ACS Sustainable Chemistry & Engineering

Research Article

pubs.acs.org/journal/ascecg

Separation of Lignin from Corn Stover Hydrolysate with Quantitative Recovery of Ionic Liquid

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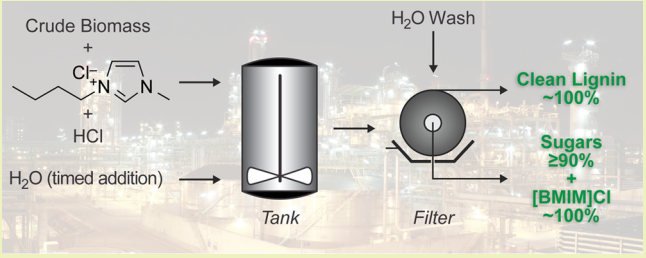
ABSTRACT: Abundant lignocellulosic biomass could become a source of sugars and lignin, potential feedstocks for the now emergent biorenewable economy. The production and conversion of sugars from biomass have been well-studied, but far less is known about the production of lignin that is amenable to valorization. Here we report the isolation of lignin generated from the hydrolysis of biomass dissolved in the ionic liquid 1-butyl-3-methylimidazolium chloride. We show that lignin can be isolated from the hydrolysate slurry by simple filtration or centrifugation, and that the ionic liquid can be recovered quantitatively by a straightforward wash with water. The isolated lignin is not only free from ionic liquid but also lacks cellulosic residues and is substantially depolymerized, making it a promising feedstock for valorization by conversion into fuels and chemicals.



KEYWORDS: *Bagasse, biorefinery, 1-butyl-3-methylimidazolium chloride, centrifugation, corn stover, filtration, techno-economics, valorization*

INTRODUCTION

Between 80 and 85% of the energy for human activity comes from the combustion of fossil fuels, especially oil.[1] The ongoing transition from a world of plentiful and cheap oil to one of tight supply and high cost will be a major challenge for humanity.[2] The accompanying release of greenhouse gases and other pollutants, which threaten both climate and health, propels the identification of alternative sources for fuels as well as bulk chemicals into a societal imperative.[3] Lignocellulosic biomass is an abundant, benign, and renewable natural resource that has the potential to meet this challenge.[4,5]

Lignocellulosic biomass is composed largely of three biopolymers: cellulose, hemicelluloses, and lignin. Cellulose is the most abundant biopolymer on the planet. Its hydrolysis yields glucose, which can be converted by either enzymic or chemical catalysts into a wide variety of important industrial compounds such as ethanol, 2-butanol, glucaric acid, lactic acid, and 5-(hydroxymethyl)furfural (HMF).[6−8] Hemicelluloses are heteropolymers that can be converted into various monosaccharides, including glucose, xylose, mannose, and galactose. These monomers can feed the manufacturing of an even wider array of useful chemicals. Finally, lignin is a heteropolymer composed of aromatic alcohols and accounts for 30% of organic carbon on Earth.[9]

Lignin has been regarded as a low-value byproduct of the papermaking and biofuels industries.[10] For example, of the 50 million tons of lignin isolated from the paper industry, 98% was burned locally for heat and electrical power, and to recover the inorganic pulping chemicals. Nevertheless, lignin has the potential to serve as a highly valuable resource for the production of aromatic chemicals and materials such as carbon fiber.[11−16] Further, in the fuels industry, the feedstock for fuel production usually accounts for 50% of total costs.[9] Utilizing the lignin to make high-value commodities, rather than merely burning it, could enhance the viability of a bioeconomy.[16]

Previously, we demonstrated that high-yields of monomeric sugars could be obtained from raw corn stover by first dissolving the biomass in a suitable ionic liquid and subsequently controlling the rate of hydrolysis with the timed additions of both a mineral acid catalyst (HCl) and water.[17] This initial work equaled or surpassed yields obtained by enzymatic hydrolysis with a reaction lasting hours instead of days. Subsequently, we reported the optimization of reaction conditions, now affording nearly quantitative yields of both glucose (92%) and xylose (95%) from stover in the ionic liquid

Received: November 16, 2014
Revised: January 22, 2015
Published: February 20, 2015

DOI: 10.1021/sc500731c
ACS Sustainable Chem. Eng. 2015, 3, 606−613

1-butyl-3-methylimidazolium chloride ([BMIM]Cl). The same reaction also afforded lignin as a solid precipitate by the end of the reaction, commensurate with its low solubility in that ionic liquid.[18,19] ^{1}H, ^{13}C HSQC NMR characterizations of the solid byproduct revealed clean and underivatized lignin structures that were free of cellulosic residue.

Even though this material presents an attractive feedstock for the development of lignin valorization routes, its large-scale production will not be realized unless a cost-effective means for recovering the ionic liquid is developed. Furthermore, our techno-economic estimates revealed that ionic liquid recovery rates should hold above ∼99% overall in order to ensure economic viability. This target surpasses all recoveries obtained to date. Dibble and co-workers used 1-ethyl-3-methylimidazolium acetate to dissolve corn stover and reduce its recalcitrance to enzymatic hydrolysis (i.e., as a pretreatment). To precipitate lignocellulose without the formation of an intractable gel, they optimized a ketone and alcohol antisolvent mixture. This route afforded polysaccharide, lignin and aliphatic-rich fractions, while recovering 89% of the original ionic liquid.[20] Later, Shi and co-workers used a thermostable and ionic liquid-tolerant enzyme cocktail to enable the one-pot pretreatment and saccharification of switchgrass.[21] This novel approach afforded 81.2% of the glucose and 87.4% of the xylose in both monomeric and oligomeric forms, and returned 90.8% of the ionic liquid after the sugar was extracted by complexation with a boronic acid.

To the best of our knowledge, no other attempts have improved meaningfully on ionic liquid recoveries or met techno-economic targets for commercial viability. Also, the recovery of ionic liquid from solids has been investigated in the context of pretreatment,[20,21] but not after a solution-phase hydrolysis. Here we report on the isolation and purification of lignin derived from biomass hydrolysis in [BMIM]Cl. Our study anticipates efficient methods for washing lignin and recovering ionic liquid in an industrial setting. In addition, we provide data on the integrity of the isolated lignin. The results highlight the ability of ionic liquids to mediate the conversion of biomass into sugars and clean lignin, feedstocks suitable for a variety of high-value applications.

RESULTS AND DISCUSSION

The hydrolysis of untreated corn stover in [BMIM]Cl using a mineral acid (HCl) catalyst provides nearly quantitative yields of glucose (92%) and xylose (95%).[3] Lignin does not dissolve in this ionic liquid, and is readily isolable by filtration. Filtration produces a wet lignin cake that retains ionic liquid in its interstitial fluid (liquor). That ionic liquid must be removed so as to produce lignin that is clean and thus amenable to valorization. Also, the removed ionic liquid must be recycled nearly quantitatively (>99%) into the process to avoid significant replacement costs and operating expenses.

Water is a low-cost solvent that is environmentally benign and dissolves [BMIM]Cl. Hence, water was chosen as the eluent for five methods used to wash lignin. Lignin was derived from corn stover and had the composition listed in Table 1. All washes were done in D_2O containing 3-(trimethylsilyl)-propionic-2,2,3,3-d_4 acid, sodium salt (TMSP-d_4), allowing for the quantification of [BMIM]Cl in solution by ^{1}H NMR spectroscopy (Figure 1). Lignin is soluble in dimethyl sulfoxide (DMSO). Adding a known concentration (1% v/v) of tetramethylsilane (TMS) to the DMSO-d_6 solvent allows for the quantification of the [BMIM]Cl retained on the lignin, again by ^{1}H NMR spectroscopy.

Table 1. NREL Composition Analysis of Corn Stover (2010 Harvest Year)[a]

component	composition (%)
glucan	31.42
xylan	18.76
galactan	1.36
arabinan	3.29
sucrose	0.57
glucose	0.93
xylose	0.00
galactose	0.20
arabinose	0.00
fructose	1.11
acetyl groups	2.22
lignin	14.31
proteins	4.79
ash	13.39

[a]The difference between a 100% mass balance and the sum of the percent compositions of individual components can be attributed to unidentified water extractives.

Wash Method 1: Wet Solids at 20 °C. Wet solids from the hydrolysis of 375 mg of corn stover were washed with five 10 mL aliquots of D_2O. Filtration times and observations for each aliquot are listed in Table 2 and shown in Figure 2A. The first aliquot of filtrate was light brown in color and cloudy due to solid particulates that were small enough to pass through the frit. Subsequent aliquots of filtrate were translucent, as expected, as particulates in the fluid "bridge" the pores in the frit, blocking additional particles but not the liquid. Also, subsequent aliquots became lighter in color, from yellow in the second aliquot, to and clear and colorless in the fifth. This color change was due to decreasing concentrations of [BMIM]Cl. Whereas the concentrations of [BMIM]Cl dropped with each addition of D_2O, filtration times increased markedly. This result could be due to the solid agglomerations breaking apart into fine lignin particles, forming a silt-like material that deters fluid movement inside the frit. Attempts with common filter paper instead of fritted glass resulted in much faster filtration rates, suggesting these results are partially dependent on the experimental setup. This filter cake was found to be 2.5 mm in thickness and 22 mm in diameter, and to have a dry mass of 89.4 mg.

An ^{1}H NMR spectrum was obtained of each aliquot of filtrate to determine the amount of eluted [BMIM]Cl. An ^{1}H NMR spectrum was also obtained of the washed solids in DMSO-d_6 to determine the amount of retained [BMIM]Cl. Approximately 455 mg of [BMIM]Cl was detected in the filtrate, whereas 0.09 mg of [BMIM]Cl was retained on the solids. Accordingly, of the total [BMIM]Cl originally retained by the solids, 99.98% was removed by this wash method. This value indicates that a straightforward water wash could remove [BMIM]Cl nearly quantitatively. The resulting stream, dilute aqueous ionic liquid, can be returned to use in the process.

The recovery of [BMIM]Cl using Wash Method 1 is promising. Still, the length of time and amount of energy required to generate and wash solids in an industrial setting could compromise utility. Accordingly, we explored other wash methods.

DOI: 10.1021/sc500731c
ACS Sustainable Chem. Eng. 2015, 3, 606−613

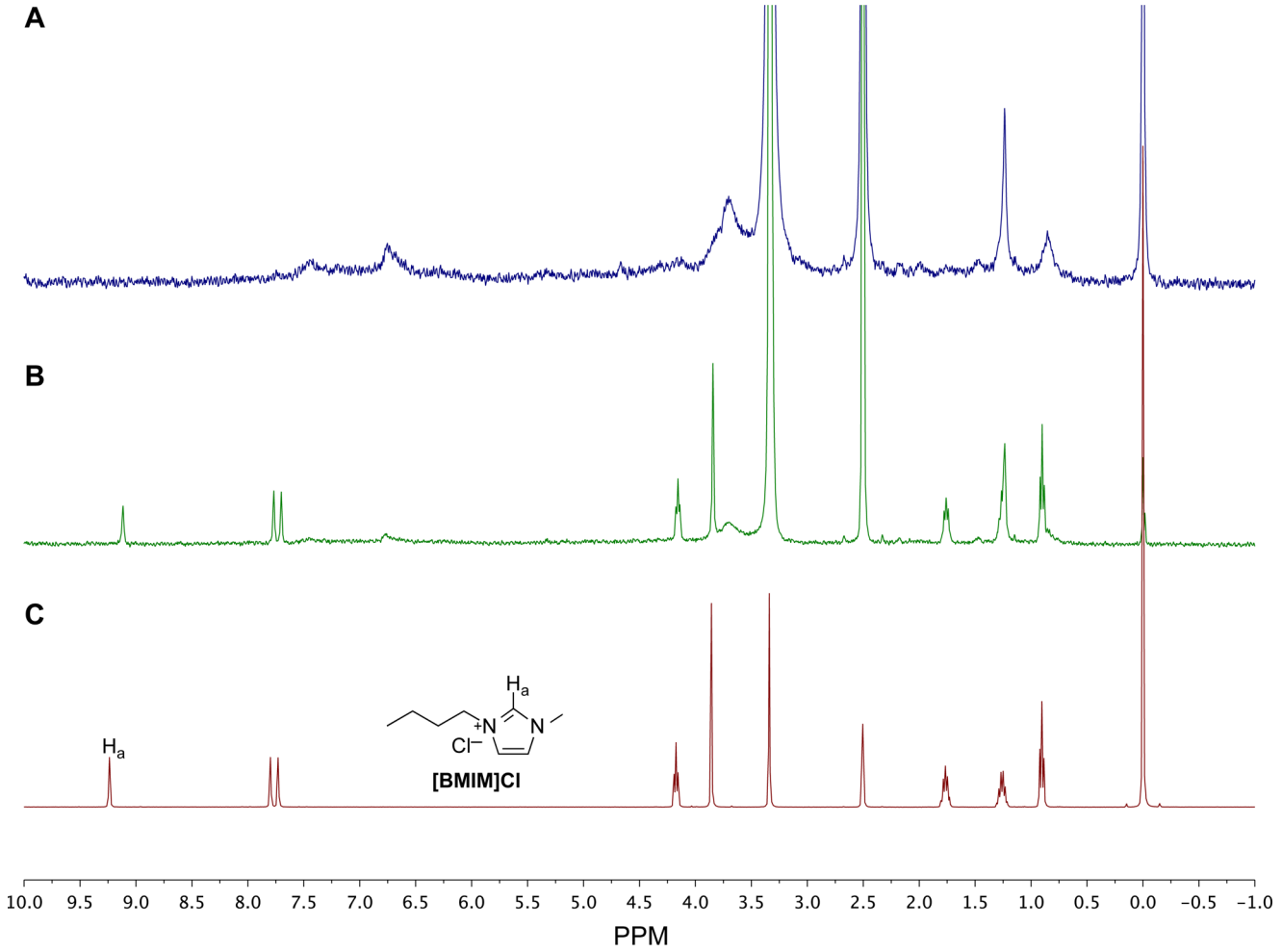


Figure 1. Representative ^{1}H NMR spectra of lignin in DMSO-d_6. (A) After removal of [BMIM]Cl using Wash Method 2. (B) Before washing of [BMIM]Cl. (C) [BMIM]Cl only (no lignin).

Table 2. Data for Washing Method 1: Wet Solids at 20 °C[a]

aliquot	filtration time (min)	[BMIM]Cl detected (mg)	observations
1	16	425.7	cloudy, some small particulates
2	12	27.2	no visible solid
3	28	2.4	no visible solid
4	37	<0.05	no visible solid
5	56	<0.05	no visible solid

[a]Aliquots of room temperature D_2O (5 × 10 mL) were poured over wet solids from a conversion of corn stover to elute retained [BMIM]Cl.

Wash Method 2: Dried Solids at 20 °C. Dried solids from a hydrolysis reaction were washed with five 10 mL aliquots of D_2O. Filtration times and observations for each aliquot are listed in Table 3 and shown in Figure 2B. Although filtration-time still increased as more filtrate passed through the solid filter cake, the overall filtration time for each of the five 10 mL aliquots of D_2O was significantly shorter for the washing of a dried pellet compared to the washing of a wet pellet, as in Wash Method 1 (Tables 2 and 3). Approximately 278 mg of [BMIM]Cl was detected in the filtrate. It is important to note that the amounts of [BMIM]Cl detected in filtrates of different wash methods are not comparable directly. More ionic liquid being detected in the Wash Method 1 is likely due to slightly less supernatant being decanted after centrifugation to isolate the solids. The key observation that distinguishes a successful wash method is the amount of [BMIM]Cl retained by the solids after washing. The ^{1}H NMR spectrum of the solids from Wash Method 2 showed no evidence of ionic liquid being retained, indicating a successful wash.

Compared to Wash Method 1, Wash Method 2 used more water to elute the same percentage of retained [BMIM]Cl. In the first, 99.98% of the retained ionic liquid was removed with 30 mL of eluent; whereas in the second, 98.53% was removed with 30 mL of eluent. When using the full 50 mL of water, however, the second method seemed to be slightly more effective because the solids did not retain any detectable [BMIM]Cl after the wash.

Wash Method 3: Wet Solids at 50 °C. Wet solids from a hydrolysis reaction were washed with five 10 mL aliquots of D_2O that had been heated to 50 °C. Filtration times and observations for each aliquot are listed in Table 4. Filtration times of the five 10 mL aliquots of warm D_2O were not significantly shorter than with ambient temperature aliquots. Additionally, because some of the D_2O evaporated during heating, the concentration of the NMR standard was unknown. Hence, NMR spectroscopy could not be used to measure the amount of [BMIM]Cl in each sample of filtrate. Nonetheless, an ^{1}H NMR spectrum of the washed solids indicated the complete removal of [BMIM]Cl. Accordingly, Wash Method 3

DOI: 10.1021/sc500731c
ACS Sustainable Chem. Eng. 2015, 3, 606−613

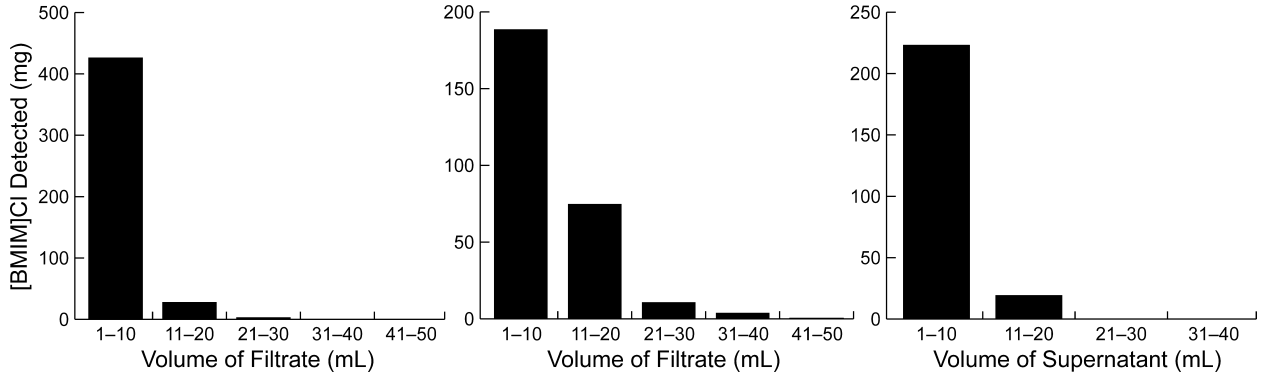


Figure 2. [BMIM]Cl in filtrate after washing solids at 20 °C. (A) Using Wash Method 1 (wet solids at 20 °C). (B) Using Wash Method 2 (dry solids at 20 °C). (C) Using Wash Method 5 (centrifugation).

Table 3. Data for Washing Method 2: Dried Solids at 20 °C[a]

aliquot	filtration time (min)	[BMIM]Cl detected (mg)	observations
1	0.5	188.6	cloudy, no large solid particulates
2	3.5	74.8	no visible solid
3	6	10.7	no visible solid
4	8	3.8	no visible solid
5	12	0.3	no visible solid

[a]Aliquots of room temperature D_2O (5 × 10 mL) were poured over dried solids from a conversion of corn stover to elute retained [BMIM]Cl.

Table 4. Data for Washing Method 3: Wet Solids at 50 °C[a]

aliquot	filtration time (min)	[BMIM]Cl detected (mg)	observations
1	14	not accurately measurable by NMR spectroscopy	some solid particulates
2	11		no visible solid
3	24		no visible solid
4	45		no visible solid
5	66		no visible solid

[a]Aliquots of D_2O (5 × 10 mL) heated to 50 °C were poured over wet solids from a conversion of corn stover to recover retained [BMIM]Cl.

was completely effective even though the warmer temperature of the filtrate did not confer the expected improvement in filtration rate.

Wash Method 4: Dried Solids at 50 °C. Dried solids from a hydrolysis reaction were washed with five 10 mL aliquots of D_2O heated to 50 °C. Filtration times and observations for each aliquot are listed in Table 5. This method had the highest filtration rate of methods 1−4, confirming that overall filtration times are significantly shortened by drying the solids prior to washing. Still, filtration times increased as more water passed through the lignin cake, similar to Wash Method 2. Here, like before, there was no evidence of [BMIM]Cl retention on washed solids, indicating an effective wash. We conclude that heating the eluent can provide a kinetic advantage to the washing of a dry but not a wet pellet.

Table 5. Data for Washing Method 4: Dried Solids at 50 °C[a]

aliquot	filtration time (min)	[BMIM]Cl detected (mg)	observations
1	1	not accurately measurable by NMR spectroscopy	cloudy, no large solid particulates
2	4		no visible solid
3	5		no visible solid
4	6.5		no visible solid
5	8		no visible solid

[a]Aliquots of D_2O (5 × 10 mL) heated to 50 °C were poured over dried solids from a conversion of corn stover to recover retained [BMIM]Cl.

Wash Method 5: Centrifugation. Centrifugation presents an alternative to filtration, having different time and energy requirements. In this method, the lignin is slurried with water, and the lignin is collected by centrifugation. ^{1}H NMR spectroscopy indicated that [BMIM]Cl was not retained on the solids following centrifugation (Figure 2C), indicating that centrifugation can be as effective as filtration as a wash method. As with filtration, >99% of the [BMIM]Cl initially retained by the solids was removed in 30 mL of wash fluid (Figure 1). Additionally, washing by centrifugation required an amount of time similar to filtration washes of dried pellets performed at 50 °C.

The small number of centrifugations possible before a pellet fails to form is a shortcoming of this wash method. This limitation could make the isolation of solids more difficult and lead to the loss of material when decanting if more than three rounds of centrifugation are necessary. This issue could be minor, as almost the entirety of the retained ionic liquid was removed after only three rounds. The scalability of this method is a worthwhile subject for a future study.

At industrial scales, centrifugation could be more cost-effective overall than vacuum filtration. Even though centrifuges are generally more expensive than vacuum filters and have higher operating and maintenance costs, the size of the centrifuge could be much smaller by achieving faster filtration and wash rates. The best choice will depend on the ultimate characteristics of the hydrolysate slurry generated in the industrial facility, and the scalability of centrifugation.

Reducing Water Use. Thus far, the ratios of wash water volume to cake liquor volume, or wash ratio, have been excessive so as to determine the maximum recoverability of ionic liquid. As quantitative recoveries of the ionic liquid became established, we shifted our focus to determining the minimal amount of wash water necessary to produce the same outcome. To this end, we measured the amount of ionic liquid remaining in the lignin cake at increasing wash ratios in an industrially relevant manner. This measure was attained by performing a series of displacement washes of the undisturbed cake with relatively small amounts of water. In an ideal

DOI: 10.1021/sc500731c
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displacement wash, one unit volume of water displaces one unit volume of liquor, recovering 100% of the liquor with a wash ratio of 1. Actual washes are less efficient, often requiring wash ratios of 3 or more.

The percentage of ionic liquid remaining in the lignin cake as a function of the wash ratio is shown in Figure 3. The results

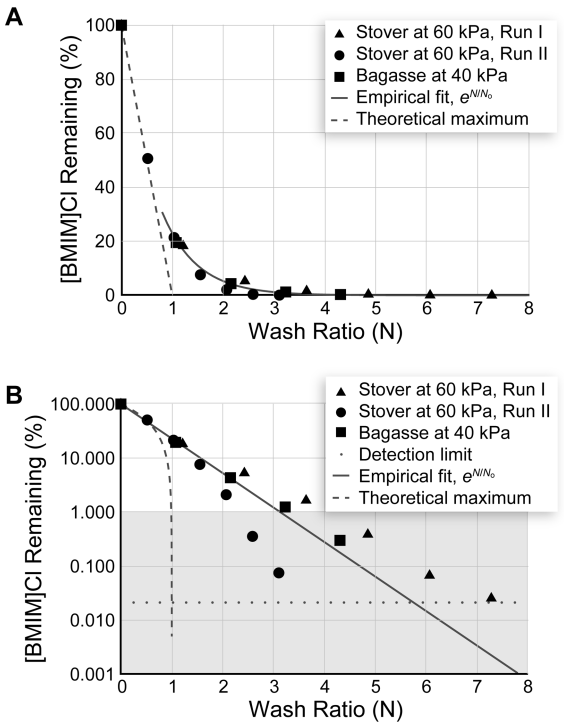

Figure 3. [BMIM]Cl in the lignin cake after washing at 20 °C. (A) Linear scale. (B) Semilogarithmic scale. The ionic liquid remaining in the cake, as quantified by high performance liquid chromatography (HPLC), is shown after successive washes with deionized water. The wash ratio is the volume ratio of wash water to cake liquor. An empirically fitted gray line is shown as a guide to the eye, and the theoretical maximum wash efficiency is depicted by the dashed gray line. The techno-economic target region for ionic liquid recovery is the shaded area in panel B. The dashed gray line marks the detection limit.

for three separate wash series, which encompass cakes derived from two biomass sources (corn stover and bagasse) and two pressure drops (40 and 60 kPa), are all comparable and resemble cake-washing curves measured for a variety of other industrial applications. The discrepancies between the series may be due to the different wash-ratio increments implemented, with smaller increments being more mass-efficient at recovering the ionic liquid. A simple exponential function of the form e^{-N/N_0}, where N_0 is a free parameter, was fitted to all data and overplotted to guide the eye. The theoretical maximum curve represents the efficiency of a perfect slug displacement wash. As such, no data points should fall to the left of this curve.

From these results, a wash ratio of 3 lowered the ionic liquid remaining in the lignin cake to 1% of the initial amount, and a wash ratio of ~6 lowered the ionic liquid to near the detection limit. A simple mass balance showed that the amount of water required to recover all the remaining ionic liquid is less than the amount required for gradual water addition in the hydrolysis reaction. Hence, the totality of the wash, which produces an aqueous stream with trace ionic liquid, can be completely recycled back to the reactor, generating no waste streams.

Cake Cracking and Discharging. Observations were also made about lignin cake cracking and ease of discharge. Cake cracking results in channels through which the wash liquid moves preferentially, creating regions of poor flow, reducing wash efficiency and liquor recovery. Importantly, we did not observe any cracks forming in the cake during filtration, washing or extended air drying. It appears that the fibrous structure of the lignin prevented any crack from forming. The last step in solid–liquid separations is discharge. We observed that the cake discharged easily, as evidenced by the ability to scrape off thin layers using a knife edge, and without disturbing underlying layers.

Lignin Integrity. Two-dimensional heteronuclear single quantum coherence (HSQC) NMR spectroscopy is a useful method for the analysis of lignin, reporting on both its purity as well as the integrity of its internal linkages.[22−25] Our ^{1}H, ^{13}C HSQC NMR data of lignin from Wash Method 2 indicates the complete removal of polysaccharides, whose peaks are absent from the spectrum (Figure 4). Major known units of lignin are present in the spectrum, such as syringyl and guaiacyl units, and *p*-coumarate esters. We note, however, that the *β*-aryl ethers, which comprise over half of the linkages in lignin, are absent from the spectrum. Lignin isolated by a similar process from a different corn stover preparation retained most *β*-aryl ethers,[25] suggestive of some variability in the biomass and ionic liquid preparations, and indicative of the high sensitivity of this particular linkage to hydrolysis. The depolymerization that we observed is consistent with previous studies in solutions of ionic liquid.[26−28] In aqueous solutions, the cleavage of C−O bonds with good yields of monomeric units was demonstrated only recently.[29] This route used aqueous formic acid and oxidized lignin to achieve >60% yields of low molecular mass fragments in mild conditions. Because the cleavage of *β*-aryl ethers affords both aromatic units and large molecular mass insoluble fragments, this route could produce feedstocks that are amenable to upgrading to aromatic molecules for conversion into bulk chemicals,[30−35,29] as well as the production of carbon fiber.[16]

We also characterized hydrolysate solids by light microscopy. Visual inspection allowed us to determine whether the hydrolysis reaction had reached completion. If not, large cellulose fibers were discernible in the solids. On the other hand, solids resulting from complete reactions were fine lignin powders lacking fibers. Washed solids were dissolved in DMSO, which resulted in a small fraction remaining out of solution and discernible under the microscope (Figure 5). We concluded by inspection that these solids were ash particles introduced during dissolution of untreated corn stover, which, according to an NREL analysis, accounts for ~13% of the mass of the corn stover used in our work (Table 1).

CONCLUSIONS

We have reported the isolation of a clean lignin fraction with quantitative recovery of ionic liquid from the biomass hydrolysate. Hydrolysates were prepared by dissolving untreated corn stover in 1-butyl-3-methylimidazolium chloride, and hydrolyzing glycosidic bonds by catalytic acid and gradual water addition. Previously, we had shown that this reaction produces monomeric xylose and glucose sugars at yields that are close to the theoretical maximum.[25] Sugars are present in a hydrolysate slurry containing undissolved solids. By filtration or

DOI: 10.1021/sc500731c
ACS Sustainable Chem. Eng. 2015, 3, 606−613

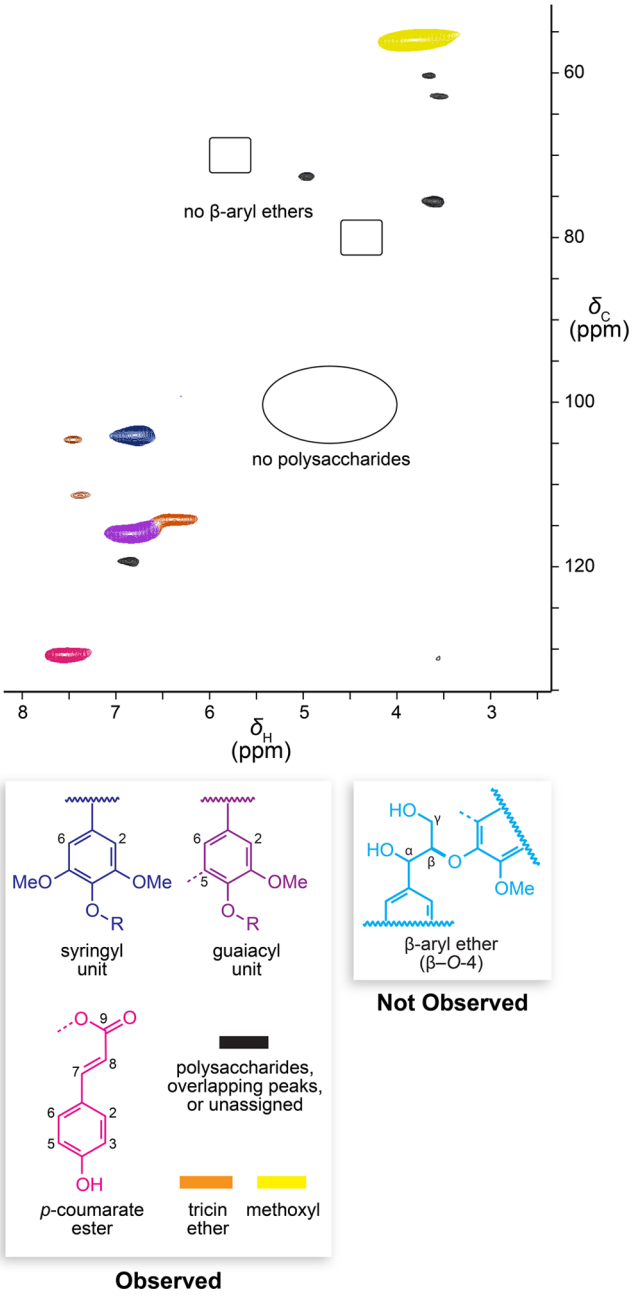

Figure 4. Representative ^{1}H, ^{13}C HSQC NMR spectrum of acetylated lignin in DMSO-d_6 following biomass conversion in [BMIM]Cl. Side chain (δ_C/δ_H 50–90/2.5–5.8) and aromatic/unsaturated (δ_C/δ_H 90–155/5.5–8.0) regions of the spectrum are shown. The integrity of several aromatic monomers, such as the syringyl, guaiacyl, and *p*-coumarate units, is preserved. *β*-Aryl ether linkages (which normally appear in the squares) are absent from the spectrum, suggesting depolymerization. Polysaccharides (which normally appear in the oval) are absent from the spectrum, indicating that cellulose, hemicelluloses, and their derived sugars have been separated from the lignin.

centrifugation of the slurry, we have obtained a cake that is composed mainly of lignin. The cake was washed with both warm- and ambient-temperature water over a fritted glass filter in order to recover the ionic liquid remaining in the cake. In nearly all cases, the amount of ionic liquid remaining dropped below detection limits, suggesting at least 99.98% recoveries from the cake and ~100% (i.e., quantitative) recoveries overall. These results show that [BMIM]Cl is not preferentially

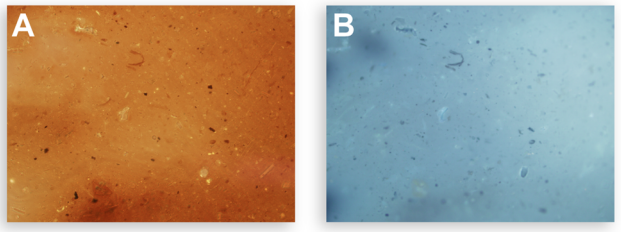

Figure 5. Solids in DMSO under 20× magnification. (A) Illumination with visible light. (B) Illumination with a mercury lamp with an Olympus U-MWU Filter Cube (excitation, 330–385 nm; emission, >420 nm).

adsorbed to solids and dissolves readily into water to enable its complete removal.

To the best of our knowledge, the ionic liquid recoveries obtained here far exceed those from pretreatments, and meet or exceed the threshold for economic viability. Solutions formed during pretreatment are highly viscous, and their dissolved polysaccharides give rise to complex rheologies that hinder solvent recovery. In addition, the most widely used ionic liquid in pretreatment, 1-ethyl-3-methylimidazolium acetate, was recently found to react chemically with sugars.[36] Carboxylate ionic liquids react with the aldehyde functionality of sugars to form adducts at the C-2 carbon of the imidazolium cation. This result would explain the consistently limited recoveries of carboxylate ionic liquids. In contrast, [BMIM]Cl is essentially inert.[36]

Subsequent experiments sought to minimize the amount of water required for the wash, concluding that a wash ratio of ~6, which could easily be recycled back into the process and would not create waste streams, is sufficient for attaining quantitative recovery. Lastly, solids displayed filtration, washing and discharging characteristics that were typical of common industrial slurries that are routinely filtered using standard equipment of reasonable size and cost. From these results, we conclude that this strategy could lead to a cost-effective and scalable biorefinery that produces both clean sugars and lignin. Further, the lignin is devoid of cellulosic residue and substantially depolymerized, making it an attractive feedstock for enabling interesting downstream valorization routes.

MATERIALS AND METHODS

Materials. Milled and sieved corn stover (Year 2010) with the composition listed in Table 1 was a gift from Dr. N. de Leon (University of Wisconsin–Madison). Sugarcane bagasse was from Dr. G. L. Gresham (Idaho National Laboratory). [BMIM]Cl was a gift from Merck KGgaA (Darmstadt, Germany). Commercial chemicals were from Sigma–Aldrich (Milwaukee, WI) and were of reagent grade or better.

Instrumentation. Hydrolysis reactions were performed with shaking by a Mini Shaker from VWR (Radnor, PA), which controlled both the stir rate (650 rpm) and the temperature (105 °C). ^{1}H NMR spectroscopy was performed with 500 and 400 MHz spectrometers from Bruker (Billerica, MA). Samples were dried under high vacuum (<0.1 Torr) with a mechanical belt-drive oil pump from Welch (Niles, IL). Filtration used a suction pump from Gast Manufacturing (Benton Harbor, MI). Centrifugation was done with an IEC HN-SII centrifuge from Damon International Equipment (now, Thermo-Fischer, Waltham, MA).

Representative Procedure for the Hydrolysis of Corn Stover. [BMIM]Cl (5.00 g) was heated to 105 °C in a 20 mL glass vial using a Mini Shaker from VWR International (Radnor, PA). Following complete liquefaction of [BMIM]Cl, corn stover (375 mg) was added

DOI: 10.1021/sc500731c
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to the vial. The mixture was allowed to stir (650 rpm) for 6 h at 105 °C, with the shaker lid firmly in place. HCl (8 M, 0.25 mL) was then added to the mixture with continued heat and stirring. After 10 min, deionized water (0.50 mL) was added to the reaction, followed by additional aliquots at 15 min (0.50 mL), 20 min (0.25 mL), 25 min (0.25 mL), 30 min (0.75 mL), and 60 min (1.00 mL). The shaker lid was replaced after each addition to prevent water evaporation. The reaction was allowed to continue for another 1 h, after which time the mixture was removed from heat and allowed to cool for 10 min. The thick, dark brown mixture was then diluted with deionized water in a falcon tube to a total volume of 50 mL. The insoluble material was separated via centrifugation (10 min at 1800 rpm, 4 °C). The supernatant was removed by decantation and stored for later use. The insoluble fraction was dried for 3 h at 20 °C under high vacuum.

Next, the insoluble material was added to molten [BMIM]Cl (2.50 g) at 105 °C. The mixture was allowed to stir (650 rpm) for 4 h. HCl (8 M, 0.13 mL) was then added to the mixture with continued heating and stirring. After 10 min, deionized water (0.25 mL) was added to the reaction mixture, followed by additional aliquots at 15 min (0.25 mL), 20 min (0.13 mL), 25 min (0.13 mL), 30 min (0.38 mL), and 60 min (0.50 mL). The reaction was allowed to continue for another 1 h, at which time the mixture was removed from heat and allowed to cool for 10 min. The mixture was then diluted with deionized water in a falcon tube to a total volume of 25 mL and subjected to centrifugation (10 min at 1800 rpm, 4 °C). The supernatant was decanted again and stored, while the insoluble fraction was saved for washing.

Quantitation of [BMIM]Cl by ^{1}H NMR Spectroscopy. [BMIM]Cl in D_2O washes was quantitated by comparing integrations of the signal from the N—C(H)=N atom of [BMIM]Cl to that from the nine ^{1}H atoms in a known concentration (0.5% w/v) of added TMSP-d_4. This signal from [BMIM]Cl is a singlet that is farther downfield (9.2 ppm) than any signal from the biomass.

Detection Limit of [BMIM]Cl by ^{1}H NMR Spectroscopy. Titers were performed to measure the lowest possible concentration of [BMIM]Cl detectable by ^{1}H NMR spectroscopy.

The first titer was performed in D_2O, which is the solvent used to wash solids and recover [BMIM]Cl retained on the solids (vide infra). Starting with a [BMIM]Cl concentration of 0.50 M, 2-fold dilutions were performed until [BMIM]Cl was no longer detectable by NMR spectroscopy at 400 MHz. This result was attained after 13 dilutions, making the detection limit for [BMIM]Cl in D_2O ∼0.25 mM.

The second titer was performed in DMSO-d_6, which is the solvent used to obtain NMR spectra of lignin and to determine the amount of [BMIM]Cl retained. Knowing the high-sensitivity of the method from the first titer, the initial concentration for this titer was 10 mM. Again, 2-fold dilutions were performed until [BMIM]Cl was no longer detectable. This result was accomplished after 8 dilutions, making the detection limit for [BMIM]Cl in DMSO-d_6 approximately 0.08 mM.

Wash Method 1: Wet Solids at 20 °C. The insoluble fraction from the two-stage hydrolysis was added to a 15 mL glass Buchner funnel with a course frit. The funnel was attached to a vacuum pump to perform filtration with suction of −75 kPa. A 10 mL solution of D_2O containing TMSP-d_4 (0.05% w/v) was poured over the solids to elute retained [BMIM]Cl. The solids were mixed with the D_2O using a small spatula to increase surface area and attempt to maximize [BMIM]Cl recovery. When the D_2O had completely passed through the filter cake and frit into the collecting flask, it was poured into a small vial for determination of [BMIM]Cl concentration. Another 10 mL aliquot of the D_2O solution was added to the funnel under suction, and the mixture was stirred once again. The wash process was repeated with 10 mL aliquots until a total of 50 mL had passed through the filter cake.

Wash Method 2: Dried Solids at 20 °C. A pellet from the conversion of corn stover in [BMIM]Cl was dried under high vacuum for 3 h before being added to the funnel for washing. The washing procedure was then performed as described in Wash Method 1.

Wash Method 3: Wet Solids at 50 °C. A pellet from the conversion of corn stover in [BMIM]Cl was transferred directly to the funnel without being dried and was washed as described in Wash Method 1, except that the D_2O was heated to 50 °C.

Wash Method 4: Dried Solids at 50 °C. A pellet from the conversion of corn stover in [BMIM]Cl was dried under high vacuum for 3 h before being added to the funnel for washing. Additionally, the D_2O was heated to 50 °C prior to being passed through the funnel. The washing procedure was then performed as described in Wash Method 1.

Wash Method 5: Centrifugation. A pellet from the conversion of corn stover in [BMIM]Cl was placed in a 25 mL tube, to which was added 10 mL of D_2O containing TMSP (0.05% w/w). The solids were suspended in the solution and subjected to centrifugation at 1800 rpm for 5 min at 4 °C. The supernatant was then decanted and analyzed by ^{1}H NMR spectroscopy. This procedure was repeated twice for a total of three rounds of centrifugation and 30 mL of D_2O wash. A fourth centrifugation was attempted with an additional 10 mL aliquot of D_2O, but after 5 min, the solids did not sediment in the bottom of the tube. The mixture was then subjected to centrifugation for 10 min, followed by an additional 20 min, but again, only a fraction of the solids formed a pellet. As a result, the mixture needed to be filtered to recover the solids and quantitate the retained [BMIM]Cl.

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Notes

The authors declare the following competing financial interest(s): Hyrax Energy, Inc. is the exclusive licensee of patents relating to ionic liquid bioprocessing that were developed at the University of Wisconsin−Madison and are owned by the Wisconsin Alumni Research Foundation (WARF). R.E.T., K.G.K., S.A.P., and R.T.R. are shareholders in Hyrax Energy, Inc.

ACKNOWLEDGMENTS

We are grateful to Dr. N. de Leon for samples of corn stover, Merck KGaA for [BMIM]Cl, and Drs. J. Ralph and B. R. Caes for contributive discussions. This work was supported by the Great Lakes Bioenergy Research Center, which is supported by the DOE Office of Biological and Environmental Research (DE-FC02-07ER64494). Biorefinery process development at Hyrax Energy, Inc. is supported by the DOE Office of Energy Efficiency and Renewable Energy (DE-SC0010126) and by the NSF Industrial Innovation and Partnerships organization (IIP-1314699). This study made use of the National Magnetic Resonance Facility at Madison, which is supported by the NIH (P41 GM103399). Microscopy was performed at the Newcomb Imaging Center, Department of Botany, University of Wisconsin−Madison.

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