Supporting Information

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SI Text

Materials and Methods. Commercial chemicals were of reagent grade or better, and were used without further purification; 1-ethyl-3-methylimidazolium chloride (99.5%, [EMIM]Cl) and 1-butyl-3-methylimidazolium chloride (99%, [BMIM]Cl) were from Solvent-Innovation; 1,3-dimethylimidazolium dimethylphosphate (≥98%), 1-ethyl-3-methylimidazolium tetrafluoroborate (97%, [EMIM]BF_4), 5-hydroxymethylfurfural, birchwood xylan (X0502, 98% xylose residues, ~95% dry solids) and Dowex® 50WX4 (200–400 mesh, H^+ form) were from Aldrich; 1-ethyl-3-methylimidazolium acetate (>90%, [EMIM]OAc), 1-ethyl-3-methylimidazolium nitrate (>99%, [EMIM]NO_3), 1-ethyl-3-methylimidazolium triflate (98.5%, [EMIM]OTf), 1-butyl-3-methylpyridinium chloride (97%), and 1-ethyl-3-methylimidazolium bromide (97%, [EMIM]Br) were from Fluka; 1-ethylpyridinium chloride (98%), 1-ethyl-2,3-dimethylimidazolium chloride (98%), and furfural were from Acros. Cellulose (medium cotton linters, C6288, ~95% dry solids) was from Sigma. Milled and sieved corn stover (~95% dry solids) was a gift from B.E. Dale and B. Venkatesh (Michigan State University, East Lansing, MI) (1), and was passed through a 40-mesh screen prior to use.

Reactions were performed in glass vessels heated in a temperature-controlled oil bath with magnetic stirring. The term "concentrated under reduced pressure" refers to the removal of water and other volatile materials using a Speed Vac concentrator system. Conductivity was measured with an Extech Instruments ExStik II conductivity meter. NMR spectra were acquired with a Bruker DMX-400 Avance spectrometer (^1H, 400 MHz; ^{13}C, 100.6 MHz) at the National Magnetic Resonance Facility at Madison.

All reaction products were analyzed by HPLC and quantified with calibration curves generated from commercially available standards. Following a typical reaction, the product mixture was diluted with a known mass of deionized water, subjected to centrifugation or filtration to remove insoluble products, and analyzed. The concentrations of products were calculated from HPLC-peak integrations and used to calculate molar yields. HPLC was performed with an Agilent 1200 system equipped with refractive index and photodiode array detectors as well as a BioRad Aminex HPX-87H column (300 × 7.8 mm; 5 mM H_2SO_4, 0.6 mL/ min, 65 °C).

Representative Procedure for Hydrolysis of Cellulose. Cellulose (18.7 mg, 104 μmol glucose units) and [EMIM]Cl (380 mg) were mixed at 105 °C for 6 h to form a viscous solution. To this solution was added aqueous HCl (1.66 M, 23.2 μL; equivalent to 3.8 mg conc. HCl), and the reaction mixture was stirred vigorously at 105 °C. During this time, the viscosity of the solution decreased dramatically. After 10 min, deionized water (80 μL) was added with stirring, followed by additional aliquots of water at 20 min (40 μL), 30 min (60 μL), and 60 min (100 μL). After a total reaction time of 3 h, the solution was diluted with water (701 μL). Insoluble materials were removed by centrifugation, and the solution was analyzed by HPLC [12.4 mg/g glucose, 88% yield; 0.34 mg/g 5-hydroxymethylfurfural (HMF), 3% yield]. In other cases, aliquots of the reaction mixture were removed periodically for HPLC analysis.

Reactions utilizing ionic liquids with melting points >105 °C (1-butyl-4-methylpyridinium chloride, 1-ethylpyridinium chloride, and 1-ethyl-2,3-dimethylimidazolium chloride) required slightly different handling. In these cases, the ionic liquid and cellulose were heated together using a heat gun until dissolution of the cellulose was achieved. Then, the mixture was heated at 105 °C for 6 h prior to the hydrolysis reaction. Although the 1-ethylpyridinium chloride solution remained liquid at this temperature, the other cellulose solutions solidified. Before addition of HCl, these solids were melted with a heat gun, and they remained liquid after the addition of aqueous HCl.

Hydrolysis of Xylan. Xylan (9.4 mg, 66 μmol xylose units) and [EMIM]Cl (188 mg) were mixed at 105 °C for several hours to form a viscous solution. To this solution was added aqueous HCl (1.66 M, 11 μL), and the reaction mixture was stirred vigorously at 105 °C. After 10 min, deionized water (40 μL) was added with stirring, followed by additional aliquots at 20 min (20 μL), 30 min (30 μL), 60 min (50 μL), and 90 min (50 μL). After a total reaction time of 3 h, the solution was diluted with water (100 μL). Insoluble materials were removed by centrifugation, and the solution was analyzed by HPLC (15.3 mg/g xylose, 77% yield).

Reaction of Glucose. Glucose (47.2 mg, 262 μmol) was dissolved in [EMIM]Cl (460 mg) and deionized water (50 μL). Conc. H_2SO_4 (5.5 μL) was added, and the resulting solution was stirred at 100 °C. Aliquots of the reaction mixture were removed periodically. Glucose recovery is based on HPLC analysis and is normalized to the initial glucose concentration. HMF molar yield is based on HPLC analysis.

Non-Chloride Ionic Liquids. Alternative ionic liquids were investigated as solvents for hydrolysis under conditions optimized with [EMIM]Cl (Table 2 in the main text). Ionic liquids that did not dissolve cellulose produced poor glucose yields. [EMIM]NO_3 and [EMIM]BF_4 failed to swell cellulose, and no glucose production was detected with these solvents. The bromide and triflate counterparts of these ionic liquids did swell cellulose, resulting in 4–5% glucose yields. On the other hand, the ionic liquids 1,3-dimethylimidazolium dimethylphosphate and [EMIM]OAc are excellent solvents for cellulose (2). Upon addition of 20 wt% water, the cellulose in the dimethylphosphate ionic liquid formed a viscous gel, and analysis of the reaction mixture revealed no glucose. Although cellulose remained dissolved in [EMIM]OAc under the reaction conditions, no glucose was produced in this solvent as well. The HCl hydrolysis catalyst in each of these solvents is buffered by dimethylphosphate and acetate, forming conjugate acids with pK_a values of 1.29 and 4.76, respectively (3, 4). These acids are probably too weak to accomplish cellulose hydrolysis under the reaction conditions (5). In contrast to other ionic liquids, chloride-containing ionic liquids such as [BMIM]Cl, 1-butyl-4-methylpyridinium chloride, and 1-ethylpyridinium chloride both dissolved cellulose and supported hydrolysis with unoptimized glucose yields ranging from 66–73%. These results suggest that ionic liquid media for cellulose hydrolysis must balance both cellulose solubility and hydrolytic activity. Chloride alone achieves this goal through its strong interactions with cellulose coupled with its weak basicity.

Representative Procedure for Recovery of Sugars and [EMIM]Cl from Hydrolyzates. Dowex® 50WX4 (75 g, 0.128 equiv) in a slurry with deionized water was placed in a jacketed column (120 cm × 1 cm, Kontes #420870-1200) maintained at 65 °C, resulting in a resin bed of 1.0 m. The resin was exchanged with $[EMIM]^+$ by passing [EMIM]Cl (64 g, 0.44 mol) in water through the column. At the end of the exchange procedure, the column effluent was neutral, signifying complete exchange of H^+ for $[EMIM]^+$. Degassed, deionized water was then passed through the column to elute any solutes.

Hydrolyzate liquids (2.741 g, 8.5 mg glucose, 17.7 mg xylose, ∼60% water) were obtained from the hydrolysis reaction of corn stover (102.3 mg) using [EMIM]Cl (1046 mg) under standard conditions. The solid residue from the reaction was reserved for a second hydrolysis reaction. A portion of the first hydrolysis liquids (2.591 g) was loaded on the top of the resin column and eluted with degassed, deionized water at a rate of 3 cm/min. Fractions were collected and analyzed by HPLC (7.5 mg glucose, 94%; 14.3 mg xylose, 86%). The fractions containing [EMIM]Cl were concentrated under reduced pressure, mixed with D_2O, and pooled, resulting in a D_2O/[EMIM]Cl solution (3.673 g). An aliquot (342.8 mg) of this solution was combined with *N,N*-dimethylacetamide (71.5 mg, 0.821 mmol), and the resulting solution was analyzed by ^1H NMR spectroscopy. Integration of the spectra revealed a 0.708:1 molar ratio of [EMIM]Cl:DMA, indicating [EMIM]Cl recovery of 913 mg (92%).

The above process was repeated with the hydrolyzate liquids (1.684 g) from the reaction of the solid residue using [EMIM]Cl (471 mg). After chromatography of a portion of the liquids (1.534 g), the ionic-liquid-containing fractions were concentrated under reduced pressure, mixed with D_2O, and pooled, resulting in a D_2O/[EMIM]Cl solution (3.261 g). An aliquot (528.4 mg) of this solution was combined with *N,N*-dimethylacetamide (79.6 mg, 0.914 mmol), and the resulting solution was analyzed by ^1H NMR spectroscopy. Integration of the spectra revealed a 0.532:1 molar ratio of [EMIM]Cl:DMA, indicating [EMIM]Cl recovery of 440 mg (103%). The combined [EMIM]Cl recovery from the two-step process was 96%.

Sugar-containing fractions from the separation process which were free of [EMIM]Cl were pooled and lyophilized to a brown residue. This residue was dissolved in deionized water (5 mL) and used for microbial growth and fermentation studies.

Bacterial Growth. *E. coli* strain KO11 was a gift from W.D. Marner (University of Wisconsin–Madison). In all cases, *E. coli* were grown at 37 °C in medium containing chloramphenicol (40 mg/L). A single colony was inoculated into Luria-Bertani medium (6) (4 mL) containing xylose (0.4 wt%). After incubation in a culture tube agitated at 250 rpm for 18 h, the cells were collected by centrifugation and resuspended in M9 minimal medium (6) (2 mL) free of any carbon source.

In a polystyrene 96-well plate, 20 wells were filled with M9 minimal medium (150 μL) containing xylose (2.62 g/L) and glucose (1.38 g/L). Ten wells were filled with M9 minimal medium (150 μL) supplemented with corn stover hydrolyzate sugars (2.62 g/L xylose, 1.38 g/L glucose, and 0.91 g/L arabinose). The remaining wells were filled with deionized water (200 μL). Each well was inoculated with the above cell suspension (5 μL), and the plate was capped with a low-evaporation lid and incubated with rapid agitation in a BioTek ELx808 Absorbance Microplate Reader. The $OD_{595\ \mathrm{nm}}$ of each well was measured every 5 min for 25 h.

Bacterial Fermentation. To maintain a low-oxygen environment, fermentation with *E. coli* was performed in a glass test tube (13 × 100 mm) fitted with a rubber stopper, which was pierced with a steel cannula. The other end of the cannula was immersed in water in a second glass test tube. The second test tube was fitted with a rubber stopper pierced with a needle for gas escape.

A single colony was inoculated into Luria–Bertani (LB) medium (4 mL) containing xylose (0.26 wt%) and glucose (0.14 wt%). After incubation in a culture tube agitated at 250 rpm for 11 h, the cells were collected by centrifugation and resuspended in fresh LB medium (4 mL). An aliquot (10 μL) of this cell suspension was added to test tubes equipped for anaerobic growth containing LB medium (1.5 mL) supplemented with either xylose (2.62 g/L) and glucose (1.38 g/L), or corn stover hydrolyzate sugars (2.62 g/L xylose, 1.38 g/L glucose, and 0.91 g/L arabinose). Each medium was tested in triplicate. Following a purge with $N_2(g)$, fermentation was performed with agitation at 250 rpm. After 12 h, the cultures were analyzed by HPLC for sugars and ethanol. The sugars were consumed completely in all cultures. The ethanol titer was compared to a theoretical yield of 0.51 g ethanol/g sugar (2.04 g/L for pure sugars or 2.25 g/L for corn stover hydrolyzate).

Yeast Growth. *P. stipitis* strain CBS 6054 was a gift from T.W. Jeffries (University of Wisconsin–Madison). All cultures of *Pichia* were grown at 30 °C. A single colony was used to inoculate YP medium (6 mL; 10 g/L yeast extract and 20 g/L peptone) containing xylose (1.2 wt%) and glucose (0.8 wt%). After incubation in a culture tube agitated at 225 rpm for 11 h, the cells in a 1-mL aliquot of the culture were collected by centrifugation. The cells were resuspended in synthetic minimal medium (0.5 mL) containing yeast nitrogen base without amino acids (6.7 g/L; Difco).

In a polystyrene 96-well plate, 10 wells were filled with synthetic minimal medium (150 μL) containing xylose (1.82 g/L), glucose (2.18 g/L), and arabinose (0.33 g/L). Five wells were filled with synthetic minimal medium (150 μL) supplemented with corn stover hydrolyzate sugars (1.82 g/L xylose, 2.18 g/L glucose, and 0.33 g/L arabinose). The remaining wells were filled with deionized water (150 μL). Each well was inoculated with the above cell suspension (10 μL), and the plate was capped with a low-evaporation lid and incubated with rapid agitation in a BioTek ELx808 Absorbance Microplate Reader. The $OD_{595\ \mathrm{nm}}$ of each well was measured every 5 min for 19 h.

Yeast Fermentation. *P. stipitis* was grown at 30 °C in YP medium (10 g/L yeast extract and 20 g/L peptone) containing the appropriate carbon source. A single colony was inoculated into medium (6 mL) containing 1.2 wt% xylose and 0.8 wt% glucose. After incubation in a culture tube agitated at 225 rpm for 11 h, the yeast suspension was added to glass test tubes containing YP medium (1.5 mL) supplemented with either xylose (3.24 g/L), glucose (3.88 g/L), and arabinose (0.58 g/L), or corn stover hydrolyzate sugars (3.24 g/L xylose, 3.88 g/L glucose, and 0.58 g/L arabinose). The sugar medium was tested in triplicate, and the hydrolyzate medium in duplicate. The test tubes were fitted with rubber stoppers pierced with needles and agitated at 150 rpm. After 52 h, the cultures were analyzed by HPLC for sugars and ethanol. The sugars were consumed completely in all cultures. The ethanol titer was compared to a theoretical yield of 0.51 g ethanol/g of glucose and xylose (3.63 g/L for pure sugars or 3.63 g/L for corn stover hydrolyzate).

Economics of Ionic Liquid Usage. In this estimation, biomass is treated in a batch process using an ionic liquid to produce sugars that are fermented into ethanol. Most of the ionic liquid is recovered and recycled for the next batch, and the remainder is replaced with fresh ionic liquid. The target for the cost of the replacement ionic liquid is $0.13/L ethanol product ($0.50/gal), which is the anticipated cost of enzymes for a process based on enzymatic hydrolysis. The fraction of the ionic liquid that must be recovered from each batch is estimated below based on inputs and assumptions about the process.

Inputs and Assumptions. Ionic liquid cost: $2.50/kg.

Monosaccharide theoretical yield: 0.73 kg monosaccharide/kg biomass (biomass with 65% polysaccharide content).

Fermentation theoretical yield: 0.51 kg ethanol/kg monosaccharide.

Hydrolysis molar yield (optimized): 90%.

Fermentation molar yield (optimized): 90%.

Ionic liquid/biomass ratio: 1 : 1.

Yield of Ethanol from Biomass.

$$\frac{0.73\ \text{kg monosaccharide}}{\text{kg biomass}} \times \frac{0.51\ \text{kg ethanol}}{\text{kg monosaccharide}} \times 0.9 \times 0.9 \times \frac{1.262\ \text{L ethanol}}{\text{kg ethanol}} = \frac{0.38\ \text{L ethanol}}{\text{kg biomass}}$$

Cost of Ionic Liquid Used to Produce 1 L of Ethanol.

$$\frac{\$2.50}{\text{kg ionic liquid}} \times \frac{1\ \text{kg ionic liquid}}{\text{kg biomass}} \times \frac{\text{kg biomass}}{0.38\ \text{L ethanol}} = \frac{\$6.58}{\text{L ethanol}}$$

Tolerable Ionic Liquid Loss.

$$\frac{\left(\frac{\$0.13}{\text{L ethanol}}\right)}{\left(\frac{\$6.58}{\text{L ethanol}}\right)} = 2.0\%$$

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Table S1. Acid-catalyzed degradation of glucose in [EMIM]Cl

H_2O, wt%	Time, min	Glucose recovery, %	HMF yield, %
0*	0	100	0
	5	100	0
	15	97	0
	30	103	0
	60	101	0
0	0	100	0
	5	49	6
	15	35	14
	30	20	11
	60	13	11
9	0	100	0
	5	80	0
	15	63	12
	30	55	13
	60	43	24
20	0	100	0
	5	92	4
	15	82	2
	30	76	5
	60	65	11
33	0	100	0
	5	93	0
	15	95	0
	30	90	3
	60	87	2

Glucose was reacted in [EMIM]Cl at 100 °C with an initial concentration of 10 wt% and a H_2SO_4 loading of 4 wt% relative to glucose. The water content is relative to the total mass of the reaction mixture. Glucose recovery is based on HPLC analysis and is normalized to the initial glucose concentration. HMF molar yield is based on HPLC analysis.

*No H_2SO_4.

Table S2. Effect of dissolution time on hydrolysis of cellulose

		Water content , wt%							
Dissolution time, h	HCl, wt%	0′	10′	20′	30′	60′	Time, h	Glucose yield, %	HMF yield, %
3	20	5	20	25	33	43	2	86	3
							3	90	5
							4	92	7
6	20	5	20	25	33	43	2	89	4
							3	93	6
							4	92	8
9	20	5	20	25	33	43	2	83	4
							3	86	5
							4	87	7

Cellulose was reacted in [EMIM]Cl at 105 °C with an initial concentration of 5 wt%. HCl loading is relative to cellulose mass. Yields are molar yields based on HPLC analysis and are relative to the glucose monomers contained in the cellulose.