Factors Affecting Wood Dissolution and Regeneration of Ionic Liquids

Bin Li,[†] Janne Asikkala,[†] Ilari Filpponen,[†] and Dimitris S. Argyropoulos*[,†,‡]

Organic Chemistry of Wood Components Laboratory, Department of Forest Biomaterials, North Carolina State University, Raleigh, North Carolina 27695-8005, and Laboratory of Organic Chemistry, Department of Chemistry, University of Helsinki, P.O. Box 55, 00014, Helsinki, Finland

Three wood species, eucalyptus grandis (E. grandis), southern pine (S. pine), and Norway spruce thermomechanical pulp (N. spruce TMP) were pretreated by dissolution in the ionic liquid (IL) 1-allyl-3-methylimidazolium chloride ([AMIM]Cl). The wood was regenerated from the ionic liquid in high yield and the recycling of the ionic liquid was nearly quantitative. The lignin contents and the efficiencies of cellulase enzymatic hydrolyses of the regenerated wood were examined offering an understanding into the IL pretreatment efficiency. The components that remained within the recycled ILs were qualitatively characterized by ^{31}P NMR spectroscopy. Wood density, pulverization intensity, and the nature of the regeneration nonsolvents were investigated as factors affecting the overall process. An increase in the wood density decreased the efficiency of the pretreatment, whereas extended pulverization periods decreased the yield of the regenerated wood after the IL pretreatment,with more glucose being released during the enzymatic hydrolysis. The yield of wood after IL pretreatment using water as the regeneration nonsolvent was found to be much higher than that of using methanol. As the reuse cycles of IL increased the wood regeneration yield increased, while certain wood components enriched within the recycled IL. The efficiency of cellulase enzymatic hydrolysis on the regenerated wood decreased with increasing reuse cycles of the IL.

Introduction

The ever growing need of producing goods from decreasing fossil based feedstock is a problem facing today's society. This issue has catalyzed the search for alternative sources of energy and feedstock for materials and chemicals with renewable resources occupying a prominent position. Furthermore, the accumulating knowledge of environmental effects and of the life cycle of products is affecting the way we view the production of materials, chemicals, and energy.

It the United States, for example, bioethanol is routinely mixed with gasoline to reduce the environmental effects of emissions in transportation and to reduce the carbon footprint of gasoline. However, most bioethanol in the United States is currently produced from corn. This has a significant impact in the amount of land used for food production, whereas the demand for food is increasing with increasing world population. Consequently, the production of biofuels from lingocellulosic materials like wood and agricultural crop feedstock could be a good choice for biofuel production, as such feedstock are still underutilized.[1]

There are inherent difficulties to release glucose from lignocellulosic materials because of the intricate nature of the lignocellulosic substrate and the way lignin and polysaccharide polymers coexist in such materials.[2] Pretreatment processes of lignocellulose are essential in order to utilize the carbohydrates in these materials especially cellulose. The purpose of the pretreatment is to open the compact structure of lignocelluloses and to improve the conversion of cellulose to glucose in the hydrolysis step.[3] Glucose can then be further fermented to bioalcohol (ethanol or butanol), but direct conversion of cellulose or lignocelluloses to bioalcohol has so far been impossible.

* Corresponding author. Tel.: (919) 515-7708. Fax: (919) 515-6302. E-mail: dsargyro@ncsu.edu.
[†] North Carolina State University.
[‡] University of Helsinki.

The various pretreatment methods available for lignocelluloses have been reviewed recently,[4−9] and they may be classified into different categories: physical (pulverization, irradiation, etc.), chemical (alkali, acid, oxidizing agents, organic solvent treatments, etc.), physicochemical (steam explosion, supercritical fluid, wet oxidation treatments, etc.), biological (lignin-degrading micro-organisms like white- and soft-rot fungi), and combinations thereof. The main components of lignocellulose are lignin, cellulose, and hemicellulose, in which the lignin, as a network polymer, binds with the carbohydrates (hemicelluloses and cellulose) to form a tight compact structure.[10] Therefore, it is nearly impossible to dissolve wood in conventional solvents in its native state. Although dissolving wood is challenging, recent discoveries have demonstrated that there are suitable media that may allow the dissolution of of lignocellulosic materials. This class of nonaqueous polar solvents are ionic liquids (IL) and they may address the enumerated problems associated with the compact nature of the lignocellulosic substrate.

Ionic liquids (IL) are by definition[11] low melting point (<100 °C) salts, which possess many advantages including very low vapor pressure, low flammability, recyclability, and low toxicity. The properties of ILs can be easily varied by changing the nature of the anion or cation of the liquid. This offers the possibility to fine-tune IL's to fit a desired application. Furthermore, careful selection of the cation and/or the anion makes ILs environmentally friendly with good solvating ability for various compounds playing a crucial role in green chemistry.[11−17]

Cellulose can be dissolved in 1-butyl-3-methyl- and 1-allyl-3methyl-imidazolium chloride ([BMIM]Cl and [AMIM]Cl, respectively) and in some imidazolium phosphate or formate salts.[18−23] Pretreatment technologies of lignocellulosic materials using ILs as a medium has become an active field in recent years.[24−30]

Chen et al. have reported that cellulose was effectively converted to glucose by cellulose enzymes after pretreatment with [BMIM]Cl. IL pretreatment was combined with steam

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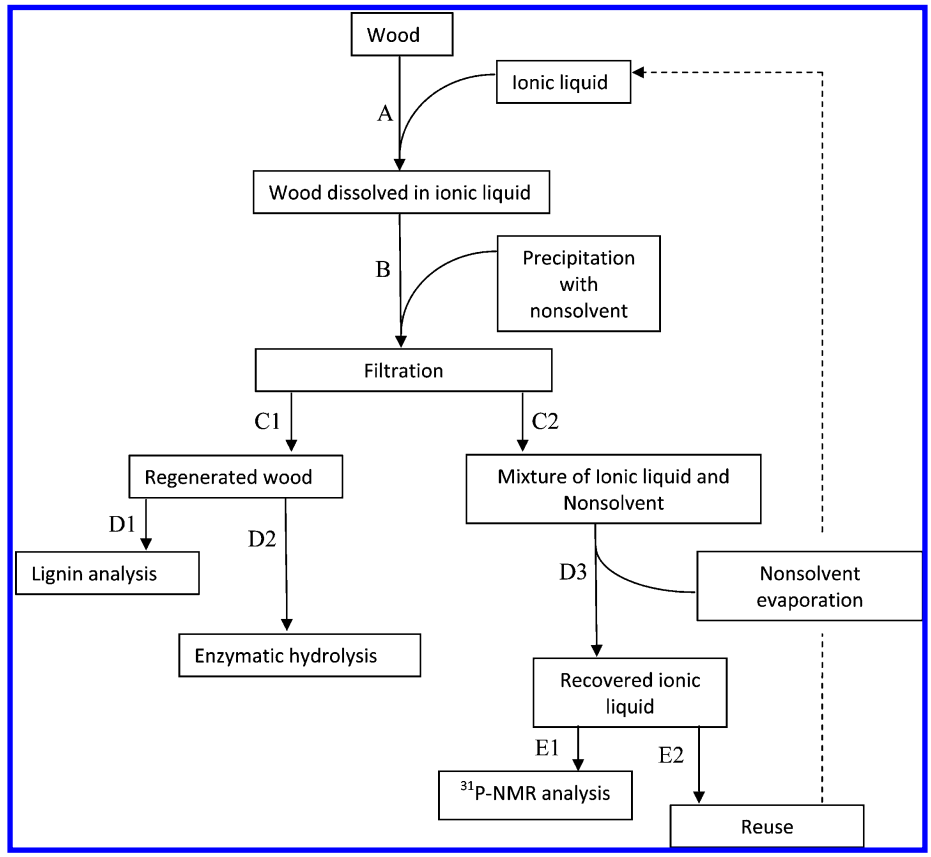
Chart 1. Schematic of the Overall Process Employed in This Study



explosion and [BIMI]Cl treatment after steam explosion gave better yield of glucose after hydrolysis.[26]

Zarvel et al. have developed high-throughput method for fast screening of dissolution power of different ionic liquids. It was found that [EMIM]Acetate was the best solvent for wood species.[31] Unfortunately the stability of [EMIM]Acetate is questionable in wood dissolution, especially if recycling of the IL solvent is desirable after dissolution. This is because wood contains naturally occurring acids (for example: glucuronic acid $pK_a = 3.18$)[32] with pK_a of a similar range as that of acetic acid ($pK_a = 4.76$).[33] As such, the acetate ion may easily become protonated forming acetic acid. This interplay of the naturally occurring acetates in wood with acetate anions of IL may seriously compromise the recycling effectiveness of acetate containing IL's.

Tan et al.[34] have developed a method for lignin extraction from lignocellulosic materials. The ionic liquid used was [EMIM] xylene sulfonate. High temperatures (170−190 °C) were used in the extraction of lignin, which required a demonstrable thermal stability for the used IL.

In our earlier work, we reported that both [BMIM]Cl and [AMIM]Cl were good solvents for softwood such as Norway spruce and southern pine, and the yield of glucose from regenerated wood was found to be significantly higher than that of untreated wood.[30]

In this paper, we have further investigated the dissolution and regeneration of three wood species (one hardwood and two softwoods) in [AMIM]Cl, and have discussed the effects of wood density, pulverization and nature of nonsolvent (for the purposes of precipitation and regeneration of wood from IL) on the efficiency of IL pretreatment (see Chart 1). Furthermore, issues related to IL recycling and reuse were investigated, since these are pivotal considerations ensuring the cost efficiency of ILs.

Experimental Section

Eucalyptus grandis, southern pine, and Norway spruce thermomechanical pulp (TMP) were the species examined and were sampled as per our earlier accounts.[35] The two softwoods (southern pine and Norway Spruce TMP) were ball-milled using a standard method reported in the literature.[3]

Unbleached Norway spruce thermomechanical pulp (TMP) was sampled in a Swedish mill. The TMP was of approximate 38% consistency and 85 mL Canadian Standard Freeness prepared by one-stage refining and a subsequent reject refining (about 20%) stage. The pulp was sampled at the press stage after the refined and refined reject pulps had been combined. This pulp currently represents a standard sample, which is the subject of Cost action E 41 entitled; "Analytical tools with applications for wood and pulping chemistry" operated by the European Union. The pulp was ground to pass a 20-mesh screen in a Wiley mill and Soxhlet extracted with acetone for 48 h. The resulting Wiley milled wood powder was air-dried and stored in a desiccator under a vacuum.

Rotary-ball milling was performed in a 5.5-L porcelain jar in the presence of 474 porcelain balls (9.4 mm in diameter), which occupied 18% of the active jar volume. One hundred grams of extractive-free wood powder were loaded into the jar, creating a porcelain ball/wood weight ratio of 16.6. The milling process was conducted at room temperature for up to 25 days with a rotation speed of 60 rpm. The pulverized samples were collected after 2, 5, and 8 days of pulverization. All wood samples used were kept in a vacuum oven at 50 °C for 48 h prior to use. After this period, the weight of the samples was found to remain constant and the moisture content was almost invariably <1%. [AMIM]Cl was synthesized by a modified literature method.[23,30]

Synthesis of 1-Allyl-3-methylimidazolium Chloride ([AMIM]Cl). This ionic liquid was synthesized by the reaction of allyl chloride with excess 1-methylimidazole to avoid the possible formation of acidic impurities. Freshly distilled allyl chloride (0.95 equivalents) was added dropwise to the solution of freshly distilled methylimidazole (1 equivalent) in dry acetone and the mixture was slowly heated to 55 °C (overnight) under a nitrogen atmosphere. After being cooled to room temperature, the acetone phase was separated and the excess methylimidazole was removed by extraction with more acetone. The crude product was added dropwise to acetone and the mixture was stirred for 5 h at room temperature (to decrease the viscosity, the mixture was allowed to be heated to 40 °C) and then the acetone phase was separated. This procedure was repeated five times. The ILs layer was separated and condensed by rotary evaporation. The crude product was further purified using active carbon in boiling methanol. After filtration and further evaporation of the volatiles, the product was dried under a vacuum at 40 °C for 48 h prior to use. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 3.83 (3 H, s), 4.73 (2H, d, $^{3}J = 6.3$ Hz), 5.10−5.20 (2H, m), 5.65−5.79 (1H, m), 7.30 (1H, s), 7.54 (1H, s), 10.10 (1H, s). ^{13}C NMR (300 MHz, $CDCl_3$) δ (ppm) 136.58, 129.92, 123.47, 121.86, 121.34, 50.98, 35.89.

Pretreatment of Wood in Ionic Liquid: Dissolution, Wood Regeneration, IL Recycling. The ionic liquid was charged into a 100 mL dried flask equipped with a mechanical stirrer, under an inert atmosphere of argon. The temperature of the dissolution process was controlled by an oil bath. The wood sample (particle size 0.1−2 mm) was then rapidly added into the ionic liquid (wood/IL 8 wt %), and the dissolution proceeded at 120 °C for 5 h with mild mechanic stirring. All wood dissolved to form a yellow or yellow/brown solution. The wood solution was gradually added into an excess of rapidly stirred regeneration solvent (distilled water or methanol). The precipitated bulky material was then filtered using a funnel (coarse glass sinter) and was washed thoroughly with additional fresh nonsolvent. A small sample of the regenerated wood was withdrawn to determine its solids content by overnight oven-drying at 110 °C allowing the regenerated wood yield to be calculated. The filtrate was condensed by rotary evaporation, dried under vacuum at 40 °C overnight to recycle the IL.

Enzymatic Hydrolysis and Glucose Determination. The regenerated wood was freeze-dried and then it was treated with cellulase (Iogen, Canada; filter paper activity) 130 FPU/mL) using a previously optimized ratio[36] of 40 FPU/g of wood. The enzymatic hydrolyses were carried out at 40 °C for 48 h using 50 mM sodium acetate buffer (pH 4.5) at 5% consistency in an orbital water bath shaker. After filtration, the solid was quantitatively collected and oven-dried overnight at 110 °C allowing for % yield determination. The filtrate from the enzymatic hydrolysis was then diluted to a volume of 250 mL using a volumetric flask. A series of 100 mL of distilled water dilutions, containing 100−1000 μL (in 100 μL intervals) of the above filtrate were prepared. Four milliliters of each dilution was then added into a test tube. A series of glucose standards with the following concentrations were also prepared: 1×10^{-6}, 2×10^{-6}, 4×10^{-6}, 6×10^{-6}, and 8×10^{-6} g/mL. In addition, the coloring reagent was prepared by mixing 1 part of reagent B with 50 parts of reagent A of BCA test kit (protein assay kit, Sigma). Reagent B is a copper solution, and reagent A is a BCA solution. The resulting solution was green in color, which should be freshly prepared for every analysis. The color was finally developed by adding (to each of the 4 mL of glucose standard solution and diluted filtrates) 1 mL of coloring reagent. The samples were then mixed using a Vortex mixer for few seconds, then allowed to react at 60 °C (protected from light by covering each tube with aluminum foil) and incubate for 2 h (stirring is not necessary). Samples containing glucose turned purple. The amount of glucose in the various samples was finally determined spectrophotometrically at 562 nm against a blank.

Table 1. Yields of Regenerated Wood and of Recycled IL after the Pretreatment of Various Wood Species with [AMIM]Cl at 120 °C for 5 h Using Water As the Nonsolvent

	wood	yield of regenerated wood (%)	yield of recovered IL (%)
1	Eucalyptus grandis	96	100
2	Southern pine	99	99
3	Norway Spruce TMP	97	98

Determination of Lignin Content. Klason lignin (acid insoluble) and acid soluble lignin contents of original and regenerated wood samples were determined according to the method published by Yeh et al.[37]

^{31}P NMR Analysis of Recycled IL. Recovered IL (0.5 g) was placed in a 5 mL vial, and then 150 μL pyridine was added and mixed by vortex to form a homogeneous solution. Next, 200−400 μL of 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane reagent was added and mixed using the vortex, allowing the formation of a yellow paste. Further additions of a solution of $Cr(acac)_3$ in $CDCl_3$ (1 mg/mL) (500 μL) followed by thorough mixing ensured a clear solution. Finally, the internal standard (N-Hydroxy-5-norbornene-2,3-dicarboxylic acid imide) solution was added, followed by another 500 μL of the solution of $Cr(acac)_3$ in $CDCl_3$ (1 mg/mL). The final solution was transferred to an NMR tube and the ^{31}P NMR spectra were recorded on a Bruker 300 MHz NMR instrument.

Results and Discussion

The pretreatment of wood was studied in accordance with the detailed flow diagram depicted in Chart 1. Initially, the wood was dissolved in ionic liquid (step A), typically an 8 wt % solution of wood in IL was used. After the wood was dissolved it was regenerated from the IL with nonsolvent (step B) and then filtered to obtain the regenerated wood (step C1) and ionic liquid with nonsolvent (step C2). The amount of lignin from the regenerated wood was determined (step D1) and the regeneration efficiency was determined via a cellulase enzymatic hydrolysis of the regenerated wood (step D2). The ionic liquid was then recycled by evaporating the nonsolvent (step D3). The accumulation of wood related components within the recycled ionic liquid was investigated using quantitative ^{31}P NMR (step E1). Furthermore, the effectiveness and the yield of recycling the IL was examined (step E2).

Wood Density Effects on IL Pretreatment. Three wood species (E. grandis, S. pine and N. Spruce TMP) were dissolved in [AMIM]Cl at 120 °C for 5 h and subsequently precipitated from the IL with water as nonsolvent (see chart 1). The regenerated wood (After step C1 in Chart 1) was nearly quantitative (>95%) and practically all the IL could be recovered (After step D3 in Chart 1; see Table 1 for details). The yield of regenerated wood and the yield of recovered IL was determined as weight percent from the original amount of wood or IL.

The data of enzymatic hydrolysis of the wood and TMP samples before and after the IL pretreatment are shown in Table 2 (entries 1−6). The yield of residual wood after enzymatic hydrolysis, yield of glucose released after enzymatic hydrolysis and efficiency of cellulose hydrolysis was calculated using eqs 1 and 2

$$\text{yield of residual wood after enzymatic hydrolysis (\%)} = (Wt_{residual}/Wt_{wood\ input}) \times 100 \quad (1)$$

$$\text{yield of glucose released (\%)} = (Wt_{glucose\ released}/Wt_{wood\ input}) \times 100 \quad (2)$$

In general, the IL pretreatment increased the amount of glucose released after the enzymatic hydrolysis. For example, during the enzymatic hydrolysis of southern pine without pretreatment, only 7 wt % glucose was released, whereas IL pretreated southern pine gave 17 wt % (2.4 fold) of glucose. The compact structure of the wood was probably partly opened during the pretreatment dissolution−regeneration procedure. Therefore, the cellulose within the wood was more efficiently degraded to glucose by the enzyme. These data are in good agreement with our previous study of Norway spruce sawdust pretreated with [AMIM]Cl.[30]

As can be seen from Table 2, the efficiency of pretreatment was different for different wood species. Here the efficiency of pretreatment was defined as the difference between the yield of wood after the enzymatic hydrolysis of original and IL-treated wood. The efficiency of pretreatment decreased with higher wood density in the following sequence: southern pine > Norway spruce TMP > eucalyptus grandis (see Figure 1). This is expected because the high-density wood has a tighter structure than low-density wood and thus is more difficult to open with the pretreatment. Therefore, pretreatment of hard wood (with high density) requires more intense conditions (higher temperatures, longer times) than that of softwood (of lower density) to reach a similar pretreatment effect.

It should be mentioned here that lower yields of polysaccharide-deficient residue were observed after the enzymatic hydrolysis of untreated wood species. In particular the residual from the enzymatic treatment of Norway Spruce TMP (78%) was found to be abnormally low considering that the preparation of powdered wood consists mostly of large particles that are known to be impenetrable to cellulolytic enzymes.[38,39] The observed yields might be due to the method used for the recovery of the enzymatically treated wood. As opposed to typically applied centrifugal separation, the samples of this study were filtered through the sintered glass funnel, which may have caused a loss of material. However, the possible overestimations made here for the efficiency of enzymatic hydrolysis will be of similar magnitude for all the examined samples and as such should not affect the overall reliability and consistency of our conclusions.

Table 2. Enzymatic Hydrolysis Data for Various Wood Species and IL Pretreated Wood Species

		Eucalyptus Grandis	Norway Spruce TMP	Southern Pine
1	wood yield % (orig. wood)	90	78	93
2	wood yield % (IL treated wood)	83	60	73
3	Δ residual[a]	7	18	20
4	yield of glucose % (orig. wood)	17	15	7
5	yield of glucose %(IL treated wood)	21	21	17
6	Δ glucose released[b]	4	6	10
7	total lignin % (orig. wood)	26[c]	28[d]	29[e]
8	hemicellulose % (orig. wood)	20[c]	26[d]	29[e]
9	cellulose % (orig. wood)	45[c]	46[d]	41[e]
10	wood air-dry density (g/cm3) (orig. wood)	0.63[f]	0.42[g]	0.27[h]

[a] Δ residual = residual (orig. wood) − residual (IL treated wood). [b] Δ glucose released = glucose released (IL treated wood) − glucose released (orig. wood). [c] See ref 42. [d] See ref 43. [e] See ref 44. [f] See ref 45. [g] See ref 46. [h] See ref 47.

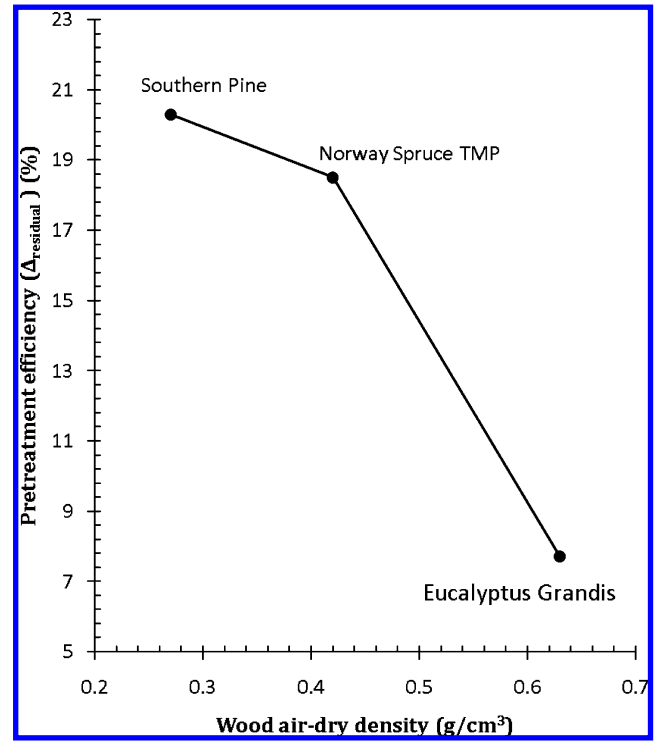


Figure 1. Effect of wood density on the pretreatment efficiency of ionic liquid.

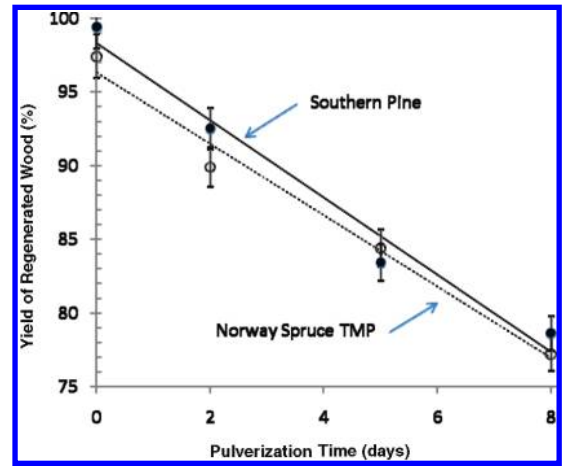


Figure 2. Effect of pulverization time on the yield of regenerated southern pine and Norway spruce TMP after IL pretreatment. Data were the average value of three duplicate experiments.

Pulverization Effects on the IL Pretreatment of Softwoods. It should be noted here that the term, "Untreated wood", implies "Willey milled original wood" without any additional treatment, like pulverization induced by ball milling or dissolution/regeneration in IL.

Two softwoods were pulverized before the IL pretreatment in order to verify the role of their accessibility toward IL within the structure of the wood. Pulverization increased the amount of glucose released from the wood during enzymatic hydrolysis. In these experiments water was used as nonsolvent in the recovery of wood. The yield of regenerated wood decreased linearly with an increase in the pulverization time. The decrease was 2% for Norway spruce TMP and 3% for southern pine per day of pulverization (see Figure 2). After 8 days of pulverization, the yield of regenerated wood decreased by about 20%.

It is known that extensive ball-milling causes degradation and chemical modification of both cellulose and lignin and thus the

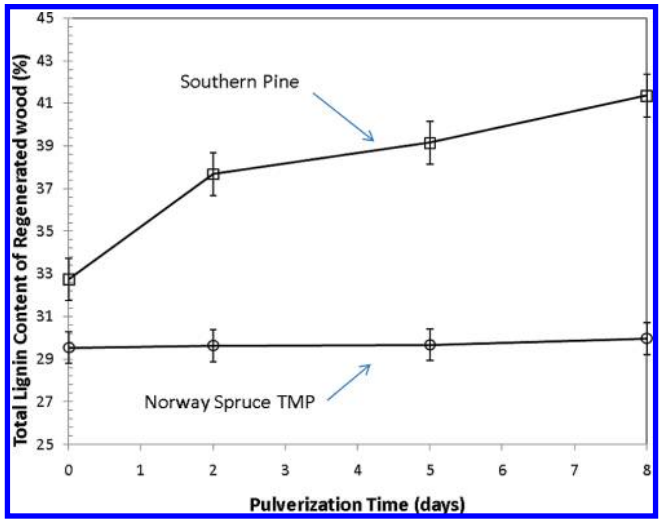


Figure 3. Combined effect of pulverization and pretreatment to lignin contents of southern pine and Norway spruce TMP. Data were the average value of three duplicate experiments.

formation of water-soluble products can not be precipitated with water.[35,40] This led to lower regenerated wood yields and lowered the recyclability of the IL.

The lignin contents of the pulverized wood after IL pretreatment were also determined (Figure 3). The lignin contents of southern pine were found to increase from 38 to 41% when the pulverization time was increased from 2 to 8 days, whereas the lignin contents of Norway spruce TMP remained nearly constant. This difference between the two softwoods might be due to the more open structure of the Norway spruce TMP than that of southern pine. Probably thermomechanical pulping opens the wood structure to some extent, causing the spruce TMP to be less responsive to the pulverization than the southern pine. The increase in lignin content with increasing pulverization time of southern pine was probably due to more hemicelluloses/cellulose remaining dissolved in the regeneration step of the pretreatment. This probably indicates that lignin is harder to degrade than hemicelluloses and cellulose components.

The identity of the components that remained in the IL after pretreatment was determined using ^{31}P NMR spectroscopy. More specifically, after the pretreatment the wood components present within the IL were phosphitylated and subjected to ^{31}P NMR. These samples showed intense signals of carboxylic acids (134.5 ppm, most probably arising from the hemicelluloses) and signals of aliphatic hydroxyl groups (150−140 ppm, arising from the cellulose and/or the dissolved hemicelluloses). In contrast only low intensity signals due to the phenolic hydroxyl groups (142−136 ppm, arising from the lignin) were detected. The main wood components present within the recovered IL were thus found to be cellulose and hemicellulose fragments, whereas most of the lignin was still present in the regenerated wood samples. This is reasonable because lignin is a cross-linked polymer, which is supposed to be more difficult to fragment with ball milling. The actual amounts of the different hydroxyl groups were calculated from the integrations of quantitative ^{31}P NMR spectra (Table 3). The concentration of aliphatic hydroxyl groups for southern pine in recovered IL (after step D3 in Chart 1) increased 2.5 fold from the original (34 μmol/g of IL) to 8-day pulverized (84 μmol/g of recovered IL). The concentration of aliphatic hydroxyl groups in IL for a Norway spruce TMP increased by 7.3 fold from the original (10 μmol/g of IL) to 8-day pulverized (73 μmol/g of recovered IL).

The enzymatic hydrolysis of pulverized softwoods before and after the IL pretreatment was also examined. (Figure 4A−D). Longer pulverization times were found to increase the yield of glucose released and decrease the amount of wood collected after enzymatic hydrolysis. The yield of wood after enzymatic hydrolysis decreased more rapidly during the initial 2 days of pulverization and a leveling off effect was observed. For example, Figure 4 A shows the yield of wood after the enzymatic hydrolysis observed for pulverized southern pine. The yield of wood without IL pretreatment decreased by 42% within the first 2 days of pulverization, whereas only 11% yield decreases were seen in the ensuing three days of pulverization. Similarly, the yield of IL pretreated wood after enzymatic hydrolysis of pulverized southern pine decreased by 26% within the first 2 days of pulverization but subsequent pulverization had nearly no effect (the yield was decreased by only about 4% during the following three days of pulverization). The amount of glucose released for pulverized southern pine was found to increase rapidly (15−16% for both before and after IL pretreatment) within the first 2 days of pulverization and was then practically constant for the remaining days of pulverization (Figure 4B). Norway spruce TMP displayed a similar tendency, as seen from Figure 4C,D. Overall, this indicates that most of the original wood structure is opened up during the first 2 days of pulverization, whereas subsequent mechanical pulverization has less of an effect. These effects were reflected on the enzymatic hydrolyses efficiencies, because they were augmented during the first 2 days of pulverization.

Comparing pulverization to IL pretreatment, pulverization (2 days) opens the wood structure more than IL pretreatment (at 120 °C for 5 h) when efficiency of enzymatic hydrolysis is used in rating enzyme accessibility. The pulverized wood with IL pretreatment gave the highest rate of cellulase hydrolysis, which demonstrated that there was a combined improvement between the pulverization and IL pretreatment. Overall, it can be deduced that a short pulverization period (<2 days) followed by an IL dissolution−regeneration cycle may offer a pre treatment that sufficiently opens up the wood structure for subsequent cellulolytic enzymatic hydrolysis.

Regeneration Nonsolvent Effect on the IL Pretreatment of Eucalyptus Grandis. Despite the described advantages of using ILs as a pretreatment media for enzymatic hydrolysis of wood they still remain relatively expensive. In an effort to reduce the costs as well as to emphasize their environmental benefits

Table 3. Concentration of Different Functional Groups Present within Recycled ILs Obtained after Wood Dissolution and Regeneration Cycles (data derived from quantitative ^{31}P NMR[35,40])

entry	wood species	aliphatic −OH (μmol/g of IL)	phenolic −OH (μmol/g of IL)	−COOH (μmol/g of IL)
1	S. Pine (orig., IL treated)	33.9	0	31.9
2	S. Pine (Ball-Milled 8days, IL treated)	83.7	0	20.1
3	N. SpruceTMP (orig., IL treated)	9.96	0	20.3
4	N. SpruceTMP (Ball-Milled 8days, IL treated)	73.0	0	33.0
5	E. Grandis (orig, clean IL treated, MeOH as nonsolvent))	61.0	0	8.61
6	E. Grandis (orig, recycled IL treated (3th cycle), MeOH as nonsolvent)	138	51.9	11.5

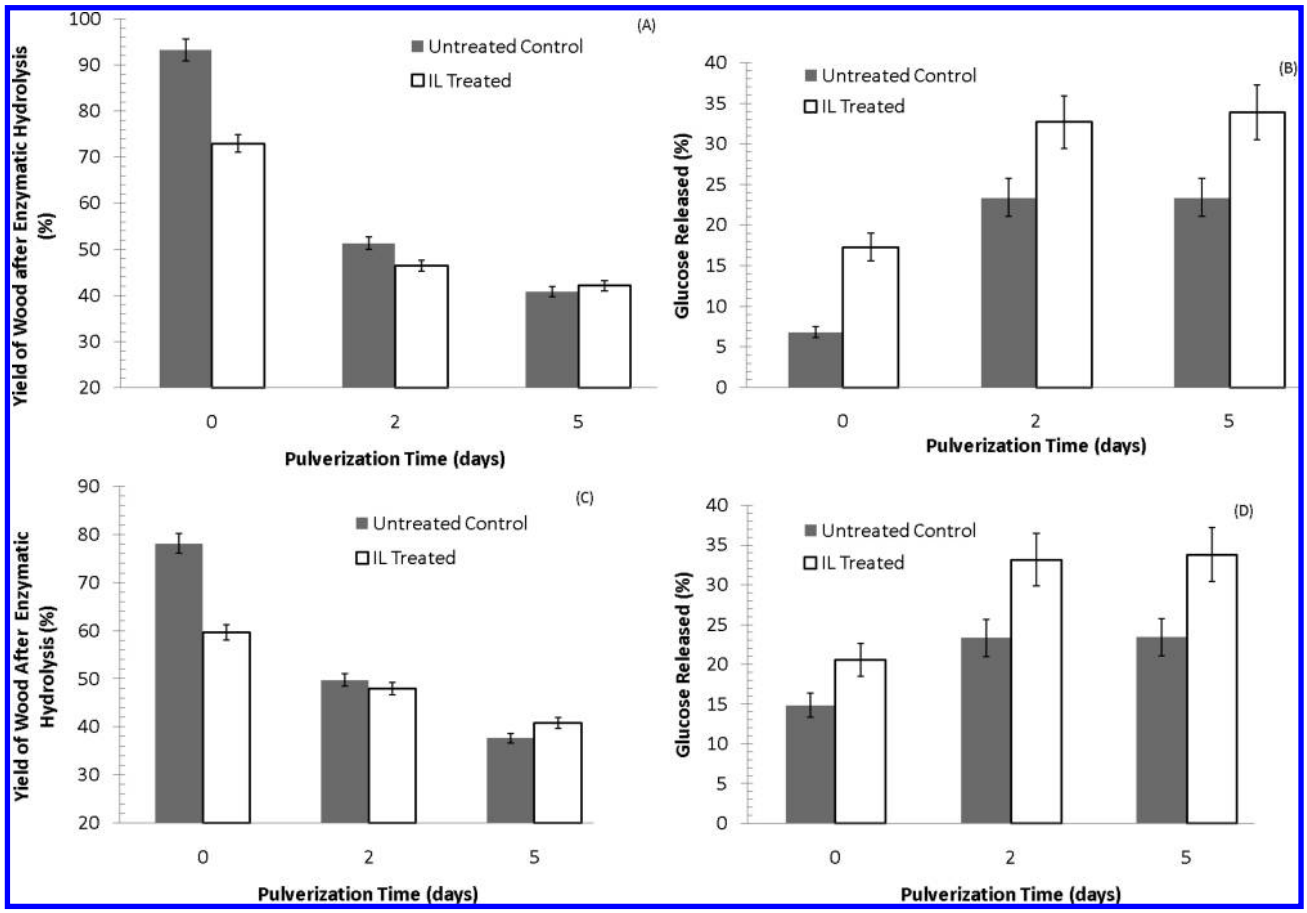


Figure 4. (A, C) Yield of wood after enzymatic hydrolysis and (B, D) the yield of glucose released for (A, B) southern pine and (C, D) Norway spruce TMP before and after IL pretreatment. Data were the average value of three duplicate experiments.

it is important to understand and document their recyclability and all associated issues with such procedures. Several factors need to be taken into consideration when efficient recycling of IL's is pursued. For instance, during the pretreatment, the choice of regeneration nonsolvent is an important consideration, which affects the yield of the regenerated wood, the yield of recycled IL and the cost of the pretreatment. During this effort, water and methanol were used and compared as the nonsolvents in wood regeneration from IL (step B in Chart 1). The yield of regenerated wood with water was higher than that of methanol at the same number of IL recycles uses (Figure 5A). The lower yields of regenerated wood obtained with methanol might be attributed to some released wood components of eucalyptus grandis (most likely tannins and extractives) dissolved in the methanol/IL mixture, which then remained within the recycled IL. Overall, for wood regeneration, water was found to be a more effective regeneration medium than methanol.

The yields of recycled IL after the pretreatment of eucalyptus grandis using different regeneration media are shown in Figure 5B (Step D3 in chart 1). After pretreating wood and recycling the IL four times, the yield of the recycled IL using methanol as the regeneration solvent was higher (96%) than when water was used during the regeneration (91%). Therefore, while methanol displayed better recovery yields of IL during the IL recycling, water was found to be a more effective nonsolvent for wood components. At this point, it is important to mention that from an environmental point of view methanol is a significantly less desirable solvent than water. Furthermore, it is likely that if the scale of the solvent recovery operation is larger, better IL recovery efficiencies are likely to be attained than those obtained for water during this effort.

The enzymatic hydrolysis of eucalyptus grandis, regenerated from IL with different nonsolvents, are shown in Figure 6A, B. The amount of glucose released from the enzymatic hydrolysis, with water as nonsolvent was always higher than that of wood regenerated with methanol as nonsolvent. Considering the higher yield of regenerated wood, the amount of glucose released and the lower cost of water; it is concluded that water is a better choice of regeneration solvent for the pretreatment of eucalyptus grandis.

Pretreatment of Eucalyptus Grandis with Recycled IL. As per our earlier data, the weight loss of wood, after pretreatment, decreased with the number of IL reuse cycles. When using water as the nonsolvent, wood was almost quantitatively precipitated (about 97%) and increasing reuse cycles did not significantly affect the wood yield (Figure 5A). However, the composition of the wood was changed with the number of IL recycles. The total lignin contents of regenerated wood were found to increase with the number of IL recycles (Figure 7). This most likely indicates that hemicelluloses remained soluble within the IL pretreatment. It is also likely that hemicelluloses and/or some cellulose degradation is taking place during the IL pretreatment process. Organic acids (such as acetic acid liberated from hemicelluloses acetates) could become enriched within the recycled IL and in turn catalyze the acidic fragmentation of the carbohydrates.[41] The details of acid catalysis on wood components in IL media will be the subject of a subsequent communication from our laboratory.

The ^{31}P NMR spectra of the phosphitylated compounds in IL from the pretreatment of eucalyptus grandis with methanol as the nonsolvent revealed the presence of carboxylic acids (9 μmol/g of IL) and aliphatic hydroxyl groups because of cellulose

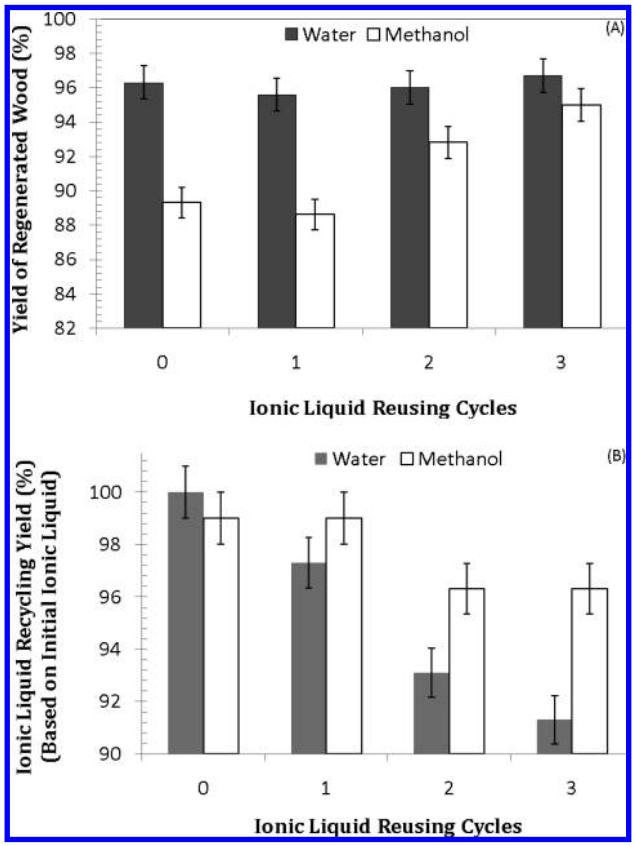


Figure 5. (A) Yield of regenerated wood and (B) the yield of IL after recycling from the pretreatment of eucalyptus grandis using different regeneration nonsolvents. Data were the average value of three duplicate experiments.

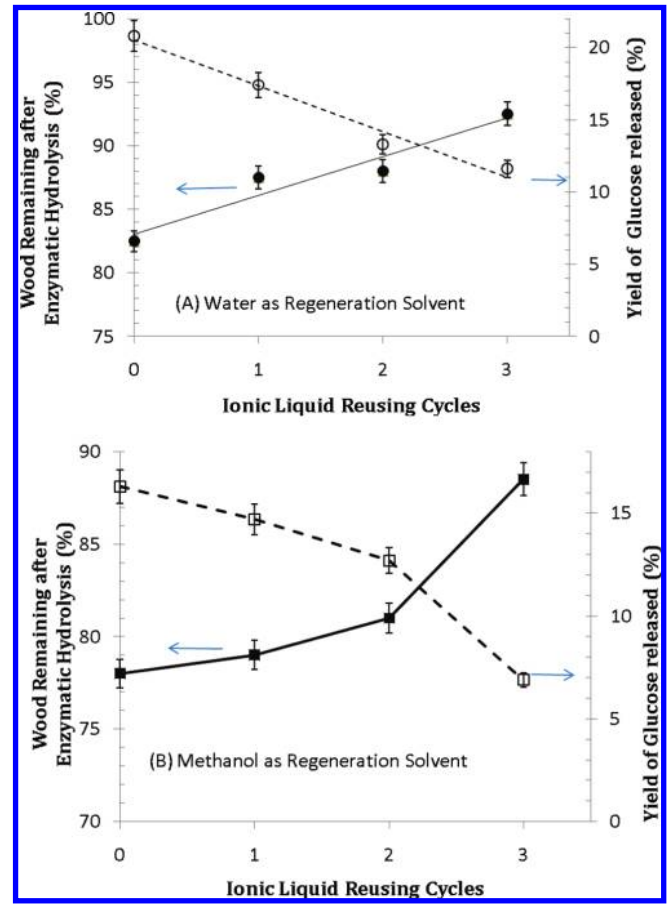


Figure 6. Effect of IL reuse cycles on the yield of wood after enzymatic hydrolysis and the yield of glucose released from IL pretreatment of eucalyptus grandis with (A) water and (B) methanol as nonsolvents. Data were the average value of three duplicate experiments..

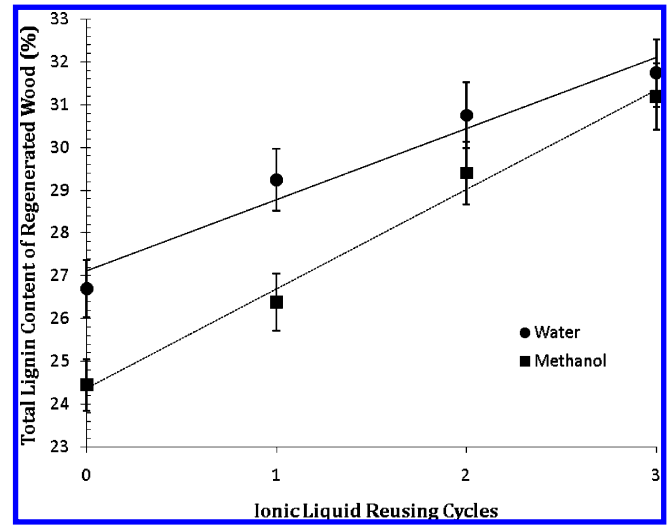


Figure 7. Lignin contents of original and IL pretreated Eucalyptus grandis with water or methanol as nonsolvent. Data were the average value of three duplicate experiments.

and/or hemicelluloses (61 μmol/g of IL). Only weak signals corresponding to the phenolic hydroxyl groups of lignin were apparent. The ^{31}P NMR spectra of the phosphitylated compounds in the recovered IL from the pretreatment of eucalyptus grandis are very similar to those of the compounds present in IL recovered from the pretreatment of the two softewoods (Table 3). The main component in IL after the pretreatment (with no IL recycling) was hemicelluloses and cellulose. However, the situation was found to be different with IL samples recycled three times during a pretreatment cycle. Significant amounts of phenolic hydroxyl groups, due to lignin were detected (52 μmol/g of IL) and the concentration of aliphatic hydroxyl groups was increased by 2.3 fold. This data was in good qualitative agreement with the data of lignin contents of regenerated wood samples (Figure 7).

The amount of glucose released by the enzymatic hydrolysis was found to decrease with the increasing number of IL reuse cycles (Figure 6). The reduced pretreatment efficiency of recycled ILs, compared to pure IL's, indicates the need to create methods of IL purification that may allow the removal of wood components from within the IL so as to preserve its pretreatment efficiency. At the same time, however, such purification protocols are to offer valuable fractionated wood components.

Conclusions

In the present paper, three wood species, two softwoods and one hardwood, were pretreated with [AMIM]Cl and then enzymatic hydrolysis was conducted. The amounts of glucose released by enzymatic hydrolyses of the regenerated wood samples were determined. Wood was regenerated in high yields from [AMIM]Cl with increased efficiency of enzymatic hydrolysis and IL recycling was realized with certain considerations. Wood species of lower density were found to have had a better response to pretreatment than wood species of higher density. Pulverization of wood resulted in lower yields of regenerated wood after pretreatment but more glucose was released during the enzymatic hydrolysis. The combination of pulverization and IL pretreatment was a good method for

increasing the rate of enzymatic hydrolysis of wood cellulose to glucose compared to previous methods. Water was found to be a better nonsolvent for the regeneration of wood from IL solutions than methanol. This is because it gave better regeneration yields of wood and higher efficiencies of cellulolytic enzymatic hydrolyses. Pretreatment of eucalyptus grandis by recycled IL's showed decreasing pretreatment efficiencies with IL reuse cycles since residual wood components accumulated within the recycled ILs. This study clearly shows that ILs require a more thorough regeneration than just water washing, in order for them to be recycled effectively.

Literature Cited

(1) Galbe, M.; Sassner, P.; Wingren, A.; Zacchi, G. Process engineering economics of bioethanol production. *Adv. Biochem. Eng. Biotechnol.* **2007**, 303–327.

(2) Sinitsyn, A.; Gusakov, A.; Vlasenko, E. Effect of structural and physico-chemical features of cellulosic substrates on the efficiency of enzymatic hydrolysis. *Appl. Biochem. Biotechnol.* **1991**, *1*, 43–59.

(3) Fan, L. T.; Lee, Y.; Gharpuray, M. M. The Nature of Lignocellulosics and Their Pretreatments for Enzymatic Hydrolysis. In *Advances in Biochemical Engineering/Biotechnology (Microbial Reactions)*; Springer: Berlin, 1982; Vol. 23, pp 157−187.

(4) Chandra, R. P.; Bura, R.; Mabee, W. E.; Berlin, A.; Pan, X.; Saddler, J. N. Substrate Pretreatment: The Key to Effective Enzymatic Hydrolysis of Lignocellulosics. In *Advances in Biochemical Engineering/Biotechnology (Biofuels)*; Springer: Berlin, 2007; Vol. 108, pp 67−93.

(5) Galbe, M.; Zacchi, G. Pretreatment of lignocellulosic materials for efficient bioethanol production. *Adv. Biochem. Eng. Biotechnol.* **2007**, 41–65.

(6) Jørgensen, H.; Kristensen, J. B.; Felby, C. Enzymatic conversion of lignocellulose into fermentable sugars: challenges and opportunities. *Biofuels, Bioprod. Biorefin.* **2007**, *2*, 119–134.

(7) Singh, P. N.; Robinson, T.; Singh, D. Pretreatment of lignocellulosic substrates. In *Concise Encyclopedia of Bioresource Technology*; Pandey, A., Ed.; CRC Press: Boca Raton, FL, 2004; pp 663−670.

(8) Mosier, N.; Wyman, C.; Dale, B.; Elander, R.; Lee, Y. Y.; Holtzapple, M.; Ladisch, M. Features of promising technologies for pretreatment of lignocellulosic biomass. *Bioresour. Technol.* **2005**, *6*, 673–686.

(9) Kumar, P.; Barrett, D. M.; Delwiche, M. J.; Stroeve, P. Methods for Pretreatment of Lignocellulosic Biomass for Efficient Hydrolysis and Biofuel Production. *Ind. Eng. Chem. Res.* **2009**, *8*, 3713–3729.

(10) Adler, E. Lignin chemistry—past, present and future. *Wood Sci. Technol.* **1977**, *3*, 169–218.

(11) Welton, T. Room-Temperature Ionic Liquids. Solvents for Synthesis and Catalysis. *Chem. Rev.* **1999**, *8*, 2071–2083.

(12) Wasserscheid, P.; Keim, W. Ionic Liquids−New Solutions for Transition Metal Catalysis. *Angew. Chem., Int. Ed.* **2000**, *21*, 3772–3789.

(13) Dupont, J.; de Souza, R. F.; Suarez, P. A. Z. Ionic Liquid (Molten Salt) Phase Organometallic Catalysis. *Chem. Rev.* **2002**, *10*, 3667–3692.

(14) Parvulescu, V. I.; Hardacre, C. Catalysis in Ionic Liquids. *Chem. Rev.* **2007**, *6*, 2015–2665.

(15) Miao, W.; Chan, T. H. Ionic-Liquid-Supported Synthesis: A Novel Liquid-Phase Strategy for Organic Synthesis. *Acc. Chem. Res.* **2006**, 897–908.

(16) Han, X.; Armstrong, D. W. Ionic Liquids in Separations. *Acc. Chem. Res.* **2007**, *11*, 1079–1086.

(17) Hardacre, C.; Holbrey, J.; Nieuwenhuyzen, M.; Youngs, T. Structure and Solvation in Ionic Liquids. *Acc. Chem. Res.* **2007**, 1146–1155.

(18) El Seoud, O. A.; Koschella, A.; Fidale, L. C.; Dorn, S.; Heinze, T. Applications of Ionic Liquids in Carbohydrate Chemistry: A Window of Opportunities. *Biomacromolecules* **2007**, *9*, 2629–2647.

(19) Swatloski, R. P.; Spear, S. K.; Holbrey, J. D.; Rogers, R. D. Dissolution of Cellose with Ionic Liquids. *J. Am. Chem. Soc.* **2002**, *18*, 4974–4975.

(20) Fukaya, Y.; Hayashi, K.; Wada, M.; Ohno, H. Cellulose dissolution with polar ionic liquids under mild conditions: required factors for anions. *Green Chem.* **2008**, 44–46.

(21) Fukaya, Y.; Sugimoto, A.; Ohno, H. Superior Solubility of Polysaccharides in Low Viscosity, Polar, and Halogen-Free 1,3-Dialkylimidazolium Formates. *Biomacromolecules* **2006**, *12*, 3295–3297.

(22) Cuissinat, C.; Navard, P.; Heinze, T. Swelling and dissolution of cellulose. Part IV: Free floating cotton and wood fibres in ionic liquids. *Carbohydr. Polym.* **2008**, *4*, 590–596.

(23) Wu, J.; Zhang, J.; Zhang, H.; He, J.; Ren, Q.; Guo, M. Homogeneous Acetylation of Cellulose in a New Ionic Liquid. *Biomacromolecules* **2004**, *2*, 266–268.

(24) Gurin, M. H. Process for biomass treatment with higher energy efficiency. U.S. 2007 161 095, 2007.

(25) Okuda, N.; Ono, Y.; Nomura, T.; Sato, M.; Miwa, H. Method for pretreatment of lignocellulose before enzymatic hydrolysis for production of sugars. JP 2006 246 711, 2006.

(26) Liu, L.; Chen, H. Enzymatic hydrolysis of cellulose materials treated with ionic liquid [BMIM]Cl. *Chin. Sci. Bull.* **2006**, *20*, 2432–2436.

(27) Li, C.; Wang, Q.; Zhao, Z. K. Acid in ionic liquid: An efficient system for hydrolysis of lignocellulose. *Green Chem.* **2008**, 177–182.

(28) Fort, D. A.; Remsing, R. C.; Swatloski, R. P.; Moyna, P.; Moyna, G.; Rogers, R. D. Can ionic liquids dissolve wood? Processing and analysis of lignocellulosic materials with 1-*n*-butyl-3-methylimidazolium chloride. *Green Chem.* **2007**, 63–69.

(29) Xie, H.; King, A.; Kilpelainen, I.; Granstrom, M.; Argyropoulos, D. S. Thorough Chemical Modification of Wood-Based Lignocellulosic Materials in Ionic Liquids. *Biomacromolecules* **2007**, *12*, 3740–3748.

(30) Kilpeläinen, I.; Xie, H.; King, A.; Granstrom, M.; Heikkinen, S.; Argyropoulos, D. S. Dissolution of Wood in Ionic Liquids. *J. Agric. Food Chem.* **2007**, *22*, 9142–9148.

(31) Zavrel, M.; Bross, D.; Funke, M.; Büchs, J.; Spiess, A. C. High-throughput screening for ionic liquids dissolving (ligno-)cellulose. *Bioresour. Technol.* **2009**, *9*, 2580–2587.

(32) Fernandes, Diniz; Jorge, M. B.; Herrington, T. M. pKa determination of weak acids over a large pH range. *J. Chem. Eng. Data* **1993**, *1*, 109–111.

(33) Lide, D. R. Section 8: Analytical chemistry. In *CRC Handbook of Chemistry and Physics*; Lide, D. R., Ed.; CRC Press: Boca Raton, FL, 2008; pp 8−42.

(34) Tan, S. S. Y.; MacFarlane, D. R.; Upfal, J.; Edye, L. A.; Doherty, W. O. S.; Patti, A. F.; Pringle, J. M.; Scott, J. L. Extraction of lignin from lignocellulose at atmospheric pressure using alkylbenzenesulfonate ionic liquid. *Green Chem.* **2009**, 339–345.

(35) Guerra, A.; Filpponen, I.; Lucia, L. A.; Saquing, C.; Baumberger, S.; Argyropoulos, D. S. Toward a Better Understanding of the Lignin Isolation Process from Wood. *J. Agric. Food Chem.* **2006**, *16*, 5939–5947.

(36) Argyropoulos, D. S.; Sun, Y.; Paluš, E. Isolation of residual kraft lignin in high yield and purity. *J. Pulp Paper Sci.* **2002**, *2*, 50–53.

(37) Yeh, T.; Yamada, T.; Capanema, E.; Chang, H.; Chiang, V.; Kadla, J. F. Rapid Screening of Wood Chemical Component Variations Using Transmittance Near-Infrared Spectroscopy. *J. Agric. Food Chem.* **2005**, *9*, 3328–3332.

(38) Blanchette, R. A.; W; Krueger, E.; Haight, J. E.; Masood, A.; Akin, D. E. Cell wall alterations in loblolly pine wood decayed by the white-rot fungus, Ceriporiopsis subvermispora. *J. Biotechnol.* **1997**, *2*−*3*, 203–213.

(39) Fukazawa, K.; Revol, J. -.F; Jurasek, L.; Goring, D. A. I. Relationship between ball milling and the susceptibility of wood to digestion by cellulase. *Wood Sci. Technol.* **1982**, *4*, 279–285.

(40) Lu, F.; Ralph, J. Non-degradative dissolution and acetylation of ball-milled plant cell walls: high-resolution solution-state NMR. *Plant J.* **2003**, *4*, 535–544.

(41) Zhuang, X.; Wang, S.; Yuan, Z.; Luo, Z.; Wu, C.; Cen, K. Analysis of cellulose hydrolysis products in extremely low acids. *Nongye Gongcheng Xuebao* **2007**, *2*, 177–182.

(42) Emmel, A.; Mathias, A. L.; Wypych, F.; Ramos, L. P. Fractionation of Eucalyptus grandis chips by dilute acid-catalysed steam explosion. *Bioresour. Technol.* **2003**, *2*, 105–115.

(43) Bertaud, F.; Holmbom, B. Chemical composition of earlywood and latewood in Norway spruce heartwood, sapwood and transition zone wood. *Wood Sci. Technol.* **2004**, *4*, 245–256.

(44) Ramsden, M. J.; Blake, F. S. R. A kinetic study of the acetylation of cellulose, hemicellulose and lignin components in wood. *Wood Sci. Technol.* **1997**, *1*, 45–50.

(45) Dickson, R.; Joe, B.; Johnston, D.; Notaras, S.; Notaras, B.; Austin, S.; Ribton-Turner, F.; Harris, P. *Pre-processing Prediction of Wood Quality in Peeler logs and Saw logs*; InnovaTek: Richland, WA, April 4 2009.

(46) Jiang, Z.; Peng, Z. *Wood Properties of the Global Important Tree Species*; Science Press: Beijing, 2001;.

(47) Sweet, M. S.; Winandy, J. E. Influence of Degree of Polymerization of Cellulose and Hemicellulose on Strength Loss in Fire-Retardant-Treated Southern Pine. *Holzforschung* **1999**, *3*, 311–317.

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