CHAPTER II
THEORETICAL BACKGROUND AND LITERATURE REVIEW

2.1 Lignocellulosic-Biomass Materials

Biomass is interested as alternative, nonpetroleum-based sources of energy. It is the only suitable and renewable primary energy resource that can provide alternative transportation fuels such as bioethanol or biodiesel in the short-term (Hamelinck *et al.*, 2005; Sun and Cheng, 2002).

Lignocellulosic materials serve as a cheap and abundant feedstock, which are required to produce fuel bioethanol at reasonable costs since lignocellulosic raw materials do not compete with food crops and they are also less expensive than conventional agricultural feedstocks (Balat, 2010). Lignocellulosic materials for fuel ethanol production can be divided into six main groups: crop residues (cane bagasse, corn stover, wheat straw, rice straw, rice hulls, barley straw, sweet sorghum bagasse, olive stones and pulp), hardwood (aspen and poplar), softwood (pine and spruce), cellulose wastes (newsprint, waste office paper and recycled paper sludge), herbaceous biomass (alfalfa hay, switchgrass, reed canary grass, coastal Bermudagrass and thimothy grass), and municipal solid wastes (MSW) (Cardona *et al.*, 2009). 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

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). Cellulose and hemicelluloses are polysaccharides that can be hydrolyzed to sugars and then fermented to bioethanol. Process of bioethanol yield from biomass is directly related to cellulose, hemicellulose, and individual sugar concentration in the feedstock. However, the lignin cannot be used for bioethanol production.

2.2.1 Cellulose

Cellulose, the major component of plant biomass (30–60 % of total feedstock dry matter), exists of D-glucose subunits, linked by β-1,4 glycosidic bonds. The cellulose in a plant consists of parts with a crystalline (organized) structure, and parts with a not well-organized, amorphous structure. Because of the β-1,4 linkage, cellulose is highly crystalline and compact making it very resistant to biological attack. The orientation of the linkages and additional hydrogen bonding make the polymer rigid and difficult to break. In hydrolysis the polysaccharide is broken down to free sugar molecules by the addition of water (Hamelinck *et al.*, 2005). This process is called saccharification which glucose, a six-carbon sugar is a product.

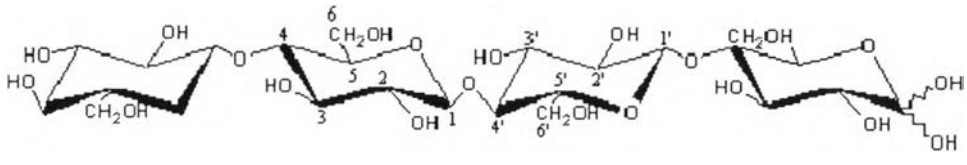

Figure 2.1 Schematic representation of a cellulose chain (Pérez and Mackie, 2001).

2.2.2 Hemicellulose

Hemicellulose (20–40 % of total feedstock dry matter) is a short, highly branched polymer of five-carbon (pentoses) and six-carbon (hexoses) sugars as shown in figure 2.2. Specifically, hemicellulose contains xylose and arabinose (five-carbon sugars) and galactose, glucose, and mannose (six-carbon sugars). Hemicellulose has a lower molecular weight than cellulose, and branches with short lateral chains that consist of different sugars, which are easy hydrolyzable polymers. Hemicellulose serves as a connection between the lignin and the cellulose fibers and gives the whole cellulose–hemicellulose–lignin network more rigidity. The dominant component of hemicellulose is glucomannan in softwood and xylan in hardwood and agricultural plants (Balat, 2010).

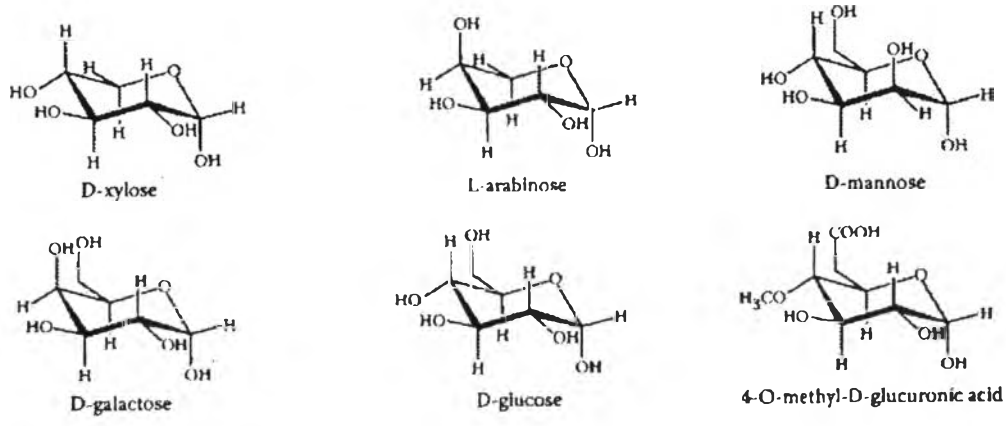

Figure 2.2 Schematic of the basic structure of hemicellulose. A, arabinose; FeA, ferulic acid; G, galactose; Glc, glucuronic acid; X, xylose (Mousdale, 2008).

2.2.3 Lignin

Lignin (15–25 % of total feedstock dry matter) is an amorphous heteropolymer consisting of three different phenylpropane units (p-coumaryl, coniferyl and sinapyl alcohol) as shown in figure 3. These are held together by different kind of linkages. Softwoods generally contain more lignin than hardwoods (Demirbas, 2008). The main purpose of having lignin is to give the plant structural support, impermeability, and resistance against microbial attack and oxidative stress. All of these make the degradation of lignin very tough. However, lignin is one of the drawbacks of using lingocellulosic-biomass materials in fermentation since it makes lignocellulose resistant to chemical and biological degradation.

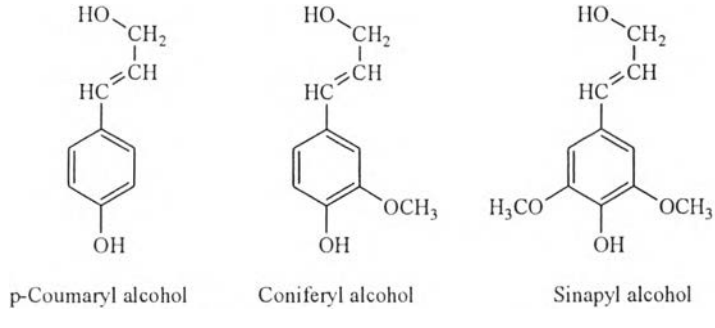

Figure 2.3 Lignin building blocks.

2.3 Sugarcane bagasse

Sugarcane bagasse (SCB) is considered to be one of the major lignocellulosic materials found in many tropical countries including Thailand. It is primarily composed of lignin (20–30 %), cellulose (40–45 %) and hemicelluloses (30–35 %). SCB is the fibrous residue left after extracting the juice from sugar cane (Saccharum officinarum) in the sugar production process (Martín *et al.*, 2007). Besides its use as a fuel for heating and power generation, its abundance and high cellulosic polysaccharide content make it suitable for ethanol production. However, like other lignocellulosic substrates, the use of bagasse as feedstock for biorefinery has been limited because the chemical structure and high pentose fraction of bagasse make it recalcitrant to enzymatic hydrolysis unless it is pretreated to a more accessible form (Buaban *et al.*, 2010).

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).

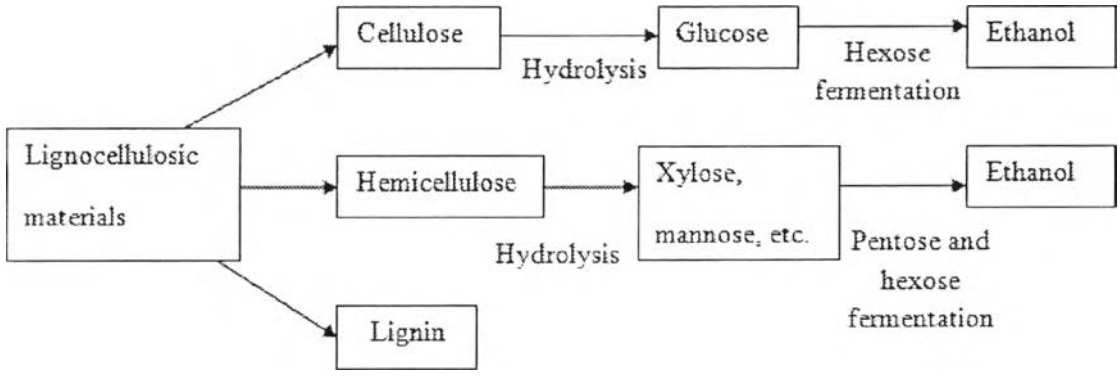

Figure 2.4 Overall view of sugar and ethanol productions from lignocellulosic materials (Eourarekullart, 2011).

2.6 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).

2.6.1 Dilute Acid Hydrolysis

Dilute acid hydrolysis is the oldest method for converting cellulose biomass to bioethanol. This process is conducted under high temperature and pressure, and has a reaction time in the range of seconds or minutes, which facilitates continuous processing. The combination of acid and high temperature and pressure makes the reactor expensive. Dilute acid hydrolysis occurs in two-stages. The first stage is performed at low temperature to maximize the yield from the hemicellulose, and the second higher temperature stage is optimized for hydrolysis of the cellulose portion of the feedstock. The primary challenge for dilute acid hydrolysis processes is how to raise glucose yields higher than 70 % in an economically viable industrial process while maintaining a high cellulose hydrolysis rate and minimizing glucose decomposition (Balat, 2010).

2.6.2 Concentrated Acid Hydrolysis

Concentrated acid hydrolysis 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, 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.

In comparison to dilute acid hydrolysis, concentrated acid hydrolysis leads to little sugar degradation and gives sugar yields approaching 100 %. The concentrated acid process offers more potential for cost reductions than the dilute acid process. However, environment and corrosion problems and the high cost of

acid consumption and recovery present a major hindrance to economic success (Balat, 2010).

2.6.3 Enzymatic Hydrolysis

Enzymatic hydrolysis is an environmentally friendly alternative that involves using carbohydrate degrading enzymes (cellulases and hemicellulases) to hydrolyze lignocelluloses into fermentable sugars. 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. The 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)

2.7 Cellulase Enzymes

Cellulase is a group of enzymes that synergistically hydrolyzes cellulose as shown in figure 2.5 (Kim, 2004). The widely accepted mechanism for enzymatic cellulose hydrolysis involves synergistic actions by endoglucanses (EG, endo-1,4-ß-D-glucanases, or EC 3.2.1.3.), exoglucanases or cellobiohydrolases (CBH, 1,4-ß-D-glucan cellobiohydrolases, or EC 3.2.1.91.), and ß-glucosidases (BGL, cellobiases or EC 3.2.1.21). EG hydrolyze accessible intramolecular ß -1,4-glucosidic bonds of cellulose chains randomly to produce new chain ends; CBH processively cleave cellulose chains at the ends to release soluble cellobiose or glucose; and BGL hydrolyze cellobiose to glucose in order to eliminate cellobiose inhibition (Zhang *et al.*, 2006). BGL complete the hydrolysis process by catalyzing the hydrolysis of cellobiose to glucose. Cellulase can be secreted extracellularly by several microbes, including bacteria from higher termites (McKendry, 2002; and Anderson, 1977) which has been widely investigated to hydrolyze cellulose.

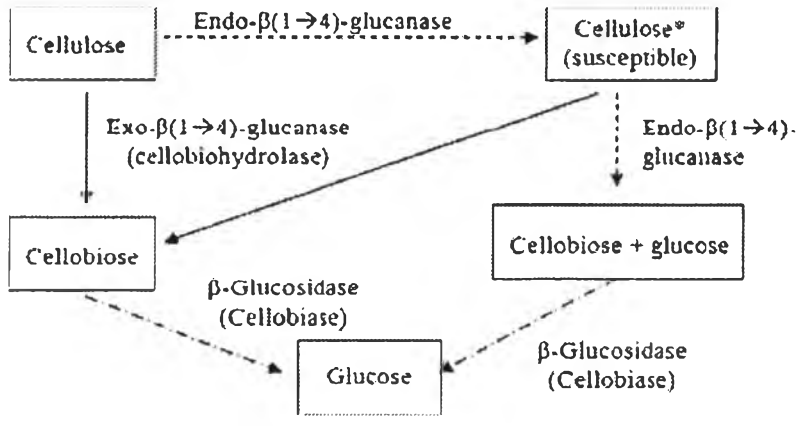

Figure 2.5 Mode of action of cellulolytic enzymes (Kim, 2004).

Ming Chen, Liming Xia, and Peijian Xue (2006) investigated the enzymatic hydrolysis of corncob and ethanol production from cellulosic hydrolysate. It was found that a high amount of cellobiose existed in the cellulosic hydrolysate, indicating poor cellobiase activity in T. reesei ZU-02 cellulase. Moreover, the accumulation of cellobiose caused severe feedback inhibition to the activities of β-1,4-endoglucanase and β-1,4-exoglucanase in cellulase, resulting in low hydrolysis yield (67.5%). Adding 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. Furthermore, fed batch hydrolysis process was also studied. They found that reducing sugar concentration reached 116.3 g $l^{-1}$with hydrolysis yields of 79.5%. Fermentation of cellulosic hydrolysate containing 95.3 g l^{-1} glucose was performed using Saccharomyces cerevisiae 316, and 45.7 g l^{-1} ethanol was obtained within 18 h.

Buaban *et al.* (2010) investigated bioethanol production from ball milled bagasse using an on-site produced fungal enzyme cocktail and xylose-fermenting Pichiastipitis. They found that ball milling for 2 h was sufficient for nearly complete transformation of cellulose structure to an accessible amorphous form. The pretreated cellulosic residues were then hydrolyzed by a crude enzyme preparation from Penicillium chrysogenum BCC4 504 containing cellulase activity combined with preparation from Aspergillus flavus BCC7179 containing complementary β-

glucosidase activity. From the result, the combination of enzyme was shown to hydrolyze pretreated bagasse efficiently. The high conversion yields of pretreated bagasse hydrolysate to ethanol were attained by separate hydrolysis and fermentation processes (SHF) and simultaneous saccharification and fermentation process (SSF) using Pichiastipitis BCC15191. From the result, ethanol concentration reached 8.4 g/l for SHF and 8.0 g/l for SSF process which show the high efficiency of this developed integrated process.

Taechapoempol (2009) investigated cellulase-producing bacteria from Thai higher termites *Microcerotermes* sp., under three different isolation conditions (aerobic anaerobic, or anaerobic/aerobic). 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. Cellulase activities (FPase, endoglucanase, and β-glucosidase) were tested at 37 °C and pH 7.2 for 24 h. 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 2.1. 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.

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 can be used to reduce 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 at 4 h. Moreover, the enzymatic hydrolysis using the mixed

strain A 002 and M 015 was also studied. It was found that the mixed strain A 002 and M 015 had an adverse effect on the glucose concentration resulting in the lower glucose concentration than that from strain A 002 and strain M 015. In addition, using no.5 Whatman filter paper structure gave the lowest glucose concentration due to the higher crystalline. While using the no. 1, 2, and 4 Whatman filter papers gave higher glucose concentration due to the lower crystalline.

Table 2.1 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	+	+	−	+
F 018	Spindle, flat, filamentous, glistering, and opaque	Light green cream	Rod	+	+	−	+

Eourarekullart (2011) investigated the conversion of corncob to sugars by enzymatic hydrolysis 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). He found that, the maximum glucose concentration of 1.08 g/l was obtained from A 002 which gave higher glucose concentration than that from strain M 015 and the optimum conditions were determined to be 37 °C, 60 mesh, and strain A 002.