

CHAPTER II

THEORETICAL BACKGROUND AND LITERATURE REVIEW

2.1 Biobutanol Production

Butanol (butyl alcohol) is an alcohol that has a four carbon primary in the formula (C₄H₉OH). Butanol is a colorless liquid with a specific odor and its vapor can cause the problem on mucid membranes and a narcotic effect in higher concentrations.

Moreover, butanol can be used to be an excellent diluent for break fluid formulations and solvent for the manufacturing of antibiotics, vitamins and hormones. An interested application of butanol is its can directly replace gasoline in the gasoline engine without modification and/or substitution because of similar characteristics (Lee *et al.*, 2008). The properties of butanol and other fuels are presented in Table 2.1 which is clearly seen that the octane number of butanol and gasoline are close.

Butanol can be produced from fermentation process by using bacteria of the genus *Clostridium*. Additionally, anaerobic conditions is needed in this process, and the main products are acetone-butanol-ethanol in the typical ratio 3:6:1. Normally, butanol which can be derived by fermentation step is called biobutanol. (Kamiński *et al.*, 2011).

Table 2.1 Properties of butanol and other fuels (Lee *et al.*, 2008)

	Properties of fuels			
	Butanol	Gasoline	Ethanol	Methanol
Energy density (MJ/L)	29.2	32	19.6	16
Air-fuel ratio	11.2	14.6	9	6.5
Heat of vaporization (MJ/kg)	0.43	0.36	0.92	1.2
Research octane number	96	91-99	129	136
Motor octane number	78	81-89	102	104

The biobutanol production process is presented in Figure 2.1. First, biobutanol can be produced from cellulosic feedstock by using pretreatment so as to disrupt complex structure of raw materials. After that, pretreated samples are

hydrolyzed by cellulose enzymes to produce C₅ and C₆ sugar and convert to biobutanol by *Clostridium beijerinckii* in fermentation step. Moreover, the high purity of biobutanol can be provided by using separation process.

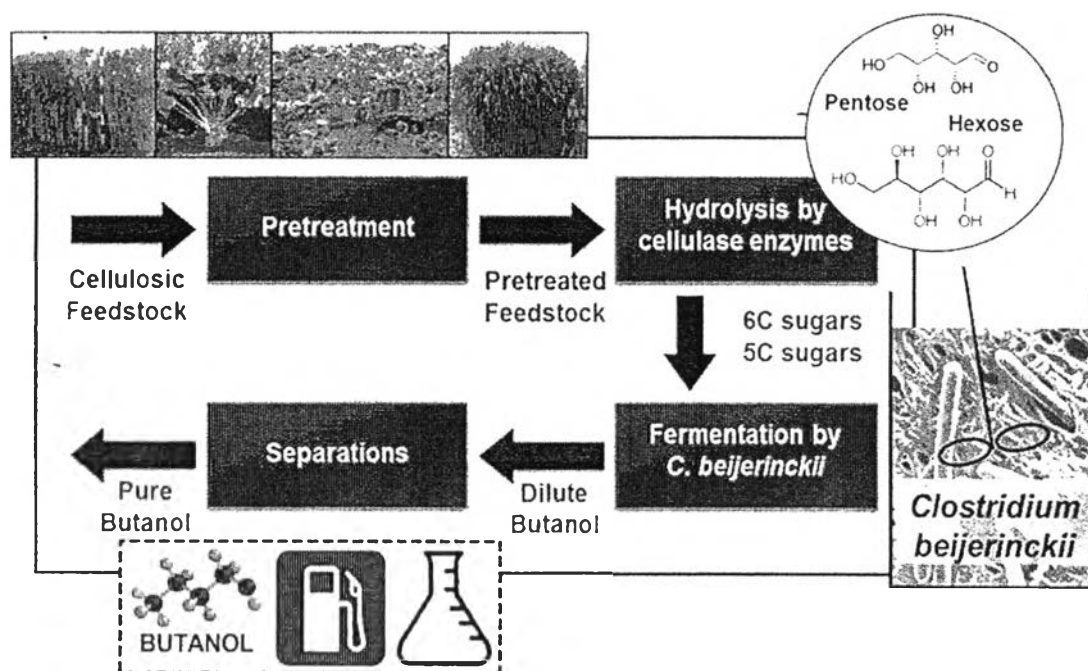


Figure 2.1 Biobutanol production process. (Using Bacteria to Produce Sustainable Fuels and Chemicals, 2012)

2.2 Lignocellulosic Biomass

Lignocellulosic biomass, the most abundant renewable bioresource, is mainly composed of three major components: cellulose (30-50 %), hemicelluloses (15-35 %), and lignin (10-30 %). Plant biomass offers the most potential platform for energy production.

2.2.1 Cellulose

Cellulose is the linkage of glucose units. It consists of a linear chain of D-glucose subunits linked to each other by β -(1,4)-glycosidic bonds presented in Figure 2.2 (A). While Figure 2.2 (B) shows cellulose fibers which are surrounded by

intra and intermolecular hydrogen making cellulose insoluble in water and the most organic solvents available (Holm and Lassi, 2011). The specific structure of cellulose favors the ordering of the polymer chains into tightly packed, highly crystalline structures that are water insoluble and resistant to depolymerization (Mosier *et al.*, 2005). Moreover, natural cellulose is muddled with hemicellulose and lignin. Consequently, lignocellulosic biomass is highly resistant hydrolysis (Ha *et al.*, 2011).

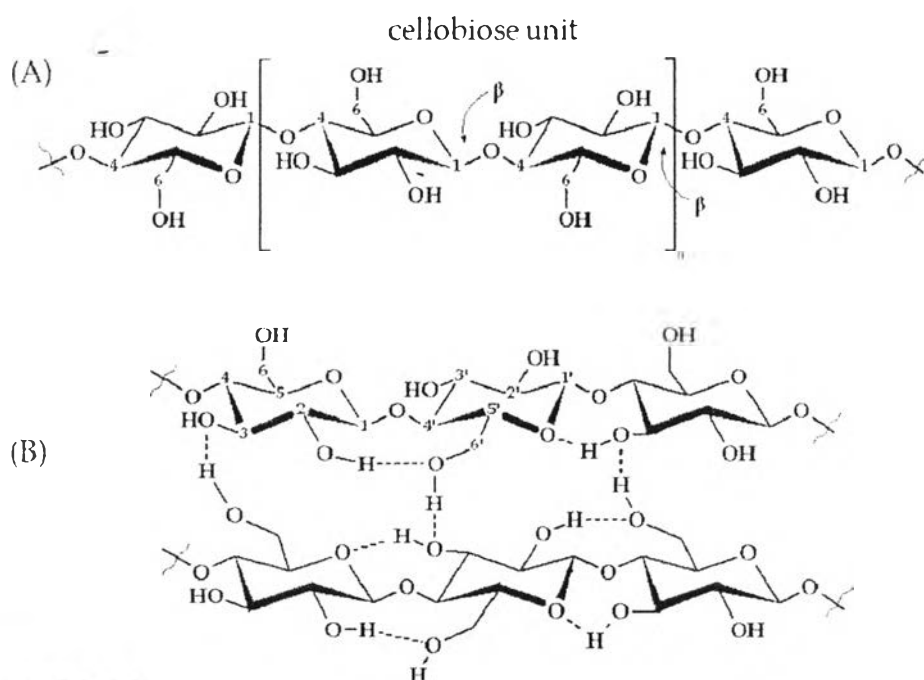


Figure 2.2 The structures of cellulose (Holm and Lassi, 2011).

2.2.2 Hemicellulose

Hemicelluloses are heterogeneous polymers of pentoses (xylose, arabinose), hexoses (mannose, glucose, galactose), and sugar acids, the structures of them are shown in Figure 2.3. Hemicelluloses are not chemically homogeneous and are completely amorphous. Xylan is the main hemicellulose in hardwoods, whereas softwood hemicelluloses contain mostly glucomannans (Saha, 2003). Hemicellulose has hydrogen-bonds to cellulose microfibrils, and forms a network that provides the structural backbone to plant cell wall (Mosier *et al.*, 2005).

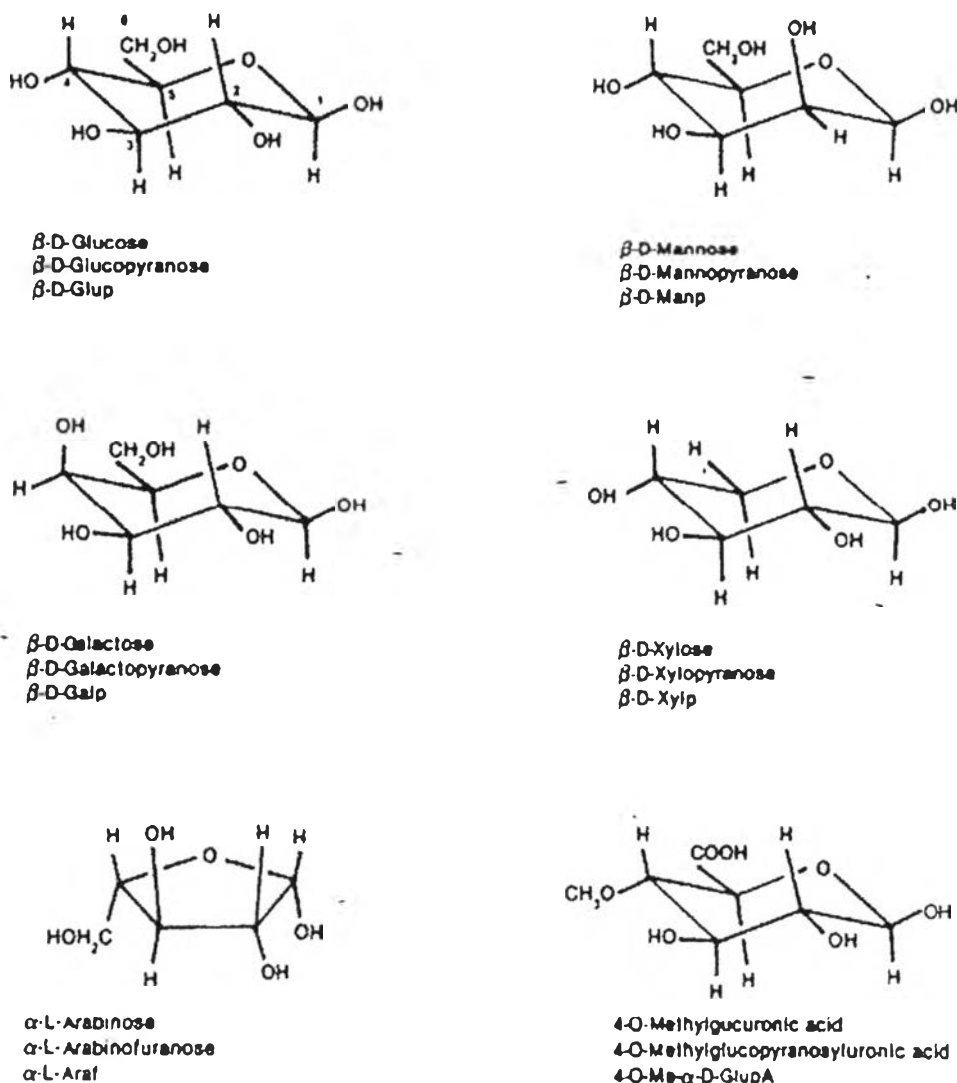


Figure 2.3 Monomer of hemicelluloses (Pettersen, 1984).

2.2.3 Lignin

Lignin is a polyphenolic polymer divided into three types of phenylpropanoid units that are considered as major precursors for biosynthesis of lignin: coniferyl, sinapyl, and *p*-coumaryl alcohol. The lignin macromolecule is linked via carbon-carbon and carbon-oxygen bonds among its phenylpropanoid building blocks with aryl ether bonds (β -O-4) being the most common and important inter unit linkage. All structures of lignin are shown in Figure 2.4. The effects of lignin on biomass enzymatic digestibility have received extensive attention. In general, the lower lignin content, a plant biomass has the higher the bioavailability of

the substrate for bioproduction. Moreover, other factors that impact biomass digestibility may be lignin ingredient, its chemical structures, and lignin-carbohydrate complex (LCC) linkages in biomass (Pu *et al.*, 2013).

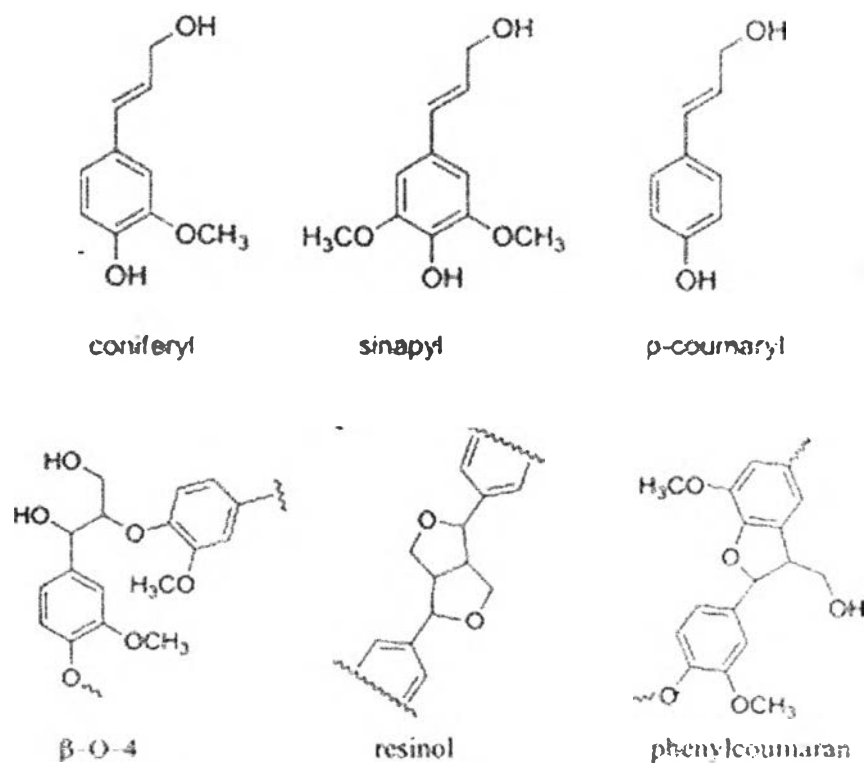


Figure 2.4 Types of lignin (Pu *et al.*, 2013).

2.3 Pretreatment of Lignocellulosic Biomass

Pretreatment is a process that can be used to modify the size and structure of biomass in order to increase the yield of hydrolyzed carbohydrate fraction to monomeric sugars (Mosier *et al.*, 2005).

The goal of lignocellulosic materials pretreatment is to break the lignin barrier, reduce the cellulose crystallinity and increase the cellulose porosity, that is presented in Figure 2.5. In addition, a pretreatment can alter its physical properties of lignocellulosic biomass such as degree of polymerization, crystallinity and the surface area leading to an enhancement of cellulases accessibility to convert cellulose to glucose (Holm and Lassi, 2011).

A high-efficiency pretreatment of biomass materials can be determined by the suitable methods for the particular biomass which can give a high yield because of a different resistant of each feedstocks, high sugar concentration production which can be derived from the biomass, the increase surface area and pore size of cell walls which can indicate the removal of complex structure of biomass, less corrosion of instruments and mild condition operation which can save the cost of pretreatment and applying to large scale for commercial production (Kumar *et al.*, 2009).

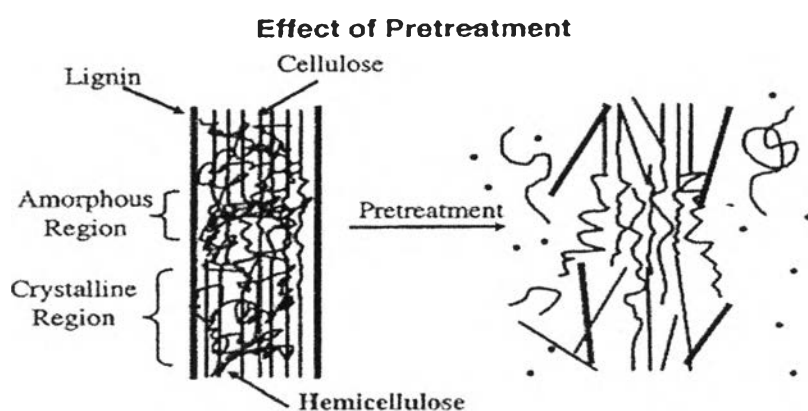


Figure 2.5 Effect of pretreatment of lignocellulosic biomass (Mosier *et al.*, 2005).

Pretreatment methods can be roughly divided into different types: physical, chemical, and biological pretreatments.

2.3.1 Physical Pretreatment

2.3.1.1 *Mechanical Comminution*

Chipping, grinding, and milling can be used to decrease cellulose crystallinity. The size of the materials is usually 10-30 mm after chipping and 0.2-2 mm after milling or grinding (Kumar *et al.*, 2009). For mechanical pretreatment, capital costs, operating costs, scale-up possibilities and depreciation of equipment are very important factors (Harmsen *et al.*, 2010). Moreover, the suitable final particle size of each agricultural materials relates to the power requirement which has to determine (Kumar *et al.*, 2009).

2.3.1.2 Pyrolysis

Pyrolysis is used for the pretreatment of lignocellulosic materials. When lignocellulosic materials is treated at temperatures higher than 300 °C, cellulose decomposes to gaseous products and residual char occurs. Fan *et al.* (1987) reported that products from pyrolysis pretreatment at mild acid hydrolysis (1 NH₂SO₄, 97 °C, 2.5 h) resulted in 80-85% conversion of cellulose to reducing sugars with more than 50% glucose. However, high operation temperature and ash production are the main problem of this method (Kumar *et al.*, 2009).

2.3.2 Physico-chemical Pretreatment

2.3.2.1 Steam Explosion (Autohydrolysis)

Steam explosion pretreatment is the most commonly used method for lignocelluloses, especially in commercial processes. In this method, high-pressure saturated steam with or without chemicals is used to heat a biomass and suddenly released after holding for few minutes and materials undergo an explosive decompression (Sun and Cheng, 2002). The typical conditions of steam explosion are 160-260 °C of temperature and pressure of 0.69-4.83 MPa. Hemicellulose degradation and lignin transformation was occurred due to high temperature leading to increasing the potential of cellulose hydrolysis (Kumar *et al.*, 2009). The advantages of this method are using less hazardous chemicals and conditions and reducing the environmental impact (Avellar and Glasser, 1998). However, the steam explosion method also has its bad points such as incomplete disruption of lignin and the generation of toxic chemicals, which may affect the downstream processes.

2.3.2.2 Liquid Hot Water Pretreatment (LWH)

In liquid hot water pretreatment, pressure is utilized to maintain water in the liquid state at elevated temperatures. Biomass is operated at high temperature mixing in water with high pressure. LHW pretreatment has the potential to enhance cellulose digestibility, sugar extraction, and pentose recovery, with the advantage of producing prehydrolyzates containing little or no inhibitor of sugar fermentation (Zheng *et al.*, 2009). In this process, 40-60% of the total biomass is dissolved, with 4-22% of the cellulose, 35-60% of the lignin and all of the hemicellulose being removed (Harmsen *et al.*, 2010).

2.3.2.3 Ammonia Fiber Explosion (AFEX)

The AFEX pretreatment method is quite similar to the steam explosion method. In AFEX pretreatment, lignocellulosic biomass is exposed to liquid ammonia at a moderate temperature (60-100°C) and a high pressure (250-300 psi) for a period of time, and then the pressure is rapidly reduced. This method provides high surface areas and results in better digestibility and enzyme accessibility (Teymouri *et al.*, 2005). There are also no generated toxic chemicals for downstream processes. However, it does not significantly remove hemicelluloses, which may reduce enzyme accessibility and final sugar yield (Zhang *et al.*, 2007).

2.3.3 Chemical Pretreatment

2.3.3.1 Ozonolysis

Ozone can be used to decrease lignin and hemicelluloses contents in many lignocellulosic materials such as wheat straw, bagasse, green hay, peanut, pine, cotton straw, and poplar sawdust. Lignin is mainly affected while hemicellulose is slightly affected, but cellulose is not. An advantage of ozonolysis pretreatment is the reactions can be operated at room temperature and normal pressure. Furthermore, ozone can be easily decomposed by using a catalytic bed or increasing the temperature. However, ozonolysis pretreatment requires a large amount of ozone, which can make the process expensive (Kumar *et al.*, 2009).

2.3.3.2 Organosolv Process

Organosolv pretreatment is a pulping technique that dissolves lignin and hemicellulose in an organic solvent such as methanol and ethanol while cellulose remains as undissolved solids. The good points of organosolv pretreatment are it can be used for all types of biomass feedstocks, it can produce high-quality lignin by-products, it is easy to recycle the solvent, and it loses the minimum of celluloses and chemicals from hemicelluloses. However, organosolv pretreatment is limited because of the high operation cost and high cost of organic solvent (Zhao *et al.*, 2009).

2.3.3.3 Acid Pretreatment

Concentrated acids, such as H₂SO₄ and HCl, have been widely used for treating lignocellulosic materials because they are powerful agents for cellulose hydrolysis (Sun and Cheng, 2002), and they need no enzymes

subsequent to the acid hydrolysis (Harmsen *et al.*, 2010). Although they are powerful agents for cellulose hydrolysis, these acids can cause toxicity, corrosion, and hazard. Moreover, reactors being resistant to corrosion are required, which makes the pretreatment process very expensive. In addition, the concentrated acid must be recovered after hydrolysis to make the process economically possible (Kumar *et al.*, 2009).

Dilute-acid hydrolysis has been successfully developed for pretreatment of lignocellulosic materials. There is a report of using dilute H₂SO₄ pretreatment that can achieve high reaction rates and improve cellulose hydrolysis (Esteghlalian *et al.*, 1997). Moreover, dilute acid effectively removes and recovers most of the hemicelluloses as dissolved sugars. And hemicelluloses removal to almost 100% for complete hemicelluloses hydrolysis gives high glucose yields due to an exposure of cellulose in biomass.

Adnan *et al.* (2013) found the best condition of dilute acid pretreatment of water hyacinth (WH) biomass which was a renewable resource for the production of bioethanol. In this study, response surface methodology had been employed for finding the optimization of temperature (°C), time (h), and different concentrations of maleic acid (MA), sulfuric acid (SA), and phosphoric acid (PA). The pretreated biomass produced 39.96 g/L, 39.86 g/L and 37.9 g/L of reducing sugars and enzymatic hydrolysis yields were 79.93, 78.71 and 75.9 % from PA, MA, and SA treated, respectively. So the effectiveness of acid for hydrolysis yield was found to be phosphoric acid > maleic acid > sulfuric acid. And the resulting sugars were fermented to ethanol, with yield 0.484 g/g of reducing sugars that was 95 % of theoretical yield (0.51 g/g glucose) by using commercial baker's yeast (*Saccharomyces cerevisiae*). Moreover, the acid pretreatment could hydrolyze all of hemicelluloses presenting in WH so the rest was pure cellulose for enzymatic saccharification that produced pure glucose for fermentation into ethanol by common Baker's yeast.

2.3.3.4 Alkaline Pretreatment

Some alkaline can be used for the pretreatment of lignocellulosic materials, and the alkaline pretreatment mainly affects the lignin content of the materials (Fan *et al.*, 1987; McMillan, 1994). Alkaline pretreatment

processes are operated at lower temperatures and pressures than other pretreatment technologies (Mosier *et al.*, 2005). However, these processes require long time of period (hours or days instead of minutes or seconds). The advantage of alkaline process to acid process is alkaline process causes less sugar degradation, and many of the caustic salts that can be recovered or regenerated (Tabil *et al.*, 2011). Alkaline pretreatments usually use sodium, potassium, calcium, and ammonium hydroxide as reactants. However, NaOH has received the most attention (MacDonald *et al.*, 1983; Fox *et al.*, 1989; Soto *et al.*, 1994). Han *et al.* (2012) studied NaOH pretreatment of wheat straw. Mixture of the wheat straw and NaOH (0.25-1.5% w/v) was treated at 121 °C and 15 psi for 0.5 to 2.5 h at a solid loading condition of 10% (w/v). The effects of reaction time, substrate concentration, temperature, and pH on the enzymatic hydrolysis was found by maintaining the enzyme/substrate ratio at 25 FPU/g. The result showed that wheat straw treated with 1.0% of NaOH for 1.5 h increased the content of cellulose by 44.52%, while decreased the content of hemicellulose and lignin by 44.15% and 42.45%, respectively. The surface structure of the wheat straw and lignin was significantly changed after pretreatment, which is more favorable for the hydrolysis reaction. The optimal condition for the cellulase hydrolysis was 30 h of reaction time, 3% of substrate, 55 °C of temperature operation and pH 5.0.

Wang (2008) studied alkaline (NaOH and $\text{Ca}(\text{OH})_2$) pretreatment of coastal Bermuda grass for enhanced reducing sugars recovery. The sample was pretreated at 121°C using 1%, 2%, and 3% (w/v) NaOH for 15, 30, 60, and 90 min. Moreover, lower NaOH concentrations (0.5% and 0.75%) and lower temperatures (50, 80, and 100°C) were also examined. $\text{Ca}(\text{OH})_2$ pretreatment of the biomass was operated at room temperature, 50, 80, and 121°C. Total reducing sugars, glucose and xylose were analyzed. The maximum total reducing sugars production was found to be pretreated at 121°C for 30 min using 1% NaOH. The highest reducing sugars yield was approximately 86% of theoretical maximum for NaOH pretreatment. And NaOH is more efficient than $\text{Ca}(\text{OH})_2$ at 121°C for improved reducing sugars yield. In case $\text{Ca}(\text{OH})_2$ pretreatment, the results showed that increasing temperature reduced the optimal pretreatment time at the same

loading and the reducing sugars production under optimal pretreatment times was enhanced by 8% of theoretical maximum from room temperature to 80°C.

2.3.3.5 Ionic Liquids Pretreatment

Ionic liquids (ILs) are new organic salts being liquids at relatively low temperatures (below 100°C) and consist of an organic cation and an inorganic anion which are shown the example in Figure 2.6. Ionic liquids exhibit interesting properties such as high thermal stabilities, broad liquid temperatures, negligible vapor pressures (Van Rantwijk and Sheldon, 2007). Moreover, no toxic or explosive gases are formed when ionic liquids is used (Anderson *et al.*, 2002). However, their physical and chemical properties such as melting points, viscosity, hydrophobicity, and hydrolysis stability are changed depending on anion and cation of ionic liquids (Huddleston *et al.*, 2001). And the found problem of ionic liquids is most of ionic liquids are viscous similar to oils that can affect the mass transfer and power requirements for the systems.

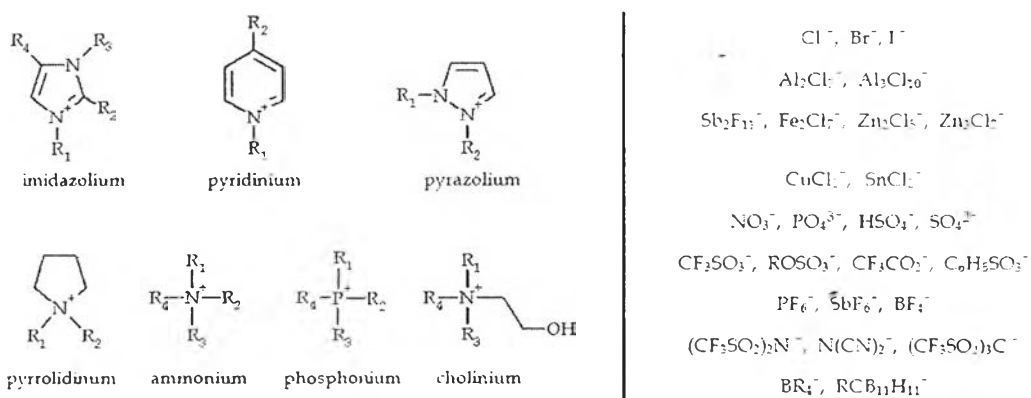


Figure 2.6 Main cations and anions in ILs (Olivier-Bourbigou *et al.*, 2010).

The interest ability of ionic liquid for pretreatment is separation of lignin and hemicelluloses from cellulose. After biomass is pretreated by using ionic liquids, dissolved cellulose can be precipitated when an anti-solvent such as water is added (Fort *et al.*, 2007). However, the ability of ionic liquids to dissolve cellulose depends on degree of polymerization and crystallinity of the native cellulose on the operating conditions (temperature, reaction time, initial

concentration of cellulose in the ILs) and the presence of contaminants (Holm and Lassi, 2011).

2.3.3.5.1 *Effect of the Anion on the Dissolution*

It is reported that the higher anion concentration will give the better solubilization and strong electronegativity (Dadi *et al.*, 2006). However, it is found that the halogen anion containing ionic liquids such as [BMIM][Br] and [BMIM][I], is not good solvents for cellulose (Zavrel *et al.*, 2009). When applying the high temperature in pretreatment process, some organohalogenides which have uncertain toxicity and are hazardous, can be produced. Nevertheless, acetate-based ionic liquids are found to be interesting because of their low melting point, lower viscosity, less toxic, and corrosion character compared to the chloride-based ionic liquids (Feng and Chen, 2008).

2.3.3.5.2 *Effect of the Cation on the Dissolution*

There is reporting that the interaction between cation and ionic liquid is weak (El Seoud *et al.*, 2007) but their role in the dissolution mechanism should not be neglected (Dadi *et al.*, 2006). The introduction of an allyl group on the imidazolium cation provides excellent improvements in dissolving of cellulose but increasing the length of the alkyl chain on the dialkyl imidazolium cation (with chloride anion) will decrease the cellulose solubilization (Olivier-Bourbigou *et al.*, 2010). Moreover, if the cation is large, the ability to form hydrogen bonds with cellulose will drop (Zhao *et al.*, 2008). Zhang *et al.* (2005) discovered that [AMIM]⁺ is more powerful in the dissolution of cellulose than [BMIM]⁺ because of its smaller size. Similarly, Kosan *et al.* (2008) showed that [EMIM][Cl] was a better alternative than [BMIM][Cl], due to the size of the cation.

2.3.3.5.3 *Dissolution Mechanism in Ionic Liquids*

Ionic liquids polar characteristics, the basic form of the ionic liquid anions and their ability to generate H-bonds are the main properties of ILs that affect the dissolution and functional modification of cellulose and carbohydrates. And the hydrogen's bonding ability that present in the IL anion is the most important promotion (Olivier-Bourbigou *et al.*, 2010). Figure 2.7 shows the mechanism of dissolution of cellulose in [BMIM][Cl]. The dissolution is started by the anion [Cl]⁻, which must be a good hydrogen bond acceptor, will form hydrogen

bond with hydrogen atoms of the hydroxyls group of cellulose while the cation $[\text{BMIM}]^+$ that has the most moderate activated hydrogen, will also form hydrogen bonding with oxygen atoms of the hydroxyls group of cellulose. From anion and cation bonding with hydrogen and oxygen, the following effects of structure of cellulose are cellulose network will break and the crystallinity of cellulose will decrease (Zhang and Zhao, 2010; Holm and Lassi, 2011).

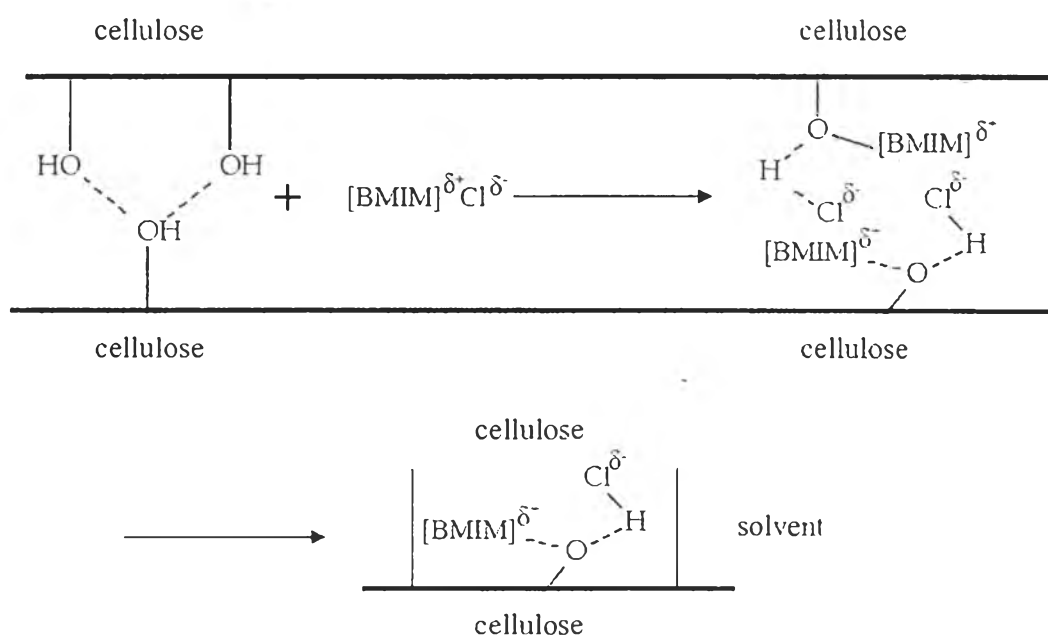


Figure 2.7 Dissolution mechanism of cellulose in $[\text{BMIM}][\text{Cl}]$ (Feng and Chen, 2008).

2.3.3.5.4 Comparison of Ionic Liquids

There were many studies about comparisons of IL types and ILs pretreatment compared to other pretreatments.

Li *et al.* (2010) studied the pretreatments of switchgrass between dilute acid (1.2% (w/w) sulfuric acid) and ionic liquid pretreatment (1-ethyl-3-methylimidazolium acetate $[\text{EMIM}][\text{Ac}]$) at 160 °C for 20 min and 160 °C for 3 h, respectively. The result showed that when compared with the untreated materials, both dilute acid and ionic liquid pretreatment removed lignin and hemicellulose and produced higher glucan content, ranging from 50.4 to 67.7%.

Ionic liquid pretreatment removed more lignin and less hemicellulose than dilute acid pretreatment, producing recovered material with lower levels of residual lignin (13.6% and 28.5%, respectively) and higher levels of residual xylan (7.6% and 4.4%, respectively). Moreover, ionic liquid-pretreated switchgrass exhibited significantly higher saccharification kinetics, with cellulose digestibility reaching 96.0% within 24 h, whereas digestibility of dilute acid pretreated switchgrass only reached 47.6% over the same time interval. Initial rates of ionic liquid were 16.7 times greater than dilute acid pretreatment and up to 54.4 times greater than untreated switchgrass.

Haykir *et al.* (2013) studied the ionic liquid pretreatment of cotton stalk via several ionic liquids; 2-hydroxy ethyl ammonium formate [HEAF], 1-allyl-3-methyl imidazolium chloride [AMIM][Cl], 1-butyl-3-methyl imidazolium chloride [BMIM][Cl], 1-ethyl-3-methyl imidazolium chloride [EMIM][Cl] and 1-ethyl-3-methyl imidazolium acetate [EMIM][Ac] at 150 °C for 30 min. The result showed that extracted lignin via ionic liquid pretreatment ranged between 31% and 46%. The extracted lignin was similar for [EMIM][Ac] and [AMIM][Cl] pretreated cotton stalk samples; 45% and 46%, respectively. Upon [HEAF] and [EMIM][Cl] pretreatments, 38% of lignin was removed from cotton stalk. And the highest biomass digestibility was obtained via [EMIM][Ac] pretreatment, which was 65% at the 72nd h of enzymatic hydrolysis, being almost 9-fold more digestible than untreated cotton stalk. The biomass digestibilities were 26%, 26%, and 25% for pretreatments conducted via [HEAF], [EMIM][Cl], and [AMIM][Cl], respectively. Furthermore, the lowest digestibility was attained for [BMIM][Cl] pretreated cotton stalk (19%).

Fu and Mazza (2011) studied the aqueous ionic liquid [EMIM][Ac] pretreatment and compared with pure ionic liquid pretreatment and dilute acid pretreatment of triticale straw. The results showed that when increasing ionic liquid concentration, an extraction of lignin was increased but the crystallinity index of the pretreated samples were decreased leading to an enhancement of sugar production. Moreover, the highest of sugar released was found by 50% ionic liquid concentration which was 81% being higher than using pure ionic liquid (67.4%) and dilute acid pretreatment (47%) because when using aqueous ionic liquid

pretreatment, more polysaccharides were retained which can be converted to more fermentable sugar.

2.3.3.5.5 Effect of Ionic Liquid and Recycled Ionic Liquid Pretreatment on Biomass Composition

To reduce the cost and amount of ionic liquids, recycle is the interesting way by evaporating water. Recycled ionic liquid gave a comparable glucose yield to original ionic liquids. Nguyen (2010) studied the efficiency of recycled [EMIM][Ac] as shown in Figure 2.8. The results showed that glucose conversion from starting ionic liquid to the 5th recycled ionic liquid were constant and clearly decreased after the 5th recycling for pure ionic liquid and ammonia-ionic liquid combined.

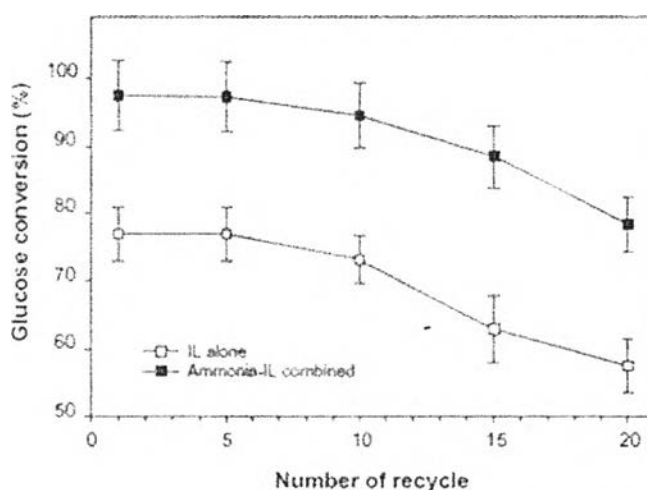


Figure 2.8 Comparison of recycling of ionic liquid in ionic liquid alone and ammonia-ionic liquid combined treatment (Nguyen *et al.*, 2010).

2.3.4 Biological Pretreatment

In biological pretreatment processes, microorganisms such as brown-, white- and soft-rot fungi are used to remove lignin and hemicellulose in waste materials (Galbe, 2007). Brown rots mainly attack cellulose, while white, and soft rots attack both cellulose and lignin. White-rot fungi are the most effective for biological pretreatment of lignocellulosic materials (Fan *et al.*, 1987). Low energy

consumption and mild environmental conditions are the advantages of biological pretreatment whereas very low rate of hydrolysis in most biological pretreatment processes is the bad point (Sun and Cheng, 2002).

2.4 Microwave Technology

In the electromagnetic spectrum, the microwave has wavelengths of 1 mm - 1 m and frequencies between 0.3 and 300 GHz located between infrared radiation and radio waves. There are reports for a long time that microwave can be used to heat materials which can be food or even organic compounds. Normally, most organic reactions have been heated using traditional heat transfer equipment such as oil baths, sand baths and heating jackets. Heat is driven into the substance, passing first through the walls of the vessel in order to reach the solvent and reactants (Hayes, 2004). However, these techniques are rather slow and a temperature gradient can develop within the sample which can occur an overheating leading to a decomposition of product, substrate and reagent. While the microwave will pass through the walls of the vessel and heat only the reactants and solvent, not the reaction vessel itself so the temperature increase will be uniform throughout the sample, which can decrease by-products formation and products decomposition (Lidström *et al.*, 2001).

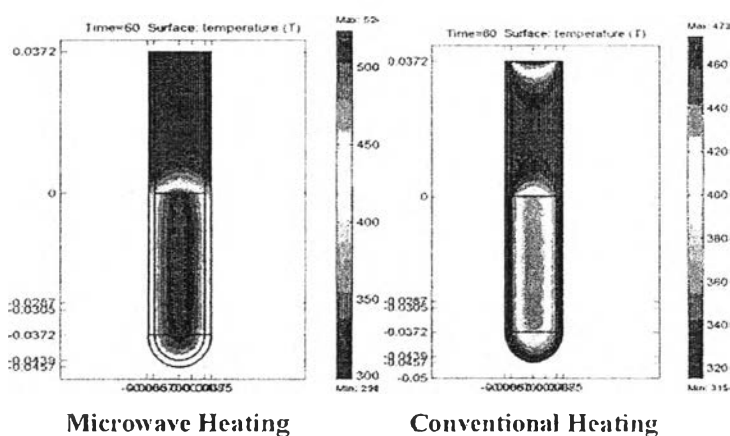


Figure 2.9 The temperature profile after 60 sec heated by microwave heating compared to treatment in oil bath. (De la Hoz *et al.*, 2005)

2.4.1 Microwave Heating for Chemical Pretreatment

Ha *et al.* (2011) studied the effect of microwave irradiation on cellulose dissolution pretreatment with ILs ([EMIM][Ac] and [BMIM][Cl]) followed by enzymatic hydrolysis. The ILs pretreatment condition was 110°C for 30 min and water was used to be an anti-solvent. The result showed that the cotton celluloses regenerated from ILs dissolution pretreatment with microwave irradiation were hydrolyzed much faster than those without microwave irradiation. After 3.5 h of enzymatic hydrolysis, 14.4 and 13.2 mg/mL of reducing sugars were released from the celluloses regenerated from [BMIM][Cl] and [EMIM][Ac] dissolution pretreatment with microwave irradiation, respectively. In contrast, only 6.4 and 5.8 mg/mL of reducing sugars were released from the celluloses regenerated from [BMIM][Cl] and [EMIM][Ac]-dissolution pretreatment alone, respectively. Moreover, IL dissolution pretreatment with microwave irradiation led to the decrease in degree of polymerization (DP) compared to a conventional heating. Regenerated cotton after [EMIM][Ac] and [BMIM][Cl] pretreatment with microwave irradiation, presented 96% and 97% reductions in DP, respectively, whereas only 19% and 26% were observed in cotton regenerated with conventional heating.

Cheng *et al.* (2011) studied the effects of NaOH concentration, time and temperature in microwave oven of rice straw pretreatment which can improve saccharification in enzymatic hydrolysis. Moreover, the comparison of conventional heating and microwave heating was also investigated in this work. -From the study found that a maximum reducing sugar yield of 69.3 g/100 g of total volatile solid rice straw (96.7% of the theoretical yield) was produced by using 50 g/L of rice straw loading pretreated by 0.5% NaOH at 140 °C for 15 min. Additionally, the structure of pretreated rice straw was changed as compared to native rice straw. Micropores and grooves were generated and the specific surface area was increased which can indicate that lignin was destroyed by NaOH and cellulose can be exposed and was favorable for cellulase enzyme to hydrolyze into the reducing sugar. And the results of rice straw after pretreatment at the same loading but different heating showed that the microwave heating used the less time pretreatment but can give more reducing sugar yield than the conventional heating when pretreated rice straw was hydrolyzed for the same condition.

2.5 Enzymatic Hydrolysis

Hydrolysis is the process that convert lignocellulosic biomass to fermentable sugars. The popular hydrolysis method is to use enzymes called cellulases. Cellulases are proteins that consist of three major groups: endoglucanase, exoglucanase and β -glucosidase. Endoglucanase will hit low crystallinity cellulose fibers while exoglucanase will hydrolyze the 1, 4-glycosidyl linkages to form cellobiose. And β -glucosidase will use for glucose production by converting cello-oligosaccharide and disaccharide cellobiose. Unless three major groups, hemicelluloses are attacked by other enzymes such as xylanase, galactomannase and glucomannase. The important reason of using enzymes in hydrolysis step is can operate at mild condition. However, the relatively unstable at high temperature, the cost of enzymes purification and the difficulty to separate from the reaction mixtures are the drawback (Alessandra *et al.*, 2012).

2.6 Response Surface Methodology (RSM)

Response surface methodology (RSM) is a mathematical collection and statistical techniques based on the fit of a polynomial equation to the experimental data, which must describe the behavior of a data set with the objective of making statistical previsions. The objective of RSM is to optimize the levels of the variables to attain the best system performance.

The simplest model which can be used in RSM is the first order model or a linear function presented in the following equation:

$$y = \beta_0 \sum_{i=1}^k \beta_i x_i + \varepsilon \quad (2.1)$$

where k is the number of variables, β_0 is the constant term, β_i is the coefficients of the linear parameters, x_i is the variables, and ε is the residual associated to the experiments.

Therefore, the responses should not present any curvature. In order to determine a critical point, a second-order or a polynomial function model must be required according to the equation presented below:

$$y = \beta_0 + \sum_{i=1}^k \beta_i x_i + \sum_{i=1}^k \beta_{ii} x_i^2 + \sum_{1 \leq i < j \leq k} \beta_{ij} x_i x_j + \varepsilon \quad (2.2)$$

where β_{ii} represents the coefficients of the quadratic parameter and β_{ij} represents the coefficients of the interaction parameters.

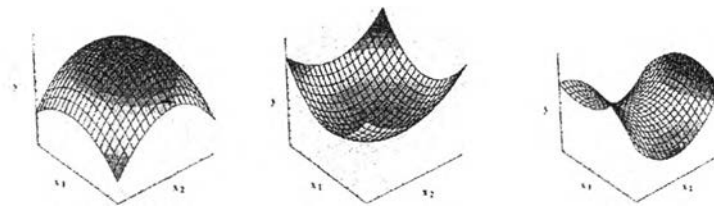


Figure 2.10 Response surface results (Bezerra *et al.*, 2008).

To estimate the parameters in Eq. (2.2), the experimental design has to assure that all studied variables are carried out at in at least three factor levels. Thus, two modeling, symmetrical response surface designs are available. The response surface results could be maximum, minimum, or saddle surfaces which were presented in Figure 2.10 (Bezerra *et al.*, 2008).

2.6.1 Full Three-Level Factorial Designs

The full three-level factorial design consists of all the combinations of the levels of the k control variables which have three levels each. If the levels are equally spaced, then the code level can be corresponded to -1, 0, 1. The number of experimental runs for this design is 3^k , which can be very large for a large k (Khuri and Mukhopadhyay, 2010). Figure 2.11 presented the full three-level factorial design for two variables.

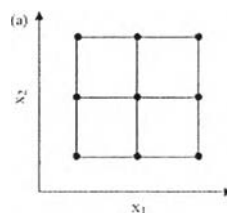


Figure 2.11 Full three-level factorial design for the optimization of two variables (Bezerra *et al.*, 2008).