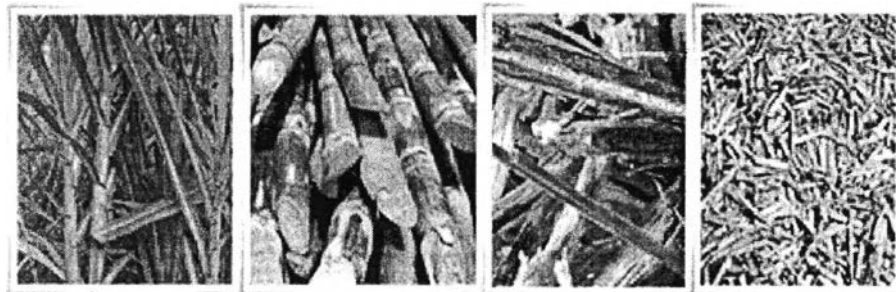


## CHAPTER II

### LITERATURE REVIEW

#### 2.1 Lignocellulosic Biomass

Currently, biofuels become alternative energy due to oil shortage, oil prices rise, and environmental concerns (Lucia, 2008; Kumar *et al.*, 2009; Luo *et al.*, 2013). It is derived from lignocellulosic biomass. Previously, the first generation biofuels come from food crop such as sugarcane, corn, and palm oil. The first generation biofuels can reduce greenhouse gas emission, oil imports, and support for agricultural industries (Sims *et al.*, 2008; Lopes *et al.*, 2013). However, the major disadvantage is rising food prices due to an increase in production of fuels. Therefore, lignocellulosic biomass is used as second generation biofuels. Lignocellulosic biomass is the most abundant and low-cost biomass for production of biofuels (Verma *et al.*, 2011). This feedstock comes from non-food crops, agricultural wastes, and forest residue, as shown in Figure 2.1. The advantage of this feedstock is a reducing of agricultural waste such as sugarcane bagasse, corn stover, and straw. Moreover, it decreases greenhouse gas emissions and do not compete with food crops (Naik *et al.*, 2010).



**Figure 2.1** Lignocellulosic biomass

([http://www.biotec.or.th/EN/images/stories/News/2010/sugarcane\\_all.jpg](http://www.biotec.or.th/EN/images/stories/News/2010/sugarcane_all.jpg)).

Lignocellulosic biomass consists of polymeric carbohydrate (cellulose and hemicellulose), aromatic polymer (lignin), pectin, inorganic compounds, protein, and

extractives (Brandt *et al.*, 2013). Major component of lignocellulosic biomass is cellulose, hemicellulose, and lignin. The composition of lignocellulosic biomass depends on its source and types of biomass (Harmsen *et al.*, 2010), as shown in Table 2.1.

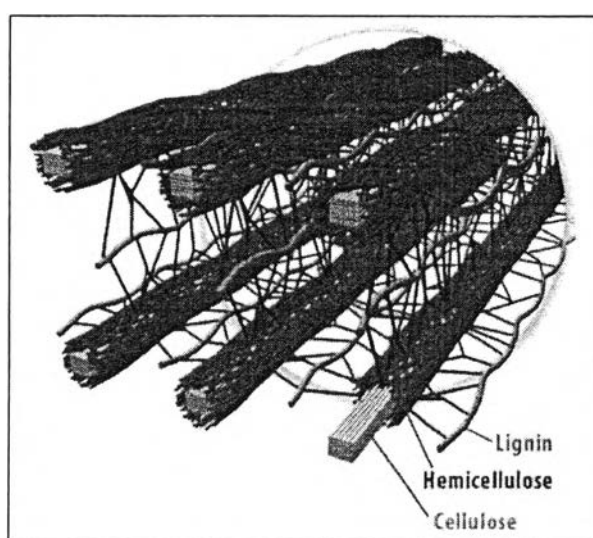
**Table 2.1** Cellulose, hemicellulose, and lignin contents in common agricultural residues and wastes (Kumar *et al.*, 2009; Luo *et al.*, 2013)

<b>Lignocellulosic material</b>	<b>Cellulose (%)</b>	<b>Hemicellulose (%)</b>	<b>Lignin (%)</b>
Hardwood 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
Solid cattle manure	1.6-4.7	1.4-3.3	2.7-5.7
Coastal bermudagrass	25	35.7	6.4
Switchgrass	45	31.4	12
Swine waste	6	28	NA

Cellulose and hemicellulose are also called holocellulosic biopolymers. They are valuable component of lignocellulosic biomass. Their applications are used

in paper and biofuels. Otherwise, lignin is also used to produce heat and electricity to paper mills and biorefineries (Varanasi *et al.*, 2013).

Generally, cellulose forms skeleton structure, but hemicellulose and lignin surround it. Hemicellulose acts as matrix and lignin cover materials. They are connected and cross-linkages occur between lignin and polysaccharides (Holm and Lassi, 2011) via ester and ether linkages (Verma *et al.*, 2011), as illustrated in Figure 2.2.



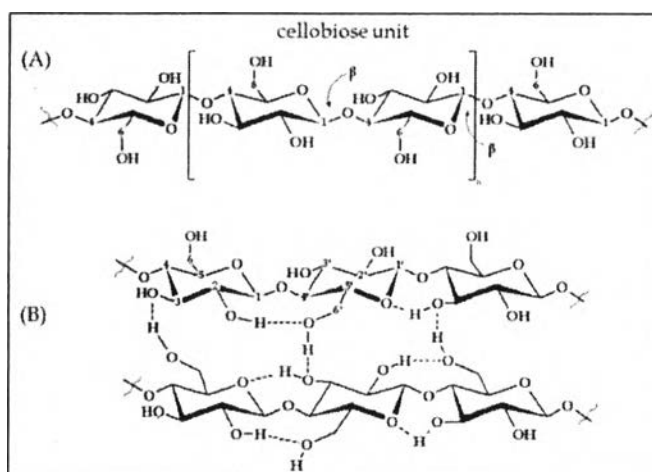
**Figure 2.2** Lignocellulosic biomass structure (Brandt *et al.*, 2013).

### 2.1.1 Cellulose

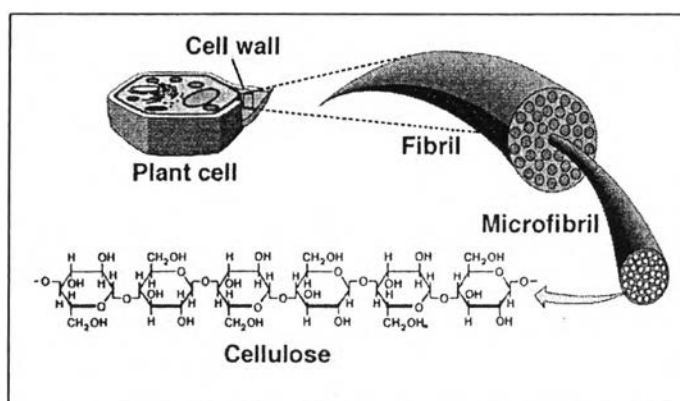
Cellulose is the main composition of lignocellulosic biomass (Brandt *et al.*, 2013) and structure of cell wall. It is a polymer of glucose because cellobiose comprise of two molecules of glucose (Harmsen *et al.*, 2010) and its monomer is glucopyranosyl. This monomer is linked by 1,4- $\beta$  glycosidic bonds (Brandt *et al.*, 2013). The polymer is arranged in long straight chains because bond between the glucose molecules. Hydroxies are on both side of the monomers. They form hydrogen bond between the molecules of cellulose and become the formation of a compound which is consisted of parallel chains attached to each other, as shown in Figure 2.3 (Harmsen *et al.*, 2010).

Cellulose is insoluble in water and organic solvents because intra- and intermolecular hydrogen bond (Holm and Lassi, 2011). Moreover, cellulose is high molecular weight and low flexibility of cellulose polymer chains (Brandt *et al.*, 2013).

In addition, cellulose composes of both crystalline and amorphous structure. Crystalline structure occur from the cluster of polymer chains that form microfibrils and change to macrofibrils (or fibril), as shown in Figure 2.4 (Harmsen *et al.*, 2010).



**Figure 2.3** The cellulose network: A is the cellulose chain and B shows inter and intra H-bonds present in cellulose (Holm and Lassi, 2011)

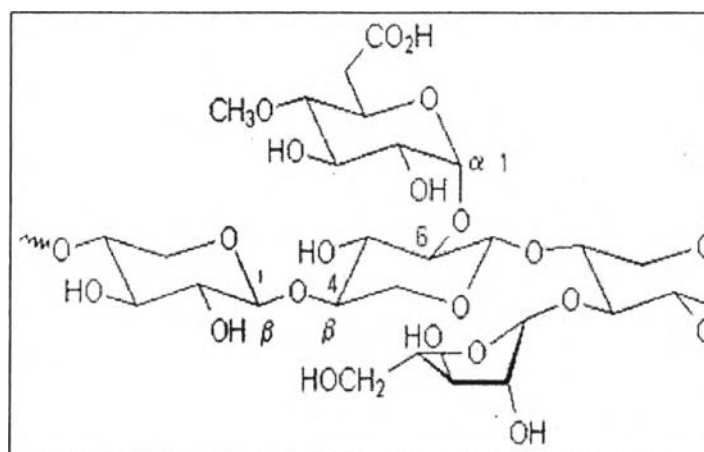


**Figure 2.4** Formation of micro- and macrofibrils (fibril) of cellulose (Harmsen *et al.*, 2010).

### 2.1.2 Hemicellulose

It is a copolymer that contains a group of polysaccharides such as arabino-xylans, gluco-mannans, galactans, and others. Normally, the common type of hemicellulose is xylan (Harmsen *et al.*, 2010) and the structure of hemicellulose is completely amorphous (Holm and Lassi, 2011) because its structure contain high branch and acetyl groups connected to the polymer chain. In addition, hemicellulose is insoluble in water at low temperature. But acid can increase the solubility of hemicellulose in water (Harmsen *et al.*, 2010).

Hemicellulose binds non-covalent to the surface of cellulose and holds the stiff cellulose in place. The replacement of hydrophobic groups such as acetyl and methyl groups increase the relation of hemicellulose to lignin and helps the cohesion between the three major lignocellulosic polymers (Brandt *et al.*, 2013). The structure of hemicellulose is shown in Figure 2.5.



**Figure 2.5** Hemicellulose structure

([http://students.chem.tue.nl/ifp19/biomass\\_files/image006.jpg](http://students.chem.tue.nl/ifp19/biomass_files/image006.jpg)).

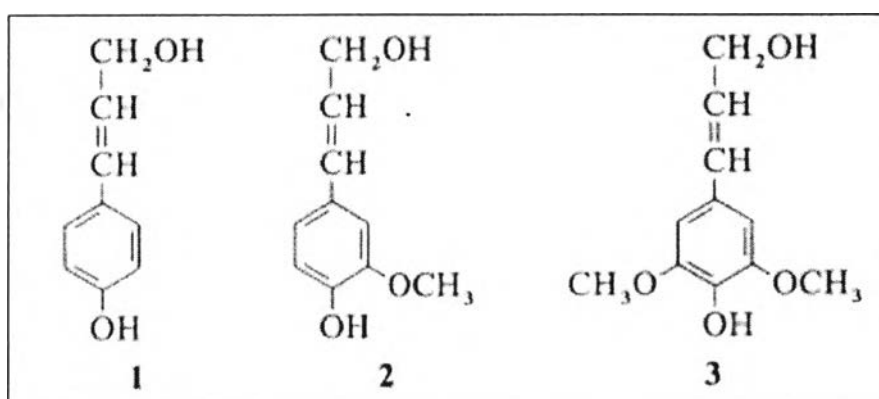
### 2.1.3 Lignin

Lignin is an aromatic and amorphous three dimensional polymer that contains three monomers : p-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol, as shown in Figure 2.6. It is insoluble in water and main fuction of lignin is protect plant cell wall from compression and bending. Futhermore, it transports

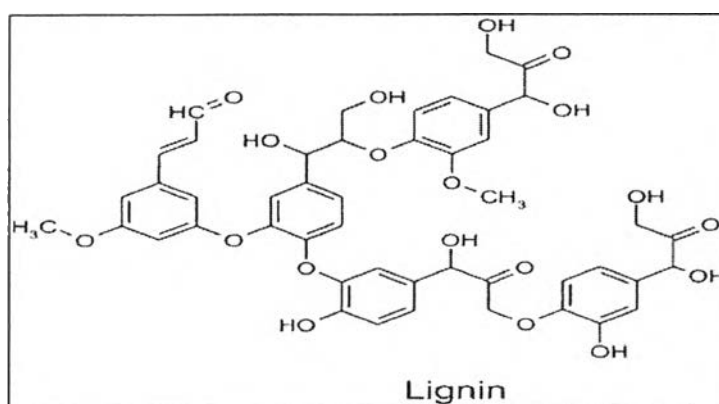
amount of water, nutrient and metabolite in the plant cell (Harmsen *et al.*, 2010). The structure of lignin is illustrated in Figure 2.7.

The composition of lignin in hardwood, softwood, and grasses is different. Hardwood contains high sinapyl alcohol, softwood contains a lot of coniferyl alcohol and grasses contain amount of p-coumaryl alcohol. The different composition is result in the delignification chemistry and biomass deconstruction.

Lignin has varies linkage such as C-O, C-C, and  $\beta$ -O-4 ether bond. The  $\beta$ -O-4 ether bond is the common linkage. It causes linear elongation of the polymer (Brandt *et al.*, 2013).



**Figure 2.6** P-coumaryl-, coniferyl-, and sinapyl alcohol: dominant building blocks of the three-dimensional polymer lignin (Harmsen *et al.*, 2010).



**Figure 2.7** Lignin structure

(<http://www.namrata.co/wp-content/uploads/2012/04/D3.bmp>).

#### 2.1.4 Napier Grass

Napier grass is one of lignocellulosic biomass and the latest energy crop which also known as Elephant grass or Uganda grass. It is a species of perennial tropical grass native to the African grasslands. Napier grass introduced into most tropical and subtropical countries such as Thailand. Moreover, It has low water and nutrient requirements. Therefore it can make use of otherwise uncultivated lands. In addition, It is extensive growth, well on the variety of soil types, and high yields throughout the year when compared to the other grasses. Harvesting can continue at an interval of 6-8 weeks for 6-7 years. The chemical composition and ethanol yield of Napier grass and energy crops are displayed in Table 2.2.

**Table 2.2** Comparison of chemical component and ethanol yield of energy crops

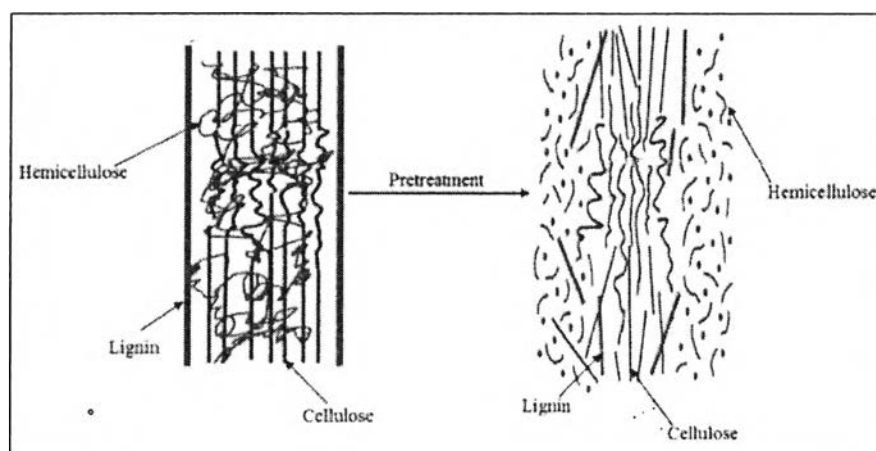
<b>Chemical component</b>	<b>Napier grass</b> (Wongwatanapaiboon <i>et al.</i> , 2012; Rengsirikul <i>et al.</i> , 2013)	<b>Switchgrass</b> (Wiseloge <i>et al.</i> , 1996; Heaton <i>et al.</i> , 2014)	<b>Miscanthus</b> (Lee <i>et al.</i> , 2007; Heaton <i>et al.</i> , 2014)	<b>Sugarcane bagasse</b> (Mathews <i>et al.</i> , 2011; Rengsirikul <i>et al.</i> , 2013)
Cellulose	35-47	28-37	43	45-55
Hemicellulose	20-26	25-34	24	20-25
Lignin	4-12	6-9	19	18-24
Total Sugar (%)	60-80	60-65	75.11	73-90
Total sugar concentration yield (KL/Rai)*	0.99-2.57	0.47-1.74	1.78	3.53
Ethanol yield (KL/Rai)	0.99-2.57	0.47-1.74	1.78	3.53

\* 100 g of glucose equals to 51.4 g of ethanol (Wasserman, 2015)

## 2.2 Pretreatment

Pretreatment is an important step to change lignocellulosic biomass to biofuels (Harmsen *et al.*, 2010) such as bioethanol, biogas, and biohydrogen (Verma *et al.*, 2011). Because of the recalcitrance property of plants cell wall, this property is resistance plants cell wall to deconstruction (Amarasekara, 2014). Then, pretreatment is a required process to break down lignin layer, reduce hemicellulose and the crystalline structure of cellulose and increase the porosity of the lignocellulosic biomass, as illustrated in Figure 2.8. Afterwards, enzyme can access to convert cellulose to fermentable sugar (Kumar *et al.*, 2009). Generally, crystalline structure is difficult to hydrolyze if it is without a pretreatment. Crystalline and amorphous form of cellulose can convert to glucose. Besides, the amorphous structure can deconstruct faster than the crystalline structure (Amarasekara, 2014).

The goals of pretreatment are: 1) increase sugar yield during enzyme hydrolysis 2) avoid the degradation of sugars that derived from hemicellulose 3) reduce the formation of fermentation inhibitors such as phenol, furfural, and HMF 4) recover of lignin for valuable coproducts and 5) be cost effective (Brodeur *et al.*, 2011).



**Figure 2.8** Schematic of the role of pretreatment in the conversion of biomass to fuel (Kumar *et al.*, 2009; Silva *et al.*, 2013).



There are many types of pretreatment. Usually, pretreatment can be classified into physical, chemical, physicochemical, and biological. Firstly, physical pretreatment reduces size of biomass and crystallinity by chipping, grinding, milling or radiation from microwave. This method is required energy that depends on particle size and crystallinity of cellulose (Brodeur *et al.*, 2011). Otherwise, physical pretreatment is commonly used in first step of pretreatment (Suppasinsatit, 2012). Secondly, chemical pretreatment use chemical to break down lignin and enzyme can access to cellulose (Amarasekara, 2014). The chemicals used in this method are acid, alkali, green solvents (ionic liquid), and others (Kumar *et al.*, 2009). The next one is physicochemical. It is a combination of physical and chemical pretreatment (Suppasinsatit, 2012). Examples of physicochemical pretreatment are steam explosion, ammonia fiber explosion (AFEX), carbon dioxide explosion, ionic liquid pretreatment, etc. The last one is biological pretreatment. It uses microorganisms (mainly fungi) to deconstruct lignin and hemicellulose and release cellulose. This method is safe and environmental friendly. In contrast, it has low rate hydrolysis and requires long time pretreatment (Brodeur *et al.*, 2011). Advantages and disadvantages of different pretreatment methods are shown in Table 2.3.

**Table 2.3** Advantages and disadvantages of different pretreatment methods of lignocellulosic biomass (Kumar *et al.*, 2009; Alvira *et al.*, 2010; Brodeur *et al.*, 2011)

Pretreatment method	Advantages	Disadvantages
Mechanical comminution	1) Reduce cellulose crystallinity	1) Power consumption higher than inherent biomass energy
Alkali	1) Efficient removal of lignin 2) Low inhibitor formation	1) High cost of alkaline catalyst 2) Alteration of lignin structure
Acid	1) High glucose yield 2) Solubilizes hemicellulose	1) High costs of acid and need for recovery 2) High costs of corrosive resistant equipment 3) Formation of inhibitors

**Table 2.3** Advantages and disadvantages of different pretreatment methods of lignocellulosic biomass (cont.) (Kumar *et al.*, 2009; Alvira *et al.*, 2010; Brodeur *et al.*, 2011)

Pretreatment method	Advantages	Disadvantages
Green solvents (Ionic liquid)	<ol style="list-style-type: none"> <li>1) Lignin and hemicellulose hydrolysis</li> <li>2) Ability to dissolve high loading of different biomass types</li> <li>3) Mild processing conditions (Low temperatures)</li> </ol>	<ol style="list-style-type: none"> <li>1) High solvent costs</li> <li>2) Need for solvent recovery and recycle</li> </ol>
Steam explosion	<ol style="list-style-type: none"> <li>1) Cost effective</li> <li>2) Lignin transformation and hemicellulose solubilization</li> <li>3) High yield of glucose and hemicellulose in two-step process</li> </ol>	<ol style="list-style-type: none"> <li>1) Partial hemicellulose degradation</li> <li>2) Acid catalyst needed to make process efficient with high lignin content material</li> <li>3) Toxic compound generation</li> </ol>
Liquid Hot Water (LHW)	<ol style="list-style-type: none"> <li>1) Separation of nearly pure hemicellulose from rest of feedstock</li> <li>2) No need for catalyst</li> </ol>	<ol style="list-style-type: none"> <li>1) High energy and water input</li> <li>2) Solid mass left over will need to be dealt with (cellulose/lignin)</li> </ol>
Ammonia Fiber Explosion (AFEX)	<ol style="list-style-type: none"> <li>1) High effectiveness for herbaceous material and low lignin content biomass</li> <li>2) Cellulose becomes more accessible</li> <li>3) Causes inactivity between lignin and enzymes</li> <li>4) Low formation of inhibitors</li> </ol>	<ol style="list-style-type: none"> <li>1) Recycling of ammonia is needed</li> <li>2) Less effective process with increasing lignin content</li> <li>3) Alters lignin structure</li> <li>4) High cost of ammonia</li> </ol>
Ammonia Recycle Percolation (ARP)	<ol style="list-style-type: none"> <li>1) Removes majority of lignin</li> <li>2) High cellulose content after pretreatment</li> <li>3) Herbaceous materials are most affected</li> </ol>	<ol style="list-style-type: none"> <li>1) High energy costs and Liquid loading</li> </ol>

**Table 2.3** Advantages and disadvantages of different pretreatment methods of lignocellulosic biomass (cont.) (Kumar *et al.*, 2009; Alvira *et al.*, 2010; Brodeur *et al.*, 2011)

Pretreatment method	Advantages	Disadvantages
CO <sub>2</sub> Explosion	<ol style="list-style-type: none"> <li>1) Increase accessible surface area</li> <li>2) Cost effective</li> <li>3) Do not form inhibitory compounds</li> </ol>	<ol style="list-style-type: none"> <li>1) Do not modify lignin or hemicelluloses</li> </ol>
Ozonolysis	<ol style="list-style-type: none"> <li>1) Reduce lignin content</li> <li>2) No toxic</li> </ol>	<ol style="list-style-type: none"> <li>1) Require large amount of ozone</li> <li>2) High cost</li> </ol>
Supercritical fluid	<ol style="list-style-type: none"> <li>1) Low degradation sugars</li> <li>2) Cost effective</li> <li>3) Increase cellulose accessible area</li> </ol>	<ol style="list-style-type: none"> <li>1) High pressure requirements</li> <li>2) Lignin and hemicellulose unaffected</li> </ol>
Organosolv	<ol style="list-style-type: none"> <li>1) Hydrolyze lignin and hemicellulose</li> </ol>	<ol style="list-style-type: none"> <li>1) Have to drain solvents from reactor, evaporated, condensed and recycle</li> <li>2) High cost</li> </ol>
Pyrolysis pulsed electrical field	<ol style="list-style-type: none"> <li>1) Produce gas and liquid at room temperature</li> <li>2) Disrupt plant cells</li> <li>3) Simple equipment</li> </ol>	<ol style="list-style-type: none"> <li>1) High temperature</li> <li>2) Need more research for ash production</li> </ol>
Biological	<ol style="list-style-type: none"> <li>1) Degrade lignin and hemicellulose</li> <li>2) Low energy consumption</li> </ol>	<ol style="list-style-type: none"> <li>1) Low rate of hydrolysis</li> <li>2) Long time pretreatment</li> </ol>
Wet oxidation	<ol style="list-style-type: none"> <li>1) Efficient removal of lignin</li> <li>2) Low formation of inhibitors</li> <li>3) Minimizes the energy demand (exothermic)</li> </ol>	<ol style="list-style-type: none"> <li>1) High cost of oxygen and alkaline catalyst</li> </ol>

### 2.3 Ionic Liquids Pretreatment

Ionic liquids pretreatment is a new physicochemical pretreatment method for ethanol production from lignocellulosic biomass (Amarasekara, 2014). Table 2.3

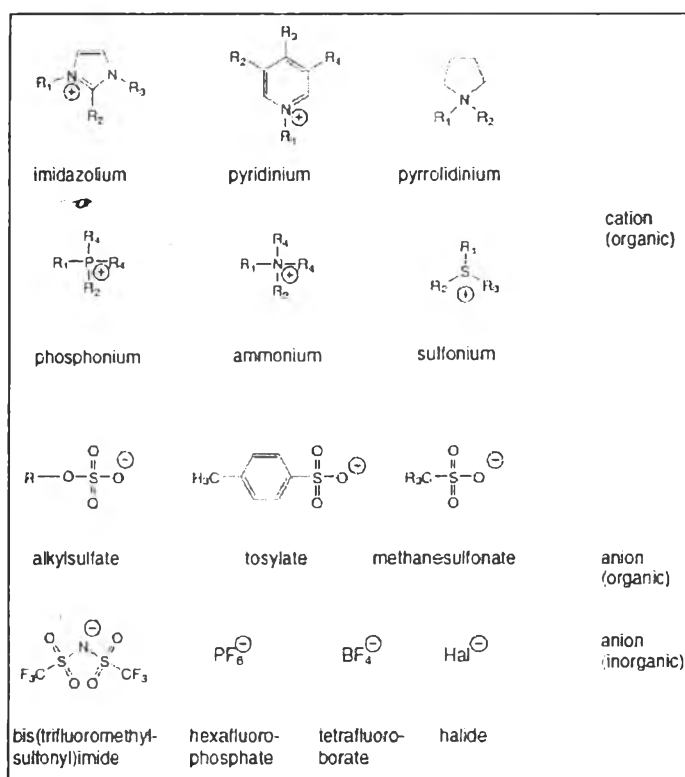
shows advantages and disadvantages of each pretreatment method. Thus, the production of bioethanol from lignocellulosic biomass requires less energy, mild condition, and green solvents can be recycled. Therefore, ionic liquids are appropriate solvent to these conditions.

### 2.3.1 Ionic Liquid

Ionic liquids (ILs) are known as green solvents for lignocellulosic pretreatment (Simmons *et al.*, 2010) because they can dissolve many types of biomass (Brodeur *et al.*, 2011), no toxic and no explosive gases are formed when used (Holm and Lassi, 2011). They consist of organic cations, inorganic or organic anions, and halide anions (Brandt *et al.*, 2013). Usually, the example of cation ionic liquids are alkylammonium, alkylphosphonium, alkylsulfonium, alkylnitride, n,n-dialkylimidazolium, and n-alkylpyridinium. The example of anion ionic liquids are  $\text{NO}_3^-$ ,  $\text{ClO}_4^-$ ,  $\text{HSO}_4^-$ ,  $\text{SO}_3^-$ ,  $\text{CF}_3\text{SO}_3^-$ ,  $(\text{CF}_3\text{SO}_2)_2\text{N}^-$ , and  $(\text{CF}_3\text{SO}_2)_3\text{C}^-$ . And the last group, halide anions contain halide-based ( $\text{Cl}^-$ ,  $\text{Br}^-$ ,  $\text{F}^-$ ), halogeno-based ( $\text{BF}_4^-$ ,  $\text{PF}_6^-$ ), and halogenoaluminium(III) (Simmons, 2013). Figure 2.9 shows common cation and anion of ionic liquids.

In addition, ionic liquids are a group of salts that are liquid form at low temperature (below 100 °C) and room temperature (Harmsen *et al.*, 2010; Holm and Lassi, 2011; Luo *et al.*, 2013). Because of the difference of molecular structure of ionic liquid, it leads to weak bond of ions so, ionic liquids exist as liquid form at room temperature (Harmsen *et al.*, 2010). Besides, their properties are also interesting, as shown in Table 2.4. From dissolution mechanism, cation and anion have to form hydrogen bond of cellulose in lignocellulosic biomass (Figure 2.10) by anion interacts with hydrogen atom and cation interacts with oxygen atom of hydroxyl group (Holm and Lassi, 2011; Lopes *et al.*, 2013). Then, dissolved cellulose was precipitated and separated from lignin and hemicellulose by anti-solvent such as water (Holm and Lassi, 2011) alcohol, and acetone (Ha *et al.*, 2011). Furthermore, the interaction between cellulose and anions are more influence than the interaction between cellulose and cations (Lopes *et al.*, 2013). For high dissolution cellulose in ionic liquids, structure of ionic liquids should be compose of good

hydrogen bond acceptor of anions, moderate hydrogen bond donator of cations and the size of cations should not too be bulky (Holm and Lassi, 2011). The effect of different anions but same cations to dissolved cellulose are  $(\text{CH}_3\text{CH}_2\text{O})_2\text{PO}_2^- \approx \text{OAc}^- > \text{SHCH}_2\text{COO}^- > \text{HCOO}^- > \text{Cl}^- > \text{Br}^- \approx \text{SCN}^-$ . Moreover, the most effective cations depend on the methylimidazolium and methylpyridinium cores, and contain allyl-, methyl-, ethyl-, or butyl- side chains (Luo *et al.*, 2013).



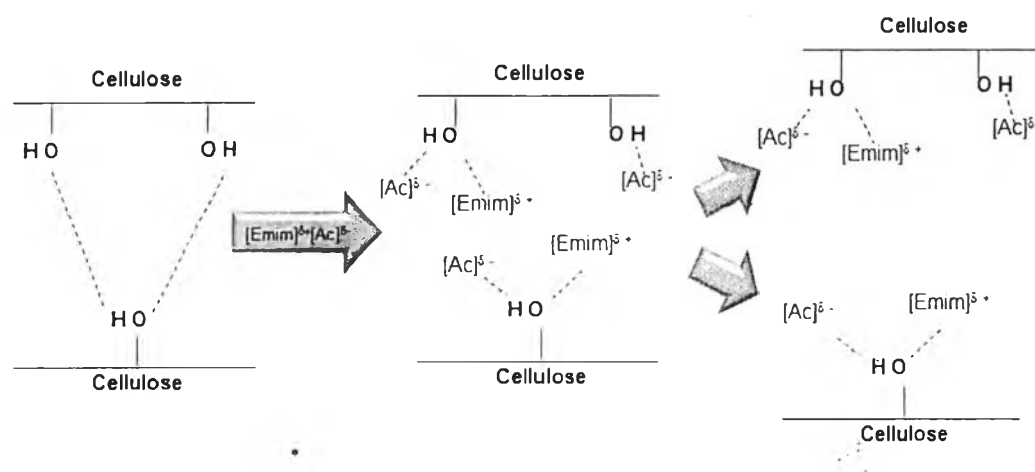
**Figure 2.9** Typical structures combine organic cations with inorganic or organic anions

(<http://www.sigmaaldrich.com/content/dam/sigma-aldrich/articles/chemfiles/volume-5-article-6/v5n6-image-3.gif>).

**Table 2.4** Advantages and disadvantages of ionic liquids (Holm and Lassi, 2011; Lopes *et al.*, 2013; Luo *et al.*, 2013; Silva *et al.*, 2013; Amarasekara, 2014)

Advantages	Disadvantages
1) Good thermal stability	1) High viscosity
2) Low vapor pressure	2) Water sensitive
3) Low melting point	3) High cost
4) High heat capacity	
5) High thermal conductivity	
6) Non-flammability	
7) High polarities	

The main advantage is they can dissolve in chemical compounds that cannot soluble in conventional solvent. The molten salts or oxide make ionic liquid high viscosity; therefore, power requirement for mixing liquid-liquid systems (Luo *et al.*, 2013). Because of their structure, structure of cation or anion can be changed. Then, solvent properties are designed (Holm and Lassi, 2011; Simmons, 2013).



**Figure 2.10** Dissolution mechanism of cellulose in [EMIM][Ac], adapted from (Feng *et al.*, 2008).

### 2.3.2 Factors of Ionic Liquids Pretreatment

The polar characteristics of ionic liquid affect the dissolution and functional modification of cellulose and carbohydrates. The interaction between ionic liquids and lignocellulosic biomass depends on the type of ionic liquid and other conditions such as temperature, pretreatment time, and water content (Lopes *et al.*, 2013). Firstly, temperature is very important factor and high temperature is preferred. It reduces pretreatment time and recalcitrant biomass by destabilizing hydrogen bond of cellulose (Lopes *et al.*, 2013; Luo *et al.*, 2013). However, it requires high energy input. In addition, pretreatment temperature depends on types of biomass and ionic liquid used (Luo *et al.*, 2013). Secondly, pretreatment time, it is related to the pretreatment temperature. The given pretreatment time may not be reached to thermo solubility limit. Thus, the high temperature reduces pretreatment time (Lopes *et al.*, 2013; Luo *et al.*, 2013). The third is water content, the moisture in biomass affects on cellulose solubility. It precipitates cellulose. Thus, the solubility of cellulose is reduced. (Fu and Mazza, 2011; Luo *et al.*, 2013). However, researchers combine ionic liquids with water for increase biomass loadings, reducing cost, and viscosity (Amarasekara, 2014). In addition, to be convenient of recycling ionic liquids, the solubility of cellulose is impossible, but the interaction between cellulose and ionic liquids is still strong in aqueous solution (Fu and Mazza, 2011).

Biomass loading is one of the important factors. Moreover, the ratio of solid to liquid influences dissolution of ionic liquids, glucose recovery, enzymatic accessibility, and regeneration efficiency are also considered as the important variables in this pretreatment (Lopes *et al.*, 2013). The ionic liquids to biomass ratio should be higher than the solubility limit (Luo *et al.*, 2013). The last one is particle size. Small particle size is suitable for pretreated samples to get higher enzymatic digestibility (Lopes *et al.*, 2013) and biomass dissolution rate (Luo *et al.*, 2013).

## 2.4 **Microwave Pretreatment**

Microwave is usually selected to improve pretreatment efficiency (Manaso *et al.*, 2013) because this technology requires a mild condition, simple, and rapid process (Budarin *et al.*, 2010). In order to increase reaction rate and energy saving,

this radiation directly reaches to materials through the interaction of molecular (Choudhary *et al.*, 2012). Generally, microwave is used in many fields such as chemical synthesis, solvent extraction, and solid state reactions. Furthermore, microwave is a commercial technology, used in food industries, composite manufacturing, and solid waste incineration (Choudhary *et al.*, 2012).

The frequency of microwave radiation is from 300 MHz to 300 GHz. It is short waves of electromagnetic energy. Normally, microwave radiation cannot breakdown chemical bond, but it can brakedown hydrogen bond (Passos *et al.*, 2013). The mechanical and thermal properties of lignocellulosic biomass is changed after used in microwave. It vibrates the hydrogen bond inside biomass. Then, hydrogen bond align itself with the magnetic field of microwave. From this cause, the chemical, physical, and biological reactions increase. Moreover, the intensity of microwave that accelerates changing biomass depends on electric field intensity, dielectric properties of biomass, and surrounding medium such as solvents (Choudhary *et al.*, 2012). At low temperature, the efficiency of microwave depends on physical properties of biomass (Budarin *et al.*, 2010). In 2011, Ha and coworkers compared microwave pretreatment with conventional pretreatment by using heating box. The cotton celluloses regenerated from ionic liquid dissolution with microwave irradiation were faster hydrolyzed than those conventional pretreatment at least 4 times. It was because microwave irradiation decreased in the degree of polymerization (DP) of cellulose dissolved in ionic liquid. Furthermore, microwave irradiation increased the cellulose dissolved in ionic liquid and decreased the amount of residual ionic liquid in regenerated celluloses.

## 2.5 Enzymatic Hydrolysis

Enzymatic hydrolysis is very important step before fermentation. In this step, the suitable enzyme acts as a catalyst to break the glycosidic bonds and release fermentable sugar from cellulosic materials (Demers *et al.*, 2009; Talebnia *et al.*, 2010). It can change organic matter containing cellulose to fuels. Sugar production from this reaction is used for producing ethanol, acetic acid, amino acids, antibiotics, and other chemicals are all potential products of these sugars (Demers *et al.*, 2009).



The main factors of enzymatic hydrolysis are substrate concentration and quality, cellulase activity, pretreatment method, reaction condition such as temperature and pH. Normally, The optimal reaction condition of enzyme is 45-50 °C and pH 4-5. At this condition, it advantages are low cost, low corrosion, and low toxic (Taherzadeh and Karimi, 2007). Moreover, it is environmental friendly. However, enzymatic hydrolysis has some drawbacks when comparing with dilute acid hydrolysis. It requires longer hydrolysis time and occurs end product inhibition. In addition, enzymes are expensive cost compared to other chemicals (Axelsson, 2011).

Cellulase is used as a cellulose hydrolysis. It is produced from fungi or bacteria. Mainly, it is from fungi because bacteria are anaerobes with a very low growth rates (Talebnia *et al.*, 2010). Cellulase is classified into three groups: endo-glucanase, exo-glucanase, and  $\beta$ -glucosidase. Firstly, endo-glucanase hydrolyzes an amorphous structure or derivative cellulose. It randomly hydrolyze at  $\beta$ -1,4-glycosidic bond. Secondly, exo-glucanase, it associates with endo-glucanase to hydrolyze microcrystalline cellulose. Finally,  $\beta$ -glucosidase, it hydrolyzes cellobiose and cellooligo-saccharides which are soluble in water (Tangsuan, 2010).

In hydrolysis process, cellulose is changed to glucose and hemicellulose is changed to pentoses and hexoses, as shown in Equations (2.1)-(2.2) (Axelsson, 2011).

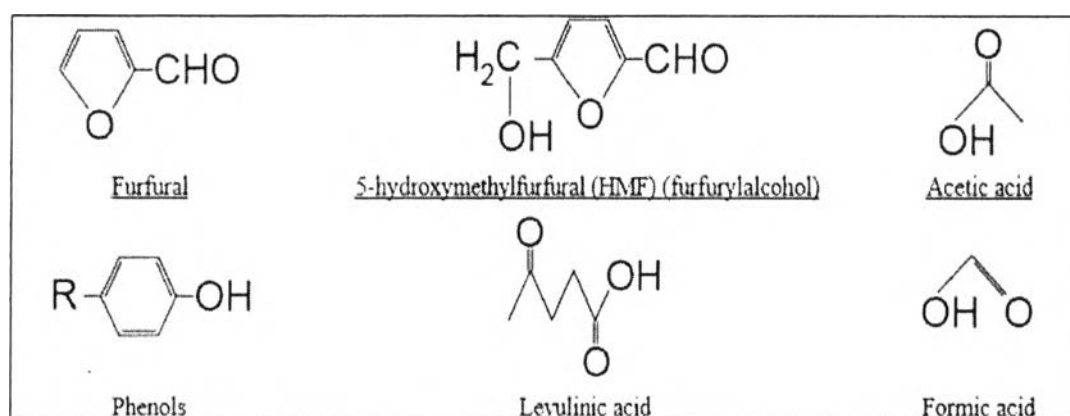


## 2.6 Fermentation

Microorganisms are used to convert monosacharides to ethanol. Types of microorganism are bacteria, yeast, and fungi. Normally, yeast is used in fermentation process. The factors of fermentation are types of microorganism, raw material, pretreatment method, hydrolysis method, and conditions such as temperature and pH (Axelsson, 2011).

The main issue of pretreatment method on fermentation process is the formation of fermentation inhibitors. These inhibitors reduce the ethanol yield and other productions. The main inhibitors are furfural, HMF (5-hydroxymethyl-furfural), acetic acid, phenols, levulinic acid, and formic acid, as illustrated in Figure 2.11. Phenolic compound has the highest toxicity followed by furfural, HMF, acetic acid, and extractives, respectively.

The degradation of pentose sugars is 5-hydroxymethyl-furfural (HMF) and hexose sugars is furfural. They affect cell growth and respiration during fermentation. To prevent degradation of HMF and furfural, the fermentation should operate at low temperature and residence time. Phenolic compounds also affect on the cell growth and respiration, but they are degraded from lignin. Otherwise, extractives are derived from the lignocellulosic structure and contain acidic resins, tanninic, and terpene acids. The last one, acetic acid is derived from acetyl groups in hemicellulose. It reduces cell pH that inhibits cell activity. The formation of extractives and acetic acid cannot prevent because they derive from the feedstock, but the solution of acetic acid is increase fermentation pH before fermentation (Harmsen *et al.*, 2010).



**Figure 2.11** Major types of inhibitors and their chemical structure (Harmsen *et al.*, 2010).

## 2.7 Response Surface Methodology (RSM)

The main objective of RSM is to optimize the relationship between a response (output variables ;  $y$ ) and input variables or factors ( $x_1, x_2, \dots, x_k$ ) (Garlapati *et al.*, 2013). The variables of RSM are quantitative variables. There are more than two input variables and at least one output variable (Tengpongsathon, 2011). The challenge of RSM is the responses ( $y$ ) and factors ( $x_1, x_2, \dots, x_k$ ) are unknown (Chen *et al.*, 2008). Mathematical and statistical techniques are used in RSM for fitting of empirical models to the experimental data (or regression analysis). Generally, linear and square polynomial functions are used in this system. The simplest model of RSM is linear function. This equation is

$$y = \beta_0 + \sum_{i=1}^k \beta_i x_i + \varepsilon \quad (2.3)$$

Where  $k$  = The number of variables

$\beta_0$  = The constant term

$\beta_1$  = The coefficients of the linear parameters

$x_i$  = The variables

$\varepsilon$  = The residual associated to the experiments

The responses are represented in three-dimensional space or contour plots, but linear function does not present curvature. Therefore, a second order model must be used. The model of second order interaction is

$$y = \beta_0 + \sum_{i=1}^k \beta_i x_i + \sum_{1 \leq i < j \leq k} \beta_{ij} x_i x_j + \varepsilon \quad (2.4)$$

Where  $\beta_{ij}$  = the coefficients of the interaction parameter

Futhermore, to determine a critical point (maximum, minimum or saddle) and the non-linear relationship between the responses and input variables, polynomial quadratic equation is used as following equation :

$$y = \beta_0 + \sum_{i=1}^k \beta_i x_i + \sum_{i=1}^k \beta_{ii} x_i^2 + \sum_{1 \leq i < j \leq k} \beta_{ij} x_i x_j + \varepsilon \quad (2.5)$$

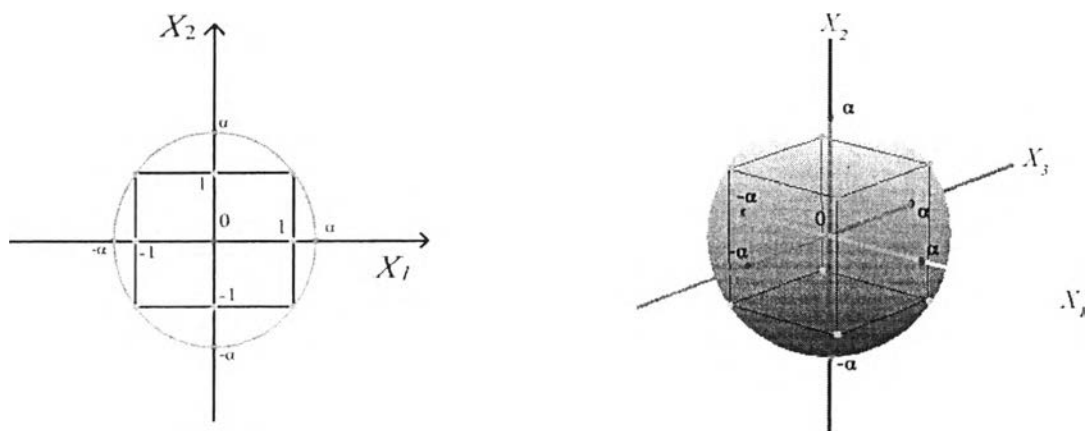
Where  $\beta_{ii}$  = the coefficients of quadratic parameter (Bezerra *et al.*, 2008)

From Equation 2.5, responses and input variables were plotted to show the main and interactive effects of the parameters on the response and to determine the optimum conditions (Garlapati *et al.*, 2013).

The major principle of RSM is 1) have to use Factorial design, Mixture design, Central Composite Design (CCD), or Plackett&Burman design and 2) value of input variables are cover studying area (Tengpongsathon, 2011).

### 2.7.1 Central Composite Design (CCD)

Normally, central composite design (CCD) includes  $2^k$  factorial design, center point, and axial point (or star point) which is  $\alpha$  value and  $-\alpha$  value on each axis as shown in Figure 2.12. There are two alpha value. One is a spherical design ( $\alpha = \sqrt{k}$ ), another is a rotatable design ( $\alpha = \left(2^{\frac{k}{4}}\right)$ ). If  $\alpha=1$  which is a special case, the design has  $3^k$ .



**Figure 2.12** Model of central composite design at  $k=2$  (left) and  $k=3$  (right)

([http://onlinecourses.science.psu.edu/stat503/sites/onlinecourses.science.psu.edu.stat503/files/lesson11/2d\\_plot.gif](http://onlinecourses.science.psu.edu/stat503/sites/onlinecourses.science.psu.edu.stat503/files/lesson11/2d_plot.gif))

([http://onlinecourses.science.psu.edu/stat503/sites/onlinecourses.science.psu.edu.stat503/files/lesson11/3d\\_plot\\_02.gif](http://onlinecourses.science.psu.edu/stat503/sites/onlinecourses.science.psu.edu.stat503/files/lesson11/3d_plot_02.gif)).

**Table 2.5** Summary of Central composite designs

(https://onlinecourses.science.psu.edu/stat503/node/59)

		<b>k = 2</b>	<b>k = 3</b>	<b>k = 4</b>	<b>k = 5</b>
<b>Central Composite Designs (CCD)</b>	Factorial points ( $2^k$ )	4	8	16	32
	Axial points ( $2*k$ )	1	6	8	10
	Center points ( $n_c$ )	5	5	6	6
	<b>Total</b>	13	19	30	48
<b>Choice of <math>\alpha</math></b>	Spherical design ( $\alpha = \sqrt{k}$ )	1.4	1.73	2	2.24
	Rotatable design ( $\alpha = 2^{\frac{k}{4}}$ )	1.4	1.68	2	2.38

The values of  $\alpha$  from spherical and rotatable design, shown in Table 2.5, are not much different. These values only show how far away the axial point should be from the center point. At center point, have to repeat 5 or 6 times because may be the optimum is in the central region. In addition to increase the variation at the center point to be approximately same as the variation at the edge for high precision (Altman, 2013).

In 2011, Fu and Mazza studied the possibility of aqueous ionic liquid pretreatment to lignocellulosic biomass and compared aqueous ionic liquid pretreatment with pure ionic liquid and dilute acid pretreatment. They selected [Emim][Ac] to treat triticale straw as a raw material at 150 °C, 90 min. They found that the sugar yield of 50% ionic liquid is the highest. It was about 81% sugar yield. When they compared pure ionic liquid with dilute acid pretreatment, pure ionic liquid (67%) was higher sugar yield than dilute acid pretreatment (47%). Furthermore, aqueous ionic liquid maintained the composition and extracted lignin of biomass, higher than pure ionic liquid and dilute acid pretreatment. So, aqueous ionic liquid could treat triticale straw and be better than pure ionic liquid and dilute acid pretreatment. Moreover, it reduces cost and be easy to recycled of ionic liquids (Fu and Mazza, 2011). In the same year; Ha and coworker compared microwave pretreatment with conventional pretreatment and studied effect of two types of ionic liquids. They used [Bmim][Cl] (1-butyl-3-methylimidazolium chloride) and [Emim][Ac] (1-ethyl-3-methylimidazolium acetate) to treat cotton cellulose at 50w 12s for microwave pretreatment and 110 °C, 30 min for conventional pretreatment.

They found that microwave pretreatment increase the solubility of cellulose and decrease degree of polymerization. Besides, microwave hydrolyzed faster than conventional pretreatment. From the effect of ionic liquids by using microwave pretreatment, [Emim][Ac] has higher the solubility of cellulose than [Bmim][Cl]. However, it hydrolyzed slower than [Bmim][Cl]. In conclusion, microwave pretreatment was a alternative method for pretreatment and [Emim][Ac] was one of the choices of ionic liquid to increase the solubility of cellulose (Ha *et al.*, 2011).

Subsequently, in 2012, Zhang and coworkers studied the effect of acid catalyst on bagasse composition. They treated sugarcane bagasse with [Bmim][Cl] at 130 °C, 30 min and added water for reducing cost and viscosity of ionic liquid. In addition, they added acid as a catalyst. Their acids were HCl, H<sub>2</sub>SO<sub>4</sub>, and FeCl<sub>3</sub>. When they compared aqueous [Bmim][Cl] with acidic aqueous [Bmim][Cl], they found that acidic aqueous [Bmim][Cl] gave higher total glucose yield than only aqueous [Bmim][Cl]. Furthermore, HCl aqueous [Bmim][Cl] gave the highest total glucose yield and followed by H<sub>2</sub>SO<sub>4</sub> and FeCl<sub>3</sub>, respectively. The water content in solution was significant. At 20% water, total glucose yield was the highest and decreased when increasing water content. Besides, when water content at >20% no formation of fermentation inhibitors or less occurring such as HMF, furfural, and acetic acid. At last, the acidic aqueous ionic liquid was an effective method for pretreatment and the optimal conditions were 20% water, 1.2% HCl, and 78.8% [Bmim][Cl] (Zhang *et al.*, 2012).

In 2013, Mood and coworkers treated barley straw by using different ionic liquids at 110 °C for 90 min. There are [Emim][Ac], [Emim][DEP] (1-ethyl-3-methylimidazolium diethyl phosphate), [Bmim][Cl] (1-butyl-3-methylimidazolium chlorides), [Mmim][DMP] (1,3-dimethyl imidazolium diethyl phosphate) and [Bmim][OTf] (1-butyl-3-methylimidazolium- trifluoromethane sulfonate). They found that [Emim][Ac] was the most effective on pretreating barley straw. It led to the highest degree of highest cellulose conversion (Mood *et al.*, 2013).

According to the literature review, the present work will study the acidic aqueous ionic liquid pretreatment on Napier grass by using microwave continuing from the previous research work (Trisinsub, 2013) with the optimal conditions at 162 °C for 48 min. Besides, two types and parts of Napier grass were

investigated and the highest fermentable sugar and the effects of acid concentration, ionic liquids-water concentration, pretreatment time and temperature on structure of Napier grass by using microwave were also studied: the [Emim][Ac] ionic liquid containing acetate-base anion was used in this study. Finally, the optimal conditions of acidic aqueous ionic liquid for Napier grass was investigated.