CHAPTER II
THEORETICAL BACKGROUND AND LITERATURE REVIEW

2.1 Lignocellulosic-Biomass Materials

Biomass is a carbon neutral resource in its life cycle and the primary contributor of greenhouse effect. Biomass is the fourth largest source of energy in the world after coal, petroleum and natural gas, providing about 14% of the world's primary energy consumption. Renewable biomass is being considered as an important energy resource all over the world. Biomass is used to meet a variety of energy needs, including generating electricity, fueling vehicles and providing process heat for industries (Bridgewater *et al.*, 1999; Bridewater, 1999). Among all the renewable sources of energy, biomass is unique as it effectively stores solar energy. It is the only renewable source of carbon that can be converted into convenient solid, liquid and gaseous fuels through different conversion processes (Ozbay *et al.*, 2001).

Lignocellulosic materials serve as a cheap and abundant feedstock, which is required to produce fuel bioethanol from renewable resources at reasonable costs. In 2007 the US Department of Energy provided more than US$1 billion toward lignocellulosic bioethanol projects, with the goal of making the fuel cost competitive at US$1.33 per gallon by 2012 (Slade *et al.*, 2009). Lignocellulosic materials can be classified in four groups based on type of resource: (1) forest residues, (2) municipal solid waste, (3) waste paper, and (4) crop residue resources. Literature reports several papers on utilization of various lignocellulosic waste materials such as rice straw (Yao *et al.*, 2007), corn stover (Wang *et al.*, 2009), switchgrass (Keshwani and Cheng, 2009), palm bagasse (Carvalho *et al.*, 2009).

2.2 Chemical Structure and Basic Components of Lignocellulosic Materials

The importance of particular type of biomass depends on the chemical and physical properties of the large molecules from which it is made. The chemical structure and major organic components in biomass are important in the development of processes for producing derived fuel and chemicals. Biomass contains varying amounts of cellulose, hemicellulose, lignin and a small amount of extractive (Bridewater, 1999).

Chemical composition of lignocellulosic materials is a key factor affecting efficiency of biofuel production during conversion processes. The structural and chemical composition of lignocellulosic materials is highly variable because of genetic and environmental influences and their interactions (Lee *et al.*, 2007). A typical chemical composition of lignocellulosic materials is 48 wt.% C, 6 wt.% H, and 45 wt.% O, the inorganic matter being a minor component (Molina-Sabio *et al.*, 2004). The proximate analysis of rice straw and wheat straw shows components as follow: volatile matter (65.47%, 75.27%), fixed carbon (15.86%, 17.71%) and ash (18.67%, 7.02%), respectively (Jenkins *et al.*, 1998).

Lignocelluloses consist mainly of cellulose, hemicellulose and lignin; these components build up about 90% of dry matter in lignocelluloses, with the rest consisting of e.g. extractive and ash (Dehkhoda, 2011).The basic structure of all woody biomass consists of three basic polymers: cellulose $(C_6H_{10}O_5)x$, hemicelluloses such as xylan $(C_5H_8O_4)m$, and lignin $[C_9H_{10}O_3(OCH_3)0.9–1.7]n$ in trunk, foliage, and bark. The proportion of these wood constituents varies between species, and there are distinct differences between hardwoods and softwoods. Cellulose + hemicellulose contents are more in hardwoods (78.8%) than softwoods (70.3%), but lignin is more in softwoods (29.2%) than hardwoods (21.7%) (Balat, 2009). The contents of cellulose, hemicelluloses, and lignin in common lignocellulosic materials are listed in Table 2.1.

Table 2.1 Contents of cellulose, hemicellulose, and lignin in common lignocellulosic materials (Saha, 2003)

Lignocellulosic material	**Cellulose (%)**	**Hemicellulose (%)**	**Lignin (%)**
Hardwood stems	40-75	10-40	15-25
Softwood stems	30-50	25-40	25-35
Corncob	45	35	15
Wheat straw	30	50	15
Rice straw	32-47	19-27	5-24
Sugarcane bagasse	40	24	25
Leaves	15-20	80-85	-
Paper	85-99	-	0-15
Newspaper	40-55	25-40	18-30
Waste paper from chemical pulps	60-70	10-20	5-10
Grasses	25-40	25-50	10-30

2.2.1 Cellulose

The major component of plant biomass (30-60% of total feed stock dry matter) is Cellulose (Hamelinck *et al.*, 2005). Cellulose is a homopolysaccharide composed of β-D-glucopyranose units linked together by (1-4)-glycosidic bonds as shown in Figure 2.1. The cellulose molecules are linear; the β-D-glucopyranose

chain units are in a chair conformation and the substituents HO-2, HO-3, and CH2OH are oriented equatorially (Sjöström, 1993). Glucose anhydride, which is formed via the removal of water from each glucose, is polymerized into long cellulose chains that contain 5000–10,000 glucose units. The basic repeating unit of the cellulose polymer consists of two glucose anhydride units, called a cellobiose unit (Mohan *et al.*, 2006).

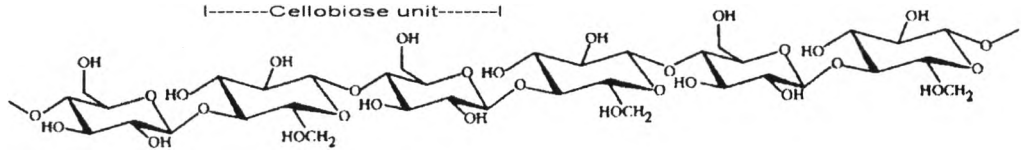


Figure 2.1 Schematic representation of a cellulose chain (Mousdale, 2008).

2.2.2 Hemicellulose

A second major wood chemical constituent is hemicellulose, which is also known as polyose. A variety of hemicelluloses usually account for 25–35% of the mass of dry wood, 28% in softwoods, and 35% in hardwoods. Hemicellulose is a mixture of various polymerized monosaccharides such as glucose, mannose, galactose, xylose, arabinose, 4-O-methyl glucuronic acid and galacturonic acid residues (Mohan *et al.*, 2006) as shown in Figure 2.2. Xylose is the predominant pentose sugar derived from the hemicellulose of most hardwood feedstocks, but arabinose can constitute a significant amount of the pentose sugars derived from various agricultural residues and other herbaceous crops, such as switchgrass, which are being considered for use as dedicated energy crops. Whereas arabinose makes only 2–4% of the total pentoses in hardwoods, arabinose represents 10–20% of the total pentoses in many herbaceous crops. Arabinose contents can be as high as 30–40% of the total pentoses in corn fiber, a by-product of corn processing (Mohagheghi *et al.*, 2002).

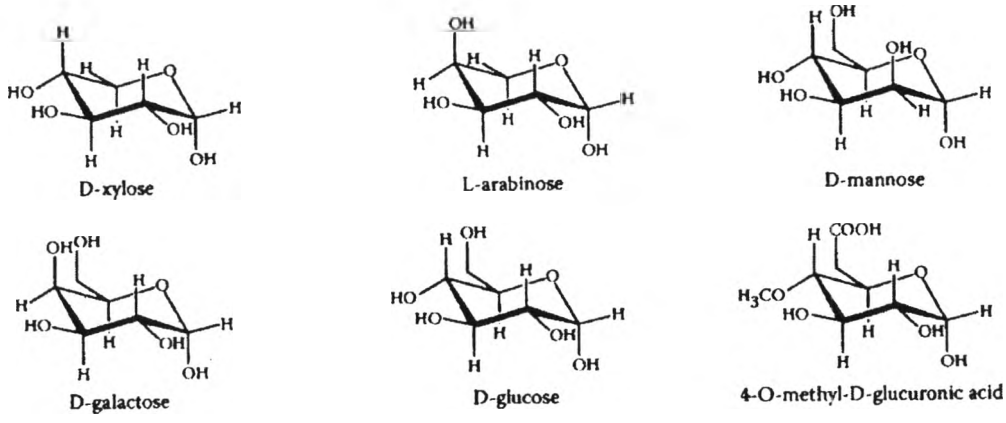
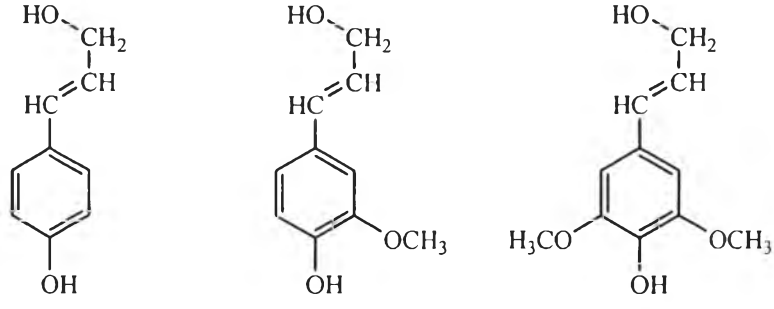


Figure 2.2 Schematic of the basic structure of hemicellulose. A, arabinose; FeA, ferulic acid; G, galactose; Glc, glucuronic acid; X, xylose (Mousdale, 2008).

2.2.3 Lignin

Lignin (15–25% of total feedstock dry matter) is an aromatic polymer synthesised from phenylpropanoid precursors. The basic chemical phenylpropane units of lignin (primarily syringyl, guaiacyl and p-hydroxy phenol) are bonded together by a set of linkages to form a very complex matrix (Demirbas, 2008). This matrix comprises a variety of functional groups, such as hydroxyl, methoxyl and carbonyl, which impart a high polarity to the lignin macromolecule (Feldman *et al.*, 1991). Softwood and hardwood lignins belong to the first and second category, respectively. Softwoods generally contain more lignin than hardwoods (Demirbas, 2008). Lignin is one of the drawbacks of using lignocellulosic-biomass materials in fermentation, as it makes lignocellulose resistant to chemical and biological degradation (Taherzadeh and Karimi, 2008). Figure 2.3 shows lignin building blocks.



p-Coumaryl alcohol Coniferyl alcohol Sinapyl alcohol

Figure 2.3 Lignin building blocks.

2.3 Rice Straw

Rice (Oryza sativa L.) is one of the major crops grown in the world. After rice is harvested from the paddy and processed, there is a large amount of rice straw (Matsumura *et al.*, 2005). This rice straw is composed of cellulose (32%-37%), hemicellulose (29%-37%) and lignin (5%-15%) (Peng *et al.*, 2008). Rice straw is one of the abundant lignocellulosic waste materials in the world. It is annually produced about 731 million tons which is distributed in Africa (20.9 million tons), Asia (667.6 million tons), Europe (3.9 million tons), America (37.2 million tons) and Oceania (1.7 million tons).

The environmentally friendly method of rice straw disposal can not only convert the agricultural waste into a value-added product, but it can also reduce our consumption of gasoline (Hideno *et al.*, 2009). Rice straw has been proposed as a large renewable resource from which bioethanol can be produced via pretreatment followed by enzymatic hydrolysis and fermentation (Yang *et al.*, 2008). This amount of rice straw can potentially produce 205 billion liters bioethanol per year, which is the largest amount from a single biomass feedstock (Karimi *et al.*, 2006).

2.4 Glucose

Glucose is a simple sugar, which is an important carbohydrate in biology. Cells utilize glucose as a source of energy and a metabolic intermediate. One of the main products of photosynthesis is glucose, which starts cellular respiration. However, glucose is useful not only biologically but also chemically. Glucose can be used as the starting raw material to produce a wide variety of chemicals and fuels. This is usually carried out with the help of microorganisms, such as fermentation of glucose to ethanol and conversion of glucose into solvents, e.g. acetone and butanol by *Clostridium acetobutylicum*. Because of the overwhelming quantity of cellulose and the renewable resource, the world will depend on it more heavily for food, fuel, chemical supplies, and raw materials in the future. There is great potential of alleviating the need for petroleum, which is fast decreasing on supply (Wang, 2009).

2.5 Sugar Production from Lignocellulosic Materials

There are several possible ways to hydrolyze lignocelluloses, as shown in Figure 2.4. The most commonly applied methods can be classified into two groups: chemical hydrolysis and enzymatic hydrolysis. In addition, there are some other hydrolysis methods, in which no chemicals or enzymes are applied. For instance, lignocelluloses may be hydrolyzed by ray or electron-beam irradiation, or microwave irradiation. However, those processes are commercially unimportant (Tacherzadeh and Karimi, 2007b).

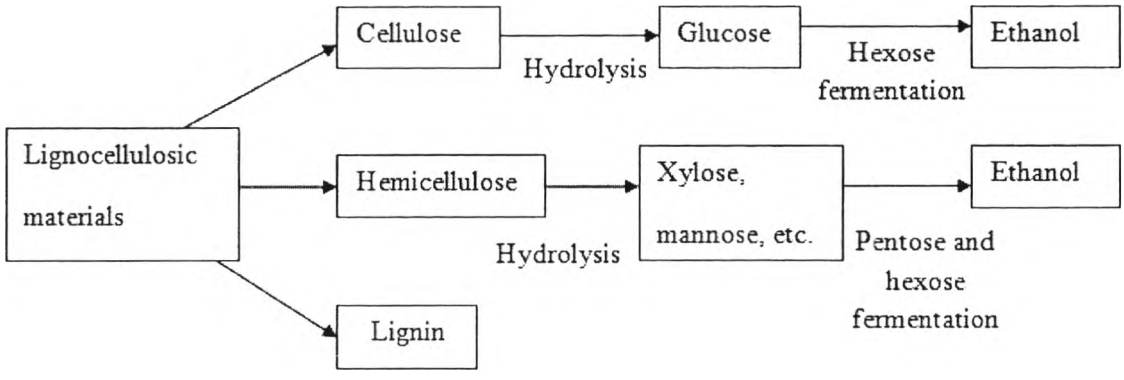


Figure 2.4 Overall view of sugar and ethanol productions from lignocellulosic materials (Eourarekullart, 2011).

2.6 Pretreatment of Lignocellulosic Materials

Pretreatment is required to alter the structural and chemical composition of feedstock in order to facilitate rapid and efficient hydrolysis of carbohydrates to fermentable sugars. In order to obtain fast enzymatic hydrolysis of feedstock with a high sugar yield, the cell structures need to be broken and porosity must be increased. Effective pretreatments must improve enzymatic hydrolysis and prevent formation of by-products that might inhibit subsequent hydrolysis and fermentation steps (Keshwani, 2009).

Cellulose, comprising glucose units, which is a main component of most agricultural residues, can be used as a promising feedstock for the future production of chemicals and fuels, including ethanol. However, direct conversion of cellulose to ethanol is not economically feasible due to the rigidity of the crystalline structure of cellulose, which results from both intra- to inter-hydrogen bondings between the cellulose chains, and so cellulose can resist chemicals and microbial enzymes (Zhu *et al.*, 2006). Hence, pretreatment steps are required to enhance the hydrolysis rate by increasing the adsorption of cellulase on the cellulose surface area, which is defined by particle size and porosity (Dadi *et al.*, 2007). To date, a number of pretreatment methods have been proposed and investigated, including dilute acid pretreatment,

steam explosion, hydrothermal process, organic solvent pretreatment, ammonia fiber explosion, and strong alkali pretreatment. The economic and environmental constraints, however, limit the applicability of these known methods, (Werner, 2006).

Taherzadeh and Karimi (2008) has summarized the prerequisites for an ideal lignocellulose pretreatment; it should: (1) production of reactive cellulosic fiber for enzymatic attack, (2) avoiding destruction of hemicelluloses and cellulose, (3) avoiding formation of possible inhibitors for hydrolytic enzymes and fermenting microorganisms, (4) minimizing the energy demand, (e) reducing the cost of size reduction for feedstocks, (5) reducing the cost of material for construction of pretreatment reactors, (6) producing less residues, and (7) consumption of little or no chemical and using a cheap chemical. Pretreatment is crucial for ensuring good ultimate yields of sugars from both polysaccharides. The goals of pretreatment of lignocellulosic material are exhibited in Figure 2.5.

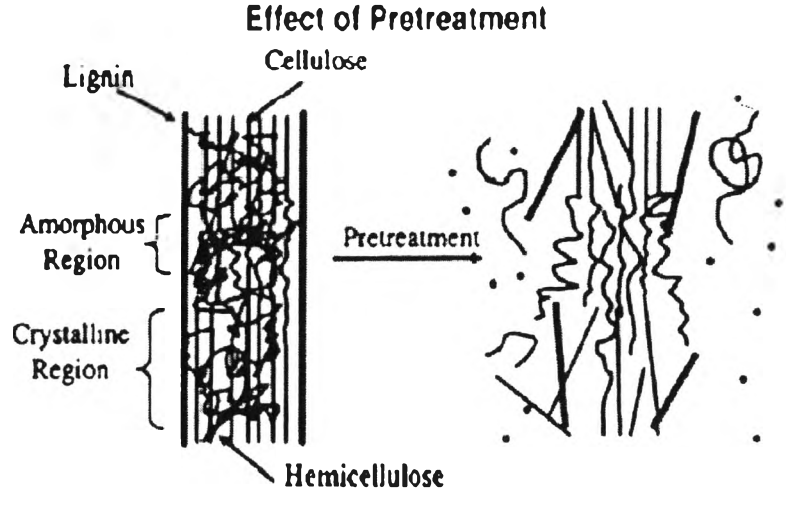


Figure 2.5 Schematic of goals of pretreatment on lignocellulosic material (Hsu *et al.*, 1980).

2.6.1 Mechanical Comminution

Lignocellulosic materials can be comminuted by a combination of chipping, grinding, and milling 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 (Kumar *et al.*, 2009; Sun *et al.*, 2002; Leustean, 2009). Vibratory ball milling was found to be more effective than ordinary ball milling in reducing cellulose crystallinity of spruce and aspen chips and in improving their digestibility (*Kumar et al.*, 2009; Zheng *et al.*, 2009). These mechanical pretreatment techniques are time-consuming, energy intensive, or expensive to process. The compression milling is apparently the only comminution process that has been tested using a production-scale apparatus (Wyman, 1996).

2.6.2 Pyrolysis

Pyrolysis has also been used for pretreating lignocellulosic materials, since biomass can be used as substrate for a fast pyrolysis for thermal conversion of cellulose and hemicelluloses into fermentable sugars with good yields (Tomas-Pejo *et al.*, 2008). When the ground cellulosic materials are treated at temperatures greater than 573 K, cellulose rapidly decomposes to produce gaseous products and residual char (Sun *et al.*, 2002; Pasha and Rao, 2009). The pyrolysis pretreatment of ground material is improved the conversion of cellulose to glucose yield from enzymatic hydrolysis (Leustean, 2009).

2.6.3 Steam Explosion

Steam explosion (autohydrolysis).Steam explosion is the most commonly used method for the pretreatment of lignocellulosic materials (McMillan, 1994). In this method, chipped biomass is treated with high-pressure saturated steam and then the pressure is swiftly reduced, which makes the materials undergo an explosive decomposition (Tomas-Pejo *et al.*, 2008). To summarize the effects of steam explosion treatment on lignocellulosics reported in the literature (Jeoh, 1998) : (1) steam explosion treatment increases crystallinity of cellulose by promoting

crystallization of the amorphous portions; (2) hemicelluloses is easily hydrolyzed by steam explosion treatment; (3) there is evidence that steam explosion promotes delignification. Steam explosion, compared to other pretreatment methods, offers potential for lower capital investment, significantly lower environmental impact, more potential for energy efficiency, less hazardous process chemicals and conditions and complete sugar recovery (Tomas-Pejo *et al.*, 2008).

2.6.4 Ammonia Fiber Explosion (AFEX)

In this process, the material is subjected to liquid ammonia at high temperature and pressure, and a subsequent fast decompression, similar to the steam explosion, which causes a fast saccharification of the lignocellulosic material (Abril *et al.*, 2009). This system does not directly liberate any sugars, but allows the polymers (hemicellulose and cellulose) to be attacked enzymatically and reduced to sugars (Dale *et al.*, 2000). It has been applied to various lignocellulosic materials, including rice straw, municipal solid waste, newspaper, sugar beet pulp, sugarcane bagasse, corn stover, switchgrass, miscanthus, apsen chips, etc. (Zheng *et al.*, 2009).

2.6.5 Carbon Dioxide Explosion

To improve lignocellulose pretreatment efficiency, the idea of using supercritical CO_2 explosion, which would have a lower temperature than steam explosion and possibly a reduced expense compared to ammonia explosion, was developed (Eourarekullart, 2011).

2.6.6 Liquid Hot-Water Pretreatment

Cooking of lignocellulosic materials in liquid hot water (LHW) is one of the hydrothermal pretreatment methods applied for pretreatment of lignocellulosic materials since several decades ago in e.g. pulp industries (Taherzadeh and Karimi, 2008). LHW subjects biomass to hot water in liquid state at high pressure during a fixed period and it presents elevated recovery rates for pentoses and generates low amount of inhibitors (Tomas-Pejo *et al.*, 2008). This pretreatment process usually has

involved temperatures of 473–503 K for up to 15 min. Around 40–60% of the total mass is dissolved in this process, with 4–22% of the cellulose, 35–60% of the lignin and all of the hemicellulose being removed (Hu *et al.*, 2008).

2.6.7 Ozonolysis

Ozonolysis involves using ozone gas to break down the lignin and hemicellulose and increase the biodegradability of the cellulose. The degradation is mainly limited to lignin. Hemicellulose is slightly affected, but cellulose is not (Kumar *et al.*, 2009). The pretreatment is usually carried out at room temperature and is effective at lignin removal without the formation of toxic by-products (Vidal and Molinier, 1998). A drawback of ozonolysis is that a large amount of ozone is required, which can make the process expensive (Kumar *et al.*, 2009).

2.6.8 Alkaline Pretreatment

Alkali pretreatment refers to the application of alkaline solutions to remove lignin and various uronic acid substitutions on hemicellulose that lower the accessibility of enzyme to the hemicellulose and cellulose (Silverstein *et al.*, 2008; Han *et al.*, 2009). These processes utilize lower temperatures and pressures compared to other pretreatment technologies. Alkali pretreatment may be carried out at ambient conditions, but pretreatment time is measured in terms of hours or days rather than minutes or seconds (Mosier *et al.*, 2005). Regardless the advantages, these methods present difficulties from the point of view of the process economy for obtaining fuels (Abril *et al.*, 2009). Sodium,potassium, calcium and ammonium hydroxide are appropriatechemicals for pretreatment. Of these four, NaOH has been studied the most (Kumar *et al.*, 2009). Dilute NaOH treatment of lignocellulosic biomass causes swelling, leading to an increase in the internal surface area, a decrease in crystallinity, separation of structural linkages between lignin and carbohydrates, and disruption of the lignin structure (Fang *et al.*, 1987).

2.6.9 Acid Pretreatment

Acid pretreatments normally aim for high yields of sugars from lignocellulosic materials. Acid pretreatment involves the use of sulfuric, nitric, or hydrochloric acids to remove hemicellulose components and expose cellulose for enzymatic digestion (Silverstein *et al.*, 2008). The acid pretreatment can operate either under a high temperature and low acid concentration (dilute acid pretreatment) or under a low temperature and high acid concentration (concentrated acid pretreatment) (Taherzadeh and Karimi, 2008). Dilute acid hydrolysis has been successfully developed for pretreatment of lignocellulosic materials. The dilute acid pretreatment works fairly well on agricultural feedstocks, such as corn stover and rice wheat straw (Zhu *et al.*, 2008). In spite of these benefits, dilute sulfuric acid has some important disadvantages (Zheng *et al.*, 2009): (1) corrosion that mandates expensive materials of construction, (2) acidic prehydrolyzates must be neutralized before the sugars proceed to fermentation, (3) gypsum has problematic reverse solubility characteristics when neutralized with inexpensive calcium hydroxide, (4) formation of degradation products and release of natural biomass fermentation inhibitors are other characteristics of acid pretreatment, (5) disposal of neutralization salts is needed, and (6) biomass particle size reduction is necessary.

2.6.10 Ionic Liquid Pretreatment

Recently, a new interesting pretreatment method, which can enhance the sequential cellulose hydrolysis rate (and is more environmentally friendly), is solvent pretreatment using ionic liquids (ILs) to reduce the crystallinity of the cellulose (Liu and Chen, 2006; Zhao *et al.*, 2009). It was reported that 1-butyl-3-methylimidazolium chloride ([BMIM]Cl), one type of effective ILs, could dissolve up to 25 wt.% of the cellulose without any degradation products. The precipitation of [BMIM]Cl-dissolved cellulose could be achieved by adding water or alcohol. After the IL-treatment step, the cellulose structure was found to change from crystalline to amorphous, which resulted in a significant increase in the enzymatic hydrolysis rate and cellulose conversion (Swatloki *et al.*, 2002).

2.6.11 Biological Pretreatment

Biological pretreatment involves microorganisms such as brown-, white- and soft-rot fungi that are used to degrade lignin and solubilize hemicellulose. White-rot fungi are the most effective biological pretreatment of lignocellulosic materials (Taherzadeh and Karimi, 2008; Kumar *et al.*, 2009; Sun and Cheng, 2002). Lee *et al.* (2007) evaluated biological pretreatment of Japanese red pine (Pinus densiflora) using three white-rot fungi (Ceriporia lacerata, Stereum hirsutum, and Polyporus brumalis). pretreatment with S. hirsutum resulted in selective degradation of the lignin rather than the holocellulose component. The advantages of biological pretreatment include low energy requirement and mild environmental conditions. However, the rate of hydrolysis in most biological pretreatment process is very low (Sun and Cheng, 2002). Advantages and disadvantages of various pretreatment processes for lignocellulosic materials are summarized in Table 2.2.

Table 2.2 Advantages and disadvantages of various pretreatment processes for lignocellulosic materials (Kumar *et al.*, 2009)

Pretreatment process	Advantages	Limitations and disadvantages
Mechanical comminution	Reduces cellulose crystallinity	Power consumption usually higher than inherent biomass energy
Steam explosion	Causes hemicellulose degradation and lignin transformation; cost-effective	Destruction of a portion of the xylan fraction; incomplete disruption of the lignin-carbohydrate matrix; generation of compounds inhibitory to microorganisms
AFEX	Increases accessible surface area, removes lignin and hemicellulose to an extent; does not produce inhibitors for down-stream processes	Not efficient for biomass with high lignin content

Pretreatment process	Advantages	Limitations and disadvantages
CO2 explosion	Increases accessible surface area; cost-effective; does not cause formation of inhibitory compounds	Does not modify lignin or hemicelluloses
Ozonolysis	Reduces lignin content; does not produce toxic residues	Large amount of ozone required; expensive
Acid hydrolysis	Hydrolyzes hemicellulose to xylose and other sugars; alters lignin structure	High cost; equipment corrosion; formation of toxic substances
Alkaline hydrolysis	Removes hemicelluloses and lignin; increases accessible surface area	Long residence times required; irrecoverable salts formed and incorporated into biomass
Organosolv	Hydrolyzes lignin and hemicelluloses	Solvents need to be drained from the reactor, evaporated, condensed, and recycled; high cost
Pyrolysis	Produces gas and liquid products	High temperature; ash production

Pretreatment process	Advantages	Limitations and disadvantages
Pulsed electrical field	Ambient conditions; disrupts plant cells;	Process needs more research
Biological	Simple equipment degrades lignin and hemicelluloses; low energy requirements	Rate of hydrolysis is very low

2.7 Hydrolysis of Lignocellulosic Materials

The carbohydrate polymers in lignocellulosic materials need to be converted to simple sugars before fermentation, through a process called hydrolysis (Taherzadeh and Karimi, 2008). Various methods for the hydrolysis of lignocellulosic materials have recently been described. The most commonly applied methods can be classified in two groups: chemical hydrolysis (dilute and concentrated acid hydrolysis) and enzymatic hydrolysis. There are some other hydrolysis methods in which no chemicals or enzymes are applied. For instance, lignocelluloses maybe hydrolyzed by gamma-ray or electron-beam irradiation, or microwave irradiation. However, those processes are commercially unimportant. Several products can result from hydrolysis of lignocellulosic material (Demirbas, 2008).

2.7.1 Concentrated Acid Hydrolysis

This process involves an acid (dilute or concentrated) pretreatment to liberate the hemicellulosic sugars while the subsequent stage requires the biomass to be dried followed by the addition of concentrated sulfuric acid (70– 90%) (Hayes, 2009).The acid concentration used in concentrated acid hydrolysis process is in the range of 10–30% (Iranmahboob *et al.*, 2002). Reaction times are typically much

longer than for dilute acid process. This process provides a complete and rapid conversion of cellulose to glucose and hemicelluloses to five-carbon sugars with little degradation. The critical factors needed to make this process economically viable are to optimize sugar recovery and cost effectively recovers the acid for recycling (Demirbas, 2008).

2.7.2 Dilute Acid Hydrolysis

The dilute acid hydrolysis process is one of the oldest, simplest and most efficient methods of producing ethanol from biomass. Dilute acid is used to hydrolyze the biomass to sugars. The first stage uses 0.7% sulfuric acid at 190 °C to hydrolyze the hemicelluloses present in the biomass. The second stage is optimized to yield the more resistant cellulose fraction. This is achieved by using 0.4% sulfuric acid at 215 1C. The liquid hydrolates are then neutralized and toxic compounds are removed before fermentation of sugar solution to ethanol (Brennan *et al.*, 1986; Harris *et al.*, 1985).

2.7.3 Enzymatic Hydrolysis

Acid hydrolysis has a major disadvantage where the sugars are converted to degradation products like tars. This degradation can be prevented by using enzymes favoring 100% selective conversion of cellulose to glucose. When hydrolysis is catalyzed by such enzymes, the process is known as enzymatic hydrolysis (Pike *et al.*, 2008). Enzymatic degradation of cellulose is generally accomplished by synergetic action of three distinct classes of cellulose enzymes that are endo-1,4-β-glucanases, exdo-1,4-β-glucanases, and β–D-glucosidases (Worasamutparkarn, 2010). Enzymatic hydrolysis of natural lignocellulosic materials is a very slow process because cellulose hydrolysis is hindered by structural parameters of the substrate, such as lignin and hemicellulose content, surface area, and cellulose crystallinity (pan *et al.*, 2006). Utility cost of enzymatic hydrolysis is low compared to acid or alkaline hydrolysis because enzyme hydrolysis is usually conducted at mild conditions (pH 4.8) and temperature (318–323 K) and does not

have a corrosion problem (Sun and Cheng, 2002). The enzymatic hydrolysis has currently high yields (75–85%) and improvements are still projected (85–95%), as the research field is only a decade young (Hamelinck *et al.*, 2005). Comparison of process conditions and performance of three cellulose hydrolysis processes is given in Table 2.3. Enzymatic hydrolysis is an environmentally friendly alternative that involves using carbohydrate degrading enzymes (cellulases and hemicellulases) to hydrolyze lignocelluloses into fermentable sugars (Keshwani and Cheng, 2009).

Table 2.3 Comparison of process conditions and performance of three hydrolysis processes (Hamelinck *et al.*, 2005)

Process	Consumables	Temperature (K)	Time	Glucose yield (%)	Available
Dilute acid	<1% H2SO4	488	3 min	50–70	Now
Concentrated acid	30–70% H2SO4	313	2–6 h	90	Now
Enzymatic	Cellulase	323	1.5 days	75-95	Now-2020

2.8 Cellulase Enzymes

Microbial degradation of lignocellulosic waste and the downstream products resulting from it is accomplished by a concerted action of several enzymes, the most prominent of which are the cellulases. For microorganisms to hydrolyze and metabolize, insoluble cellulose, extracellular cellulases must be produced which are either free or cell associated. Three major types of cellulase activities are recognized (Lyn, 1996; and Sheehan, 1999).

(1) Endoglucanases (1,4-β-D-glucanohydrolases)

(2) Exoglucanases

(a) Cellodextrinases (1,4-β-D-glucan glucanohydrolases)

(b) Cellobiohydrolases (1-β-D-glucan cellobiohydrolases)

(3) β-Glucosidases (β-glucoside glucohydrolases)

Endoglucanases cut at random at internal amorphous sites in the cellulose polysaccharide chain generating oligosaccharides of various lengths and consequently shorter chains appear. Exoglucanases act in a processive manner on the reducing and non-reducing ends of the cellulose chains liberating either glucose (glucanohydrolases) or cellobiose (cellobiohydrolase) as major products. Exoglucanases can also act on microcrystalline cellulose peeling of cellulose chains from the microcrystalline structure (Sheehan, 1999). β-Glucosidases hydrolyze soluble cellodextrins and cellobiose to glucose. These three hydrolysis processes occur simultaneously as shown in Figure 2.6.Cellulase, which is secreted extracellularly by several microbes, including bacteria from higher termites (McKendry, 2002; and Anderson, 1977) has been widely investigated to hydrolyze cellulose.

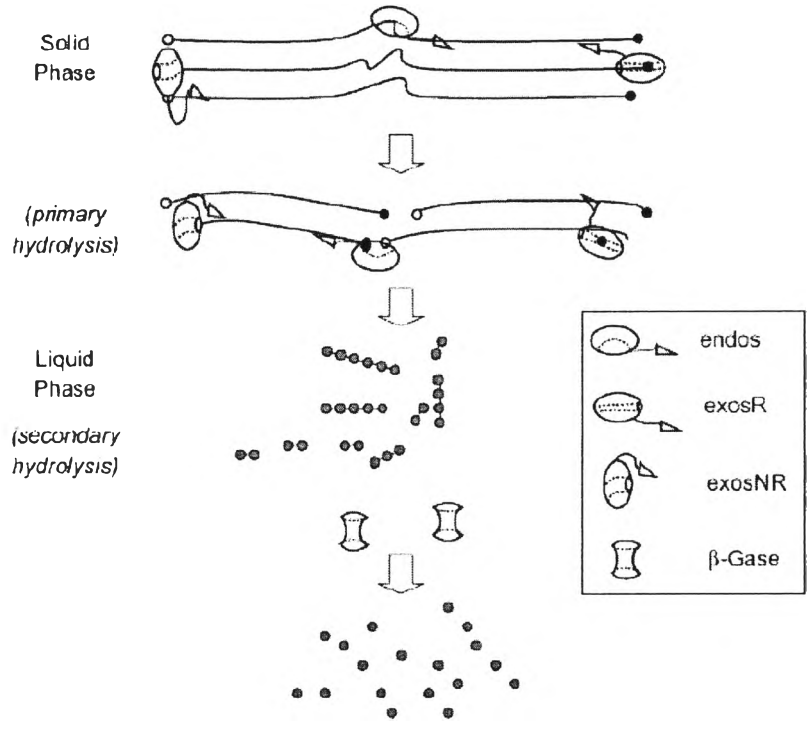


Figure2.6 Mechanistic scheme of enzymatic cellulose hydrolysis by *Trichoderma* non-complexed cellulase system (Zhang *et al.*, 2006).

There are different factors that affect the enzymatic hydrolysis of cellulose, namely, substrates, cellulase activity, reaction conditions (temperature, pH as well as other parameters), and a strong product inhibition. To improve the yield and rate of enzymatic hydrolysis, research has been focused on optimizing the hydrolysis process and enhancing the cellulase activity (Sun and Cheng, 2002). The rate of enzymatic hydrolysis of cellulose is dependent upon several structural features of the cellulose. The cellulose features known to affect the rate of hydrolysis include: (1) molecular structure of cellulose, (2) crystallinity of cellulose, (3) surface area of cellulose fiber, (4) degree of swelling of cellulose fiber, (5) degree of polymerization, and (6) associated lignin or other materials (Detry, 1982). A low substrate concentration gives low yield and rate, and a high cellulase dosage may increase the costs disproportional (Hamelinck *et al.*, 2005).

Ken-Lin Chang (2010) studied enzymatic conversion with freeze pretreatment of rice straw by cellulase from *Aspergillus niger* (Sigma Chemical Co.) and xylanase from *Trichoderma viride* (Sigma Chemical Co.) at -20°C. The freeze pretreatment was found to significantly increase the enzyme digestibility of rice straw from 48% to 84%. This indicated that freeze pretreatment was highly effective for enzymatic hydrolysis and low environmental impact. According to the results, enzymatic hydrolysis of untreated rice straw with 150 U cellulase and 100 U xylanase for 48 h yielded 226.77 g kg^{-1} and 93.84 g kg^{-1} substrate-reducing sugars respectively. However, the reducing sugar yields from freeze pretreatment under the same conditions were 417.27 g kg^{-1} and 138.77 g kg^{-1} substrate, respectively. In addition, hydrolyzates analysis showed that the highest glucose yield obtained during the enzymatic hydrolysis step in the present study was 371.91 g kg^{-1} of dry rice straw, following pretreatment.

Roslan (2011) investigated the production of bioethanal from rice straw using cellulase by local *Aspergillus* sp. The rice straw was pretreated by few cycles of wet disc milling prior saccharified it using the crude cellulase produced from rice straw by locally isolated *Aspergillus* sp. in solid state fermentation. This crude cellulase was measured to have activity 6.3 FPU g^{-1} rice straw. The saccharification released glucose from total cellulose more than 90%. Then, the saccharified product was subjected to fermentation by yeast. The highest bioethanol yield produced from the fermentation was 0.102 g g^{-1} rice straw which isequivalent to 62.1% of theoritical buthanol yield. This sugested that the use of crude cellulase from rice straw onto rice straw can lead to a good yield of bioethaol, provided an efective pretreatment was used.

Taechapoempol (2009) studied cellulase-producing bacteria from Thai higher termites *Microcerotermes* sp., under three different isolation conditions (aerobic anaerobic, or anaerobic/aerobic). He found that only three effective isolates of A 002, M 015, and F 018 out of forty-seven cellulase-producing bacteria isolated from the termites had the highest hydrolysis capacity value (HC value). Identification from the 16s rRNA gene sequencing method revealed that all of the effective isolates

were Bacillus subtilis. The cellulase activities (F phase, endoglucanase, and β-glucosidase) of A 002, M 015, and F 018 were also tested at 37°C and pH 7.2. The results showed that the isolate M 015 exhibited the highest endoglucanase activity whereas the isolate F 018 gave the highest FPase and β-glucosidase activities. The microbiological characteristics of the three effective isolates are summarized in Table 2.4. Furthermore, these effective isolates were tested for their toxic tolerance to [BMIM]Cl. All of the isolates were able to tolerate the [BMIM]Cl in the concentration range of 0.1 to 1.0 vol.%, and no growth retardation in the lag phases, except that the isolate A 002 had a growth retardation in the [BMIM]Cl concentration range of 0.5 to 1.0 vol.%, was observed.

Table 2.4 Characteristics of isolates A 002, M 015, and F 018 by microbiological methods (Taechapoempol, 2009)

Isolate	Colonial appearance	Pigmentation	Cell shape	Gram's staining	Spore forming	Oxidase test	Catalase test
A 002	Circular, flat, entire, rough, and membranous	Light brown cream	Rod	+	+	−	+
M 015	Spindle, raised, entire, glistering, and opaque.	Light brown cream	Rod	+	+	−	+

Isolate	Colonial appearance	Pigmentation	Cell shape	Gram's staining	Spore forming	Oxidase test	Catalase test
F 018	Spindle, flat, filamentous, glistering, and opaque	Light green cream	Rod	+	+	−	+

Worasamutprakarn (2010) investigated conversion of cellulose to glucose by using three effective isolates (strain A 002, M 015, and F 018), isolated from Thai higher termites Microcerotermes sp with [BMIM]Cl ionic liquid pretreatment. For the pretreatment step, it was found that [BMIM]Cl could be effectively used to decrease the crystallinity of cellulose chains with optimum conditions of 5:100 cellullose-to-[BMIM]Cl ratio and 100°C. The crystallinity of cellulose chains decreased about 90 % after the pretreatment. However, about 5 % of [BMIM]Cl was remained in the pretreated cellulose even after washing by deionized water. The results from enzymatic hydrolysis showed that strain F 018 produced the highest glucose concentration at 0.59 g/L after 4 h operation. In addition, using no.5 Whatman filter paper with high crystalline structure gave the lowest glucose concentration. While using the no. 1, 2, and 4 Whatman filter papers with lower crystallinity gave higher glucose concentration.

Eourarekullart (2011) investigated the enzymatic hydrolysis of corncob with two effective isolates (strain A 002 and M 015), from Thai higher termites, *Microcerotermes* sp. under different particle size of corncob (40 and 60 mesh) and hydrolysis temperature (30 and 37 °C). From the results, the glucose concentration from the strain A 02 was significantly higher than that from strain M 015 and both bacteria strains hydrolyzed corncob very well in the 65 modified DSMZ broths medium 2. The maximum amount of glucose which was 1.08 g/L can be obtained from the hydrolysis reaction with strain A 002 bacteria and 60 mesh size of corncob at 37°C.