CHAPTER II
LITERATURE REVIEW

2.1 Cellulose

2.1.1 Characteristic and Properties of Cellulose

Cellulose is a glucan polymer of D-glucopyranose units, which are linked together by β-1,4-glucosidic bonds. Cellulose molecules are oriented in parallel alignment and form both intra- and intermolecular hydrogen bonds. These interactions cause packing density of cellulose increases until crystalline regions are formed. These parts are directly involved the resistant to enzymatic hydrolysis (Rowell *et al.*, 2000). Most plant-derived cellulose has high crystalline and may contain as much as 80% crystalline regions. Figure 2.1 shows structure of cellulose unit.

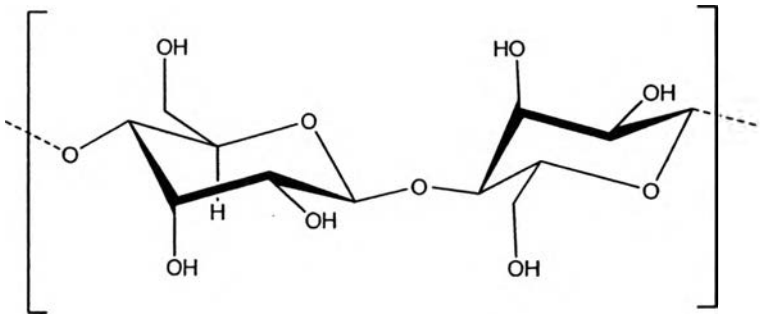

Figure 2.1 Cellulose structure.

2.1.2 Cellulose Utilization

Cellulose can be used in many applications especially it is the major component of paper, cardboard and also textiles that are made from cotton, linen, and other plant fibers.

Cellulose can be modified by other chemicals for producing other materials such as cellophane, a thin transparent film, and rayon, an important fiber that has been used for textile industries. In laboratory usage, cellulose can be used as

the stationary phase for thin layer chromatography. Nitrocellulose or cellulose nitrate is used in smokeless gunpowder.

In addition, cellulose can be used to make hydrophilic and highly absorbent sponges as well as water-soluble adhesive and binders such as methyl cellulose and carboxymethyl cellulose, which are used in wallpaper paste. Microcrystalline cellulose and powdered cellulose are used as inactive fillers in tablets and as thickeners and stabilizers in processed foods.

2.1.3 Cellulose Conversion

Cellulose is an abundant renewable resource that is composed glucose units. Therefore, cellulose is considered as one major of glucose feedstocks, which can be used for energy productions like ethanol or hydrogen from fermentation processes. However, direct using of glucose from cellulose does not have enough efficient. The two additional processes are required to separate cellulose from biomass and breaking cellulose chains into simple sugar (glucose).

2.1.3.1 Pretreatment

After the biomass is shredded, chipped, grounded, milled or pulverized, it is sent to pretreatment part for separating lignin and hemicellulose out from cellulose. Several methods such as steam explosion, hydrothermal, and dilute acid pretreatment can be used.

a) Steam explosion pretreatment

This method uses high-pressure steam for a short period of time to expose biomass and then quickly decompress it to an atmosphere pressure. This depressurization causes the rapid expansion of water from the steam and the biomass explodes into a pulp. This method can use some chemicals like sulfur dioxide or ammonia as catalysts for kinetic rate enhancement.

b) Hydrothermal pretreatment

Biomass is added to water and then temperature and pressure are increased.

c) Dilute acid pretreatment

This process uses dilute sulfuric acid at high temperature and pressure to hydrolyze other parts like hemicellulose and remaining lignin out of

cellulose and also help cellulose more accessible. There are three different processing technologies used in the dilute acid treatment: countercurrent processing, two-temperature processing, and pressurized hot wash.

2.1.3.2 Hydrolysis

After the cellulose has been exposed through a pretreatment process, then this cellulose is depolymerized to glucose units by using various processes—concentrated acid hydrolysis, diluted acid hydrolysis, and enzymatic hydrolysis.

a) Concentrated acid hydrolysis

After the pretreatment step, the dried cellulose is decrystallized by reactions between cellulose and concentrated sulfuric acid. The resulting gelatin is then diluted with water and heated to release glucose. Glucose is separated out from sulfuric acid by using either a membrane or chromatography column, and this sulfuric acid is recycled for process cost reduction.

b) Diluted acid hydrolysis

This process is similar with the diluted acid pretreatment, except that the temperature of the reaction is raised to 215°C for breaking cellulose.

c) Enzymatic hydrolysis

This process uses biologically-produced enzyme, cellulase, to break down the pretreated cellulose to glucose instead of using acid.

2.2. Cellulase

2.2.1 Cellulase

Cellulase or cellulolytic enzyme is the group of enzyme, that can hydrolyze β-1,4-glycosidic linkage in cellulose via synergistic actions by three different enzymes: endoglucanase (1,4-β-D-glucan-4-glucanohydrolase, E.C. 3.2.1.4) randomly hydrolyzes cellulose chain at intramolecule β-1,4-glucosidic bonds to produce new chain ends; exoglucanase (1,4-β-D-glucan cellobiohydrolase, E.C. 3.2.1.91) cleaves the chain ends of cellulose and release cellobiose or glucose as product; and β-glucosidase (E.C. 3.2.1.21) hydrolyzes produced cellobiose to glucose

(Zhang *et al.*, 2006). The mechanism of the three hydrolysis processes is shown in Figure 2.2.

In the primary hydrolysis step, endoglucanase and exoglucanase are directly involved in this reaction. The reaction takes place at the surface of substrates and then soluble sugars, which have a degree of polymerization (DP) up to 6, are released into the liquid phase. This reaction step, enzymatic depolymerization step, is considered as the rate-limiting step for the whole cellulose hydrolysis process. Secondary hydrolysis takes place in the liquid phase by β-glucosidase. This enzyme hydrolyzes cellobiose from primary hydrolysis into glucose. During cellulose hydrolysis, the changing of solid substrate characteristics is varied with time, including changes in a number of cellulose chain ends resulting from endoglucanase and exoglucanase, and changes in cellulose accessibility resulting from substrate consumption and cellulose fragmentation. Synergistic between endoglucanase and exoglucanase causes changes at the substrate's surface along the time, resulting in rapid changes in hydrolysis rates.

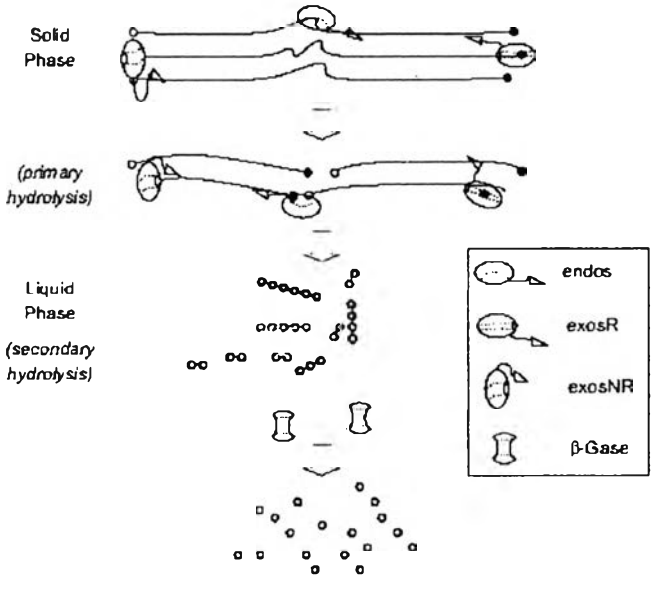

Figure 2.2 Mechanistic scheme of enzymatic cellulose hydrolysis by *Trichoderma*, non-complexed cellulase system (Zhang *et al.*, 2006).

2.2.2 Cellulase-Producing Bacteria

Cellulase-producing bacteria can be classified to several groups as shown in Table 2.1 (Lynd *et al.*, 2002): (1) fermentative anaerobes, typically Gram-positive (*Clostridium, Ruminococcus*, and *Caldicellulosiruptor*) but a few Gram-negative bacteria are also found; (2) aerobic Gram-positive bacteria (*Cellulomonas* and *Thermobifida*); and (3) aerobic gliding bacteria (*Cytophaga* and *Sporocytophaga*).

A bacteria group is classified by using oxygen as growth factor and different in cellulolytic strategy between the anaerobic and aerobic groups.

Anaerobic bacteria degrade the cellulose by cellulosome, a complexed cellulase system. Several anaerobic species that utilize cellulose do not release measurable amounts of extracellular cellulose. Most of anaerobic cellulolytic species grow optimal on cellulose when attach to the substrate. Cellulolytic anaerobes are similar to other fermentative anaerobes in their low cell yields.

Aerobic bacteria utilize the cellulose through the production of substantial amounts of extracellulase cellulase enzymes at the cell surface. Although many aerobic bacteria adhere to cellulose, physical contact between cells and cellulose does not appear to be necessary for cellulose hydrolysis. Cellulolytic aerobes produce higher cell yields as compare with anaerobes.

On the other hand, cellulose-utilizing bacteria, which can live either aerobic or anaerobic condition, are called facultative bacteria. These aerobes are still required some investigation about genus of bacteria and mechanisms that use for cellulose degradation (Lynd *et al.*, 2002).

Table 2.1 Some cellulase-producing bacteria, which are classified by oxygen relationship

Conditions	Bacteria	Representative species	Gram reaction
Aerobic	*Acidothermus*	*A. cellulolyticus*	+
	Bacillus	*B. pumilis*	+
	Caldibacillus	*C. cellovorans*	+
	Cellulomonasc	*C. flavigena, C. uda*	+
	Cellvibrio	*C. fulvus, C. gilvus*	-
	Cytophaga	*C. hutchinsonii*	-
	Erwinia	*C. carotovora*	-
	Micromonospora	*M. chalcae*	+
	Pseudomonas	*P. fluorescens* var. *cellulosa*	-
	Sporocytophaga	*S. myxococcoides*	-
	Streptomyces	*S. reticuli*	+
	Thermobifida	*T. fusca*	+
Anaerobic	*Acetivibrio*	*D. cellulolyticus*	-
	Anaerocellum	*D. thermophilum*	+
	Butyrivibrio	*B. fibrisolvens*	+
	Caldicellulosiruptor	*C. saccharolyticum*	-
	Clostridium	*C. thermocellum,* *C.cellulolyticum*	+
	Eubacterium	*E. cellulosolvens*	+
	Fervidobacterium	*F. islandicum*	-
	Fibrobacter	*F. succinogenes*	-
	Halocella	*H. cellulolytica*	-
	Ruminococcus	*R. albus, R. flavefaciens*	+

2.2.3 Isolation of Cellulase-Producing Bacteria from Termite

In the past, cellulase-producing bacteria were investigated for a long time. Several sources from environment (pool, soil, and mud) or animal species like cattle, crayfish, and insect, are selected for cellulolytic bacteria determination (Lynd *et al.*, 2002).

Termite is one group of social insects, which can decompose cellulose in dead plant into its food. From phylogenetic identification, termite can be classified into two subgroups: lower termites and higher termites. Lower termites are termites from families Mastotermitidae, Hodotermitidae, Kalotermitidae, Rhinotermitidae and Serritermitidae, which use symbiotic protozoans in the hind intestine for cellulose digestion. Higher termites are termites from family Termitidae, which use cellulolytic bacteria in their hindgut for cellulose digestion (Mo *et al.*, 2004). There are several reports showed the possibility to isolate cellulolytic bacteria from termites.

Wenzel *et al.* (2002) isolated 119 cellulolytic strains from the gut of *Z. angusticollis*, which were assigned to 23 groups of aerobic, facultative anaerobic or microaerophillic cellulolytic bacteria.

Bakalidou *et al.* (2002) isolated novel cellulolytic bacteria, *Cellulosimicrobium* strain $MX5^T$ from hindgut of the Australia termite *Mastotermes darwiniensis*. The isolate was found as a facultative anaerobe, which had a Gram-positive cell wall profile.

2.3 Ionic liquids

Ionic liquids (ILs) have been accepted as a new chemical group, which can be used in various applications. ILs are known as salts that are liquid at room temperature. These salts are made of organic cation and inorganic anion. The structure of ILs is similar to the typical salts—for example, sodium chloride or potassium chloride. However, ILs are different from typical salts because salts melt at higher temperature, 800°C (the melting point of sodium chloride is 801°C) but most of ILs can melt and remain in the liquid phase at room temperature. The upper

limit of melting temperature for classification as "IL" is 100°C and higher melting salts are referred as molten salts.

Recently, researchers have discovered the ways for using ILs in several applications such as using ILs as solvent instead of volatile organic solvents, supporting enzyme-catalyzed reactions, hosting a variety of catalysts, purification of gases, homogeneous and heterogeneous catalysis, biological reactions media, and removal of metal ions (Keskin *et al.*, 2007).

2.3.1 Structure and Synthesis of ILs

Because ILs are comprised from cation and anion, the composition and specific properties of ILs depend on the type of cation and anion, size, geometry and charge distribution. By combining various kinds of cation and structures, it is estimated that 10^{18} ILs can be designed (Keskin *et al.*, 2007). The details of cation and anion are discussed below.

2.3.1.1 Cation

The cations of ILs are generally a bulk organic structure with low symmetry. Most ILs are based on ammonium, sulfonium, phosphonium, imidazolium, pyridinium, thiazolium, oxazolium, and pyrazolium cations. However, the most widely used cations are imidazolium, pyridinium, phosphonium, and ammonium. Generally, the cation has an influence on the hydrophobicity or hydrogen bonding ability and length of the alkyl chain in cation influences viscosity, surface tension, and density. The commonly used cations in ILs are shown in Figure 2.3.

Chiappe and Pieraccini (2005) studied the properties of a series of imidazolium cation based ILs. The result shows that the melting temperature of ILs decreases as the size and asymmetry of the cationic increases but increasing the branching on alkyl chain increases the melting temperature.

2.3.1.2 Anion

The anions in ILs also influence the properties of ILs. The types of IL anions can be classified in two types: ILs containing fluorous anions such as PF_6^-, BF_4^-, $CF_3SO_3^-$, $(CF3SO_3)_2N^-$ and ILs with non-fluorous anions such as $AlCl_4^-$

, Cl^-, CH_3COO^- and SCN^-. Usually, the anion controls thermal stability, water miscibility, and solvation. The commonly used anions are shown in Figure 2.3.

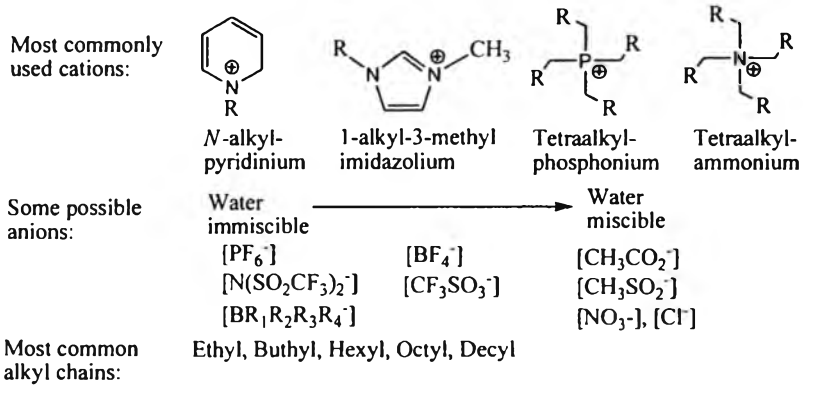

Figure 2.3 Most commonly used cation structures and possible anion types (Keskin *et al.*, 2007).

2.3.1.3 Synthesis

ILs are synthesized by using three basic methods: metathesis reaction, acid-base neutralization, and direct combination. Many alkylammonium halides can be synthesized by the metathesis reaction of appropriate halogenoalkane and amine. Pyridinium and imidazolium are also synthesized by metathesis reaction. Acid-base neutralization is suitable for synthesizing monoalkylamonium nitrate salts by neutralization of aqueous solutions of amine and nitric acid. After neutralization, ILs are undergone vacuumed to remove excess water. The last method, direct combination, is used to synthesize halogenoaluminate and chlorocuprate ILs by combination between halide salt and metal halide (Keskin *et al.*, 2007).

2.3.2 Basic Properties of Ionic Liquids

2.3.2.1 Melting Temperature of Ionic Liquids

ILs can remain in the liquid phase at room temperature and their melting temperature is lower than 100°C. Because of poor combination between bulky asymmetric organic cations and smaller inorganic counterparts, the lattice

energy between ion pairs is lower than typical salt. Hence, ILs can melt at temperature below 100°C.

In addition, carbon numbers in the alkyl chain also influence of the melting temperature of ILs. From observation, melting temperature decreases from the methyl substitution to the butyl to hexyl compound and then increases (Marsh *et al.*, 2004).

2.3.2.2 Density of Ionic Liquids

Generally, ILs are always denser than water. Their density values are ranged between 1 to 1.6 g cm^{-3}. Because of the effect of alkyl chain lengths, the density is also decreased with the increase in the length of the alkyl chain.

2.3.2.3 Viscosity in Ionic Liquids

Most ILs are viscous fluids, which have the viscosity more than oil and also higher than conventional organic solvents about two to three orders of magnitude. However, high viscosity of ILs is not always desirable because of these high viscosity fluids are able to give negative effects of mass transfer and power requirements for mixing in the heterogeneous liquid-liquid systems. Viscosity depends on the length of the alkyl chain. From the experiments, the viscosity of $[C_nMIM]PF_6$ is increased from n equal 4, 6 and 8, respectively, and the range of viscosity is 450–682 mPa.s (Marsh *et al.*, 2004).

The melting temperature, density and viscosity are representing in the Table 2.2.

Table 2.2 Physicochemical properties of some selected ion liquids sorted by anion (Berthod *et al.*, 2008)

Code	Cation name	MW. (Da)	Melting point (°C)	Density (25°C)	Viscosity (25°C) (cP)	Sol. in water
		Bis(trifluoromethylsulfonyl) amide				
EMIM NTfO2	1-Ethyl-3-methyl imidazolium	391	−17	1.52	18	n
EEIM NTfO2	1,3-Diethyl imidazolium	405	14	1.452	35	n
		Dicyanamide				
BMIM DCA	1- Butyl-3-methyl imidazolium	205	−6	1.06	37	s
		Tetrafluoroborate				
EMIM BF4	1-Ethyl-3-methyl imidazolium	197.8	6	1.248	66	s
BMIM BF4	1-Butyl-3-methyl imidazolium	225.8	−82	1.208	233	s
		Hexafluorophosphate				
BMIM PF6	1-Butyl-3-methyl imidazolium	284	10	1.373	400	18 g l^{-1}
HMIM PF6	1-Hexyl-3-methyl imidazolium	312	−61	1.304	800	n
		Chloride				
EMIM Cl	1-Ethyl-3-methyl imidazolium	146.5	89	1.12*	Solid	s
BMIM Cl	1-Butyl-3-methyl imidazolium	174.5	65	1.10*	Solid	s
		Perfluoroalkylsulfate				
EMIM TfO	1-Ethyl-3-methyl imidazolium	260	−9	1.39	45	s
BMIM TfO	1-Butyl-3-methyl imidazolium	288	16	1.29	90	s

Table 2.2 (Continued)

Code	Cation name	m.w. (Da)	Melting point (°C)	Density (25°C)	Viscosity (25°C) (cP)	Sol. in water
		Formate (methanoate)				
EAF	Ethyl ammonium	91	−10	0.990	11.5	s
PAF	Propyl ammonium	105	−10	0.979	18	s
		Acetate (ethanoate)				
EMIM Act	1-Ethyl-3-methyl imidazolium	170	−20	1.03	91	s
BMIM Act	1-Butyl-3-methyl imidazolium	198	−20	1.06	525	s
		Thiocyanat				
BA SCN	Butylammonium	132	20.5	0.949	97	s
DPA SCN	Dipropylammonium	160	5.5	0.964	86	s
		Nitrate				
EA NO3	Ethylammonium	108	12.5	1.122	32	s
PA NO3	Propylammonium	122	4	1.157	67	s

Density and viscosity values at 25°C except an asterisk (*) indicates that the given value corresponds to the salt melting temperature. Solubility in water at room temperature: n = non-soluble (two phases form); s = soluble; p = partly soluble. TfO = triflate anion or trifluoromethyl sulfate.

2.3.2.4 Solvent Properties of Ionic Liquids

ILs are good solvents for several substances: organic, inorganic, organometallic compounds, bio-molecules, and metal ions. Because of poor coordination between cation and anion, ILs are highly polar but non-coordinating solvents. ILs are also called as green solvents because they are non-volatile even though in vacuum condition, non-flammable, non-explosive; feasible to recycle and repeated reuse.

Several advantages of ILs are over conventional organic solvents, which make them environmentally compatible, are described: (1) ILs have the ability to dissolve many different organic, inorganic and organometallic

materials; (2) ILs are highly polar; (3) ILs do not evaporate since they are in very low pressure condition; (4) ILs are thermal stable, approximately up to 300°C; (5) ILs have high thermal conductivity; (6) ILs are immiscible with many organic solvents; (7) ILs are nonaqueous polar alternatives for phase transfer processes; and (8) the solvent properties of ILs can be tuned for a specific application by varying the anion and cation combinations (Keskin *et al.*, 2007).

2.3.2.5 Air and Moisture Stability of Ionic Liquids

Many ILs are both air and moisture stable even some are hydrophobic. Most imidazolium and ammonium salts are hydrophilic, which are able to form hydration with moisture or water. The hydrophobic of IL increases with an increase in the alkyl chain length.

2.3.2.6 Toxicology of Ionic Liquids

The toxicity data of ILs have been limited until now. Although ILs have extremely low vapor pressure and they will not cause air pollution, however, they can be harmful the environment as they enter. Most ILs are water soluble and they can enter to aquatic environment by accidental spills or effluents. The most commonly used ILs, [BMIM]PF_6 and [BMIM]PF_4, are decomposed in the presence of water and hydrofluoric and phosphoric acids are formed as released products (Ganske and Bornscheuer, 2005).

There is a relation between the order of toxicity and alkyl chain length of the cation. For alkyl methyl imidazolium ILs with C_4 as side chain shows moderated toxicity, whereas the C_{12}, C_{16}, and C_{18} species are very highly toxic. While pyridinium, phosphonium and ammonium species with C_4 side chain also shows moderated toxic but C_6 and longer side chains show significant increasing of toxicity (Keskin *et al.*, 2007).

2.3.3 Cellulose-Dissolution Ionic Liquids

ILs are suggested for the dissolution of cellulose. There are many researches that study about the solubility of cellulose in various ILs. Table 2.3 shows details about solubility of cellulose in some ionic liquids.

Table 2.3 Solubility of cellulose in some ionic liquids (Novoselov *et al.*, 2007)

Ionic liquid	Solubility
1-Butyl-3-methylimidazolium chloride	Soluble [a]
1-Allyl-3-methylimidazolium chloride	Soluble
1-Allyl-3-butylimidazolium chloride	Soluble
1,3-Diallylimidazolium chloride	Soluble
1-Butyl-2,3-diethylimidazolium chloride	Dissolves slowly
1-Allyl-3-propargylimidazolium chloride	Dissolves slowly
1-Butyl-2,3-dimethylimidazolium thiocyanate	Insoluble
1-Butyl-3-methylimidazolium saccharinate	Insoluble
1-Butyl-3-methylimidazolium tosylate	Insoluble
1-Butyl-3-methylimidazolium hydrogensulfate	Insoluble
1-Allyl-3-methylimidazolium dicyanamide	Insoluble
1-Allyl-3-butylimidazolium dicyanamide	Insoluble
1-Allyloxy-3-methylimidazolium dicyanamide	Insoluble
1-Allyloxy-3-methylimidazolium chloride	Insoluble

[a] Cellulose is considered as soluble if its concentration in the given solvent of 3% and over can be attained.

Swatloki *et al.* (2002) studied the dissolution of cellulose in different types of ILs and experiment conditions. From the result, 1-butyl-3-methylimidazolium chloride ([BMIM]Cl) was one of effective ILs, which is able to use for dissolution cellulose pulp, Figure 2.3. This can be dissolved in [BMIM]Cl to 10 wt.% at 100°C and up to 25 wt.% when using microwave as the heating source. From this result, they speculated that the high chloride concentration and activity in [BMIM]Cl is highly effective in breaking the intra- and interhydrogen bond of cellulose chains.

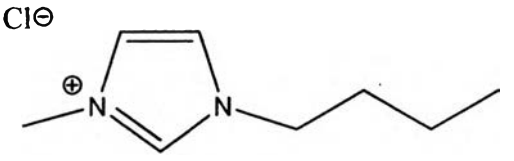

Figure 2.4 Structure of 1-butyl-3-methylimidazolium chloride, [BMIM]Cl.

However, the solubility of cellulose of [BMIM]Cl was decreased as 1 wt.% water (approximatly 0.5 mole fraction H_2O) was added to the system. From the addition, cellulose pulp was precipitated, and this was called "regenerated cellulose". The regenerated cellulose and initial dissolving pulp were characterized by scanning electron microscopic (SEM) and thermogravimetric analysis (TGA). SEM showed the changing of cellulose morphology after it was dissolved in [BMIM]Cl, Figure 2.5. Figure 2.6 shows dissolved pulp that was regenerated from [BMIM]Cl. The curve of initial cellulose sample was rapid decomposition in temperature range from 350–360°C. The regenerated cellulose exhibited a lower onset temperature for decomposition.

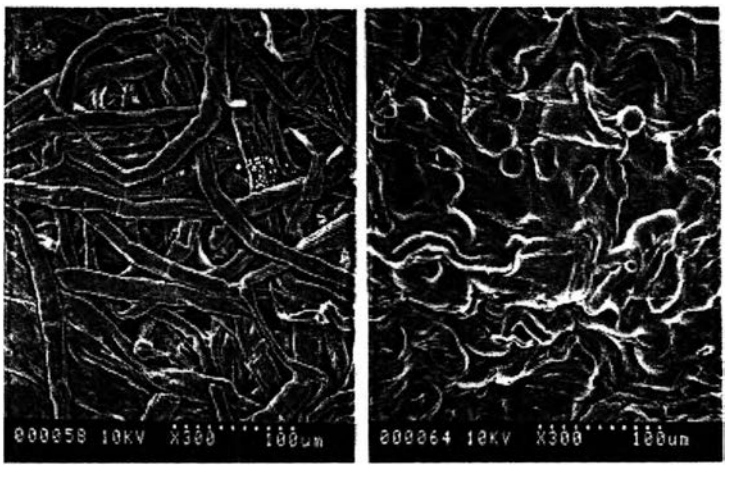

Figure 2.5 SEM micrographs of the initial dissolving pulp (left) and after dissolution in [BMIM]Cl and regeneration into water (right) (Swatloki *et al.*, 2002).

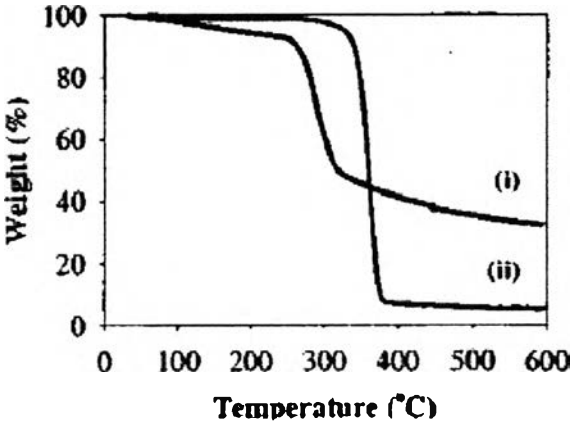

Figure 2.6 Thermal decomposition profiles of (i) regenerated cellulose and (ii) original dissolving pulp. Samples were heated in platinum sample containers under a nitrogen atmosphere at 10°C min^{-1} (Swatloki *et al.*, 2002).

Novoselov *et al.* (2007) used computer modeling to study the dissolution of cellulose in [BMIM]Cl. The dissolution took place because chloride ion attacked the hydroxyl group of cellulose and then cleavaged both intra- and interhydrogen bond of cellulose (O_2H_2---O_6 and O_6H_5'---O_3''). After that, chloride ion formed bond between the H_2 and H_5 atoms so that cellulose was able to dissolve in [BMIM]Cl. The mutual arrangement of molecules in the course of solvation of cellulose with [BMIM]Cl is shown in Figure 2.7.

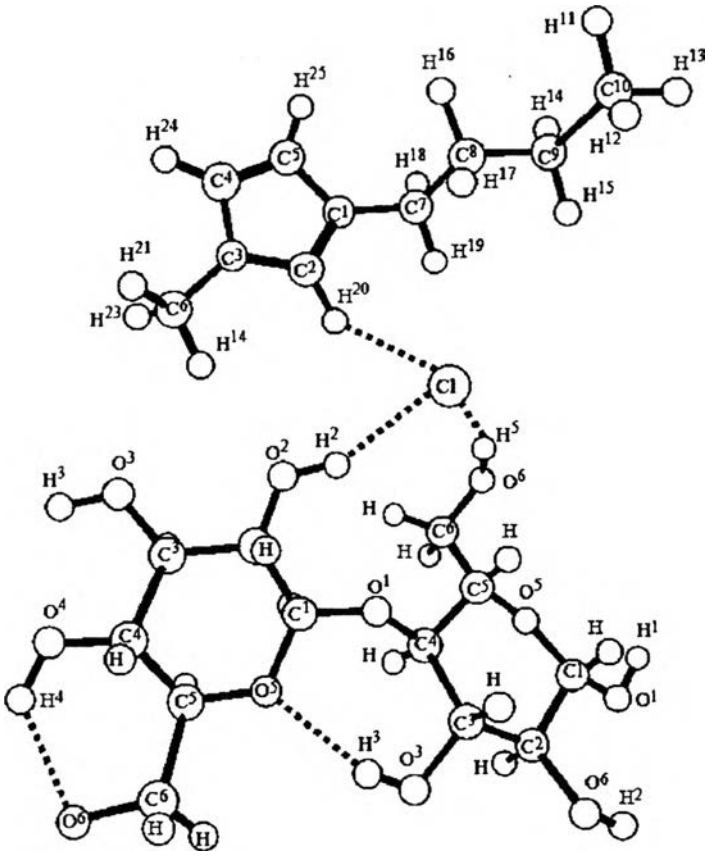

Figure 2.7 Complex of 1-butyl-3-methylimidazolium chloride with cellobiose (Novoselov *et al.*, 2007).

Moreover, they also described the decreasing of cellulose dissolution in [BMIM]Cl when water is added to it. From modeling, they suggested that electron density of the solvent molecule was transferred to the water molecule, which caused the dipole moment of [BMIM]Cl to decrease and then decrease the dissolving power of [BMIM]Cl.

Dadi *et al.* (2007) studied the morphology changing of cellulose after dissolution in [BMIM]Cl and regenerated in water by using X-ray diffraction (XRD), Figure 2.8. The XRD patterns showed the peaks of regenerated cellulose at 5, 10, 15 and 30 wt.% compared to non-dissolved cellulose. The heights of the peak at 2θ = 22.5° of regenerated cellulose at every percent weight was lower than untreated cellulose. Decreasing of height referred to the amount of crystalline or crystalline

index that remained in the cellulose structure. Thus, [BMIM]Cl is an effective solvent that can convert crystalline cellulose to amorphous cellulose.

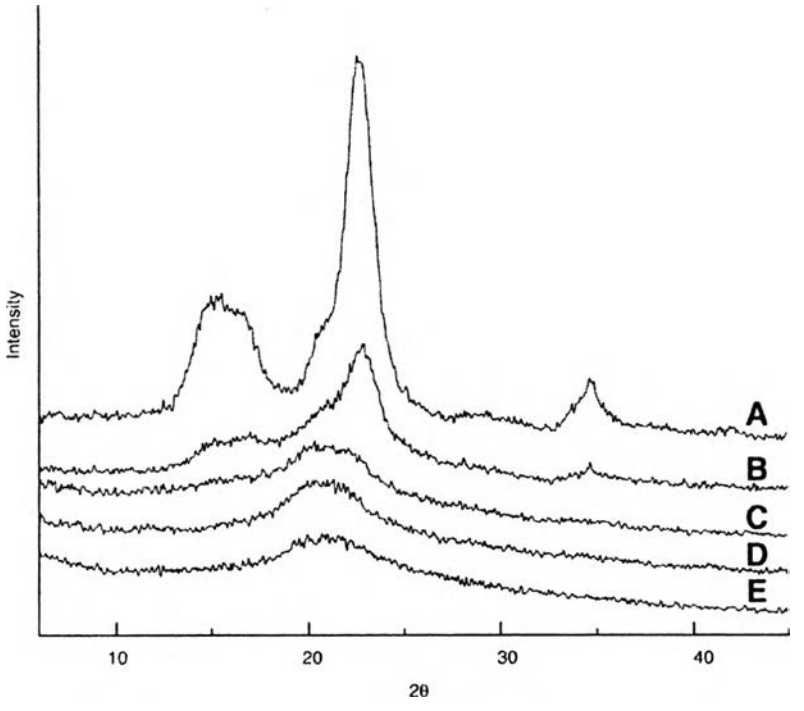

Figure 2.8 XRD patterns for IL-treated and untreated-cellulose. Untreated cellulose **(A)**, exhibited a significantly greater degree of crystallinity than that of regenerated samples **(B–E)**. Cellulose samples were incubated in [BMIM]Cl at 120°C for 30 min and precipitated with deionized water. Samples B–E correspond to 30, 15, 10, and 5 wt.% (Dadi *et al.*, 2007).

However, Swatloski *et al.* (2002) also determined the trace of ionic liquid, [BMIM]Cl, in regenerated cellulose. They found that approximately 76 μg of [BMIM]Cl per gram of cellulose (76 ppm) remained within the regenerated cellulose.

In addition, Dadi *et al.* (2007) also studied the enzymatic hydrolysis rate of cellulose by commercial cellulase, *Trichoderma reesei,* after pretreatment with [BMIM]Cl. The result showed the cellulose that was treated in [BMIM]Cl had higher hydrolysis rate than untreated cellulose at the same incubation conditions and enzyme loading. Furthermore, the amount of reducing sugar that released from

hydrolysis reaction was also higher than untreated cellulose at the same time, Figure 2.9.

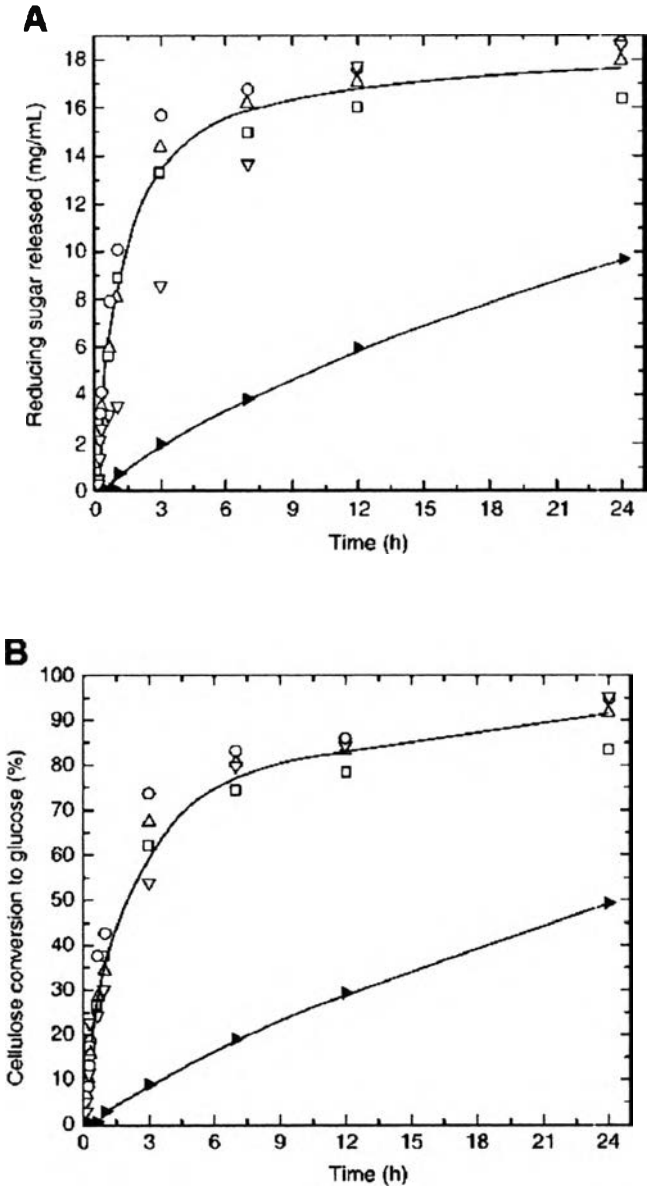

Figure 2.9 Cellulose samples of 5% (□), 10% (○), 15% (Δ), and 30% (∇) were incubated for 30 min in [BMIM]Cl at 120°C, and precipitated with deionized water. Hydrolysis rates of IL incubated samples by *T. reesei* are compared with that of untreated Avicel (▲). **(A)** Total soluble sugars (measured using a DNSA assay) and **(B)** Percent cellulose conversion to glucose (measured by HPLC) (Dadi *et al.*, 2007).

Liying and Hongzhang. (2006) also studied the effect of [BMIM]Cl that influenced enzymatic hydrolysis from commercial cellulase in wheat straw. The result showed that wheat straw, which was treated by [BMIM]Cl, had lower degree of polymerization and hydrolysis rate was higher than untreated wheat straw.

2.3.4 Tolerance of Bacteria in Imidazolium Cation Based Ionic Liquids

There are some research papers that revealed the study of tolerance of several bacteria in presence of ILs.

Matsumoto *et al.* (2004) studied the tolerance of nine lactic producing bacteria at 5 vol.% of various ILs: the haxafluorophosphates of 1-butyl-, 1-hexyl- and 1-octyl-3-methylimidazolium, [BMIM]PF_6, [HMIM]PF_6 and [OMIM]PF_6, respectively, in MRS medium. The result showed that nine lactic producing bacteria were able to tolerate and produce lactic acid in the presence of ionic liquid. However, the amount of viable cells of bacteria and lactic acid were lower as compared with bacteria that were not contained in ILs. The lactic acid producing activities of the bacteria were generally decreased with increasing alkyl chain length in the imidazolium cation moiety.

Ganske and Bornscheuer (2005) studied the effect of 1-butyl-3-methylimidazolium tetrafluoroborate, [BMIM]BF_4 and 1-butyl-3-methylimidazolium haxafluorophosphates, [BMIM]PF_6 on the growth of *Escherichia coli*, *Pichia pastoris* and *Bacillus cereus,* the microorganisms used in the laboratory in biotransformations and enzyme production, at different concentrations of ionic liquids (0.1, 1, 4, and 10 vol.%). The result shows that [BMIM]BF_4 was toxic for all bacteria at 1 vol.% while [BMIM]PF_6 was toxic for *Escherichia coli* and inhibits growth of *Bacillus cereus* of at 1 vol.% but *Pichia pastoris* was unaffected at 10 vol.%.