CHAPTER II

LITERATURE REVIEW

2.1 Characterization of Lignocellulosic Materials

Lignocellulosic materials contain a mixture of carbohydrate polymers (cellulose and hemicellulose) and lignin. The carbohydrate polymers are tightly bound to lignin mainly through hydrogen bonding, but also through some covalent bondings. The contents of cellulose, hemicelluloses, and lignin in common lignocellulosic materials are listed in Table 2.1 (Saha, 2003).

Table 2.1 Contents of cellulose, hemicellulose, and lignin in common lignocellulosic materials (Saha, 2003)

Lignocellulosic material	Cellulose (%)	Hemicellulose (%)	Lignin (%)
Hardwood stems	40-75	10-40	15-25
Softwood stems	30-50	25-40	25-35
Corncob	45	35	15
Wheat straw	30	50	15
Rice straw	32-47	19-27	5-24
Sugarcane bagasse	40	24	25
Leaves	15-20	80-85	-
Paper	85-99	-	0-15
Newspaper	40-55	25-40	18-30
Waste paper from chemical pulps	60-70	10-20	5-10
Grasses	25-40	25-50	10-30

2.1.1 Cellulose

Cellulose is the main component of most lignocellulosic materials. Cellulose is a linear polymer and glucosyl residues linked by β-1,4 bonds. However, each glucose residue is rotated 180° relative to its neighbors so that the basic repeating unit is in fact cellobiose, a dimer of a two-glucose unit. As glucose units are linked together into polymer chains, a molecule of water is lost, which makes the chemical formula $C_6H_{10}O_5$ for each monomer unit of "glucan". The parallel

polyglucan chains form numerous intra- and intermolecular hydrogen bonds, which result in a highly ordered crystalline structure of native cellulose, interspersed with less-ordered amorphous regions (Tacherzadeh and Karimi, 2007b). Figure 2.1 shows structure of cellulose chain.

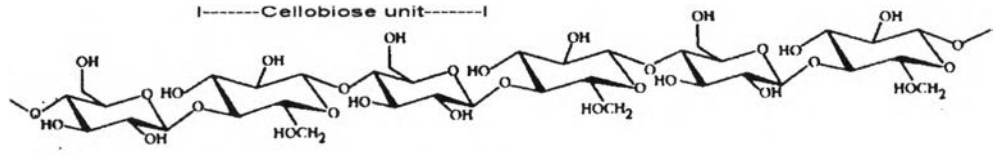

Figure 2.1 Schematic representation of a cellulose chain (Mousdale, 2008).

2.1.2 Hemicellulose

Hemicelluloses are heterogeneous polymers of pentoses (e.g. xylose, and arabinose), hexoses (e.g. mannose, glucose, and galactose), and sugar acids. Hemicelluloses are relatively easily hydrolyzed by acids to their monomer components consisting of glucose, mannose, galactose, xylose, arabinose, and small amounts of rhamnose, glucuronic acid, methyglucuronic acid, and galacturonic acid. Xylans are the most abundant hemicelluloses. Xylans of many plant materials are heteropolysaccharides with homopolymeric backbone chains of 1,4-linked β-D-xylopyranose units. Xylans from different sources, such as grasses, cereals, softwood, and hardwood, differ in composition. Besides xylose, xylans may contain arabinose, glucuronic acid or 4-*O*-methyl ether, and acetic, ferulic, and *p*-coumaric acids. The degree of polymerization of hardwood xylans (150-200) is higher than that of softwoods (Saha, 2003). Figure 2.2 shows basic structure of hemicellulose.

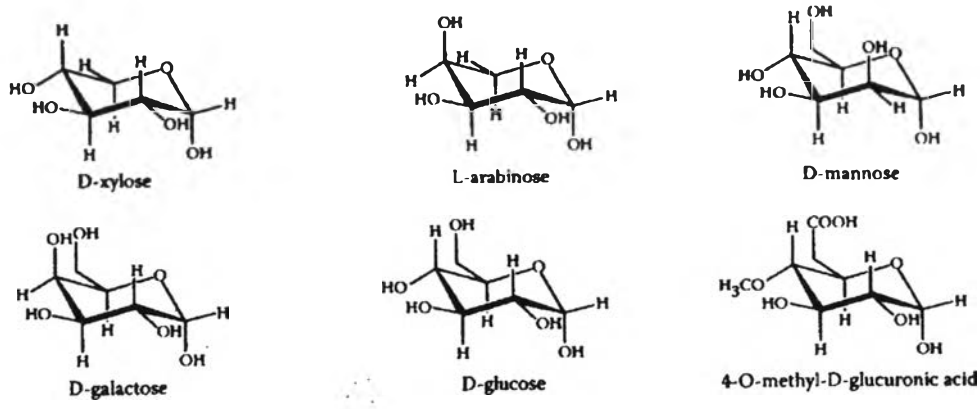

Figure 2.2 Schematic of the basic structure of hemicellulose. A, arabinose; FeA, ferulic acid; G, galactose; Glc, glucuronic acid; X, xylose (Mousdale, 2008).

Yoon (1998) reported the effect of hemicellulose on enzymatic digestibility. Hemicellulose adsorbs cellulase enzyme and the adsorbed enzyme is unavailable for cellulose hydrolysis. Hemicellulose in a lignocellulosic substrate physically is also known to block the contact between cellulase enzyme and cellulose. It is, therefore, concluded that hemicellulose could be an important barrier to enzymatic hydrolysis of cellulose and its removal is a prerequisite for complete hydrolysis of cellulose in biomass.

2.1.3 Lignin

Lignin is an aromatic polymer with the substituents connected by both ether and carbon-carbon linkages. It is composed of three principal building blocks: *p*-coumaryl alcohol (p-hydroxyphenyl propanol), coniferyl alcohol (guaiacyl propanol), and sinapyl alcohol (syringyl propanol) (Figure 2.3).

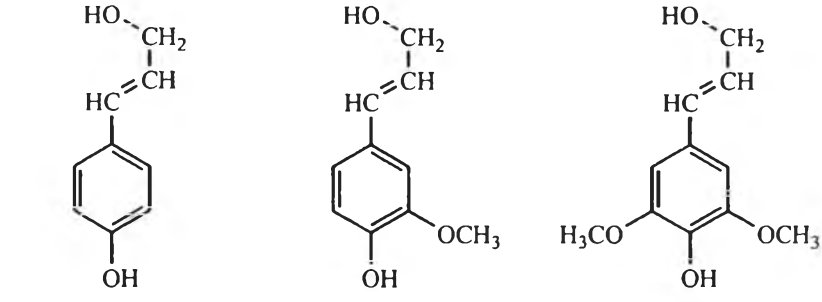

Figure 2.3 Lignin building blocks.

2.2 Sugar Production from Lignocellulosic Materials

There are several possible ways to hydrolyze lignocelluloses, as shown in Figure 2.4. The most commonly applied methods can be classified into two groups: chemical hydrolysis and enzymatic hydrolysis. In addition, there are some other hydrolysis methods, in which no chemicals or enzymes are applied. For instance, lignocelluloses may be hydrolyzed by ray or electron-beam irradiation, or microwave irradiation. However, those processes are commercially unimportant (Tacherzadeh and Karimi, 2007b).

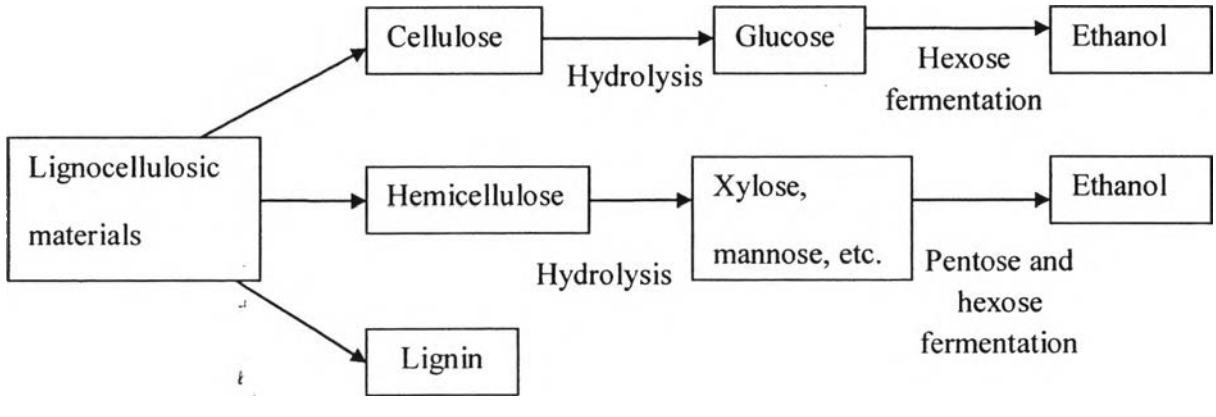

Figure 2.4 Overall view of sugar and ethanol productions from lignocellulosic materials.

Sugar substrates (such as sugarcane juice and molasses), starchy materials (such as wheat, corn, barley, potato, and cassava), and lignocellulosic materials (such as forest residuals, straws, and other agricultural by-products) are being considered as the raw materials for sugar production. The dominating sugars available or produced from these popular raw materials are

- Glucose, fructose, and sucrose in sugar substances
- Glucose in starchy materials
- Glucose from cellulose and either mannose or xylose from hemicellulose of lignocellulosic materials

2.3 Pretreatment of Lignocellulosic Materials

Pretreatment is a necessary element in bioconversion of ligcellulosic materials to fuels and chemicals. After the biomass is shredded and chipped, milling and pretreatment are required to separate the cellulose from the hemicellulose and the lignin. The goals of the pretreatment process are to remove lignin and hemicellulose, reduce the crystallinity of cellulose, and increase the porosity of the lignocellulosic materials. Pretreatment methods can be divided into different categories: physical (milling and grinding), physicochemical (steam pretreatment/autohydrolysis, hydrothermolysis, and wet oxidation), chemical (alkali, dilute acid, oxidizing agents, and organic solvents), biological, electrical, or a combination of these. Table 2.2 lists some important methods for pretreatment of lignocellulosic biomass.

Table 2.2 Methods for pretreatment of lignocellulosic biomass (Saha, 2003)

Method	Example
Thermo-mechanical	Grinding, milling, shearing, extrusion
Autohydrolysis	Steam pressure, steam explosion, supercritical carbon dioxide explosion
Acid treatment	Dilute acid (H_2SO_4, HCl), concentrated acid (H_2SO_4, HCl)
Alkali treatment	Sodium hydroxide, ammonia, alkaline hydrogen peroxide
Organic solvent treatment	Methanol, ethanol, butanol, phenol

Sun *et al.* (2002) reported that the purpose of the pretreatment was to remove lignin and hemicellulose, reduce cellulose crystallinity, and increase the porosity of the materials. Pretreatment must meet the following requirements: (1) improve the formation of sugars or the ability to subsequently form sugars by enzymatic hydrolysis; (2) avoid the degradation or loss of carbohydrate; (3) avoid the formation of byproducts inhibitory to the subsequent hydrolysis and fermentation processes; and (4) be cost-effective.

The following pretreatment technologies are promising for cost-effective pretreatment of lignocellulosic biomass for biological conversion to fuels and chemicals.

2.3.1 Physical Pretreatment

2.3.1.1 Mechanical Comminution

Comminuting of lignocellulosic materials through a combination of chipping, grinding, and/or milling can be applied to reduce cellulose crystallinity. The size of the materials is usually 10-30 mm after chipping and 0.2-2 mm after milling or grinding. Vibratory ball milling was found to be

more effective than ordinary ball milling in reducing cellulose size (Kumar *et al.*, 2009).

2.3.2 Physicochemical Pretreatment

2.3.2.1 Steam Explosion

Steam explosion is the most commonly used method for the pretreatment of lignocellulosic materials (Kumar *et al.*, 2009). In this method, biomass is treated with high-pressure saturated steam, and then the pressure is suddenly reduced, which makes the materials undergo an explosive decompression. The process causes hemicellulose degradation and lignin transformation due to high temperature, thus increasing the potential of cellulose hydrolysis.

2.3.2.2 Ammonia Fiber Explosion (AFEX)

Ammonia fiber explosion (AFEX) is a physicochemical pretreatment process, in which lignocellulosic biomass is exposed to liquid ammonia at high temperature and pressure for a period of time, and then the pressure is suddenly reduced. The AFEX process is very similar to steam explosion. The AFEX technology has been used for the pretreatment of many lignocellulosic materials, including alfalfa, wheat straw, and wheat chaff.

2.3.2.3 Carbon Dioxide Explosion

To improve lignocellulose pretreatment efficiency, the idea of using supercritical CO_2 explosion, which would have a lower temperature than steam explosion and possibly a reduced expense compared to ammonia explosion, was developed.

2.3.3 Chemical Pretreatment

2.3.3.1 Ozonolysis

Ozone treatment is one way of reducing the lignin content of lignocellulosic wastes. The degradation is mainly limited to lignin. Hemicellulose is slightly affected, but cellulose is not. Ozonolys is pretreatment has an advantage that the reactions are carried out at room temperature and normal pressure. Furthermore, the fact that ozone can be easily decomposed by using a catalytic bed or increasing

the temperature means that the process can be designed to minimize environmental pollution (Kumar *et al.*, 2009).

2.3.3.2 Acid Hydrolysis

Concentrated acids, such as H_2SO_4 and HCl, have been used to treat lignocellulosic materials. Pretreatment with acid hydrolysis can result in improvement of enzymatic hydrolysis of lignocellulosic biomasses to release fermentable sugars. Although they are powerful agents for cellulose hydrolysis, concentrated acids are toxic, corrosive, hazardous, and thus require reactors that are resistant to corrosion, which makes the pretreatment process very expensive.

2.3.3.3 Alkaline Hydrolysis

Alkali pretreatment processes utilize lower temperatures and pressures than other pretreatment technologies. Alkali pretreatment can be carried out at ambient conditions, but pretreatment times are on the order of hours or days rather than minutes or seconds. Compared with acid processes, alkaline processes cause less sugar degradation, and many of the caustic salts can be recovered and/or regenerated.

2.3.4 Biological Pretreatment

In particular, physical and thermochemical processes require abundant energy for biomass conversion. Biological treatment using various types of rot fungi, which is a safe and environmentally friendly method, is increasingly being advocated as a process that does not require high energy for lignin removal from a lignocellulosic biomass, despite extensive lignin degradation (Okano *et al.*, 2005). In biological pretreatment processes, microorganisms, such as brown-, white-, and soft-rot fungi, are used to degrade lignin and hemicellulose in waste materials (Galbe and Zacchi, 2007). All of the advantages and disadvantages of the above explained pretreatment methods are summarized in Table 2.3.

Table 2.3 Advantages and disadvantages of various pretreatment methods

Type	Pretreatment	Advantages	Disadvantages
Physical pretreatment	Mechanical comminution[a]	Improve the digestibility of biomass	Require exorbitant amount of energy
Physico-chemical pretreatment	Steam explosion[b] (autohydrolysis)	1. Low energy requirement compared to mechanical comminution 2. No recycling or environmental costs	1. Formation of inhibitory compounds 2. Destruction of xylan fraction 3. Incomplete disruption of the lignin-carbohydrate matrix
	Ammonia fiber explosion[b] (AFEX)	1. Significantly improve saccharification rates of various herbaceous crops 2. Not produce inhibitors for downstream biological process 3. Not require small particle size for efficacy	Not very effective for biomass with high lignin content
	CO_2 explosion[a]	1. More cost effective than ammonia fiber explosion 2. No formation of inhibitory Compounds	Low yield compared to steam or ammonia explosion
Chemical pretreatment	Ozonolysis[a]	1. Effectively remove lignin 2. Not produce toxic residues for the downstream process 3. Carry out at room temperature and pressure	Large amount of ozone required, making the process expensive
	Acid hydrolysis[b]	1. Achieve high xylan-to-xylose conversion yields (less severe conditions) 2. Significant improve cellulose hydrolysis	1. Higher cost than some physico-chemical pretreatment 2. Need neutralization of pH
	Alkaline hydrolysis[b]	1. Decrease degree of polymerization and crystallinity 2. Separation of structural linkages between lignin and carbohydrates 3. Disruption of lignin structure	No effect for soft woods with lignin content greater than 26%
Biological pretreatment[a]		1. Low energy requirement 2. Mild environmental conditions	Very low hydrolysis rate

[a] Kumar *et al.*, (2009), [b] Dimian and Bildea (2008)

2.4 Hydrolysis of Lignocellulosic Materials

2.4.1 Enzymatic Hydrolysis

Enzymatic hydrolysis of cellulose and hemicelluloses can be carried out by highly specific cellulose and hemicellulase enzymes (glycosyl hydrolases). Enzymatic degradation of cellulose to glucose is generally accomplished by synergistic action of three distinct classes of enzymes:

- 1,4-β-D-glucan-4-glucanohydrolases or endo-1,4-β-glucanases, which are commonly measured by detecting the reducing groups released from carboxymethylcellulose (CMC).
- Exo-1,4-β-D-glucanases, including both 1,4-β-D-glucan hydrolases and 1,4-β-D-glucan cellobiohydrolases. 1,4-β-D-glucan hydrolases liberate D-glucose, and 1,4-β-D-glucan cellobiohydrolases liberate D-cellobiose.
- β-D-glucoside glucohydrolases or β-D-glucosidases, which release D-glucose from cellobiose and soluble cellodextrins, as well as an array of glycosides.

There is a synergy between exo-exo, exo-endo, and endo-endo enzymes, which has been demonstrated several times. Substrate properties, cellulose activity, and hydrolysis conditions (e.g. temperature and pH) are the factors that affect the enzymatic hydrolysis of cellulose. To improve the yield and rate of enzymatic hydrolysis, there has been some research focused on optimizing the hydrolysis process and enhancing cellulose activity. Substrate concentration is a main factor that affects the yield and initial rate of enzymatic hydrolysis of cellulose. At low substrate levels, an increase in substrate concentration normally results in an increase in the yield and reaction rate of the hydrolysis. However, high substrate concentration can cause substrate inhibition, which substantially lowers the rate of hydrolysis, and the extent of substrate inhibition depends on the ratio of total substrate to total enzyme (Lee *et al.*, 1994).

2.4.2 Chemical Hydrolysis

Chemical hydrolysis involves exposure of lignocellulosic materials to a chemical for a period of time, at a specific temperature, and results in sugar monomers from cellulose and hemicellulose polymers.

2.4.2.1 Concentrated-Acid Hydrolysis

Hydrolysis of lignocelluloses by concentrated sulfuric or hydrochloric acids is a relatively old process. Concentrated-acid processes are generally reported to give higher sugar and ethanol yields, compared to dilute-acid processes. Furthermore, they do not need a very high pressure and temperature. Although this is a successful method for cellulose hydrolysis, concentrated acids are toxic, corrosive, and hazardous, and these acids require reactors that are highly resistant to corrosion (Lee *et al.*, 1994).

2.4.2.2 Diluted-Acid Hydrolysis

Diluted-sulfuric acid hydrolysis is a favorable method for either the pretreatment before enzymatic hydrolysis or the conversion of lignocellulose to sugar. This pretreatment method gives high reaction rate and significantly improves enzymatic hydrolysis. Depending on the substrate used and the conditions applied, up to 95 % of the hemicellulosic sugars can be recovered by dilute-acid hydrolysis from the lignocellulosic feedstock (Galbe and Zacchi, 2007).

Enzymatic hydrolysis of corncob and ethanol fermentation from cellulosic hydrolysate were investigated. After corncob was pretreated, the cellulosic residue was hydrolyzed by cellulase from Trichoderma reesei ZU-02, and hydrolysis yield was found to be 67.5 %. Poor cellobiase activity in T. reesei cellulase restricted the conversion of cellobiose to glucose, and the accumulation of cellobiose caused severe feedback inhibition to the activities of β-1,4-endoglucanase and β-1,4-exoglucanase in cellulose system. Supplementing cellobiase from Aspergillus niger ZU-07 greatly reduced the inhibitory effect caused by cellobiose, and the hydrolysis yield was improved to 83.9 % with an enhanced cellobiase activity of 6.5 CBU/$g_{substrate}$ (Chen *et al.*, 2007).

Chunmei *et al.* (2010) investigated biohydrogen production from corncob using natural anaerobic microflora. They found mechanism of

degrading corncob for hydrogen production by chemical composition analysis, Fourier transform infrared spectroscopy (FTIR), and X-ray diffraction (XRD). The amorphous domains of cellulose and hemicellulose were hydrolyzed into fermentable saccharine through acid pretreatment, and the microorganisms had a devastating effect on the crystalline of the cellulose. The hydrogen yield from pretreated corn cob was much higher than that from raw corncob.

The effect of varying initial particle sizes on enzymatic hydrolysis rates and rheological properties of sawdust slurries was investigated. Slurries with four particle size ranges (33 μm < $x \leq$ 75 μm, 150 μm < $x \leq$ 180 μm, 295 μm < $x \leq$ 425 μm, and 590 μm < $x \leq$ 850 μm) were subjected to enzymatic hydrolysis using an enzyme dosage of 15 filter paper units per gram of cellulose at 50 °C and 250 rpm in shaker flasks. At lower initial particle sizes, higher enzymatic reaction rates and conversions of cellulose to glucose were observed. After 72 h, 50 and 55% more glucose was produced from the smallest size particles than the largest size ones, for initial solids concentration of 10 % and 13 % (w/w), respectively. The effect of initial particle size on viscosity over a range of shear was also investigated. For equivalent initial solids concentration, smaller particle sizes result in lower viscosities such that at a concentration of 10 % (w/w), the viscosity decreased from 3000 cP for 150 μm < $x \leq$ 180 μm particle size slurries to 61.4 cP for 33 μm < $x \leq$ 75 μm particle size slurries. Results indicate particle size reduction may provide a means for reducing the long residence time required for the enzymatic hydrolysis step in the conversion of biomass to ethanol (Dasari *et al.* 2007).

Swatloki *et al.* (2002) studied the dissolution of cellulose in different types of ionic liquids (ILs) and experimental conditions. The solubility of cellulose in [BMIM]Cl ionic liquid decreased as 1 wt.% water (approximately 0.5 mole fraction of H_2O) was added to the system. From the water addition, cellulose pulp was precipitated, and this was called "regenerated cellulose". The initial dissolving pulp, and regenerated cellulose were characterized by scanning electron microscopic (SEM) and thermogravimetric analysis (TGA). SEM showed the change of cellulose morphology after it was dissolved in [BMIM]Cl, as shown in Figure 2.5

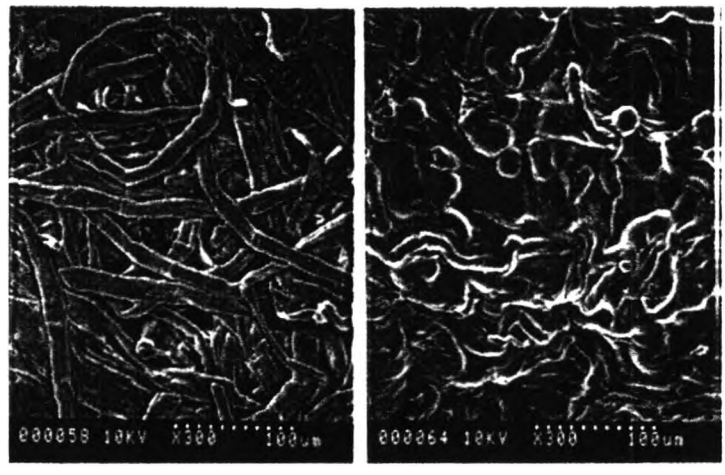

Figure 2.5 SEM micrographs of the initial dissolving pulp (left) and after dissolution in [BMIM]Cl and regeneration into water (right) (Swatloki *et al.*, 2002).

Kumar *et al.* (2009) reported that the factors affecting the hydrolysis of cellulose included porosity (accessible surface area) of the biomass materials, cellulose fiber crystallinity, and content of both lignin and hemicellulose. The presence of lignin and hemicellulose makes the accessibility of cellulase enzymes and acids to cellulose more difficult, thus reducing the efficiency of the hydrolysis process. Pretreatment is required to alter the size and structure of the biomass, as well as its chemical composition, so that the hydrolysis of the carbohydrate fraction to monomeric sugars can be achieved rapidly and with greater yields. The hydrolysis process can be significantly improved by removal of lignin and hemicellulose, reduction of cellulose crystallinity, and increase of porosity through pretreatment processes.

Taechapoempol (2009) isolated cellulose-producing bacteria from Thai higher termites *Microcerotermes sp.* under different isolation conditions, and found that three effective isolates, which had the highest HC value, were A002, M015, and F018. Identification from the DNA base composition revealed that all effective isolates were Bacillus subtilis. Moreover, these three bacteria were tested for their toxic tolerance to [BMIM]Cl. All isolates were able to tolerate 0.1–1.0 vol % [BMIM]Cl, and no growth retardation in the lag phases, except A 002, which had growth retardation at 0.5–1.0 vol% [BMIM]Cl, was observed.

Worasamutprakarn (2010) investigated the conversion of cellulose to glucose by using cellulase-producing bacteria isolated from higher termites with [BMIM]Cl ionic liquid pretreatment. For the pretreatment step, it was found that [BMIM]Cl could be effectively used to decrease the crystallinity of cellulose chains with optimum conditions at 5:100 cellullose-to-[BMIM]Cl ratio and 100 °C. The crystallinity of cellulose chains were decreased about 90 % after the pretreatment. However, about 5 % of [BMIM]Cl was remained in the pretreated cellulose even after washing by deionized water. The results from enzymatic hydrolysis showed that strain F 018 provided the highest glucose concentration at 0.59 g/l after 4 h operation. Moreover, the no.5 Whatman filter paper with high crystalline structure gave the lowest glucose concentration, while the No. 1, 2, and 4 Whatman filter papers with lower crystallinity gave higher glucose concentration.