Chapter 4
Application of Ionic Liquids in the Conversion of Native Lignocellulosic Biomass to Biofuels

Marcel Lucas, Gregory L. Wagner and Kirk D. Rector

4.1 Introduction

Lignocellulosic biomass could become an abundant source of liquid fuels and commodity chemicals that could satisfy energy needs in transportation and alleviate concerns about rising greenhouse gas emissions. The variety of potential feedstocks, which includes wood, agricultural wastes, forest products, grasses, and algae, reduces the pressure on food crops, in particular corn, for the production of ethanol [1, 2]. Wood is composed of three main components: cellulose, hemicellulose, and lignin. Cellulose is a polymer of glucose. Hemicellulose is a branched polymer of different monosaccharides. Lignin is a branched polymer with *p*-hydroxyphenyl, guaiacyl, and syringyl units [3]. The conversion of lignocellulosic biomass to biofuels consists in the hydrolysis of cellulose and hemicellulose into fermentable sugars, followed by the fermentation of these sugars into ethanol and commodity chemicals. The access of enzymes to cellulose is severely restricted by the complex structure of the wood cell wall and the recalcitrance of lignin.

Conversion of native biomass to biofuels therefore requires a pretreatment step that should separate the three main components of wood, improve access of enzymes to cellulose, and decrease cellulose crystallinity. Kraft pulping has been the dominant process to produce purified cellulose substrates for papermaking, but it involves toxic chemicals and requires large amounts of water [4]. Other biomass pretreatments, such as acid hydrolysis, steam explosion, alkaline hydrolysis, and ammonia fiber explosion, are energy-intensive and also involve toxic chemicals [5]. Pretreatment is the most expensive step in the biomass conversion process, and could represent a fifth of the total cost [1, 2].

M. Lucas · G. L. Wagner · K. D. Rector (✉)
Chemistry Division, Los Alamos National Laboratory,
Los Alamos, NM 87545, USA
e-mail: kdr@lanl.gov

C. Baskar et al. (eds.), *Biomass Conversion*,
DOI: 10.1007/978-3-642-28418-2_4, © Springer-Verlag Berlin Heidelberg 2012

Recently, room-temperature ionic liquids (ILs) have been considered as potential solvents for the dissolution and pretreatment of biomolecules and biomass [4, 6–11]. It was found that the solubility of cellulose in 1-butyl-3-methylimidazolium chloride ([BMIM][Cl]) could reach 25 wt% [9]. ILs are salts with melting temperatures below 100°C, characterized by an extremely low vapor pressure, high thermal stability, and low flammability. Their physicochemical properties, such as glass transition and melting temperatures, thermal stability, refractive index, and polarity depend on their chemical composition and structure [12–14]. The multitude of possible anion–cation combinations and the blending of multiple ILs provide great flexibility when tailoring an IL for a specific application [15]. ILs have numerous promising applications in catalysis [16–20], electrochemistry, separations of gases, liquids, and impurities [21]. The positive effect of ionic liquids on catalysis was partially attributed to the stabilization of reactive intermediates and catalytically active oxidation states [17]. Promising studies on cellulose dissolution and regeneration led to an intense effort to develop an effective IL pretreatment for the direct pretreatment and dissolution of native biomass [4, 6–11].

In this chapter, the dissolution of native biomass in ILs will be reviewed. In Sect. 4.2, the different factors affecting biomass solubility in ILs will be reviewed. The mechanisms involved in the biomass delignification and cellulose dissolution will be discussed in Sect. 4.3. Section 4.4 will focus on the compatibility of ILs with cellulases and the different strategies developed for the stabilization of enzymes in ILs. Section 4.5 will deal with the recycling and biodegradability of ILs. Finally, in Sect. 4.6, applications of biomass pretreatment with ILs (other than fuel production) in the making of composite materials, the biomedical field, the production of commodity chemicals, and biochemical sensing will be reviewed.

4.2 Pretreatment of Native Biomass

4.2.1 Cellulose and Lignin Composition in Biomass

Great variability in lignocellulosic biomass feedstocks is observed in wood or non-wood plants: differences in fiber dimensions, lignin, and cellulose content across different species [22]. Enzymatic hydrolysis of 1,100 natural *Populus trichocarpa* trees resulted in a wide range of sugar yields that depended on the lignin content and the ratio of syringyl and guaiacyl units in lignin. Among the 1,100 samples, the lignin content ranged from 15.7 to 27.9 wt%, while the syringyl-to-guaiacyl unit ratio ranged from 1.0 to 3.0 [23]. Even in the same plant, differences were observed between the mature sections at the base and the younger sections at the top [24].

Due to this great diversity of chemical composition and the complex structure of native biomass, effective methods for the dissolution or hydrolysis of purified

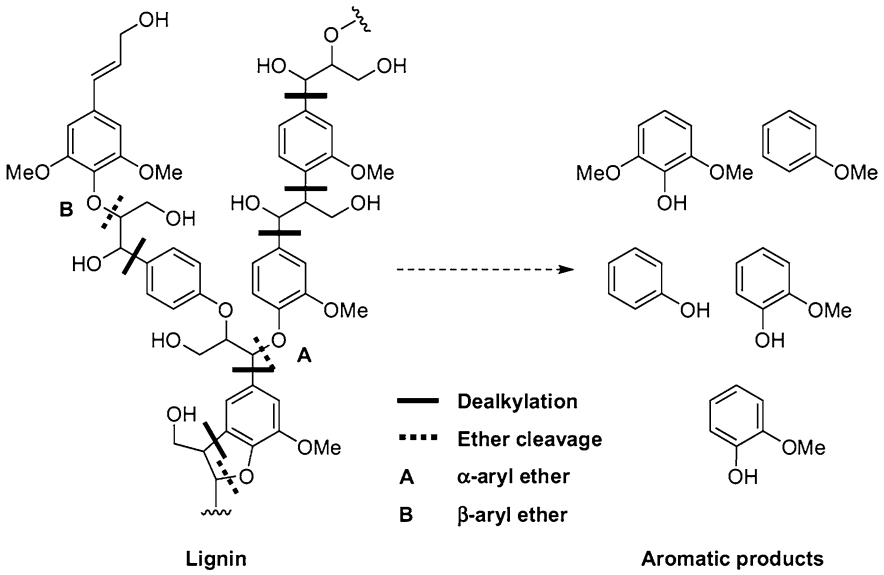

Fig. 4.1 Generalized chemical structure of lignin and schematic for its conversion into monomeric aromatic products. Reactions which cleave aryl–ethers and aryl–alkyl linkages would enable conversion of lignin into valuable aromatic chemicals. Reprinted from [28], copyright (2011), with permission from Elsevier

cellulose or glucose oligomers can fail to translate to native biomass. In lignin, each type of linkages in the constituting monolignols provides a possible pathway for biomass delignification (Fig. 4.1) [25–28]. Developing a unique IL pretreatment that would be suitable for multiple feedstocks represents a tremendous challenge.

4.2.2 Dissolution of Biomass in Ionic Liquids

A wide variety of biomass feedstock/IL combinations has been studied for their potential in biomass pretreatment. Multiple wood species have been studied: poplar [29], spruce [7, 30–34], eucalyptus [31, 32], pine [4, 6, 7, 31, 32, 35–37], maple [25, 38], *Metasequoia glyptostroboides* [16], red oak [36], common beech [34], cork [39], and Japanese fir [40]. Other biomass feedstocks currently under investigation include grasses, such as switchgrass [41, 42], *Miscanthus* grasses [26, 43], and agricultural wastes, such as corn stovers [6, 33, 35, 43–45], wheat straw [27] and rice straw [6, 35, 46].

Among the most successful and widely used ILs in native wood pretreatment are the imidazolium-based ILs with the chloride or acetate anion. The ILs 1-allyl-3-methylimidazolium chloride ([AMIM][Cl]) and [BMIM][Cl] could dissolve

maple wood flour at solubilities above 30 g/IL kg at 80°C under nitrogen atmosphere after 24 h [25]. Ball-milled pine powder and spruce sawdust (size 0.1–2 mm) were completely dissolved in [BMIM][Cl] and [AMIM][Cl] at a weight ratio of up to 8% at 80–110°C in 8 h with mechanical stirring [7]. [AMIM][Cl] was able to dissolve completely 5 wt% of spruce, silver fir, beech, chestnut wood chips (particle size 1–2 mm) at 90°C in 12 h, whereas the same wood samples were only partially dissolved in 1-ethyl-3-methylimidazolium chloride ([EMIM][Cl]), [BMIM][Cl], and 1,3-dimethylimidazolium dimethylphosphate ([MMIM][Me_2PO_4]) in the same conditions [34]. The IL 1-ethyl-3-methylimidazolium chloride ([EMIM][Cl]) can partially dissolve wheat straw and pine wood particles (<1 mm, 5 wt%) at 100°C in 24 h, [BMIM][Cl] can only partially dissolve wheat straw, and 1-ethyl-3-methylimidazolium acetate ([EMIM][OAc]) could dissolve neither [47]. Ground pine, poplar, eucalyptus, and oak were dissolved in [BMIM][Cl] with a 5 wt% solubility at 100°C. After 24 h, about 45 wt% of the cellulosic material was extracted from the native biomass. The extraction rates were higher for softwoods, such as pine and poplar. ^{13}C Nuclear Magnetic Resonance (NMR) confirmed the presence of dissolved polysaccharides in the wood/IL mixture [4].

[EMIM][OAc] dissolved spruce, beech, chestnut completely (5 wt%), but not silver fir [34]. In another study, [EMIM][OAc] could dissolve 5 wt% of red oak (particle size 0.125–0.250 mm) completely in 25 h at 110°C, while it took 46 h to dissolve 5 wt% of southern yellow pine in the same conditions [36]. Pretreatment of maple wood flour with [EMIM][OAc] or 1-butyl-3-methylimidazolium acetate ([BMIM][OAc]) at 90°C increased significantly the sugar yield and the amount of extracted lignin [38]. Pretreatment with 1-butyl-3-methylimidazolium methyl sulfate ([BMIM][$MeSO_4$]) at the same temperature for the same duration resulted in sugar yields comparable to the untreated wood flour and an amount of extracted lignin lower than with [EMIM][OAc] or [BMIM][OAc]. This was explained by the fact that [BMIM][$MeSO_4$] only delignified the middle lamella and not the primary cell wall and cellulose-rich secondary cell wall. Also, the [EMIM][OAc] or [BMIM][OAc] pretreatment for 12 h reduced the wood fiber diameter from an average of 250 μm in the untreated flour to about 17 μm. Pretreatment with [BMIM][$MeSO_4$] had no effect on the wood fiber diameter [38].

Other combinations of IL/native wood were studied. Dry wood (*Metasequoia glyptostroboides*, 60 mesh sawdust) was partially dissolved in 1-butyl-3-allylimidazolium chloride ([BAIM][Cl]) or 1-methyl-3-allylimidazolium chloride ([MAIM][Cl]) at a weight ratio from 4.5:1 to 10.5:1 (60–90°C for 10–40 min) [16]. Phenyl-containing ionic liquids were synthesized to see if the aromatic π-systems would be better at disrupting the strong π–π interactions between aromatic groups in lignin. Indeed, after the wood was dissolved in 1-benzyl-3-methylimidazolium chloride ([BzMIM][Cl]), the solution was clear, free of any residual lignin [7]. Ball-milled poplar was soluble in 1-allylpyridinium chloride, cyanomethylpyridinium chloride, and pyridinium chloride within 1 h at 60°C with solubilities ranging from 35 to 80 mg/g [29].

In addition to woods, ILs could at least partially dissolve or delignify other feedstocks, including leaves and agricultural wastes. Triticale straw, flax shives, and wheat straw were soluble in [EMIM][OAc] and [BMIM][Cl] [27]. The dissolution of shredded oil palm fronds in [BMIM][Cl] was studied for temperatures ranging from 60 to 100°C [22]. Lignin was extracted from bagasse using the IL 1-ethyl-3-methylimidazolium alkylbenzenesulfonate at high temperatures (170–190°C) [48]. Rice straw powder (<2 mm) could be dissolved in [BMIM][Cl], [EMIM][Cl], and [EMIM][OAc] completely in 24 h at 130°C. The amount of regenerated cellulose and glucose after enzymatic hydrolysis was highest for [EMIM][OAc] [46]. Milled corn cob had solubilities above 30 g/kg at 130°C in 1-methyl-3-methylimidazolium dimethylphosphite ([MMIM][DMP]), 1-ethyl-3-methylimidazolium diethylphosphate ([EMIM][DEP]), 2-ethyl-3-methylimidazolium dimethylphosphite ([EMIM][DEP]), [BMIM][Cl], and 1-butyl-1-methylpyrrolidinium chloride ([BMPy][Cl]). Pretreatment with chloride ILs resulted in the doubling of reducing sugar yield after enzymatic hydrolysis [44].

4.2.3 Effect of Ionic Liquid Chemical Composition

The nature of the anion played a major role in the dissolution of biomass. For example, [EMIM][OAc] was more effective than [EMIM][Cl] in the dissolution of southern yellow pine [36]. The chloride anion combined with the [BMIM] cation was effective in the dissolution of maple wood flour. Substitution of the chloride anion with the tetrafluoroborate or hexafluorophosphate anions made the maple wood flour insoluble [25]. Maple wood flour pretreated in [EMIM][OAc] and [BMIM][OAc] at 90°C for 6 h resulted in a decrease in cellulose crystallinity, higher glucose, and xylose yields. In contrast, pretreatment with [BMIM][$MeSO_4$] had little effect on the biomass cell structure, sugar yields, and cellulose crystallinity, compared to untreated wood flour [38].

The ability to dissolve biomass was related to the anion basicity. [EMIM][OAc] was a better solvent than [BMIM][Cl] for southern yellow pine and red oak (particle size 0.125–0.250 mm), due to the increased basicity of the acetate anion and also its lower viscosity and melting point [36]. Cork powder remained insoluble in [EMIM][Cl] and [BMIM][Cl] after 4 h at 100°C. Replacing the chloride anion with a lactate or ethanoate anion improved the cork dissolution significantly. ILs based on the cholinium cation and alkanoate anions were more effective in the cork dissolution. Among the alkanoate anions included in the study, the increasing alkyl chain length (ethanoate, butanoate, hexanoate) led to an increase in biomass dissolution efficiency, attributed to an increase in the basicity of the anion [39].

The structure of the cation plays a role in the melting point of the IL. Alkyl groups on the imidazole tend to lower the melting temperature, and enhance wood liquefaction and processability of the wood solution. For example, wood dissolution was more effective in 1-butyl-3-allylimidazolium chloride ([BAIM][Cl]), then in 1-methyl-3-allylimidazolium chloride ([MAIM][Cl]) [16].

4.2.4 Effect of Temperature

Most IL pretreatments were conducted at high temperatures ranging from 70 to 190°C [7, 22, 25, 27, 36, 37, 43, 48–50]. Higher sugar yields are usually reported for pretreatments at higher temperatures for longer times [7, 22, 25, 27, 50]. Wood dissolution at temperatures above 100°C was faster in [BMIM][Cl] and [AMIM][Cl] [7]. Short pretreatments at higher temperatures resulted in higher glucose yields from oil palm fronds [22]. Increasing temperatures from 50 to 130°C during the dissolution of maple wood flour in [EMIM][OAc] increased the amount of extracted lignin and reduced the recovered wood flour [25]. In another study, increasing the temperatures from 70 to 150°C increased the solubility of lignin in triticale straw residues. The cellulose and hemicellulose content in the residues decreased with higher temperatures. The IL treatment at higher temperatures also resulted in higher glucose yields after enzymatic hydrolysis. More than 95% of the initial cellulose extracted above 130°C after 11 h was hydrolyzed [27]. This can be partially explained by the fact that, at higher temperatures, the self-diffusion coefficients of the IL anions and cations increase dramatically [49].

Another possible explanation of the benefits of high temperatures on biomass pretreatment is the improved access of enzymes to cellulose. The surface area, pore size distribution, and pore volume of switchgrass (3 wt%, 40 mesh) pretreated with [EMIM][OAc] at 110–160°C for 3 h, were measured by nitrogen porosimetry. The switchgrass pretreated at 160°C adsorbed significantly more gas than the untreated sample or the one treated at 120°C, with a specific surface area 30 times higher (15.8 m^2/g at 160°C, 0.7 m^2/g at 120°C, and 0.5 m^2/g for the untreated sample). The increased surface area and pore volume were correlated with an increase in the initial rates of enzymatic hydrolysis. After 30 min of hydrolysis with cellulases from *Trichoderma reesei*, the concentration of reducing sugars in the broth was 2.84 g/L for a 3-h IL treatment at 110°C and 7.44 g/l for the sample treated at 160°C. The lignin removal efficiency also increased from 25% at 110°C to 74% at 160°C. The improved delignification and sugar yields observed for pretreatments above 150°C was attributed mostly to the softening or melting of lignin. The average glass transition temperature of lignin is around 165°C, but varies considerably depending on its chemical composition and the ratio of monolignol units [50].

Cellulose degradation was reported when IL pretreatment is conducted at higher incubation time and higher temperature, leading to lower sugar yields after enzymatic hydrolysis [22]. There was evidence of degradation of the IL and cellulose, when pine wood chips were pretreated in [EMIM][OAc] at 110°C for 16 h. The appearance of additional peaks on ^{13}C NMR spectra of the pine/IL solution was attributed to the generation of glucose oligomers and the degradation of [EMIM][OAc] [36].

4.2.5 Effect of Density

[AMIM][Cl] dissolved *Eucalyptus grandis*, southern pine sawdust (particle size 0.1–2 mm) and Norway spruce thermomechanical pulp almost completely in 5 h at 120°C. IL pretreatment of southern pine improved the glucose yield after enzymatic hydrolysis from 7 to 17 wt%. But the improvement of the glucose yield after IL pretreatment was found to decrease with increasing wood density. Higher density wood (*Eucalyptus grandis*) requires an IL pretreatment at a higher temperature than low-density wood (southern pine) to achieve the same pretreatment efficiency [31]. Hardwoods such as red oak usually have a higher density than softwoods such as pine, but softwoods also tend to have higher lignin content. Lignin in softwoods is also rich in guaiacyl units, while lignin in hardwoods is a mixture of guaiacyl and syringyl units [36]. Similarly, wheat straw (low lignin content) could be dissolved in [EMIM][OAc] with acetic acid at a lower temperature (100°C) than pine wood (higher lignin content) (120°C) for the same particle size (<1 mm) [47].

4.2.6 Viscosity

Generally, the high viscosity of the IL affects negatively the overall efficiency of the pretreatment [7]. The IL viscosity depends on the IL chemical composition and temperature. For example, [AMIM][Cl] has a lower viscosity than [BMIM][Cl], which enabled wood dissolution at a lower temperature (80°C instead of 110°C). Dissolution in ILs with aromatic substituents, such as [BzMIM][Cl] and 1-methyl-3-*m*-methoxylbenzylimidazolium chloride, required higher temperatures (130°C) to achieve the same wood solubility, which was attributed to their higher melting temperatures and viscosities [7]. However, the viscosity of the wood/IL mixture also increases with the wood dissolution over time with the accumulation of extracted products and generation of by-products [4]. The viscosity of cellulose solutions in [EMIM][OAc] or [BMIM][Cl] was found to increase with the cellulose concentration [51, 52].

One way to decrease the viscosity of the wood/IL mixture is to increase the temperature, but this solution is energy-intensive and can accelerate the degradation of the IL [22]. Another method consists in the addition of a co-solvent with lower viscosity. The viscosity of a wood/[BMIM][Cl] mixture was reduced by the addition of deuterated dimethyl sulfoxide, which had no noticeable effect on the wood dissolution efficiency [4]. In another study, [BzMIM][Cl] was blended with [AMIM][Cl] to reduce its viscosity without significant efficiency loss. Biomass dissolution could occur at a lower temperature and even at room temperature in the less viscous [AMIM][Cl] [7].

During wood dissolution in [BAIM][Cl] and [MAIM][Cl] with $AlCl_3$ as a catalyst, increased acidity led to a decrease in viscosity, which was attributed to the formation of $AlCl_4^-$ and $Al_2Cl_7^-$ that weakens the hydrogen bonds in ionic liquids [16].

4.2.7 Acid Hydrolysis

Several acids served as catalysts with [BMIM][Cl] for the hydrolysis of corn stalk: hydrochloric acid, sulfuric acid, nitric acid, phosphoric acid, and maleic acid. Overall, hydrochloric acid was the most efficient catalyst. Sulfuric and nitric acids were also efficient, but required a higher loading to achieve the same yield in reducing sugars. At the same temperature (100°C), reactions with phosphoric and maleic acids were much slower than with the other acids, even at high loadings. The combination of hydrochloric acid (7 wt%) and [BMIM][Cl] was efficient in the hydrolysis of corn stalk, rice straw, pine wood, and bagasse [6]. Faster degradation of cellulose and hemicellulose was also observed at higher temperatures and for longer pretreatment times for *Eucalyptus grandis* [32]. The weight loss increased with the amount of hemicellulose, which was higher in softwoods (spruce and pine). More carbohydrates (polysaccharides and lignin) were hydrolyzed as the acid concentration increased [32]. Trifluoroacetic acid (0.2 wt%) also served as an acid catalyst in the dissolution of loblolly pine in [BMIM][Cl] at 120°C. Its effect was similar to sulfuric acid H_2SO_4 at the same molar concentration. After a 2-h treatment, 62 wt% of the loblolly pine was converted to soluble products. No further increase in the yield was seen after a 4-h treatment [53]. The addition of $AlCl_3$ led to a decrease in pH in a mixture of wood (*Metasequoia glyptostroboides*) and [BAIM][Cl] and [MAIM][Cl], which accelerated the dissolution of wood at a lower temperature. The amount of insoluble residues in the IL and pH decreased with increasing $AlCl_3$ amount. The selection of the metal chloride affected the pH and the liquefaction efficiency: $AlCl_3$ led to lower pH than $SnCl_2$ and $FeCl_3$. The stronger acidity led to higher liquefaction efficiency [16]. These results were consistent with a previous study in which the initial acid hydrolysis rates of cellobiose increased with increasing acid strength. The conversion of cellobiose to glucose was much faster for acids with negative pKa values, such as methanesulfonic acid ($pKa = -1.9$) and sulfuric acid ($pKa = -3$) [26].

From these results, it was argued that biomass does not dissolve in ILs directly, but that it needs to be hydrolyzed first before the dissolution of the hydrolysis products. Pine wood and wheat straw (mesh size smaller than 1 mm) were dissolved in [EMIM][OAc] with acetic acid as catalyst. After dissolution, a drop in pH was observed with formation and accumulation of acetic acid in the IL/biomass solution. The addition of acetic acid to [EMIM][OAc] accelerated the dissolution of wheat straw. After dissolution and addition of water, the precipitate contained an amount of lignin that increased with the amount of acetic acid added, suggesting that acetic acid also acted as a co-solvent for lignin [47].

Indeed, IL pretreatments with acid may increase the yield of reducing sugars following enzymatic hydrolysis, but they also promote the degradation of cellulose and hemicellulose when conducted at higher temperatures and for longer times [6, 32, 47, 53]. Faster degradation of cellulose and hemicellulose was observed at

higher temperatures and for longer pretreatment times for *Eucalyptus grandis* [32]. For the acid hydrolysis of loblolly pine in [BMIM][Cl], the yield of monosaccharides reached a maximum after 2 and 0.5 h of pretreatment at 120 and 150°C, respectively [53]. Similarly, the yield of reducing sugars after hydrolysis of corn stalk in [BMIM][Cl] with HCl reached a maximum for an incubation time of 30 min at 100°C [6]. High performance liquid chromatography (HPLC) of residues from the acid-catalyzed pretreatment of loblolly pine in [BMIM][Cl] showed that the monosaccharides from biomass reacted by dehydration to form other compounds, such as 5-hydroxymethylfurfural and furfural [53]. ^{31}P NMR spectra of the recycled IL after pretreatment of *Eucalyptus grandis* exhibited signatures from 5-hydroxymethylfurfural, acetol, 2-methoxy-4-methylphenol, catechol, and acetic acid [32]. Fourier-transform infrared (FTIR) spectroscopy of corn stalk after pretreatment in [BMIM][Cl] with sulfuric acid showed the functionalization of lignin with sulfonic groups [6]. The generation of these by-products reduces the total reducing sugar yield, can affect the enzymatic hydrolysis of the remaining cellulose and complicate the recycling of the IL.

4.2.8 Catalysts

In addition to acids, other catalysts such as Li salts (LiCl, LiBr, LiAc, $LiNO_3$, or $LiClO_4$) were added to enhance the dissolution of cellulose in [EMIM][OAc]. It was believed that the lithium cation can disrupt the hydrogen bonding network in cellulose [54].

Two polyoxometalates, an acidic form $H_5[PV_2Mo_{10}O_{40}]$ and an [EMIM][OAc] compatible form [1-ethyl-3-methylimidazolium]$_4$H$[PV_2Mo_{10}O_{40}]$, were prepared and used as catalysts for the dissolution of southern yellow pine (particle size <0.125 mm, 5 wt%) in [EMIM][OAc] at 110°C [55]. The addition of 0.5 wt% acidic polyoxometalate reduced the time for complete dissolution of pine from 46 to 15 h. The regenerated cellulose contained significantly less lignin, with limited losses in cellulose. The [EMIM]-compatible form improved delignification, but with greater cellulose losses in the regenerated cellulose [55].

4.2.9 Pretreatment with Ammonia

An ammonia pretreatment prior to IL dissolution improved delignification of biomass and enhanced recyclability. The rice straw (particle size 2–5 mm) was first treated with ammonia (10%) at 100°C for 6 h. After filtering and drying steps, it was dissolved in [EMIM][OAc] at 130°C for 24 h. The ammonia pretreatment step reduced the time for complete dissolution in IL from 24 to 6 h. It increased slightly the amount of regenerated cellulose after the IL treatment for <24 h. The major improvement was the significant increase in the glucose conversion rate of

97% (compared to the amount of regenerated cellulose) with the ammonia pretreatment, compared to the 78% rate without ammonia pretreatment. This improvement allowed for a significant reduction in the amount of cellulases necessary for cellulose hydrolysis: despite a reduction of the cellulase concentration by a factor 10, the cellulose conversion rate remained at 83% [46].

4.2.10 Microwave Heating and Ultrasounds

The complete dissolution of native biomass in ILs using conventional heating (oil bath) may take several hours at high temperatures. In order to reduce the energy costs associated with heating, a commercial microwave oven was used to heat the wood/IL mixture before it was heated using a conventional oil bath. The application of 100 pulses of 3 s reduced the time necessary to dissolve pine sawdust (particle size 0.125–0.250 mm) completely from 46 to 16 h [36]. Microwave irradiation also accelerated the production of 5-hydroxymethylfurfural and furfural directly from milled corn stalks, rice straws, and pine wood, reducing the reaction time down to a few minutes [35].

Ultrasounds can also accelerate the complete dissolution of cellulose in [BMIM][Cl] and [AMIM][Cl] from several hours to several minutes [56]. The exposure of pine sawdust (particle size 0.125–0.250 mm) to 1 h of ultrasound at 40°C before IL treatment reduced the time necessary to dissolve the sample from 46 to 23 h [36].

4.2.11 Biomass Size Reduction

The dissolution of Norway spruce in [BMIM][Cl] or [AMIM][Cl] depended on the size of the biomass. Whereas ball-milled powder and spruce sawdust (size 0.1–2 mm) were completely dissolved at 80°C in several hours, it took several weeks to dissolve wood chips ($5 \times 5 \times 1$ mm^3) at 130°C in the same ILs. In general, dissolution was fastest for ball-milled wood, followed by sawdust (particle size 0.1–2 mm), thermomechanical pulp fibers, and wood chips [7]. A similar size effect was observed for southern pine and red oak wood chips [36], and rice straw [46]. Ball-milling of Norway spruce TMP and southern pine increased the glucose yield after IL pretreatment and enzymatic hydrolysis, by opening access to the wood structure for enzymes. The same effect, which became more significant with milling time, was also observed for corn stovers. The molecular weight of ball-milled corn stovers decreased with increasing milling time [33].

The size reduction effect could be explained by the increase of effective surface area and the improved access of enzymes to the biomass cellulose. However, feedstock size reduction through mechanical grinding is energy-intensive [36]. Also, ball-milling for several days can lead to significant degradation and chemical

modification of cellulose and lignin, as well as the generation of soluble species that reduces the recyclability of the IL [31, 33, 57]. It was reported that extensive ball-milling causes cleavage of aryl–ether linkages in lignin and the generation of phenolic hydroxyl groups [57].

4.2.12 Comparison with Other Pretreatments

Few studies have directly compared the efficiency of IL pretreatments to other pretreatments, such as the ammonia or organosolv pretreatment. Rice straw particles were pretreated with [EMIM][OAc] (1 g biomass in 20 ml of [EMIM][OAc] at 130°C for 24 h) or ammonia (1 g biomass in 10 ml of 10 vol.% ammonia at 100°C for 6 h). In these conditions, the amount of cellulose regenerated was comparable for the two pretreatments. However, the conversion rate of cellulose to glucose was significantly higher with the IL pretreatment and the improvement due to IL was most remarkable for larger particles (>10 mm) [46].

In another study, switchgrass was subjected to an acid pretreatment (3 wt% biomass in 1.2% sulfuric acid heated at 160°C for 20 min) or an IL pretreatment with [EMIM][OAc] (3 wt% biomass heated at 160°C for 3 h). Analysis of the recovered biomass after IL pretreatment showed lower lignin content and higher hemicellulose content, compared to the recovered biomass after acid pretreatment. X-ray diffraction measurement of the cellulose crystallinity showed a significant decrease in crystallinity after IL pretreatment, whereas the acid pretreatment caused an increase in crystallinity, which was attributed to the preferential breakdown of the amorphous cellulose during the acid pretreatment. Scanning electron microscopy showed that the cell wall structure was mostly preserved during the acid pretreatment, while the IL pretreatment left no fibrous structure. For the same enzyme loading, the enzymatic hydrolysis had faster kinetics and higher reducing sugar yields after the IL pretreatment. After a 24 h saccharification process, 96% of the cellulose was hydrolyzed for the IL-pretreated sample, while only 48% were hydrolyzed for the acid-pretreated sample [41].

4.2.13 Water Adsorption as an Issue

The wide range of biomass solubility in ILs reported in the literature could be partially explained by the contamination with water, which can significantly affect their physicochemical properties [58]. Even hydrophobic ILs, such as 1-ethyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide ([EMIM][Tf_2N]), are hygroscopic and can contain up to 25% of water (molar ratio) when exposed to an environment with a relative humidity of 81% [59]. Water can also be produced during the reaction of biomass with IL by the hydrolysis of acetate groups [47]. Traces of water can be detected by ^{1}H NMR or IR spectroscopy [60]. They can be

quantified by Karl-Fischer titration or gravimetrically [59]. Water can be removed in a vacuum oven or in a freeze dryer [7, 61]. Its presence complicates the IL recycling and removal is energy-intensive.

Addition of water above 4 wt% to loblolly pine wood before its pretreatment in [BMIM][Cl] led to a notable decrease in soluble products, monosaccharides, and 5-hydroxymethylfurfural. This was attributed to the competition with the cellulose hydroxyl groups to form hydrogen bonds with Cl^- ions [53]. The presence of water reduced the solubility of wood in ILs and the yield of sugars released in the dissolution of maple wood flour in [BMIM][OAc] and [EMIM][OAc] [7]. Water also prevented the IL from effectively reducing the cellulose crystallinity [38]. Water can also prevent the formation of by-products such as 5-hydroxymethylfurfural during the dissolution of cellulose in [EMIM][Cl] catalyzed by HCl or H_2SO_4 [62].

The IL hygroscopicity is the result of the adsorption of water on the IL surface, diffusion from the surface and/or the formation of complexes through hydrogen bonding [59, 63, 64]. The hygroscopicity depends on the IL composition and structure [65]. Adsorption would depend on the charge distribution and structure of the cation and anion, while diffusion would be affected by the IL viscosity [59]. The length of alkyl chains and substitution on the cation ring (*e.g.*, pyridinium, imidazolium) affected the mutual solubility of the IL with water [65, 66]. For ILs with the [EMIM] cation, water uptake increased with different anions in the following order: dicyanamide < diethyl phosphate < chloride < acetate [61].

4.2.14 Presence of Impurities

As-produced commercial ILs can contain halides, water, organics, and unreacted salts from their synthesis [60]. The presence of impurities could explain differences in performance between identical ILs from different manufacturers [67]. The presence of residual chloride salts can dramatically increase the viscosity of the IL and decrease its density. ^{1}H NMR studies suggested that the viscosity increase may be due to the increase in hydrogen bonding between the chloride anion and the protons of the imidazolium cation [68]. Halides in a few ILs, such as 1-butyl-3-methylimidazolium tetrafluoroborate ([BMIM][BF_4]), can be removed by water washing, but the water excess would have to be removed under vacuum or by distillation [60].

Impurities, such as methyl imidazole (source of imidazolium-based ILs), can affect the pH of the solution and reduce ion concentrations [69]. The mixture of water with commercially available [EMIM][Cl] (equal weight) has a pH around 7, whereas the mixture of purified [EMIM][Cl] and water (equal weight) has a pH of 5.12. The addition of methyl imidazole to the purified IL brought the pH back to around 7. The lower pH obtained with the purified was attributed to the enhanced water dissociation and the resulting higher ion concentrations. Ab initio simulations predicted enhanced water dissociation at a high ionic strength (high

IL content) or at a high dielectric constant (high water content). The ions from the enhanced water dissociation in purified ILs could effectively catalyze the conversion of cellulose to sugars without the addition of acid catalysts [69].

4.3 Mechanism of Delignification and Cellulose Dissolution

4.3.1 Analytical Techniques

Advances in a variety of analytical techniques have provided valuable insight into the mechanisms involved in delignification and cellulose dissolution. Optical and fluorescence microscopy enabled the study of wood expansion (Fig. 4.2) [70, 71] and switchgrass dissolution [42] in ionic liquids at the micron scale. Distinct autofluorescence from cellulose and lignin signatures distinguish the cellulose-rich cell walls and the lignin-rich cell corners and middle lamellae in poplar and switchgrass [42, 70, 71]. Optical and scanning electron microscopies have been particularly useful in visualizing IL interacting with heterogeneous native biomass. They revealed structural changes after dissolution, regeneration, and chemical functionalization [22, 30, 36, 38, 41, 42, 44, 72].

X-ray diffraction provided an insight into structural changes occurring at the atomic scale in cellulose during its dissolution and regeneration [7, 36, 38, 71, 73]. It was used to monitor in situ the loss of cellulose crystallinity in poplar, ramie fibers [71], switchgrass, eucalyptus, and pine wood [73]. After the regeneration of dissolved pine and spruce sawdust, X-ray diffraction revealed a change of crystal structure from the native cellulose I to the cellulose II structure [7, 36]. Neutron scattering was used to estimate the surface roughness of switchgrass, eucalyptus, and pine after their IL pretreatment [73].

These structural changes were supplemented by analyses of the biomass chemical composition. The distinct Raman signatures of cellulose and lignin have made hyperspectral Raman imaging a powerful tool to map the chemical composition of native biomass [74] and its evolution during pretreatments [70, 71]. IR spectroscopy was commonly used to assess purity [4, 39, 42], loss of hemicelluloses/lignin after the dissolution [36, 42], chemical functionalization [6, 7, 30, 48], cleavage of β–O–4 bonds in lignin models [75, 76]. It can also probe the interactions between the anion and cation in the IL and the hydrogen bonding network [77]. FTIR spectroscopy combined with principal component analysis was used to distinguish lignins from bagasse, softwoods, and hardwoods [78]. Efforts were made to use IR spectroscopy as a method to quantify glucose and cellobiose in [EMIM][OAc]. The IR absorption of multiple bands in glucose and cellobiose was found to vary with concentration and empirical nonlinear relations between the absorbance and the concentration were derived [79].

Optical absorption spectroscopy offers a quick way to quantify the saccharification of purified substrates, such as Avicel, and native biomass.

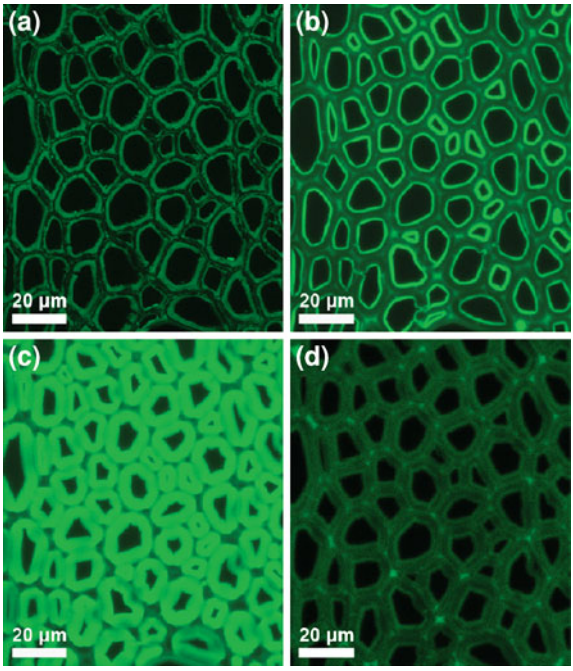

Fig. 4.2 Autofluorescence images of poplar wood cells **a** before [EMIM][OAc] pretreatment, **b** 15 min into the pretreatment, **c** after 3 h pretreatment, and **d** 20 min after rinsing with deionized water. All images were collected in the same conditions. The brightness was increased for image **d** for clarity. Reprinted with permission from [70]. Copyright 2011 American Chemical Society

The 2,4-dinitrosalicyclic reagent acid assay has been widely used to quantify reducing sugars, including glucose [26, 41, 44, 45, 80]. However, on native substrates (municipal solid waste, paper mill wastes, or agricultural wastes), the method suffers from the interference from other chemicals and impurities [78, 81]. Due to the variety and heterogeneity of native biomass, it has also been difficult to find adequate standards to establish Beer–Lambert relations between the absorbance and the sugar concentration, particularly for lignin which has different ratio of syringyl and guaiacyl units [78]. ILs, such as [BMIM][Cl], also absorb strongly in the UV range. Optical absorption analyses are also complicated by chemical alterations of the biomass during pretreatments [78].

Analytical techniques, such as mass spectrometry [44, 69], HPLC [26, 43, 44, 46], high-performance anion-exchange chromatography (HPAEC) [41, 45, 50], have been used to identify hydrolysis products. HPLC and HPAEC can quantitate the amount of reducing sugars produced during cellulose hydrolysis. Size exclusion chromatography was used to determine the molecular weight distribution of milled woods and their dissolution products in ILs [33].

Another widely used analytical technique is NMR. The variety of isotopes available (^{1}H, ^{13}C, ^{31}P, $^{35/37}$Cl) has made NMR spectroscopy a versatile method to characterize chemical functionalization [7, 30, 82], assess purity of products [32], identify/quantify hydrolysis/dissolution products [3, 4, 31, 32, 36], study the structure of milled native biomass (poplar, switchgrass) [29] and lignin [83], and study hydrogen bonding in cellulose dissolution [84, 85].

The combination of all these techniques has provided a wealth of information at multiple length scales about the chemical composition and structure of the pre-treated biomass. Quantitation of reaction products allowed for the optimization of reaction conditions, such as temperature and IL composition, and revealed the critical factors affecting the delignification and hydrolysis of cellulose.

4.3.2 Purified Cellulose Substrates and Lignin Models

Due to the complexity and variability of native biomass, early studies on possible mechanisms have focused on purified cellulose/lignin substrates [3, 14, 28, 67, 86, 87], oligomers of glucose and lignin models [26, 28, 75, 76]. Indeed, biomass is a complex heterogeneous substrate constituted of cellulose, hemicellulose, and lignin at varying ratios depending on the biomass feedstock. Cellulose can have several different crystalline structures [71]. Lignin is a branched polymer composed of different types of aryl–ether units and bonds that ionic liquids can cleave (aryl–ethers and aryl–alkyl linkages) [75]. The composition of lignin can affect its structure. Hardwood lignins have usually a higher ratio of syringyl/guaicyl units, giving them a more linear structure. In contrast, softwoods contain mostly guaiacyl phenolic units, giving them a branched structure [32].

The dissolution of Avicel cellulose was studied in different 1-alkyl-3-methylimidazolium chloride ILs prepared with alkyl chains of various lengths (2–10 atoms). It was found that Avicel cellulose was more soluble with alkyl chains with an even number of carbon atoms [61]. The depolymerization of cellulose was studied also in [BMIM][Cl] using an acid resin as a catalyst [88, 89]. It was proposed that the hydrolysis of cellulose is initiated with the protonation of the oxygen atom in the glycosidic bond. The glycosidic bond then breaks to form a cyclic carbocation, followed by a nucleophilic attack of water to add a hydroxyl group [89].

The cleavage of a particular type of linkage was studied on specifically designed lignin models with the desired linkage. For example, the IL 1-H-3-methylimidazolium chloride was effective in the cleavage of the β–O–4 bond in guaiacylglycerol-β-guaiacyl ether and veratrylglycerol-β-guaiacyl ether [75]. The cleavage of the same lignin models in [BMIM][Cl] required the presence of metal chloride catalysts, such as $FeCl_3$, $CuCl_2$, and $AlCl_3$ [76]. The reactivity of 2-methoxy-4-(2-propenyl)phenol (similar to guaiacyl unit), 4-ethyl-2-methoxyphenol (alkyl substitution), and 2-phenylethyl phenyl ether (with β-aryl ethers linkage) was studied in 1-ethyl-3-methylimidazolium triflate and [EMIM][Cl] with metal chlorides and acid catalysts [28].

Another study focused on the dissolution of pine kraft lignin. It was found soluble at temperatures above 50°C in 1,3-dimethylimidazolium methylsulfate ([MMIM][$MeSO_4$]), 1-hexyl-3-methylimidazolium trifluoromethanesulfonate ([HMIM][CF_3SO_3]), [BMIM][$MeSO_4$]. However, it was insoluble in 1-butyl-3-methylimidazolium hexafluorophosphate ([BMIM][PF_6]) even at 120°C. The

anion in imidazolium-based ILs affected the solubility dramatically: the methylsulfate anion was more effective than the chloride and bromide anions at dissolving lignin [3].

4.3.3 Swelling

Numerous ILs cause the swelling of native biomass, which was seen as a good indicator of biomass solubility. Swelling occurs even at room temperature in poplar exposed to [EMIM][OAc]. The cross-sectional area of poplar cell walls expanded by 60 to 100% in 3 h in [EMIM][OAc]. After rinsing with deionized water, the wood structure contracted almost immediately [70, 71]. Significant swelling was also observed in *Miscanthus* switchgrass heated at 100°C in [EMIM][Cl] for 20 h [26]. The magnitude of wood swelling depended on the IL used in the pretreatment.

The swelling of pine (*Pinus radiata*) sapwood chips (dimensions $10 \times 10 \times 5$ mm^3) was studied in ILs consisting of the [BMIM] cation and several different anions. Little swelling was observed in 1-butyl-3-methylimidazolium trifluoromethanesulfonate ([BMIM][CF_3SO_3]) even at 120°C. The ILs 1-butyl-3-methylimidazolium dicyanamide ([BMIM][$N(CN)_2$]) and [BMIM][$MeSO_4$] led to a swelling along the tangential direction (tangent to tree rings) of about 8%, which is larger than with just water (5%). The most dramatic swelling was observed in the tangential direction with [BMIM][Me_2PO_4] and [BMIM][OAc] and the magnitude was temperature-dependent, from 15% at 90°C to 20% at 120°C [37]. Little expansion was observed in pine chips along the radial direction. [BMIM][$N(CN)_2$] and [BMIM][$MeSO_4$] caused the expansion along the axial direction, while 1-butyl-3-methylimidazolium dimethylphosphate ([BMIM][Me_2PO_4]) and [BMIM][OAc] caused the reduction in the axial direction, most likely due to partial dissolution. The different swelling rates among ILs were attributed to the temperature-dependent viscosity [37].

However, swelling induced by the IL does not necessarily mean that the IL is a good solvent for cellulose and biomass. The swelling and dissolution of pine wood pulp fibers were studied in [BMIM][Cl], 1-allyl-3-methylimidazolium bromide ([AMIM][Br]), and butenylmethylimidazolium bromide. While the fibers swelled and dissolved in [BMIM][Cl], they swelled homogeneously in [AMIM][Br] and butenylmethylimidazolium bromide without dissolution. Both ILs penetrated the fibers quickly but did not disrupt the hydrogen bonding [90].

4.3.4 Regeneration and Reduction of Cellulose Crystallinity

Cellulose can be regenerated from the IL/biomass solution with an anti-solvent, such as acetone, water, dichloromethane, or acetonitrile, in excess [4, 7, 36].

Lignin and the IL can be washed away with NaOH [48]. Lignin can be precipitated with HCl [48] or H_2SO_4 [27]. Cellulose and lignin can also be extracted separately: cellulose was precipitated with ethanol and lignin with water after dissolution of wheat straw and pine wood with [EMIM][Cl] [47]. After regeneration, cellulose usually becomes amorphous or changes into its cellulose II structure [7, 36].

Changes in crystallinity have been characterized in purified cellulose substrates and native biomass by X-ray diffraction [8, 10, 25, 38, 71, 91]. The intensity of the (002), (110), and (1–10) reflections from cellulose usually decrease in intensity after the IL pretreatment and regeneration, indicating a loss in crystallinity [2, 14, 17, 67, 78, 149]. This was the case for cellulose in maple flour dissolved in [BMIM][OAc] and [EMIM][OAc] [38], and in spruce sawdust dissolved in [AMIM][Cl] [7]. The lower crystallinity led to improved access for hydrolytic enzymes and an improved glucose yield after hydrolysis [7].

However, it is possible for cellulose to retain its native cellulose I structure. Upon application of [EMIM][OAc] on poplar microtome section, the (002), (110), and (1-10) reflections of cellulose I disappeared and the diffraction pattern was dominated by a diffuse ring from the [EMIM][OAc]. When the pretreated sample was exposed to water, the IL was expelled from the wood and the diffraction pattern of cellulose was gradually recovered (Fig. 4.3). This recovery of cellulose I is in contrast to studies on biomass dissolution at high temperatures, where the regenerated cellulose is either amorphous or in the cellulose II phase. This is explained by the partial solubilization of cellulose microfibrils. Those microfibrils that retained their cellulose I structure acted as nucleation sites for cellulose I recrystallization after expulsion of [EMIM][OAc] [71].

4.3.5 Hydrogen Bonding

The dissolution of cellulose was usually attributed to the ability of the IL to disrupt the hydrogen-bond network in cellulose by forming hydrogen bonds with cellulose. For example, in [AMIM][Cl], the chloride anion is a hydrogen-bond acceptor, while the proton at the 2-position of the imidazolium ring is a hydrogen-bond donor [32]. NMR studies of [BMIM][Cl] showed that the chloride anion has an active role in the solubility of cellulose through hydrogen bonding with the hydroxyl groups of cellulose [84]. Density-functional theory calculations showed that the anions in imidazolium-based ILs tended to form hydrogen bonds with the O2 and O3 hydroxyl groups of cellulose. The strength of the hydrogen bonds increased for the following anions in the order: hexafluorophosphate < tetrafluoroborate < alkyl phosphate < acetate. The trend matched the one observed in the dissolution of cellulose in the corresponding imidazolium-based ILs, where cellulose solubility was highest with the acetate anion [25, 92]. The strong hydrogen bonding ability of ILs means that they can disrupt the hydrogen bonding network in lignocellulosic biomass by displacing the lignocellulose components to form stronger hydrogen bonds [34].

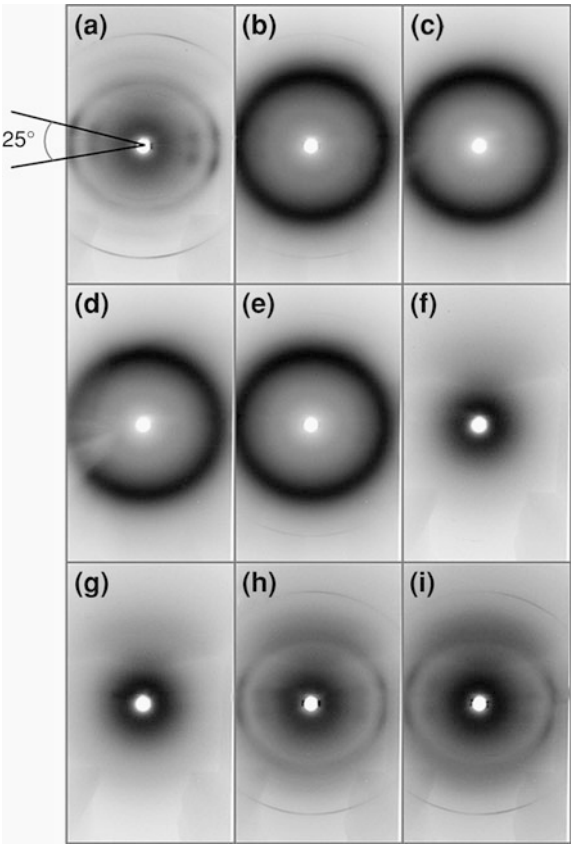

Fig. 4.3 A time series of X-ray diffraction images recorded from a radial section of *Poplar* sp. As [EMIM][OAc] is applied and then expelled with water; **a** Untreated sample, **b–e** application of [EMIM][OAc], **f–i** application of water. The fiber diffraction direction is approximately vertical. Note the presence of two superimposed equators in **a**, **h**, and **i** with a relative orientation of approximately 25°. Reprinted from [71], copyright (2011), with permission from Elsevier

Molecular dynamics simulations were conducted to study the interaction between [EMIM][OAc] with glucose oligomers (5–20 units). The total interaction energy between [EMIM][OAc] with cellulose (around −75 kcal/mol) was larger than the one between water and cellulose (around −50 kcal/mol) and the one between methanol and cellulose (around −45 kcal/mol). The difference between [EMIM][OAc] and water/methanol became larger with the cellulose chain length [49]. The acetate anion is also a hydrogen-bond acceptor, with the potential to form hydrogen bonds with the three hydroxyl groups of each unit of cellulose. The strength of these hydrogen bonds (14 kcal/mol) was estimated to be three times higher than the hydrogen bonds in water (5 kcal/mol) and methanol (4 kcal/mol). The simulations showed that the imidazolium cation interacts strongly with the glucose ring structure via van der Waals forces. Also, the interactions between [EMIM][OAc] and cellulose led to conformation changes in the cellulose chains, which can explain the loss in crystallinity and structural changes in regenerated cellulose [49].

The hydrogen bonding ability of ILs was probed by IR spectroscopy. ILs were prepared with the same anion $[Tf_2N]^-$ and different cations with increasing hydrogen bonding ability: 1,2,3-trimethylimidazolium, 1,3-dimethylimidazolium, 1,2-dimethylimidazolium, and 1-methylimidazolium. The increasing strength of hydrogen bonds was indicated by a shift of the IR absorption band below 150 cm^{-1} toward higher wave numbers. This band shifted from 62 cm^{-1} for the 1,2,3-trimethylimidazolium cation to 101 cm^{-1} for the 1-methylimidazolium cation. There was a linear relationship between the measured peak position and the average interaction energies in IL clusters from ab initio calculations. Ab initio calculations also showed that the interaction energy is minimal for the 1,2,3,4,5-pentamethylimidazolium cation where all protons were substituted by methyl groups and the hydrogen bonding ability was reduced [77].

Formation of hydrogen bonding between [EMIM][OAc] and cellobiose was also studied by ^1H NMR. A broadening of the OH resonances was observed as the molar ratio between [EMIM][OAc] and cellobiose was increased, which was explained by the interaction between the O atoms in the hydroxyl groups and the protons of the imidazolium ring. The accompanying downshift of the OH resonances was attributed to the hydrogen bonding between the acetate anion and the hydrogen atoms in the cellulose hydroxyl groups. NMR spectra of [EMIM][OAc] with increasing cellobiose concentration indicated that the strongest hydrogen bonding between the imidazolium cation and cellobiose involves the proton at the 2-position of the imidazolium ring. The next strongest hydrogen bonds involve the protons at the 4- and 5-position, which are much weaker hydrogen-bond donors [84]. When all the hydroxyl groups in cellobiose were acetylated, the NMR spectra of the [EMIM][OAc]/cellobiose octaacetate remained unchanged as the IL concentration increased. This showed that hydrogen bonding between cellobiose and the IL cation/anion is the main reason cellobiose dissolves in [EMIM][OAc]. In order to dissolve cellulose effectively, it was proposed that the IL must have an anion that is a good hydrogen acceptor, and a cation that is a moderate hydrogen-bond donor and not too large [84].

4.3.6 Empirical Solvent Polarity Scales

There have been attempts to describe the variety of solvation interactions, in which ILs are involved (for example: hydrogen bonding, dispersive, dipolar, ionic), by a set of empirical parameters that could be used to predict reaction products, yields, kinetics, and solubility [37, 38, 93–105]. The empirical parameters are determined by mixing the IL with a dye or a probe molecule. The interactions of the IL with the dye/probe are then characterized by absorption spectroscopy [37, 38, 87, 97–101] or gas chromatography [93, 94, 96].

The set of solvent polarity parameters developed by Kamlet and Taft [102–105] has been widely used to predict cellulose and biomass solubility in IL [25, 37, 38, 87, 106–108]. The three parameters α, β, and π^* characterize the IL hydrogen-bond acidity (ability to donate hydrogen bonds), hydrogen-bond basicity (ability to accept), and polarity, respectively [102–104]. They are measured by absorption spectroscopy with mixtures of IL with three different solvatochromic dyes: (2,6-dichloro-4-(2,4,6-triphenyl-1-pyridinio)phenolate, 4-nitroaniline, and *N*,*N*-diethyl-4-nitroaniline [37, 87, 107].

The parameter α depends mostly on the IL cation. All three protons in the imidazolium cation can form hydrogen bonds, giving the cation α values between 0.45 and 0.63 [37, 49, 107]. Ammonium cations can have higher α values than the imidazolium cation (1.10) [107]. As for the parameter β, it depends mainly on the anion. The parameter β was measured for a series of ILs with the [BMIM] cation: it was highest for the acetate anion (1.20) [37], followed by the anions dimethylphosphate (1.12) [37], chloride (0.83) [37], dicyanamide (0.60) [37], trifluoromethanesulfonate (0.48) [37], tetrafluoroborate (0.38) [101], hexafluorophosphate (0.21) [101], and hexafluoroantimonate (0.15) [107].

In general, ILs with high hydrogen-bond basicity were best suited for cellulose dissolution. They usually have an anion with high basicity, such as chloride, acetate, formate, and diethylphosphate [25, 37, 38, 106, 108]. ILs with bromide, biscyanamide or thiocyanate anions have lower hydrogen-bond basicity. Cellulose swells in these ILs but are only partially dissolved [37, 106]. ILs with tetrafluoroborate, hexafluorophosphate, and trifluoromethanesulfonate anions are generally poor solvents for cellulose [37, 96, 106]. It was confirmed that cellulose is soluble in [BMIM][Cl], but not in [BMIM][BF_4] and [BMIM][PF_6], which can be explained by the much larger hydrogen-bond basicity of [BMIM][Cl] [96].

The predictions made from Kamlet-Taft parameters on biomass pretreatment efficiency were tested on maple wood flour (5 wt%) with the IL/wood mixture heated at 90°C. Two ILs, [BMIM][OAc] ($\beta = 1.18$) and [BMIM][$MeSO_4$] ($\beta = 0.60$), were included in the study. The parameter β was tuned in the range 0.60–1.18 by the addition of water and the preparation of [BMIM][OAc]/[BMIM][$MeSO_4$] mixtures. Upon addition of 10 wt% water, β for [BMIM][OAc] decreased from 1.18 to 0.98, while for [BMIM][$MeSO_4$], it only decreased from 0.60 to 0.57. After IL pretreatment and enzymatic hydrolysis, the glucose yield, xylose yield, crystallinity, and lignin extracted from wood was measured as a function of the IL parameter β. The yield of glucose and xylose released increased linearly with β. The lignin extraction efficiency and crystallinity of the pretreated wood were stable for $\beta < 0.84$. For $\beta > 0.84$, the lignin extraction efficiency increased with β and the crystallinity decreased dramatically with β [38].

4.4 Compatibility with Cellulases

4.4.1 General Toxicity of Ionic Liquids

There is a general scarcity of toxicology data and studies on ILs [109]. Tests were developed to measure the toxicity on unicellular and multicellular organisms. A measure of toxicity is the concentration (EC_{50}) of the IL that induces a 50% decrease of the organism viability. These tests are, however, expensive and time-consuming, severely restricting the number of ILs/organisms that can be tested. Effective screening is necessary to focus resources on a limited number of IL structures. Also, the IL interactions with the organisms or culture medium are not fully understood, which potentially affects the interpretation of the results. The IL may change the chemical composition of the culture medium, its pH, and cause interferences with widely used spectrophotometric methods [110].

The survival rate or microorganisms, invertebrates and human cell lines was assessed as a function of the ionic liquid concentration [60, 111–114]. The toxicity from 1-alkyl-3-methyl-imidazolium ILs was found to increase as the length of the alkyl chain increased [60, 115]. The IL cation mostly determines the toxicity of the IL. Only minimal effect from the anion was observed [60, 113, 115]. The toxicity of ILs was also assessed on *Clostridium* sp., a bacterium capable to ferment sugars. No growth was observed above concentrations of 58 mM in [EMIM][OAc], 56 mM in [EMIM][DEP], and 54 mM in [MMIM][DMP]. But at low concentration below 15 mM, [EMIM][OAc] stimulated the growth and glucose fermentation by pH modulation in the culture medium [116].

It should be pointed out that IL toxicity can also come from the formation of by-products from the acid hydrolysis of biomass, such as furfurals, which are known to reduce cell viability and inhibits fermentation [117].

4.4.2 Deactivation of Cellulases in ILs

Cellulose hydrolysis is the result of the synergistic action of three different types of cellulases: endoglucanases that cleave β-1,4-glycosidic bonds on cellulose chains, cellobiohydrolases that convert long cellulose chains into cellobiose, and β-glucosidases that convert cellobiose into glucose [118, 119]. The mechanisms underlying cellulase activity on a heterogeneous substrate, such as lignocellulosic biomass, is still under investigation [72, 119]. Multiple models have been developed to understand the multiple steps involved in cellulose hydrolysis: adsorption of cellulases on the substrate, formation of the enzyme–substrate complex, location and hydrolysis of β-glycosidic bonds, desorption of the enzyme, synergy between endoglucanases, cellobiohydrolases, and β-glucosidases [119].

Once biomass is regenerated from its IL solution, it can still contain traces of IL that can reduce cellulase activity [72]. Several studies have focused on the stability

of commercial cellulases in various ILs and their saccharification yields on purified cellulose substrates and native biomass. Celluclast 1.5L (cellulases from *Trichoderma reesei*) and Novozyme 188 (β-glucosidase from *Aspergillus niger*) retained 76 and 63% of their original activity on carboxymethylcellulose after incubation at 50°C for 24 h in 15 and 20% [EMIM][OAc] solutions, respectively [120]. The activity of Celluclast 1.5L was also assessed on α-cellulose in [MMIM][DMP], [AMIM][Cl], [BMIM][Cl], and [EMIM][OAc] at a 10 vol.% concentration. The activity in these ILs was between 70 and 85% lower than the activity in sodium acetate buffer at pH 4.8 [67]. An increase in the IL concentration led to an increase in the IL viscosity by a factor of 4 [67]. The activity of cellulases from *Trichoderma reesei* on cellulose azure was found to decrease dramatically with low concentrations (22 mM) of [BMIM][Cl] or [BMIM][BF_4] [121]. No saccharification of Avicel cellulose was observed with cellulases from *Trichoderma reesei* in 60 vol.% [EMIM][DEP] [122]. The activity of cellulases from *Aspergillus niger* decreased with incubation time in [BMIM][Cl] and [BMIM][Cl] concentration [123]. It is important to note at this point that variations of 20% in cellulase activity were observed between different Celluclast 1.5L lots from the same manufacturer [67].

Despite the partial deactivation of cellulases in ILs, reducing sugar yields were still higher after IL pretreatment with low residual IL concentrations, due to the improved access of enzymes to the cellulose in biomass. For example, the cellulase mixture of Celluclast 1.5L and Novozyme 188 still converted 45% of the cellulose contained in a solution of 0.6% [EMIM][OAc]-pretreated yellow poplar with 15% [EMIM][OAc]. The conversion rate was much higher than for the untreated yellow poplar (11%) [120]. The activity of the same mixture was also assessed on purified cellulose substrates: an Avicel solution in [EMIM][OAc] and untreated Avicel in citrate buffer. After enzymatic hydrolysis for 24 h at 50°C, 91% of the [EMIM][OAc]-pretreated Avicel was converted to glucose, while only 49% of the untreated Avicel was converted [120]. With cellulases from *Trichoderma reesei* in 20 vol.% [EMIM][DEP], 70% of the cellulose was converted to cellobiose or glucose, a conversion rate that was higher than the untreated Avicel (about 33%). A comparison with [EMIM][OAc] using the same procedure yielded conversion rates that were half of those with the diethylphosphate anion [122].

The stability of another commercial cellulase, GC 220, a mixture of endogluconases and cellobiohydrolases from *Trichoderma reesei* was assessed in eight different ionic liquids. With the exception of tris-(2-hydroxyethyl)methylammonium methylsulfate (HEMA), the fluorescence of the trytophyl marker on the cellulases was quenched in the other ILs that included several imidazolium-based ILs, suggesting denaturation of the enzymes. The cellulase activity was measured spectroscopically in a citrate buffer (pH 4.8) and in the eight ILs using cellulose azure as the substrate. Cellulase activity was detected only in the ILs 1-methylimidazolium chloride ([MIM][Cl]) and HEMA, but it was significantly lower than in the buffer. The cellulases remained active even after 2 h in these two ILs at 65°C [124].

The tolerance of cellulases produced by *Penicillium janthinellum* to ionic liquids was tested by incubating the extracted enzymes in an aqueous solution of [BMIM][Cl] of concentration ranging from 10 to 50%, and then measuring their residual activity on different substrates (filter paper Whatman no. 1, carboxymethylcellulose, xylan solution or *p*-nitro phenyl *β*-D-glucopyranoside). After incubation in 10% ionic liquid for 5 h, the cellulases retained at least 80% of their activity on all substrates. At a higher concentration of 50%, the residual activity decreased significantly to reach below 20% for all substrates [125].

The tolerance of cellulase-producing bacteria from termites to [BMIM][Cl] was studied by characterizing their growth in [BMIM][Cl] at concentrations ranging from 0.1 to 10 vol.%. The three bacteria that were the most effective at cellulase production could tolerate [BMIM][Cl] at concentrations smaller than 1.0 vol.%. No growth was observed for concentrations larger than 5 vol.%. For two of the bacteria, the growth rates were unchanged for concentrations smaller than 1.0 vol.%. [118].

Cellulases are deactivated in ILs through multiple mechanisms. Stability and unfolding of the cellulases were studied by differential scanning calorimetry. Thermal unfolding was irreversible in the citrate buffer with a broad transition peak between 60 and 75°C. The ILs [MIM][Cl] and HEMA improved the stability of the cellulases with the shift of the transition temperatures above 75°C. The low activity in HEMA compared to the buffer was attributed to the high viscosity of HEMA [124]. Cellulase activity also decreased when the viscosity of the enzyme solution without IL was increased with polyethylene glycol [67].

Deactivation was attributed to the dehydrating environment introduced with [BMIM][Cl] that causes the denaturation of the enzyme. This conclusion was supported by fluorescence spectra of the cellulase in [BMIM][Cl] and various denaturants, such as the surfactant sodium dodecylsulfate and urea [121]. Cellulase deactivation in [BMIM][Cl] was similar to the deactivation in NaCl solutions at high concentrations above 0.35 M, suggesting that interactions between the enzymes and the IL charged species also play a role in the denaturation of the enzymes [67, 121]. Enzyme activity can be recovered when the IL was diluted with buffer solution [67].

4.4.3 Temperature and pH Dependence of Cellulase Activity

Cellulases operate optimally at a specific temperature, and the introduction of IL can shift the optimal temperature. One of the cellulase identified by metagenomics exhibited an optimum activity at 55°C in McIllvaine buffer (0.2 M Na_2HPO_4 with 0.1 M citric acid, pH 6.5). In 1-ethyl-3-methylimidazolium trifluoroacetate ([EMIM][TfAc]) and 1-butyl-1-methylpyrrolidinium trifluoromethanesulfonate ([BMPy][CF_3SO_3]), the optimum temperature shifted to 37 and 20°C, respectively [126].

Increasing the temperature from 50 to 60–70°C can also accelerate the deactivation of cellulases from *T. reesei* in [EMIM][OAc] [91]. The stability of mixtures of Celluclast 1.5 l and Novozyme 188 was tested in the presence of [EMIM][OAc] at concentrations ranging from 5 to 30% (volume/volume) in citrate buffer (pH 4.8) with poplar and Avicel [120]. When the hydrolysis was conducted at 4°C for a [EMIM][OAc] concentration of 30%, the activity of the cellulase mixture after 24 h remained above 70% of the activity without [EMIM][OAc]. At 50°C, the drop in cellulase activity dropped further to 31% of the control activity after 24 h in a 30% [EMIM][OAc] solution [120].

Enzyme activity is also pH-dependent [126, 127]. Celluclast 1.5 l hydrolyzes cellulose at an optimum pH between 4.5 and 5. No cellulase activity was observed for pH values below 2 or above 8 [127]. Three cellulases identified with metagenomic libraries have optimal pH values of 5, 7, and 7.5 [126]. The oxidation of *o*-phenylenediamine by lignin peroxidase was most effective at pH 3.2 [128]. A deviation from the optimal pH induced by the introduction of ILs can cause the deactivation of cellulases. The pH of the wood/IL mixture is affected not only by the IL concentration but also IL composition and structure [129]. Increasing the concentration of 1,3-dimethylimidazolium dimethylphosphate [MMIM][DMP] from 0 to 0.5 vol.% in the enzyme solution led to an increase of the pH from 4.8 (optimum for hydrolysis) to 6.5 [67]. Mixtures of water with ILs based on an imidazolium cation and a BF_4^- anion have a pH that increases with the length of the alkyl chain on the cation. The addition of hydroxyl groups increases the acidity of the IL [129]. The pH can also vary during the biomass reaction with the IL. Measurements in wheat straw and pine wood solution in [EMIM][Cl], [BMIM][Cl], and [EMIM][OAc] showed a drop in pH over time. A HPLC analysis showed the formation and the accumulation of acetic acid, which comes from the hydrolysis of acetate groups in the biomass [47].

4.4.4 Effect of High Pressure

The activity of commercial cellulases extracted from *Trichoderma viride* and *Aspergillus niger* on carboxymethylcellulose and Avicel generally increased at high pressure up to 500 MPa (above atmospheric pressure) [130]. The activity of cellulases from *Aspergillus niger* was assessed on carboxymethylcellulose in 10% [BMIM][Cl] at 30°C at hydrostatic pressures up to 675 MPa (above atmospheric pressure). The activity increased by 70% at a pressure of 100 MPa, compared to the activity at atmospheric pressure; then decreased for pressures above 200 MPa. The activity at 600 MPa was comparable to the one at atmospheric pressure. Although the cellulases lost 50% of their activity in 10% [BMIM][Cl] at atmospheric pressure, their activity in 10% [BMIM][Cl] at 100 MPa is about 85% of the one in acetate buffer at atmospheric pressure. This result suggests that high pressure can limit the de-activation of cellulase in ILs [123].

4.4.5 Identification of Cellulases Resistant to Ionic Liquids

Traces of ILs can be significantly reduced with multiple washing with water. However, in order to reduce water use, the extraction or development of cellulases capable to hydrolyze biomass at high IL loading (>5 vol.%) is necessary [72]. An intense effort is underway to find compatible combinations of cellulases and ILs. A few cellulases were found to tolerate high concentrations of ILs. Endogluconases isolated from thermophilic organisms *Thermatoga maritima* and *Pyrococcus horikoshii* retained 50 and 95% of their activities on carboxymethylcellulose after incubation in 15 vol.% [EMIM][OAc] for 30 min at 80°C, respectively. In contrast, commercial cellulases from *Trichoderma viride* were de-activated in 10 vol.% [EMIM][OAc] at 37°C. The activity of cellulases from *Thermatoga maritima* and *Pyrococcus horikoshii* only decreased by 34% and 11% on IL-pretreated corn stovers in 15 vol.% [EMIM][OAc] for 14 h at 80°C, compared to the hydrolysis without IL. The sugar yields were much higher than in the untreated corn stovers. The unfolding temperature of the cellulases, measured by differential scanning calorimetry, decreased with increasing [EMIM][OAc] concentration [131].

High-throughput techniques are necessary to screen the wide variety of IL/cellulase combinations. The activity of Celluclast 1.5L was assessed on numerous cellulose substrates with varying crystallinity in different buffers and at various pH using 96-well plates [127]. In a similar high-throughput approach, IL/biomass solutions were pipetted into wells in a 96-well plate filled with the anti-solvent to be regenerated before addition of the enzyme cocktail. This avoids the handling of solid regenerated biomass, which is difficult to pipette [72]. The amount of reducing sugars can then be assessed spectroscopically with the dinitrosalicylic acid reagent [80]. This high-throughput technique can not only identify quickly the cellulases capable to tolerate ILs, but also assess the effect of pH and substrate concentration [127].

Cellulases resistant to IL were identified using metagenomics, where microbial DNA was extracted from organisms found in a natural environment and then cloned in a host bacterium (for example, *Escherichia coli*) [126, 132–134]. Using metagenomics, 24 cellulase clones were identified and their activity was tested in six different ILs on carboxymethylcellulose at 37°C for 30 min. One of the isolated enzymes retained about 40% of its cellulase activity in 30 vol.% of 1-ethyl-3-methylimidazolium trifluoromethanesulfonate ([EMIM][CF_3SO_3]), [EMIM][TfAc], and [BMPy][CF_3SO_3]. However, no activity was observed on Avicel cellulose. Longer incubation of the cellulases in 60 vol.% any IL for 17 h deactivated the cellulases [126].

4.4.6 Designing New Ionic Liquids Suitable for Cellulose Dissolution and Cellulase Activity

The origin of enzyme deactivation in ILs was studied by comparing enzyme activity in different ILs. The effect of IL chemical composition, structure, and

functionalization were studied to design new ionic liquids suitable for cellulose dissolution and cellulase activity. It was found that ionic liquids with a higher molecular weight would maintain enzyme (lipase) activity at a high level. Adding a longer alkyl chain on the imidazolium cation would accomplish that, but this would lead to a higher viscosity, which has a negative effect on cellulose dissolution. To reduce the viscosity, the long alkyl chain was substituted by oxygenated chains, such as poly(ethylene glycol) and poly(propylene glycol). A long oxygenated chain reduced the cellulose solubility, so an optimum chain length was determined to maintain both the cellulase activity and cellulose solubility high. The imidazolium cation with the best overall performance was derived from triethylene glycol monomethyl ether. The oxygen atoms introduced are believed to form hydrogen bonds with cellulose, facilitating its dissolution. Adding a longer alkyl or oxygenated chain on the other side of the imidazolium cation led to a significant decrease in cellulose solubility, which was attributed to the reduction of hydrogen bonding with cellulose due to steric hindrance. The acetate anion led to higher cellulase activity and cellulose solubility than the chloride anion [135].

4.4.7 Stabilization of Cellulases in Microemulsions and by Immobilization

In addition to the development of new IL-resistant enzymes, a variety of methods have been developed to stabilize enzymes in ILs [136]. Most of them have been applied to cellulases. One of these methods is the preparation of microemulsions with ILs that can reduce the dehydration effect of ILs on enzymes. To reduce toxicity and deactivation, microemulsions of water in [BMIM][PF_6] were stabilized using the surfactant Triton X-100. Lignin peroxidases from *Phanerochaete chrysosporium* and laccases from *Trametes versicolor* could oxidize 2,6-dimethoxyphenol and *o*-phenylenediamine in these microemulsions. The highest activities for lignin peroxidases and laccases were 13 and 33 μmol/l/min, respectively. In contrast, the same enzymes had negligible catalytic activity in pure or water-saturated [BMIM][PF_6] [128].

Another approach to stabilize cellulases is their immobilization on a substrate. For example, immobilization on a poly(ethylene glycol) substrate increased activity of cellulases from *Trichoderma reesei* in 0.05 M citrate buffer and [BMIM][Cl], compared to the free enzyme [121]. Cellulase from *Trichoderma reesei* immobilized on 150 μm particles suffered no inhibition in 20 vol.% of [MMIM][DMP], *N*,*N*-dimethylethanolammonium lactate, and *N*,*N*-dimethylethanolammonium acetate. In contrast, reducing sugar yields decreased in 20 vol.% of [MMIM][$MeSO_4$], [BMIM][Cl], [BMIM][PF_6], and [BMPy][Cl], by 36, 28, 37, and 34%, respectively [44].

Celluclast immobilized onto a polymeric support (Amberlite XAD4) was coated with the hydrophobic ILs 1-butyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide ([BMIM][Tf_2N]) or butyltrimethylammonium

bis(trifluoromethylsulfonyl)imide ([N1114][Tf_2N]). The activity of these coated cellulases were assessed as a function incubation time in [BMIM][Cl] and in [N1114][Tf_2N]/[BMIM][Cl] mixtures at different molar ratios. The hydrophobic IL coating slowed the deactivation effect from [BMIM][Cl]. It was believed that the hygroscopicity of water-immiscible ILs can keep the enzymes hydrated and prevent their unfolding. The polymeric support may act as a water reservoir to preserve the cellulase activity. The cellulase activity decreased with increasing [N1114][Tf_2N] concentration, most likely due to the restricted access of cellulose to the coated enzyme [137].

4.5 Recycling

4.5.1 How Green are ILs?

Complete life cycle assessments of ILs have been attempted to estimate the cumulative energy demand (for synthesis and disposal), the environmental impact and the economic viability (cost of chemicals, energy, disposal, personnel, equipment, and processing). Due to the complexity of the IL life cycle and the limited data available, life cycle assessments have remained a challenge. Previous attempts have instead focused on the optimization of single steps in the IL life cycle, such as the supply of materials and the IL synthesis [138, 139]. It was found that the IL synthesis is expensive [21], and requires large quantities of materials, solvents, energy, and also generates toxic emissions [138]. Therefore, their recycling and biodegradability are crucial not only for their economic viability but also to reduce their environmental impact [138].

4.5.2 Recycling Attempts

After cellullose dissolution in ILs and its regeneration with an anti-solvent, the IL is usually filtered (or centrifuged) and washed with ethanol, acetone, ethyl ether, or water several times to remove by-products of wood degradation. Due to the IL low vapor pressure [60], the excess can be removed with a rotary evaporator, possibly at high temperatures, before the IL reuse [25, 31, 46, 55, 89]. It can also be separated with ethyl ether, then dissolved in acetonitrile/ethyl acetate and frozen for 24 h. The IL is then placed in a vacuum over at 90°C for 8 h before reuse [16].

Using these recycling procedures, the reuse of ILs for the pretreatment of native biomass through multiple cycles was reported. [BAIM][Cl] and [MAIM][Cl] could dissolve *Metasequoia glyptostroboides* wood sawdust without any efficiency loss after five cycles [16]. After five cycles, [EMIM][OAc] only lost 10% of its efficiency to dissolve maple wood flour [25]. The dissolution of rice straw in

[EMIM][OAc] was repeated for 20 cycles with no reported efficiency loss. The cellulose recovery even increased over time due to the accumulation of dissolved cellulose residues that can be recovered in later cycles [46]. ILs, such as [BMIM][PF_6] and [BMIM][BF_4], were successfully recycled through multiple reaction cycles. Their recyclability was attributed mainly to their low solubility in some organic solvents or water. They can thus be extracted with an organic solvent or washed with water [60].

The IL recyclability is limited by the formation and accumulation of by-products or impurities. The degradation of cellulose was reported in reactions conducted at high temperatures [22, 36] or with acid catalysts [6, 32, 53]. The dehydration of free monosaccharides could lead to the formation of 5-hydroxymethylfurfural and furfural [53]. After the IL use for the dissolution of native biomass, ^{31}P NMR spectra revealed signatures from 5-hydroxymethylfurfural, acetol, 2-methoxy-4-methylphenol, catechol, and acetic acid [32]. Even if these by-products can be avoided, the lignin extracted from the biomass accumulated in the recycled ILs with the increasing number of cycles [25, 32]. There was also accumulation of hemicelluloses, which are polar and have good affinity with polar ILs, such as 1-allyl-3-methylimidazolium chloride [32].

Wood naturally contains acid groups that can become free by hydrolysis and generate acids in the IL solution, such as acetic acid (pKa = 4.76) and glucuronic acid (pKa = 3.18) [31]. The generation of strong acids can protonate the acetate anion in [EMIM][OAc], for example, reduce the IL dissolution efficiency and complicate its recovery for reuse [31, 36]. Recycling is further limited by the high viscosity of ILs, which complicates handling and purification steps [36]. Therefore, efficient methods to separate the different dissolved products are necessary for the recycling of ILs [53].

Recycling efficiency depends on the anti-solvent used for the regeneration of wood after dissolution. Using water as the anti-solvent resulted in a higher yield of regenerated wood than using methanol [31]. The glucose yield after enzymatic hydrolysis after four cycles was also higher using water as an anti-solvent. In the case of *E. grandis*, the lower yield with methanol was explained by the larger amount of extractives dissolved in the methanol/IL mixture. However, after four recycling cycles, the recycled IL yield was 96% with methanol as the anti-solvent and 91% with water. At an industrial scale, water is preferable to methanol, since it is cheaper and more benign environmentally [31].

Another possibility is to replace the anti-solvent by an aqueous solution of phosphate, carbonate, or sulfate. The addition of a K_3PO_4 solution to the biomass solution led to the precipitation of the dissolved biomass and the appearance of a biphasic system with an IL-rich phase and a salt-rich phase. The extracted IL can then be dried and reused [43]. Phenylboronic acid and naphthalene-2-boronic acid were used to extract more glucose, xylose, cellobiose from IL/corn stover solutions after enzymatic hydrolysis in order to improve recyclability [45].

4.5.3 Biodegradability

The potential persistence, accumulation in soils and water and biodegradability of ILs was assessed using standardized methods, such as the closed bottle test or the CO_2 headspace test [60, 111, 114]. Studies on IL biodegradability included the major types of cations: ammonium, imidazolium, phosphonium, and pyridinium ions [111]. The widely used 1-butyl-3-methylimidazolium IL remains stable after 28 days in an aqueous suspension with waste-water microorganisms [60]. It was found that ILs with halogens, branched alkyl chains, pyridine rings, aliphatic ethers are usually more resistant to biodegradation [111]. With these observations, efforts are underway to develop biodegradable ILs. Current strategies include replacing branched alkyl chains with linear alkyl chains, functionalization to enable enzymatic hydrolysis, and the incorporation of phenyl rings [60].

4.6 Applications

4.6.1 Applications of Purified Cellulose Substrates

Considering their abundance, recyclability, and biodegradability, purified cellulose substrates have a great potential in composite materials for biomedical applications, tissue engineering, and sensing. The dissolution of cellulose in ILs increased their processability to facilitate their mixing with other composite components, chemical functionalization, and their extrusion to form materials with the desired shape.

Materials based on purified cellulose or cellulose composites have been developed in ionic liquids using various techniques [14, 140], including mixing multiple components with dissolved cellulose in ionic liquids [140–146], grafting different monomer units onto cellulose to create copolymers [147, 148], and dissolving cellulose into polymerizable ionic liquids [149]. The development of these composites enhanced the mechanical properties [141–144], thermal stability [141, 148], magnetic properties [143], and solubility in dimethyl sulfoxide and dimethylformamide [147], compared to pure cellulose. Films of carbon nanotubes coated with cellulose served as effective scaffolds for the growth of HeLa cells [145]. The biocompatibility of activated charcoal was significantly enhanced with a heparin-cellulose coating deposited in ionic liquids [146]. The regeneration of cellulose after dissolution in ILs using supercritical CO_2 [148] or liquid nitrogen freeze drying [149] enabled the formation of micro- and nanoporous cellulose foams, that can be used for insulation, catalysis or as scaffolds for tissue engineering.

Cellulose dissolution in ILs also enabled the immobilization of chemical reagents [150, 151], drugs [152], and enzymes on solid substrates [153, 154]. Enzymes immobilized on a solid substrate still served as effective catalysts, while

their stabilization and reuse were improved by immobilization [153, 154]. The immobilization of 1-(2-pyridylazo)-2-naphthol was exploited to detect Zn, Mn and Ni ions at concentrations as low as 10^{-6} mol/l [150]. The immobilization of calix [4] arenes on cellulose was used in nitrogen oxide NO_x sensing [151].

Cellulose derivatives, including acetates, carboxymethylates, benzoylates, sulfonates, phthalates, have been synthesized by the dissolution of cellulose in ILs followed by their chemical functionalization. These derivatives are widely used in coatings, films, membrane separation, textiles, and composites [14, 155–158]. Cellulose was directly converted to 5-hydroxymethylfurfural using $CrCl_2$, $CrCl_3$, or $RuCl_3$ as a catalyst [95, 159, 160], and to hexitols using Ru nanoparticles [161]. The molecule 5-hydroxymethylfurfural is believed to be the building block for a wide variety of commodity chemicals. Its derivatives have potential applications as resins, polymers, herbicides, pharmaceuticals, plasticizers, and solvents. [95].

Hydrogen was successfully produced from glucose and cellulose in ILs using Ru as a catalyst. The use of $^{13}C_6$-glucose in the reaction revealed that glucose decomposed into formic acid, which then decomposed into H_2 and CO_2 [162].

4.6.2 Applications of Native Biomass

The main application of the pretreatment of native biomass remains the production of liquid fuels. However, the valorization of lignin and hemicellulose may enhance the economic viability of IL-based processes through the production of commodity chemicals [106]. Ionic liquids have been used as the solvent for the synthesis of 5-hydroxymethylfurfural. Corn stovers were converted into 5-hydroxymethylfurfural in [EMIM][Cl] using $CrCl_2$ as a catalyst [163]. Pine wood and rice straw were also used as feedstocks using [BMIM][Cl] under microwave irradiation with $CrCl_3$ as the catalyst [35]. Acorns, with a high starch content, were successfully converted into 5-hydroxymethylfurfural in 1-octyl-3-methylimidazolium chloride, using mixtures of chromium halides ($CrCl_2$, $CrCl_3$, $CrBr_3$, CrF_3) as catalysts [164].

Chemical functionalization of native wood sawdust was performed by dissolution of the biomass in an IL, followed by its reaction at high temperature or room temperature. For example, poplar sawdust reacted with octanoyl chloride, butyryl chloride and lauroyl chloride in [BMIM][Cl] to produce esterified wood [165, 166]. The addition of an acetic anhydride–pyridine mixture to a solution of spruce led to wood acetylation [7]. Milled fir wood was dissolved in [BMIM][Cl] and also reacted with acetic anhydride with pyridine for acetylation [40]. Norway spruce sawdust and thermomechanical pulp reacted with acetyl chloride, benzoyl chloride, acetic anhydride, phenyl isocyanate, and lauroyl chloride after dissolution in [BMIM][Cl] to produce acetylated, benzoylated, lauroylated, and carbanilated wood derivatives. Thermogravimetric analyses and differential scanning calorimetry showed that the thermal properties of spruce were affected with the appearance of a clear glass transition [30, 167].

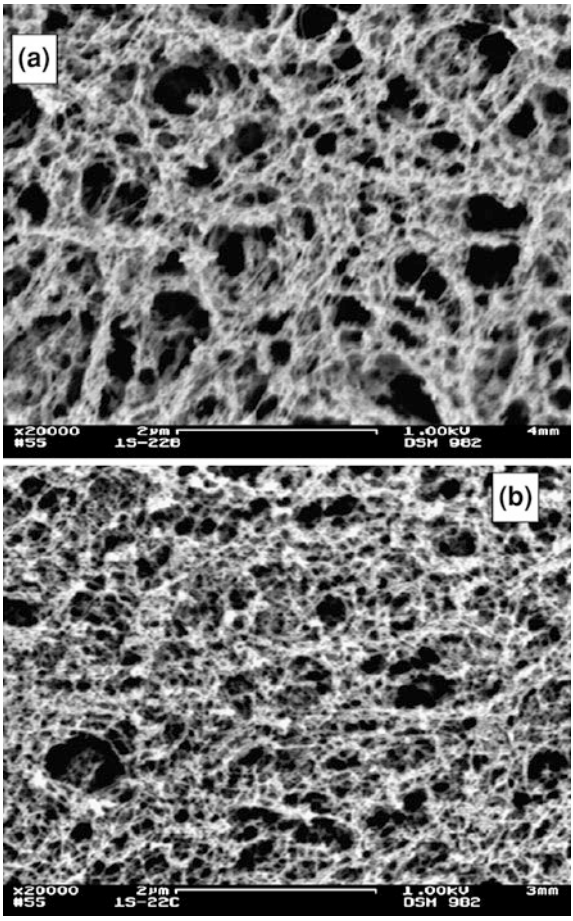

Fig. 4.4 Scanning electron micrographs of aerogels produced from spruce wood, coagulated in baths containing **a** 10 wt% ethanol and **b** 90 wt% ethanol. Reprinted from [168], copyright (2011), with permission from Elsevier

In another study, spruce thermomechanical pulp reacted with benzoyl chloride and lauroyl chloride in [BMIM][Cl] with pyridine to produce benzoylated and lauroylated spruce. The spruce derivatives were then added to poly(styrene) and poly(propylene) to extrude composite fibers and sheets. Thermogravimetric analyses showed an enhanced thermal stability of the composites compared to the spruce thermomechanical pulp [167]. The modifications of the chemical and thermal properties could improve the processability of wood and increase its compatibility to other polymers for the fabrication of composite materials.

Aerogels from milled spruce wood were prepared by dissolving the wood in [BMIM][Cl] at 130°C for more than 4 h. The hot solution was immersed in an ethanol bath at room temperature. The obtained gel was then transferred into a cell where it is immersed in ethanol and liquid CO_2 at 70 bars for 2–3 h. The mixture was heated above the supercritical temperature when the pressure was released to obtain the dry aerogel. Scanning electron micrographs of the aerogels revealed an

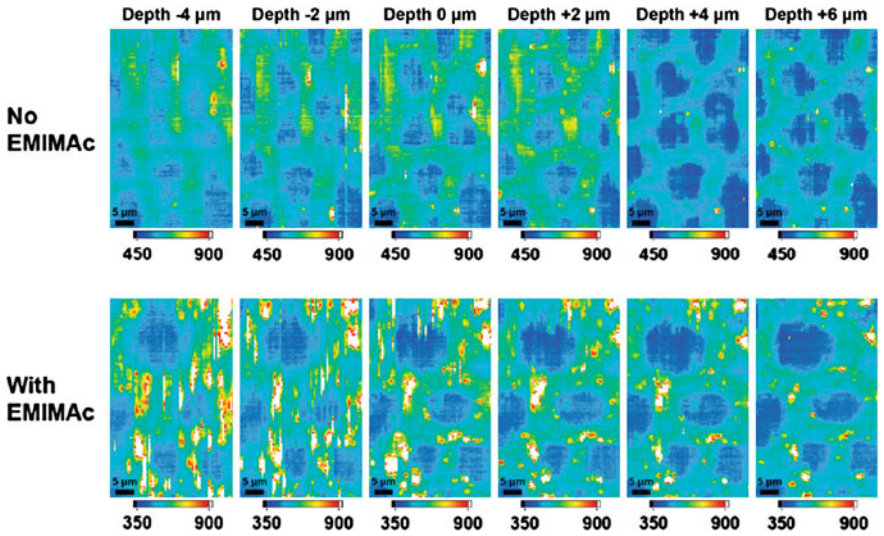

Fig. 4.5 Raman images at different depths after deposition of nanoparticles and rinsing on the untreated wood sample and the sample pretreated with [EMIM][OAc]. The depth 0 µm corresponds to the surface of the sample. Reprinted with permission from [70]. Copyright 2011 American Chemical Society

open pore structure, with pore sizes ranging from 100 nm to 4 µm depending on the feedstock and the reaction conditions (Fig. 4.4) [168].

Composite fibers derived from native Southern yellow pine, oak, and bagasse were prepared by dissolving them in [EMIM][OAc] at temperature above 175°C for 10–30 min. The solution was spun into fibers by extruding the solution into a water bath. The selection of the feedstock affected the thickness and surface roughness of the resulting fiber. The thickness was higher when the biomass dissolution in IL was incomplete. Fibers made from pine pretreated with NaOH were thinner and their surface was smoother, possibly due to the lower hemicellulose and lignin content. Dissolution of bagasse at higher temperatures (185°C for 10 min) improved the processability and the maximum tensile stress applied to the fibers before breaking. The fibers with the highest tensile stresses at failure had the highest cellulose content and were derived from biomass with the highest cellulose content: bagasse with a 58% cellulose content (wt% of biomass) and oak with 49% cellulose content [169]. Wool keratin fibers, a polymer of amino acids, and cellulose were also dissolved at high temperatures (above 100°C) in [BMIM][Cl] and successfully extruded into composite fibers using methanol as the anti-solvent. The composite fibers were less brittle than the pure regenerated wool keratin fibers [170].

All applications mentioned above required the dissolution of biomass at high temperatures, typically above 100°C. A more economical and less energy-intensive strategy is to exploit the swelling of the biomass upon exposure to IL. Poplar

wood was shown to swell at room temperature when exposed to [EMIM][OAc], with cell walls cross-sectional areas expanding by 60–100% in 3 h. After rinsing with deionized water, the wood structure contracted almost immediately [70, 71]. The rinsing of the swollen biomass with a suspension of nanoparticles allowed for the incorporation of materials inside the wood structure without prior dissolution. As a proof of concept, gold nanoparticles of 100 nm diameter were incorporated into poplar and confocal surface-enhanced Raman microscopy showed that the nanoparticles were up to 4 μm deep into the cell wall structure (Fig. 4.5). The incorporation of materials/chemicals into natural and paper products have numerous applications in the development of effective biomass pretreatments, isotope tracing, sensing, and imaging [70].

4.7 Conclusions

In the past decade, numerous ILs have been synthesized to improve the pretreatment and dissolution of native biomass. The application of advanced analytical techniques have provided an insight into the mechanisms involved in the biomass dissolution and the improved access of enzymes to cellulose for a more efficient conversion to fermentable sugars. Advances on the development of IL-tolerant cellulases would enable the pretreatment of biomass and the hydrolysis of cellulose in one step, and therefore improve the economic viability of IL pretreatment of biomass. Biomass dissolution in ILs also has potential applications in composites, tissue engineering, chemical functionalization and sensing. Improved extraction processes are still necessary to optimize efficiency and recycle the ILs. Issues, such as corrosion due to ILs, their full environmental impact and disposal, remain unresolved.

Acknowledgments This study was funded by a Laboratory Directed Research and Development grant from Los Alamos National Laboratory (20080001DR).

References

1. Huber GW, Iborra S, Corma A (2006) Synthesis of transportation fuels from biomass: chemistry, catalysts, and engineering. Chem Rev 106:4044–4098. doi:10.1021/cr068360d
2. Banerjee S, Mudliar S, Sen R, Giri B, Satpute D, Chakrabarti T, Pandey RA (2010) Commercializing lignocellulosic bioethanol: technology bottlenecks and possible remedies. Biofuels Bioprod Biorefin 4:77–93. doi:10.1002/bbb.188
3. Pu Y, Jiang N, Ragauskas AJ (2007) Ionic liquid as a green solvent for lignin. J Wood Chem Technol 27:23–33. doi:10.1080/02773810701282330
4. Fort DA, Remsing RC, Swatloski RP, Moyna P, Moyna G, Rogers RD (2007) Can ionic liquids dissolve wood? Processing and analysis of lignocellulosic materials with 1-n-butyl-3-methylimidazolium chloride. Green Chem 9:63–69. doi:10.1039/b607614a

5. Hendriks ATWM, Zeeman G (2009) Pretreatments to enhance the digestibility of lignocellulosic biomass. Bioresour Technol 100:10–18. doi:10.1016/j.biortech.2008.05.027
6. Li C, Wang Q, Zhao ZK (2008) Acid in ionic liquid: an efficient system for hydrolysis of lignocellulose. Green Chem 10:177–182. doi:10.1039/b711512a
7. Kilpeläinen I, Xie H, King A, Granstrom M, Heikkinen S, Argyropoulos DS (2007) Dissolution of wood in ionic liquids. J Agric Food Chem 55:9142–9148. doi:10.1021/jf071692e
8. Dadi AP, Varanasi S, Schall CA (2006) Enhancement of cellulose saccharification kinetics using an ionic liquid pretreatment step. Biotechnol Bioeng 95:904–910. doi:10.1002/bit.21047
9. Swatloski RP, Spear SK, Holbrey JD, Rogers RD (2002) Dissolution of cellose with ionic liquids. J Am Chem Soc 124:4974–4975. doi:10.1021/ja025790m
10. Dadi AP, Schall CA, Varanasi S (2007) Mitigation of cellulose recalcitrance to enzymatic hydrolysis by ionic liquid pretreatment. Appl Biochem Biotechnol 136–140:407–422. doi:10.1007/s12010-007-9068-9
11. Li C, Zhao ZK (2007) Efficient acid-catalyzed hydrolysis of cellulose in ionic liquid. Adv Synth Catal 349:1847–1850. doi:10.1002/adsc.200700259
12. Huddleston JG, Visser AE, Reichert WM, Willauer HD, Broker GA, Rogers RD (2001) Characterization and comparison of hydrophilic and hydrophobic room temperature ionic liquids incorporating the imidazolium cation. Green Chem 3:156–164. doi:10.1039/b103275p
13. Castner EW Jr, Wishart JF (2010) Spotlight on ionic liquids. J Chem Phys 132:120901. doi:10.1063/1.3373178
14. Pinkert A, Marsh KN, Pang S, Staiger MP (2009) Ionic liquids and their interaction with cellulose. Chem Rev 109:6712–6728. doi:10.1021/cr9001947
15. Castiglione F, Raos G, Appetecchi GB, Montanino M, Passerini S, Moreno M, Famulari A, Mele A (2010) Blending ionic liquids: how physico-chemical properties change. Phys Chem Chem Phys 12:1784–1792. doi:10.1039/b921816e
16. Xie H, Shi T (2010) Liquefaction of wood (*Metasequoia glyptostroboides*) in allyl alkyl imidazolium ionic liquids. Wood Sci Technol 44:119–128. doi:10.1007/s00226-009-0273-2
17. Lee JW, Shin JY, Chun YS, Jang HB, Song CE, Lee SG (2010) Toward understanding the origin of positive effects of ionic liquids on catalysis: Formation of more reactive catalysts and stabilization of reactive intermediates and transition states in ionic liquids. Acc Chem Res 43:985–994. doi:10.1021/ar9002202
18. Welton T (2004) Ionic liquids in catalysis. Coord Chem Rev 248:2459–2477. doi:10.1016/j.ccr.2004.04.015
19. Welton T (1999) Room-temperature ionic liquids: solvents for synthesis and catalysis. Chem Rev 99:2071–2084. doi:10.1021/cr980032t
20. Hallett JP, Welton T (2011) Room-temperature ionic liquids: solvents for synthesis and catalysis. Part 2. Chem Rev 111:3508–3576. doi:10.1021/cr1003248
21. Joglekar HG, Rahman I, Kulkarni BD (2007) The path ahead for ionic liquids. Chem Eng Technol 30:819–828. doi:10.1002/ceat.200600287
22. Tan HT, Lee KT, Mohamed AR (2011) Pretreatment of lignocellulosic palm biomass using a solvent-ionic liquid [BMIM]Cl for glucose recovery: an optimisation study using response surface methodology. Carbohydr Polym 83:1862–1868. doi:10.1016/j.carbpol.2010.10.052
23. Studer MH, DeMartini JD, Davis MF, Sykes RW, Davison B, Keller M, Tuskan GA, Wyman CE (2011) Lignin content in natural *Populus* variants affects sugar release. Proc Natl Acad Sci U S A 108:6300–6305. doi:10.1073/pnas.1009252108
24. Ververis C, Georghiou K, Christodoulakis N, Santas P, Santas R (2004) Fiber dimensions, lignin and cellulose content of various plant materials and their suitability for paper production. Ind Crops Prod 19:245–254. doi:10.1016/j.indcrop.2003.10.006

25. Lee SH, Doherty TV, Linhardt RJ, Dordick JS (2008) Ionic liquid-mediated selective extraction of lignin from wood leading to enhanced enzymatic cellulose hydrolysis. Biotechnol Bioeng 102:1368–1376. doi:10.1002/bit.22179
26. Vanoye L, Fanselow M, Holbrey JD, Atkins MP, Seddon KR (2009) Kinetic model for the hydrolysis of lignocellulosic biomass in the ionic liquid, 1-ethyl-3-methyl-imidazolium chloride. Green Chem 11:390–396. doi:10.1039/b817882h
27. Fu D, Mazza G, Tamaki Y (2010) Lignin extraction from straw by ionic liquids and enzymatic hydrolysis of the cellulosic residues. J Agric Food Chem 58:2915–2922. doi:10.1021/jf903616y
28. Binder JB, Gray MJ, White JF, Zhang ZC, Holladay JE (2009) Reactions of lignin model compounds in ionic liquids. Biomass Bioenergy 33:1122–1130. doi:10.1016/j.biombioe.2009.03.006
29. Jiang N, Pu Y, Samuel R, Ragauskas AJ (2009) Perdeuterated pyridinium molten salt (ionic liquid) for direct dissolution and NMR analysis of plant cell walls. Green Chem 11:1762–1766. doi:10.1039/b913609f
30. Hendriks ATWM, Zeeman G (2009) Pretreatments to enhance the digestibility of lignocellulosic biomass. Bioresour Technol 100:10–18. doi:10.1016/j.biortech.2008.05.027
31. Li B, Asikkala J, Filpponen I, Argyropoulos DS (2010) Factors affecting wood dissolution and regeneration of ionic liquids. Ind Eng Chem Res 49:2477–2484. doi:10.1021/ie901560p
32. Li B, Filpponen I, Argyropoulos DS (2010) Acidolysis of wood in ionic liquids. Ind Eng Chem Res 49:3126–3136. doi:10.1021/ie1000983
33. Zoia L, King AWT, Argyropoulos DS (2011) Molecular weight distributions and linkages in lignocellulosic materials derivatized from ionic liquid media. J Agric Food Chem 59:829–838. doi:10.1021/jf103615e
34. Zavrel M, Bross D, Funke M, Büchs J, Spiess AC (2009) High-throughput screening for ionic liquids dissolving (ligno-)cellulose. Bioresour Technol 100:2580–2587. doi:10.1016/j.biortech.2008.11.052
35. Zhang Z, Zhao ZK (2010) Microwave-assisted conversion of lignocellulosic biomass into furans in ionic liquid. Bioresour Technol 101:1111–1114. doi:10.1016/j.biortech.2009.09.010
36. Sun N, Rahman M, Qin Y, Maxim ML, Rodríguez H, Rogers RD (2009) Complete dissolution and partial delignification of wood in the ionic liquid 1-ethyl-3-methylimidazolium acetate. Green Chem 11:646–655. doi:10.1039/b822702k
37. Brandt A, Hallett JP, Leak DJ, Murphy RJ, Welton T (2010) The effect of the ionic liquid anion in the pretreatment of pine wood chips. Green Chem 12:672–679. doi:10.1039/b918787a
38. Doherty TV, Mora-Pale M, Foley SE, Linhardt RJ, Dordick JS (2010) Ionic liquid solvent properties as predictors of lignocellulose pretreatment efficacy. Green Chem 12:1967–1975. doi:10.1039/c0gc00206b
39. Garcia H, Ferreira R, Petkovic M, Ferguson JL, Leitão MC, Gunaratne HQN, Seddon KR, Rebelo LPN, Pereira CS (2010) Dissolution of cork biopolymers in biocompatible ionic liquids. Green Chem 12:367–369. doi:10.1039/b922553f
40. Qu C, Kishimoto T, Kishino M, Hamada M, Nakajima N (2011) Heteronuclear single-quantum coherence nuclear magnetic resonance (HSQC NMR) characterization of acetylated fir (*Abies sachallnensis* MAST) wood regenerated from ionic liquid. J Agric Food Chem 59:5382–5389. doi:10.1021/jf200498n
41. Li C, Knierim B, Manisseri C, Arora R, Scheller HV, Auer M, Vogel KP, Simmons BA, Singh S (2010) Comparison of dilute acid and ionic liquid pretreatment of switchgrass: biomass recalcitrance, delignification and enzymatic saccharification. Bioresour Technol 101:4900–4906. doi:10.1016/j.biortech.2009.10.066
42. Singh S, Simmons BA, Vogel KP (2009) Visualization of biomass solubilization and cellulose regeneration during ionic liquid pretreatment of switchgrass. Biotechnol Bioeng 104:68–75. doi:10.1002/bit.22386

43. Shill K, Padmanabhan S, Xin Q, Prausnitz JM, Clark DS, Blanch HW (2010) Ionic liquid pretreatment of cellulosic biomass: enzymatic hydrolysis and ionic liquid recycle. Biotechnol Bioeng 108:511–520. doi:10.1002/bit.23014
44. Li Q, Jiang X, He Y, Li L, Xian M, Yang J (2010) Evaluation of the biocompatibile ionic liquid 1-methyl-3-methylimidazolium dimethylphosphite pretreatment of corn cob for improved saccharification. Appl Microbiol Biotechnol 87:117–126. doi:10.1007/s00253-010-2484-8
45. Brennan TCR, Datta S, Blanch HW, Simmons BA, Holmes BM (2010) Recovery of sugars from ionic liquid biomass liquor by solvent extraction. Bioenerg Res 3:123–133. doi:10.1007/s12155-010-9091-5
46. Nguyen TAD, Kim KR, Han SJ, Cho HY, Kim JW, Park SM, Park JC, Sim SJ (2010) Pretreatment of rice straw with ammonia and ionic liquid for lignocellulose conversion to fermentable sugars. Bioresour Technol 101:7432–7438. doi:10.1016/j.biortech.2010.04.053
47. van Spronsen J, Tavares Cardoso MA, Witkamp GJ, de Jong W, Kroon MC (2011) Separation and recovery of the constituents from lignocellulosic biomass by using ionic liquids and acetic acid as co-solvents for mild hydrolysis. Chem Eng Process 50:196–199. doi:10.1016/j.cep.2010.12.010
48. Tan SSY, MacFarlane DR, Upfal J, Edye LA, Doherty WOS, Patti AF, Pringle JM, Scott JL (2009) Extraction of lignin from lignocellulose at atmospheric pressure using alkylbenzenesulfonate ionic liquid. Green Chem 11:339–345. doi:10.1039/b815310h
49. Liu H, Sale KL, Holmes BM, Simmons BA, Singh S (2010) Understanding the interactions of cellulose with ionic liquids: a molecular dynamics study. J Phys Chem B 114:4293–4301. doi:10.1021/jp9117437
50. Arora R, Manisseri C, Li C, Ong MD, Scheller HV, Vogel K, Simmons BA, Singh S (2010) Monitoring and analyzing process streams towards understanding ionic liquid pretreatment of switchgrass (*Panicum virgatum* L.). Bioenerg Res 3:134–145. doi:10.1007/s12155-010-9087-1
51. Sescousse R, Le KA, Ries ME, Budtova T (2010) Viscosity of cellulose-imidazolium-based ionic liquid solutions. J Phys Chem B 114:7222–7228. doi:10.1021/jp1024203
52. Gericke M, Schlufter K, Liebert T, Heinze T, Budtova T (2009) Rheological properties of cellulose/ionic liquid solutions: from dilute to concentrated states. Biomacromolecules 10:1188–1194. doi:10.1021/bm801430x
53. Sievers C, Valenzuela-Olarte MB, Marzialetti T, Musin I, Agrawal PK, Jones CW (2009) Ionic-liquid-phase hydrolysis of pine wood. Ind Eng Chem Res 48:1277–1286. doi:10.1021/ie801174x
54. Xu A, Wang J, Wang H (2010) Effects of anionic structure and lithium salts addition on the dissolution of cellulose in 1-butyl-3-methylimidazolium-based ionic liquid solvent systems. Green Chem 12:268–275. doi:10.1039/b916882f
55. Sun N, Jiang X, Maxim ML, Metlen A, Rogers RD (2011) Use of polyoxometalate catalysts in ionic liquids to enhance the dissolution and delignification of woody biomass. ChemSusChem 4:65–73. doi:10.1002/cssc.201000272
56. Mikkola JP, Kirilin A, Tuuf JC, Pranovich A, Holmbom B, Kustov LM, Murzin YD, Salmi T (2007) Ultrasound enhancement of cellulose processing in ionic liquids: from dissolution towards functionalization. Green Chem 9:1229–1237. doi:10.1039/b708533h
57. Ikeda T, Holtman K, Kadla JF, Chang HM, Jameel H (2002) Studies on the effect of ball milling on lignin structure using a modified DFRC method. J Agric Food Chem 50:129–135. doi:10.1021/jf010870f
58. Nishi N, Kawakami T, Shigematsu F, Yamamoto M, Kakiuchi T (2006) Fluorine-free and hydrophobic room-temperature ionic liquids, tetraalkylammonium bis(2-ethylhexyl) sulfosuccinates, and their ionic liquid–water two-phase properties. Green Chem 8:349–355. doi:10.1039/b511529a
59. Di Francesco F, Calisi N, Creatini M, Melai B, Salvo P, Chiappe C (2011) Water sorption by anhydrous ionic liquids. Green Chem 13:1712–1717. doi:10.1039/c1gc15080d

60. Scammells PJ, Scott JL, Singer RD (2005) Ionic liquids: the neglected issues. Aust J Chem 58:155–169. doi:10.1071/CH04272
61. Vitz J, Erdmenger T, Haensch C, Schubert US (2009) Extended dissolution studies of cellulose in imidazolium based ionic liquids. Green Chem 11:417–424. doi:10.1039/b818061j
62. Binder JB, Raines RT (2010) Fermentable sugars by chemical hydrolysis of biomass. Proc Natl Acad Sci U S A 107:4516–4521. doi:10.1073/pnas.0912073107
63. Tran CD, De Paoli Lacerda SH, Oliveira D (2003) Absorption of water by room-temperature ionic liquids: effect of anions on concentration and state of water. Appl Spectrosc 57:152–157. doi:10.1366/000370203321535051
64. Wang Y, Li H, Han S (2006) A theoretical investigation of the interactions between water molecules and ionic liquids. J Phys Chem B 110:24646–24651. doi:10.1021/jp064134w
65. Chapeaux A, Simoni LD, Stadtherr MA, Brennecke JF (2007) Liquid phase behavior of ionic liquids with water and 1-octanol and modeling of 1-octanol/water partition coefficients. J Chem Eng Data 52:2462–2467. doi:10.1021/je7003935
66. Freire MG, Carvalho PJ, Gardas RL, Marrucho IM, Santos LMNBF, Coutinho JAP (2008) Mutual solubilities of water and the $[C_n mim][Tf_2N]$ hydrophobic ionic liquids. J Phys Chem B 112:1604–1610. doi:10.1021/jp7097203
67. Engel P, Mladenov R, Wulfhorst H, Jäger G, Spiess AC (2010) Point by point analysis: how ionic liquid affects the enzymatic hydrolysis of native and modified cellulose. Green Chem 12:1959–1966. doi:10.1039/c0gc00135j
68. Seddon KR, Stark A, Torres MJ (2000) Influence of chloride, water, and organic solvents on the physical properties of ionic liquids. Pure Appl Chem 72:2275–2287. doi:10.1351/pac200072122275
69. Zhang Y, Du H, Qian X, Chen EYX (2010) Ionic liquid-water mixtures: enhanced K_w for efficient cellulosic biomass conversion. Energy Fuels 24:2410–2417. doi:10.1021/ef1000198
70. Lucas M, Macdonald BA, Wagner GL, Joyce SA, Rector KD (2010) Ionic liquid pretreatment of poplar wood at room temperature: swelling and incorporation of nanoparticles. ACS Appl Mater Interfaces 2:2198–2205. doi:10.1021/am100371q
71. Lucas M, Wagner GL, Nishiyama Y, Hanson L, Samayam IP, Schall CA, Langan P, Rector KD (2011) Reversible swelling of the cell wall of poplar biomass by ionic liquid at room temperature. Bioresour Technol 102:4518–4523. doi:10.1016/j.biortech.2010.12.087
72. Bharadwaj R, Wong A, Knierim B, Singh S, Holmes BM, Auer M, Simmons BA, Adams PD, Singh AK (2011) High-throughput enzymatic hydrolysis of lignocellulosic biomass via in situ regeneration. Bioresour Technol 102:1329–1337. doi:10.1016/j.biortech.2010.08.108
73. Cheng G, Varanasi P, Li C, Liu H, Melnichenko YB, Simmons BA, Kent MS, Singh S (2011) Transition of cellulose crystalline structure and surface morphology of biomass as a function of ionic liquid pretreatment and its relation to enzymatic hydrolysis. Biomacromolecules 12:933–941. doi:10.1021/bm101240z
74. Sun L, Simmons BA, Singh S (2011) Understanding tissue specific compositions of bioenergy feedstocks through hyperspectral Raman imaging. Biotechnol Bioeng 108:286–295. doi:10.1002/bit.22931
75. Jia S, Cox BJ, Guo X, Zhang ZC, Ekerdt JG (2010) Cleaving the β–O–4 bonds of lignin model compounds in an acidic ionic liquid, 1-h-3-methylimidazolium chloride: An optional strategy for the degradation of lignin. ChemSusChem 3:1078–1084. doi:10.1002/cssc.201000112
76. Jia S, Cox BJ, Guo X, Zhang ZC, Ekerdt JG (2011) Hydrolytic cleavage of β–O–4 ether bonds of lignin model compounds in an ionic liquid with metal chlorides. Ind Eng Chem Res 50:849–855. doi:10.1021/ie101884h
77. Wulf A, Fumino K, Ludwig R (2010) Spectroscopic evidence for an enhanced anion–cation interaction from hydrogen bonding in pure imidazolium ionic liquids. Angew Chem Int Ed 49:449–453. doi:10.1002/anie.200905437

78. Kline LM, Hayes DG, Womac AR, Labbé N (2010) Simplified determination of lignin content in hard and soft woods via UV-spectrophotometric analysis of biomass dissolved in ionic liquids. BioResources 5:1366–1383
79. Kiefer J, Obert K, Fries J, Bösmann A, Wasserscheid P, Leipertz A (2009) Determination of glucose and cellobiose dissolved in the ionic liquid 1-ethyl-3-methylimidazolium acetate using Fourier transform infrared spectroscopy. Appl Spectrosc 63:1041–1049. doi:10.1366/000370209789379367
80. Miller GL (1959) Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal Chem 31:426–428. doi:10.1021/ac60147a030
81. Rivers DB, Gracheck SJ, Woodford LC, Emert GH (1984) Limitations of the DNS assay for reducing sugars from saccharified lignocellulosics. Biotechnol Bioeng 26:800–802. doi:10.1002/bit.260260727
82. Ebner G, Schiehser S, Potthast A, Rosenau T (2008) Side reaction of cellulose with common 1-alkyl-3-methylimidazolium-based ionic liquids. Tetrahedron Lett 49:7322–7324. doi:10.1016/j.tetlet.2008.10.052
83. King AWT, Zoia L, Filpponen I, Olszewska A, Xie H, Kilpeläinen I, Argyropoulos DS (2009) In situ determination of lignin phenolics and wood solubility in imidazolium chlorides using ^{31}P NMR. J Agric Food Chem 57:8236–8243. doi:10.1021/jf901095w
84. Remsing RC, Swatloski RP, Rogers R, Moyna G (2006) Mechanism of cellulose dissolution in the ionic liquid 1-*n*-butyl-3-methylimidazolium chloride: a ^{13}C and $^{35/37}$Cl NMR relaxation study on model systems. Chem Commun 2006:1271–1273. doi:10.1039/b600586c
85. Zhang J, Zhang H, Wu J, Zhang J, He J, Xiang J (2010) NMR spectroscopic studies of cellobiose solvation in [EMIM][OAc] aimed to understand the dissolution mechanism of cellulose in ionic liquids. Phys Chem Chem Phys 12:1941–1947. doi:10.1039/b920446f
86. Kahlen J, Masuch K, Leonhard K (2010) Modelling cellulose solubilities in ionic liquids using COSMO-RS. Green Chem 12:2172–2181. doi:10.1039/c0gc00200c
87. Fukaya Y, Hayashi K, Wada M, Ohno H (2008) Cellulose dissolution with polar ionic liquids under mild conditions: required factors for anions. Green Chem 10:44–46. doi:10.1039/b713289a
88. Dwiatmoko AA, Choi JW, Suh DJ, Suh YW, Kung HH (2010) Understanding the role of halogen-containing ionic liquids in the hydrolysis of cellobiose catalyzed by acid resins. Appl Catal A Gen 387:209–214. doi:10.1016/j.apcata.2010.08.032
89. Rinaldi R, Meine N, vom Stein J, Palkovits R, Schüth F (2010) Which controls the depolymerization of cellulose in ionic liquids: the solid acid catalyst or cellulose? ChemSusChem 3:266–276. doi:10.1002/cssc.200900281
90. Cuissinat C, Navard P, Heinze T (2008) Swelling and dissolution of cellulose. Part IV: free floating cotton and wood fibres in ionic liquids. Carbohydr Polym 72:590–596. doi:10.1016/j.carbpol.2007.09.029
91. Zhao H, Jones CL, Baker GA, Xia S, Olubajo O, Person VN (2009) Regenerating cellulose from ionic liquids for an accelerated enzymatic hydrolysis. J Biotechnol 139:47–54. doi:10.1016/j.jbiotec.2008.08.009
92. Guo J, Zhang D, Duan C, Liu C (2010) Probing anion–cellulose interactions in imidazolium-based room temperature ionic liquids: a density functional study. Carbohydr Res 345:2201–2205. doi:10.1016/j.carres.2010.07.036
93. Armstrong DW, He L, Liu YS (1999) Examination of ionic liquids and their interaction with molecules, when used as stationary phases in gas chromatography. Anal Chem 71:3873–3876. doi:10.1021/ac990443p
94. Abraham MH (1993) Scales of solute hydrogen-bonding: their construction and application to physicochemical and biochemical processes. Chem Soc Rev 22:73–83. doi:10.1039/CS9932200073
95. Zakrzewska ME, Bogel-Łukasik E, Bogel-Łukasik R (2011) Ionic liquid-mediated formation of 5-hydroxymethylfurfurals—a promising biomass-derived building block. Chem Rev 111:397–417. doi:10.1021/cr100171a

96. Anderson JL, Ding J, Welton T, Armstrong DW (2002) Characterizing ionic liquids on the basis of multiple solvation interactions. J Am Chem Soc 124:14247–14254. doi:10.1021/ja028156h
97. Carmichael AJ, Seddon KR (2000) Polarity study of some 1-alkyl-3-methylimidazolium ambient-temperature ionic liquids with the solvatochromic dye, Nile Red. J Phys Org Chem 13:591–595. doi:10.1002/1099-1395(200010)13:10<591:AID-POC305>3.0.CO;2-2
98. Reichardt C (1965) Empirical parameters of the polarity of solvents. Angew Chem Int Ed Engl 4:29–40. doi:10.1002/anie.196500291
99. Bonhôte P, Dias AP, Papageorgiou N, Kalyanasundaram K, Grätzel M (1996) Hydrophobic, highly conductive ambient-temperature molten salts. Inorg Chem 35:1168–1178. doi:10.1021/ic951325x
100. Aki SVNK, Brennecke JF, Samanta A (2001) How polar are room-temperature ionic liquids? Chem Commun 2001:413–414. doi:10.1039/B008039J
101. Muldoon MJ, Gordon CM, Dunkin IR (2001) Investigations of solvent–solute interactions in room temperature ionic liquids using solvatochromic dyes. J Chem Soc Perkin Trans 2(2001):433–435. doi:10.1039/B101449H
102. Kamlet MJ, Taft RW (1976) The solvatochromic comparison method. I. The β-scale of solvent hydrogen-bond acceptor (HBA) basicities. J Am Chem Soc 98:377–383. doi:10.1021/ja00418a009
103. Taft RW, Kamlet MJ (1976) The solvatochromic comparison method. 2. The α-scale of solvent hydrogen-bond donor (HBD) acidities. J Am Chem Soc 98:2886–2894. doi:10.1021/ja00426a036
104. Kamlet MJ, Abboud JL, Taft RW (1977) The solvatochromic comparison method. 6. The π^* scale of solvent polarities. J Am Chem Soc 99:6027–6038. doi:10.1021/ja00460a031
105. Kamlet MJ, Abboud JLM, Abraham MH, Taft RW (1983) Linear solvation energy relationships. 23. A comprehensive collection of the solvatochromic parameters, π^*, α, and β, and some methods for simplifying the generalized solvatochromic equation. J Org Chem 48:2877–2887. doi:10.1021/jo00165a018
106. Stark A (2011) Ionic liquids in the biorefinery: a critical assessment of their potential. Energy Environ Sci 4:19–32. doi:10.1039/c0ee00246a
107. Crowhurst L, Mawdsley PR, Perez-Arlandis JM, Salter PA, Welton T (2003) Solvent–solute interactions in ionic liquids. Phys Chem Chem Phys 5:2790–2794. doi:10.1039/b303095d
108. Fukaya Y, Sugimoto A, Ohno H (2006) Superior solubility of polysaccharides in low viscosity, polar, and halogen-free 1,3-dialkylimidazolium formates. Biomacromolecules 7:3295–3297. doi:10.1021/bm060327d
109. Kralisch D, Stark A, Körsten S, Kreisel G, Ondruschka B (2005) Energetic, environmental and economic balances: spice up your ionic liquid research efficiency. Green Chem 7:301–309. doi:10.1039/b417167e
110. Nicola Wood, Stephens G (2010) Accelerating the discovery of biocompatible ionic liquids. Phys Chem Chem Phys 12:1670–1674. doi:10.1039/b923429b
111. Coleman D, Gathergood N (2010) Biodegradation studies of ionic liquids. Chem Soc Rev 39:600–637. doi:10.1039/b817717c
112. Docherty KM, Kulpa CF (2005) Toxicity and antimicrobial activity of imidazolium and pyridinium ionic liquids. Green Chem 7:185–189. doi:10.1039/b419172b
113. Frade RFM, Matias A, Branco LC, Afonso CAM, Duarte CMM (2007) Effect of ionic liquids on human colon carcinoma HT-29 and CaCo-2 cell lines. Green Chem 9:873–877. doi:10.1039/b617526k
114. Pham TPT, Cho CW, Yun YS (2010) Environmental fate and toxicity of ionic liquids: a review. Water Res 44:352–372. doi:10.1016/j.watres.2009.09.030
115. Romero A, Santos A, Tojo J, Rodríguez A (2008) Toxicity and biodegradability of imidazolium ionic liquids. J Hazard Mater 151:268–273. doi:10.1016/j.jhazmat.2007.10.079
116. Nancharaiah YV, Francis AJ (2011) Alkyl-methylimidazolium ionic liquids affect the growth and fermentative metabolism of *Clostridium* sp. Bioresour Technol 102:6573–6578. doi:10.1016/j.biortech.2011.03.042

117. Almeida JRM, Bertilsson M, Gorwa-Grauslund MF, Gorsich S, Lidén G (2009) Metabolic effects of furaldehydes and impacts on biotechnological processes. Appl Microbiol Biotechnol 82:625–638. doi:10.1007/s00253-009-1875-1
118. Taechapoempol K, Sreethawong T, Rangsunvigit P, Namprohm W, Thamprajamchit B, Rengpipat S, Chavadej S (2011) Cellulase-producing bacteria from Thai higher termites, *Microcerotermes* sp.: enzymatic activities and ionic liquid tolerance. Appl Biochem Biotechnol 164:204–219. doi:10.1007/s12010-010-9128-4
119. Bansal P, Hall M, Realff MJ, Lee JH, Bommarius AS (2009) Modeling cellulase kinetics on lignocellulosic substrates. Biotechnol Adv 27:833–848. doi:10.1016/j.biotechadv.2009.06.005
120. Wang Y, Radosevich M, Hayes D, Labbé N (2011) Compatible ionic liquid-cellulases system for hydrolysis of lignocellulosic biomass. Biotechnol Bioeng 108:1042–1048. doi:10.1002/bit.23045
121. Turner MB, Spear SK, Huddleston JG, Holbrey JD, Rogers RD (2003) Ionic liquid salt-induced inactivation and unfolding of cellulase from *Trichoderma reesei*. Green Chem 5:443–447. doi:10.1039/b302570e
122. Kamiya N, Matsushita Y, Hanaki M, Nakashima K, Narita M, Goto M, Takahashi H (2008) Enzymatic in situ saccharification of cellulose in aqueous-ionic liquid media. Biotechnol Lett 30:1037–1040. doi:10.1007/s10529-008-9638-0
123. Salvador ÂC, da C. Santos M, Saraiva JA (2010) Effect of the ionic liquid [bmim]Cl and high pressure on the activity of cellulose. Green Chem 12:632–635. doi:10.1039/b918879g
124. Bose S, Armstrong DW, Petrich JW (2010) Enzyme-catalyzed hydrolysis of cellulose in ionic liquids: a green approach toward the production of biofuels. J Phys Chem B 114:8221–8227. doi:10.1021/jp9120518
125. Adsul MG, Terwadkar AP, Varma AJ, Gokhale DV (2009) Cellulases from *Penicillium Janthinellum* mutants: solid-state production and their stability in ionic liquids. BioResources 4:1670–1681
126. Pottkämper J, Barthen P, Ilmberger N, Schwaneberg U, Schenk A, Schulte M, Ignatiev N, Streit WR (2009) Applying metagenomics for the identification of bacterial cellulases that are stable in ionic liquids. Green Chem 11:957–965. doi:10.1039/b820157a
127. Jäger G, Wulfhorst H, Zeithammel EU, Elinidou E, Spiess AC, Büchs J (2011) Screening of cellulases for biofuel production: online monitoring of the enzymatic hydrolysis of insoluble cellulose using high-throughput scattered light detection. Biotechnol J 6:74–85. doi:10.1002/biot.201000387
128. Zhou GP, Zhang Y, Huang XR, Shi CH, Liu WF, Li YZ, Qu YB, Gao PJ (2008) Catalytic activities of fungal oxidases in hydrophobic ionic liquid 1-butyl-3-methylimidazolium hexafluorophosphate-based microemulsion. Colloids Surf B 66:146–149. doi:10.1016/j.colsurfb.2008.05.016
129. Cui X, Zhang S, Shi F, Zhang Q, Ma X, Lu L, Deng Y (2010) The influence of the acidity of ionic liquids on catalysis. ChemSusChem 3:1043–1047. doi:10.1002/cssc.201000075
130. Murao S, Nomura Y, Yoshikawa M, Shin T, Oyama H, Arai M (1992) Enhancement of activities of cellulases under high hydrostatic pressure. Biosci Biotechnol Biochem 56:1366–1367. doi:10.1271/bbb.56.1366
131. Datta S, Holmes B, Park JI, Chen Z, Dibble DC, Hadi M, Blanch HW, Simmons BA, Sapra R (2010) Ionic liquid tolerant hyperthermophilic cellulases for biomass pretreatment and hydrolysis. Green Chem 12:338–345. doi:10.1039/b916564a
132. Rees HC, Grant S, Jones B, Grant WD, Heaphy S (2003) Detecting cellulase and esterase enzyme activities encoded by novel genes present in environmental DNA libraries. Extremophiles 7:415–421. doi:10.1007/s00792-003-0339-2
133. Grant S, Sorokin DY, Grant WD, Jones B, Heaphy S (2004) A phylogenetic analysis of Wadi el Natrun soda lake cellulase enrichment cultures and identification of cellulase genes from these cultures. Extremophiles 8:421–429. doi:10.1007/s00792-004-0402-7
134. Voget S, Steele HL, Streit WR (2006) Characterization of a metagenome-derived halotolerant cellulase. J Biotechnol 126:26–36. doi:10.1016/j.jbiotec.2006.02.011

135. Zhao H, Baker GA, Song Z, Olubajo O, Crittle T, Peters D (2008) Designing enzyme-compatible ionic liquids that can dissolve carbohydrates. Green Chem 10:696–705. doi:10.1039/b801489b
136. Zhao H (2010) Methods for stabilizing and activating enzymes in ionic liquids—a review. J Chem Technol Biotechnol 85:891–907. doi:10.1002/jctb.2375
137. Lozano P, Bernal B, Bernal JM, Pucheault M, Vaultier M (2011) Stabilizing immobilized cellulase by ionic liquids for saccharification of cellulose solutions in 1-butyl-3-methylimidazolium chloride. Green Chem 13:1406–1410. doi:10.1039/c1gc15294g
138. Righi S, Morfino A, Galletti P, Samorì C, Tugnoli A, Stramigioli C (2011) Comparative cradle-to-gate life cycle assessments of cellulose dissolution with 1-butyl-3-methylimidazolium chloride and *N*-methyl-morpholine-*N*-oxide. Green Chem 13:367–375. doi:10.1039/c0gc00647e
139. Kralisch D, Reinhardt D, Kreisel G (2007) Implementing objectives of sustainability into ionic liquids research and development. Green Chem 9:1308–1318. doi:10.1039/b708721g
140. Kosan B, Michels C, Meister F (2008) Dissolution and forming of cellulose with ionic liquids. Cellulose 15:59–66. doi:10.1007/s10570-007-9160-x
141. Wu RL, Wang XL, Li F, Li HZ, Wang YZ (2009) Green composite films prepared from cellulose, starch and lignin in room-temperature ionic liquid. Bioresour Technol 100:2569–2574. doi:10.1016/j.biortech.2008.11.044
142. Zhang H, Wang Z, Zhang Z, Wu J, Zhang J, He J (2007) Regenerated-cellulose/multiwalled-carbon-nanotube composite fibers with enhanced mechanical properties prepared with the ionic liquid 1-allyl-3-methylimidazolium chloride. Adv Mater 19:698–704. doi:10.1002/adma.200600442
143. Sun N, Swatloski RP, Maxim ML, Rahman M, Harland AG, Haque A, Spear SK, Daly DT, Rogers RD (2008) Magnetite-embedded cellulose fibers prepared from ionic liquid. J Mater Chem 18:283–290. doi:10.1039/b713194a
144. Takegawa A, Murakami M, Kaneko Y, Kadokawa J (2010) Preparation of chitin/cellulose composite gels and films with ionic liquids. Carbohydr Polym 79:85–90. doi:10.1016/j.carbpol.2009.07.030
145. Li L, Meng L, Zhang X, Fu C, Lu Q (2009) The ionic liquid-associated synthesis of a cellulose/SWCNT complex and its remarkable biocompatibility. J Mater Chem 19:3612–3617. doi:10.1039/b823322e
146. Park TJ, Lee SH, Simmons TJ, Martin JG, Mousa SA, Snezhkova EA, Sarnatskaya VV, Nikolaev VG, Linhardt RJ (2008) Heparin–cellulose–charcoal composites for drug detoxification prepared using room temperature ionic liquids. Chem Commun 2008:5022–5024. doi:10.1039/b809791g
147. Zhu J, Wang WT, Wang XL, Li B, Wang YZ (2009) Green synthesis of a novel biodegradable copolymer base on cellulose and poly(*p*-dioxanone) in ionic liquid. Carbohydr Polym 76:139–144. doi:10.1016/j.carbpol.2008.10.004
148. Hao Y, Peng J, Li J, Zhai M, Wei G (2009) An ionic liquid as reaction media for radiation-induced grafting of thermosensitive poly (*N*-isopropylacrylamide) onto microcrystalline cellulose. Carbohydr Polym 77:779–784. doi:10.1016/j.carbpol.2009.02.025
149. Kadokawa J, Murakami M, Kaneko Y (2008) A facile method for preparation of composites composed of cellulose and a polystyrene-type polymeric ionic liquid using a polymerizable ionic liquid. Compos Sci Technol 68:493–498. doi:10.1016/j.compscitech.2007.06.004
150. Egorov VM, Smirnova SV, Formanovsky AA, Pletnev IV, Zolotov YA (2007) Dissolution of cellulose in ionic liquids as a way to obtain test materials for metal-ion detection. Anal Bioanal Chem 387:2263–2269. doi:10.1007/s00216-006-1114-x
151. Hines JH, Wanigasekara E, Rudkevich DM, Rogers RD (2008) Calix[4]arenes immobilized in a cellulose-based platform for entrapment and detection of NO_x gases. J Mater Chem 18:4050–4055. doi:10.1039/b803289k
152. Viswanathan G, Murugesan S, Pushparaj V, Nalamasu O, Ajayan PM, Linhardt RJ (2006) Preparation of biopolymer fibers by electrospinning from room temperature ionic liquids. Biomacromolecules 7:415–418. doi:10.1021/bm050837s

153. Turner MB, Spear SK, Holbrey JD, Daly DT, Rogers RD (2005) Ionic liquid-reconstituted cellulose composites as solid support matrices for biocatalyst immobilization. Biomacromolecules 6:2497–2501. doi:10.1021/bm050199d
154. Bagheri M, Rodríguez H, Swatloski RP, Spear SK, Daly DT, Rogers RD (2008) Ionic liquid-based preparation of cellulose-dendrimer films as solid supports for enzyme immobilization. Biomacromolecules 9:381–387. doi:10.1021/bm701023w
155. Erdmenger T, Haensch C, Hoogenboom R, Schubert US (2007) Homogeneous tritylation of cellulose in 1-butyl-3-methylimidazolium chloride. Macromol Biosci 7:440–445. doi:10.1002/mabi.200600253
156. Abbott AP, Bell TJ, Handa S, Stoddart B (2005) *O*-Acetylation of cellulose and monosaccharides using a zinc based ionic liquid. Green Chem 7:705–707. doi:10.1039/b511691k
157. Cao Y, Wu J, Meng T, Zhang J, He J, Li H, Zhang Y (2007) Acetone-soluble cellulose acetates prepared by one-step homogeneous acetylation of cornhusk cellulose in an ionic liquid 1-allyl-3-methylimidazolium chloride (AmimCl). Carbohydr Polym 69:665–672. doi:10.1016/j.carbpol.2007.02.001
158. Heinze T, Dorn S, Schöbitz M, Liebert T, Köhler S, Meister F (2008) Interactions of ionic liquids with polysaccharides—2: cellulose. Macromol Symp 262:8–22. doi:10.1002/masy.200850202
159. Qi X, Watanabe M, Aida TM, Smith R (2010) Fast transformation of glucose and di-/polysaccharides into 5-hydroxymethylfurfural by microwave heating in an ionic liquid/catalyst system. ChemSusChem 3:1071–1077. doi:10.1002/cssc.201000124
160. Kim B, Jeong J, Lee D, Kim S, Yoon HJ, Lee YS, Cho JK (2011) Direct transformation of cellulose into 5-hydroxymethyl-2-furfural using a combination of metal chlorides in imidazolium ionic liquid. Green Chem 13:1503–1506. doi:10.1039/c1gc15152e
161. Zhu Y, Kong ZN, Stubbs LP, Lin H, Shen S, Anslyn EV, Maguire JA (2010) Conversion of cellulose to hexitols catalyzed by ionic liquid-stabilized ruthenium nanoparticles and a reversible binding agent. ChemSusChem 3:67–70. doi:10.1002/cssc.200900235
162. Taccardi N, Assenbaum D, Berger MEM, Bösmann A, Enzenberger F, Wölfel R, Neuendorf S, Goeke V, Schödel N, Maass HJ, Kistenmacher H, Wasserscheid P (2010) Catalytic production of hydrogen from glucose and other carbohydrates under exceptionally mild reaction conditions. Green Chem 12:1150–1156. doi:10.1039/c002910f
163. Binder JB, Raines RT (2009) Simple chemical transformation of lignocellulosic biomass into furans for fuels and chemicals. J Am Chem Soc 131:1979–1985. doi:10.1021/ja808537j
164. Lee JW, Ha MG, Yi YB, Chung CH (2011) Chromium halides mediated production of hydroxymethylfurfural from starch-rich acorn biomass in an acidic ionic liquid. Carbohydr Res 346:177–182. doi:10.1016/j.carres.2010.11.009
165. Yuan TQ, Sun SN, Xu F, Sun RC (2010) Homogeneous esterification of poplar wood in an ionic liquid under mild conditions: characterization and properties. J Agric Food Chem 58:11302–11310. doi:10.1021/jf103050t
166. Yuan T, Sun S, Xu F, Sun R (2011) Homogeneous butyrylation and lauroylation of poplar wood in the ionic liquid 1-butyl-3-methylimidazolium chloride. Bioresour Technol 102:4590–4593. doi:10.1016/j.biortech.2010.12.102
167. Xie H, Jarvi P, Karesoja M, King A, Kilpelainen I, Argyropoulos DS (2009) Highly compatible wood thermoplastic composites from lignocellulosic material modified in ionic liquids: preparation and thermal properties. J Appl Polym Sci 111:2468–2476. doi:10.1002/app.29251
168. Aaltonen O, Jauhiainen O (2009) The preparation of lignocellulosic aerogels from ionic liquid solutions. Carbohydr Polym 75:125–129. doi:10.1016/j.carbpol.2008.07.008
169. Sun N, Li W, Stoner B, Jiang X, Lu X, Rogers RD (2011) Composite fibers spun directly from solutions of raw lignocellulosic biomass dissolved in ionic liquids. Green Chem 13:1158–1161. doi:10.1039/c1gc15033b
170. Xie H, Li S, Zhang S (2005) Ionic liquids as novel solvents for the dissolution and blending of wool keratin fibers. Green Chem 7:606–608. doi:10.1039/b502547h