Chapter 3
Lignocellulose Pretreatment by Ionic Liquids: A Promising Start Point for Bio-energy Production

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3.1 Introduction

The impacts of climate change are forcing governments to limit greenhouse gas (GHG) emission through the utilization of sustainable energy, such as solar energy, wind energy, hydrogen energy, etc. Being well recognized as one of the sustainable energy alternatives to petroleum fuels, biofuels are developed from biomass, which are storage of solar energy via photosynthesis by nature. All countries have put the development of biofuels at the top of their agenda on the road to a clean energy system. Traditionally, biofuels were usually produced from corn, sugarcane, and so on. They are recognized as food sources for human and animals. Recently, the overdevelopment of biofuels has simulated concerns about food-based biofuels, and it was regarded as potential threat of food security and strains on natural resources [1]. As the most abundant biomass on the planet, lignocellulose is mainly consisted of cellulose, hemicellulose, and lignin [2]. The utilization of lignocellulosic resources was regarded as one pathway for production of biofuels without occupying plowland and contributing to the greenhouse effect. Additionally, nowadays almost all alternative energy sources have low-energy return on investment (EROI) values, because they require high-energy input [3]. Therefore, the development of energy-efficient conversion technologies is a challenge during the biofuel industrialization process.

Lignocellulosic biomass, primarily being a complex mixture of cellulose, hemicellulose, and lignin, is naturally resistant to breakdown by pests, disease, and weather. This inherent recalcitrance makes the production of monosugars or other valuable chemicals from lignocellulose expensive and inefficient. It is well

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recognized that cellulose crystallinity, covalent interactions between lignin and polysaccharides, and robust hydrogen bond in cellulose microfibrils must be broken before cellulose and hemicellulose are converted to sugars efficiently through pretreatment processes [4]. Lignocellulose pretreatment, which involves many physical, chemical, structural, and compositional changes, is considered to be a central unit in an efficient and economic conversion of lignocellulosic biomass into fuels and chemicals. Presently, there are quite a lot of various physical-, chemical- and biological-based pretreatment technologies for lignocellulosic biomass available [5]. However, they still suffer from different problems, such as hash conditions, high cost, and low efficiency. Sometimes, an integration of different pretreatment strategies is needed aiming to a more efficient pretreatment.

The full dissolution of cellulose and lignocellulose in ionic liquids (ILs) was accompanied by the destruction of cellulose crystallinity and inter (or inner) hydrogen-bonding network, partially deconstruction of covalent bonds between carbohydrate and lignin, and decrease in the lignin content in cellulose rich products, all of which are beneficial factors for further chemical or biological conversion of carbohydrates into monosugars and chemicals. This chapter aims to provide an up-to-date progress in the understanding of the fundamental sciences and its relation to enzymatic hydrolysis with the ILs-based strategies at this start point of lignocellulose biorefinery.

3.2 Ionic Liquids: Good Solvents for Biomass

For a long time, the full dissolution biomass is one of the biggest barriers for the homogeneous utilization of biomass. In 2002, Rogers et al. first reported that 1-methyl-3-butyl-imidazlium chloride was able to dissolve cellulose with capability of 10–25 wt% depending on heating methods [6]. Since then, the soluble behaviors of most of carbohydrates and biopolymers have been studied [7], such as chitin, chitosan [8, 9], lignin [10], silk fibroin [11], and wool keratin [12]. In 2007, Kilpelainen et al. first investigated the details of woody lignocellulosic materials in ILs. It was found that the lignocellulosic materials were harder to dissolve compared to the soluble behavior of cellulose by ILs, which needed higher temperature, longer time, and more intense stirring. As a result, a 7 wt% spruce wood solution was achieved at 130°C in 8 h. Further study showed that the 1-methyl-3-ethyl-imidazolium acetate was a better solvent for lignocellulosic materials. After the dissolution of cellulose or wood in ILs, it was anticipated that all the chemical bonds and functional groups on these biopolymers are totally open to external chemicals and catalysts, which rationally facilitates the conversion of chemical bonds and functional groups. All of the pioneering work has stimulated a growing research effort in this field to investigate the potential of this new homogenous platform [13]. Another reason for the passion in biomass utilization in ILs is that the process is the combination of the application of biorenewable resources as raw materials and sustainable solvents for the production of valuable materials and chemicals, which will contribute to the foundation of bio-based sustainable chemical industry [14].

3.2.1 Relationship Between Ionic Liquids' Structure and Solubility

Cellulose is a polysaccharide consisting of linear chains of several 100–10,000 β(1-4) linked D-glucose units [2]. The chains are assembled in both parallel and anti-parallel ways via hydrogen bonds, which adds more rigidity to the structure, and a subsequent packaging of bound-chains into microfibrils forms the ultimate building materials of nature. The formed rigid structure determines the insolubility characteristic in conventional solvents and thus limits its full exploitation of the potential of cellulose as feedstock. The story of dissolution of cellulose in ionic liquids may go back to a published patent by Charles Graenacher [15], in which they reported that molten benzylpyridinium chloride or *N*-ethylpyridinium chloride in the presence of nitrogen-containing bases was able to dissolve cellulose. However, the potential of ILs for biomass processing was only recognized seriously till the discovery of imidazonium-based ionic liquids by Rogers et al. in 2002 [6]. It was found that the solubility of ionic liquids to cellulose is related to the anions in ILs, with the order of solubility of $Cl^- > Br^- > SCN^-$, all of which have the same cation of 1-methyl-3-butyl imidazonium, and the BF_4^-, PF_6^- based ILs cannot dissolve cellulose. Furthermore, microwave irradiation could promote the dissolution both in dissolving rates and solubility of ILs [6]. Since then, with the aim to develop more efficient, economic and 'greener' ionic liquids for cellulose processing, a lot of ILs have been synthesized and screened for the dissolution of cellulose and other biopolymers through tuning the structure of cations or anions, and using cheap and renewable resource as raw materials for the ILs synthesis [7]. Whereas, the imidazonium cation-based ILs companied with Cl^-, acetate, formate, and dimethyl phosphate anions present better performance than that of quaternary ammonium, pyrrolidinium, phosphonium-based ILs [16]. For example, Zhang et al. reported that 1-allyl-3-methylimidazolium chloride ([Amim]Cl) was a high-efficient ILs for cellulose dissolution and derivation with advantages of low melting point and low viscosity [17]. Ohno et al. reported low viscosity, polar and halogen-free 1,3-dialkylimidazolium formats, and acetate ionic liquids which have superior solubility of various polysaccharides under mild conditions (10 wt% at even 60°C) [18]. Fukaya et al. found that alkylimidazolium salts containing dimethyl phosphate, methyl methylphosphonate, or methyl phosphonate have the potential to dissolve cellulose under mild conditions. Especially, *N*-ethyl-*N*-methylimidazolium methylphosphonate enabled the preparation of cellulose solution (10 wt%) and rendered soluble cellulose (2–4 wt%) without pretreatments and heating [19]. Due to the excellent solubility of phosphonate-derived ionic liquids to cellulose, it was found that 1-ethyl-3-methylimidazolium phosphinate could extract polysaccharides (or cellulose) from bran even without heating [20].

Lignocellulose, mainly composed of cellulose, lignin, hemicellulose, and extractives, represents an abundant carbon-neutral renewable resource. The three-dimensional cross-linked lignin network binds the whole wood architecture together, which determines their comparatively harder solubility in solvents than cellulose [2]. In 2007, Kilpelainen et al. first reported the details of the dissolution of woody lignocellulosic materials and defined the various variables that determine its solubilization efficiency in ILs [21, 22]. By stirring the mixtures mechanically, an up to 8 wt% wood solution was obtained by simple mixing of dried wood sawdust and thermo-mechanical fiber samples with the ILs ([Amim]Cl or [Bmim]Cl) at 80–120°C. The results showed that the solubility of wood-based lignocellulosic material is related to several key factors, such as ILs' structure, size of lignocellulosic materials, water content of both the ILs and lignocellulosic materials, etc. Interestingly, an introduction of a phenyl group into ionic liquids ([Benzlymim]Cl) could result in a completely transparent, amber-colored but viscous solution. It was estimated that coulombic interactions, such as H bonding, p–p stacking, and van der Waals interactions in ILs can be up to 600 kJ/mol, whereas H-bonds (for water) or van der Waals forces are generally around 40 kJ/mol [23]. It was proposed that a cationic moiety with an electro-rich aromatic π-system may create stronger interactions for polymers capable of undergoing π–π and n–π interactions according to the Abraham salvation equation [16, 21].

Further in-depth study of the influence of ionic liquids' structure on their solubility by Doherty et al., especially, on the effect of anions, demonstrated the relationship between the Kamlet–Taft α, β, and π^* solvent polarity parameters of different ILs ([Emim][OAc], [Bmim][OAc], and [Bmim][$MeSO_4$]) and effective pretreatments of lignocellulosic biomass. The β parameter provides an excellent predictor for fermentable sugar yields ($\beta > 1.0$, resulting in >65% glucose yields after 12 h cellulose hydrolysis following pretreatment) [24]. The hydrogen bond accepting ability of the anions of the ILs, as characterized by ^1H NMR and the β parameter of the ILs, are closely linked to the solubility of cellulose, which was also supported by other work [25, 26].

3.2.2 *Molecular Level Understanding of the Interaction of Ionic Liquids and Lignocellulose: The Key for Lignocellulose Pretreatment*

Although ILs have been demonstrated to be highly effective solvents for the dissolution of cellulose and lignocellulosic biomass, to date, the mechanism of this dissolution process remains not well understood. There is no definitive rationale for selecting ionic liquids that are capable of dissolving these biopolymers. Most current work is based on the hypothesis that cellulose insolubility is due to the strong intermolecular hydrogen bonds between cellulose chains. The dissolution of cellulose by a solvent is dependent on the destruction of these hydrogen bonds.

The outstanding solubility of ionic liquids to cellulose is due to the hydrogen basicity of anions, which can disrupt the hydrogen-bonding network among cellulose and lead to the dissolution. So far, there have been a few theoretical and experimental studies, including molecular dynamic studies and NMR analyses.

In 2007, Remsing et al. reported that $^{35/37}Cl$ NMR relaxation measurements could be employed to study Cl–H hydrogen bonds in [Bmim] Cl [27]. It was found that the solvation of cellulose by the ionic liquid 1-*n*-butyl-3-methylimidazolium chloride ([Bmim]Cl) involves hydrogen bonding between the carbohydrate hydroxyl proton and the chloride ion in a 1:1 stoichiometry. Their further study demonstrated that the anions in these ILs are involved in specific interactions with the solutes, and govern the solvation process by analysis of $^{35/37}Cl$ and ^{13}C relaxation data for sugar solutions in both imidazolium chlorides and [Emim] [OAc] [28]. Variable-temperature NMR spectroscopy was also applied in the investigation on the dissolution mechanism of cellulose in 1-ethyl-3-methylimidazolium acetate ([Emim] [OAc]) in DMSO-d_6. The results confirmed that the hydrogen bonding of hydroxyls with the acetate anion and imidazolium cation of EmimAc is the major force for cellulose dissolution in the ILs. The relatively small acetate anion favors the formation of a hydrogen bond with the hydrogen atoms of the hydroxyls, while the aromatic protons in the bulky cation imidazolium, especially H_2, prefer to associate with the oxygen atoms of hydroxyls with less steric hindrance [29].

Since glucose is one of the main repeating units of polysaccharides, a better understanding of the interaction mechanism of glucose with ILs will provide in-depth understanding of the interaction of ILs and polysaccharides. In 2006, Youngs et al. investigated the molecular dynamics simulations of the solvation environment of isolated glucose monomers in a chloride-based IL (1, 3-dimethylimidazolium chloride); the results revealed that the sugar prefers to bind to four chloride anions. Coordination shells involve only three anions, two of which are bridging chlorides. The low value of chloride: glucose ratio explains the unexpected high solvation degree of glucose in ILs [30]. Few glucose–glucose hydrogen bonds, but chloride anions hydrogen bonding to different glucose molecules simultaneously were found, partially explain the high solubility of glucose/cellulose in ILs [31].

As a natural polymer, cellulose is significantly amphiphilic and hydrophobic interactions are important for explaining the solubility pattern of cellulose. Lindman et al. presented strong evidence that cellulose is amphiphilic and that the low aqueous solubility must have a marked contribution from hydrophobic interactions [32]. Thus, we should reconsider the molecule interaction between lignocellulosic biomass and ILs. Liu et al. developed an all-atom force field for 1-ethyl-3-methylimidazolium acetate [Emim][OAc] and the behavior of cellulose in this IL was examined using molecular dynamics simulations of a series of (1–4) linked β-D-glucose oligomers (degree of polymerization $n = 5$, 6, 10, and 20). They found that there is strong interaction energy between the polysaccharide chain and the IL, and the conformation (β-(1, 4)-glycosidic linkage) of the cellulose was altered. The anion acetate formed strong hydrogen bonds with hydroxyl groups of the cellulose, and some of the cations were found to be in close contact with the polysaccharides through hydrophobic interactions. These results supported the fact that the cations play a significant role in the dissolution of cellulose in anion acetate ILs [33]. Guo et al. calculated geometries, energies, IR characteristics, and electronic properties of the cellulose-anion (acetate, alkyl phosphate, tetrafluoroborate and hexafluorophosphate) complexes using density functional theory calculations (DFT). They found that the strength of interactions of anions with cellulose follows the order: acetate anion > alkyl phosphate anion > tetrafluoroborate anion > hexafluorophosphate anion, and the favorable sites of cellulose for the chloride anion attack are around the O_2 and O_3 hydroxyls [34].

Singh et al. reported that autofluorescent mapping of plant cell walls was used to visualize cellulose and lignin in pristine switchgrass (*Panicum virgatum*) stems to determine the mechanisms of biomass dissolution during ionic liquid pretreatment. Swelling of the plant cell wall, attributed to the disruption of inter- and intramolecular hydrogen bonding between cellulose fibrils and lignin, followed by complete dissolution of biomass, was observed without using imaging techniques that require staining, embedding, and processing of biomass [35]. This could be applied to the elucidation of structural information of wood and wood components.

3.3 Toward Better Understanding of the Wood Chemistry in Ionic Liquids

The increasing research attention onto the utilization of biomass as feedstock for the production of sustainable materials and chemicals has been directed toward an in-depth understanding of plant cell wall natural structures and their constituents, which are consisted mainly with cellulose, hemicellulose, and lignin. A better understanding of these issues, on one hand, can guide the development of new efficient pretreatment technologies and robust catalysts for the catalytic separation and conversion of biopolymers; on the other hand, can provide new avenues to rationally designing of bio-energy crops with improved processing properties by either reducing the amounts of lignin present or providing a lignin that is easier to degrade. Traditionally, the elucidation of wood structure (lignin) usually follows the destruction-analysis process (e.g. Klason method) due to the insolubility of lignocellulose in conventional organic solvents, and the information obtained does not represent the natural structure of lignin.

Lignin is a complex aromatic chemical polymer present most commonly in wood. As an integral part of the secondary cell walls of plants, it is one of the most abundant organic polymers on the earth, exceeded only by cellulose. In 2007, Jiang et al. investigated the solubility of lignin in different ionic liquids, and the results showed that the order of lignin solubility for varying anions was: $[MeSO_4]^- > Cl^- > Br^- \gg [PF_6]^-$. This result indicated that the solubility of lignin is principally influenced by the anions of ILs [36]. Further ^{13}C NMR analysis of lignin and lignin model compounds presented that ^{13}C signals using ILs as the solvent is shifted up-field by δ 0.1–1.9 ppm in comparison to ^{13}C NMR data acquired using dimethyl sulfoxide (DMSO) as the solvent.

The full dissolution of lignocellulosic materials in ILs provided a new homogeneous media without degradation of their components for the structural analysis of plant cell wall and lignin. Kilpelainen et al. demonstrated that the fully acetylated Norway spruce in ionic liquids was soluble in $CDCl_3$, which allowed the first recording of the solution state ^{1}H NMR spectra of intact acetylated wood. The careful integration of β–O–4 signals for lignin in the ^{1}H NMR spectrum yielded a value of 7.3%, which was in good agreement with the anticipated value of 8% [21]. Further in situ quantitative ^{31}P NMR analysis of spruce dissolved in ionic liquids showed the presence of 13.3 mmol/g hydroxyl groups. This value was close to the theoretically calculated value of 15.7 mmol/g based on traditional methods [37]. Analysis of different pulverization degrees provided semi-empirical data to chart the solubility of Norway spruce in IL [amim] Cl, and further method refinement afforded an optimized method of analysis of the lignin phenolic functionalities, without prior isolation of the lignin from the wood fiber [38, 39].

ILs not only can be used as solvents for catalysis and biomass dissolution, but also can be used as solvents for nuclear magnetic resonance analysis directly. Ragauskas et al. synthesized a series of perdeuterated pyridinium ILs for the direct dissolution and NMR analysis of plant cell walls. Due to the high melting point of pyridinium salts, a co-solvent DMSO-d_6 was used to reduce the viscosity of the resulting mixtures, for example, a mixture of 1:2 [Hpyr] Cl-d_6/DMSO-d_6 was able to dissolve Poplar up to 8 wt% at 80°C in 6 h. Further in situ ^{1}H NMR and ^{13}C NMR analysis showed the full structural map of signals from cellulose, hemicellulose, and lignin. For example, the signals at δ 61.5, 74.1, 75.8, 76.9, 80.1, and 103.0 ppm were in part attributed to cellulose. Whereas, the lignin methoxyl group corresponding to the signals at δ 57 ppm and δ 58–88 ppm could be attributed, in part, to C_β in β–O–4, C_γ/C_α in β–O–4, β-5, and β–β. The signal at δ 106 ppm was attributed to C2/6 resonance of syringyl-like lignin structures, and between 110 and 120 ppm to C2, C5, and C6 resonance of guaiacyl-like lignin structures. The properties and easy preparation of perdeuterated pyridium molten salt [Hpyr]Cl-d_6 offer significant benefits over imidazolium molten salts for NMR analysis of plant cell walls; furthermore, the use of non-ball-milled samples in this study can provide a more efficient and accurate characterization of lignin in the plant cell walls compared with the results from traditional methods [40]. Although lignin can provide a renewable source of phenolic polymers, a high lignin content has proved to be a major obstacle not only in the processing of plant biomass to biofuels, but also in other processes such as chemical pulping and forage digestibility. Therefore, precise analytic techniques for efficient lignin content assessment of a large number of samples are in high demand. Further study from Ragauskas's group reported a linear extrapolation method for the measurement of lignin content by the addition of a specific amount of isolated switchgrass lignin to the biomass solution, and the integration ratio changes could be measured in the quantitative ^{1}H NMR spectra with non-deuterated DMSO as the internal standard. The results showed comparable lignin contents as the traditional Klason lignin contents. They demonstrated that this direct dissolution and NMR analysis of biomass provided a new venue for rapidly assessing the lignin contents in large numbers of "new" plants in biofuel research [41].

3.4 Ionic Liquids Pretreatment Technology for Enzymatic Production of Monosugars

Lignocellulose provides a key sustainable source of biomass for transformation into biofuels and bioenergy products. However, lignocellulosic biomass is recalcitrant to biotransformation to sugars and other value-added products by microbial or enzymatic methods, which limits its use and the economically viable bioconversion [4]. The main goal of pretreatments is to increase the enzyme accessibility and improve the digestibility of cellulose and hemicellulose. Thus, different pretreatment methods and conditions (milling, irradiation, microwave, steam explosion, ammonia fiber explosion, supercritical CO_2 and its explosion, alkaline hydrolysis, liquid hot-water pretreatment, organosolv processes, wet oxidation, ozonolysis, dilute- and concentrated-acid hydrolyses, biological and green ILs pretreatments) should be investigated according to the process configuration selected for the subsequent hydrolysis and fermentation steps at low-energy consumption [42]. The lignocellulose crystalline should be accessible to enzymatic surface reactive area, and lignin is the main obstructive factors on enzymatic hydrolysis [43–45].

After the dissolution of cellulose and lignocellulosic materials into ILs, it is possible to recover the samples by simply adding a nonsolvent, such as water or ethanol into the solution. The X-ray spectra of the regenerated material showed that the X-ray diffraction signals from the crystalline regions of cellulose disappeared after the dissolution–regeneration process with a fully amorphous material obtained, and the crystalline form of cellulose was transformed completely from cellulose I to cellulose II after regeneration from ILs. Such a transformation is anticipated to allow a greater accessibility for the hydrolytic enzymes to rapidly penetrate and hydrolyze the cellulose and hemicellulose. Therefore, the ILs-based pretreatment technologies for monosugars production have received much attention recently. Pioneering investigations in this field suggest that cellulose regenerated from IL solutions is subject to faster saccharification than untreated substrates, and it was found that the initial enzymatic hydrolysis rates were approximately 50-fold higher for regenerated cellulose as compared to untreated cellulose (Avicel PH-101) [46–49].

This pretreatment technology has also been applied to lignocellulosic materials. It was found that under unoptimized conditions, about 60% of the theoretical amount of glucose was enzymatically released from spruce when predissolved in [Amim] Cl and regenerated by the addition of water as an anti-solvent [21]. Since then, with the aim to develop an economic and efficient pretreatment technology, a lot of efforts have been devoted into optimizing this new technology by modifying the structures of ILs, using different origins of lignocellulosic materials and simplifying the sample regeneration and ILs recycle processes.

Due to the good solubility of 1-ethyl-3-methyl-imidazolium diethyl phosphate to cellulose and lignocellulose, Li et al. reported that the enzymatic hydrolysis of wheat straw pretreated by 1-ethyl-3-methyl-imidazolium diethyl phosphate at 130°C for 30 min is enhanced significantly, and the yield of reducing sugars reached 54.8% [50]. In 2010, Nguyen demonstrated that the integration of ammonia and [Emim][OAc] for rice straw pretreatment could reduce the cost of pure ILs pretreatment technology, which may make this emerging technology more adaptable to industrial application in enzymatic hydrolysis of different biomass. It was found that the combination treatment exhibited a synergy effect for rice straw with 82% of the cellulose recovery and 97% of the enzymatic glucose conversion. This cooperative effect showed over 90% of the glucose conversion even with a reduced enzyme usage and incubation time. Furthermore, the ILs could be recycled more than 20 times. Compared with the conventional pretreatments of ILs, this combined method for lignocellulosic biomass pretreatment was more economical and ecofriendly [51].

Besides using different ILs for the pretreatment, the integration of energy-efficient heating ways into the pretreatment will increase the efficiency, such as ultrasonic and microwave technology, which are widely used in chemistry reaction and biology researches. In 2010, Yang et al. reported that a new approach for in situ enzymatic saccharification of cellulose in ILs (ILs)-aqueous media was presented in which ultrasonic pretreatment was used to enhance the conversion of cellulose. Under optimized reactive conditions, higher conversion (95.48%) of cellulose was obtained in the media of aqueous-[Mmim] [DMP] by conducting the pretreatment of cellulose with ultrasonic heating, whereas the conversion of untreated cellulose was 42.77%. Further analysis of the pretreated sample showed that the application of ultrasonic resulted in the depolymerization of cellulose, which led to more efficient saccharification [52]. Microwave irradiation on cellulose dissolution pretreatment with ILs can not only enhance the solubility of cellulose in ILs, but significantly decrease the degree of polymerization of regenerated cellulose after IL dissolution pretreatment as well. The rate of enzymatic hydrolysis of cotton cellulose was increased by at least 12-fold after IL dissolution pretreatment at 110°C and by 50-fold after IL dissolution pretreatment with microwave irradiation [53]. Accordingly, the amount of reducing sugars released from regenerated cellulose and [Bmim] [Cl] and [Emim] [OAc] with microwave irradiation were 17.1 and 15.6 mg/ml after 24 h, respectively. This implied that an approximately threefold enhancement in the cellulose hydrolysis yield could be achieved using IL dissolution pretreatment associated with microwave irradiation compared to that of untreated cotton cellulose (5.0 mg/ml after 24 h).

It is essential to minimize sugar losses, to increase solids concentration, and to decrease the cost of the pretreatment step in the biomass conversion. In order to increase sugar yields, efficient conversion and utilization of hemicellulosic sugars have become an important task and an opportunity to reduce the cost of bioenergy production [54]. It is also one of the biggest challenges for biomass pretreatment with ionic liquids, because the IL-pretreated xylan did not show distinct advantages on its enzymatic saccharification. On the contrary, some ILs may cause xylan degradation and loss during the dissolution and regeneration steps [55].

Lignin is not only one of the important components in lignocellulosic materials, but also a main barrier for enzymatic hydrolysis of lignocellulose biomass. One main purpose of biomass pretreatment is to partially remove lignin from the lignocellulosic materials. In 2009, Tan et al. demonstrated that 1-ethyl-3-methylimidazolium alkylbenzenesulfonates IL ([Emim] [ABS]) could extract lignin from sugarcane plant waste, and a 93% extraction yield was achieved [56]. Further study by Lee et al. showed that IL [Emim] [OAc] could effectively extract lignin from triticale straw, flax shives, and wheat straw, and in the meantime cellulose digestibility of the recovered residues was significantly enhanced. The ionic liquid [Bmim] Cl was less efficient than [Emim] [OAc] for delignification of straw. It was found that higher temperatures and longer extraction time are beneficial for improved lignin extraction and cellulose hydrolysis of the residues, for example, 52.7% of acid insoluble lignin in triticale straw was extracted by [Emim][OAc] at 150°C after 90 min, yielding >95% cellulose digestibility of the residue in only 2 h. The results implied that the outstanding performance of ionic liquids-based pretreatment technology for enzymatic hydrolysis of lignocellulose is attributed to both the deconstruction of crystal structure of cellulose and the delignification during the dissolution and regeneration process.

Despite the efficiency of ILs-based pretreatment technology, the high cost of ILs and their recycle ability are the main challenges regarding to the scale-up industry application of this emerging technology. Most recently, Shill et al. successfully developed a three-phase system consisting of [Emim] [OAc], water, and cellulose forms following dissolution of biomass in the IL and subsequent addition of an aqueous concentrated phosphate solution. This process partially separated lignin from the cellulose in Miscanthus, and enhanced the rate of hydrolysis of the precipitated cellulose. ILs and concentrated phosphate solution were recycled and reused [57]. The design presented an ideal concept process for the biomass pretreatment with ionic liquids.

3.5 Ionic Liquids Pretreatment Technology for Chemical Production of Monosugars

Hydrolysis of cellulose to glucose is virtually an essential step in any practical cellulosic biofuel production via a biological route. However, for a long time, the heterogeneous acidic hydrolysis of cellulose to the production of glucose took the dominant position due to the limit of cellulose solvent. However, the traditional acid hydrolysis of lignocellulose was inefficient and cost-intensive. Considering the full dissolution of cellulose in ILs, it is rational to expect that the dissolution process could break internal and external supramolecular structures among the cellulosic fibers, which will facilitate the interaction between the cellulose and external catalysts and reactants, thus a new hydrolysis behavior of cellulose will be envisioned in ILs.

In 2007, Li et al. first reported the hydrolysis behavior of cellulose in ILs in the presence of mineral acids [58]. The results showed that catalytic amounts of mineral acid were sufficient to stimulate the hydrolysis reaction. For example, when the acid/cellulose mass ratio was set to 0.46, yields of total reducing sugar (TRS) and glucose were 64 and 36%, respectively, after 42 min at 100°C. In fact, excess acid loading in the ILs system was detrimental in terms of sugar yields because side reaction tended to occur which consumed the hydrolysis products. Preliminary kinetic study indicated that the cellulose hydrolysis catalyzed by H_2SO_4 followed a consecutive first-order reaction sequence, where $k1$ for TRS formation and $k2$ for TRS degradation were $0.073\ \mathrm{min}^{-1}$ and $0.007\ \mathrm{min}^{-1}$, respectively. Their further study on the hydrolysis behavior of lignocellulose in ILs demonstrated that hydrochloric acid was also an effective catalyst [59]. TRS yields were up to 66, 74, 81, and 68% for hydrolysis of corn stalk, rice straw, pine wood, and bagasse, respectively, in the presence of only 7 wt% catalyst at 100°C under an atmospheric pressure within 60 min. Under those conditions, the constants for $k1$ and $k2$ were 0.068 and $0.007\ \mathrm{min}^{-1}$, respectively, for the hydrolysis of corn stalk. Similar work was also done by Li et al. using different woody lignocellulosic materials, and it was found that the acidic pretreatment of woody biomass species (*Eucalyptus grandis*, Southern pine and Norway spruce) in [Amim] Cl resulted in the near-complete hydrolysis of cellulose, hemicellulose and a significant amount of lignin [60]. Acid-catalyzed conversion of loblolly pine wood was also investigated in [Bmim] Cl and almost identical results were achieved [61].

Toward a better understanding of the acidic hydrolysis behavior of cellulose in ILs, Vanoye et al. investigated the kinetics of the acid-catalyzed hydrolysis of cellobiose in the ILs 1-ethyl-3-methylimidazolium chloride ([Emim]Cl), which was usually studied as a model for general lignocellulosic biomass hydrolysis in ILs systems. The results showed that the rates of the two competitive reactions, polysaccharide hydrolysis, and sugar decomposition, varied with acid strength, and that for acids with an aqueous p*K*a below approximately zero. It was found that the hydrolysis reaction was significantly faster than the degradation of glucose, thus allowing hydrolysis to be performed with a high selectivity in glucose, which was consisted with the results obtained in Li's work [62]. It was expected that the higher the degree of polymerization (DP) value of cellulose, the longer the reaction time will be required for a satisfactory glucose yield, while more TRS will be observed with a shorter reaction time in ILs, which implies that cellulose hydrolysis in ILs catalyzed by mineral acids most likely follows a random hydrolysis mechanism, as observed with the concentrated-acid system [58]. It was proposed that both endoglycosidic and exoglycosidic scissions occur during the hydrolysis process, but the endoglycosidic product, oligoglucoses, is the major one at the initial stage, which was usually observed in traditional heterogeneous hydrolytic systems. Since then, a lot of mineral acids, organic acids, and solid acids have been applied for the homogeneous hydrolysis of cellulose and lignocellulosic materials in ionic liquids. The results have been summarized in Table 3.1 [63, 64].

Among all these significant contributions into the production of monosugars from biomass with the ILs platform, it is worthy of mention that, in 2010, Zhang et al. demonstrated that under relatively mild conditions ($\leq$140°C, 1 atm) and in the absence of acid catalysts, such as HCl, H_2SO_4, the dissolved cellulose in [Emim] Cl could be converted into reducing sugars in up to 97% yield. Their combined study of experimental methods and ab initio calculations demonstrated that the K_w value of water in the mixture was up to three orders of magnitude higher than that of the pure water under ambient conditions. Such high K_w values are typically achievable under high temperature or subcritical conditions, which is responsible for the remarkable performance in the absence of acid catalysts. They hypothesized that the increased $[H^+]$ was attributed to the enhanced water auto ionization by ionic liquids. This process will be affected by the electrostatic environment of the solution, the broad dielectric medium of the solvent, and the temperature. Comparative ab initio calculations based on the thermodynamic cycle shows that IL-water mixture exhibits higher concentrations of both $[H^+]$ and $[OH^-]$ than pure water, thus enabling the acid- and base-catalyzed reactions [70].

Under homogeneous conditions, the physical barriers of cellulose (such as crystallinity, morphology, surface area, and other physical features) are not present. But the recycling of the acidic catalysts is one of the main drawbacks of the conventional acid-catalyzed reaction processes. Separation processes represent more than half of the total investment in equipment for the chemical and fuel industries, while the introduction of heterogeneous catalysis made the catalyst separation easy after the reaction for industrial processes [72]. After the dissolution of cellulose in ionic liquids, different solid acid catalysts have also been investigated for the hydrolysis of cellulose. In 2008, Rinaldi et al. reported that a solid acid (Amberlyst 15 DRY) catalyzed hydrolysis of cellulose and (ligno)cellulose in ILs [73, 74]. In these studies, depolymerized cellulose was precipitated and recovered by addition of water to the hydrolytic system, and the DP value was estimated by gel-permeation chromatography. It was found that the size of recovered cellulose fibers became successively smaller over time, resulting in a colloidal dispersion for the material recovered after 5 h. The depolymerization of cellulose proceeded progressively, resulting in the formation of soluble oligosaccharides if the reaction was carried out over a long time. For example, cellooligomers consisted of approximately 10 anhydroglucose units (AGU) which were seen after 5 h. The phenomena observed in these studies further supported the proposed hydrolytic pathway in ILs by Li et al. [58]. It was interesting to observe that there was an induction period for the production of glucose, and further titration results of the ILs separated from a suspension of Amberlyst 15DRY in [Bmim]Cl suggested that proton was progressively released into the bulk liquid

Table 3.1 Catalytic hydrolysis of (ligno)cellulose into monosugars in ionic liquids

Raw materials	Acids	Ionic liquids	Regeneration solvent	TRS yield (%)	Sugar yield	References
Avicel	H_2SO_4	[Bmim]Cl	Water	73	32% glucose	[58]
α-cellulose	H_2SO_4	[Bmim]Cl	Water	63	39% glucose	[58]
Spruce	H_2SO_4	[Bmim]Cl	Water	71	28% glucose	[58]
Sigmacell	H_2SO_4	[Bmim]Cl	Water	66	28% glucose	[58]
Corn stalk	HCl	[Bmim]Cl	Water	66	–	[59]
Rice straw	HCl	[Bmim]Cl	Water	74	–	[59]
Pine wood	HCl	[Bmim]Cl	Water	81	–	[59]
Bagasse	HCl	[Bmim]Cl	Water	66	–	[59]
Eucalyptus grandis	HCl	[Amim]Cl	Water\methanol\ethanol	95^a		[60]
Southern pine	HCl	[Amim]Cl	Water\methanol\ethanol	67^a		[60]
Norway spruce	HCl	[Amim]Cl	Water\methanol\ethanol	82^a		[60]
Thermomechanical pulp	HCl	[Amim]Cl	Water\methanol\ethanol	82^a		[60]
Cellulose	HCl	[Emim]Cl	Water	–	89% glucose	[65]
Corn stover	HCl	[Emim]Cl	Water	70–80	–	[65]
Miscanthus grass	CH_3SO_3H	[Emim]Cl	Water	–	68% glucose	[62]
Cellobiose	$H_3PW_{12}O_{40}$	–	Water^b	96^c	51% glucose	[66]
Cellulose	$Sn_{0.75}PW_{12}O_{40}$	–	Water^b	23	100%^{c}	[66]
Lignocellulose	$H_3PW_{12}O_{40}$	–	Water^b	32	82%^{c}	[65]
Cellulose	Nafion$^®$ NR50	[Bmim]Cl	Water	35	–	[67]
α-Cellulose	HY zeolite	[Bmim]Cl	Water	46.9	34.9%	[68]
Avicel cellulose	HY zeolite	[Bmim]Cl	Water	47.5	36.9%	[68]
Spruce cellulose	HY zeolite	[Bmim]Cl	Water	44.4	34.5%	[68]
Sigmacell cellulose	HY zeolite	[Bmim]Cl	Water	42.4	32.5%	[68]
β-Cellulose	HY zeolite	[Bmim]Cl	Water	–	12.5%	[68]

(continued)

Table 3.1 (continued)

Raw materials	Acids	Ionic liquids	Regeneration solvent	TRS yield (%)	Sugar yield	References
Cellulose	$Si_{33}C_{66}$-673-SO_3H	–	Water	90[c]	50% glucose	[69]
Microcrystalline cellulose	$FeCl_2$	$[(CH_2)_4SO_3Hmim][HSO_4]$	Diethyl\ether\water	84.42 [c]	10.24%	[64]
Western red cedar	O_2	[Emim][Cl]	DMSO	N.C.[d]	N.C.	[63]
Cellulose	Proton acid	[R(D)MIM]Cl-water	Water	97	N.C.	[70]
Loblolly pine wood	TFA	[Bmim]Cl	Water	79 [c]	N.C.	[61]
Corn stover	Boronic acids	[Emim][OAc]	Hot water	N.C.	<97% glucose	[71]

[a] Carbohydrates were hydrolyzed at 1.4–1.5 mol of HCl/g wood acid concentration

[b] The reaction was carried out in aqueous solution

[c] TRS selectivity

[d] *N.C* not characterized

within an hour upon through an ion-exchange process involving $[\text{Bmim}]^+$ of the ionic liquid and H^+ species of the solid acid.

The design of solid catalysts, that are suitable for both heterogeneous and homogeneous conversion, is one of the most top challenges for biomass utilization [75]. It was found that the H^+ species and reaction media are highly related to their catalytic activity toward the hydrolysis of cellulose. For example, Shimizu et al. developed $H_3PW_{12}O_{40}$ and $Sn_{0.75}PW_{12}O_{40}$ for the hydrolysis of lignocellulose, which showed higher TRS yield than conventional H_2SO_4 in water [66]. Other solid acids, such as Nafion® NR50, sulfonated silica/carbon nanocomposites, have also been studied for the hydrolysis of cellulose in ILs. It was found that the crystalline cellulose was partially loosened and transformed to cellulose II from cellulose I, then to glucose assisted by Nafion® NR50. Afterwards, a catalyst was recycled and the residual (hemi) cellulose solid, which could be hydrolyzed into monosugars by enzymes, was separated by adding antisolvents [67]. Due to the presence of strong, accessible Brønsted acid sites and the hybrid surface structure of sulfonated silica/carbon nanocomposites, it was found that a 42.5% glucose yield was achieved after three recycles of this catalyst in ILs [69].

Solid acid-catalyzed hydrolysis of cellulose in ILs was greatly promoted by microwave heating. The results showed that H-form zeolites with a lower Si/Al molar ratio and a larger surface area exhibited better performance than that of the sulfated ion-exchanging resin NKC-9. The introduction of microwave irradiation at an appropriate power significantly reduced the reaction time and increased the yields of reducing sugars. A typical hydrolysis reaction with Avicel cellulose produced glucose in around 37% yield within 8 min [68].

Monosugars are intermediates linking the sustainable biomass and clean energies, such as bioethanol and microbial biodiesel. In 2010, Binder et al. first investigated the fermentation potential of sugars produced from cellulose in ILs after separation of ILs by ion-exclusion chromatography. The results showed that adding water gradually to a chloride ionic liquid-containing catalytic HCl led to a nearly 90% yield of glucose from cellulose and 70–80% yield of sugars from untreated corn stover. Ion-exclusion chromatography allowed the recovery of the ILs and delivered sugar feedstocks that support the vigorous growth of ethanologenic microbes. This simple chemical process presents a full pathway from biomass to bio-energy based on the ionic liquids platform, although the development of more economic technologies for the recovery and separation of the ILs and sugars is still in high demand [65].

Recent work has demonstrated that the recovery of sugars from ILs could be fulfilled by extraction based on the chemical affinity of sugars to boronates such as phenyl boronic acid and naphthalene-2-boronic acid [71]. 90% of mono- and di-saccharides could be extracted up by boronate complexes from aqueous ILs solutions, pure ILs systems, or hydrolysates of corn stove-containing ILs.

3.6 Enzymatic Compatible Ionic Liquids for Biomass Pretreatment

Although ILs have proven to be ideal solvents for biomass pretreatment and homogeneous chemical catalytic conversion of biomass into monosugars, the process still suffered a shortage of high cost cellulose regeneration. Considering the fact that ILs are also regarded as ideal solvents for biocatalysis due to their unique advantages compared to conventional solvents, researchers are devoting to develop an integrated process of pretreatment and enzymatic hydrolysis in one batch, which will eliminate the need to recover the regenerated lignocellulosic materials, and will lead to a more economic and environmentally friendly conversion process for bio-energy production [5]. It is rational to postulate that ILs are potentially ideal media for the enzymatic conversion of cellulose and lignocellulosic materials into sugar. However, carbohydrate-dissolving ILs are typically composed of Cl^-, dca^-, $HCOO^-$, ^-OAc, i.e., anions which form strong hydrogen bonds with the carbohydrate. These interactions facilitate the dissolution of biomass, but denaturation of enzymes can be a problem which hinders the enzymatic conversion of dissolved cellulose in ILs. To overcome this obstacle, the design and synthesis of enzyme-compatible ionic liquids which are capable of dissolving cellulose, and do not considerably deactivate enzymes is essentially necessary. In addition, factors such as IL polarity, IL network, ion kosmotropicity, viscosity, hydrophobicity, the enzyme dissolution, surfactant effect, etc., may also influence the catalytic performance of enzymes [76]. To improve the enzyme solubility and activity in ILs, various attempts have been made, including immobilized enzymes, microemulsions, whole cells catalysis, multi-phase partitioning (TPP) reaction, the use of additives ($NaHCO_3$, Na_2CO_3, or triethylamine), enzyme-coated microcrystals, and lipase lyophilization with cyclodextrins [77].

In 2008, Kamiya et al. first reported a one-batch enzymatic process for the saccharification of cellulose in aqueous-IL [1-methyl-3-methyl-imidazolium] [Diethyl phosphate] system, which showed initial information on the potential of [1-methyl-3-methyl-imidazolium] [Diethyl phosphate] as the solvent for in situ pretreatment and enzymatic hydrolysis of lignocellulosic materials in ILs media [78]. Further study by Yang et al. with the diethyl phosphate-based ionic liquids showed that ultrasonic pretreatment could enhance the in situ enzymatic saccharification of cellulose in aqueous-ionic liquid media, as a result 95.5% conversion of cellulose could be obtained [79]. Furthermore, they also found that the pretreatment of corn cob in 1-methyl-3-methylimidazolium dimethylphosphite ([Mmim]DMP) in view of its biocompatibility with both lignocellulose solubility and cellulase activity (more than 70% saccharification rate), did not bring negative effects on saccharification, cell growth, and accumulation of lipid of *R. opacus* ACCC41043 [80].

It is well recognized that ILs can be designed with different cation and anion combinations, which allows the possibility of tailoring reaction solvents with specific desired properties, and these unconventional solvent properties of ILs provide the opportunity to carry out many important biocatalytic reactions that are impossible in traditional solvents. In order to avoid denaturing enzyme, Zhao et al. designed a series of glycol-substituted cation and acetate anion ILs that are able to dissolve carbohydrates but do not considerably inactivate the enzyme (immobilized lipase B from *Candida Antarctica*). The ILs could dissolve more than 10% (wt) cellulose and up to 80% (wt) D-glucose. The transesterification activities of the lipase in these ILs are comparable with those in hydrophobic ILs [81]. Garcia et al. reported a class of biocompatible and biodegradable cholinium-based ILs, the cholinium alkanoates, which showed a highly efficient and specific dissolution of the suberin domains from cork biopolymers. These results are almost more efficient than any system reported so far [82]. However, they did not perform the in situ conversion experiments in these ILs. Bose et al. employed tryptophyl fluorescence and DSC to investigate the reactivity and stability of a commercial mixture of cellulases in eight ILs. Only 1-methylimidazolium chloride (mim Cl) and tris-(2-hydroxyethyl) methylammonium methylsulfate (HEMA) provided a medium hydrolysis [83]. Although we can conclude that high concentrated ILs can make the enzyme lose its activity, there are still many new ILs or enzymes that show good biocompatability or IL-tolerance. These results provide us a green approach to the production of biofuels. At present, it is evident that the pretreatment of lignocellulose in ILs is a good choice for the fast enzymatic hydrolysis of cellulose.

With the aim to search for cellulose hydrolyzing enzymes that are stable in ILs, in 2009, Pottkamper et al. applied metagenomics for the identification of bacterial cellulases that are stable in ILs. By screening metagenomic libraries, 24 novel cellulase clones were identified and tested for their performance in the presence of ILs. Most enzyme clones showed only very poor or no activities. Three enzyme clones,(*i.e.,*. pCosJP10, pCosJP20, and pCosJP24) were moderately active and stable in the presence of 1-butyl-1-methyl-pyrrolidinium trifluoromethanesulfonate. The corresponding genes of these environment-derived cosmids were similar to known cellulases from *Cellvibrio japonicus* and a salt-tolerant cellulase from an uncultured microorganism. It was found that the most active protein (CelA$_{10}$) belonged to GH5 family cellulases and was active at IL concentrations of up to 30% (v/v). Recombinant CelA$_{10}$ was extremely tolerant to 4 M NaCl and KCl. In addition, improved cellulase variants of CelA$_{10}$ were isolated in a directed evolution experiment employing SeSaM-technology. The analysis of these variants revealed that the N-terminal cellulose binding domain played a pivotal role for IL resistance [84]. Meanwhile, Datta et al. found that both hyperthermophilic enzymes were active on [Emim] [OAc] pretreated Avicel and corn stover. Furthermore, these enzymes could be recovered with little loss in activity after exposure to 15% [Emim] [OAc] for 15 h. These results demonstrated the potential of using IL-tolerant extremophilic cellulases for hydrolysis of IL-pretreated lignocellulosic biomass and for biofuel production [85].

3.7 Conclusions and Prospects

Abundant lignocellulose biomass has the potential to become a sustainable source of fuels and chemicals. It needs to realize that this potential requires the economical conversion of recalcitrant lignocellulose into useful intermediates, such as sugars. With the development of biotechnology, the fermentation of sugar can lead to production of various bio-energy and value-added chemicals, such as bioethanol and biodiesel. Therefore, the development of an efficient pretreatment of biomass for monosugars production is the entry point of bio-based chemical industry. Ionic liquids have unique properties compared with conventional organic solvents. The full dissolution of cellulose and lignocellulose in ILs allows a full map of homogenous utilization of them in association with advanced catalytic and separation technologies. Bearing all of these significant progresses in our mind, from in-depth understanding of the dissolution mechanism, chemically catalytic and enzymatic hydrolysis, to in situ pretreatment-enzymatic hydrolysis, a clear pathway and potential to the production of bio-energy and chemicals from biomass in ILs has been illustrated. To take the full advantage of the opportunities afforded by ILs in biomass processing and conversion, there are still a number of challenges ahead on their potential industrial applications [77], for example:

1. The design and preparation of cheaper, non-toxic, enzyme-compatible ILs capable of dissolving cellulose, on the basis of in-depth understanding of dissolution mechanism of cellulose in ILs;
2. Hydrolytic dynamic study of cellulose in ILs, which will provide in-depth information and knowledge for the design and development of high-efficient catalysts;
3. Integration of sustainable energy methodologies, advanced catalytic technologies, and separation technologies into the ILs platforms;
4. Development of efficient and facile separation technologies for recovery of ILs and separation of hydrolyzed sugars for downstream applications;
5. Metabolism of ILs by microorganism and gene modification of microorganism aiming to increase their tolerance to ILs.

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