Ionic liquid solvent properties as predictors of lignocellulose pretreatment efficacy†

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Effective pretreatment of lignocellulosic biomass is vital to its bioconversion to a usable liquid fuel. A growing body of work has focused on using room temperature ionic liquids (RTILs) to pretreat lignocellulose for subsequent fermentation. However, little is known about the physicochemical parameters of RTILs that promote effective pretreatment. In this work we examine the relationship between the Kamlet–Taft α, β, and π^* solvent polarity parameters of different RTILs ([Emim][OAc], [Bmim][OAc], and [Bmim][$MeSO_4$]) and effective pretreatment of lignocellulosic biomass. We find the β parameter is an excellent predictor of pretreatment efficacy. Acetate containing RTILs ($\beta > 1.0$) remove >32% of lignin from maple wood flour and significantly reduce cellulose crystallinity, resulting in >65% glucose yields after 12 h cellulase hydrolysis. Pretreatment in [Bmim][$MeSO_4$] ($\beta = 0.60$) results in the removal of only 19% of the wood flour's lignin with no decrease in crystallinity, and no improvement in sugar yield over untreated wood flour. The addition of water and the dilution of the acetate anion with the methyl sulfate anion decrease the β value and subsequently have a negative impact on lignin extraction, cellulose crystallinity, and sugar yields.

Introduction

The worldwide demand for liquid fuels is projected to grow 51% by 2030,[1] and tremendous effort is being applied to meet this demand from renewable sources. The production of biofuels from lignocellulosic feedstocks, such as agricultural waste, wood, or energy crops, provides a means to meet this demand in manner that reduces the emission of greenhouse gases.[2] In contrast to the present biofuels paradigm, these feedstocks do not contribute to the world's food supply, and using them avoids the food *vs.* fuel debate. Lignocellulosic biomass is predominantly comprised of three subcomponents: the semi-crystalline polysaccharide cellulose, the amorphous multicomponent polysaccharide hemicellulose, and the amorphous phenolpropanoid polymer lignin. These three biopolymers are the primary constituents of plant cell walls, with cellulose being present in the form of microfibrils approximately 4 nm in diameter, which bundle to form larger macrofibrils on the order of 500 nm in diameter. These fibrils are contained within a protective sheath of lignin and hemicellulose and form the plant cell wall. Plant cells, which have dimensions on the order of tens to hundreds of microns, are cemented together by a lignin-rich region known as the middle lamella.[3,4] The combined effect of the chemistry and structure of lignocellulosic biomass results in a relatively stable material that is very recalcitrant to chemical and biological degradation.

The conversion of lignocellulosic biomass into biofuels has been performed either biochemically[5–7] or thermochemically,[8,9] and comparisons of these two routes have shown little difference in terms of economic and environmental impact.[10] This work focuses on process improvements aimed at the biochemical route, which can be divided into three main steps: (1) feedstock pretreatment; (2) polysaccharide hydrolysis to monosaccharides; and (3) fermentation of sugars to a combustible liquid fuel. Of these three processes, the pretreatment step is widely considered to be the most critical because of its impact on cost[11,12] and downstream processing.[13] The goal of pretreatment is to increase the accessibility and reactivity of the polysaccharide portion of lignocellulose without substantive destruction of polysaccharides to maximize the release of fermentable sugars. Such destruction not only reduces process yields, but also can lead to the formation of microbial inhibitors, such as furfurals, which can negatively impact the fermentation step.[14] A large number of pretreatment processes have been developed, and can be categorized as physical (milling), chemical (acid or alkaline hydrolysis), physicochemical (steam explosion, ammonia fiber explosion, supercritical fluids), and biological (white rot fungi); these processes have been reviewed extensively,[7,15–19] and a common theme in such pretreatments is the removal of lignin

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and/or hemicellulose to enhance accessibility of enzyme to the cellulose fraction. The removal of hemicellulose results in higher glucose yields from cellulose,[20,21] but the ultimate loss of fermentable sugar contained in hemicellulose illustrates the need for continued development of innovative pretreatment processes.

Room Temperature Ionic Liquids (RTILs) are salts that melt below 100 °C and exhibit many properties of interest to green processing such as high thermal stability and negligible vapor pressure.[22,23] The near limitless potential to combine anions and cations in order to tailor solvent properties has led to RTILs being termed "designer solvents"[24] and RTILs have found applications in chemical transformations,[25] separations,[26,27] and enzymatic reactions.[28–30] RTILs have been shown to dissolve cellulose,[31,32] lignin[33,34] and whole wood.[35–37] For example, the dissolution of microcrystalline cellulose (MCC) and wheat straw in 1-butyl-3-methylimidazolium chloride ([Bmim][Cl]), and 1-allyl-3-methylimidazolium chloride ([Amim][Cl]), and regeneration using counter solvents led to reduced crystallinity and enhanced saccharfication.[38–40] A series of RTILs designed to have a lower viscosity than [Bmim][Cl], comprised of imidazolium and ammonium based cations with alkyloxyl chains and acetate anions, were shown to be as effective at enhancing the saccharification of MCC as [Bmim][Cl].[41] Through the construction of a cellulase adsorption isotherm, these authors showed that RTIL-treated MCC provides greater enzyme accessibility than untreated MCC. The efficacy of a given RTIL in the dissolution of biomass and its subcomponents has been linked to both the selection of the anion[42] and the cation.[32] Pretreatment of wood in the RTIL 1-ethyl-3-methylimidazolium acetate ([Emim][OAc]) reduces both lignin content and the crystallinity resulting in conversion of >90% cellulose in wood to glucose by cellulase from *Trichoderma viride*.[43] The set of solvent polarity parameters defined by Kamlet and Taft have often been used to characterize an RTIL's hydrogen bond acidity (α), hydrogen bond basicity (β), and polarizability (π^*).[44] The reduction in crystallinity of wood pretreated with [Emim][OAc] may be due to hydrogen bond basicity of the acetate anion as reflected in the Kamlet–Taft β parameter. This is supported by the literature, with the cellulose solvent [Bmim][Cl] having a $\beta = 0.95$ and the non-cellulose solvent [Bmim][PF_6] having a $\beta = 0.44$.[45] However, to date, there has been no direct study of the relationship between the solvent polarity parameters for a set of RTILs and their ability to reduce cellulose crystallinity, selectively remove lignin and increase enzyme generated fermentable sugar yields.

Establishing a relationship between readily measurable solvent polarity parameters of RTIL and the measurable outcomes of biomass treated with these solvents is critical to advance the continued design and selection of RTILs for biomass pretreatment. Due to the high cost of RTILs, solvent recycling will be necessary to process biomass on an industrial scale. As the RTIL endures multiple processing cycles it will inevitably take on water and other contaminants, and a thorough understanding of the impact of these agents on pretreatment is necessary. Herein, we correlate lignin extraction into RTIL, reduction in cellulose crystallinity, and cellulase catalyzed fermentable sugar yields to the physicochemical properties of the RTIL, as represented by the Kamlet–Taft solvent polarity parameters. Alteration of these parameters by addition of small volume fractions of water to a set of 1,3 dialkylimidizolium RTILs ([Emim][OAc], [Bmim][OAc] & [Bmim][$MeSO_4$]) on lignin extraction, biomass crystallinity and enzymatic hydrolysis of biomass into monomeric sugars is examined. Binary mixtures consisting of [Bmim][OAc] and [Bmim][$MeSO_4$] are also studied as pretreatment media to determine the effect of anion composition on efficacy of pretreatment. As a result, for the first time, a quantitative solvent parameter has been uncovered that can be used to fine-tune RTIL composition for optimal pretreatment of lignocellulosic biomass.

Results and discussion

Aqueous and organic solvents used in pretreatment strategies are not effective in enhancing the yield of fermentable sugars from lignocellulose hydrolysis unless harsh treatment conditions are used, *e.g.*, steam explosion or the presence of strongly acidic or basic conditions.[46,47] Moreover, only a small number of traditional solvents and conditions are able to remove/disrupt lignin and increase the accessibility of cellulose,[48,49] while autohydrolysis by compressed liquid hot water must operate at high temperature (>190 °C) in order to achieve high yields.[50,51] As opposed to organic solvents, RTILs show promise solvents capable of pretreatment at moderate temperature (<100 °C), and through combinatorial synthetic approaches that enable stable interactions of anions and cations, a staggering number of RTILs can be designed for use as potential pretreatment solvents.[52] We have focused on three RTILs ([Emim][OAc], [Bmim][OAc], and [Bmim][$MeSO_4$]), which cover two imidazolium cations and two distinct anions, as well as on the effect of addition of water concentration on lignin extraction and subsequent enzymatic hydrolysis of cellulose and hemicellulose. Maple wood flour was used as our biomass, as it represents a particularly recalcitrant form of lignocellulose (hardwood) and thus is a challenging model for optimization of RTILs used in a pretreatment strategy. A commercial cellulase (consisting of both cellulase and xylanase activities) from *T. viride* was used as our model enzyme.

Establishment of pretreatment time

Pretreatment of maple wood flour in [Emim][OAc] at 90 °C is sufficient to significantly reduce lignin content and cellulose crystallinity index while resulting in substantial sugar yields after enzymatic hydrolysis.[43] This result was confirmed in the current work for times of 6, 12, and 24 h in both [Bmim][OAc] and [Emim][OAc] (Table 1, Fig. S1–S5†). Yields and rates of enzymatic hydrolysis of wood flour were significantly enhanced following pretreatment in [Bmim][OAc]; substantial improvement in both were obtained after 6 h pretreatment in the RTIL. More modest improvement in the yields and rates of enzymatic hydrolysis of cellulose and xylan were obtained following 12 and 24 h pretreatment. Increased enzymatic degradability of the polysaccharides was concomitant with a dramatic reduction in the crystallinity index (CrI) of the cellulose.

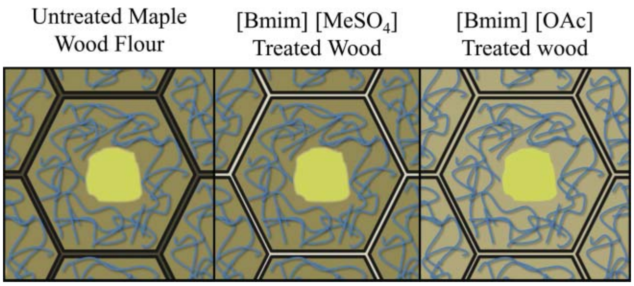
The importance of reducing CrI to subsequent enzymatic hydrolysis of the polysaccharides was evident when the model substrate, microcrystalline cellulose (MCC) was used. The saccharification rate and glucose yield from MCC was far superior to that of untreated maple wood flour, presumably due to increased enzyme access to cellulose in the absence of

Table 1 Summary of fermentable sugar yields, saccharification rates, lignin extraction, and crystallinity index of maple wood flour pre-treated with [Bmim][OAc] and [Emim][OAc] at 90 °C for 6, 12, and 24 h

Pretreatment Conditions			Sugar yield (%)		Saccharification rate/mg ml^{-1} h^{-1}			
Feedstock	RTIL	Time/h	Glucose	Xylose	Glucose	Xylose	Extracted lignin (%)	Crystallinity index (Crl)
Maple wood flour	None	0	4.5	2.6	0.11	0.03	0.0	66
microcrystalline cellulose		0	35	N/A	0.93	N/A	N/A	85
Maple wood flour	[Bmim][OAc]	6	64	57	2.1	0.91	25	37
		12	74	65	2.5	1.11	35	39
		24	74	64	2.4	1.12	49	35
Maple wood flour	[Bmim][OAc]	6	59	45	1.5	0.52	26	49
		12	65	56	2.0	0.83	32	41
		24	70	64	2.1	0.91	37	35
Maple wood flour	[Bmim][MeSO$_4$]	12	1.1	5.1	0.06	0.04	19	66

lignin and hemicellulose. In contrast, the saccharification rates and glucose yields of pretreated wood flour, with as little as 25% of its lignin removed, were superior to MCC, illustrating the impact of crystallinity reduction. Thus, both reduction in CrI and increased lignin extraction serve together to afford the greatest increase in fermentable sugar yield and saccharification rate.

In addition to reducing the cellulose CrI of wood flour and increasing the subsequent enzymatic hydrolysis rates and yields of the cellulose and xylan fractions, the RTIL pretreatment for 6 h was also able to increase dramatically the extraction of lignin, and this continued up to 24 h with nearly 50% of the lignin extracted from the wood flour. Results with [Emim][OAc] were somewhat less striking, indicating that the physicochemical properties of the RTIL affect the pretreatment effectiveness. The impact of the RTIL is clearly evident using [Bmim][MeSO$_4$]. The glucose and xylose yields of wood flour treated with [Bmim][MeSO$_4$] were similar to untreated wood flour, and the CrI of wood flour treated in this RTIL was only marginally higher than that of untreated wood. Approximately 20% of the lignin was extracted, ~3 g L^{-1}, which is far below the solubility of lignin in [Bmim][MeSO$_4$] (>300 g L^{-1}).[34] The relatively low extraction of lignin into an RTIL that is capable of solubilizing a high concentration of lignin indicates that [Bmim][MeSO$_4$] may not have full access to the lignin present in the wood flour, and the lignin extraction is restricted to the lignin rich middle lamella region while excluded from lignin present in the primary and secondary cell walls (Scheme 1).



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$_4$]-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).

The effect of added water and binary RTIL mixtures on pretreatment outcomes

The glucose and xylose yields of wood flour pretreated at 90 °C for 12 h in [Bmim][OAc] and [Emim][OAc] decline as water is blended into the RTIL solvents, even with as little as 5–10% (w/w) added (Fig. 1, S6–S8†). This takes place simultaneously to an increase in both the CrI of the pretreated wood flour and its lignin content (Fig. 2 and 3). Untreated wood flour displays a strong peak at $2\theta = 22.5°$, corresponding to the (002) crystal plane of cellulose, as well as a composite peak corresponding to the (101) and (10$\bar{1}$) crystal planes at $2\theta = 15.5°$. The intensity corresponding to the (002) plane decreases dramatically for wood flour treated with [Bmim][OAc] or [Emim][OAc], and no distinct peak remains for the (101) and (10$\bar{1}$) planes. With the addition of water to these RTILs, their ability to disrupt the crystallinity of cellulose within wood flour is greatly diminished, as evidenced by the gradual restoration of peak intensities to the levels of untreated wood flour seen in Fig. 3. Water has very little effect on [Bmim][MeSO$_4$] pretreatment outcomes. SEM micrographs of maple wood flour untreated and treated in each of the three RTILs, as well as treated in each of the RTIL's with 10% (w/w) added water are shown in Fig. 4. Images of untreated wood flour display fiber diameters as large as 250 μm, but after treatment with either [Bmim][OAc] or [Emim][OAc] the fiber diameter decreases to approx. 17 μm. This visual evidence of inter-crystalline swelling combined with the evidence of intra-crystalline disruption seen in the diffractograms (Fig. 3) confirms that [Bmim][OAc] or [Emim][OAc] cause dramatic morphological changes in wood flour. Images of wood flour treated with [Bmim][MeSO$_4$], however, display no fiber size reduction when compared with untreated wood flour, consistent with the lack of effectiveness of this RTIL.

Comparison of the images of wood flour treated with RTILs containing 10% (w/w) added water display differences between the RTILs containing acetate and methyl sulfate. The image

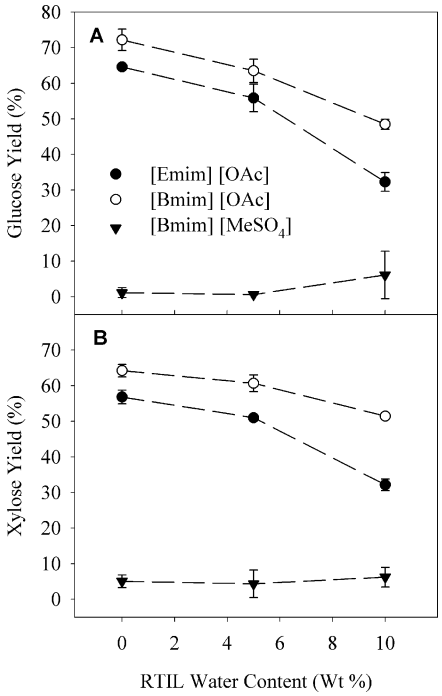


Fig. 1 Yields of glucose (A) and xylose (B) as a function of RTIL water content for [Emim][OAc], [Bmim][OAc], and [Bmim][$MeSO_4$].

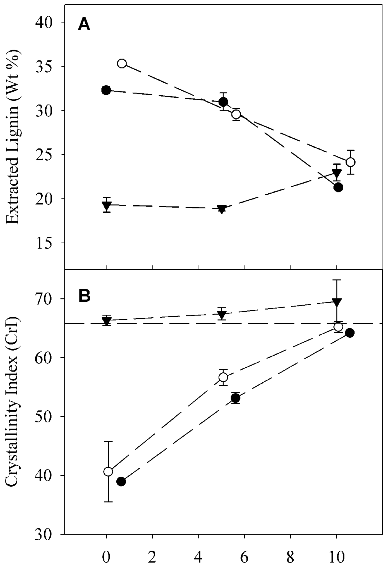


Fig. 2 Extracted lignin (A) and CrI (B) from maple wood flour as a function of RTIL water content for [Emim][OAc], [Bmim][OAc], and [Bmim][$MeSO_4$]. Dashed line in B indicates CrI of untreated wood flour.

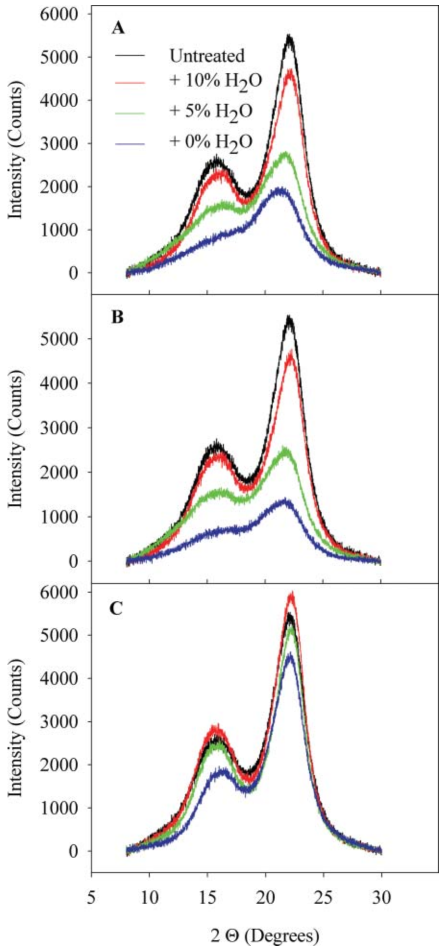


Fig. 3 Diffractograms of maple wood flour treated in [Bmim][OAc] (A), [Emim][OAc] (B), and [Bmim][$MeSO_4$] (C) with 0%, 5% & 10% (w/w) added water.

of [Bmim][$MeSO_4$] containing 10% (w/w) added water is indistinguishable from the image with no water added, whereas images of the acetate RTILs with this added water content display evidence of moderate inter-crystalline swelling, much as if the fibers have begun to unravel, but are still largely intact. The visual evidence is consistent with the diffractograms, where the peak intensity of maple wood flour treated with RTIL containing 10% (w/w) added water is only slightly reduced compared to peak intensity of untreated maple wood flour. Therefore, the impact of the added water is to reduce the ability of the acetate RTILs to affect both intra- and inter-crystalline swelling, as well as fiber size reduction.

It is noteworthy that the extracted lignin from wood flour treated in each of the three RTILs with 10% (w/w) added water is similar, ranging from 21 to 24% (w/w). The difference in CrI is also small: 64 for the acetate containing RTILs and 70 for [Bmim][$MeSO_4$], while the difference in fermentable sugar yields is quite large, with [Bmim][OAc] yielding 48% and 51% glucose and xylose respectively, [Emim][OAc] yielding 32% glucose and xylose, and [Bmim][$MeSO_4$] yielding 6% glucose and xylose. The influence of CrI on fermentable sugar yield has been shown to be coupled to the amount of feedstock de-lignification,[53] with enzymatically-generated sugar yields from biomass with $>90\%$ of lignin removed showing little sensitivity to CrI, while biomass with $<10\%$ of lignin removed being more sensitive to CrI. In the present data, with 21 to 24% of the lignin removed and a difference in CrI of 6 between the acetate and $MeSO_4$ containing RTILs, some sensitivity of sugar yield to CrI is expected, but factors, such as the degree of inter-crystalline swelling, are likely playing a role.

In addition to added water, the effectiveness of RTIL pre-treatment can be influenced by generating blended mixtures

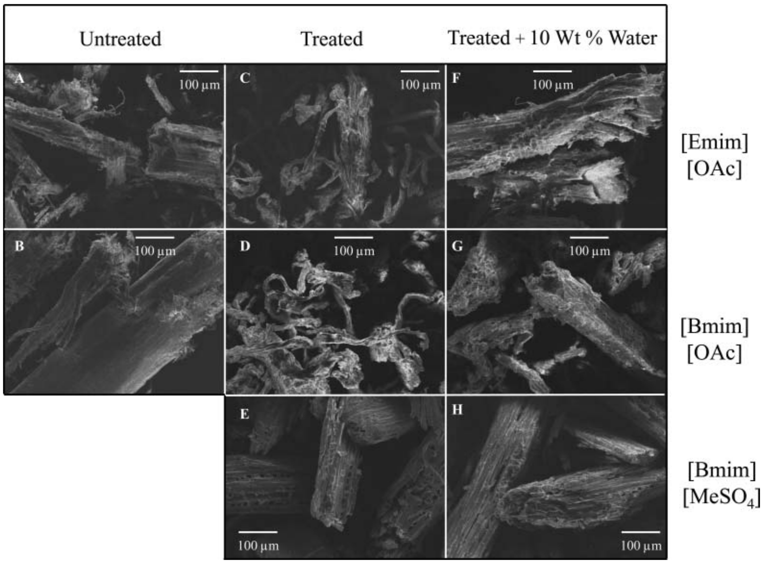


Fig. 4 SEM Images of untreated maple wood flour (A and B), maple wood flour pretreated in [Emim][OAc] (C), [Bmim][OAc] (D), and [Bmim][$MeSO_4$] (E), [Emim][OAc] with 10% (w/w) added water (F), [Bmim][OAc] with 10% (w/w) added water (G), and [Bmim][$MeSO_4$] with 10% (w/w) added water (H). All images are the same scale.

of RTILs. As shown in Fig. 5, the extent and initial rate of cellulose and xylan hydrolysis increases as the $MeSO_4$ anion is replaced with the acetate anion in this binary mixture of [Bmim][OAc]/[Bmim][$MeSO_4$]. Correspondingly, the CrI decreases and the extracted lignin content increases as acetate content increases and $MeSO_4$ content decreases.

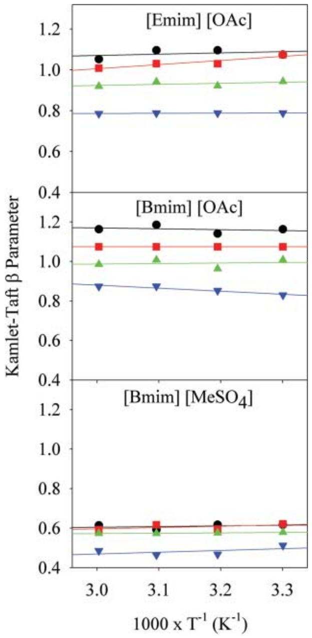


Fig. 5 Kamlet–Taft β parameter temperature dependence of [Emim][OAc], [Bmim][OAc], and [Bmim][$MeSO_4$] with 0% (●), 5% (■), 10% (▲), and 20% (w/w) (▼) added water.

Measurement of Kamlet–Taft parameters

Differences in the effectiveness of RTILs suggest that specific physicochemical properties of an RTIL strongly influence lignocellulose pretreatment. To date, essentially all research performed has been phenomenological, although properties of the RTILs can be assessed quantitatively. For this reason, we set out to establish fundamental parameters that can be used to fine-tune RTIL pretreatment effectiveness. One set of parameters comes from Kamlet–Taft, which provides a quantitative measure of solvent polarizability (π^* parameter), hydrogen bond donator (HBD) capacity (α parameter), hydrogen bond acceptor (HBA) capacity (β parameter). All three parameters are determined spectrophotometrically using a series of dyes (eqn (1)–(3)), where $v(\mathrm{DENA})_{\max}$ and $v(\mathrm{4NA})_{\max}$ are the maximum wavelengths of *N*,*N*-diethyl-4-nitroaniline and 4-nitroaniline, respectively, in kilokeyser (kK, 10^{-3} cm $^{-1}$), and $E_T(30)$ is $28\,591/\lambda_{\max}$ where $\lambda_{\max}$ is the maximum wavelength of Reichardt's dye in nm:

$$\pi^* = \frac{v(\mathrm{DENA})_{\max} - 27.52}{-3.183} \tag{1}$$

$$\alpha = \frac{E_T(30) - 14.6(\pi^* - 0.23) - 30.31}{16.5} \tag{2}$$

$$\beta = \frac{1.035 \times v(\mathrm{DENA})_{\max} - v(\mathrm{4NA})_{\max} + 2.64}{2.8} \tag{3}$$

Since all three parameters are temperature dependent,[54,55] we have taken measurements between 30 and 60 °C (Fig. 5, 6 & S9–S12), which were then extrapolated to estimate the value at 90 °C to match our pretreatment temperature (Table 2).

The α, β, and π^* parameters of water are reported to be 1.02–1.17, 0.14-0.18, and 1.09, respectively.[56–59] The addition of water to RTILs has the greatest impact on the β parameter; dropping 17% upon the addition of 10% (w/w) water in the case of [Bmim][OAc]. This drop is indicative of water's relatively poor HBD capacity. No clear trend is observed in the α parameter, and the π^* parameter only modestly increases as water is added. Reichardt's dye did not produce a signal

Table 2 Kamlet–Taft α, β, and π* parameters extrapolated to 90 °C

	Kamlet Taft Parameter Extrapolated to 90 °C		
Solvent	*α*	*β*	*π**
[Bmim][OAc]	0.57	1.18	0.89
[Bmim][OAc] + 5% H_2O	0.53	1.07	0.93
[Bmim][OAc] + 10% H_2O	0.55	0.98	0.94
[Bmim][OAc]	0.53	1.06	0.97
[Bmim][OAc] + 5% H_2O	0.50	0.96	1.02
[Bmim][OAc] + 10% H_2O	0.56	0.91	0.99
[Bmim][$MeSO_4$]	ND	0.60	1.00
[Bmim][$MeSO_4$] + 5% H_2O	ND	0.58	0.99
[Bmim][$MeSO_4$] + 10% H_2O	ND	0.57	1.03
25%[Bmim][OAc]/75%[Bmim][$MeSO_4$]	0.55	0.84	0.97
50%[Bmim][OAc]/50%[Bmim][$MeSO_4$]	0.61	0.98	0.95
75%[Bmim][OAc]/25%[Bmim][$MeSO_4$]	0.60	1.04	0.95

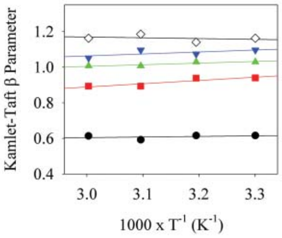


Fig. 6 Kamlet–Taft *β* parameter temperature dependence of [Bmim][OAc]/[Bmim][$MeSO_4$] binary solution; wt% [Bmim][OAc] – 0% (●), 25% (■), 50% (▲), 75% (▼), 100% (w/w) (◇).

in [Bmim][$MeSO_4$], so *α* was not determined for this RTIL. Among RTILs the *β* parameter displays the greatest variation, as illustrated by its sensitivity to the anion composition in the binary [Bmim][OAc]/[Bmim][$MeSO_4$] mixture. The impact of anion selection on *β* parameter is well reported in literature.[44]

The addition of water to the acetate-containing RTILs reduces their HBA capacity, as seen by a decrease in the *β* parameter. A large HBA capacity is required for RTILs to effectively dissolve cellulose,[31] hence to disrupt the crystallinity of cellulose in lignocellulose a solvent must also possess a large HBA capacity. The inability of [Bmim][$MeSO_4$] to disrupt wood flour crystallinity is not surprising considering the solvent's low *β* parameter (0.60), which decreases with added water. The trend observed in the [Bmim][OAc]/[Bmim][$MeSO_4$] binary mixture may also be explained by the Kamlet–Taft parameters. The nature of the anion has the largest impact on the *β* parameter; the acetate *β* parameter is ~50% higher than that for the $MeSO_4$, while the *α* and *π** parameters vary by only ~10%. The strong anion composition dependence of the *β* parameter also coincides with greater crystallinity disruption and higher sugar yields from the pretreated lignocellulose (Fig. 7). Specifically, we evaluated the relationship between the Kamlet–Taft *β* parameter and CrI using a sigmoidal fit (R^2 = 0.87) to take under consideration the threshold CrI of untreated wood flour at CrI = 66 (Fig. 8). The onset of CrI reduction occurred in solvents with $\beta \geq 0.84$. The correlation using a sigmoidal fit between the *β* parameter and extracted lignin (R^2 = 0.85) suggests that a relationship also exists, with a sharp increase in extracted lignin occurring when $\beta \geq 0.84$.

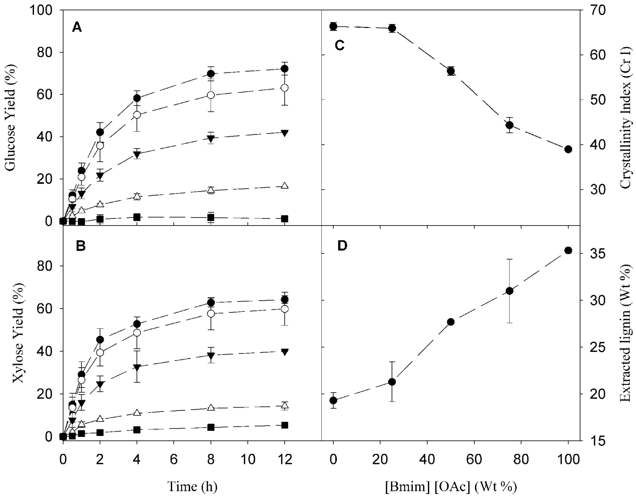


Fig. 7 Enzymatic timecourse of glucose (A) and xylose (B) release from maple wood flour following pretreatment in blended [Bmim][OAc]/[Bmim][$MeSO_4$]; (● 100% (w/w) [Bmim][OAc], ○ 75% (w/w) [Bmim][OAc], ▼ 50% (w/w) [Bmim][OAc], Δ 25% (w/w) [Bmim][OAc], ■ 0% (w/w) [Bmim][OAc]). CrI (C) and extracted lignin (D) from maple wood flour as a function of [Bmim][OAc] weight percent in RTIL blend.

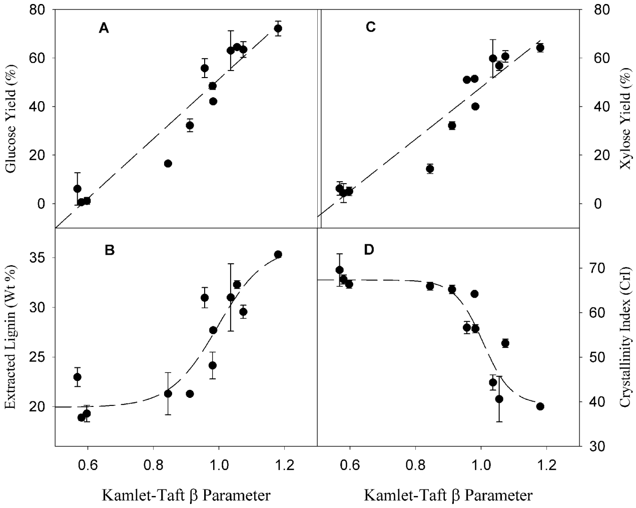


Fig. 8 Correlation of *β* parameter extrapolated to 90 °C with glucose yield (A), extracted lignin (B), xylose yield (C) and CrI (D) for the series of RTILs studied.

These results suggest the following mechanism of high β RTIL ability to affect lignin extraction coupled with reduced cellulose CrI. As the RTILs open up the lignocellulose structure, more access to lignin contained within the fiber is allowed, resulting in greater lignin extraction, as seen in Scheme 1. The *β* parameter of a given RTIL shows good linear correlation with the glucose yield (R^2 = 0.94) and the xylose yield (R^2 = 0.93) from wood flour pretreated in that solvent. In addition to the ability of an RTIL to reduce CrI and lignin content, other factors such the ability to promote inter-crystalline swelling and reduction in fiber size may play important roles in determining the efficacy of an RTIL as a pretreatment solvent for lignocellulosic biomass.

Experimental

Chemicals and reagents

1-Butyl-3-methylimidazolium acetate ([BMIM][OAc]), 1-butyl-3-methylimidazolium methyl sulfate ([BMIM][$MeSO_4$]), 1-ethyl-3-methylimidizolium acetate ([EMIM][OAc]), cellulase from *Trichoderma viride*, sodium citrate, citric acid, sodium hydroxide, sulfuric acid, 4-nitroaniline, Reichardt's dye #30, microcrystalline cellulose, glucose, and xylose were purchased from Sigma-Aldrich (St. Louis, MO, USA) and used without modification. The water content of [BMIM][OAc], [EMIM][OAc], and [BMIM][$MeSO_4$] was determined by Karl Fisher titration to be 0.68% (w/w), 0.09% (w/w), and 0.02% (w/w), respectively. *N*,*N*-Diethyl-4-nitroaniline was purchased from Frinton laboratories (Hainesport, NJ, USA). Indulin AT, a purified softwood kraft lignin from pine, was provided by MeadWestvaco (Charleston, SC, USA). Depol 740 L was graciously donated by Biocatalysts Ltd. (Cardiff, Wales, UK). Maple wood flour was graciously donated by P. J. Murphy Forest Products (Montvale, NJ, USA).

Kamlet–Taft parameter measurement

4-Nitroaninline, *N*,*N*-diethyl-4-nitroaniline, and Reichardt's dye solutions were prepared in ethanol to a concentration of 1 mg mL^{-1}. Two microlitres each 4-nitroaniline and *N*,*N*-diethyl-4-nitroaniline and 20 µL of Reichardt's dye were pipetted into three separate vials and the ethanol was evaporated under a stream of dry nitrogen. Dye concentrations of 12 µM, 8 µM, and 28 µM respectively, were obtained by adding 1.25 mL of the appropriate RTIL to each vial and mixing on a rotary shaker at 200 RPM for 20 min. The absorbance spectra at 30, 40, 50, and 60 °C of each RTIL/dye sample was measured between 350 and 700 nm using a spectrophotometer equipped with temperature control (Molecular Dynamics model Spectramax M5; Sunnyvale, CA, USA) and the maximum wavelength recorded. Using linear regression extrapolation, the value of each parameter at 90 °C was estimated.

Wood flour pretreatment

A slurry of 5% (w/w) maple wood flour in RTIL was prepared by combining 500 mg of wood flour with 10 g of RTIL in a round bottomed flask. When RTIL/water mixtures or binary RTIL mixtures were used the solvent was blended prior to addition to the wood flour. The flask was then placed in an oil bath and heated at 90 °C for 6, 12 or 24 h with magnetic stirring. After heating, an additional 20 g of RTIL was stirred into the pretreated slurry to reduce its viscosity and the undissolved solid fraction was separated from the RTIL by vacuum filtration. By using additional RTIL as diluents we avoided precipitation of the dissolved biomass portion. The recovered solid fraction was washed extensively with water and dried at 95 °C overnight. In the case of [Bmim][$MeSO_4$] with no added water it was necessary to use 20 g of [Bmim][$MeSO_4$] with 10% H_2O added to reduce the viscosity to permit vacuum filtration, which may have caused some dissolved biomass to come out of solution. All 12 h pretreatments were duplicated.

Enzymatic hydrolysis

Enzymatic hydrolysis reactions were performed in 10 mL of 50 mM pH 5 citrate buffer at 50 °C in 20 mL vials placed on a rotary shaker set to 200 rpm. Wood flour (200 mg) was combined with cellulase from *T. viride* (1.5 mg mL^{-1}) and Depol 740 L (0.05 mL mL^{-1}) with a combined activity 8.5 filter paper units (FPU) per mL in each reactor. Aliquots (200 µL) were removed at 0.5, 1, 2, 4, 8, and 12 h and placed in a 95 °C block heater for 5 min to quench the enzymatic reactions. Glucose, xylose, and cellobiose were quantified by HPLC (Shimadzu model LC-Ai; Kyoto, Japan) equipped with a refractive index detector and a styrene divinylbenzene resin column (Aminex HPX-87P; 300 × 7.8 mm, 8% cross linking; Bio-Rad; Hercules, CA, USA) column operated at 85 °C. The mobile phase consisted of deionized water with a flow rate of 0.6 mL min^{-1}. Glucose and xylose yields are calculated at each time point as follows:

$$\text{Sugar yield}_i = \frac{\text{Released sugar}(t)_i}{\text{Sugar content of untreated wood}_i} \tag{4}$$

where *i* indexes the measured sugar, either glucose or xylose. Initial hydrolysis rates are calculated from the linear regression slope through the initial two hours of the reaction.

Measurement of extracted lignin content

A sample of the liquid fraction of the RTIL wood slurry obtained after filtration was diluted 100-fold with 0.1 N NaOH, and its absorbance at 280 nm was measured spectrophotometrically (Shimadzu model UV-2401 PC; Kyoto, Japan). The measurement was corrected for RTIL background absorbance and the extracted lignin content was calculated from a Kraft lignin standard. The initial lignin content of the wood flour was taken as the sum of the acid insoluble and acid soluble lignin of untreated wood as determined according to NREL/TP-510-42618.[60]

Biomass crystallinity

All treated and untreated wood samples were analyzed by wide angle X-ray scattering on a D8 Discovery diffractometer (Brüker, Madison WI). All scans were in the range 8° ≤ 2θ ≤ 30° at a scan speed of 0.1° min^{-1} and a step size of 0.01°. The cellulose crystallinity index (CrI) was determined *via* eqn (5):

$$\text{CrI} = \frac{\sum I_{\text{Cr}}}{I_{\text{Tot}}} \times 100 \tag{5}$$

where $\sum I_{\text{Cr}}$ is the summation of the integrated intensity of the crystalline peaks and I_{Tot} is the integrated intensity of the entire sample. The crystalline peaks correspond to (101) at $2\theta \approx 14.75°$, $(10\bar{1})$ at $2\theta \approx 16.5°$, (021) at $2\theta \approx 20.65°$, and (002) at $2\theta \approx 22.5°$. Curve fitting was used to separate the intensities of the four crystalline peaks from the amorphous signal generated by maple wood flour.

Conclusion

In summary, readily measurable parameters describing the solvent polarity of RTILs can be measured and used to predict the efficacy of lignocellulosic biomass pretreatment. In particular the, Kamlet–Taft β parameter provides an excellent predictor for CrI reduction, lignin extraction, and fermentable sugar yields following pretreatment, with higher β values corresponding to more desirable pretreatment outcomes. Such a predictive relationship will likely aid in the rational design of RTILs for lignocellulosic biomass pretreatment, as well as benefit the recycling and reuse of RTILs in biomass pretreatment by providing an in-process evaluation of solvent quality.

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