CHAPTER II

LITERATURE REVIEW

2.1 Biofuel Production

Biofuels are fuel derived from biomass which is substance based on biological and/or organic matter. There are several types of biomass including: wood, herbaceous plant, agricultural wastes, and forestry wastes that can produce biofuels (Wyman *et al.*, 2005). Biofuels have advantages over petroleum and coal fuel because it is recyclable and renewable energy. In addition, there are various states of fuel including solid, liquid, and gas. This advantage allows biofuels to be applied in many purposes of use. Moreover, emission of carbon dioxide is relatively less than that of conventional petroleum fuels. Nowadays, biofuels are utilized only 15% share of energy consumption, which make many researchers attempt to study more on this topic. According to oil price spikes, the higher demand on energy security and concern of greenhouse gas emissions from fossil fuels, biofuels are gaining public and scientific attention (http://www.vcharkarn.com/varticle/374#P1).

Ethanol is a biofuel-additive for gasoline. It is produced from lignocellulosic biomass. There have two reasons indicated that ethanol is attracted interest as an alternative liquid fuel especially for transportation. First, the oil crisis stressed the dependence on the petroleum supply. The petroleum shortage can be reduced by the use of alternative fuels from renewable resources like ethanol, which is produced by lignocellulosic biomass. Second, ethanol production process uses clean energy from renewable resources, which not accumulates net carbon dioxide added in the atmosphere. Additionally, the exhaust emissions from ethanol process and toxic compounds of ethanol are lower than those from petroleum (Olsson and Hahn-hagerdal, 1996). Ethanol is always mixed with gasoline at the rates of 5%, 10% and 85%. A total of 85% ethanol can be used in specific engines whereas mixing 5% and 10% can be used without any engine modifications (Wyman *et al.*, 1994). Bioethanol is a high octane number biofuels, which is produced from fermentation of corn, potatoes, grain (wheat, barley and rye), sugar beet, sugar cane, and vegetable residues (Icoz *et al.*, 2009).

Butanol is another type of gasoline additive. Biobutanol is expected to play a major role in next-generation of biofuels, considering its many advantages. The butanol's advantages are: (1) content closer energy to that of gasoline than ethanol so consumers face less of a compromise on future fuel demand; (2) used in higher blend concentrations than ethanol without requiring specially adapted vehicles. There is the potential in the future to increase the maximum allowable use in gasoline up to a 16% volume; (3) easily added to conventional gasoline, due to its low vapor pressure; (4) co-blend synergy of vapor pressure with biobutanol and gasoline containing ethanol, which facilitates ethanol blending (http://www.bp.com). Table 2.1 shows some specifications of ethanol, butanol, and gasoline (http://www.epa.gov).

Table 2.1 Specifications of ethanol, butanol, and gasoline
(http://www.epa.gov/air/caaac/mstrs/March2007/Wolf.pdf)

	Ethanol	1-Butanol	Gasoline
Sp. Gravity, 60/60 F	0.794	0.814	0.720-0.775
Heating Value(MJ/l)	21.1-21.7	26.9-27.0	32.2-32.9
RON	106-130	94	95
MON	89-103	80-81	85
Rvp@5%/10%(psi)	31/20	6.4/6.4	<7.8/15
Oxygen(%wt)	34.7	21.6	<2.7

Biofuels can be produced from various methods. There are two main platforms used to produce biofuels from biomass, which are gasification and carbohydrate platform. However, in this work concentrates on the carbohydrate platform which using lignocellulosic material as a feedstock. Biofuels production from lignocellulosic material consists of four mainly steps. Prehydrolysis or

pretreatment converts some carbohydrate polymers into fermentable sugars and improves cellulase enzyme accessibility in hydrolysis step. For pretreated hemicellulose, it is ready to be fermented to produce ABE (acetone, butanol, and ethanol) because fermentable sugars such as five carbon sugars like xylose, rhamnose, and arabinose and six carbon sugars like glucose, mannose, and galactose are gained right after pretreatment process. The hydrolysis process can be significantly improved by removal of lignin and hemicellulose, reduction of cellulose crystallinity, and increase of porosity through pretreatment processes. Hydrolyzed cellulose and hemicellulose were fermented to biofuels like acetone, ethanol, and butanol by using an anaerobic bacterium in fermentation step. Then the products were sent to biofuels separation step in order to separate the desired product from others (as shown in Figure 2.1). The factors that affect the hydrolysis step of cellulose including porosity of the lignocellulosic biomass, cellulose fiber crystallinity and hemicellulose, and lignin content (McMillan, 1994). The composition of cellulose, hemicellulose, and lignin in common agricultural residues are listed in Table 2.2 (Sun and Cheng, 2002).
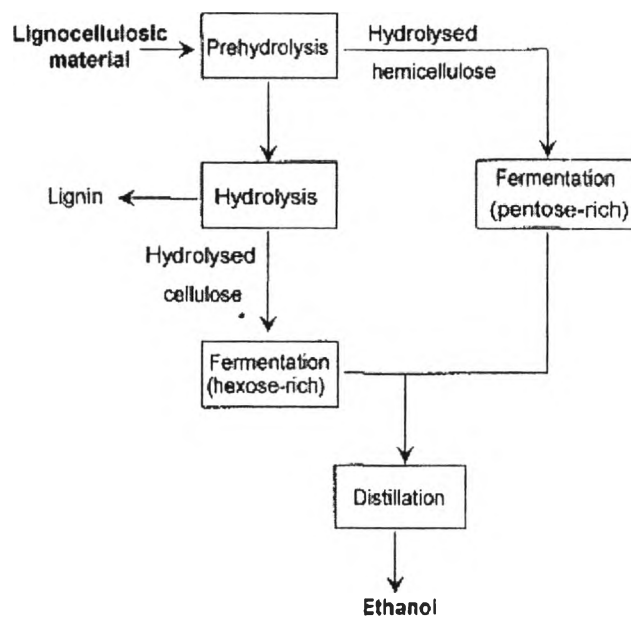

Figure 2.1 General flowchart for ethanol production from lignocellulosic material (Olsson and Hahn-hagerdal, 1996).

Table 2.2 Contents of cellulose, hemicelluloses, and lignin in common agricultural residues and wastes (Sun and Cheng, 2002)

Lignocellulosic materials	Cellulose (%)	Hemicellulose (%)	Lignin (%)
Hardwoods stems	40-55	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 pulps	60-70	10-20	5-10
Primary wastewater solids	8-15	NA	24-29
Swine waste	6.0	28	NA
Solid cattle manure	1.6-4.7	1.4-3.3	2.7-5.7
Coastal Bermuda grass	25	35.7	6.4
Switch grass	45	31.4	12.0

2.2 Composition of Lignocellulosic Biomass

Wood, grass, forestry waste, agricultural residues, and municipal solid waste are lignocellulosic biomass. They consist of three major components: cellulose (36–61%), hemicellulose (13–39%) and lignin (6–28%), as shown in Figure 2.2 (Olsson and Hahn-hagerdal, 1996).
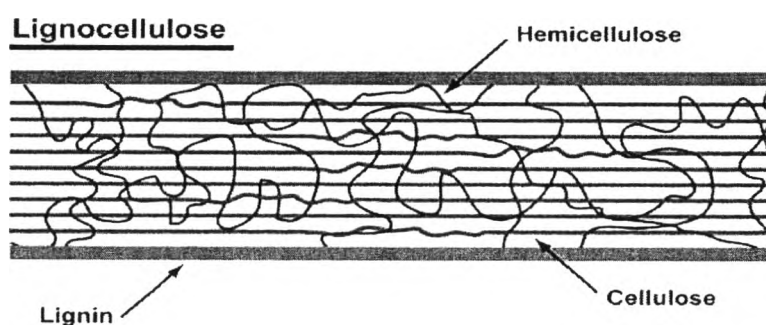

Figure 2.2 Lignocellulose consists of cellulose, hemicelluloses, and lignin. (http://askbluey.com/Image?q=Lignocellulosic+Biomass)

2.2.1 Cellulose

Cellulose is an organic compound with the formula $(C_6H_{10}O_5)_n$. It is the structural component in the rigid cell walls of plants. For industrial usage, cellulose is used to produce paper, paperboard, and card stock that made from wood pulp, cotton, linen, and plant fibers. The structure of cellulose consists of linear polysaccharide polymer with repeating units of β–D–glucose connected by β–1–4 glycosidic bonds (Fengel and Wegener, 1984), as shown in Figure 2.3. Glycosidic bonds can easily be brokendown by strong aqueous acids. Cellulose is a highly crystalline material (Fan *et al.*, 1982). The order of crystalline arrangement depends on inter-molecular and intra-molecular of OH groups that react with hydrogen atoms of adjacent glucose unit. Higher order of crystalline arrangement means cellulose is difficult to extent and hydrolyze in the enzymatic hydrolysis process.
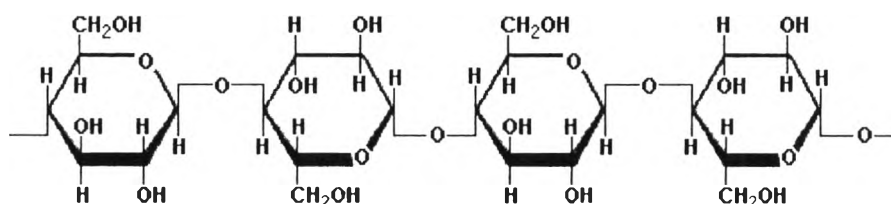

Figure 2.3 Structure of cellulose chain.
(http://sites.google.com/site/leachbiostuff/problem-set-1)

2.2.2 Hemicelluloses

Hemicelluloses can be found in fruit, plant stems, and grain hulls. However, hemicelluloses are indigested, they can be fermented by bacteria and yeasts. Hemicelluloses are branched polysaccharides consisting of many different kinds of sugars such as five-carbon sugars (e.g. Pentose, D-xylose, and L-arabinose) and six-carbon sugars (e.g. D-mannose, D-glucose, and D-galactose) (Saka, 1991). Hemicelluloses have a low molecular weight and branches with short lateral chains of them are easily hydrolyzed than cellulose (Fengel and Wegener, 1984). Hemicelluloses use as a connection between the cellulose and the lignin which can help cellulose-hemicelluloses-lignin network more rigidity (Laureano-Perez *et al.*,

2005). In lignocellulosic biomass, hemicelluloses are the most thermal-chemically sensitive (Levan *et al.*, 1990). Undergo thermal-chemical pretreatment, the side groups of hemicelluloses are firstly reacted, follow by their back bone (Sweet and Winandy, 1999). The solubility of hemicelluloses increases with increasing temperature. Whereas hemicelluloses contain higher molecular polymers, their solubility could not be predicted due to uncertain melting points (Gray *et al.*, 2003). The solubilization of hemicelluloses not depends on temperature but also depends on moisture content and pH (Fengel and Wegener, 1984).

Since hemicelluloses are heteropolymer so the structure and composition of hemicelluloses are widely various. Arabinoxylan is one type of hemicelluloses that comprise of xylan backbone made up of β–1,4–linked D–xylose units with arabinose side chains (as shown in Figure 2.4). Arabinoxylan is polysaccharides which can be found in the bran of grasses and grains such as wheat, rye, and barley. The appearance of arabinose side chains reduces hydrogen bonding. As a result, hemicelluloses have low crystallinity. Another type of hemicelluloses is glucomannan (as shown in Figure 2.5). The structure of glucomannan is mainly composes of straight-chain copolymer that consists of D-mannose and D-glucose linked in β-(1$\rightarrow$4) position in a ratio of 1.6:1. The xylan of hemicelluloses can be extracted well in an acid environment, whereas glucomannan can scarcely be extracted (Balaban and Ucar, 1999).
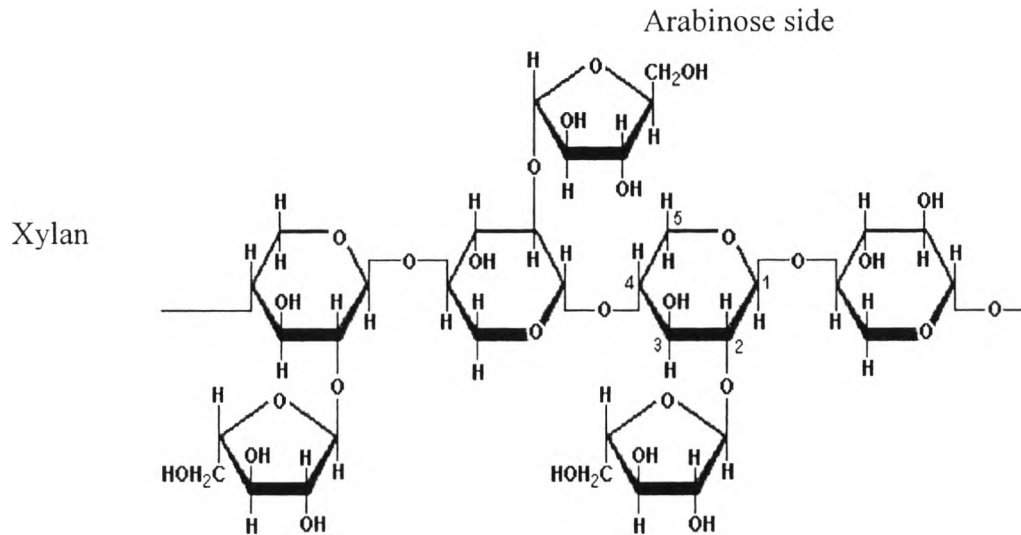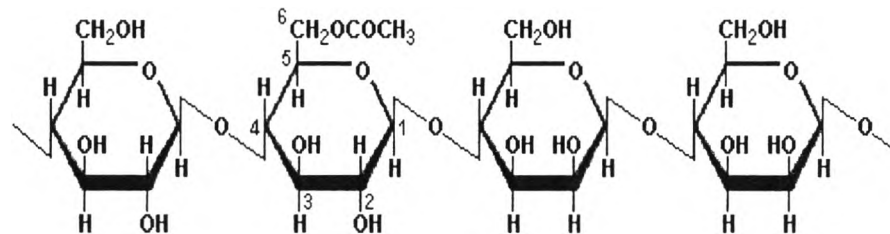
Arabinose side

Figure 2.4 Structure of arabinoxylan.

(http://www.scientificpsychic.com/fitness/carbohydrates2.html)

Figure 2.5 Structure of glucomannan.

(http://www.scientificpsychic.com/fitness/carbohydrates2.html)

2.2.3 Lignin

Lignin can be found from wood and a whole part of secondary cell walls of plants. Lignin always acts as plant structure support, impermeability, and resistance against microbial attack and oxidative stress. It is an amorphous heteropolymer that not dissolve in water and optically inactive which make the lignin degradation very difficult (Fengel and Wegener, 1984). Lignin is synthesized from phenylpropanoid precursors, namely, coniferyl alcohol, sinapyl alcohol, and p-coumaryl alcohol (Adler, 1977) (as shown in Figure 2.6). The lignin's solubilization depends on p-coumaryl, coniferyl, sinapyl alcohol or combinations of them (Grabber,

2005). These phenylpropanoid precursors are called monolignols. The various types of carbon-carbon bond and ether bond between individual monolignols make the formation of haphazard linkages in the lignin complex structure. The degree of lignin polymerization is perplexing to gauge owing to the molecule consists of various type of substructures which has random arrangement. Furthermore, the complex structure of lignin makes it difficult to process and modification. Lignin is provided into two catagories: guaiacyl lignins and guaiacyl-syringyl lignins. Guaiacyl lignins have a methoxy-group in the 3-carbon position where as guaiacyl-syringyl lignins have a methoxy-group in both 3-carbon and 5-carbon positions.
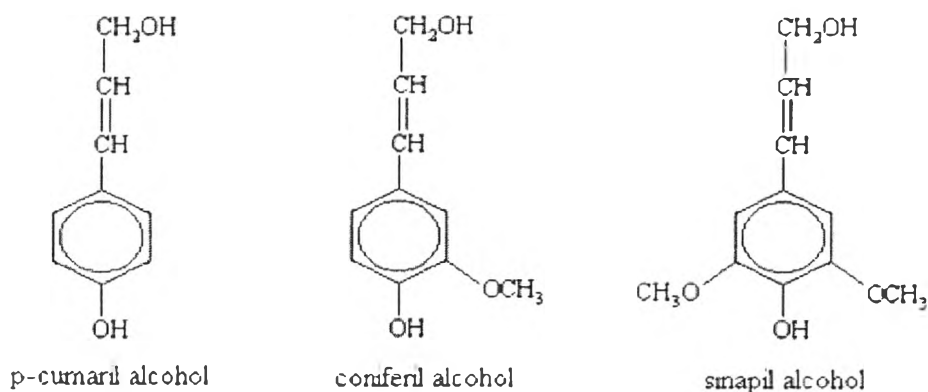
CH_2OH
|
CH
||
CH

OH

p-cumaril alcohol

CH_2OH
|
CH
||
CH

OCH_3

OH

conifenl alcohol

CH_2OH
|
CH
||
CH

CH_3O OCH_3

OH

snapil alcohol

Figure 2.6 Structure of monolignols.
(http://www.engin.umich.edu/dept/che/research/savage/energy.html)

2.3 Pretreatment of Lignocellulosic Biomass

Bioethanol production from lignocellulosic biomass consists of four major steps, including pretreatment, hydrolysis, fermentation, and ethanol separation, respectively. The lignocellulosic biomass to value-added products such as ethanol is always hindered by the structural and chemical of biomass. The physical and chemical structures of native lignocellulosic biomass recalcitrate enzymatic hydrolysis of cellulose (Zheng *et al.*, 2009). For example, fermentable sugars, which are hydrolyzed from cellulose and hemicelluloses, are trapped inside the crosslinking structure of the lignocellulosic biomass. Therefore, the pretreatment step is essential

to remove lignin and hemicellulose and render the cellulose amenable to enzymatic in hydrolysis step.

The purpose of the pretreatment is to modify the crystalline polysaccharides form to a more reactive amorphous form. An effective pretreatment is characterized by several criteria (as shown in Figure 2.7): (1) improve the ability of sugars formation by enzymatic hydrolysis; (2) limit the formation of by product inhibitors to the subsequent enzymatic hydrolysis and fermentation processes; (3) avoid the degradation or loss of carbohydrate; (4) minimizing energy input and (5) being cost–effective (Zheng *et al.*, 2009).
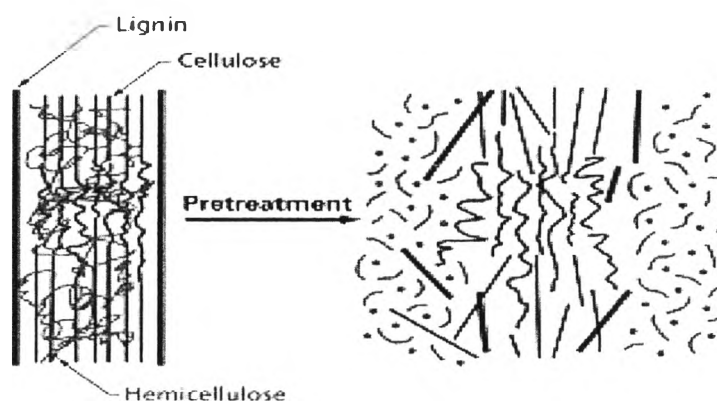

Figure 2.7 Schematic of goals of pretreatment on lignocellulosic material (Mosier *et al.*, 2005).

Various pretreatment technologies have been studied to improve structural and chemical of lignocellulosic biomass such as physical pretreatment, chemical pretreatment, and biological pretreatment (Zheng *et al.*, 2009). This work focused on the chemical pretreatment.

Chemical pretreatment was originally developed in the paper industry for delignification of cellulosic materials to produce high quality paper products. The primary goal of chemical pretreatment is improving the biodegradability of cellulose by removing hemicelluloses and lignin, and reducing degree of polymerization and crystallinity. There are various chemical pretreatment techniques, including catalyzed steam-explosion, acid, alkaline, ammonia fiber/freeze explosion, pH-

controlled liquid hot water, and ionic liquids pretreatment. However, this thesis mainly discuss only on the acid pretreatment.

The goal of acid pretreatment is to solubilize hemicellulose and to make the celloluse better accessible (Hendriks and Zeeman, 2009). Acid pretreatment method can be derived into two categories: concentrated acid and dilute acid pretreatments.

Concentrated acid preltreatment can remove hemicelluloses, which is combined with hydrolysis of cellulose. Although concentrated acid is mostly effective for cellulose hydrolysis, concentrated acid is toxic, corrosive, hazardous. Additionally, pretreatment with concentrated acid requires expensive reactor that can resist to corrosion. After hydrolysis the concentrated acid must be recovered and recycled to render the process economically feasible (Siver and Zacchi, 1995). Therefore, it has phased out gradually.

Dilute acid pretreatment has been successfully developed for pretreatment of lignocellulosic material. Several dilute acids such as dilute sulfuric acid, dilute nitric acid, dilute hydrochloric acid, dilute phosphoric acid, and peracetic acid have been used for pretreatment. From all of acid-based pretreatment, sulfuric acid is the best one for pretreatment because of its inexpensive and effective (Zheng et al., 2009). The dilute sulfuric acid pretreatment can enhance reaction rate and improve cellulose hydrolysis (Esteghlalian et al., 1997). Under moderate temperature, saccharification has low yield due to sugar decomposition. Therefore, dilute acid pretreatment under high temperature is favorable for cellulose hydrolysis (McMillan, 1994). Although dilute acid pretreatment makes a lot of advantages, it also has important disadvantage. Disadvantage of sulfuric acid pretreatment is the formation of degradation products and release of natural biomass fementation inhibitors like furfural, HMF, and phenolic compound.

2.4 Enzymatic Hydrolysis

Enzyme, acid, and alkali can be used to hydrolyze lignocellulosic materials to reducing sugar in hydrolysis step. However, enzymatic hydrolysis has less operating cost and higher utility than acid and alkali hydrolysis because the enzymatic is operated at mild condition (pH 4.8 at temperature range 45°C–50°C). Moreover, enzymatic hydrolysis can save the operating cost since it does not have corrosive problem (Duff and Murry, 1996). Cellulase enzymes are highly specific to enzymatic hydrolysis of cellulose (Beguin and Aubert, 1994). Cellulases are a mixture of various enzymes. There are at least three groups in celluloses structure that are related in hydrolysis process (1) endoglucanase (EG, endo-1,4-D-glucanohydrolase, or EC 3.2.1.4) has performance to attack low crystallinity regions in cellulose fiber and create free chain-ends; (2) exoglucanase or cellobiohydrolase (CBH, 1,4-β-D-glucan cellobiohydrolase, or EC 3.2.1.91.) can remove cellobiose units from free chain-ends; (3) β-glucosidase (EC) 3.2.1.21) can hydrolyze cellobiose to glucose (Coughlan and Ljungdahl, 1988).

There are several factors that affect the efficiency of enzymatic hydrolysis process. The first factor that resisted enzymatic hydrolysis is crystallinity of cellulose. The second is degree of cellulose polymerization. The third is surface area of substrate. And the last is lignin content. The researchers claimed that the crystallinity factor affect to 1-h enzymatic hydrolysis only (Chang and Holtzapple, 2000; Koullas et al., 1992; Laureano-Perez et al., 2005; Puri, 1984). Caulfield and Moore (1974) reported that reduced particle size and increased surface area can improve efficiency of enzymatic hydrolysis than reduced crystallinity. Grethlein (1985); Grous et al. (1986); Thompson et al. (1992) reported that the main limiting enzymatic hydrolysis step is the pore size of the substrate which is related to the active size that enzyme can operate. Grous et al. (1986) suggested that drying lignocelluloses can cause pore structural collapse so drying moisture content is need prior to enzymatic hydrolysis step. Lignin content can obstruct enzymatic hydrolysis by blocking cellulase acessibility to cellulose because of its structure which acts as a shield. Therefore, remove lignin can increase the rate of hydrolysis step (McMillan, 1994).

2.5 Mechanisms of Inhibition

In order to achieve fermentation process, dilute acid pretreatment and enzymatic hydrolysis are required to pretreat lignocellulosic biomass, respectively. However, after dilute acid pretreatment, the lignocellulosic hydrolysates not only contain fermentable sugars but also carry a wide range of toxic compounds, which inhibit the growth of microorganism used for fermentation. The composition of toxic compounds such as weak acid, furfural, hydroxymethylfurfural, and phenolic compound like vanillin which can inhibit microorganism growth and it depends on the chemistry of the pretreatment process and the type of lignocellulosic biomass. The effect of inhibiting compounds on fermentation are shown in Table 2.3.

Table 2.3 Effect of inhibiting compounds on fermentation (Olsson and Hahn-Hagerdal,1996)

Group of inhibitors	Inhibitor	Concentration (g l^{-1})	Microorganism	% inhibition of growth (g) or fermentation (f)
Compounds released during pretreatment	Acetic acid	1.4	*Saccharomyces cerevisiae*	50% (f); pH = 4.5
	Acetic acid	4.3	*S. cerevisiae*	50% (f); pH = 5.5
	Acetic acid	8.0	*Pichia stipitis*	98% (f); pH = 5.1
	Acetic acid	8.0	*P. stipitis*	25% (f); pH = 6.5
Sugar degradation products	Furfural	1.0	*P. stipitis*	47% (g); 71% (f)
	5-hydroxymethyl furfural	3.0	*P. stipitis*	69% (g); 90% (f)
Lignin degradation products	Cinnamaldehyde	1.0	*S. cerevisiae*	100% (f)
	p-hydroxybenzaldehyde	0.4	*Klebsiella pneumoniae*	68% (g)
	p-hydroxybenzaldehyde	1.0	*S. cerevisiae*	48% (f)
	Syringaldehyde	0.5	*K. pneumoniae*	40% (g)
	Syringaldehyde	0.22	*P. stipitis*	72% (f)
Fermentation products	Acetaldehyde	5.0	*S. cerevisiae*	80% (g)
	Ethanol	120	*S. cerevisiae*	100% (g)
	Formic acid	2.7	*S. cerevisiae*	80% (g)
	Lactic acid	38	*S. cerevisiae*	80% (g)
Remaining	Chromium	0.1	*Pachysolen tannophilus*	95% (f)
	Copper	0.04	*P. tannophilus*	29% (f)
	Iron	0.5	*P. tannophilus*	45% (f)
	Nickel	0.05	*P. tannophilus*	92% (f)

2.5.1 Weak Acid

Depending on acid dissociation constant, Ka, acid can be divided into two parts: strong acid and weak acid. Weak acid is an acid that is partially ionized. It can release only partial amount of its hydrogens to the solution. Weak acid occurs

from degradation of hemicelluloses. Weak acids, such as acetic, formic, octanoic and levulinic, are commonly used as food preservation owing to performance of cell-growth inhibition, as shown in Figure 2.8 (Brown and Booth, 1991). The growth-inhibition effecting microorganisms results from inflow of undissociated acid concentration (Axe and Bailey, 1995; Stouthamer, 1979; Verduyn *et al.*, 1990; Verduyn *et al.*, 1992; Warth, 1988). Since undissociated acid penetrates the cell membrane and intracellularly dissociates owing to the higher intracellular pH, It is very sensitive with pH during fermentation process. Some researches showed that acetic, formic, and levulinic acid can affect to the ethanol yield in fermentation process (Larsson *et al.*, 1998). Low acid concentration (<100 mmol l^{-1}) were shown to increase ethanol yield in fermentation process at pH 5.5, while the ethanol yield decreases at higher concentration. From this result, aliphatic carboxylic acids (>200 mmol l^{-1}) have been shown to relative with inhibitor fermentation (Nilvebrant *et al.*, 1997).

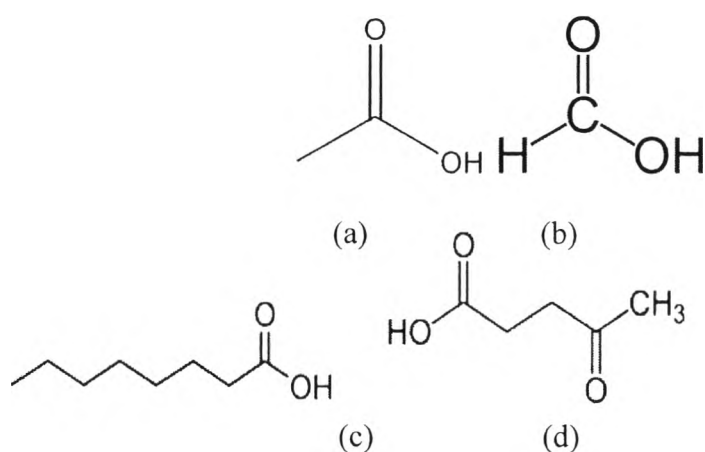**Figure 2.8** Structure of (a) Acetic acid, (b) Formic acid, (c) Octanoic acid, and (d) Levulinic acid. (http://en.wikipedia.org/wiki)

2.5.2 Furfural

Furfural is an aromatic aldehyde with the formula OC_4H_3CHO (as shown in Figure 2.9). It is colorless liquid, viscous, and odorous. Normal boiling point of furfural is 160°C. When furfural exposes to atmosphere, it becomes dark brown or black. Furfural is readily soluble in polar organic solvents. However, it is

only slightly dissolves in either water or alkanes. In Chemical part, furfural participates in the same kinds of reactions as other aldehydes and other aromatic compounds. Under temperature above 250°C furfural can be decomposed by heat into furan and carbon monoxide. While under the presence of acid and heat, furfural is irreversibly solidified into a hard thermosetting resin (http://www/answers.com).
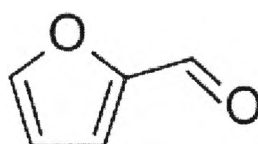
Figure 2.9 Molecular structure of furfural.
(http://en.wikipedia.org/wiki/Furfural)

Furfural is produced commercially by the acid hydrolysis of pentosan polysaccharides, which is a polymer of five-carbon sugars, from non-food residues of food crops and wood wastes. Pentosans xylan, arabinan and pentosan are precursors of furfural, which content in the lignocellulosic biomass in a portion of 25–40%. (http://www.greatvistachemicals.com). When lignocellulosic biomass heated with acid, hemicelluloses were hydrolyzed to five-carbon sugars called xylose. Under the same conditions of heat and acid, xylose, and other five-carbon sugars were dehydrated. This reaction makes these material lost three water molecules to become furfural, as shown in Figure 2.10 (http://www.answer.com/topic/furfural).
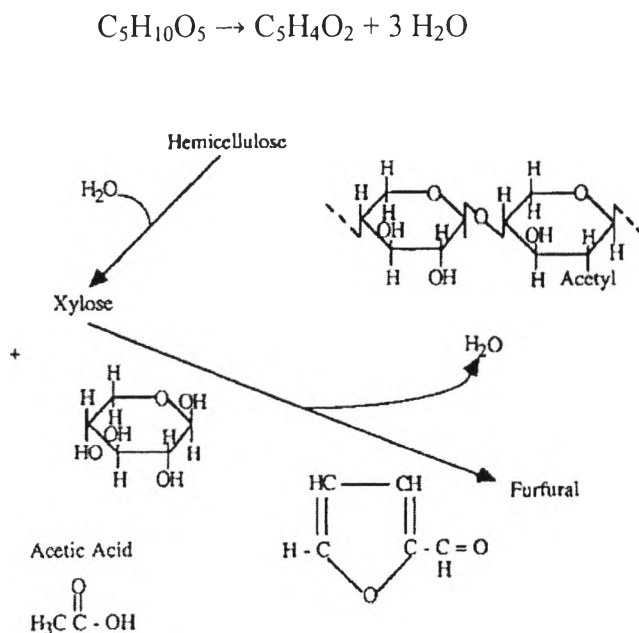
$$C_5H_{10}O_5 \rightarrow C_5H_4O_2 + 3\ H_2O$$

Figure 2.10 Acid-catalyzed pathway for the hydrolysis of hemicelluloses to xylose and the degradation of xylose to furfural (Weil *et al.*, 2002).

There are several mechanisms for furfural toxicity like chemical reactivity with cellular components damage to the cellular membrane, and inhibition of metabolism. The toxicity of furfural appears to be a function of its hydrophobic (Palmqvist *et al.*, 1999).

Furfural is used in many manufactures such as the furan industry, an intermediate in the synthesis of pharmaceuticals, agricultural chemicals, stabilizers, and fine chemicals. However, the major application of furfural is used as a feedstock for furfuryl alcohol production, which is used in the furan resin productions. Moreover, furfural is used as a solvent for refining lubricating oils, butadiene extraction and the furfural-based production of tetrahydrofuran. Hydroxymethyl-furfural is an organic compound. It is colourless and water-soluble. HMF molecule is a one type of furan derivative which is containing both aldehyde and alcohol groups in one molecule (as shown in Figure 2.11). Moreover, HMF is a biomass-derived platform chemical compound which can be produced alternative polymers or liquid biofuels. Liquid biofuels that produced by HMF undergo chemical processes can be used to potential alternatives like ethanol. HMF synthesis is directly made from

cellulose raw material which contents glucose and fructose via the Maillard reaction. The reaction occurs when sugars react with amino acids or proteins (Mauron, 1981). The main factors which influence to Millard reaction are reaction time, the temperature, the precursor concentration, and pH. The Millard reaction occurs in food which is heated. In addition, this reaction occurs in the human body and influences a wide variety of physiological functions. Tetrahydrofuran is used as a commercial solvent and is converted in starting materials for the nylon preparation (http://www.greatvistachemicals.com).

2.5.3 Hydroxymethylfurfural (HMF)

HMF toxicity has been reported to be converted at a lower rate than furfural, which might be due to lower membrane permeability. This reason causes a longer lag-phase in growth (Larsson *et al.*, 1998). HMF practically disappear in fresh food; however, it is naturally created in sugar-containing food during heat-treatments like milk, fruit juices, and honey (http://en.wikipedia.org/wiki/Hydroxymethyl-furfural).
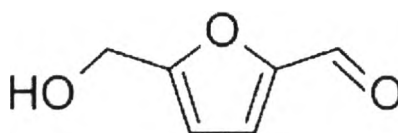

Figure 2.11 Molecular structures of Hydroxymethylfurfural.
(http://en.wikipedia.org/wiki/Hydroxymethylfurfural)

2.5.4 Phenolic Compounds

Phenolic compounds are chemical compounds, which are consisting of a hydroxyl functional group connected with aromatic hydrocarbon group. The simple form of phenolic compounds is phenol with formula C_6H_5OH (as shown in Figure 2.12). The solubility of phenolic compound depends on the composition of the liquid (Palmqvist and Hahn-Hagerdal, 2000). However, phenol has hydroxyl group like alcohol, it is not classified as alcohol owing to some unique properties. Phenol has higher acidity due to the aromatic ring's tight coupling with the oxygen and weak bond between the oxygen and hydrogen atom. The acidity of the hydroxyl

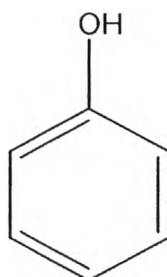group in phenol is commonly medium between the acidity of aliphatic alcohols and carboxylic acids (http://en.Wikipedia.org/ wiki/Phenols).

Figure 2.12 Molecular structures of Phenol.
(http://en.wikipedia.org/wiki/Phenol)

Partial breakdown of lignin generates phenolic compounds (Bardet *et al.*, 1985; Lapierre *et al.*, 1983; Sears *et al.*, 1971). Another report shown that phenolic compounds are formed undergo carbohydrate degradation (Popoff and Theander, 1976; Suortti, 1983). The low molecular weight of phenolic compounds has been investigated to inhibit lignocellulosic hydrolysates in fermentation process (Buchert *et al.*, 1989; Clark and Mackie, 1984). When a willow hemicelluloses hydrolysate removed phenolic compounds by treatment with the lignin-oxidising enzyme laccase, inhibition of fermentation has been shown to decrease (Jonsson *et al.*, 1998).

2.5.1.1 Vanillin

Vanillin is a phenolic aldehyde which is a one type of phenolic compound, an organic compound with the formula $C_8H_8O_3$ (as shown in Figure 2.13). Vanillin has three-functional groups include aldehyde, ether, and phenol (http://en. wikipedia.org /wiki/Vanillin).
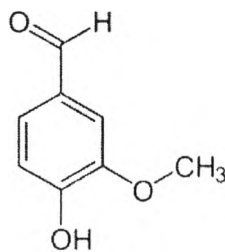
Figure 2.13 Molecular structures of Vanillin.
(http://en.wikipedia.org/wiki/Vanillin)

Vanillin production is a biomass-based process which uses a by-product of the pulp and paper from lignin oxidation. Some researches showed that vanillin was formed by the quaiacylpropane units of lignin degradation, which has been detected in hydrolysates from willow (Jonsson *et al.*, 1998), poplar (Ando *et al.*, 1986), red oak and pine (Clark and Mackie, 1984). Natural vanillin can be produced by the microbial transformation of ferulic acid. Ferulic acid was metabolized by bacteria belonging to different genera to sole carbon source which was producing vanillin. Vanillin has been found to be less toxic than 4-hydroxybenzoic acid and it had no toxic effect at concentration lower than 1 g/L (Ando *et al.*, 1986). Vanillin has a wide range of applications in food industry, perfume industry, and pharmaceutical chemical. Vanillin is used as fragrance in food preparation, intermediate in the productions of herbicides, antifoaming agents or drugs, ingredient of household products such as air-fresheners and floor polished.

2.6 Literature Review

Conversion of abundant lignocellulosic biomass to biofuels as transportation fuels presents a viable option for improving energy security and reducing greenhouse emissions. Unlike fossil fuels, which come from plants that grew millions of years ago, biofuels are produced from plants grown today. They are cleaner-burning than fossil fuels, and the short cycle of growing plants and burning fuel made from them does not add CO_2 to the atmosphere. Lignocellulosic materials such as agricultural

residues (e.g., wheat straw, sugarcane bagasse, corn stover), forest products (hardwood and softwood), and dedicated crops (switchgrass, salix) are renewable sources of energy. These raw materials are sufficiently abundant and generate very low net greenhouse emissions (kumar *et al.*, 2009).

Corn fiber, a waste product used for animal feed, is an interesting candidate for additional sugar production enhancing ethanol yield. Dry corn is composed of approximately 30% glucan from starch and cellulose and 25% xylan from hemicellulose. There are three general steps required to convert the polysaccharides, cellulose, and hemicellulose in corn fiber to fermentable sugars. First, the fiber is pretreated to change the chemically resistive crystalline polysaccharides to more reactive amorphous form. Second, the pretreated fiber is converted to monosachharides by enzymetic or acid hydrolysis. Last, the sugars obtained are fermented to value added products such as ethanol (Weil *et al.*, 2002).

Due to its compact crystalline structure formed mainly by inter- and intra-molecular hydrogen bonds, cellulose is found difficult to be hydrolyzed into fermentable sugars. The factors that affect the hydrolysis of cellulose include porosity (accessible surface area) of the waste materials such as corncob, cellulose fiber crystallinity, and lignin and hemicellulose content. The presence of lignin and hemicellulose makes the access of cellulase enzymes to cellulose difficult, resulting in reducing the efficiency of the hydrolysis. Therefore, removing lignin and hemicellulose, reduction of cellulose crystallinity, and increasing porosity in pretreatment processes that can dramatically improve the hydrolysis and lead to better yield of fermentable sugars. The purpose of the pretreatment is to remove lignin and hemicellulose, reduce cellulose crystallinity and increase the porosity of the materials. Pretreatment must achieve the following requirements: (i) improve the formation of sugars or the ability to subsequently form sugars by enzymatic hydrolysis; (ii) avoid the degradation or loss of carbohydrate; (iii) avoid the formation of byproducts inhibitory to the subsequent hydrolysis and fermentation processes; and (iv) be cost-effective. Physical, physico-chemical, chemical, and biological processes have been used for pretreatment of lignocellulosic materials (Palmqvist and Hahn-Hagerdal, 2000).

Chemical pretreatment is widely studied and applied recently, especially acid hydrolysis. Concentrated acids, such as H_2SO_4 and HCl, have been used to treat lignocellulosic materials. Despite they are powerful agents for cellulose hydrolysis, concentrated acids are usually toxic, corrosive, and hazardous. They require reactors that are resistant to corrosion. In addition, the concentrated acid must be recovered after hydrolysis in order to make the process economically feasible. Dilute acid hydrolysis has been successfully developed for the pretreatment of lignocellulosic materials since it can achieve high reaction rates and significantly improve cellulose hydrolysis. At moderate temperature, saccharification suffered from low yields because of sugar decomposition. High temperature in dilute acid treatment is favorable for cellulose hydrolysis (Sun and Cheng, 2002).

Sulfuric acid is widely used in dilute acid hydrolysis. Redding *et al.*, (2011) studied the parameters that can affect sugar yield before and after hydrolysis step, which are sulfuric acid concentration (0.3%, 0.6%, 0.9%, and 1.2% (w/w)), temperature (120 °C, 140 °C, 160 °C, and 180 °C), pretreatment duration (5, 15, 30, and 60 min) and the formation of furfural and HMF (sugar degradation products) in prehydrolysis step was observed. The relationship of temperature, sulfuric acid concentration, and time has already been investigated by using modified Arhenius equations. Before hydrolysis step, xylose is found to be a major component in prehydrolysate liquor, while arabinose and galactose concentrations are negligible. The highest xylose yield in the prehydrolysate liquor was 83% under pretreatment condition of 1.2% sulfuric acid, 140 °C for 30 min. In hydrolysis step, the hydrolysate was analyzed for mainly glucose monomer. The highest yield of glucose in the hydrolysate was 95% at 0.6% sulfuric acid at 160 °C for 30 min. The glucose yield is increased by the higher level of removing hemicelluloses in form of either xylose or furfural generation. It was shown that furfural and HMF are inhibitor compounds in the fermentation process, which are generated under harsh condition (high temperature). However, the optimal condition giving the highest total sugar and ethanol yield was favorable in high temperature, which was 1.2% sulfuric acid, 140 °C for 30 min.

Another interesting candidate using in dilute acid hydrolysis is phosphoric acid, H_3PO_4. Zhang *et al.*, (2010) studied the differences in crystallinity and surface

morphology between pretreated with phosphoric acid, P-MCC (Pretreated Microcrystalline cellulose), and untreated one, MCC, by using XRD and AFM, respectively. It was also reported that after the pretreatment with phosphoric acid, the corresponding sugar yields resulted from enzymatic hydrolysis of P-MCC were increased. Because phosphoric acid is non-corrosive, nontoxic, safe to be used and inexpensive compared to other mineral acids, it has been investigated as a solvent for the dissolution of crystalline cellulose over the last 80 years.

In the phosphoric acid reaction, the hydroxyl groups of cellulose were esterified by phosphoric acid forming cellulose phosphate (Cellulose–O–PO_3H_2), and the remaining hydroxyl groups on cellulose chains, hydrogen ion, and water could form hydrogen bond with each other. Cellulose phosphate could be reverted to free phosphoric acid and amorphous cellulose without any significant substitution or recrystallization through the phosphoric acid regeneration process by water. With the treatment of phosphoric acid, the typical peaks of amorphous cellulose at the 2θ of 12° (101) and 20.32° (101) can be seen gradually. With an increase in reaction time, more and more crystalline cellulose was converted to amorphous cellulose. The diffraction peak of 002 was shifted to lower diffraction angle. Crystalline peaks, such as 101, 101, and 004 peaks disappeared gradually with reaction time.

For Atomic Force Microscope (AFM) results, the fiber surface of P-MCC is uneven and rough. There are depressions in the amplitude map of P-MCC, which were due to traces of cellulose dissolved by phosphoric acid. Appearance of the rod-like crystalline cellulose arranged orderly could be seen. The structural of crystal is clearly and compact in MCC.

In addition, phosphoric acid pretreatment was also found to enhance the adsorption activity, which leads to higher efficiency in following enzymatic hydrolysis.

Another work studied the optimal conditions for phosphoric acid pretreatment (Gamez *et al.*, 2006). The interest in the use of H_3PO_4 is that after neutralization of hydrolysates with NaOH, the salt formed is sodium phosphate. This salt can be remained in the hydrolysates because it is nutrient of microorganisms. Hence, the filtration is not required with the consequent advantages: improve the economic of the process by avoiding the filtration to remove the salts and less the

amount of nutrient is needed for fermentation, and is friendly with the environment (the salt formed is not waste). The highest xylose concentration was 17.6 g/L in the experiment carried out at 4% H_3PO_4 for 300 min. Xylose concentration was always increasing with time except when the highest concentration of phosphoric acid was applied (6% H_3PO_4). In this case, xylose concentration decreased from 14.7 g/L at 180 min to 13.6 g/L at 300 min.

It was found that the rate of xylose release increased with the phosphoric acid concentration. For example, xylose concentrations in hydrolysates at 60 min of reaction were 6.1, 7.3, and 8.6 g/L using H_3PO_4 concentrations of 2%, 4%, and 6%, respectively.

It was observed that acetic acid is generated in the hydrolysis of the acetyl groups of the hemicelluloses. The acetic acid concentration increased quickly with time and phosphoric acid concentration during the first 60 min. After that, the rate of release was very low but the acetic acid concentration was not decreased indicating that no decomposition reactions appear. Acetic acid can be an inhibitor of microbial growth from 4 to 10 g/L by going through the cellular membranes and decrease intracellular pH, which affect to the metabolism of the cells. However, the average furfural concentration was 0.5 g/L, a 2.6% of the potential furfural concentration. This indicates that the decomposition of pentoses to furfural is low in treatment using phosphoric acid.

In conclusion, the optimal conditions selected were 122 °C, 4% H_3PO_4 and 300 min. Using these conditions, 17.6 g of xylose/L, 2.6 g of arabinose/L, 3.0 g of glucose/L, 1.2 g furfural/l, and 4.0 g acetic acid/L, were obtained. The efficiency in these conditions was 4.46 g sugars/g inhibitors and the mass fraction of sugars in dissolved solids in liquid phase was above 55%.