemical stability, thermal stability, high reaction rates, low volatility with potentially minimal environmental impact, and non-flammable property. However, it is high solvent costs and need for solvent recovery and recycles.

2.3.2.5 Oxidative Delignification

Delignification of lignocellulose can also be achieved by treatment with an oxidizing agent such as hydrogen peroxide, ozone, oxygen or air. Wet oxidation operates with oxygen or air in combination with water at elevated temperature and pressure. This method has been proven to be efficient for treating lignocellulosic materials because the crystalline structure of cellulose is opened during the process (Harmsen *et al.*, 2010).

2.3.3 Physico-chemical Pretreatments

2.3.3.1 Steam-Explosion

In this method, biomass is treated with high-pressure saturated steam and high temperature for a period of time, and then the pressure is quickly reduced, which makes the materials are disrupted that increases the accessibility of the cellulose to the enzymes during hydrolysis. The higher temperature of process causes hemicellulose degradation and lignin transformation, hence the potential of cellulose hydrolysis increase. The steam-explosion pretreatment process has been a proven technique for the pretreatment of different biomass feedstocks. It is able to generate complete sugar recovery while utilizing a low capital investment, low

environmental impacts concerning the chemicals and conditions being implemented, and has a higher potential for optimization and efficiency (Brodeur *et al*, 2011).

2.3.3.2 Ammonia Fiber/Freeze Explosion (AFEX)

In the AFEX process, biomass is treated with liquid ammonia at high temperature and pressure for a period time and then the pressure is suddenly released. This rapid expansion of the ammonia gas causes swelling of the biomass feedstock, creating a disruption in the lignin-carbohydrate linkage, hemicellulose hydrolysis and ammonolysis of glucuronic cross-linked bonds, and partial decrystallization of the cellulose structure, all leading to a higher accessible surface area for enzymatic attack pH-controlled liquid hot water (Brodeur *et al.*, 2011).

The several advantages include: lower moisture content, lower formation of sugar degradation products due to moderate conditions, 100 percent recoveryof solid material, and the ability for ammonia to lessen lignin's effect on enzymatic hydrolysis. A number of disadvantages can be seen in the form of costs due to recycle and treatment of chemicals that are being used, less effective process with increasing lignin content and high cost of ammonia.

2.3.3.3 Carbon Dioxide Explosion

This method is a supercritical carbon dioxide explosion that CO_2 formscarbonic acid when dissolved in water, the acid increases the hydrolysis rate. CO_2 was suggested to be helpful in hydrolyzing hemicellulose as well as cellulose. Moreover, the low temperature prevents any appreciable decomposition of monosaccharides by the acid (Kumar *et al.*, 2009). A positive effect was found for lignocellulosic material with a high moisture content (>40%), but the pretreatment was not effective enough to compensate for the high capital costs for high-pressure equipment (Harmsen *et al.*, 2010).

2.2.4 Biological

Microorganisms can also be used to treat the lignocelluloses and enhance enzymatic hydrolysis whichdegrade lignin and hemicellulose but small part of cellulose. In biological pretreatment processes, microorganisms such as brown-, white-, and soft-rot fungi are used for this purpose. Low energy requirement, no chemical requirement, and mild environmental conditions are the mainadvantages of

biological pretreatment. However, the treatment rate is very low in most biological pretreatment processes (Taherzadeh, and Karimi, 2008).

Table 2.3 Summary of various processes used for the pretreatment of lignocellulosic biomass (Brodeur *et al.*, 2011; Kumar *et al.*, 2009)

Pretreatment process	Advantages	Disadvantages
Mechanical Comminution	- Reduces cellulose crystallinity	- Power consumption usually higher than inherent biomass energy
High energy radiation	- Increase of specific surface area of cellulosic biomass - Decrease of the degrees of polymerization and crystallinity of cellulose - Hydrolysis of hemicellulose and partial depolymerization of lignin	- Slow - Expensive -Some composition of lignocellulosic material might be decomposition at high temperature
Alkaline	- Removes lignin(major) and hemicelluloses(minor) - Increases accessible surface area - Low inhibitor formation	- Long residence times required - High cost of alkaline catalyst - Alteration of lignin structure
Acid	- Hydrolyzes hemicellulose to xylose and other sugars - Alters lignin structure	- High costs of acids and need for recovery - High costs of corrosive resistant equipment -Formation of inhibitors

Table 2.3 (Cont.) Summary of various processes used for the pretreatment of lignocellulosic biomass (Brodeur *et al.*; Kumar *et al.*, 2009).

Pretreatment process	Advantages	Disadvantages
Organosolv	- Removes lignin(major) and hemicelluloses(minor)	- Solvents need to be drained from thereactor, evaporated, condensed, and recycled - High cost
Ionic Liquids	- Hydrolysis of lignin and hemicellulose - Ability to dissolve high loadings of different biomass types - Mild processing conditions (low temperatures)	- High solvent costs - Need for solvent recovery and recycle
Oxidation delignification	- Removal of lignin - Dissolve hemicelluloses - Decrystallization cellulose	- Low yield of fermentable sugars
Steam explosion	- Causes hemicellulose degradation and lignintransformation - Cost-effective	- Partial hemicellulose degradation - Acid catalyst needed to make process efficient with high lignin content material - Toxic compound generation

Table 2.3 (Cont.) Summary of various processes used for the pretreatment of lignocellulosic biomass (Brodeur *et al.*, 2011; Kumar *et al.*, 2009).

Pretreatment process	Advantages	Disadvantages
Ammonia fiber/freeze explosion	- Increases accessible surface area, removes lignin(major)and hemicellulose(minor) to an extent - Causes inactivity between lignin and enzymes - Does not produce inhibitors for downstream processes	- Recycling of ammonia is needed - Less effective process with increasing lignin content - No separation takes place - High cost of ammonia
Carbon dioxide explosion	- Increases accessible surface area - Low degradation of sugars - Cost-effective; does not cause formationof inhibitory compounds	- High pressures requirements - Lignin and Hemicellulose unaffected
Biological	- Degrades lignin and hemicelluloses - Low energy requirements -No chemical requirement - Mild environmental conditions	

Table 2.4 Effect of various pretreatment methods on the chemical composition and chemical/physical structure of lignocellulosic biomass (Brodeur *et al.*, 2011)

Pretreatment	Increases Accessible Surface Area	Decrystallizes Cellulose	Removes Hemicellulose	Removes Lignin	Alters Lignin Structure
Steam explosion	H		H		L
Liquid Hot Water	H	ND	H		L
Dilute Acid	H		H		H
AFEX	H	H	L	H	H
Lime	H	ND	L	H	H
Green Solvents	H	H	L	H or L	L
Supercritical Fluid	H	H	H		L

H: high effect, L: Low effect, ND: not determined, * Depends on the chemical nature of the solvent.

Of the several pretreatment techniques, alkali pretreatment is much attractive as above-mentioned. Chang *et al.* (1998) used lime (calcium hydroxide) as a pretreatment agent for short pretreatment times (1–3 h) and high temperatures (85–135°C). Under these conditions, the reducing sugar yield of the pretreated bagasse increased from 153 to 659 mg Eq glucose/g dry biomass, and that of the pretreated wheat straw increased from 65 to 650 mg Eq glucose/g dry biomass. Ga´spa´r *et al.* (2007) used alkali pretreatment which appeared to be the most promising in breaking the ester bonds between lignin, hemicellulose, and cellulose by avoiding fragmentation of the hemicellulose polymer.

Microwave irradiation has been widely used in many areas because of its high heating efficiency, high selectivity taking short time, and easy operation. Hu and Wen (2008) reported that 99% of the theoretical reducing sugar yield was obtained after 30 min pretreatment of 50 g/l switch grass at 190°C with microwave heating and enzymatic hydrolysis.

Generally, some useful component might be decomposed at high temperature during pretreatment. Microwave technique can easily combine with chemical reaction and accelerate the chemical reaction rate (Zhu *et al.*, 2005). As a result, a combination of microwave treatment and alkali treatment might be an alternative way for pretreating corn cob. Treating by microwave/alkali showed more weight loss, higher cellulose content and lower moisture, lignin, and hemicellulose content than that by alkali alone (Zhu *et al.*, 2005). They also concluded that the enzymic hydrolysis of rice straw treated by microwave/alkali had a higher rate and produced a hydrolysate with higher glucose content and lower xylose content, which is more suitable for subsequent fermentation.

Hu and Wen (2008) showed that the physical structure changes of the switchgrass pretreated by different methods were imaged by scanning electron microscope (Figure. 2.9). When switchgrass was treated by microwave, many granules appeared on the surface (2.9C), indicating partial break down of the lignin structure, although those lignin may still "stay" on the particle surfaces. For the switchgrass presoaked in alkali andtreated by microwave, the materials became "thinner and striated" (2.9D), suggesting that lignin was dissolved, and cellulose/hemicellulose were more exposed.

In addition, Cheng *et al.,* (2011) discussed about microwave and alkali pretreatment that microwave can heat rice straw uniformly from insides and efficiently breaks down the lignin-hemicellulose structure, destroys the crystal structure of cellulose and degrade the hemicelluloses into reducing sugar. Rice straw pretreated by NaOH can generate micropores and grooves, which was then exploded by microwave heating due to high temperature and pressure.
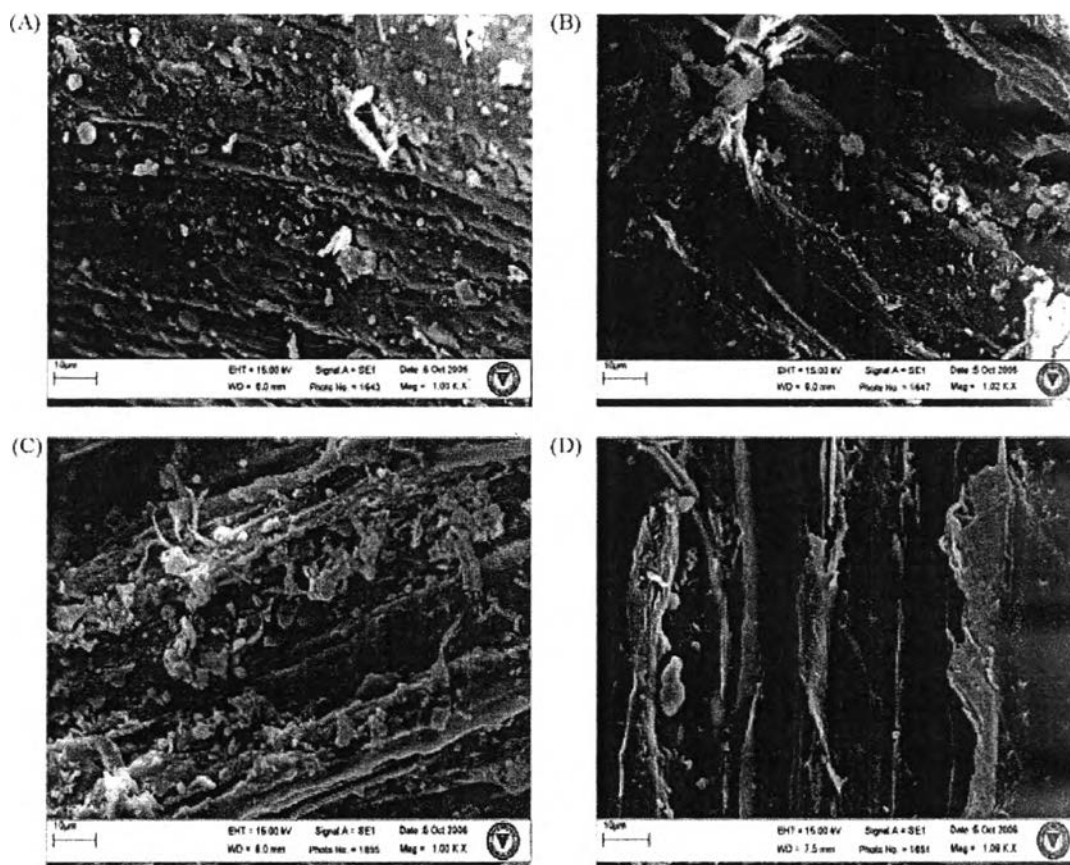
Figure 2.9 Scanning electron microscope images of switchgrass (1000×). (A) Raw switchgrass without treatment; (B) material after being mixed with water and treatedwith conventional heating at 190°C, 100 g/L solid content for 30 min; (C) material after being mixed with water and treated with microwave heating at 190°C, 100 g/L solid content for 30 min; (D) material after being presoaked in alkali solution (0.1 g alkali/g biomass) for 2 h, and then treated with microwave heating at190°C, 100 g/L of solid content for 30 min.

Carrillo *et al.*, (2005) observed that alkali pretreatment increased the total sugar yield on the enzymtic hydrolysis. This is due to the swelling and hydrolysis of lignin and other hemicellulose during the NaOH alkali pretreatment.

Zhu *et al.*, (2006) used the simultaneous saccharification and fermentation (SSF) of the microwave-assisted alkali and conventional alkali pretreated wheat straw to ethanol. They observed that production of ethanol from microwave-assisted pretreated wheat straw had lower enzyme loading, shorter reaction time, and could

achieve higher ethanol concentration and yield than the conventional alkali pretreated wheat straw.

The effectiveness of alkaline pretreatment varies, depending on the substrate and treatment conditions. Using alkali chemicals (NaOH, Ca(OH)$_2$ and KOH) to pretreat rice straw in 24 h at 25°C, it was found that NaOH (6% chemical loading, g/g dry rice straw) was the best alkali chemical to achieve 85% increase of glucose yield by enzymatic hydrolysis (Zheng *et al.*,2009).

Lime (calcium hydroxide) pretreatment has regained interest as one of the promising pretreatment technologies being studied. This pretreatment has low formation of fermentation inhibitors, increases pH, and provides a low-cost alternative for lignin solubilization, removing approximately 33% of lignin and 100% of acetyl groups. The action of lime is slower than other pretreatments but its low cost and safe handling make it attractive, as reported by Rabelo *et al.*(2008).

Chen *et al.*, (2009) pretreated corn fiber by 1% and 2% of NaOH and KOH. The cell wall was disrupted by dissolving hemicellulose and lignin, by hydrolyzing uronic and acetic acid esters and by swelling cellulose and decreasing the crystallinity of cellulose, increasing the biodegradability of the cell wall because cleavage of the bonds between lignin and cellulose. According to results the alkaline pretreatment of corn fiber has a great potential, due to hemicellulose polymer obtained is a highly valuable product and high yields of it (about 80%) can be recovered with 1% and 2% (w/w) alkaline solutions.

2.4 Enzymic Hydrolysis

In the process, cellulose chains are broken down into glucose and xylose sugar molecules by enzymes known as cellulase. It refers to a class of enzymes produced by fungi, bacteria, and protozoans that catalyze the hydrolysis of cellulose. There are a number of cellulase enzymes that are capable of degrading cellulose, of which the most intensively studied is the enzyme complex derived from filamentous fungus *Trichoderma Reesei.* Champagne and Li (2009) indicated that enzyme complexes generally consist of three components: endo-β-glucanase (EG) which attach to regions of low crystallinity in the cellulose fiber, generating free chain ends;

exo-β-glucanase (EG), which attach to regions of low crystallinity in the cellulose fiber, generating free chain ends; cellobio-hydrolase (CBH), which degrade cellulose molecules further by removing cellobiose units from the free chain; and, β-glucosidase which hydrolyze cellobiose to produce glucose, as shown in Figure 2.10.

They informed that the enzymatic hydrolysis of cellulose takes place in three stages: (1) the adsorption of the cellulase enzyme complex onto the surface of the cellulose structure; (2) the degradation of cellulose into mono sugars; and (3) the desorption of cellulose. The enzymatic hydrolysis of biomass is to proceed in typical processes; the crystalline structure of cellulose needs to be disrupted, accessible area increased and the lignin and hemicellulose separated from the cellulose before treatment with enzymes (Brodeur *et al.*, 2011).
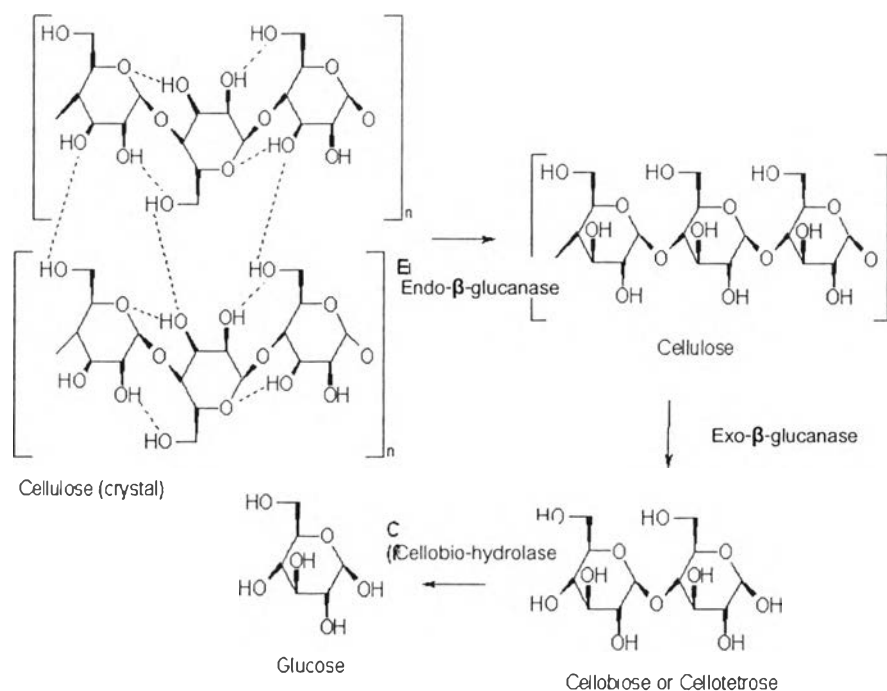
Figure 2.10 The mechanism of the hydrolysis of cellulose
(http://www.answers.com/topic/cellulase).

The cellulose enzyme used was a commercial Trichodermareesei cellulase (formerly called Trichodermaviride cellulase) which was supplemented with

antibiotics tetracycline (40 mg ml^{-1}) and cycloheximide (30 mg ml^{-1}) to prevent microbial contamination reported by Zhu *et al.,* (2005).

Kaar and Holtzapple (2000) recommended enzyme loading for the enzymic saccharification of pretreated corn stover by using *T. reesei* is 10 FPU (g dry biomass)$^{-1}$ and the recommended hydrolysis temperature is 40°C. Ultimate digestion of pretreated corn stover produced conversions of 88, 88, and 92% for the glucan, xylan, and arabinan, respectively. They also reported that sugar yield from enzymatic hydrolysis was independent of the solid content within a range from 60 to 200 g/L. It might be caused by the different energy absorption by samples with different solid contents. For samples with low solid content, a relatively high proportion of water will favor the adsorption of microwave irradiation energy because the energy adsorption is based on oscillation of water molecules. At low solid content (and high water loading) will receive more energy, which eventually facilitates the disruption of the crystalline cellulose. As a result, higher sugar yields wasobtained from samples at low solid content (Hu and Wen, 2008).