CHAPTER II
LITERATURE REVIEW

2.1 Biobutanol Production

Biobutanol can be produced by Acetone–Butanol–Ethanol (ABE) fermentation process, as shown in Figure 2.1. This process has been improved by using various strains of the bacterium either *Clostridium acetobutylicum* or *Clostridium Beijerinckii* and different substrates such as corn and molasses for many years. However, these substrates have high cost resulting in high price of butanol. Therefore, to produce butanol by using biomass as a feedstock is another choice to reduce butanol price.

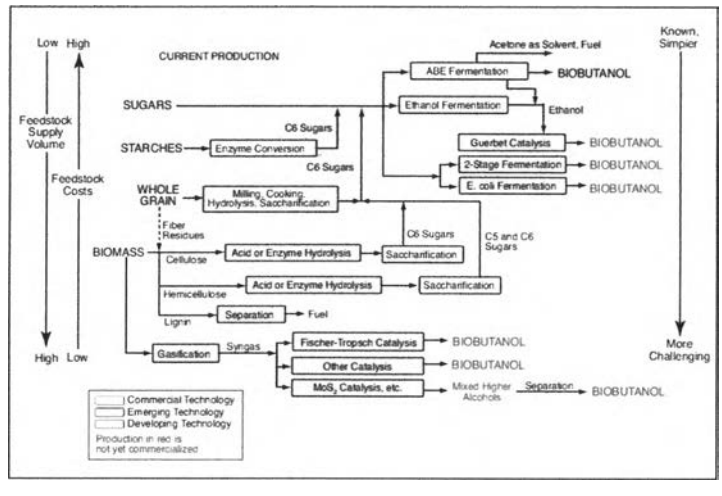

Figure 2.1 ABE fermentation process (Cascone, 2008).

Butanol is a four carbon alcohol. It contains more hydrogen and carbon. Butanol has several advantages. For example, butanol is easier to blend with gasoline and other hydrocarbon products and is safer to handle since butanol is less volatile and explosive, has high flash point and low vapor pressure. It can be shipped and distributed through existing pipelines and filling stations. An 85 % butanol/gasoline blends can be used in unmodified petrol engines and it is cleaner burning than ethanol (Nigam and Singh, 2011).

2.2 Lignocellulosic Biomass

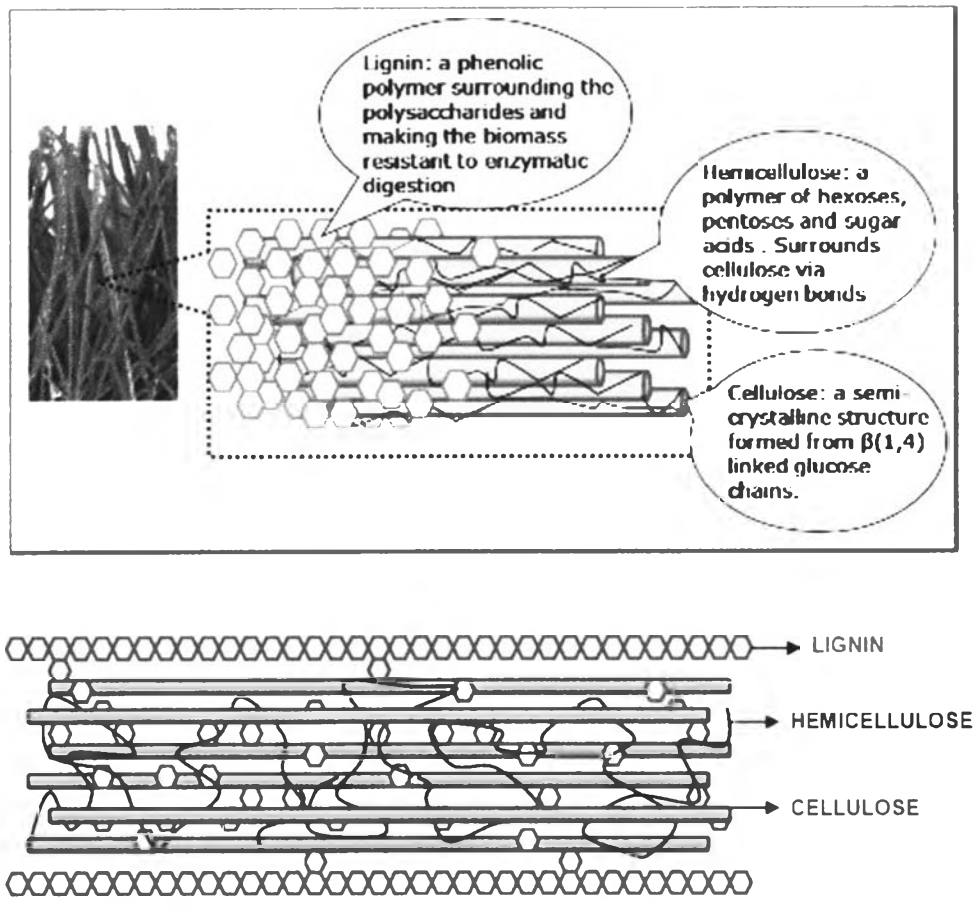

Figure 2.2 Representation of lignocellulose structure showing cellulose, hemicellulose, and lignin fractions (Mussatto *et al.*, 2010).

Lignocellulosic biomass such as agricultural waste and crop residue resources are one of the major renewable resources for fuels and chemicals. Lignocellulosic biomass involve mainly of cellulose, hemicellulose, and lignin that are closely associated in a complex crystalline structure, as shown in Figure 2.2. Basically, lignin and hemicellulose surrounds cellulose which forms a skeleton. The complex structure limited the enzymatic hydrolysis accessibility. Table 2.1 shows the composition of various lignocellulosic biomass.

Table 2.1 Composition of representative lignocellulosic feedstocks (Menon *et al.*, 2012)

Feedstocks	Carbohydrate composition (% dry wt)		
	Cellulose	Hemicellulose	Lignin
Bamboo	49–50	18–20	23
Banana waste	13	15	14
Corncobs	32.3–45.6	39.8	6.7–13.9
Corn stover	35.1–39.5	20.7–24.6	11.0–19.1
Cotton stalk	31	11	30
Rice straw	29.2–34.7	23–25.9	17–19
Rice husk	28.7–35.6	11.96–29.3	15.4–20
Wheat straw	35–39	22–30	12–16
Grasses	25–40	25–50	10–30
Sugarcane bagasse	25–45	28–32	15–25
Nut shells	25–30	22–28	30–40

2.2.1 Cellulose

Cellulose is the main component of plant biomass (Balat, 2011). Cellulose is a homopolymer of cellobiose which consists of two glucose and joined by β–1,4 glycosidic linkage. Because the long cellulose chains are connected via H–bond and van der walls bond; therefore, cellulose is packed into microfibrils that have highly crystalline structure. The crystalline structure is less degradable and less soluble (Taherzadeh and Karimi, 2008). The structure of cellulose is shown in Figure 2.3. In addition, the chemical pretreatment is the way to disrupt the crystalline structure of cellulose in order to enhance the rate of hydrolysis.

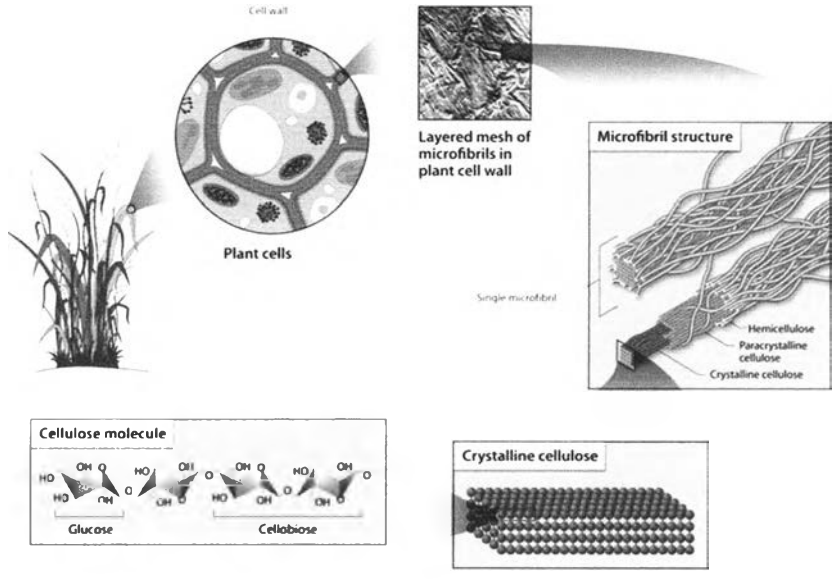

Figure 2.3 The structure of cellulose (Menon *et al.*, 2012).

2.2.2 Hemicellulose

In contrast to cellulose, hemicelluloses are heteropolymer of five–carbon sugar (mainly xylose and arabinose) and six–carbon sugar (glucose, galactose, and mannose), as shown in Figure 2.4. Hemicellulose has branches chains and amorphous nature which are readily hydrolyzed to sugar compare to cellulose (Lee *et al.*, 2007). Moreover, mannose is a major sugar in softwoods and xylose is a main sugar in hardwoods and agriculture residues such as corncobs (Taherzadeh and Karimi, 2008).

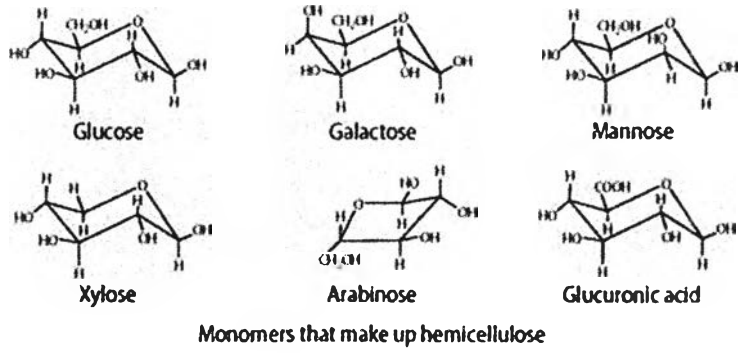

Figure 2.4 Monomers of hemicelluloses (Taherzadeh and Karimi, 2008).

2.2.3 Lignin

Lignin is an aromatic polymer and very complex molecules which contains of more functional groups such as carbonyl, methoxyl, and hydroxyl (Demirbas, 2008). More specifically, sinapyl alcohol, coniferyl alcohol, and p–coumaryl alcohol basis are encountered, as shown in Figure 2.5 (Harmsen *et al.*, 2010). Moreover, Lignin inhibits the enzymatic hydrolysis and makes lignocellulosic biomass resistant to biological and chemical degradation (Taherzadeh and Karimi, 2008).

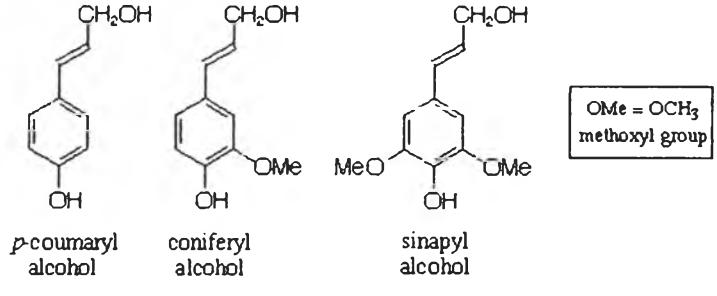

Figure 2.5 Phenyl propane units (Taherzadeh and Karimi, 2008).

2.3 Pretreatment of Lignocellulosic Biomass

Pretreatment is necessitated to alter the structure of lignocellulosic biomass to make cellulose more accessible to the enzymes which change lignocellulosic biomass into sugars. Pretreatment can be increased the efficiency and decreased the cost via research and development (Mosier *et al.*, 2003a,b). The objective of lignocellulosic biomass pretreatment is to remove lignin and solubilize hemicellulose. As a result, pretreatment can expose more cellulose to the enzyme; therefore, the ability of enzymatic hydrolysis is increased and high sugar production was obtained (Kumar *et al.*, 2009). Normally, pretreatment process can be classified into three groups, including physical, chemical, and biological pretreatment.

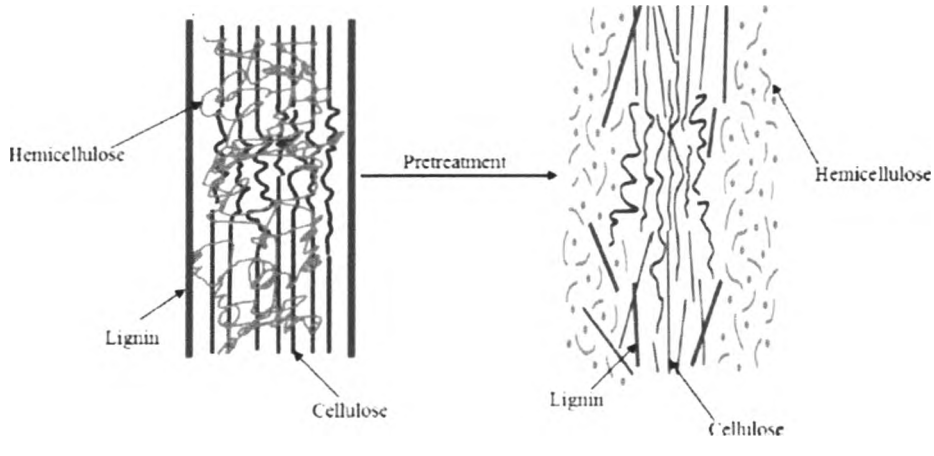

Figure 2.6 Schematic of the role of pretreatment (Kumar *et al.*, 2009).

2.3.1 Physical Pretreatment

Physical pretreatment is used to reduce the size of lignocellulosic biomass in order to increase the surface area such as chipping, grinding, and milling. Milling or grinding can decrease the size of lignocellulosic biomass to 0.2–2 mm and 10–30 mm after chipping (Kumar *et al.*, 2009, Sun and Cheng, 2002, Leustean, 2009). Power requirements of this process depend on the characteristics of biomass and the final size of lignocellulosic biomass. Physical pretreatment is required long time, more energy, and high cost (Balat, 2011).

2.3.2 Physico–Chemical Pretreatment

2.3.2.1 Steam Explosion (Autohydrolysis)

Lignocellulosic biomass is treated with high–pressure saturated steam, then the pressure is rapidly decreased, which causes the biomass go through an explosive decomposition. Steam explosion is the most usually used technique for the pretreatment of lignocellulosic biomass. Crystallinity of cellulose is increased by this method. Furthermore, steam explosion easily solubilizes the hemicelluloses and promotes lignin removal (Jeoh, 1998).

2.3.2.2 Ammonia Fiber Explosion

Ammonia fiber explosion (AFEX) is the alkaline physico–chemical pretreatment processes. Liquid ammonia is used at high pressure and temperature, result in fast decomposition (Abril *et al.*, 2009). The process makes cellulose and hemicellulose to be attacked the enzyme (Balat, 2011).

2.3.2.3 Liquid Hot–water Pretreatment

Liquid hot water (LHW) pretreatment is the hydrothermal pretreatment of lignocellulosic biomass (Taherzadeh and Karimi, 2008). LHW exposes lignocellulosic biomass to hot water in liquid state at high pressure and it gives higher recovery rates for pentoses and produces small amount of inhibitors (Tomas *et al.*, 2008).

2.3.3 Chemical Pretreatment

Chemical pretreatments were used to improve the biodegradable of cellulose by solubilizing hemicellulose and removing lignin. Chemical pretreatment is the most famous pretreatment method and can be classified into alkali, acid, liquid hot–water, organic acids, and ionic liquids pretreatment.

2.3.3.1 Acid Pretreatment

Acid pretreatment of lignocellulosic biomass provides high reaction rate and produces the great sugar yield (Karimi *et al.*, 2006). Acid pretreatment includes the concentrated and diluted acids in order to fracture the rigid structure of the lignocellulosic biomass and solubilize hemicellulose and expose cellulose for enzymatic hydrolysis (Silverstein *et al.*, 2008). Dilute H_2SO_4 is the most frequently used for many type of lignocellulosic biomass such as corn

stover, switchgrass, and corncobs. However, the other acid such as H_3PO_4, HNO_3, and HCl is considered to apply for pretreatment (Zhang *et al.*, 2007). The acid pretreatment increases hemicellulose solubilization rate when compare with steam explosion or liquid hot water pretreatment; therefore, the enzymatic hydrolysis of cellulose is enhanced. The removal of hemicellulose by acid pretreatment followed by removal of lignin by alkali pretreatment results in pure cellulose (Menon *et al.*, 2012).

The dilute acid pretreatment was examined for peanut shells, cassava stalks, rice hulls, and sugarcane bagasse (Martin *et al.*, 2007). The 2 % (w/w) H_2SO_4 pretreatment was operated at 122 °C for 20, 40, or 60 min at a solid–to–liquid ratio of 1:10. The result showed that the long reaction time can increase the sugar production. Cara *et al.* (2008) studied dilute acid pretreatment of olive tree biomass. Pretreatment was performed at 0.2, 0.6, 1.0, and 1.4 % (w/w) H_2SO_4 and temperature range 170–210 °C. It was found that 83 % of sugars from hemicellulose were recovered with 1 % H_2SO_4 at 170 °C.

2.3.3.2 Alkaline Pretreatment

Alkali pretreatment represents to remove lignin that lower the enzyme accessibility to the hemicellulose and cellulose (Han *et al.*, 2009) Alkaline pretreatment is performed at lower temperatures and pressures compared to other pretreatment methods but pretreatment time is very long (Mosier *et al.*, 2005). NaOH, KOH, $Ca(OH)_2$, and NH_4OH are suitable for alkaline pretreatment. However, NaOH has been studied the most (Kumar *et al.*, 2009).

Wang (2009) studied NaOH pretreatment of Coastal Bermuda grass. Coastal Bermuda grass was pretreated with NaOH 0.5 % to 3 % (w/v) from 15 to 90 min at 121 °C. The result showed that 30 min was appropriate time to remove lignin with concentration of NaOH of equal or over 1 % but it was no difference in removal of lignin between 2 and 3 % NaOH.

Joshua *et al.* (2012) investigated the production of ABE from algae biomass. They found that the acid followed by alkaline pretreatment produced 8.92 g/l of soluble sugars, while non–pretreated algae had only 0.73 g/l of soluble

sugar. These result revealed that the pretreatment can enhance the sugars production.

Ponthein and Cheirsilp (2011) studied the pretreatment of palm pressed fiber by hydrothermal with acid and alkaline to remove lignin and get high cellulose content fiber. The result indicated that the pretreatment with NaOH followed by H_2SO_4 gave highest cellulose content and reduced the amount of hemicellulose and lignin more than pretreatment with NaOH or H_2SO_4 alone. Moreover, Zhu *et al.* (2006) also found that the pretreatment of rice straw by alkali and acid increased cellulose content up to 75–80 %. The amount of hemicellulose and lignin content also significantly decreased to 3 and 3–5 %, respectively. While the pretreatment with alkaline or acid alone gave similar lignin and hemicellulose content at 7–23 and 7–15 %, respectively.

2.3.3.3 Ozonolysis

Ozonolysis includes consuming ozone gas to remove lignin and hemicellulose and improve the biodegradability of the cellulose. The pretreatment is performed at room temperature (Vidal *et al.*, 1988). However, the high amount of ozone is required result in high process cost (Kumar *et al.*, 2009).

2.3.4 Biological Pretreatment

Biological pretreatment includes microorganisms such as white–,brown–, and soft–rot fungi which are used to degrade lignin and solubilize hemicellulose. This pretreatment require low energy but the hydrolysis rate is very low (Sun *et al.*, 2002).

2.3.5 Microwave Pretreatment

Microwaves lie between radio wave frequencies (RF) and infrared (IR) frequencies in the electromagnetic (EM) spectrum, as shown in Figure 2.7. Frequencies of microwaves are 0.3 GHz to 300 GHz and wavelengths of 1 m to 1 mm. Solids, liquids, and gases are able to work with microwave radiation and be heated. Heat within the lignocellulosic biomass that increases in temperature is generated by microwave absorption (Clark and Sutton, 1996).

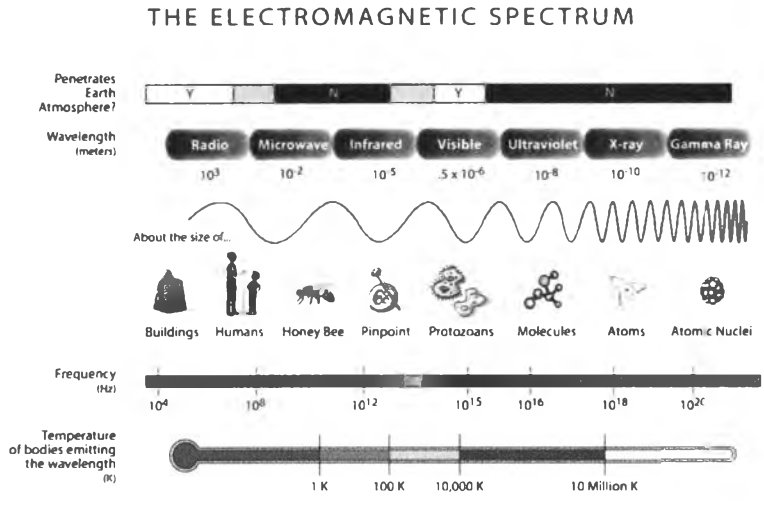

Figure 2.7 The electromagnetic spectrum with applications at various frequencies (https://mynasadata.larc.nasa.gov/ElectroMag.html).

The pretreatment with microwave radiation is a high energy efficiency and environmental friendly method because microwave pretreatment can enhance cellulosic biomass reactivity that has a direct interaction between heated lignocellulosic biomass and electromagnetic field to create heat. Moreover, the lignocellulosic biomass is heated internally. Consequently, the heating is rapid and volumetric. On the other hand, the conventional pretreatment is heated externally such as the electrical coils around autoclave reactor (Ooshima *et al.*, 1984).

Microwave pretreatment has the following benefits compared with conventional heating methods (Jones *et al.*, 2002).

- Greater heating rates
- No direct interaction between heated lignocellulosic biomass and heating source.
- Selective heating is reached
- Greater control of the heating or drying process
- Decreased equipment size and waste

Table 2.2 shows the benefits and challenges of microwave processing

Table 2.2 Benefits and challenges of microwave processing (Clark and Sutton, 1996)

Benefits	• Cost savings (time and energy, reduced floor space) • Rapid heating of thermal insulators (most ceramics and polymers) • Precise and controlled heating (instantaneous on/off heating) • Selective heating • Volumetric and uniform heating (due to deep energy penetration) • Short processing times • Improved quality and properties • Synthesis of new materials • Processing not possible with conventional means • Reduction of hazardous emissions • Increased product yields • Environmentally friendly (clean and quiet) • Self–limiting heating in some materials • Power supply can be remote • Clean power and process conditions
Challenges	• Heating low–loss poorly absorbing materials • Controlling accelerated heating (thermal runaway) • Exploiting inverted temperature profiles • Eliminating arcing and controlling plasmas • Efficient transfer of microwave energy to work piece • Compatibility of the microwave process with the rest of the process line • Reluctance to abandon proven technologies • Timing • Economics

Antonio *et al.* (2005) studied thermal effect of microwave radiation. The result revealed that microwave radiation was rapid and volumetric, with the whole material heated simultaneously. Whereas, conventional heating was slow and started into the material from the surface. The temperature profile as shown in Figure 2.8

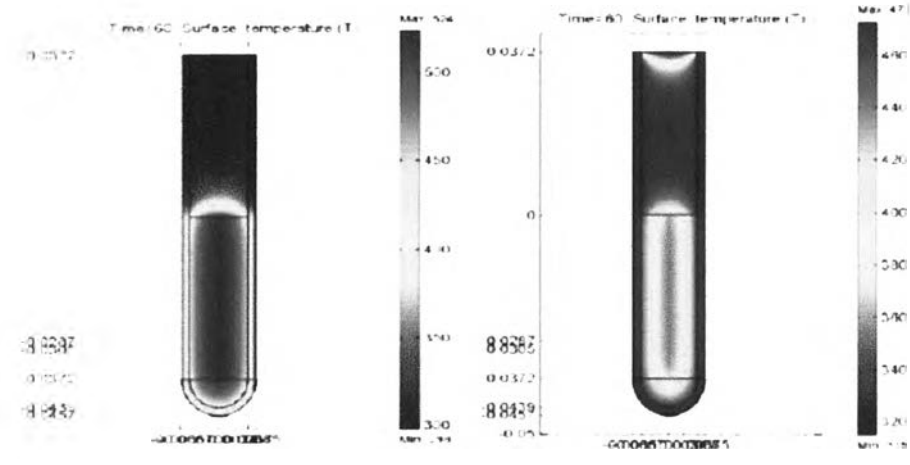

Figure 2.8 The temperature profile after 60 sec as affected by microwave radiation (left) compared to treatment in oil bath (right).

Hu and Wen (2008) studied microwave/H_2O pretreatment followed by enzymatic hydrolysis of switchgrass. The result showed that the yield of total sugar including xylose and glucose from microwave radiation was 34.5 g/100 g biomass which was 53 % higher than conventional pretreatment. In addition, microwave/alkaline pretreatment was investigated. The 0.1 g/g of alkali loading gave the sugar yield higher than conventional pretreatment. From the SEM images showed that microwave pretreatment can disrupt the recalcitrant structures of switchgrass more than conventional pretreatment. The optimum conditions of microwave pretreatment was 190 °C with solid–to–liquid ratio 50 g/l for 30 min. The microwave/alkaline pretreatment followed by enzymatic hydrolysis gave sugar yield 58.7 g/100 g biomass. The results can be concluded that microwave/ alkali pretreatment was an efficient method to enhance the enzymatic hydrolysis of switchgrass.

Zhu *et al.* (2005) also investigated microwave/alkali pretreatment and enzymatic hydrolysis of wheat straw. The results indicated that the weight loss and wheat straw composition had no effect to pretreatment time and microwave power. The microwave/alkali pretreatment eliminated lignin and solubilized hemicellulose with shorter pretreatment time compared with the conventional/alkali pretreatment. In addition, microwave/alkali pretreatment followed by enzymatic hydrolysis gave the glucose concentration, hydrolysis rate, and total sugar higher than conventional/alkali pretreatment.

Zhu *et al.* (2006) investigated rice straw pretreatment with microwave/alkali, microwave/acid/alkali, and microwave/acid/alkali/H_2O_2 followed by enzymatic hydrolysis. The result showed that xylose could be recovered via microwave/acid/alkali and microwave/acid/alkali/H_2O_2 pretreatment. Moreover, microwave/acid/alkali/H_2O_2 pretreatment gave the highest glucose concentration and hydrolysis rate.

In our group, Ploypradith P. (2010) studied the NaOH pretreatment with microwave on corncobs. The optimum conditions were found at 2 % NaOH at 100 °C for 30 min which could reduce lignin by 66.27 % and increase in surface area by 38.31 %. And the highest glucose concentration can reach up to 32.53 g/l and total sugar of 42.93 g/l was released. Moreover, microwave assists NaOH can produce total sugar concentration at shorter pretreatment time and lower pretreatment temperature compared with autoclave. In addition, total sugar concentration of microwave was higher than that of conventional heating.

Wanitwattanarumlug B. (2011) also studied the pretreatment of corncobs using microwave and potassium hydroxide. The highest sugar yield of 34.79 g/l was obtained from the corn cobs pretreated by microwave and 2 % KOH at 120 °C for 25 min. The results indicated that microwave–assisted alkali pretreatment was an efficient way to develop the enzymatic hydrolysis accessibility.

There are many research work related with pretreatment methods to improve its conversion. Among them, the microwave–assisted chemical pretreatment is a more effective to enhance the enzymatic hydrolysis by accelerating the reaction. In this study, the combined pretreatment of corncobs with microwave was conducted. A two–stage pretreatment using 2 % NaOH at 100 °C for 30 min the

optimal condition of NaOH from Ploypradith P. (2010) and followed by H_2SO_4 pretreatment. In this work, NaOH was used to separate lignin in the first stage and the effect of temperature, reaction time, and solid–to–liquid ratio (SLR) were determined in the second stage of two–stage pretreatment by response surface methodology (RSM).

2.4 Inhibitors from Biomass Pretreatment

The pretreatment process generates numerous by–products that inhibit the growth of microorganism and fermentation. However, the generation of by–product depends on feedstock and pretreatment method (Jonsson *et al.*, 2013). Especially, acid pretreatment that solubilizes hemicellulose leading to the formation of pentoses, hexoses, and inhibitors such as feruric acid, acetic acid, 2–furaldehyde (furfural), formic acid, and furoic acid. Furthermore, cellulose also degrades hexoses to 5–hydroxymethylfurfural (HMF), and levulinic acid. Other aldehydes and phenol can be formed by degration of lignin. Figure 2.9 shows the inhibitors that are generated during pretreatment (Liu and Blaschek, 2010). Although more than 100 compounds were detected as inhibitors, many have not been well studied (Liu *et al.*, 2004).

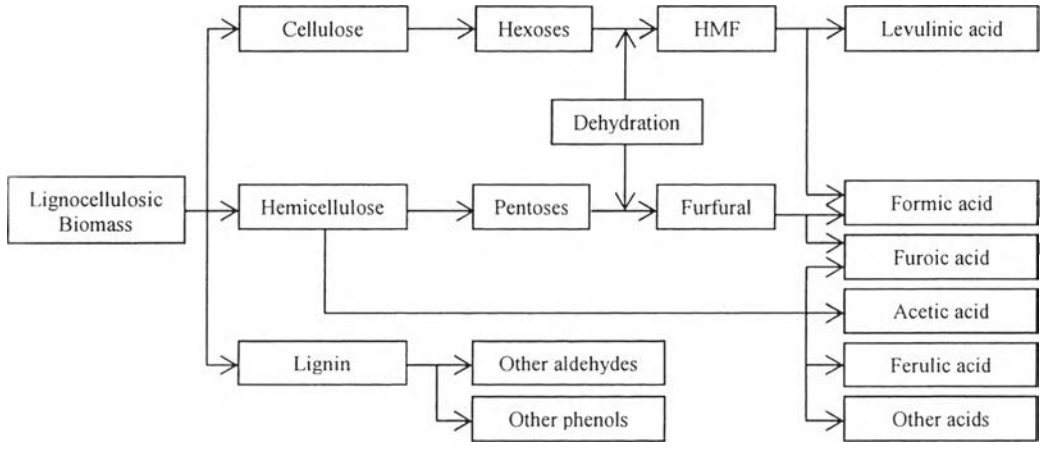

Figure 2.9 The degradation product of lignocellulosic biomass during pretreatment (Liu and Blaschek, 2010).

Inhibitors can be classified base on chemical functional groups into 4 groups as aldehydes, ketones, phenols, and organic acids. Some studies have investigated that the low molecular weight compounds have more toxic to microbes than high molecular weight due to easier to transport (Sierra *et al.,* 1991).

2.4.1 Aldehyde Inhibitors

Aldehyde inhibitors are compounds with one or more aldehyde functional groups with a furan ring, a benzene ring or a phenol structure. For example, furfural and HMF which contain a furan ring and an aldehyde functional group. Other aldehyde inhibitors include 4–hydroxy-benzaldehyde, vanillin (Klinke *et al.,* 2002), syringaldehyde, and other compounds having a benzene ring or a phenol–based structure including isovanillin, ortho–vanillin, and coniferylaldehyde (Liu and Blaschek, 2010). The structure of aldehyde inhibitors are shown in Figure 2.10.

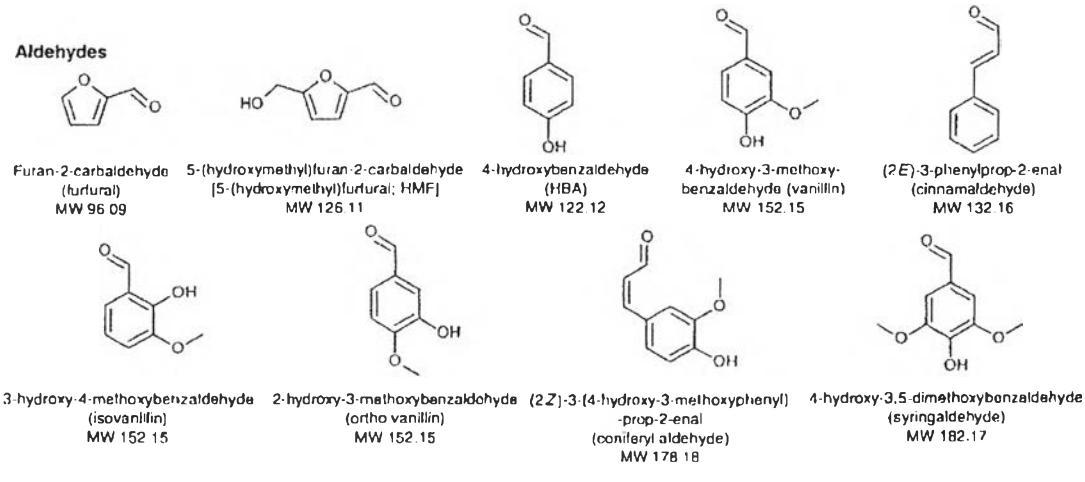

Figure 2.10 The structure of aldehyde inhibitors (Liu and Blaschek, 2010).

2.4.2 Ketone Inhibitors

Ketone inhibitors include 4–hydroxyacetopheone and the closely related compounds acetovanillone and acetocsyringone. These compounds all share a common ketone functional group (Klinke *et al.*, 2003). The structure of ketone inhibitors are shown in Figure 2.11.

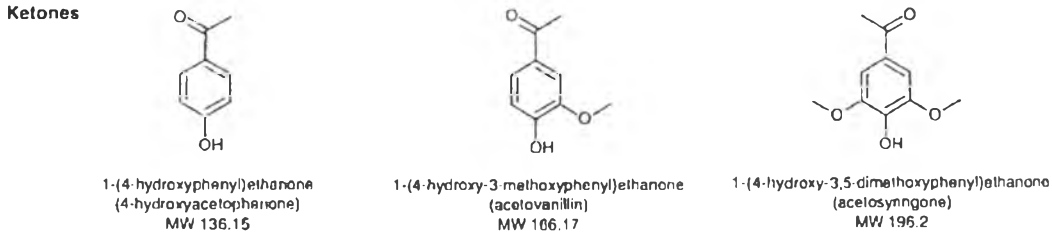

Figure 2.11 The structure of ketones inhibitors (Liu and Blaschek, 2010).

2.4.3 Phenol–based Inhibitors

Phenol–based inhibitors are grouped together including phenol, benzene–l,2–diol (catechol), benzene–1,4–diol (hydroquinone), 4–ethylbenzene–l,2–diol (ethylcatechol), 2–methylphenol, 3–methylbenzene–l,2–diol (methylcatechol), 2–methoxyphenol (guaiacol), 4–(hydroxymethyl)–2–methoxyphenol (vanillyl alcohol), and 2,6–dimethoxybenzene–1,4–diol (Klinke *et al.*, 2002). The structure of phonols inhibitors are shown in Figure 2.12.

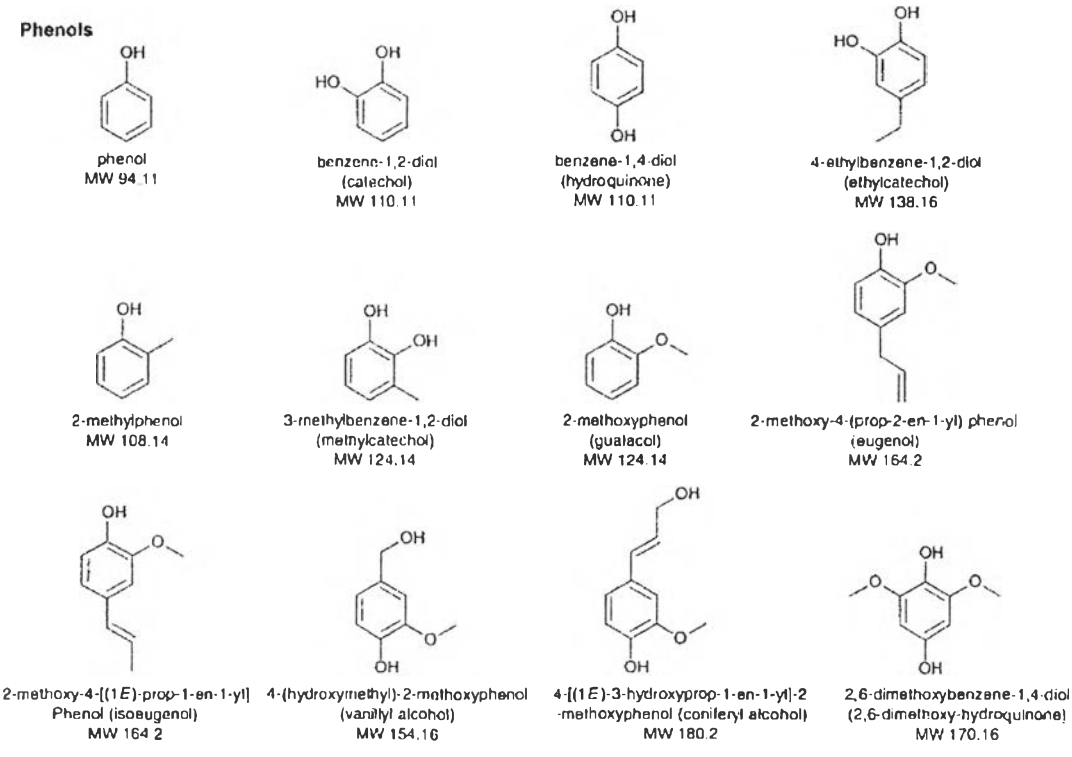

Figure 2.12 The structure of phenols inhibitors (Liu and Blaschek, 2010).

2.4.4 Organic acid Inhibitors

Organic acid inhibitors include simple acids as well as furoic acid with a furan ring that was considered as being a furan inhibitor. Moreover, many previously recognized phenolic compounds are now grouped as members of the organic acid inhibitor class based on their functional structure. Inhibitory compounds of this class all contain a carboxyl functional group and include acetic acid, formic acid, levulinic acid, caproic acid, furoic acid, 4–hydroxybenzoic acid, 3–hydroxybenzoic acid, 2–hydroxybenzoic acid, 2,5–dihydroxybenzoic acid, protocatechic acid, vanillic acid, gallic acid, syringic acid ,4-hydroxycinnarnic acid, ferulic acid, homovanillic acid, guaiaclyglycolic acid, and sinapic acid. These inhibitors are thought to be exert their inhibitory actions via their carboxyl functional groups. The structure of organic acid inhibitors are shown in Figure 2.13.

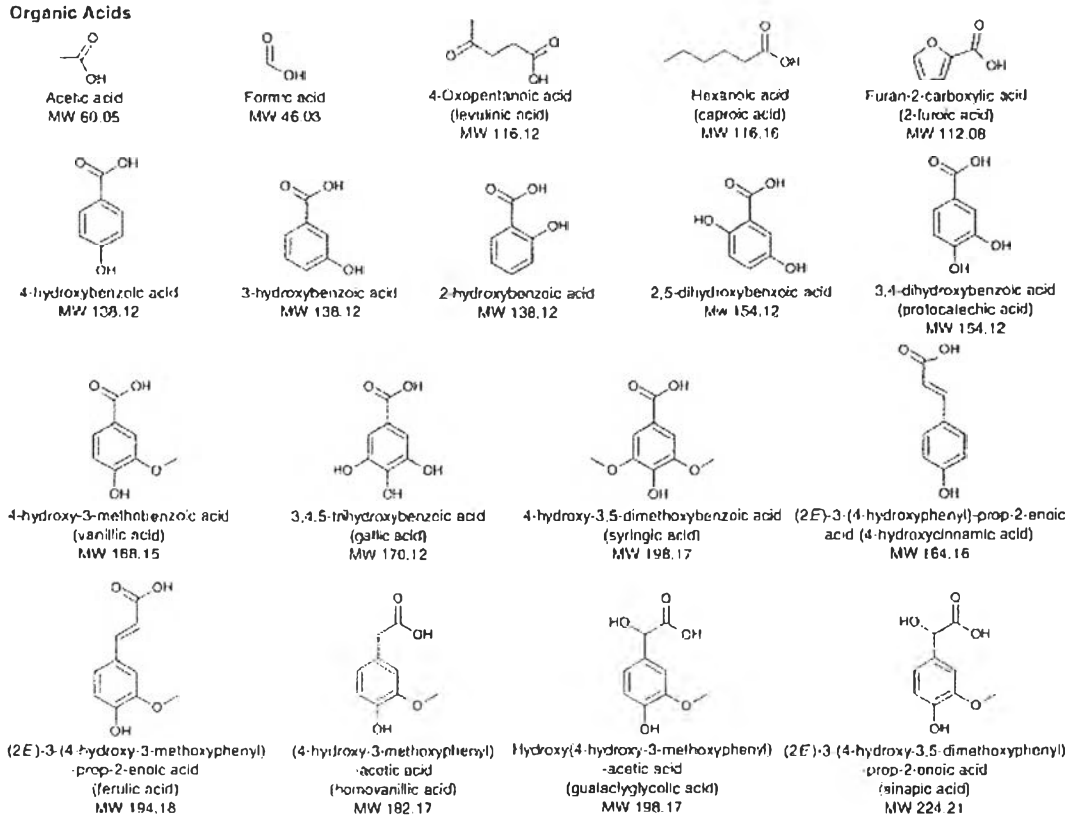

Figure 2.13 The structure of organic acid inhibitors (Liu and Blaschek, 2010).

2.5 Butanol Fermentation Inhibitors

Pretreatment has been seen as a preferred method that make the enzyme in enzymatic hydrolysis step highly digests biomass in order to produce high amount of reducing sugar for further ABE fermentation. However, the toxic compounds including weak acids, furan derivatives, phenolic compounds, vanillic aldehyde, and tannin are generated during pretreatment (Eva and Bärbel, 2000); therefore, no microorganism can efficiently produce butanol from lignocellulosic biomass (Weber *et al.*, 2010) due to the inhibitors affect cell growth and ABE production.

Ezeji *et al.* (2007) studied the impact of inhibitors that generated from H_2SO_4 pretreatment on ABE concentrations. The results showed that syringaldehyde, ferulic, and ρ–coumaric acids were potent inhibitors of ABE production by *Clostridium beijerinckii* BA101 as shown in Figure 2.14. In general,

ferulic and coumaric acids inhibit microorganism by damaging the hydrophobic sites of the bacteria cells because ferulic and coumaric acids are phenolic acids and phenolic compounds that affect membrane permeability (Heipieper et al., 1994). Furthermore, the authors observed that furfural and HMF (3 g/l) were not inhitory to *Clostridium beijerinckii* BA101. However, the combination of furfural and HMF affects the culture negatively. In addition, the production of salt, sulfate, which is result of sulfuric acid used for pretreatment was also toxic to *Clostridium beijerinckii* BA101.

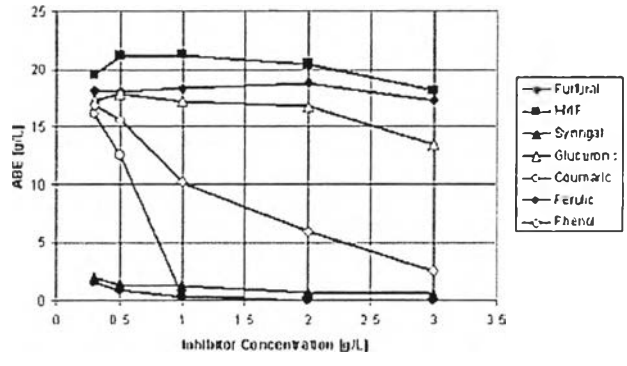

Figure 2.14 The effect of inhibitors generated during 0.5 % H_2SO_4 pretreatment of corn fiber on ABE concentrations (Ezeji *et al.*, 2007).

2.6 Detoxification Method (Chandel *et al.*, 2011)

Since inhibitors from pretreatment process can be problematic for fermentation, the removal of inhibitors from hydrolysates is necessary to enhance microbial growth and fermentation efficiency. Nevertheless, inhibitors depend on type of pretreatment and feedstock. The most detoxification methods are physical, chemical, and biological (Chandel *et al.*, 2011).

2.6.1 Physical Methods

2.6.1.1 *Evaporation*

The evaporation under vacuum can remove volatile compounds for example, furfural, acetic acid, and vanillin from hydrolysate of lignocellulosic biomass. However, evaporation retains the non–volatile toxic compounds such as lignin derivatives and extractives in the hydrolysates. A study by Wilson *et al.* (1989) found a decrease in the concentration of furfural, vanillin, and acetic acid by 100 %, 29 %, and 54 %, respectively, compared with the concentrations in the hydrolysate. Likewise, Larsson *et al.* (1999) studied the removal of furfural and HMF using vacuum evaporation from wood hydrolysate. The results showed that furfural and HMF were reduced 90 %, 4 %, respectively.

2.6.1.2 *Membrane separations*

Adsorptive micro porous membranes have surface functional groups attached to their internal pores, that remove the cell wall derived inhibitors from acid hydrolysates. Grzenia *et al.* (2010) applied the membrane extraction for inhibitors removal from sulfuric acid hydrolysate of corn stover. The results showed that acetic acid, formic acid, levulinic acid, HMF, and furfural were eliminated.

2.6.2 Chemical Methods

2.6.2.1 *Neutralization*

The neutralization of acid hydrolysates is required step before fermentation because of low pH. Alkali ($Ca(OH)_2$ or NaOH) is used for hydrolysates neutralization (pH in the range of 6–7). Phenolics and furfural may be removed by precipitation.

2.6.2.2 *Overliming*

It was reported that overliming is the most cost effective method for detoxifying soft wood hydrolysates. Detoxification after pretreatment and enzymatic hydrolysis or before fermentation by alkali treatment begins by adding lime (NaOH or $Ca(OH)_2$) to adjust the pH of the hydrolysate to a high value (in the range of 9–11) followed by pH readjustment to 6.6 with H_2SO_4. Adjustment of pH with $Ca(OH)_2$ has been reported to increase the fermentability more than that

with NaOH. The total amount of phenolic compounds was more efficiently decreased by $Ca(OH)_2$. However, it has been shown that monovalent ions such as Na^+ affect the ethanol productivity negatively, whereas Ca^{2+} does not. However, acetic acid and sugars were not removed by treatment process with NaOH or $Ca(OH)_2$. Moreover, a heating step in the overliming procedure (leading to some evaporation) improves fermentability (Larsson, 1999). Furthermore, Ethanol productivity was more than twice as high after treatment with $Ca(OH)_2$ compared with NaOH. The total concentration of phenolic compounds was affected by over-liming detoxification due to phenolics were most efficiently removed with this method (Larsson, 1999).

2.6.2.3 *Activated Charcoal Treatment*

Activated charcoal is a cost effective method with high capacity to absorb compounds without affecting the amount of sugar in hydrolysate (Chandel *et al.*, 2007). The activated charcoal treatment efficiency depends on pH, temperature, contact time, and the activated charcoal taken and the liquid hydrolysate volume ratio (Prakasham *et al.*, 2009).

2.6.2.4 *Ion Exchange Resins*

Ion exchange resins treatment was used to remove lignin–derived inhibitors, furfurals, and acetic acid. The ion–exchange resins based separation of fermentative inhibitors may not be cost effective (Lee *et al.*, 1999).

2.6.3 Biological Methods

The biological methods for detoxification are less side reactions, environmental friendly, smaller amount of energy requirements, and more feasible (Parawira and Tekere, 2011). The microorganisms and the enzymes have potential to alter the chemical nature of inhibitors. However, the slow reaction time and the loss of fermentable sugars make this method unattractive (Yang and Wyman, 2008).

2.7 **Response Surface Methodology (RSM)** (Carley *et. al.*, 2004)

Response Surface Methodology (RSM) is a statistical and mathematical technique beneficial for developing, improving, and optimizing processes. A low–order polynomial is appropriate to use. The first–order model is proper when interested in approximating the true response surface over a relatively small region of the independent variable space.

In general, the first–order model is expressed as following equation

$$Y_i = a_0 + \sum_{i=1}^{k} a_i x_i$$

Where Y_i is the response; x_i is the input variables, which influence the response variable Y_i; a_0 is the offset term; a_i is the *i*th linear coefficient.

The curvature in the true response surface is often strong enough that the first–order model is inadequate. A second–order model will be required. The following equation was used to correlate the dependent and independent variables of second–order model.

$$Y_i = a_0 + \sum_{i=1}^{k} a_i x_i + \sum_{i=1}^{k} a_{ii} x_i^2 + \sum_{i}^{k} \sum_{j}^{k} a_{ij} x_i x_j$$

Where Y_i is the response; x_i, x_j are the input variables, which influence the response variable Y_i; a_0 is the offset term; a_i is the *i*th linear coefficient; a_{ii} is the quadratic coefficient and a_{ij} is the *ij*th interaction coefficient.

The second–order model is generally applied in RSM for some reasons:

1. The second–order model is very flexible because of a wide variety of functional forms; therefore, it will work well as an approximation to the true response surface.
2. It is easy to estimate the parameters in the second–order model.
3. Second–order models perform well in solving real response surface problems.