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# Room Temperature Ionic Liquids as Emerging Solvents for the Pretreatment of Lignocellulosic Biomass

Mauricio Mora-Pale,<sup>1</sup> Luciana Meli,<sup>1</sup> Thomas V. Doherty,<sup>2</sup> Robert J. Linhardt,<sup>1,3,4</sup> Jonathan S. Dordick<sup>1,3</sup>

<sup>1</sup>Department of Chemical and Biological Engineering, Center for Biotechnology and Interdisciplinary Studies (CBIS), Rensselaer Polytechnic Institute, 110 8th Street, Troy, New York 12180; telephone: 518-276-2899; fax: 518-276-2207; e-mail: dordick@rpi.edu

<sup>2</sup>Department of Material Science and Engineering, Center for Biotechnology and Interdisciplinary Studies (CBIS), Rensselaer Polytechnic Institute, Troy, New York

<sup>3</sup>Department of Biology, Center for Biotechnology and Interdisciplinary Studies (CBIS), Rensselaer Polytechnic Institute, Troy, New York

<sup>4</sup>Department of Chemistry and Chemical Biology, Center for Biotechnology and Interdisciplinary Studies (CBIS), Rensselaer Polytechnic Institute, Troy, New York

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**ABSTRACT:** Room temperature ionic liquids (RTILs) are emerging as attractive and green solvents for lignocellulosic biomass pretreatment. The unique solvating properties of RTILs foster the disruption of the 3D network structure of lignin, cellulose, and hemicellulose, which allows high yields of fermentable sugars to be produced in subsequent enzymatic hydrolysis. In the current review, we summarize the physicochemical properties of RTILs that make them effective solvents for lignocellulose pretreatment including mechanisms of interaction between lignocellulosic biomass subcomponents and RTILs. We also highlight several recent strategies that exploit RTILs and generate high yields of fermentable sugars suitable for downstream biofuel production, and address new opportunities for use of lignocellulosic components, including lignin. Finally, we address some of the challenges that remain before large-scale use of RTILs may be achieved.

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**KEYWORDS:** room temperature ionic liquids; lignocellulosic biomass; cellulose; hemicellulose; lignin; pretreatment

## Introduction

Over the last three decades, there has been growing interest in the application of room temperature ionic liquids

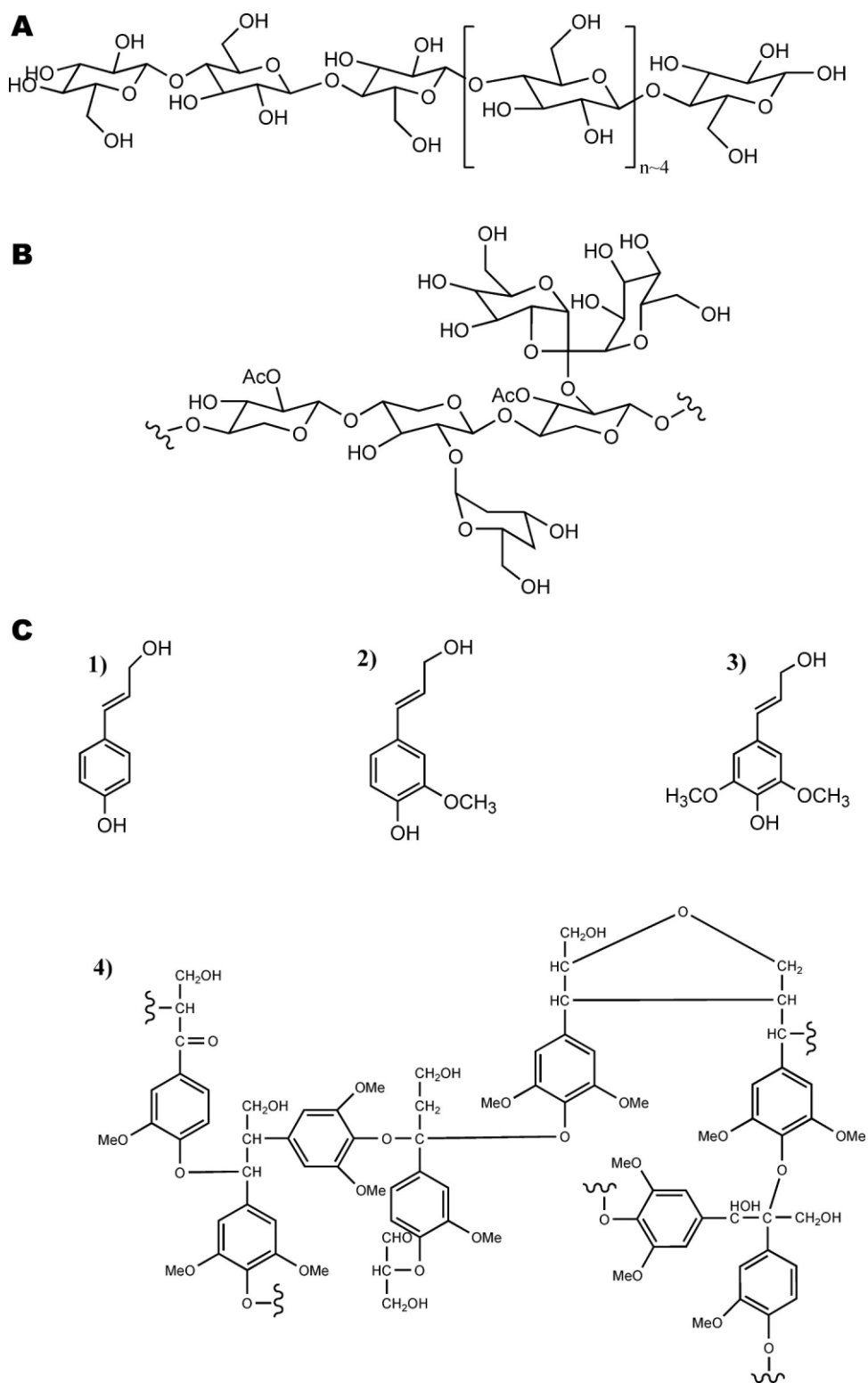
(RTILs) to the development of new technologies in chemical and biological transformations, separations, and more recently biomass pretreatment. RTILs consists of an organic cation and an organic or inorganic anion, the combination of which affords a vast number of synthetically accessible pairs, estimated to be as high as  $10^9$ . This tremendous compositional breadth allows solvent properties to be tailored to specific applications in electrochemical reactions (Dymek et al., 1984; Wilkes et al., 1982), absorption-spectroscopy (Appleby et al., 1986), and ion-exchange chromatography (Stuff 1991). RTILs have also been used in biocatalysis, particularly as non-aqueous alternatives to organic solvents (Moniruzzaman et al., 2010). More recently, RTILs have been used as solvents for lignocellulosic biomass processing with the aim of developing alternatives for lignocellulosic pretreatment (Brandt et al., 2010; Dadi et al., 2006; Kim et al., 2003; Lee et al., 2009; Nguyen et al., 2010; Pezoa et al., 2010; Singh et al., 2009; Zavrel et al., 2009; Zhao et al., 2009a).

Lignocellulosic biomass is comprised predominantly of three subcomponents: the semi-crystalline polysaccharide cellulose, the amorphous multicomponent polysaccharide hemicellulose, and the amorphous phenylpropanoid polymer lignin (Fig. 1). The conversion of lignocellulosic feedstocks, such as agricultural waste, wood, or energy crops, to liquid fuels such as ethanol and butanol, and more recently hydrocarbons, may provide a renewable means to meet future fuel demand that does not consume edible commodities, and concomitantly reduce the emission of greenhouse gases (Perlack et al., 2005). The recalcitrance

Correspondence to: J.S. Dordick

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**Figure 1.** Chemical structures of: (A) cellulose; (B) hemicellulose; and (C) lignin. Lignin monomers are: (C1) *p*-hydroxyphenyl alcohol; (C2) coniferyl alcohol; and (C3) synapyl alcohol.

of lignocellulose presents challenges for chemical and biological conversion into usable energy. The pretreatment stage is, therefore, critical to the overall process because it must increase the accessibility and reactivity of polysaccharides by deconstructing the three-dimensional structure of lignocellulose, and breaking down the semi-crystalline cellulose and hemicellulose without significant degradation of polysaccharide.

The most common pretreatment methods have historically used dilute acid hydrolysis and the overall biochemical process for biofuel production includes three main stages: (1) feedstock pretreatment; (2) polysaccharide hydrolysis to monomeric sugars; and (3) fermentation of released sugars to a combustible liquid fuel. Unfortunately, acid hydrolysis (even in dilute form) results in a significant amount of polysaccharide decomposition and in the formation of microbial inhibitors (e.g., furfural and hydroxymethylfurfural formed from the acid-catalyzed condensation of xylose and glucose, respectively), which negatively impact downstream fermentation. As a result, a number of alternatives to dilute acid hydrolysis have been developed, which are categorized as physical, chemical, physicochemical, and biological. A detailed assessment

of lignocellulosic pretreatment strategies is beyond the scope of this review. However, the reader is directed to a recent review by Dale and coworkers (da Costa Sousa et al., 2009). Table I summarizes some of these approaches along with their key advantages and disadvantages. While all of the listed pretreatments result in enhanced enzymatic saccharification of the residual polysaccharide, an effective process that is inexpensive with low capital costs and minimal impact on downstream processing has yet to be developed.

The very high solvating properties of RTILs have been exploited in the dissolution of cellulose (Kosan et al., 2008; Swatloski et al., 2002), lignin (Lateef et al., 2009), and even whole wood (Fort et al., 2007; Honglu and Tiejun, 2006; Kilpelainen et al., 2007), and such solvating power has led to a number of studies where RTILs have been considered for biomass pretreatment. Herein, we provide an overview of recent scientific and technological advances on the use of RTILs in lignocellulosic biomass pretreatment, including their critical unique solvating power. We highlight current unsolved problems and provide a perspective on where innovative new opportunities may be applied to solve challenges in this area.

**Table I.** Advantages and disadvantages among common pretreatment processes.

Pretreatment	Category	Advantages	Disadvantages
Mechanical disruption (milling) (Datta et al., 2010; Li et al., 2010b; Sousa et al., 2009)	Physical	Cost effective for herbaceous and agricultural residues	Energy requirement is high for hardwoods
Steam explosion (Clark and Mackie, 1987; Kaar et al., 1998; Schultz et al., 1983)	Physicochemical	Cost effective for hardwood feedstocks	Hemicellulose fraction is partially degraded. Inhibitory sugar degradation products can be formed due the release of acetic acid
Ammonia fiber expansion (Chundawat et al., 2010; Holtzapple et al., 1991; Jin et al., 2010; Krishnan et al., 2010; Lau et al., 2010; Murnen et al., 2007; Teymouri et al., 2004, 2005)		Cellulose crystallinity may be reduced depending on pretreatment conditions. Short residence time (up to 15 min). Lignin is delocalized from the cell wall structure. High digestibility (>90%) for herbaceous and agricultural residues. Hydrolyzates are highly fermentable (low inhibition)	Requires ammonia recycling system. Low digestibility reported for hard woods
Acid hydrolysis (Foust et al., 2009; Herrera et al., 2003; Li et al., 2008; Lloyd and Wyman, 2005; Shuai et al., 2010; Sun and Cheng, 2005; Torget et al., 1991; Um et al., 2003; Xiang et al., 2003)	Chemical	Residence times are short, lignin and hemicellulose are removed	Reaction vessel corrosion. Formation of inhibitory sugar degradation products
Alkaline hydrolysis (Azzam, 1989; Gould, 1985; Kim and Holtzapple, 2005; Zhang et al., 2010)		Cellulose crystallinity is reduced, Decrease in the polymerization degree of carbohydrates, lignin is removed	Digestibility enhancement is low in softwoods
High temperature organic solvent pretreatment (Ni and VanHeiningen, 1996; Williamson, 1987; Zhao et al., 2009b; Zhu et al., 2009)		Lignin is removed. Organic solvents can be recovered by distillation	Unremoved solvents may inhibit biologic processing. Organic solvents recovery requires energy consumption. Efficient control because of inherent fire and explosion hazard
Degradation by white-rot and soft-rot fungi (Hatakka and Uusi-Rauva, 1983; Keller et al., 2003; Lee, 1997; Muller and Trosch, 1986; Sousa et al., 2009; Zhu et al., 2006)	Biological	Organisms will degrade lignin. Energy requirement is low. Reaction conditions are mild	Long reaction times (2–6 weeks)

## Solvation Properties of RTILs

By definition, an RTIL is a salt that melts below 100°C and exhibits high thermal stability, high electrical conductivity, wide electrochemical window (the range within which cations and anions are inert toward electrochemical oxidation and reduction), and negligible vapor pressure. The tunable properties of RTILs (e.g., viscosity, melting point, polarity, and hydrogen bond basicity) depend on the selection of the anion and cation. Additionally, RTIL toxicity and corrosivity need to be considered for potential applications in lignocellulosic biomass pretreatment (Mäki-Arvela et al., 2010).

Empirical relationships have been developed that provide correlations between the solvation properties of solvents and specific measurable parameters that allow quantitative analysis of RTILs in terms of their critical molecular solvation properties. The most widely applied approach uses the linear solvation energy relationships (LSER) developed by Acree and Abraham (2006), Kamlet-Taft (Crowhurst et al., 2006; Fukaya et al., 2006), and Hildebrand (Marciniak, 2010; Swiderski et al., 2004), and further refined by Hansen to include more complex interactions between solute and solvent (Hansen, 2007). These LSERs provide correlations between a given solubility property, such as solvatochromic shifts or partition coefficients, with several additive terms that represent specific solubility interactions.

The Abraham solvation model (Eq. 1) describes a three-step solvation process: a cavity of suitable size is created in the solvent, the solvent molecules reorganize around the cavity, and the solute is introduced into the cavity and solute-solvent interactions are allowed to take place (Anderson et al., 2002). Each solute has a different solvent-solute interaction influenced by various acidic, basic, electron-donating, electron-withdrawing, and aromatic functional groups (Abraham et al., 1991; Revelli et al., 2010).

$$\log K_L = c + rR_2 + s\pi_2^H + a\alpha_2^H + b\beta_2^H + l\log L \quad (1)$$

$R_2$  is the excess of molar refraction calculated from the solute refractive index,  $\pi^H$  is the solute dipolarity/polarizability,  $\alpha^H$  and  $\beta^H$  denote the solute hydrogen bond acidity and basicity, respectively, and  $L$  is the solute-gas partition coefficient at 25°C. The parameters for RTILs described in Equation (1) are:  $\log K_L$  is the solubility coefficient,  $c$  is a constant,  $s$  denotes dipolarity/polarizability,  $a$  hydrogen bond basicity,  $b$  hydrogen bond acidity dispersion forces, and  $l$  describes dispersion forces.

The Kamlet-Taft parameters ( $\alpha$ ,  $\beta$ , and  $\pi^*$ ) are temperature-dependent solvent polarity scales (Lee and Prausnitz, 2010; Trivedi et al., 2010) and similar as described for Abraham equation. For RTILs, Kamlet-Taft parameters can be easily measured by uv/vis spectroscopy in the presence of solvatochromic dyes; the most common being Reichardt's dye (RD), *N,N*-diethyl-4-nitroaniline (DENA), and 4-nitroaniline (4NA). The Kamlet-Taft  $\pi^*$  parameter (Eq. 2) provides a measure of the solvent dipolarity/

polarizability ratio, the  $\alpha$  parameter (Eq. 3) provides a measure of solvent's ability to act as a hydrogen bond donor, and the  $\beta$  parameter (Eq. 4) provides a measure of the solvent's ability to serve as a hydrogen bond acceptor. The Kamlet-Taft  $\pi^*$  is determined using the wavelength corresponding to the absorbance maximum of DENA ( $\nu_{\max}^{\text{DENA}}$ ) in kiloheser.

$$\pi^* = \frac{\nu_{\max}^{\text{DENA}} - 27.52}{-3.183} \quad (2)$$

The Kamlet-Taft  $\alpha$  is determined using the  $\pi^*$  value and the wavelength corresponding to maximum absorbance of RD ( $\lambda_{\text{RD}}$ ) in nm.

$$\alpha = \frac{[E_T(30) - 14.6(\pi^* - 0.23) - 30.31]}{16.5} \quad (3)$$

where  $E_T(30) = 28592/\lambda_{\max}^{\text{RD}}$ . The Kamlet-Taft  $\beta$  parameter is determined using ( $\nu_{\max}^{\text{DENA}}$ ) and the wavelength corresponding the maximum absorbance of 4NA ( $\nu_{\max}^{\text{4NA}}$ ) in kiloheser.

$$\beta = \frac{[1.035\nu_{\max}^{\text{DENA}} - \nu_{\max}^{\text{4NA}} + 2.64]}{2.8} \quad (4)$$

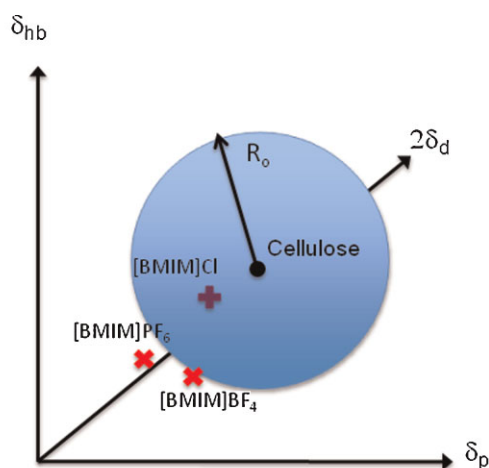
The Hildebrand solubility parameter ( $\delta_H$ ) is defined as the square root of the cohesive energy density (CED) and it provides a measure of the degree of interaction among chemical species:

$$\delta_H = (\text{CED})^{1/2} = \left(\frac{\Delta U}{V}\right)^{1/2} = \left[\frac{(\Delta H^{\text{vap}} - RT)}{V}\right]^{1/2} \quad (5)$$

where  $V$  is the molar volume,  $\Delta U$  is the internal energy,  $\Delta H^{\text{vap}}$  is the enthalpy of vaporization,  $R$  is the gas constant, and  $T$  is the absolute temperature.  $\delta_H$  has been widely used as an indicator of solubility, wherein species with similar  $\delta_H$  values are likely to be miscible. This single parameter approach works particularly well for non-polar compounds. For more polar solvents like RTILs, however, the solubility behavior can be more accurately predicted by using multicomponent solubility parameters, such as those proposed by Hansen (2007) which discriminate between different types of interactions that contribute to intermolecular attraction (e.g.,  $\delta_d$  = dispersive component,  $\delta_{\text{hb}}$  = hydrogen bonding, and  $\delta_p$  = polar interactions):

$$\delta_H = [\delta_d^2 + \delta_p^2 + \delta_{\text{hb}}^2]^{1/2} \quad (6)$$

The parameters can help us to create a three-dimensional model in which a spherical volume of solubility can be generated for each compound (Fig. 2); the coordinates at the center of the sphere are ( $\delta_d$ ,  $\delta_p$ ,  $\delta_{\text{hb}}$ ), and a fourth parameter,  $R_o$ , determines the maximum difference in affinity tolerable for solubility to take place. The difference in energy between



Chemical	$d_d$ (MPa) <sup>1/2</sup>	$d_p$ (MPa) <sup>1/2</sup>	$d_{hb}$ (MPa) <sup>1/2</sup>	$R_a$ (MPa) <sup>1/2</sup>
Microcrystalline cellulose	19.4	26.2	23.2	-
[BMIM]Cl	19.1	20.7	20.7	6.0
[BMIM]PF <sub>6</sub>	21	17.2	10.9	15.6
[BMIM]BF <sub>4</sub>	23	19	10	16.7

**Figure 2.** Schematic representation of the HSP sphere for microcrystalline cellulose. [BMIM]Cl is expected to lie within the sphere of solubility based on its HSP values. The non-solvents [BMIM]PF<sub>6</sub> and [BMIM]BF<sub>4</sub> have much lower  $d_{hb}$  and  $d_p$  values than cellulose and should thus be located outside the solubility sphere. The lower panel shows the HSP for microcrystalline cellulose (Archer, 1991) and three [BMIM]-based ionic liquids (Hansen, 2009).

components 1 and 2 is calculated as follows:

$$R_a^2 = 4(\delta_{d1} - \delta_{d2})^2 + (\delta_{p1} - \delta_{p2})^2 + (\delta_{hb1} - \delta_{hb2})^2 \quad (7)$$

For example, microcrystalline cellulose has Hansen solubility parameter (HSP) values that are comparable to that of 1-butyl-3-methylimidazolium [BMIM]Cl (Fig. 2), yet quite distinct from those of [BMIM]PF<sub>6</sub> and [BMIM]BF<sub>4</sub>. This is qualitatively consistent with the observed high solubility of pulp cellulose in [BMIM]Cl, and its insolubility in the latter two ionic liquids (Swatloski et al., 2002). The use of HSPs, upon generation of a larger dataset, may be a practical approach to predict synergistic mixtures of ionic liquids that are capable of dissolving lignocellulosic biomass and some of its components. It may also be necessary to consider the effect of ionic interactions in the predictive power of HSP for ionic liquids (Tsiptsias et al., 2010).

A major concern with RTILs as solvents is their high viscosities, which can represent a barrier to mass and phase transfer. For example, alkylimidazolium chlorides typically have viscosities >2,000 cp at 25°C (Fukaya et al., 2006). However, far lower viscosities are observed for RTILs possessing phosphonate, carboxylate, and formate anions

(as low as 66 cp at 25°C). Moreover, an allyl group in the alkyl chain of the cation (e.g., [AMIM]Cl) lowers the solvent melting point and viscosity (Fukaya et al., 2006; Mizumo et al., 2004). Some of the most common RTILs used as solvents for lignocellulose biomass or its subcomponents are summarized in Table II and extensively elsewhere (Mäki-Arvela et al., 2010).

## RTILs as Pretreatment Candidates for Lignocellulosic Biomass

### Interaction of RTILs with Lignocellulosic Subcomponents

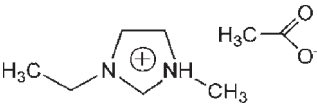
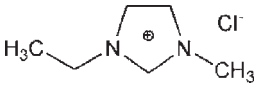
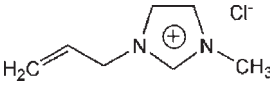
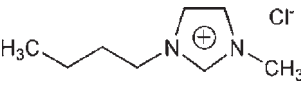
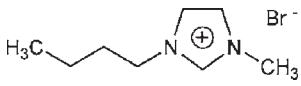
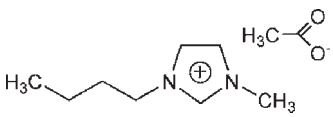
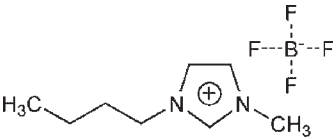
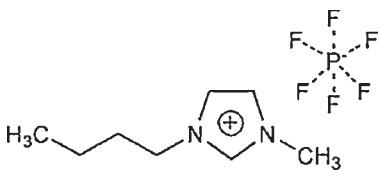
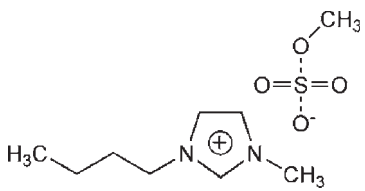
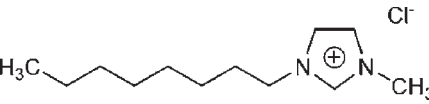
The extremely diverse range of RTILs that can be synthesized has fostered various screening approaches to identify ionic liquids with the ability to solubilize the polysaccharide or lignin components of lignocellulose. In a pioneering study involving various cellulose sources, including pulps, fibrous cellulose, and filter papers, Rogers and co-workers examined a range of 1-alkyl-3-methylimidazolium cations and inorganic anions for their abilities to dissolve cellulose (Swatloski et al., 2002). The [BMIM]Cl was found to be the most effective RTIL capable of dissolving up to 25% (w/w) cellulose; replacing the Cl<sup>-</sup> with Br<sup>-</sup> or SCN<sup>-</sup> resulted in substantially lower cellulose dissolution while BF<sub>4</sub><sup>-</sup> and PF<sub>6</sub><sup>-</sup> were unable to dissolve cellulose. A larger 1-octyl chain reduced the effectiveness of the RTIL. Similar results were observed for Cl<sup>-</sup>-containing RTILs (Mäki-Arvela et al., 2010) for dissolution of cotton linters and Kraft cellulose aided by high-power ultrasound to enhance dissolution.

High-throughput systems have been developed to identify effective RTILs that solubilize lignocellulose components, based on transmitted light and light scattering measurements. Zavrel et al. (2009) screened more than 20 RTILs to identify optimal solvents for microcrystalline cellulose. Despite the fact that chloride-based ionic liquids were known to be effective solvents for cellulose, [EMIM]OAc was found to be a good solvent for cellulose for up to 4% (w/w) at 80°C (Fig. 3). Comparisons among other ionic liquids showed high rates of dissolution of cellulose at 50 and 80°C. The ability of [EMIM]OAc, as well as Cl<sup>-</sup>-containing ionic liquids, to dissolve cellulose indicate their capacity to interact with cellulose through hydrogen bonds and disrupting the crystalline structure of the polysaccharide.

Many efforts have focused on the design and synthesis of new ionic liquids with particular properties such as reduced viscosity (Fukaya et al., 2006; Gericke et al., 2009) and higher hydrogen bond accepting capacity (Fukaya et al., 2006). For example, a series of 1,3-dialkylimidazolium formates were produced having significantly lower viscosity than chloride-based RTILs. Their ability to dissolve cellulose and other polysaccharides under mild conditions was greater than Cl<sup>-</sup>-based RTILs due to their strong hydrogen bond accepting capacity (determined by Kamlet-Taft parameters) (Fukaya et al., 2006). Interaction of RTILs and cellulose



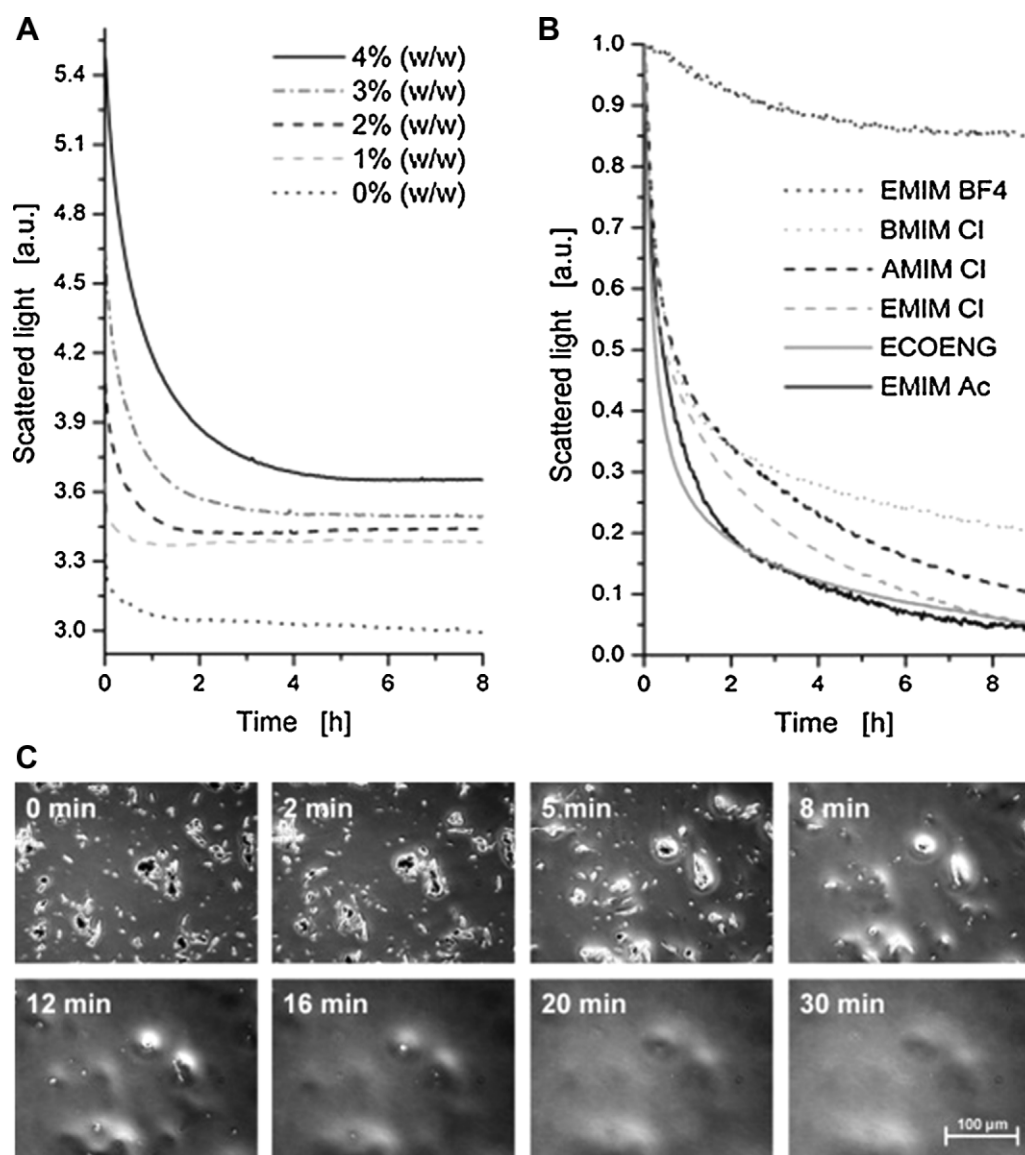
**Table II.** RTILs used for the dissolution of lignocellulosic biomass subcomponents and biomass pretreatment.

RTIL	Structure	Uses
1-Ethyl-3-methylimidazolium acetate [EMIM]OAc		Dissolution of cellulose (Kosan et al., 2008; Vitz et al., 2009; Zavrel et al., 2009) Extraction of lignin from maple wood flour (Lee et al., 2009) Dissolution of a variety of carbohydrates such as sugars, starch, and cellulose (Zhao et al., 2008)
1-Ethyl-3-methylimidazolium chloride [EMIM]Cl		Dissolution of cellulose (Kosan et al., 2008; Vitz et al., 2009; Zavrel et al., 2009)
1-Allyl-3-methylimidazolium chloride [AMIM]Cl		Dissolution of cellulose (Fukaya et al., 2008; Zavrel et al., 2009; Zhang et al., 2005) Dissolution of hard wood and softwoods (Kilpelainen et al., 2007) Extraction of lignin from maple wood flour (Lee et al., 2009)
1-Butyl-3-methylimidazolium chloride [BMIM]Cl		Dissolution of cellulose (Erdmenger et al., 2007; Heinze et al., 2005; Kosan et al., 2008; Swatloski et al., 2002; Vitz et al., 2009; Zavrel et al., 2009) Pretreatment of cellulose for enhancing enzymatic hydrolysis (Dadi et al., 2006) Dissolution of hard wood and softwoods (Fort et al., 2007; Kilpelainen et al., 2007) Extraction of lignin from maple wood flour (Lee et al., 2009)
1-Butyl-3-methylimidazolium bromide [BMIM]Br		Dissolution of cellulose (Swatloski et al., 2002; Vitz et al., 2009; Zavrel et al., 2009) Dissolution of lignin (Pu et al., 2007)
1-Butyl-3-methylimidazolium acetate [BMIM]OAc		Extraction of lignin from maple wood flour (Doherty et al., 2010)
1-Butyl-3-methylimidazolium tetrafluoroborate [BMIM]BF <sub>4</sub>		Dissolution of cellulose (Swatloski et al., 2002; Zavrel et al., 2009) Extraction of lignin from maple wood flour (Lee et al., 2009)
1-Butyl-3-methylimidazolium hexafluorophosphate [BMIM]PF <sub>6</sub>		Dissolution of cellulose (Swatloski et al., 2002; Zavrel et al., 2009) Extraction of lignin from maple wood flour (Lee et al., 2009)
1-Butyl-3-methylimidazolium methylsulfate [BMIM]MeSO <sub>4</sub>		Extraction of lignin from maple wood flour (Doherty et al., 2010) Dissolution of lignin (Pu et al., 2007)
1-Octyl-3-methylimidazolium chloride [OMIM]Cl		Dissolution of cellulose (Swatloski et al., 2002; Vitz et al., 2009; Zavrel et al., 2009)

cause swelling of the cellulose fibers; transmission optical microscopy images show the division of cellulose fibers in segments followed by fragmentation of fibers (Cuissinat et al., 2008a,b). Molecular dynamic simulations confirm the

role of the anion on cellulose dissolution, while cations only weakly interact with cellulose (Youngs et al., 2006).

Lignin is a biopolymer derived from *p*-hydroxycinnamyl, coniferyl, and sinapyl alcohols (Fig. 1C) present in all woody



**Figure 3.** **A:** Dissolution profiles of different weight percentages of microcrystalline cellulose in [EMIM]OAc at 60°C; **B:** Comparison of ionic liquids ability to dissolve 4% w/w microcrystalline cellulose at 80°C. **C:** In situ microscopy pictures of [EMIM]OAc with 1% w/w microcrystalline cellulose (Zavrel et al., 2009—reproduced by permission of Bioresource Technology; doi:10.1016/j.biortech.2008.11.052).

plants and representing the second most abundant renewable resource (Pu et al., 2007). A number of ionic liquids have been tested for dissolution of lignin (Pu et al., 2007) which include 1,3-dimethylimidazolium methylsulfate ([MMIM]MeSO<sub>4</sub>), 1-hexyl-3-methylimidazolium trifluoromethanesulfonate ([HMIM]CF<sub>3</sub>SO<sub>3</sub>), 1-butyl-2,3-dimethylimidazolium tetrafluoroborate ([BM<sub>2</sub>IM]BF<sub>4</sub>), 1-butylmethylpyridinium hexafluorophosphate ([BMPy]PF<sub>6</sub>), and 1-butyl-3-methylimidazolium cations ([BMIM]<sup>+</sup>) with a range of anions (MeSO<sub>4</sub><sup>-</sup>, Cl<sup>-</sup>, Br<sup>-</sup>, and PF<sub>6</sub><sup>-</sup>). As with dissolution of cellulose, anions play a critical role in dissolving lignin; non-coordinating anions (BF<sub>4</sub><sup>-</sup> and PF<sub>6</sub><sup>-</sup>) exhibited no or very limited ability to dissolve residual softwood Kraft lignin. MeSO<sub>4</sub><sup>-</sup>-containing ionic liquids

could significantly dissolve lignin at 50°C and partially at room temperature. For [BMIM]-based ionic liquids, the order of lignin solubility was found to be MeSO<sub>4</sub><sup>-</sup> > Cl<sup>-</sup> Br<sup>-</sup> >> PF<sub>6</sub><sup>-</sup>. Based on the solubility of lignin in RTILs, acid-catalyzed depolymerization reactions have been attempted in RTILs. However, the complete depolymerization of lignin without self-condensation still represents a challenge (Binder and Raines, 2009; Li et al., 2009).

### Interaction of RTILs with Whole Wood

Imidazolium-based ionic liquids have been used extensively for dissolution of hardwoods and softwoods (Fort et al., 2007; Kilpelainen et al., 2007). For example, [BMIM]Cl has



**Table III.** Dissolution behavior of wood-based lignocellulose in different RTILs.

RTIL	Temperature (°C)	Spruce	Silver fir	Common beech	Chestnut	Norway spruce sawdust	Norway spruce TMP	Southern pine TMP
[AMIM]Cl	90	+	+	+	+			
[AMIM]Cl	110					+		
[AMIM]Cl	80					±		
[AMIM]Cl	130						+	±
[EMIM]Cl	90	±	±	±	±			
[BMIM]Cl	90	±	±	±	±			
[BMIM]Cl	110					+		
[BMIM]Cl	130						+	±
[BZMIM]Cl	130							±
[BZMIM]Cl	130						±	
[BZ-OME-MIM]Cl	130							
[Benzylmim]Cl	130							±
ECOENG	90	±	±	±	±			
[EMIM]OAc	90	+	±	+	+			

Data from Kilpelainen et al. (2007) and Zavrel et al. (2009).  
 +, complete dissolution; ±, partial dissolution.

been used to partially dissolve untreated pine, poplar, eucalyptus, and oak. The cellulose component can be reconstituted by precipitation and its purity and physical properties were shown to be comparable to that of pure cellulose subjected to similar treatment. A broader screening of imidazolium-based ionic liquids ([BMIM]Cl, [AMIM]Cl, [BzMIM]Cl, [Bz-OMe-MIM]Cl, and 1-benzyl-3-methylimidazolium dicyanoamide [BzMIM]DCA) was performed for dissolution of different wood samples (Kilpelainen et al., 2007; Table III). Not surprisingly, the kinetics of wood dissolution strongly depended on the biomass particle size (wood powder > sawdust > TMP fibers ≫ wood chips), which is consistent with the presence of the complex and compact structure of wood cell walls, and its constituent lignin, cellulose, and hemicellulose that inhibit the internal diffusion of the RTIL. Zavrel et al. (2009) tested the dissolution of three different types of wood chips (spruce, silver fir, common beech, and chestnut) in six different ionic liquids (Table III). [AMIM]Cl completely dissolved each of the aforementioned wood chips, while [EMIM]OAc was able to dissolve three types of wood and just partially the silver fir. For [EMIM]Cl, [BMIM]Cl, and 1,3-dimethylimidazolium-dimethylphosphate (ECOENG) partial dissolution was observed.

The interaction of RTILs with lignocellulose involves ionic,  $\pi$ - $\pi$ , and hydrogen bonding interactions. Competition for hydrogen bonding allows RTILs to disrupt the three-dimensional network of lignocellulose (Moulthrop et al., 2005) and can be attributed to the nature of the anions (Dadi et al., 2006; Kilpelainen et al., 2007; Liu et al., 2005; Remsing et al., 2006; Swatloski et al., 2002) although cations may also interact with the hydroxyl groups of cellulose (Dadi et al., 2006; Zhang et al., 2005).  $\pi$ - $\pi$  interactions between cations and the aromatic rings of lignin may also play a role in the dissolution of lignin (Kilpelainen et al., 2007). For example, [AMIM]Cl contains  $\pi$  electrons within its imidazolium ring as well as its side chain, and thus

its cation is able to interact with the polyphenolic structure of lignin and simultaneously the anion forms hydrogen bonds disrupting the crystalline structure of cellulose and the interactions between lignin and polysaccharides; the latter may explain why [AMIM]Cl was able to dissolve essentially any type of wood tested. Biomass solubilization during RTIL pretreatment of switch grass with [EMIM]OAc by auto-fluorescent mapping of plant cell walls revealed the swelling of the plant cell wall, which is attributed to the disruption of inter- and intramolecular hydrogen bonding between cellulose and lignin, leading to a complete dissolution of biomass (Singh et al., 2009). The effectiveness of RTILs that interact with wood can also be explained through the Kamlet-Taft parameters (Doherty et al., 2010). In particular the Kamlet-Taft  $\beta$  parameter appears to predict the ability of an RTIL to disrupt the hydrogen bond network between lignin and polysaccharides as well as the crystalline structure of cellulose I (Kilpelainen et al., 2007; Lee et al., 2009).

### Extraction with RTILs

An alternative to completely dissolving biomass is to selectively dissolve just a single biomass component. For example, it has been observed that acetate-based ionic liquids selectively extract lignin from recalcitrant maple wood flour (Lee et al., 2009). Table IV summarizes data from the pretreatment of maple wood flour in [EMIM]OAc; over a range of temperatures from 50 to 130°C, extraction of total lignin increased from 19% to 63%, while extracted xylan and cellulose remained nearly constant over the entire temperature range. Similar results were observed at 90°C for up to 70 h. Interestingly, even when cellulose was not dissolved, its crystalline structure decreased considerably. Specifically, crystallinity index, calculated from X-ray diffraction spectra, of maple wood flour showed a decrease from 63 to ~30.

**Table IV.** Effect of incubation time and temperature on composition, crystallinity index (CrI), and enzymatic hydrolysis of maple wood flour.

Time/temperature (h)/(°C)	Extracted lignin (%)	Cellulose CrI	Cellulose (mg)	Xylan (mg)	Lignin (mg)
Untreated	0	63	10.3	5.7	3.5
0.5/90	16	56	9.1	5.1	2.9
1.5/90	28	47	9.0	5.0	2.5
5.0/90	42	43	8.8	4.9	2.0
8.0/90	44	40	8.8	5.0	1.9
14/90	51	42	8.8	5.0	1.7
19/90	54	46	8.9	5.0	1.6
32/90	70	41	9.2	5.1	1.0
42/90	77	43	8.9	4.7	0.8
70/90	86	38	8.6	4.4	0.5
1.5/50	19	57	8.7	5.1	2.8
1.5/70	21	52	9.0	5.0	2.7
1.5/90	28	47	9.0	5.0	2.5
1.5/110	44	38	8.7	4.8	1.9
1.5/130	63	30	8.7	4.2	1.3

The composition values for cellulose, xylan, and lignin were taken after RTIL pretreatment (Lee et al., 2009).

The reduction in crystallinity of wood pretreated with [EMIM]OAc may be due to the hydrogen bond basicity of the acetate anion as reflected in the Kamlet-Taft  $\beta$  parameter (Lungwitz et al., 2010). Analogous results were observed with [BMIM]OAc during the selective extraction of lignin from maple wood flour (Doherty et al., 2010). Determinations of Kamlet-Taft  $\beta$  parameters of [EMIM]OAc and [BMIM]OAc (1.06 and 1.18, respectively) indicate that an RTIL's ability to serve as an H-bond acceptor (high  $\beta$ -value) is important for selective lignin extraction, with the anion having the greater impact on the  $\beta$  parameter (Lee and Prausnitz, 2010). The strong anion composition dependence of the  $\beta$  parameter also coincides with reduced cellulose crystallinity in the lignocellulose that remains undissolved in the RTIL (Doherty et al., 2010). The combination of high extracted lignin with reduced cellulose crystallinity as the  $\beta$ -value increases (Fig. 4) results in a highly degradable polysaccharide fraction of the residual lignocellulose.

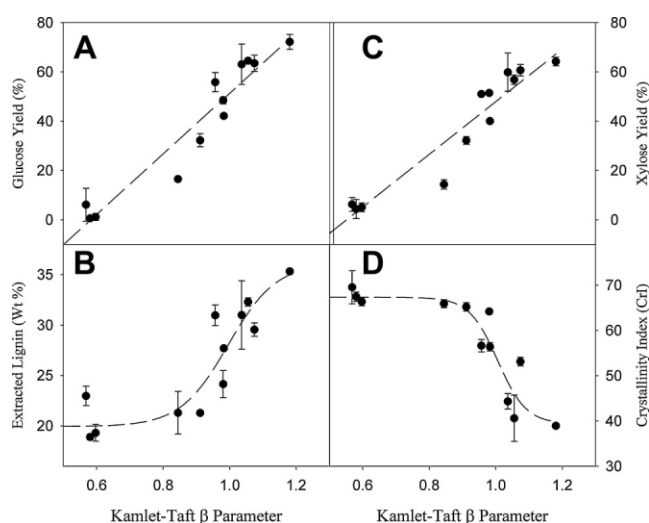
## Impact of RTIL Pretreatment on Enzymatic Conversion of Lignocellulose into Fermentable Sugars

### Reconstituted Lignocellulose

A majority of RTIL-based pretreatment strategies involve the complete dissolution of lignocellulose followed by selective precipitation of cellulose and hemicellulose with water prior to enzymatic hydrolysis. Based on the ability of chloride-based ionic liquids to dissolve wood and its subcomponents, [AMIM]Cl was used to pretreat eucalyptus, southern pine, and Norway spruce thermochemical pulp (Li et al., 2010a). After regeneration with water and subsequent enzymatic hydrolysis (40 FPU/g wood, 40°C, 48 h) the release of fermentable sugars was 1.5–2.5 greater than untreated wood, showing that the disruption of the

hydrogen bonding network of wood led to increased accessibility of cellulases for the reconstituted wood and increased yield of fermentable sugars (cellulose and xylose).

Similar results were obtained by Li et al. (2009) who used reconstituted wheat straw following dissolution in [EMIM]OAc, [BMIM]Cl, and several phosphoric RTILs including 1-ethyl-3-methyl imidazolium diethyl phosphate ([EMIM]DEP), 1-ethyl-3-methyl butylpyridinium diethyl phosphate ([EMBy]DEP), and 1-ethyl-3-methyl imidazolium dibutyl phosphate ([EMIM]DBP). Phosphate-containing RTILs are less viscous, more thermally stable, less corrosive, less toxic, and less hygroscopic than chloride-containing ionic liquids. After 1 h pretreatment at 100°C



**Figure 4.** Correlation of Kamlet-Taft  $\beta$  parameter extrapolated to 90°C with: (A) glucose yield; (B) extracted lignin; (C) xylose yield; and (D) CrI for a series of RTILs ([EMIM]OAc, [BMIM]OAc, and [BMIM]MeSO<sub>4</sub>) at different water concentrations (0%, 5%, and 10% w/w; Doherty et al., 2010—reproduced by permission of The Royal Society of Chemistry; <http://dx.doi.org/10.1039/C0CG00206B>).

and complete dissolution of wheat straw, [EMIM]DEP gave the highest yields of reducing sugars (~50%) following enzymatic hydrolysis, emerging as an attractive alternative for larger-scale applications as a result of the use of phosphate-containing ionic liquids. The increase of fermentable sugars after RTIL pretreatment is not just due to the reduced crystallinity of the reconstituted cellulose, but also increased accessibility of the hemicellulose, which is entrapped within the cellulosic matrix (Zhao et al., 2009a). Comparison between conventional acid pretreatment and ionic liquid pretreatment (with [EMIM]OAc) on switchgrass showed high conversions of hydrolyzed cellulose (24 h enzymatic reaction yields of 96% for RTIL pretreatment versus 47% for dilute acid pretreatment) and higher amounts of reducing sugars (5 mg/mL vs. 4 mg/mL for RTIL and dilute acid, respectively), as well as considerably shorter processing time (~15 h for RTIL vs. >72 h for dilute acid pretreatment; Li et al., 2010b).

### Residual Lignocellulose Following Selective Lignin Extraction

As described above, extraction of lignin occurs simultaneous with the reduction in residual cellulose crystallinity. Lee et al. (2009) demonstrated that this residual cellulose was highly susceptible to cellulase- and xylanase-catalyzed hydrolysis to fermentable sugars. From a limited set of ionic liquids tested, [EMIM]OAc was found to be highly selective in extracting lignin from maple wood flour and in the process reduce the crystallinity of cellulose and increased the accessibility of both the residual cellulose and hemicellulose fractions to enzymatic hydrolysis (Fig. 5A and B). Nearly complete hydrolysis of both the cellulose and hemicellulose fractions was achieved in <24 h using a commercial cellulase from *Trichoderma viridae*, which contained both cellulases and xylanases. Subsequent studies resulted in identification of [BMIM]OAc as a highly effective pretreatment solvent for shorter pretreatment times. After 12 h pretreatment at 90°C, ca. 75% of maple wood flour's cellulose was hydrolyzed to glucose (*Trichoderma viride* cellulase 34 U/mL, 37°C, 11 h) and 65% of the wood's xylan was hydrolyzed to xylose compared with 4.5% and 2.6% of the untreated wood's cellulose and xylan hydrolyzed, respectively (Doherty et al., 2010).

The effectiveness of RTILs in pretreating maple wood flour was studied mechanistically by Doherty et al. (2010). As described above, high Kamlet-Taft  $\beta$ -value correlate well with high-lignin extraction efficiency and reduction of cellulose crystallinity (Fig. 4B and D). Scanning electron microscopy images revealed clear swelling of maple wood flour after pretreatment with [BMIM]OAc and [EMIM]OAc (Fig. 5C). Similar images were observed after pretreatment of switchgrass with [EMIM]OAc and regeneration of lignocellulose (Singh et al., 2009). These results suggest that high  $\beta$ -value RTILs open up the lignocellulose structure, enabling greater access to lignin contained within

the fiber and resulting in greater lignin extraction (Scheme 1).

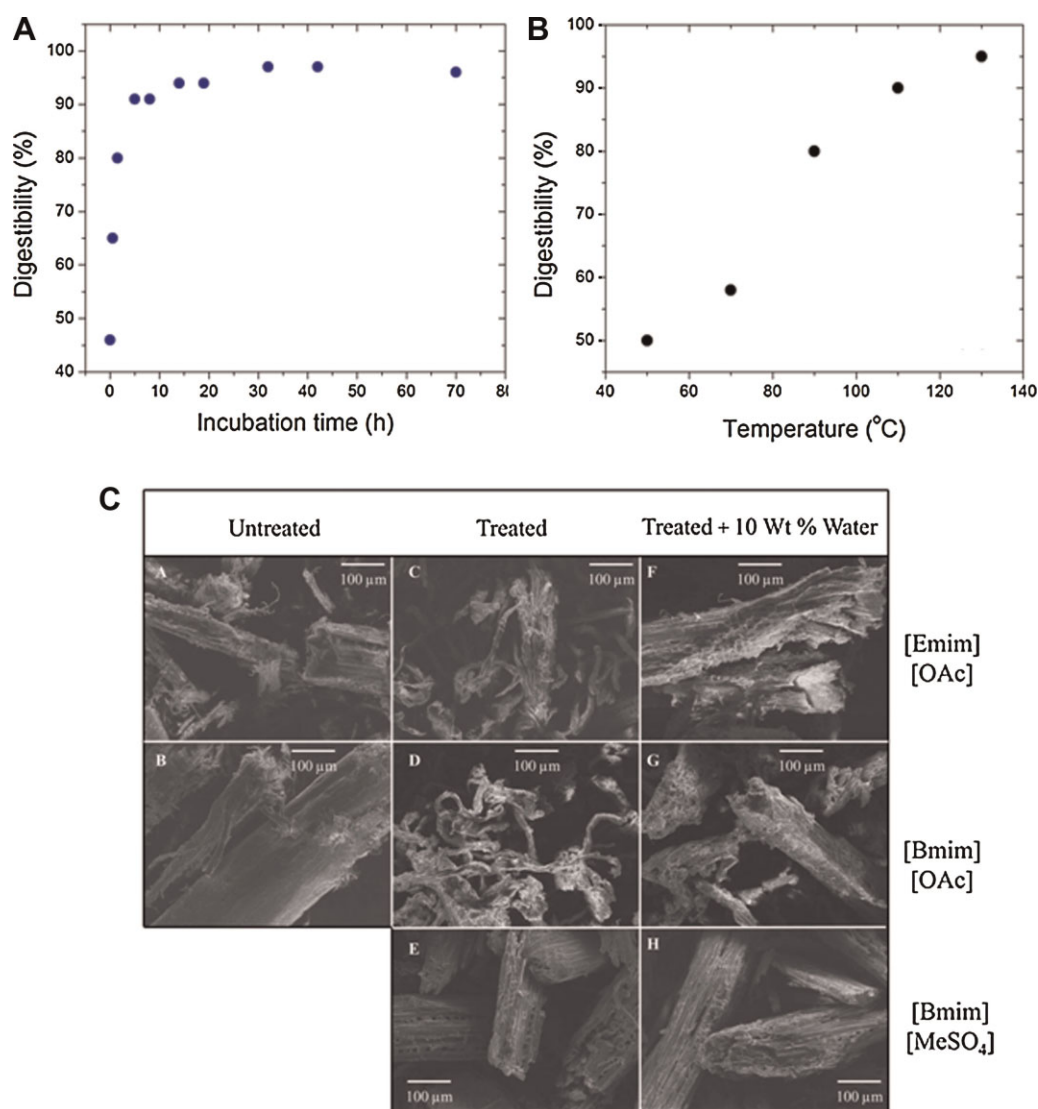
### Combined Pretreatment Strategies

Binder and Raines (2010) reported a high-yielding chemical process for the hydrolysis of lignocellulosic biomass into monosaccharides. First, they evaluated dilute acid hydrolysis of microcrystalline cellulose in [EMIM]Cl and observed that gradual addition of water prevented precipitation of polysaccharides during hydrolysis and also reduced the production of 5-hydroxymethylfurfural. The timing of water addition was critical for high glucose yields. The same water-dilution process used for microcrystalline cellulose was then used in a two-step hydrolysis of corn stover. During the first step, corn stover was mixed with [EMIM]Cl and hydrolyzed with 10% (w/w) HCl at 105°C. The first stage produced yields of 71% and 42% xylose and glucose, respectively. Addition of up to 70% (v/v) water precipitated unhydrolyzed polysaccharides and lignin, which were then dissolved in fresh [EMIM]Cl and subjected to an identical second stage. The combination of the two stages resulted in 70% and 79% yields of glucose and xylose, respectively, and subsequent ethanol fermentation was performed using an engineered *E. coli* KO11 strain, which produces ethanol selectively, and by yeast fermentation using *Pichia stipitis*. This novel chemical hydrolysis method provides flexibility for an integrated lignocellulosic biomass process because the RTIL makes polysaccharides readily accessible for chemical hydrolysis in combination with controlled addition of water that prevents precipitation and formation of microbial inhibitors. However, the effectiveness of this approach has yet to be extended to more recalcitrant feedstocks.

Aqueous ammonia pretreatment has been applied to remove lignin from lignocellulosic materials (Kim et al., 2003; Kim and Lee, 2007; Wyman et al., 2005) and it serves as an auxiliary step to improve the solubility of biomass and decrease the dissolving time in RTILs. For example, Nguyen et al. (2010) treated rice straw (RS) with ammonia (1 g of RS in 10 mL of 10% (v/v) ammonia solution, incubated at 100°C for 6 h) followed by incubation with a set of ionic liquids ([EMIM]Cl, [EMIM]OAc, and 1-ethyl-3-methylimidazolium hydrogen sulfate [EMIM]Su). Glucose conversions of 50% and 70% were obtained for individual ammonia and [EMIM]OAc pretreatments, respectively, using a mixture of cellulases. Importantly, after combined pretreatment with ammonia and RTIL, conversions reached ~80% using one-tenth the enzyme concentration. Moreover, using same amounts of enzyme, conversions were close to 100% with the combined pretreatment.

### Future Perspectives

The use of RTILs as pretreatment solvents for lignocellulosic biomass conversion into liquid fuels and other value added



**Figure 5.** A: Effect from incubation time on digestibility of pretreated maple wood flour with [EMIM]OAc at 90°C, and (B) effect of pretreatment temperature on digestibility of pretreated maple wood flour. Both graphs were made from data reported by Lee et al. (2009). C: SEM images of untreated maple wood flour (A and B), maple wood flour pretreated in [EMIM]OAc (C), [BMIM]OAc (D), and [BMIM]MeSO<sub>4</sub> (E) and with 10% (w/w) added water in [EMIM]OAc (F), [BMIM]OAc (G), and [BMIM]MeSO<sub>4</sub> (H) (Doherty et al., 2010—reproduced by permission of The Royal Society of Chemistry; <http://dx.doi.org/10.1039/C0GC00206B>).

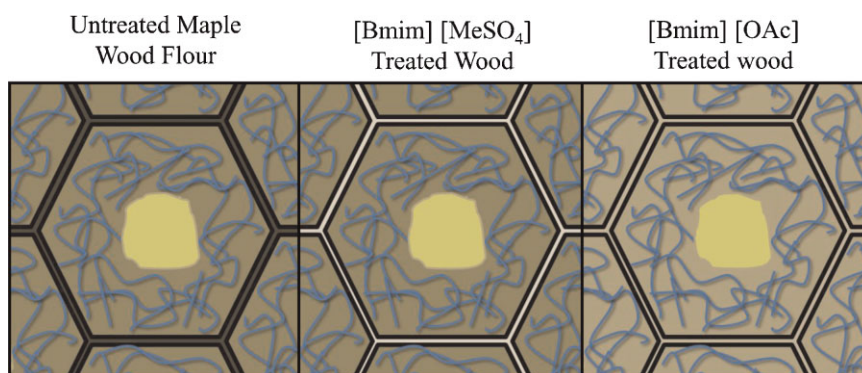
products is still in its infancy. Many hurdles remain to be addressed, not the least of which is the cost of RTILs, but also a lack of mechanistic information on the effect of the RTIL on the core constituents of lignocellulose.

### Recycling and Regeneration of RTILs

The high cost of RTILs necessitates their efficient recycling and regeneration, particularly for the extremely large volumes required for biomass pretreatment. Currently, RTILs are produced in kg scale and therefore are offered at high prices; however, BASF expects to see prices standard quality ionic liquids with growing demands in tons

quantities around \$40/kg. Lee et al. (2009) observed that [EMIM]OAc can be recycled at least four times without any deleterious effect on lignin extraction or generation of fermentable sugars following enzymatic hydrolysis of the residual lignocellulose. A side benefit of this extraction process is the potential to provide a highly pristine lignin. Recycling, however, cannot be performed indefinitely. Removal of lignin and other extractives is critical, and this requires a potentially complex set of steps including precipitation by antisolvents such as deionized water or alcohols. This would then be followed by filtration, adsorption of impurities by activated carbon, organic solvent washing, evaporation, and purification with neutral-activated alumina (Revelli et al., 2010). The large





**Scheme 1.** Lignin distribution in RTIL treated maple wood flour. Plant cell is depicted as a hexagon with the primary cell wall outlined in black and the secondary cell wall in brown. Cellulose fibrils are depicted in blue and the vacuole is shown in yellow. The middle lamella is depicted in brown between the cells. Untreated maple wood (left)—native lignin distribution in middle lamella, primary cell wall, and secondary cell wall. [BMIM] [MeSO<sub>4</sub>] treated wood (center)—delignification of middle lamella (light brown), native lignin distribution maintained in primary and secondary cell walls. [BMIM] [OAc] (right)—partial delignification of middle lamella (light brown) and secondary cell wall (light brown) (Doherty et al., 2010—the scheme is reproduced with permission by permission of the Royal Society of Chemistry; <http://dx.doi.org/10.1039/C0GC00206B>).

number of steps to achieve the regeneration of the RTIL is a critical problem that must be addressed.

Nevertheless, some options may exist. The use of supercritical (SC) fluids to extract RTIL-soluble polymers may be attractive. The solvent properties of SC fluids can be easily tuned by temperature and pressure, and isolation of the extracted compounds can be accomplished by bringing the solvent to subcritical conditions. Another option is the use of anion exchange resins for isolation of the ionic liquid as a salt (Bonhote et al., 1996), which could be accompanied by at least partial precipitation of the lignin. Moreover, if the newly formed ionic liquid is hydrophobic (e.g., [BMIM]PF<sub>6</sub>), one may envision the use of a temperature-controlled, micellization-transfer-demicellization “shuttle” between the ionic liquid and an aqueous phase using poly(ethylene oxide)-containing (PEO) block copolymers, as described by Lodge and coworkers (Bai et al., 2008). For lignin extraction, micelles formed from a block copolymer containing a lignin-compatible block and a PEO block could serve as nanocarriers for the lignin, which could be transferred from the ionic liquid to an aqueous phase upon contact due to the preferential affinity of the micelles for water in a certain temperature range. Since PEO exhibits a lower critical solution temperature in water, a subsequent decrease in the temperature of the aqueous phase would improve the solvent quality of water for PEO and should thus cause demicellization, and precipitation of the lignin.

Another promising recycling strategy has been developed by Blanch and coworkers (Shill et al., 2010). They recovered [EMIM]OAc and [BMIM]OAc via a three-phase system formed by adding concentrated solutions of K<sub>3</sub>PO<sub>4</sub> and K<sub>2</sub>HPO<sub>4</sub> (40%) after dissolution of miscanthus in RTILs. The phases included a salt-rich aqueous phase, a solid phase rich in cellulose, and an RTIL-rich phase that contains most of the lignin. This process decreases the amount of water in the RTIL that must be evaporated before recycling, which would reduce energy costs. In particular, the addition

of K<sub>3</sub>PO<sub>4</sub> solution was advantageous because the resulting high pH resulted in further pretreatment of the miscanthus leading to higher yields of released sugars. For example, enzymatic hydrolysis of miscanthus pretreated in [EMIM]OAc at 70°C for 44 h, and precipitated with K<sub>3</sub>PO<sub>4</sub> solution yielded ~100% cellulose hydrolysis in 48 h, whereas the pretreated miscanthus precipitated with K<sub>2</sub>HPO<sub>4</sub> solution only yielded ~65% cellulose hydrolysis. Apparently, the basicity of K<sub>3</sub>PO<sub>4</sub> cleaves the lignin favoring its partition into the RTIL phase. Recycling of RTILs (with solubilized lignin) showed lower efficacy after further pretreatments (complete conversion was achieved in 25 h after pretreatment at 140°C for 1 h in fresh [EMIM]OAc, while complete conversion was reached in 48 h after pretreatment of recycled RTIL at 140°C for 1 h). However, remaining water was not removed from the RTIL and it may cause the loss of the efficacy on the pretreatment, as was previously demonstrated by Dordick and coworkers (Doherty et al., 2010).

### Enzymatic Reactions in RTILs

The ability of RTILs to decrease cellulose crystallinity and enhance accessibility to cellulases raises the possibility to consider single-step pretreatment-enzymatic hydrolysis. Nearly all RTILs that dissolve cellulose are highly chaotropic, and hence highly denaturing to proteins. Nonetheless, in the presence of a sufficient concentration of water, cellulase activity may be preserved. For example, cellulases from *Penicillium janthinellum* were isolated and tested in five RTILs. The enzymes remained sufficiently stable for 1 h in up to 50% (w/w) [BMIM]Cl/H<sub>2</sub>O and 50% (w/w) [BMP]Cl/H<sub>2</sub>O; however, the enzymes were nearly fully inactive after 4 h incubation in the RTILs (Adsul et al., 2009). Cellulases from the hyperthermophilic bacterium *Thermatoga maritima* and a hyperthermophilic archaeon *Pyrococcus*

horikoshii (overexpressed in *E. coli*) showed little loss of activity in 15% [EMIM]OAc after 15 h; the commercial cellulase from *Trichoderma viride* lost 100% of its activity under identical conditions (Datta et al., 2010). Recently, a cellulase from *T. reesei* was found to be stable in tris-(2-hydroxyethyl) methylammonium methylsulfate (HEMA) for periods longer than 2 h at temperatures as high as 115°C (Bose et al., 2010). However, expressing cellulase variants in bacteria or yeast is time consuming and expensive, and often results in low activity (Okada et al., 2000; Walter and Schrempf, 1995; Wang et al., 1994).

Recently, Clark and coworkers developed a cell-free, high-throughput cellulase expression platform to express a library of 54 bacterial and archaeal endoglucanases (EG) from constructs containing chimeric genes encoding different combinations of catalytic domains (CD) with and without cellulose-binding modules (CBM) at either the C- or N-terminal (Kim et al., 2010). Addition of CBM increased the activity of enzymes against pretreated crystalline cellulose in [EMIM]OAc. The same library of chimeric enzymes were tested against lignocellulosic materials (miscanthus and corn stover) pretreated in [EMIM]OAc and ammonia fiber explosion. However, the effectiveness of CBM depended on the source of the cellulase. In all cases, the specific cellulose hydrolysis rates of pretreated materials were 2- to 50-fold higher than untreated materials. Moreover, the rates of cellulose hydrolysis of corn stover pretreated in [EMIM]OAc were 2- to 5-fold higher than ammonia fiber expansion (AFEX) pretreated corn stover, revealing the advantage of RTIL pretreatment over the more conventional process. EG chimeras with the *A. cellulolyticus*, *S. lividans*, and *B. fibrisolvens* CD's containing the C-terminal CBM exhibited high activity on pretreated corn stover and miscanthus, resulting in a promising set of enzymes that can be tested in RTIL–water mixtures.

The opportunity to use a suite of enzymes in RTILs to both pretreat and convert lignocellulose into fermentable sugars remains a significant, yet likely attainable, challenge. One might imagine that improved cellulases can be generated, using now common methods of enzyme evolution to yield enzymes with sufficient stability in higher concentrations of suitable RTILs. Fortunately, to swell the cellulose and thus provide accessibility of the enzyme to the cellulose fibers does not require neat RTIL. Indeed, some water is necessary to aid in cellulase binding to the cellulose chains, to provide sufficient dynamics for these enzyme complexes to perform their endo- and exoglycosidase function, and of course to serve as co-substrate for hydrolysis to occur. A similar study could be undertaken with xylanases to generate pentoses from the hemicellulose fraction. In addition to yielding xylose and other hemicellulose constituents, xylanase treatment could also remove critical parts of the cell wall that further blocks cellulase diffusion to the cellulose (Remond et al., 2010; Valls et al., 2010).

Enzymatic delignification may be dramatically improved in RTILs. Because lignin is attached to the cell wall through ester and ether bridges to the hemicellulose fraction,

xylanases may aid in the extraction of lignin out of the biomass. In aqueous solutions, enzymatic delignification is seriously hampered by both enzyme accessibility to the hemicellulose and the poor solubility of the lignin once some critical linkages are broken. Lignin solubilizing RTILs (such as [EMIM]OAc) may prove useful here. Ferulic acid esterases are common and provide a natural mechanism to hydrolyze ester linkages between the xylose 5-OH moiety and the small number of ferulic acid residues present in lignin (Anderson et al., 2002; Benoit et al., 2006; Buanafina et al., 2008). However, a more significant enzymatic activity would require an etherase, which can selectively split ether linkages present between lignin and hemicellulose (Otsuka et al., 2003; Sonoki et al., 2002). As a side benefit, etherases may also cause the controlled depolymerization of the lignin, thereby assisting in its removal and solubilization into the RTIL. While no etherase is known that can hydrolytically break the lignin-hemicellulose linkages, it is not far-fetched to consider that myriad protein engineering methodologies could be applied to endow structurally related enzymes (e.g., epoxide hydrolases) with the ability to serve as etherases.

### Lignin as a Renewable Resource

Lignin represents a vastly underutilized resource. Despite being the second most abundant polymer on earth (after cellulose), ~98% of lignin is burned as a source of energy (Lora and Glasser, 2002; Thielemans et al., 2002), primarily in the pulp and paper industry. With the rapidly growing interest in generating fuels from lignocellulose, a large amount of lignin will be made available. This will undoubtedly spur new concepts for using lignin as a resource for value added products. The field of lignochemicals deserves its own review; however, within the context of the current review it is important to consider that lignin released from lignocellulose via RTIL pretreatment is likely to be far more “pristine” than Kraft lignin. While Kraft lignin has been studied as a source of renewable phenolic-derived compounds, such as lignin- and formaldehyde-based adhesives that are used in the production of veneer plywood (Klašnja and Kopitović, 1992; Mahendran et al., 2010) and for ion exchange separations (Zoumpoulakis and Simitzis, 2001), pristine lignin obtained from RTIL pretreatment offers a material that consists of a highly functionalized phenolic-based three-dimensional network that may find more general application in phenol-formaldehyde replacements, conversion into liquid hydrocarbon fuels following hydrogenative depolymerization, or possibly into specific low molecular weight chemicals. Along these lines, the generation of novolak and resole resins in the absence of formaldehyde or other chemical crosslinker has already been advanced using lignin as a starting material. For example, *p*-cresol can be incorporated into Kraft lignin by peroxidase-catalyzed copolymerization in 50–70% dioxane–water mixtures (Popp et al., 1991). These results suggest that



isolated pristine lignin in RTILs ([EMIM]OAc and [BMIM]OAc) can also be copolymerized with phenols catalyzed by peroxidases under optimal conditions in a RTIL/water media.

Pristine lignin may be depolymerized into smaller oligomers or monomers (e.g., via oxidation, acidic and alkaline hydrolysis, thermochemical hydrogenation; Binder and Raines, 2009; Crestini et al., 2006; Kleinert and Barth, 2008a,b; Kleinert et al., 2009; Matsushita and Yasuda, 2005; Yuan et al., 2010). A key challenge is to perform these transformations in RTILs where pristine lignin is extracted from the biomass. Along these lines, several studies on depolymerization of lignin have been performed in RTILs to exploit them as reaction media using homogenous and heterogeneous catalysis. A number of compounds (e.g., vanillin, syringol, syringaldehyde, and 2,6-dimethoxy-*p*-benzoquinone) were generated following oxidative depolymerization of lignin (using Mn(NO<sub>3</sub>) as a catalyst) in four different RTILs (1-ethyl-3-methylimidazolium methylsulfonate, [EMIM]MeSO<sub>3</sub>; 1-ethyl-3-methylimidazolium ethylsulfonate [EMIM]EtSO<sub>3</sub>; 1-ethyl-3-methylimidazolium trifluoromethylsulfonate, [EMIM]CF<sub>3</sub>SO<sub>3</sub>; and 1-methyl-3-methylimidazolium methylsulfate [MMIM]MeSO<sub>4</sub>) (Stark et al., 2010). Such manganese-catalyzed routes mimic natural manganese peroxidases, such as that from *Ceriporiopsis subvermisporea*, a white-rot fungus that is capable of slowly degrading lignin.

## Conclusions

Lignocellulosic biomass pretreatment in RTILs is a promising alternative, with comparable or superior yields of fermentable sugars, than conventional pretreatments. The broad number of RTILs that can be synthesized allows the design of solvents with specific physicochemical properties that play a critical role interacting with lignocellulosic biomass subcomponents. Today, these interaction mechanisms are better understood. However, future challenges rely on the ability to make this process economically attractive. This might be achieved by optimizing large-scale pretreatment conditions, performing post-pretreatment steps in RTILs, reusing RTILs, recycling the RTILs with reduced energy consumption and enhancing process efficiency, and producing high-value products in addition to ethanol (e.g., products traditionally derived from petroleum, fine chemicals or fuels with higher energy value). Moreover, the potential high-value of lignin suggests that it might instead be used in the large-scale diversified manufacture of high-value chemicals, traditionally obtained from petroleum.

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