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Simulated Moving Bed Chromatography: Separation and Recovery of Sugars and Ionic Liquid from Biomass Hydrolysates

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Since the industrial revolution, fossil fuels have served as an energy source for humankind. Today, they supply the vast majority of the world's energy and chemicals.[1] Nonetheless, global and socioeconomic concerns mandate a reduction in our dependence on these reserves.[2] Cellulose, the most abundant organic molecule in the world, is the primary component of lignocellulosic biomass and could serve as a renewable and sustainable energy resource. Cellulose is, however, recalcitrant, having a highly crystalline structure and being intermingled with hemicelluloses and lignin in biomass materials.[3] A transition from fossil fuels to cellulose as an energy resource requires accessing and transforming the cellulose embedded within biomass.

Monosaccharides can result from the hydrolysis of cellulose and hemicelluloses (Scheme 1). Then, chemical and biological methods can access a variety of useful compounds.[4] Although enzymes can catalyze the hydrolysis of cellulose and hemicelluloses,[4d,f,5] low substrate accessibility and encasing lignin confound this approach[6] and mandate the use of a pretreatment.

Some ionic liquids—salts that melt near ambient temperature—can solvate cellulosic material.[7] Specifically, ionic liquids containing chloride and other simple anions disrupt the network of inter- and intrapolymer hydrogen bonds that endow cellulose with its high crystallinity. In this manner, dissolution in ionic liquid can itself be a pretreatment en route to monosaccharides.[8]

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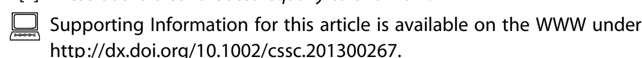
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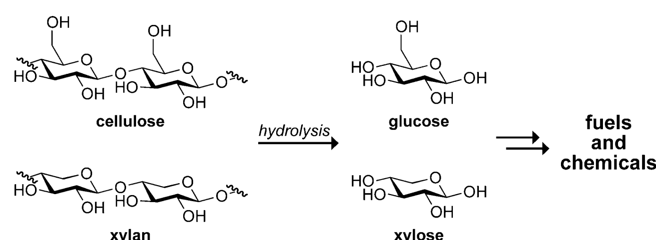

Scheme 1. Hydrolysis of cellulose and the hemicellulose xylan into glucose and xylose, which can be transformed into fuels and chemicals.

High yields of sugars can be obtained from raw biomass directly in the ionic liquid 1-ethyl-3-methylimidazolium chloride ([EMIM]Cl), by adding acid to catalyze hydrolysis and water commensurate with its consumption.[9] Separation of the [EMIM]Cl from the sugars was accomplished using ion-exclusion chromatography, resulting in >95% recovery of the ionic liquid, and 94% and 88% recovery of glucose and xylose, respectively. This separation method is not, however, amenable to large-scale implementation.

Simulated moving bed (SMB) chromatography is a continuous separation method that is currently used for large-scale separations in the petrochemical, food, mining, and pharmaceutical industries.[10] In recent years, SMB chromatography has been used increasingly for the separation of biomass components.[11] SMB chromatography emulates countercurrent separation, wherein the mobile phase flows in the opposite direction of the solid phase (Figure 1). The solid phase is represented by the individual columns connected in series; the mobile phase is represented by inlet streams of feed and eluent, and outlet streams of raffinate and extract. Valves between the columns are switched open or closed at timed intervals to introduce the inlet streams and withdraw the outlet streams between the separation zones, simulating counterclockwise rotation of the columns. Under appropriate conditions, continuous separation of sample components can be achieved with extremely high purity and recovery. The optimization of flow rates and column switch times enables a higher level of separation to occur with less solvent consumption, making SMB chromatography a powerful tool for separating binary mixtures.

Herein, we report on the high-yielding recovery (>99%) of pure ionic liquid and sugars using optimized SMB chromatography to purify the hydrolysis products of raw biomass on

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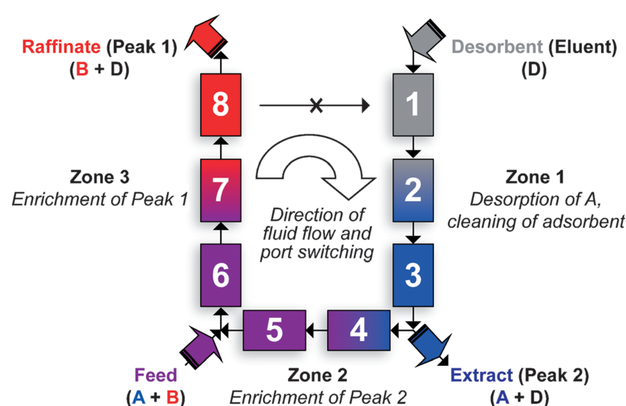

Figure 1. Schematic of three-zone (3-2-3) simulated moving bed (SMB) chromatography.

a multigram scale. We also valorize the residual lignin from this process.

We began by seeking a chemical method for the multigram-scale saccharification of biomass. 1-Butyl-3-methyl-imidazolium chloride ([BMIM]Cl) has been reported to have a higher capacity to dissolve cellulose than [EMIM]Cl,[7d] allowing less ionic liquid to be used and thus lessening cost.[12] We quickly discovered, however, that using the optimized hydrolysis conditions determined previously for hydrolysis in [EMIM]Cl[9] provided drastically lower yields in [BMIM]Cl (<20% glucose and xylose). These low yields necessitated reoptimization of the hydrolysis reaction conditions.

Reoptimization began by using pure cellulose in [BMIM]Cl. As the acid-catalyzed hydrolysis of cellulose can often lead to undesirable byproducts such as 5-hydroxymethylfurfural and humins,[13] fine control of water addition is necessary to halt reactivity beyond hydrolysis. Believing that the low glucose and xylose yields were a result of the sugars' being transformed into these byproducts, we varied the amounts and times of water addition. Still, achievable yields were not comparable to those in the process developed in [EMIM]Cl. Next, we tested the hypothesis that the cellulose lacks time to hydrolyze; however, extending the reaction time did not improve the yields. We then attempted the hydrolysis reaction using a higher concentration of acid than that used in the process using [EMIM]Cl. A screening of different concentrations of HCl did result in increased glucose yields, and a 6 M HCl solution was optimal, giving a glucose yield of 69% (Table 1).

Having optimized conditions for the hydrolysis of cellulose in [BMIM]Cl, we next sought an optimal separation strategy using SMB chromatography to separate [BMIM]Cl from glucose and xylose. The first step was to determine their retention times on the adsorbent beds with single-column experiments. We exchanged the resin in an ion-exclusion column with [BMIM]Cl in water, and determined the approximate retention times with single injections of [BMIM]Cl, glucose, and xylose. These experiments showed that [BMIM]Cl was least adsorbed, followed by glucose, and then xylose in accordance with published data.[11c] A follow-up single column experiment used a mixture of [BMIM]Cl (3.637 min) and glucose (5.195 min), giving the void fraction, fast peak-time of retention, and slow peak-time of retention (Supporting Information, Figures S1 and S2). These values were entered into the SMB chromatography parameter calculator—based on the Triangle model[14]—for determining which internal flow would achieve optimal separation of the ionic liquid and sugars. A representative example of the SMB chromatography parameter calculator worksheet for 3-zone separations is shown in Figure S3. When we applied these parameters to the separation of pure glucose and xylose from pure [BMIM]Cl, the 3-zone separation gave the best recovery and purification of the sugars and ionic liquid. Additionally, varying both concentration (Table S1) and flow rates (Table S2) enabled modulation of the amount of [BMIM]Cl and sugars recovered in the raffinate and extract fractions, respectively.

Table 1. Acid hydrolysis of cellulose in [BMIM]Cl.

[HCl] [M]	Glucose molar yield[a] [%]
2	9
4	31
6	69
8	56
10	50
12	50

[a] Yields are based on HPLC analysis and are relative to glucose monomers in the cellulose.

Next, we attempted to utilize our conversion and separation strategies on biomass material in the form of raw corn stover. Preliminary experiments used 20 parts [BMIM]Cl relative to the corn stover (i.e., a 5 wt% solution). As biomass is comprised of more than cellulose, we again screened varying acid concentrations to ensure that the requisite higher acid concentration would not result in a build-up of unwanted side products via breakdown of hemicelluloses and lignin and their subsequent polymerization to humins. An HCl concentration of 8 M was optimal for maximal yields of glucose (35%) and xylose (78%) in a single-stage hydrolysis reaction (Table 2). These results are comparable with those reported previously in [EMIM]Cl for glucose (42%) and xylose (71%).[9]

Then, we sought to take advantage of the superior capacity of [BMIM]Cl to dissolve cellulose by decreasing the loading of the ionic liquid relative to the corn stover. We tested three dif-

Table 2. Acid hydrolysis of biomass in [BMIM]Cl.

[HCl] [M]	Glucose molar[a] yield [%]	Xylose molar[a] yield [%]
2	4	43
4	20	79
6	24	83
8	35	78
10	32	79
12	38	62

[a] Yields are based on HPLC analysis and are relative to glucose and xylose monomers in the corn stover.

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Table 3. Optimization of biomass loading in [BMIM]Cl.

Biomass[a]	Amount of corn stover [wt%]	Glucose yield[b] [%]	Xylose yield[b] [%]
corn stover	5.0	42	85
corn stover	7.5	39	83
corn stover	10.0	33	82
AFEX-treated corn stover	5.0	47	88
AFEX-treated corn stover	7.5	32	85
AFEX-treated corn stover	10.0	10	75

[a] AFEX: Ammonia fiber expansion. [b] Yields are based on HPLC analysis and are relative to glucose and xylose monomers in the corn stover.

ferent concentrations using single-stage hydrolysis reactions. Interestingly, the concentration could be increased from 5 to 7.5 wt% corn stover in [BMIM]Cl and still access comparable sugar yields (Table 3). A 10 wt% solution, however, began to show decreasing glucose yields. Hence, for higher productivity, we chose the 7.5 wt% solution for future experiments. We were also interested in applying our system to ammonia fiber expansion (AFEX) pretreated corn stover, a process that uses liquid ammonia under high pressures and temperatures to decrystallize cellulose, mildly depolymerize and redistribute the lignin, and increase micropore size and number in cell walls, providing increased access to the sugars.[15] Hydrolysis of the AFEX pretreated corn stover provided comparable yields of glucose (47%) and xylose (88%) to those from untreated corn stover (Table 3). This finding indicates that, as reported previously for hydrolysis in [EMIM]Cl,[9] hydrolysis in [BMIM]Cl does not require any pretreatment of biomass material to access the sugars in high yields. Furthermore, subjecting the corn stover and AFEX-treated corn stover hydrolysates to purification from the [BMIM]Cl with SMB chromatography enabled >95% isolation of sugars in the [BMIM]Cl raffinate of both hydrolysates.

These optimized conditions led us to test our system on a larger, multigram scale. To do so, we increased our loadings and were gratified to discover that a two-stage hydrolysis reaction on corn stover enabled access to high yields of glucose (92%) and xylose (95%). The two-stage hydrolysis effectively doubled the glucose yields by compensating for the slower rate of hydrolysis of cellulose than hemicelluloses containing xylose. Purification using SMB chromatography again afforded >95% ionic liquid in the raffinate. For this process to be industrially viable, it would need to recover the ionic liquid nearly quantitatively.[16] Hence, we resubjected our extract, which contained traces of ionic liquid, to SMB chromatography and were able to achieve an overall recovery of 99.5% of the [BMIM]Cl in the raffinate fractions. Thus, using a two-stage hydrolysis reaction followed by SMB chromatography, ionic liquids were recovered in yields that enable their use as an industrial solvent in a viable biomass conversion process.

After successfully purifying [BMIM]Cl in the raffinate fraction, we were interested in recycling it for use in subsequent reactions. Hence, we subjected the raffinate to lyophilization to facilitate the removal of the aqueous eluent from the ionic liquid. When we performed a hydrolysis reaction using the recycled [BMIM]Cl and fresh corn stover, we found that our yields were reduced significantly compared to those using fresh [BMIM]Cl (Table S3). This decrease was most likely due to the hygroscopy of the ionic liquid, resulting in a retention of water in the [BMIM]Cl. This retention was evident by inspecting the [BMIM]Cl, which is a white solid but becomes a yellow oil upon water absorption. We suspect that the retained water hinders the dissolution of cellulose. In an effort to increase hydrolysis and offset the water retention, we employed a greater concentration of HCl, but were unsuccessful in obtaining increased yields. Hence, while >99% recovery of the [BMIM]Cl is possible, further purification to remove the majority of retained water would be necessary for its use in subsequent hydrolysis reactions.

The conditions of the hydrolysis reaction expose an ionic liquid to an aqueous mineral acid at a temperature of 105 °C. These conditions could lead to degradation of the ionic liquid, precluding its continued use.[17] To assay for degradation, we subjected [BMIM]Cl to aqueous 0, 4, 8, or 12 M HCl at a temperature of 105 °C for 30 days. ^{1}H NMR spectra of the samples revealed no apparent differences compared to untreated [BMIM]Cl (Figure S4). We conclude that [BMIM]Cl maintains its integrity under the conditions of the hydrolysis reaction.

We were also interested in testing the viability of our purified sugars for use as feedstocks for microbial growth. It is imperative that sugars be free of any contaminants that would hinder growth, necessitating their clean separation from the ionic liquid, which can be toxic.[8b] We tested our sugars using *Escherichia coli* strain KO11, a microbe engineered to use hexose and pentose sugars as feedstocks for ethanol production.[18] The *E. coli* cells did not grow in M9 minimal medium, which contains only inorganic matter. But by using the corn stover hydrolysate sugars as the carbon source, we were able to achieve robust growth of *E. coli* strain KO11 (Figure 2). Furthermore, the growth rate was comparable to that of *E. coli* strain KO11 using an analogous mixture of commercial glucose and xylose as the carbon source. Thus, the SMB chromatography process is able to recover a hydrolysate suitable for microbial consumption.

Finally, we realized that our hydrolysis strategy provides the opportunity to recover residual lignin. Many extant hydrolysis processes produce a lignin that may have little value other

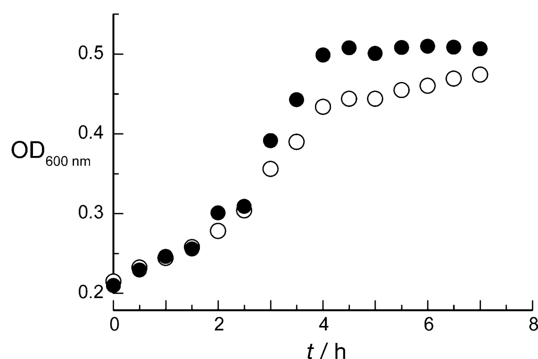

Figure 2. Comparison of aerobic growth rates of *E. coli* strain KO11 in M9 minimal medium with ●) hydrolysate sugars purified by SMB chromatography, or ○) commercial glucose and xylose as the sole carbon source.

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than for burning to provide process energy. Lignin is, however, a potential source of aromatic chemicals, and efforts are being made to access its aromatic constituents.[19,20] Optimal economics requires valorization of the lignin component. Hence, we evaluated the recovered lignin for any degradation resulting from the acid hydrolysis reaction. Using 2D-NMR spectroscopy, we found that our recovered lignin showed little sign of degradation and contained fewer polysaccharides than a lignin sample recovered from a typical cellulase-mediated saccharification process (Figure 3). In particular, the most hydrolytically sensitive interunit linkages, the β-aryl ethers, remain prominent. Perhaps surprisingly, *p*-coumarate esters remained intact and associated with the lignin, attesting to the mild nature of the hydrolysis.

In conclusion, we demonstrate the potential of the industrial application of SMB chromatography as a purification technique for the recovery of both sugar products and ionic liquid solvent from biomass hydrolysates. By using 8 M HCl in a two-stage hydrolysis reaction, we access both glucose and xylose sugars in high yields from raw corn stover biomass. SMB chromatography proved able to separate and recover the [BMIM]Cl and sugars at nearly quantitative levels. Although an additional step would be required to dry the [BMIM]Cl for further use in hydrolysis reactions, its purity and integrity assures its continued viability for biomass dissolution. Furthermore, the recovered hydrolysate sugar products are able to be used as feedstocks for microbes, as demonstrated by using *E. coli* KO11 to produce ethanol, and the residual lignin appears to largely retain its native structure, suggesting that it could be more readily valorized. This technology could lend itself to application in an industrial biorefinery to convert large quantities of raw biomass to contaminant-free sugars whilst recycling ionic liquid solvents for continued use.

Experimental Section

General considerations

Commercial chemicals were from Sigma–Aldrich (Milwaukee, WI), were of reagent grade or better, and were used without further purification. 1-Butyl-3-methylimidazolium chloride (98%, [BMIM]Cl) was a gift from Merck KGaA (Darmstadt, Germany). Cellulose (medium cotton linters, C6288, ca. 95% dry solids) was from Sigma Chemical (St. Louis, MO). Milled and sieved corn stover (batch 2008), which consisted of glucan (35.1 wt%), xylan (22.2 wt%), arabinan (3.1 wt%), Klason lignin (16.2 wt%), ash (5.7 wt%), ethanol extractives (2.6 wt%), soluble sugars (3.7 wt%), and total water + ethanol extractives (10.2 wt%), and AFEX pre-treated corn stover (batch #740–140), which consisted of glucan (35.7 wt%), xylan

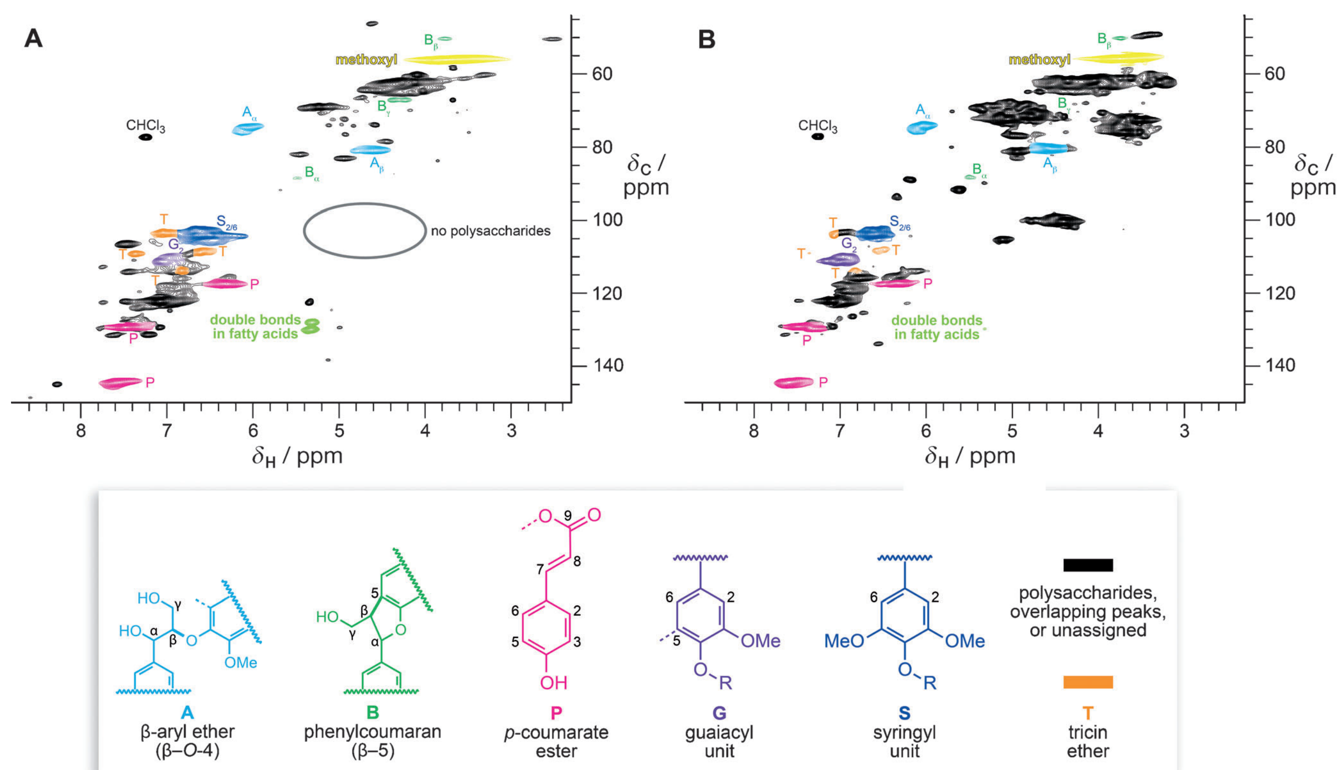

Figure 3. 2D HSQC NMR analysis of the integrity of acetylated residual lignin after a **A**) chemical, or **B**) enzymatic biomass→sugar conversion process. Side-chain (δ_C/δ_H 50–90/2.5–5.8) and aromatic/unsaturated (δ_C/δ_H 90–155/5.5–8.0) regions in the spectrum of corn stover after treatment with **A**) our biomass→sugar conversion process, or **B**) a typical cocktail of cellulases.[21,22] The lignin in panel A appears to be largely intact—the labile β-aryl ethers (cyan, A) are prominent, as are the phenylcoumarans (green, B), both prominent interunit linkage types in lignin. Aromatic guaiacyl units (purple, G), syringyl units (blue, S), as well as newly identified tricin ethers (orange, T) and *p*-coumarate esters (magenta) are readily seen, although there is considerable overlap—signals are only colored/assigned when they are reasonably well resolved. Notably, in panel A there are no polysaccharide anomeric signals (region circled), indicating that the polysaccharides have been hydrolyzed completely.

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(21.2 wt%), arabinan (2.6 wt%), acid-insoluble lignin (17.4 wt%), ash (5.9 wt%), acetyl content (2.4 wt%), total extractives (10.5 wt%), and other (4.3 wt%), were gifts from N. de Leon and B. E. Dale (Great Lakes Bioenergy Research Center). Dowex 50WX4-400 columns were from Semba Biosciences (Madison, WI). Hydrolysis reactions were performed in 20 mL glass vials shaking at 600 rpm in a temperature-controlled Mini Shaker from VWR (Radnor, PA). The term "high vacuum" refers to vacuum ($<$0.1 Torr) achieved by a Welch mechanical belt-drive oil pump.

Analytic methods

Hydrolysis reaction products were analyzed by HPLC and quantified using calibration curves generated from commercially available standards. Product concentrations were calculated from HPLC-peak integrations, which were then used to calculate molar yields. During a reaction, an aliquot of the reaction mixture was removed, diluted with a known mass of deionized water, clarified by centrifugation at 12000 rpm for 5 min, and subjected to analysis by HPLC. HPLC was performed with a 1200 system from Agilent Technologies (Santa Clara, CA) equipped with refractive index and photodiode array detectors. Glucose, xylose, and [BMIM]Cl were analyzed by ion-exclusion chromatography with an Aminex HPX-87H column (300×7.8 mm) from Bio-Rad (Hercules, CA) using a 5 mM H_2SO_4 mobile phase at a flow rate of 0.6 mL min^{-1} at 65 °C.

Representative procedure for the hydrolysis of cellulose

[BMIM]Cl (4 g in each of 6 vials) was heated with mixing at 105 °C until melted. Cellulose (250 mg with 1.54 mmol glucose units) was added to each vial. The mixtures were stirred vigorously at 105 °C for 6 h to achieve complete dissolution. To each mixture was added 0.20 mL of aqueous HCl (2, 4, 6, 8, 10, or 12 M). After 10 min, deionized water (0.80 mL) was added to each mixture with continued stirring, followed by additional aliquots of water at 20 min (0.40 mL), 30 min (0.60 mL), and 60 min (1.00 mL). After a total reaction time of 3 h, the solutions were diluted with water (6.00 mL) and allowed to cool to ambient temperature. Insoluble materials were removed by centrifugation and an aliquot of the solution was used for HPLC analysis.

Representative procedure for the two-step hydrolysis of corn stover

[BMIM]Cl (5 g in each of 6 vials) was heated with mixing at 105 °C until melting of the [BMIM]Cl. Corn stover (0.375 g with 0.81 mmol glucose units and 0.63 mmol xylose units) was added to each vial. The mixtures were stirred vigorously at 105 °C for 6 h to dissolve the saccharides completely. To each mixture was added 0.25 mL of aqueous 8 M HCl. After 10 min, deionized water (0.50 mL) was added to each mixture with continued stirring, followed by additional aliquots of water 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). After 2 h, the solutions were allowed to cool to ambient temperature and diluted to a total volume of 25 or 50 mL with deionized water to yield a [BMIM]Cl concentration of 200 or 100 mg mL^{-1}, respectively. Insoluble material was allowed to sediment and then removed as a pellet with centrifugation. Aliquots of the supernatant were passed through a 0.45 μm filter and analyzed by HPLC.

The pellet from the first hydrolysis reaction was dried under high vacuum overnight. [BMIM]Cl (3 vials of 5 g) was heated with mixing to 105 °C until melting of the [BMIM]Cl. The solid brown pellet was split and added (3 vials of 0.600 g), and the reaction mixtures were stirred vigorously at 105 °C for 4 h to dissolve the saccharides completely. To each mixture was added 0.25 mL of aqueous 8 M HCl. After 10 min, 0.50 mL of deionized water was added to each mixture with continued stirring. Additional aliquots of water were added 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). After 2 h, the reaction mixtures were allowed to cool to ambient temperature and diluted to a total volume 25 or 50 mL with deionized water to yield a [BMIM]Cl concentration of 200 or 100 mg mL^{-1}, respectively. Insoluble material was allowed to sediment and then removed as a pellet with centrifugation and used for lignin analysis (see below). Aliquots of the supernatant were passed through a 0.45 μm filter and analyzed by HPLC.

Parameter calculations for simulated moving bed chromatography

Retention times and isotherm data were collected with single-column experiments using a Dowex 50WX4–400 ion-exclusion column (25×1 cm) exchanged with [BMIM]Cl (22 g) dissolved in deionized water (125 mL). At the end of the exchange procedure, the column effluent was neutral, indicating an exchange of H^+ by $[BMIM]^+$. Approximate retention times were determined with single injections of [BMIM]Cl, glucose, and xylose. The mobile phase used for these experiments was deionized water at a flow rate of 2 mL min^{-1} with column temperatures at 22, 50, or 65 °C. Subsequent single-column experiments used mixtures of [BMIM]Cl and glucose. Data obtained from these experiments were used to determine retention times and isotherms. SMB chromatography parameters such as switch times and flow rates were calculated and optimized with the SMBC Parameter Calculator worksheet (available online at www.sembabio.com). Isotherm calculations were performed with ChromWorks software.

Simulated moving bed chromatography

SMB chromatography was performed with an Octave 10 Chromatography System from Semba Biosciences. Eight Dowex 50WX4–400 columns (10×1 cm) were connected in series and fluid flow paths were controlled through a pneumatic valve system. The columns were exchanged with [BMIM]Cl as indicated above, and deionized water or 5 mM H_2SO_4 was used as the desorbent. Fluid flow was controlled with 4 independent pumps capable of flow rates from 0.05 to 10 mL min^{-1} and supplied with the Octave 10 system. Each column had 9 valve positions with inlet access to all 4 pumps, 4 outlet lines, and an intercolumn shutoff valve that controlled the flow to the next column in series. Pump flow rates and valve operation to achieve SMB chromatography protocols were controlled by SembaPro software. All SMB experiments were performed at ambient temperature.

Ionic liquid stability

[BMIM]Cl (512 mg) was heated at 105 °C with stirring at 400 rpm to melt the ionic liquid. A 4.2 μL solution of 12 M HCl was added at 1 wt% relative to the ionic liquid. The mixture was heated at 105 °C with stirring at 400 rpm for 30 days. Then, an aliquot was removed for ^{1}H NMR analysis in CD_3OD at ambient temperature using a Bruker DMX-400 Avance spectrometer (^{1}H, 400 MHz) at the National Magnetic Resonance Facility at Madison (NMRFAM).

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Bacterial growth

E. coli strain KO11 was a gift from D.H. Keating (University of Wisconsin-Madison). An *E. coli* freezer stock was grown on a Luria-Bertani agar plate containing chloramphenicol (15 $mg\,L^{-1}$) for 12 h at 37 °C. A single colony was picked and used to inoculate Luria-Bertani medium (5 mL) containing xylose (20 $g\,L^{-1}$) in a culture tube at 37 °C and 250 rpm for 18 h. The cells were collected by centrifugation (4000 rpm for 5 min at 4 °C) and resuspended in M9 minimal medium (2 mL) containing M9 salts (1×), $MgSO_4$ (1 mM), $CaCl_2$ (0.1 mM), and thiamine (0.1 $\mu g\,mL^{-1}$).
In a polystyrene 96-well plate, 33 wells were filled with the M9 minimal medium (190 μL). A 10 μL solution of commercial sugars (8.6 $mg\,mL^{-1}$ glucose, 10.0 $mg\,mL^{-1}$ xylose) was added to 11 wells. A 10 μL solution of recovered hydrolysate sugars (8.5 $mg\,mL^{-1}$ glucose, 9.8 $mg\,mL^{-1}$ xylose), which contained a trace of [BMIM]Cl (30–50 $mg\,mL^{-1}$), was added to another 11 wells. Finally, 10 μL of additional M9 minimal medium was added to the remaining 11 wells. An aliquot (10 μL) of the above-cell suspension was used to inoculate 10 wells from each of the 3 sets, leaving 1 well without added bacteria as a negative control. The plate was capped with a low-evaporation lid and incubated with rapid agitation in a warm room at 37 °C. The $OD_{600\ nm}$ of each well was measured every 30 min for 7 h using an Infinite M1000 Absorbance Microplate Reader from Tecan (Männedorf, Switzerland).

Lignin analysis

The pellet collected by centrifugation after a two-stage hydrolysis reaction was rinsed with water to remove residual [BMIM]Cl. The residue was then dissolved in dichloromethane, acetylated, and analyzed by NMR spectroscopy, as described previously.[23]

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