CHAPTER II
THEORETICAL BACKGROUND AND LITERATURE REVIEW

2.1 Lignocellulosic Biomass

Lignocellulosic biomass refers to agricultural and forestry residues, municipal solid wastes, energy crops, solid animal wastes, and biowaste streams, which are composed of cellulose, hemicellulose, and lignin. As shown in Figure 2.1, the major fraction is cellulose (35-50%). The next largest fraction is hemicellulose (20-35%), and the third largest fraction is lignin (15-25%). The cellulose and hemicellulose, which typically comprise two thirds of the dry lignocellulosic materials, are polysaccharides. Both hemicellulose and lignin provide a protective sheath around the cellulose, so the utilization of the embedded polysaccharides can be accomplished by the hydrolysis step (Mosier *et al.*, 2005; Laser *et al.*, 2002; Lynd, 1996; Kuhad and Singh, 1997; Sun and Cheng, 2002).

Lignocellulosic biomass can improve energy security, reduce trade deficits, decrease urban air-pollution, and contribute little, if any, net carbon dioxide accumulation to the atmosphere (Wyman, 1994). Lignocellulosic biomass can also be converted to ethanol, which is a great alternative energy source and a safer alternative to methyl tertiary butyl ether (MTBE), the most common additive to gasoline used to provide cleaner combustion (McCarthy and Tiemann, 1998). Figure 2.2 shows the production of ethanol from lignocellulosic biomass. Ethanol from lignocellulosic biomass is produced mainly via biochemical routes. The three major steps involved are pretreatment, enzymatic hydrolysis, and fermentation. Biomass is pretreated to improve the accessibility of enzymes. After pretreatment, biomass undergoes enzymatic hydrolysis for conversion of polysaccharides into monomer sugars, such as glucose and xylose. Subsequently, sugars are fermented to ethanol by the use of different microorganisms (www.bioenergyconsult.com).

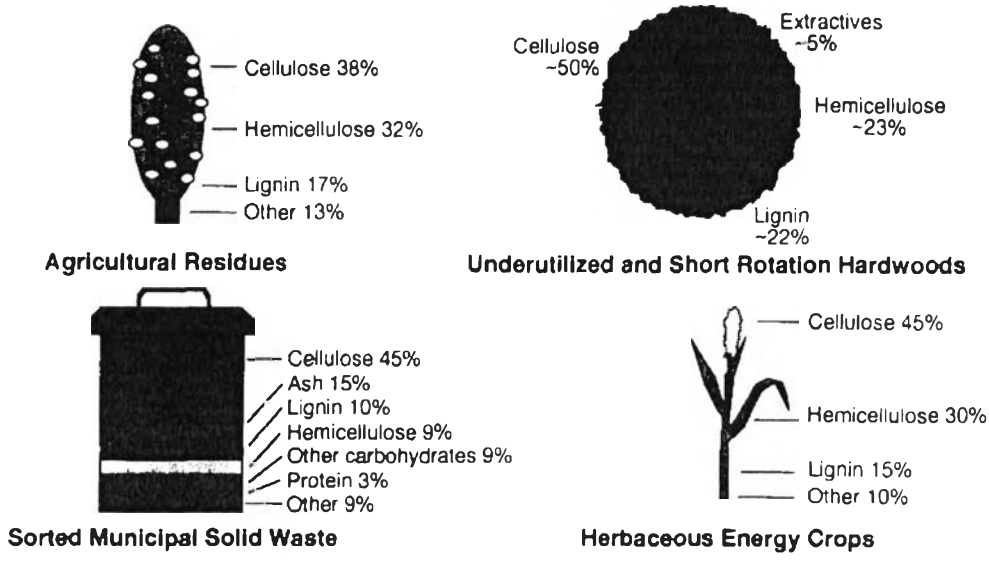


Figure 2.1 Various types of lignocellulosic biomass showing very similar composition with the major fraction being cellulose with a lesser amount of hemicellulose and lignin (Wyman, 1993; Wyman and Goodman, 1993a,b).

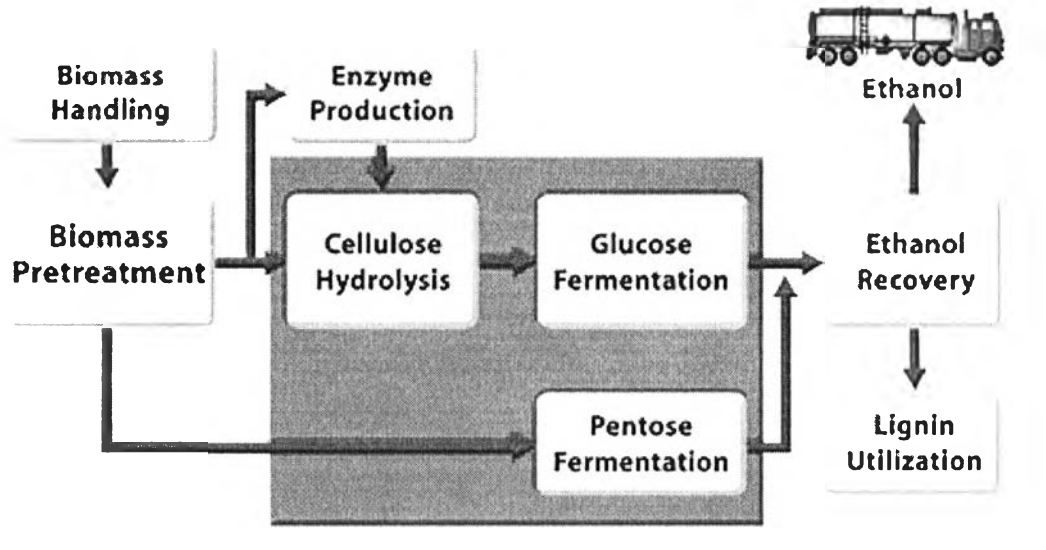


Figure 2.2 Production of ethanol from lignocellulosic biomass (www.ethanolrfa.org).

2.2 Chemical Structure and Basic Components of Lignocellulosic Materials

2.2.1 Cellulose

Cellulose is a linear polymer of glucose linked through α-1,4-linkages and is usually arranged in microcrystalline structures (Malherbe and Cloete, 2002). The repeating unit that has been established through the linkage and constitutes cellulose chain is called cellobiose. Cellulose in biomass is present in both crystalline and amorphous forms. Crystalline cellulose comprises the major proportion of cellulose, whereas a small percentage of unorganized cellulose chains form amorphous cellulose. Cellulose is more susceptible to enzymatic degradation in its amorphous form (Bĕguin and Aubert, 1994). Figure 2.3 shows structure of single cellulose molecule.

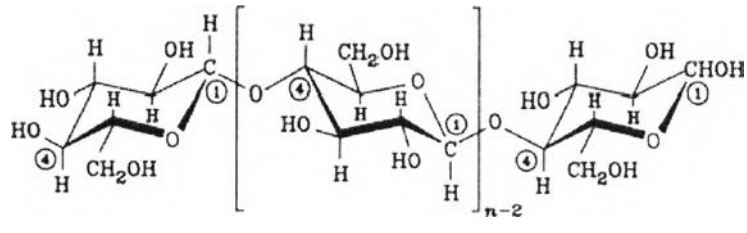


Figure 2.3 Structure of single cellulose molecule (Harmsen *et al.*, 2010).

2.2.2 Hemicellulose

Hemicellulose is a polysaccharide with a lower molecular weight than cellulose. It is formed from D-xylose, D-mannose, D-galactose, D-glucose, L-arabinose, 4-O-methyl-glucuronic, D-galacturonicand D-glucuronic acids as shown in Figure 2.4. Sugars are linked together by β-1,4- and sometimes by β-1,3-glycosidic bonds. The main difference between cellulose and hemicellulose is that hemicellulose has branches with short lateral chains consisting of different sugars and cellulose consists of easily hydrolysable oligomers (Murad and Azzaz, 2010). Hemicelluloses are classified according to the sugar that composes the backbone. In softwoods mannan (main sugar is mannose) is present, and in hardwoods and grasses is xylan (Jørgensen *et al.*, 2007; CCRC, 2007). Xylan has a backbone of 1,4-β-linked

xylose residues, several of which are acetylated (Kuhad *et al.*, 1997). Despite homoxylans (linear and unsubstituted) having been reported, the most common is to find heteroxylans, which possess small ramifications containing other sugars or acids. According to the abundance of the other residues, xylans are further classified into subgroups. Grasses are composed mainly of glucuronoarabinoxylans, having arabinose and glucuronic acid as the most abundant substituents (Carpita *et al.*, 2001; Saha, 2003).

Heteroxylans can crosslink with each other, adsorb to cellulose, bind to structural proteins and lignin, forming part of the matrix, in which cellulose is embedded. It forms a physical barrier to microbial and enzymatic attack on cellulose. Its acetyl content has the added effect of sterically hindering enzyme activity, interfering with substrate recognition. That applies not only to cellulose, but also to the enzymatic hydrolysis of hemicellulose itself (Chang and Holtzapple, 2000). Hemicellulose has weaker bonds than cellulose, which can be extensively broken using suitable kinds of pretreatments, such as dilute acid hydrolysis. Removing hemicellulose by these methods greatly facilitates subsequent cellulose digestion (Saha *et al.*, 2005).

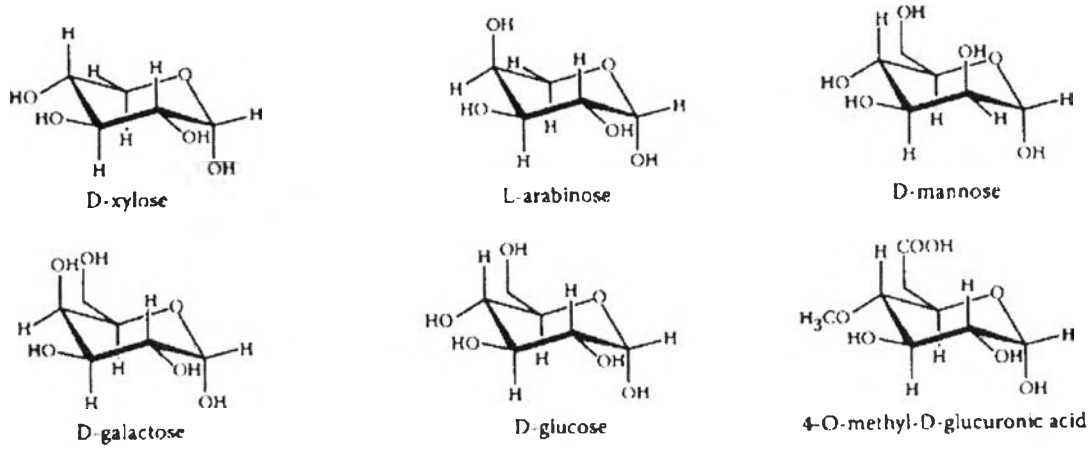
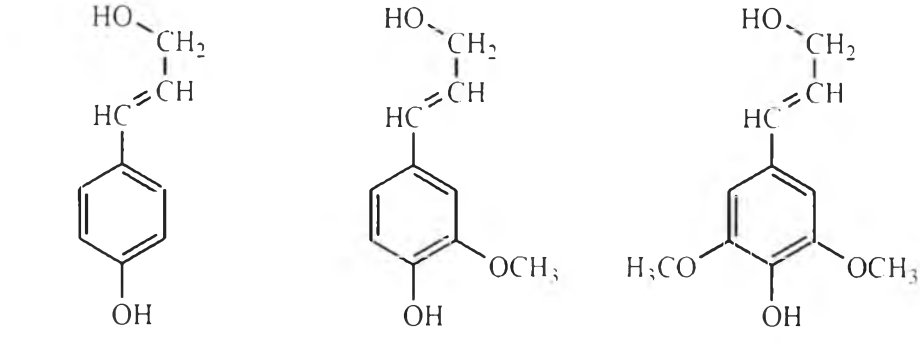


Figure 2.4 Schematic representation of hemicellulose structure. A, arabinose; FeA, ferulic acid; G, galactose; Glc, glucuronic acid; X, xylose (Mousdale, 2008).

2.2.3 Lignin

Lignin is a complex, large molecular structure containing cross-linked polymers of phenolic monomers, and it is an amorphous three-dimensional polymer with phenylpropane units as the predominant building blocks (Harmsen *et al.*, 2010). It is present in the primary cell wall, imparting structural support, impermeability, and resistance to microbial attack (Perez *et al.*, 2002). In general, herbaceous plants such as grasses have the lowest contents of lignin, whereas softwoods have the highest lignin contents (Kumar *et al.*, 2009). The presence of lignin in the biomass makes it more difficult for enzyme and acid accessibility to cellulose because of its highly ordered crystalline structure, thus the efficiency of hydrolysis process is lower.



p-Coumaryl alcohol Coniferyl alcohol Sinapyl alcohol

Figure 2.5 P-coumaryl- (1), coniferyl- (2) and sinapyl alcohol (3): dominant building blocks of the three-dimensional polymer lignin (Harmsen *et al.*, 2010).

2.3 Corncob

Corncob is a concentrated agricultural waste, which can be produced from corn industries and often causes environmental problems due to the lack of effective utilization (Chen *et al.*, 2007). Corncob approximately consists of 45% cellulose, 35% hemicellulose, and 15% lignin, as shown in Table 2.1. Due to its chemical properties and physical characteristics, corncob is suitable as a feedstock for energy generation (Saliu and Sani, 2012). Figure 2.6 shows the picture of corncobs. In addition, ethanol is one of the renewable energy sources, which can be produced by

the conversion of corncob to fermentable sugar. Crofcheck and Montross (2004) found a greater yield of glucose (i.e., ethanol) from corncob than other corn residues such as stalks or leaves plus husks.

Table 2.1 Cellulose, Hemicellulose, and Lignin contents in common agricultural residues and waste (Kumar *et al.*, 2009)

lignocellulosic material	cellulose(%)	hemicellulose(%)	lignin(%)
hardwood stems	40-50	24-40	18-25
softwood stems	45-50	25-35	25-35
nut shells	25-30	25-30	30-40
corn cobs	45	35	15
Grasses	25-40	35-50	10-30
Paper	85-99	0	0-15
wheat straw	30	50	15
sorted refuse	60	20	20
Leaves	15-20	80-85	0
cotton seed hairs	80-95	5-20	0
Newspaper	40-55	25-40	18-30
waste papers from chemical pulp	60-70	10-20	5-10
primary wastewater solids	8-15		
solid cattle manure	1.6-4.7	1.4-3.3	2.7-5.7
coastal bermudagrass	25	35.7	6.4
Switchgrass	45	31.4	12
swine waste	6	28	na

Figure 2.6 Corncob as a feed stock for ethanol production (www.replacingoil.com).

Chen *et al.* (2007) studied enzymatic hydrolysis of corncob and ethanol production from cellulosic hydrolysate. After corncob was protreated by 1% H_2SO_4 at 108 °C for 3 h, the cellulosic residue was hydrolyzed by cellulose from *Trichoderma reesei* ZU-02 and the hydrolysis yield was 67.5%. Due to the poor cellubiase activity in *T. reesei* cellulose, it leads to the restriction of the conversion of cellobiose to glucose and the accumulation of cellobiose caused severe feedback inhibition to the activities of β-1,4-endoglucanase and β-1,4-exoglucanase in cellulose system. Supplementation of cellobiase from *Aspergillus niger* ZU-07 greatly reduced the inhibitory effect caused by cellobiose, and the hydrolysis yield was improved to 83.9% with enhanced cellobiase activity of 6.5 CBU/g. Fed-batch hydrolysis process was started with a bath hydrolysis containing 100 g/L substrate, with adding cellulosic residue at 6 and 12 h twice to get a final substrate concentration of 200 g/L. After 60 h of reaction, the reducing sugar concentration reached 116.3 g/L with a hydrolysis yield of 79.5%. Further fermentation of cellulosic hydrolysate containing 95.3 g/L glucose was performed using *Saccharomyces cerevisiae* 316, and 45.7 g/L ethanol was obtained within 18 h. The research results are meaningful in fuel ethanol production from agricultural residue instead of grain starch.

2.4 Sugarcane Bagasse

Sugarcane bagasse (SCB) is one of many future feed stocks for ethanol production especially in tropical countries. This fibrous residue can be obtained after extracting juice from sugarcane. In general, about 1 ton of sugarcane generates 280 kg of bagasse or about 50% of this residue is used in distillery plants as a source of energy (Cardona *et al.*, 2009). Therefore, SCB becomes an interesting feedstock for an alternative energy source. Figure 2.7 shows the picture of crushed bagasse.

SCB consists of cellulose (40-45%), hemicellulose (30-35%), and lignin (20-30%) (Peng *et al.*, 2009). Bagasse also has low ash content so it offers numerous advantages compared with other agricultural residues such as paddy straw, rice straw, and wheat straw. Pretreatment of bagasse has often been found useful to improve its digestibility and easy access for microbial attack (Alani and Smith, 1988; Doran *et al.*, 1994). This helps to fractionate cellulose, hemicellulose, and lignin and also to open the cellulose structure.

Figure 2.7 Sugarcane bagasse (SCB) wastes (Loh *et al.*, 2013).

2.5 Pretreatment of Lignocellulosic Materials

Lignocellulosic materials have to be pretreated prior to hydrolysis to improve the accessibility of the biomass as shown in Figure 2.8. For this pretreatment, several processes are available: mechanical treatment for size reduction (e.g. chopping, milling, grinding), hydrothermal treatment (e.g. uncatalysed steam treatment with or without steam explosion, acid catalyzed steam treatment, liquid hot water treatment) and chemical treatment (e.g. dilute acid, concentrated acid, lime, NH_3, H_2O_2). Diverse advantages and drawbacks are associated with each pretreatment method (Mosier *et al.*, 2005; Hendriks and Zeeman, 2009; Chen and Qui, 2010; Telebnia *et al.*, 2010).

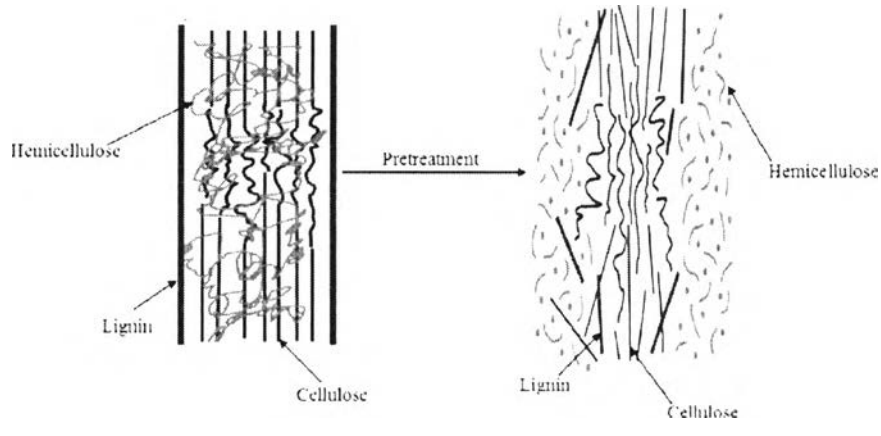


Figure 2.8 Schematic of the role of pretreatment in the conversion of biomass to fuel (Kumar *et al.*, 2009).

2.5.1 Physical Pretreatments

Physical pretreatments alter the physical structure of biomass, referring to reducing crystallinity, increasing pore size, and reducing degree of polymerization. In simpler term, the purpose of a pretreatment is to breakdown the lignocellulosic structure to its monosaccharide components for use as fermentation substrates. They can involve mechanical methods (milling), radiation or pyrolysis. One of the

advantages of physical pretreatment is that it is relatively insensitive to the physical and chemical characteristics of the biomass employed (Carvalho, 2009).

Comminuting of lignocellulosic materials through a combination of chipping, grinding, and/or milling can be applied in order to reduce cellulose crystallinity, making it easily access by cellulases. The final target size of the lignocellulosic materials is usually from 2 to 0.2 mm when physical pretreatment has been used. It is also thought that it disrupts lignin-carbohydrate complexes, which further aids the enzymatic hydrolysis (Mais *et al.*, 2002). One limitation of the physical pretreatments is the fact that it is unable to remove lignin. This restricts the access of enzymes and inhibits their activity, preventing the hydrolysis of cellulose from getting close to the theoretical values.

2.5.2 Chemical Pretreatments

2.5.2.1 Acid hydrolysis

Acid hydrolysis can be classified into two types: concentrated acid hydrolysis and dilute acid hydrolysis (prehydrolysis). Concentrated hydrolysis has been used to treat lignocellulosic materials by liberating the hemicellulosic sugars, while the subsequent stage requires the biomass to be dried followed by the addition of concentrated acid (70-90%) (Hayes, 2009). Although they are powerful agents for cellulose hydrolysis, concentrated acids are toxic, corrosive, hazardous, and require reactors that are resistant to corrosion (Sun and Cheng, 2002). In addition, the concentrated acid must be recovered after hydrolysis to make the process economically feasible (Sivers and Zacchi, 1995). Dilute acid hydrolysis has been successfully developed for pretreatment of lignocellulosic materials. This method can achieve high reaction rates and significantly improve cellulose hydrolysis (Esteghlalian *et al.*, 1997). There are two types of dilute acid pretreatment processes: high temperature (Temperature > 160 °C), continuous-flow process for low solid loading (5-10% weight of substrate/weight of reaction mixture) and low temperature (Temperature < 160 °C), batch process for high solid loading (10-40%) (Brennan *et al.*, 1986; Converse *et al.*, 1989; Cahela *et al.*, 1983; Esteghlalian *et al.*, 1997). High temperature in dilute acid treatment is favorable for cellulose hydrolysis (McMillan, 1994). Recently developed dilute acid hydrolysis processes use less

severe conditions and achieve high xylan to xylose conversion yields. Achieving high xylan to xylose conversion yields is necessary to achieve favorable overall process economics because xylan accounts for up to a third of the total carbohydrate in many lignocellulosic materials (Hinman *et al.*, 1992).

2.5.2.2 Alkaline hydrolysis

Alkaline hydrolysis is the process that uses alkaline solutions such as NaOH, $Ca(OH)_2$(lime) or ammonia to remove part of the lignin and hemicellulose, and also helps to improve the accessibility of enzyme to the cellulose depending on catalyst used. Alkaline pretreatment can be divided into two major groups: i) Pretreatments that use sodium, potassium, or calcium hydroxide; and ii) Pretreatments that use ammonia. The mechanism of alkaline hydrolysis is the saponification of intermolecular ester bonds crosslinking hemicellulose and lignin. The removal of these linkages substantially increases biomass porosity and causes cellulose swelling (Tarkow and Feist, 1969). Alkaline-based methods are more effective for lignin solubilization compared to acid or hydrothermal processes, except ammonia recycling percolation treatment, which yield biomass solids mostly containing cellulose (Florbela *et al.*, 2008). Furthermore, alkaline hydrolysis processes utilize lower temperatures and pressures than other pretreatment technologies (Mosier *et al.*, 2005) and this pretreatment method can be carried out at ambient conditions, but pretreatment times are on the order of hours or days rather than minutes or seconds. Compared with acid processes, alkaline processes cause less sugar degradation, and many of the caustic salts can be recovered and/or regenerated (Kumar *et al.*, 2009). However, the suitability of pretreatment technologies can differ from species to species of biomass (Kim, 2004).

2.5.3 Physico-Chemical Pretreatments

2.5.3.1 Steam explosion

Steam explosion was developed in 1925 by W.H. Mason for hardboard production (Mason, 1926). Since the use of the process has been exploded to include applications such as ruminant feed production and hardwood pulping. Several studies applying steam explosion for pretreatment of various biomass feedstocks. Schultz *et al.* (1984) compared the effectiveness of steam explosion pretreatment on mixed hardwood chips, rice hulls, corn stalks, and sugarcane bagasse.

Steam explosion at 240-250 °C and 1 min increased enzymatic hydrolysis rates of hardwood chips, rice hulls, and sugarcane bagasse to about the same rate as filter paper. The steam exploded samples showed no increase in acid hydrolysis rates as compared to untreated samples. The study also found no differences in hydrolysis rates for sample stored for 8 months prior to enzyme hydrolysis and samples exploded shortly before enzyme hydrolysis. Chornet and Overend (1988) describe steam explosion as being a thermomechanochemical process. The breakdown of structural components is aided by heat in the form of stem (thermo), shear forces due to the expansion of moisture (mechano), and hydrolysis of glycosidic bonds (chemical). In the reactor, steam under high pressure penetrates the lignocellulosic structures by diffusion. The steam condenses under the high pressure thereby "wetting" the material. The wet biomass is exploded when the pressure inside the reactor is suddenly released. Typically, the material is driven out of the reactor through a small nozzle by the induced force. Several phenomena occur at this point. First, the condensed moisture within the structure evaporates instantaneously due to the sudden decrease in pressure. The expansion of the water vapor exerts a shear force on the surrounding structure. If this shear force is high enough, the vapor will cause the mechanical breakdown of the lignocellulosic structure. The process description highlights the importance of optimizing the two governing factors: retention time, and temperature. Temperature governs the steam pressure within the reactor. Higher temperatures translate to higher pressures, therefore increasing the difference between reactor pressure and atmospheric pressure. The pressure difference is in turn proportional to the shear force of the evaporating moisture (Jeoh, 1998). The limitations of steam explosion include the formation of degradation products that may inhibit downstream processes (Aparicio *et al.*, 2006).

2.5.3.2 Ammonia fiber explosion (AFEX)

In the AFEX process, biomass is treated with liquid ammonia at high temperature and pressure (Teymouri *et al.*, 2005). The effective parameters in the AFEX process are ammonia loading, temperature, water loading, blowdown pressure, time and number of treatments. A typical AFEX process is carried out with 1-2 kg ammonia/kg dry biomass at 90 °C during 30 min. It reduces the lignin content and removes some hemicellulose while decrystallising cellulose. The cost of

ammonia and especially of ammonia recovery drives the cost of the pre-treatment (Holtzapple *et al.*, 1991), even though ammonia can be easily recovered due to its volatility.

2.5.3.3 Liquid hot water (LHW)

LHW pretreatment utilizes pressurized hot water at a pressure less than 5 MPa and in the temperature range of 170-230 °C for several minutes followed by decompression up to atmospheric pressure. Bagasse, corn stalk, straw of wheat, rice, and barley pretreated by LHW could achieve 80-100% conversion of hemicellulose to produce 45-65% xylose (Sun and Cheng, 2002; Cardona and Sánchez, 2007).

Table 2.2 Advantages and Disadvantages of various pretreatments ([a]Kumar *et al.*, 2009; [b]Dimian and Bildea, 2008)

Type	Pretreatment	Advantages	Disadvantages
Physical pretreatment	Mechanical comminution[a]	Improve the digestibility of biomass	Require exorbitant amount of energy
Physico-chemical pretreatment	Steam explosion[b] (autohydrolysis)	1. Low energy requirement compared to mechanical comminution 2. No recycling or environmental costs	1. Formation of inhibitory compounds 2. Destruction of xylan fraction 3. Incomplete disruption of the lignin-carbohydrate matrix
	Ammonia fiber explosion[b] (AFEX)	1. Significantly improve saccharification rates of various herbaceous crops 2. Not produce inhibitors for downstream biological process 3. Not require small particle size for efficacy	Not very effective for biomass with high lignin content
	CO_2 explosion[a]	1. More cost effective than ammonia fiber explosion 2. No formation of inhibitory Compounds	Low yield compared to steam or ammonia explosion

Table 2.2 (cont.) Advantages and Disadvantages of various pretreatments ([a]Kumar *et al.*, 2009; [b]Dimian and Bildea, 2008)

Chemical pretreatment	Ozonolysis[a]	1 Effectively remove lignin 2 Not produce toxic residues for the downstream process 3 Carry out at room temperature and pressure	Large amount of ozone required, making the process expensive
	Acid hydrolysis[b]	1 Achieve high xylan-to-xylose conversion yields (less severe conditions) 2 Significant improve cellulose hydrolysis	1 Higher cost than some physico-chemical pretreatment 2 Need neutralization of pH
	Alkaline hydrolysis[b]	1 Decrease degree of polymerization and crystallinity 2 Separation of structural linkages between lignin and carbohydrates 3 Disruption of lignin structure	No effect for soft woods with lignin content greater than 26%
Biological pretreatment[a]		1 Low energy requirement 2 Mild environmental conditions	Very low hydrolysis rate

2.6 Enzymatic Hydrolysis

Enzymatic hydrolysis of cellulose and hemicellulose can be carried out by highly specific cellulose and hemicellulase enzymes (glycosylhydrolases) (Rabinovich *et al.*, 2002a).

2.6.1 Enzymatic Hydrolysis of Cellulose

Enzymatic hydrolysis can be considered as a part of the biological pretreatments. Enzymatic hydrolysis of cellulose consists of cellulase enzyme adsorption onto the surface of the cellulose, biodegradation of cellulose to fermentable sugars, and desorption of the cellulase. Enzymatic degradation of cellulose to glucose is generally accomplished by synergistic action of at least three major classes of enzymes: endo-glucanases, exo-glucanases, and β-glucosidase. These enzymes are usually called together cellulase or cellulolytic enzymes and the products of the hydrolysis are usually reducing sugars including glucose as shown in Figure 2.9 (Wyman 1996; Duff and Murray, 1996). The reaction pathway shows the progression from the cellulose substrate to the glucose product through the activity of

three enzymes: endocellulase, exocellulase and β-glucosidase. The endocellulase and exocellulase enzymes incrementally cleave cellobiose molecules off of the long chain polysaccharide molecules. This cellobiose intermediate then becomes the substrate in the hydrolysis reaction with β-glucosidase, producing the final glucose product (Demers *et al.*, 2009).

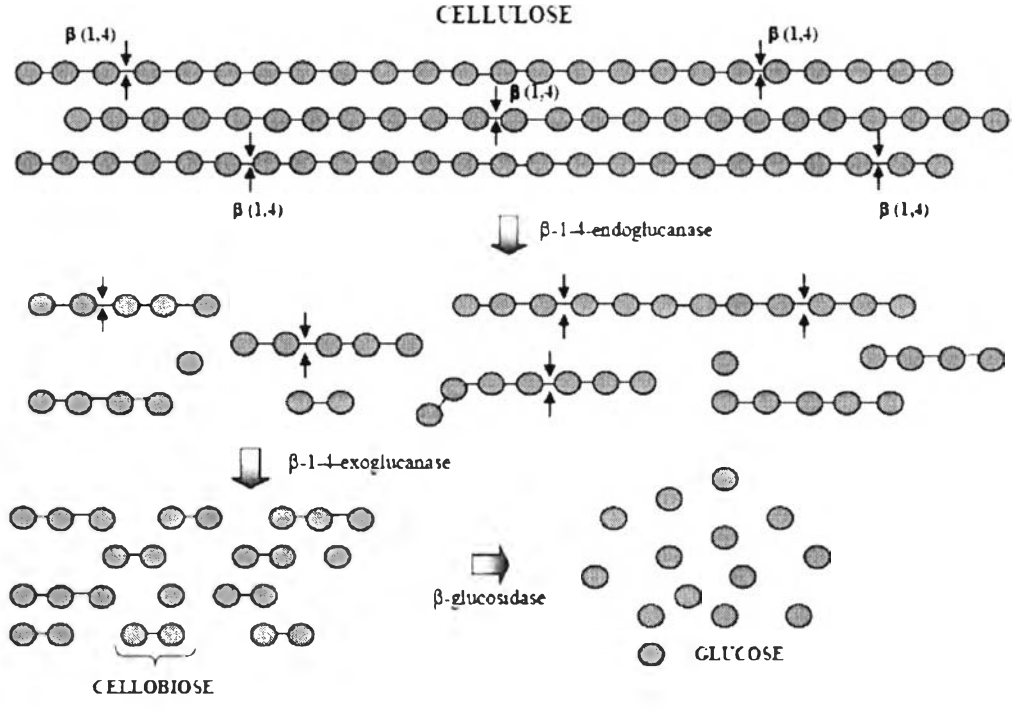


Figure 2.9 Reaction pathways from cellulose to glucose (Mussatto and Teixeira, 2010)

The hydrolysis is performed under mild conditions (e.g. pH 4.5-5.0 and temperature 40-50 °C). Therefore, one may expect low corrosion problems, low utility consumption, and low toxicity of the hydrolysates as the main advantages of this process (Taherzadeh and Karimi, 2008).

2.6.2 Enzymatic Hydrolysis of Hemicelluloses

Although similar types of enzymes are required for hemicellulose hydrolysis, more enzymes are required for its complicated degradation because of its greater complexity compared to cellulose. Therefore, xylanase is the best studied (Kuhad *et al.*, 1997). If hemicellulose fraction is not used effectively, it adds

substantial costs for waste disposal to the process and results in significant loss potential revenue from a fraction of the feedstock, which was paid for (Wyman, 1994).

2.7 Cellulase Enzymes

Cellulases have many applications in various industries such as chemicals, fuel, food, wine, animal feed, textile, laundry, pulp and paper, and agriculture. Cellulase enzyme can be produced by several organisms, mostly by fungi and bacteria, including aerobes, anaerobes, mesophiles, thermophiles, and extremophiles (Bahkali, 1996; Magnelli and Forchiassin, 1999; Shin *et al.*, 2000; Immanuel *et al.*, 2006). Aerobic fungi and bacteria generally produce extracellular cellulases. Bacterial cellulases are constitutively produced, whereas fungal cellulase is produced only in the presence of cellulose (Suto and Tomito, 2001).

However, the enzymatic hydrolysis rate of the cellulose is relatively low because of its high crystallinity (Taechapempol *et al.*, 2011). Most animals cannot hydrolyze cellulose because they do not have cellulolytic enzymes. By contrast, termites can efficiently hydrolyze cellulose with the aid of intestinal microbiota (Breznak and Brune, 1994).

2.8 Cellulose Hydrolysis with Enzyme from Termites

Taechapoempol *et al.* (2011) studied cellulase-producing bacteria from Thai higher termites, *Microcerotermes* sp.: enzymatic activities and ionic liquid tolerance. The three highest hydrolysis-capacity-value isolates of *Bacillus Subtilis* (A 002, M 015, F 018) obtained from Thai higher termites, *Microcerotermes* sp., under different isolation conditions (aerobic, anaerobic, and anaerobic/aerobic) that possessed the highest HC value were tested comparatively for cellulose activities (FPase, endoglucanase, and β-glocosidase) at 37 ^{o}C and pH 7.2 for 24 h. Their tolerance to an ionic liquid, 1-butyl-3-methylimidazaolium chloride ([BMIM]Cl), was also investigated. All the effective isolates were identified as *B. subtilis* by the 16S rRNA gene sequencing method. The results showed that the isolate M 015

provided the highest endoglucanase activity and the isolate F 018 provided the highest FPase and β-glocosidase activities. The characteristics of isolates A 002, M 015, and F 018 by microbiological methods were shown in Table 2.3. In addition, 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 was observed, except that the isolate A 002 had a growth retardation in the [BMIM]Cl concentration range of 0.5 to 1.0 vol%. Therefore, the cellulose-degrading ability of these isolates can potentially be used as a pretreatment step for alcohol fermentation.

Table 2.3 Characteristics of isolates A 002, M 015, and F 018 by microbiological methods (Taechapoempol *et al.*, 2011)

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	+	+	–	+
F 018	Spindle, flat, filamentous, glistering, and opaque	Light green cream	Rod	+	+	–	+

Wongskeo *et al.* (2012) studied production of glucose from the hydrolysis of cassava residue using bacteria isolates from Thai higher termites. They studied the possibility of using cassava residue consists of 49.66% starch, 21.47% cellulose, 12.97% hemicellulose, and 21.86% lignin as a raw material to produce glucose using enzymatic hydrolysis. In this research, the two effective isolates (strain A 002 and strain M 015), *Mircorerotermes* sp., from Thai higher termites were used to determine their hydrolysis activity of cassava residue at 37 °C. The effects of particles size (40 mesh and 60 mesh) were also studied. The results showed that the optimum condition for maximum glucose concentration was 1.51 g/L at 10 h using 60 mesh sizes of cassava residue and strain A002 as a bacteria cell at 37 ^{o}C.

Eourarekullart (2011) studied conversion of corncob to sugars by microbial hydrolysis. They investigated the effects of particle size of corncob (40 and 60 mesh), hydrolysis temperature (30 °C and 37 °C), hydrolysis time, and strains of bacteria isolated from Thai higher termites (strain A 002 and M 015). In the experiments, 1.6 g of corncob obtained from River Kwai International Food Industry Co., Ltd., 4-7 g of bacteria cells, and 1 L of production medium were used. Cellulose, hemicellulose, lignin, and extractive contents of the raw corncob were 47.37%, 31.26%, 17.06%, and 3.32 % respectively. The result showed that the maximum amount of glucose from the hydrolysis reaction was 1.08 g/L, which can be obtained from the bacteria stain A002 with 60 mesh corncob sizes at 37 °C.

Worasamutprakarn (2010) studied the conversion of cellulose to glucose by microbes isolated from higher termites. Due to the high crystallinity of cellulose, a pretreatment step is required in order to reduce the crystallinity and increase the accessibility of cellulose. An ionic liquid, [BMIM]Cl, was used in the experiment. The effects of cellulose-to-[BMIM]Cl ratio, temperature, and time on the dissolution of cellulose were studied. For the pretreatment step, it was found that [BMIM]Cl can be used to reduce the crystallinity of cellulose with the optimum condition at the 5:100 cellulose-to-[BMIM]Cl ratio, and the crystallinity of cellulose was decreased about 90% after treating with ionic liquid at 100 °C, although the ionic liquid still remained in the pretreated cellulose at about 5% after washing by deionized water. For the enzymatic hydrolysis step, the three effective isolates (strain A 002, M 015, and F018) from Thai higher termites, *Microcerotermes* sp. on the glucose production from cellulose were also investigated. The results showed that strain F 018 provided the highest glucose concentration from the pretreated cellulose about 0.59 g/L at 4 h and the glucose concentration from the untreated cellulose was lower than the pretreated cellulose. In addition, the structures of cellulose in the enzymatic hydrolysis were investigated by using no.5 Whatman filter paper. The result showed that the hydrolysis of No.5 Whatman filter paper resulted in the lowest glucose concentration, while using No. 1, 2, and 4 Whatman filter papers showed the higher glucose concentration.

Hokittikul (2013) studied glucose production from corncob by microbial hydrolysis. The microbial hydrolysis of corncob to glucose by using cellulase-producing bacteria isolated from Thai higher termites, *Microcerotermes* sp. Corncob consisted of 45.73% cellulose, 42.66% hemicellulose, and 7.99% lignin. In the experiments, each reactor contained corncob particles, bacteria cells, and production medium. The effects of particle size (40 mesh, 60 mesh, 80 mesh), temperature (30 °C and 37 °C), and strains of bacteria (A 002 and M 015) on the glucose concentration were also studied. Furthermore, glucose production using the isolated bacteria compared with that using commercial enzyme. From the preliminary result, the optimum condition of microbial hydrolysis using the bacterial isolates from Thai higher termites was found to be 60 mesh of corncob particle, with stain A 002 at 37 °C.