ACS Sustainable Chemistry & Engineering

Research Article

pubs.acs.org/journal/ascecg

Facile and Simple Pretreatment of Sugar Cane Bagasse without Size Reduction Using Renewable Ionic Liquids−Water Mixtures

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Supporting Information

ABSTRACT: In this work, sugar cane bagasse pretreatment by renewable cholinium amino acids ionic liquids ([Ch][AA] ILs) and subsequent enzymatic hydrolysis of the residues were conducted. Six ILs tested were found to be effective for sugar cane bagasse pretreatment. Upon pretreatment using these ILs, the enzymatic digestion of this lignocellulosic biomass was improved significantly due to extensive delignification. The IL cholinium lysine ([Ch][Lys]) displayed excellent pretreatment efficiency with sugar cane bagasse of various sizes as the substrates. The addition of water into [Ch][Lys] did not exert a negative effect on pretreatment effectiveness, although the delignification capacity of the IL decreased. The sugar yields of 80% for glucose and 84% for xylose were obtained in the enzymatic hydrolysis after the sugar cane bagasse without size reduction was pretreated with a biomass loading of 5 wt % by 50 g 50% [Ch][Lys]−water mixture at 90 °C for 6 h. A simple and atom-economic preparation approach to ILs was successfully developed for lignocellulosic biomass pretreatment, which may significantly reduce ILs costs, and the reactant contaminations in the IL−water mixture had no detrimental effect on the pretreatment efficiency.

KEYWORDS: *Cholinium amino acids ionic liquids, Delignification, Enzymatic hydrolysis, Lignocellulosic biomass, Particle size*

INTRODUCTION

With the frequent eruption of the energy crisis, significant increase in fuel prices, and increasing concerns about global warming in recent years, use of renewable, clean, and abundant lignocellulosic biomass such as agricultural and forestry residues has attracted considerable interest for the production of fuels and platform chemicals.[1,2] Sugar cane bagasse is one of the most abundant agro-industrial byproducts. Approximately 100 million tons annually of this lignocellulosic biomass are produced, and more than 50% of the total productivity is contributed by China, Brazil, India, and Thailand.[3,4] Unfortunately, currently, most of the sugar cane bagasse is burned to produce energy, particularly in China, which not only wastes the valuable bioresource but also results in environmental problems. Indeed, sugar cane bagasse is a promising raw material for biorefinery because there is a high content of fiber and a low content of ash in this biomass.[4] More importantly, this lignocellulosic biomass is a byproduct from the sugar industry, and therefore, the labor- and energy-extensive collection of the feedstock is avoided, which is vital for cost-effective production of low value biofuels. However, conversion of lignocellulosic biomass into fermentable sugars remains challenging because it is highly recalcitrant to chemical and biological degradation due to the complex heteromatrix structures. Therefore, pretreatment, which results in disruption of the crystalline structure of cellulose and/or removal of hemicellulose and lignin, is an essential step for overcoming the native recalcitrance of lignocellulosic biomass to degradation.

A variety of pretreatment methods including physical, physicochemical, chemical, and biological approaches are constantly developed to meet the increasing demand for low cost, high efficiency, environmental friendliness, etc.[5,6] Ionic liquids (ILs) has emerged as a promising type of solvents for lignocellulosic biomass pretreatment because of their strong dissolution ability toward biomass components under mild conditions, high chemical and thermal stability, nonflammability, and recyclability.[7,8] However, traditional imidazolium and pyridinium ILs are nonrenewable and have been demonstrated to be highly toxic and poorly biodegradable,[9,10] which will undoubtedly limit their large-scale applications. In addition, the presence of low quantities of water in these ILs was reported to be considerably detrimental to the dissolution ability of these ILs toward cellulose as well as the pretreatment efficiency.[11−13] Therefore, extensive drying of raw materials and ILs is generally necessary prior to pretreatment, resulting in a significant increase in energy consumption. This motivated an

Received: December 19, 2012
Revised: March 19, 2013
Published: March 20, 2013

Table 1. Pretreatment of Sugar Cane Bagasse Using Various ILs and Subsequent Enzymatic Hydrolysis of the Residues

pretreatment[a]				composition of residues (%)[b]				enzymatic hydrolysis of residues[c]			
								initial saccharification rate (mg mL^{-1} h^{-1})		sugar yield (%)	
ILs (%)	lignin extracted (%)	residues recovery (%)	cellulose CrI (%)[d]	cellulose	xylan	AIL	ASL	glucose	xylose	glucose	xylose
untreated	0	100	43.4	35.8	13.5	24.5	0.73	0.14	0.01	25.5	6.3
[Ch][OAc]	12.4	85.4	58.8	40.3	12.7	24.9	0.62	0.48	0.12	41.3	29.1
[Ch][Lys]	61.5	56.6	75.5	61.4	14.7	13.9	0.55	1.58	0.55	80.7	56.1
[Ch][Gly]	63.3	53.8	70.9	59.4	15.1	13.4	0.48	1.60	0.55	75.9	50.3
[Ch][Ala]	58.4	55.1	66.7	61.8	15.3	14.6	0.52	1.54	0.52	71.0	50.1
[Ch][Ser]	49.8	60.6	69.5	57.4	15.4	16.6	0.53	1.15	0.44	74.1	58.9
[Ch][Thr]	44.9	65.3	70.8	49.9	15.3	17.8	0.48	1.47	0.54	71.9	66.1
[Ch][Pro]	60.6	60.8	66.3	52.6	15.2	13.4	0.53	1.19	0.44	71.0	63.5

[a]A total of 300 mg samples of sugar cane bagasse (including the rind and pith, comminuted with size of 100 mesh) was incubated in 6 g of ILs under N_2 with magnetic stirring at 90 °C for 6 h. [b]Determined via the NREL protocol. Results are expressed as a percentage of the residues. AIL, acid-insoluble lignin; ASL, acid-soluble lignin. [c]Reaction conditions: 20 mg recovered sugar cane bagasse, 7 mL citrate buffer (50 mmol L^{-1}, pH 4.8), 17.5 U mL^{-1} cellulase from *Trichoderma reesei*, 50 °C, 200 rpm. Values calculated from the time courses shown in Figure S1 of the Supporting Information. [d]Values calculated from XRD spectra in Figure S2 of the Supporting Information.

intense interest in the development of moisture-tolerant ILs pretreatment processes.[14,15]

Recently, the synthesis of novel ILs from renewable biomaterials and their use for biomass pretreatment have received more attention.[16−21] Our group has reported on a group of novel renewable cholinium amino acids ([Ch][AA]) ILs;[16] most of them have been found to be effective for rice straw pretreatment.[17] The pronounced pretreatment effectiveness of these novel biological ILs can be attributed to selective removal of lignin rather than the decrease in cellulose crystallinity.[17] Hence, this kind of ILs-mediated pretreatment is expected to be highly tolerant to moisture because the presence of water cannot result in a significant decrease of lignin solubility in ILs. A substantial amount of xylan (50%) in the native rice straw was lost after pretreatment by the IL cholinium lysine ([Ch][Lys]) at 90 °C for 5 h[17] because xylan is a thermo-, acid-, and base-sensitive biopolymer.[22] Xylan loss would not only reduce the yield of fermentable reducing sugar but also have a negative effect on the downstream lignin separation and ILs reuse. Adjustment of pretreatment severity, especially the alkalinity of the solvent, by adding water is feasible for the reduction of xylan loss.[15] In addition, the addition of water can not only significantly reduce the viscosity of ILs, which makes handling easier, but also can enhance mass transfer. Besides, the pretreatment process will be more cost-effective and environmentally friendly due to the replacement of significant quantities of expensive ILs with cheaper and greener water.

Particle size of lignocellulosic biomass is one of the important factors affecting pretreatment economy because size reduction will consume substantial amounts of energy. Generally, size reduction is beneficial for enzymatic digestion of biomass because of the remarkable increase in the accessible surface area and/or disruption of the crystalline structure of cellulose.[23,24] Interestingly, it has been reported that favorable particle size of lignocellulosic biomass is ILs-[25] or materials-dependent[26] for efficient pretreatment. In this work, the effect of sugar cane bagasse size on the pretreatment effectiveness of [Ch][AA] ILs−water mixtures and subsequent enzymatic hydrolysis was investigated. The preparation of ILs, especially purification, is usually time consuming and arduous. Therefore, this work also evaluated the pretreatment effectiveness of ILs prepared through a simple and atom-economic process.

EXPERIMENTAL SECTION

Reagents. Cellulase/xylanase from *Trichoderma reesei* was bought from Sigma−Aldrich (U.S.A.) (unit definition: one unit will liberate 1.0 μmol of glucose from Sigmacell cellulose Type 20 in 1 h at pH 5.0 at 37 °C) and was used as received. The [Ch][AA] ILs [Ch][Lys], cholinium glycine ([Ch][Gly]), cholinium alanine ([Ch][Ala]), cholinium serine ([Ch][Ser]), cholinium threonine ([Ch][Thr]), and cholinium proline ([Ch][Pro]) were prepared as described by us.[16] Cholinium acetate ([Ch][OAc]) was synthesized with a similar method. Fresh sugar cane bagasse obtained locally was air-dried at room temperature for 2 days, mechanically powdered or cut into various sizes, and stored in −20 °C before use. Other chemicals were of the highest purity commercially available.

Pretreatment of Biomass with ILs−Water Mixtures. Sugar cane bagasse pretreatment was carried out as described recently with slight modification.[17] Briefly, sugar cane bagasse samples were incubated under N_2 in various ILs−water mixtures with a biomass loading of 5 wt % stirred with a magnetic stirrer; IL content, temperature, and time period are stated for each experiment. Then, the suspension was diluted with water and filtered. With the corresponding IL as the control, the filtrate was decanted to a vial for the determination of lignin content at 280 nm with the extinction coefficient of 2.19 L g^{-1} cm^{-1} (Shimadzu UV 2550, Japan). The residues were washed with distilled water until the filtrate was colorless. Then, the residues were lyophilized and stored in a sealed bag at −20 °C prior to use.

Compositional Analysis of Sugar Cane Bagasse. Cellulose, xylan, and lignin contents of sugar cane bagasse samples were determined according to the standard NREL analytical procedure.[27] Briefly, the samples were treated successively with 72% sulfuric acid at 30 °C for 1 h and 4% acid at 121 °C for 1 h. The sugar contents were determined by HPLC (Waters 515) equipped with a Bio-Rad Aminex HPX-87H column and a refractive index detector (Waters 2410). The mobile phase consisted of a 5 mmol L^{-1} sulfuric acid aqueous solution with a flow rate of 0.5 mL min^{-1}. The column temperature was 65 °C. The cellulose and xylan contents were calculated from glucose and xylose contents multiplied by conversion factors of 0.90 and 0.88, respectively.[17] The content of acid-insoluble lignin was determined gravimetrically by using filtering crucibles. The content of acid-soluble lignin was measured spectrophotometrically at 320 nm using the extinction coefficient of 30 L g^{-1} cm^{-1}.

The losses of cellulose and xylan were calculated as follows

$$\text{Loss of polysaccharide} = \left(1 - \frac{\text{Residues recovery} \times \text{polysaccharide content in residues}}{\text{Native polysaccharide content}}\right) \times 100$$

Enzymatic Hydrolysis. Enzymatic hydrolysis was conducted at 50 °C in a 50 mL vial containing 20 mg biomass and 17.5 U mL^{-1} cellulase in 7 mL citrate buffer (50 mmol L^{-1}, pH 4.8). The reaction was stirred at 200 rpm, and aliquots (200 μL) were withdrawn at specified time intervals and boiled for 5 min to quench the enzymatic reaction. After centrifugation (18,000 *g*, 10 min), the glucose and xylose concentrations were measured by HPLC. All reactions were performed in duplicate.

Glucose and xylose yields were calculated as follows

$$\text{Sugar yield (\%)} = \frac{\text{Released sugar amount}}{\text{Theoretic sugar amount in native biomass}} \times 100$$

The initial saccharification (sugar release) rates were calculated from the sugar released in 30 min.

Fourier Transform Infrared Spectroscopy (FTIR) Analysis. Samples were pressed uniformly with KBr into pellets for FTIR analysis in transmission mode using a Bruker Optics Vector 33 system (Bruker, Germany). Spectra were recorded over the range of 400−4000 cm^{-1} with a spectral resolution of 0.3 cm^{-1}.

X-ray Diffraction (XRD) Analysis. The samples were scanned on a D8 ADVANCE diffractometer (Bruker, Germany) from 2θ = 5−60° with a scan speed of 0.07°/min and a step size of 0.04° at 40 kV and 40 mA. The cellulose crystallinity index (CrI) was determined by the deconvolution method:[28] $CrI = \Sigma(A_{Cr})/(A_{Tol}) \times 100$, where A_{Cr} is the summation of the integrated areas of the crystalline peaks and A_{Tol} is the summation of all the integrated areas. The crystalline peaks correspond to (101) at $2\theta \approx 14.75°$, ($10\bar{1}$) at $2\theta \approx 16.5°$, (021) at $2\theta \approx 20.65°$, and (002) at $2\theta \approx 22.5°$. Curve fitting was used to separate the four crystalline peaks from the amorphous signal.

RESULTS AND DISCUSSION

Sugar Cane Bagasse Pretreatment with Various ILs. Six [Ch][AA] ILs with low viscosity and high solubility toward lignin were used for sugar cane bagasse pretreatment (Table 1), with a typical cholinium alkanoate IL, [Ch][OAc], as the comparison. It could be found that the pretreatment by [Ch][OAc] at 90 °C for 6 h removed only 12.4% lignin from sugar cane bagasse and thus afforded the residues with low contents of polysaccharides, although this IL has proved to be effective for bamboo powder pretreatment recently,[29] and the sugar yields (41.3% for glucose and 29.1% for xylose) were also not satisfactory in the enzymatic hydrolysis. Interestingly, all tested [Ch][AA] ILs showed strong delignification ability for sugar cane bagasse, although the lignin content in this biomass is much higher than that in rice straw (25% vs 18%).[17] For example, lignin of 44.9−63.3% was removed from sugar cane bagasse after pretreatment using these [Ch][AA] ILs. As shown in Table 1, the delignification capacity of these ILs showed a clear dependence on the anion structures. The basicity of the ILs as well as other solvent properties might play a role in the pretreatment of the lignocellulosic biomass.[19] In addition, selective delignification may occur mainly in the cell corner and compound middle lumen of cell walls, which has been visualized by transmission electron microscopy.[18] Accordingly, the composition of the lignocellulosic biomass changed significantly after pretreatment. After pretreatment by [Ch][Lys], for example, the cellulose content in sugar cane bagasse increased from 35.8% to 61.4%, while the content of acid-insoluble lignin decreased from 24.5% to 13.9%. The changes in chemical composition after pretreatment were also characterized by FTIR analysis (Figure 1). As compared to the native

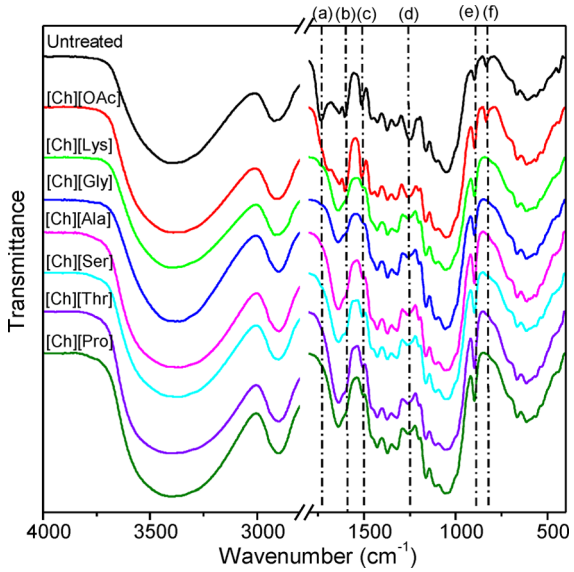

Figure 1. FTIR spectra of the untreated and pretreated sugar cane bagasse. Regions of the spectra mentioned in the text are annotated as follows: (a) 1732 cm^{-1}, (b) 1604 cm^{-1}, (c) 1513 cm^{-1}, (d) 1249 cm^{-1}, (e) 898 cm^{-1}, and (f) 834 cm^{-1}.

biomass, the changes in the spectrum of sugar cane bagasse pretreated by [Ch][OAc] were slight, especially in the characteristic peaks, which is in good agreement with the similar composition of the native and this IL-pretreated biomass (Table 1). Nonetheless, the spectra of sugar cane bagasse changed drastically after pretreatment by [Ch][AA] ILs. The peaks at 1604 cm^{-1} (lignin aromatic skeletal vibrations plus a C=O stretch) nearly disappeared, and the intensity of the peaks at 1513 cm^{-1} (lignin aromatic skeletal vibrations)[30] was greatly reduced upon pretreatment. In addition, the peaks at 834 cm^{-1} assigned to a C−H out-of-plane vibration in lignin[31] completely disappeared in the recovered residues. This indicated the removal of large quantities of lignin. Also, the disappearance of the peaks at 1732 cm^{-1} suggested the cleavage of ester linkages between lignin and carbohydrates[32] or the extensive deacetylation in hemicellulose.[33] In addition, the breakage of ester linkages between lignin and hemicellulose after pretreatment was further confirmed by the reduced peak intensity at 1249 cm^{-1} (C−O stretching signals in lignin and hemicellulose).[34] The peaks at 898 cm^{-1} can be attributed to the β-glycosidic linkages between the sugar units in cellulose and hemicellulose, and the increase in intensity demonstrated the presence of predominant polysaccharides in the recovered residues.[32]

Enzymatic saccharifications of the residues are presented in Table 1. The initial saccharification rates of pretreated sugar cane bagasse were improved greatly as compared to that of the native biomass (Table 1, 8.2- to 11.4-fold increase in initial rate of glucose release and 44.0- to 55.0-fold increase in initial rate of xylose release). In addition, the digestibility of polysaccharides (Figure S1, Supporting Information, 82−93% for cellulose and 80−89% for xylan) and the yields of sugars (Table 1, 71−80% for glucose and 50−66% for xylose) were also drastically improved upon pretreatment. The significant enhancement in the enzymatic hydrolysis could be ascribed

dx.doi.org/10.1021/sc300172v | ACS Sustainable Chem. Eng. 2013, 1, 519−526

Table 2. Effect of Substrate Size on Sugar Cane Bagasse Pretreatment and Subsequent Enzymatic Hydrolysis

							enzymatic hydrolysis of residues[c]			
pretreatment[a]			composition of residues (%)[b]				initial saccharification rate (mg mL^{-1} h^{-1})		sugar yield (%)	
substrate size (mm)	lignin extracted (%)	residues recovery (%)	cellulose	xylan	AIL	ASL	glucose	xylose	glucose	xylose
untreated[d]	0	100	39.5	12.1	22.7	0.68	0.14	0.02	28.4	9.4
5 × 3 × 2	50.4	54.8	66.7	14.9	13.0	0.34	1.49	0.35	82.1	45.3
8 × 6 × 3	47.2	57.5	66.5	15.4	13.2	0.32	1.47	0.28	84.8	46.2
14 × 8 × 5	47.5	57.9	63.6	16.8	14.2	0.36	1.48	0.33	83.8	48.5
20 × 10 × 6	44.6	58.5	61.4	16.0	13.8	0.39	1.36	0.31	79.6	53.4
30 × 25 × 8	44.7	58.3	60.7	16.5	14.5	0.41	1.48	0.32	82.1	47.7

[a]A total of 300 mg samples of sugar cane bagasse (pith of sugar cane bagasse) was incubated in 6 g of [Ch][Lys] under N_2 with magnetic stirring at 90 °C for 6 h. [b]Determined via the NREL protocol. Results are expressed as a percentage of the residues. AIL, acid-insoluble lignin; ASL, acid-soluble lignin. [c]Reaction conditions: 20 mg recovered sugar cane bagasse, 7 mL citrate buffer (50 mmol L^{-1}, pH 4.8), 17.5 U mL^{-1} cellulase from *Trichoderma reesei*, 50 °C, 200 rpm. [d]Size of 5 mm × 3 mm × 2 mm. Values calculated from the time courses shown in Figure S3 of the Supporting Information.

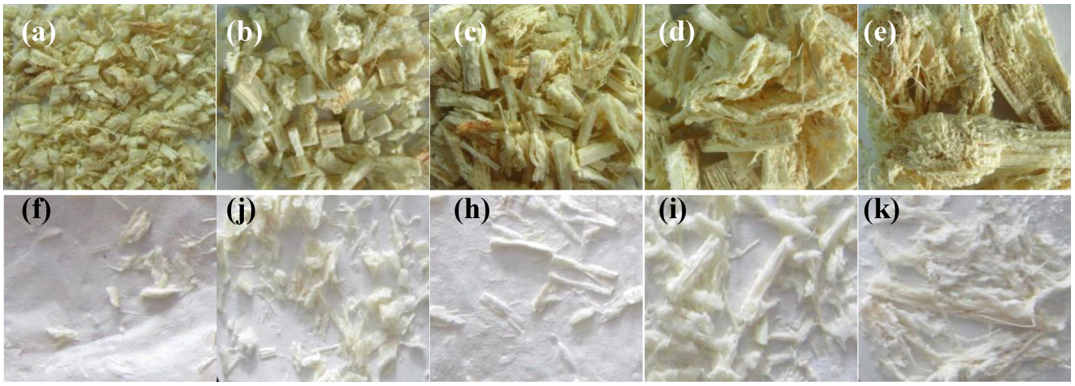

Figure 2. Pictures of sugar cane bagasse pith with different particle size before treatment (a, b, c, d, e) and after treatment (f, j, h, i, k). (a) (f) 5 mm × 3 mm × 2 mm, (b) (j) 8 mm × 6 mm × 3 mm, (c) (h) 14 mm × 8 mm × 5 mm, (d) (i) 20 mm × 10 mm × 6 mm, and (e) (k) 30 mm × 25 mm × 8 mm.

to the considerable removal of lignin rather than the decrease in cellulose CrI because pretreatment led to net increase in cellulose CrI of the samples (Table 1 and Figure S2, Supporting Information), possibly due to the considerable removal of amorphous components such as lignin. This is consistent with our recent results.[17] On the basis of the high delignification capacity and good sugar yields, [Ch][Lys] was used for subsequent studies.

Effect of Sugar Cane Bagasse Size. Particle size not only exerts an effect on enzymatic digestibility of lignocellulosic biomass but also is a crucial factor affecting the pretreatment economy because comminution or size reduction is an energy intensive process.[35] Obviously, it is desirable to directly treat large size biomass to furnish highly digestible residues. However, maximal particle sizes for pretreatment were reported to be material- and method-dependent;[35] interestingly, Bahcegul's recent work proved that different ILs favored different lignocellulosic biomass particle sizes.[25] To establish a low-cost sugar cane bagasse pretreatment process, the effect of particle size on sugar cane bagasse pretreatment was investigated (Table 2).

Our preliminary study showed that no decrease in sugar yields was found in the enzymatic hydrolysis after sugar cane bagasse with particle size ranging from 20 to 100 mesh was pretreated by [Ch][Lys] (data no shown). Inspired by the results, the particle size of sugar cane bagasse was further increased, and its effect on pretreatment and subsequent enzymatic hydrolysis was presented in Table 2. In order to obtain raw materials of relatively uniform structure and size, the pith of sugar cane bagasse was selected to evaluate the influence of biomass sizes because its composition is relatively homogeneous and its size is prone to being controlled. The pictures of the native biomass and recovered residues are shown in Figure 2. It was found that the morphology of sugar cane bagasse altered drastically after pretreatment. The original structure of the native biomass was disrupted completely, and the samples became whiter and looser, possibly due to extensive delignification. As shown in Table 2, the delignification capacity of the IL weakened with the increase in sugar cane bagasse size. For example, the lignin extractability was much lower for sugar cane bagasse of larger sizes than that for 100 mesh biomass (44.6−51.5% vs 61.5%).

There was an apparent correlation between digestibility of polysaccharides without pretreatment and biomass size in the enzymatic hydrolysis of the native biomass (Figure S3, Supporting Information). For example, the cellulose digestibility was much higher for the native biomass of 5 mm × 3 mm × 2 mm than for the native biomass of 30 mm × 25 mm × 8 mm (28% vs 15%). However, after pretreatment, enzymatic hydrolysis of polysaccharides in the residues did not show a clear dependence on the biomass size. In all cases, highly digestible residues were produced after pretreatment, although the delignification degrees were different. It has been reported that complete removal of lignin is not necessary for the

Table 3. Effect of IL Content on Sugar Cane Bagasse Pretreatment and Subsequent Enzymatic Hydrolysis

pretreatment[a]			composition of residues (%)[b]				enzymatic hydrolysis of residues[c]			
							initial saccharification rate (mg mL^{-1} h^{-1})		sugar yield (%)	
IL content (wt %)	lignin extracted (%)	residues recovery (%)	cellulose	xylan	AIL	ASL	glucose	xylose	glucose	xylose
untreated	0	100	39.5	12.1	22.7	0.68	0.11	0.08	14.9	9.2
0	4.6	90.2	42.4	13.2	23.3	0.63	0.26	0.11	14.9	29.9
5	26.9	72.8	52.5	15.7	21.6	0.55	1.25	0.28	72.8	46.4
20	32.2	64.4	56.2	17.4	18.7	0.46	1.46	0.35	76.7	57.0
50	38.6	59.6	61.9	17.9	16.8	0.52	1.49	0.29	83.1	51.6
80	45.0	59.1	61.1	17.6	15.9	0.50	1.62	0.40	83.8	51.0
100	44.7	58.3	60.7	16.5	14.5	0.41	1.48	0.32	82.1	47.7

[a]A total of 400 mg samples of sugar cane bagasse (pith of sugar cane bagasse) with the size of around 30 mm × 25 mm × 8 mm was incubated in 8 g [Ch][Lys]−water mixture under N_2 with magnetic stirring at 90 °C for 6 h. [b]Determined via the NREL protocol. Results are expressed as a percentage of the residues. AIL, acid-insoluble lignin; ASL, acid-soluble lignin. [c]Reaction conditions: 20 mg recovered sugar cane bagasse, 7 mL citrate buffer (50 mmol L^{-1}, pH 4.8), 17.5 U mL^{-1} cellulase from *Trichoderma reesei*, 50 °C, 200 rpm. Values calculated from the time courses shown in Figure S5 of the Supporting Information

achievement of good cellulose digestibility.[17,36,37] Therefore, more than 44% lignin removal might be practicable for the production of readily biodegradable residues. In the enzymatic digestion of the residues, comparable initial saccharification rates (1.36−1.49 mg mL^{-1} h^{-1} of initial glucose release rate and 0.28−0.35 mg mL^{-1} h^{-1} of initial xylose release rate) and sugar yields (79.6−84.8% for glucose and 45.3−53.4% for xylose) were achieved (Table 2).

Effect of IL Content. [Ch][Lys]−water mixtures were evaluated as the solvents for sugar cane bagasse pretreatment (Table 3). Only 4.6% lignin was extracted from sugar cane bagasse after pretreatment by water at 90 °C for 6 h, although hot water proved to be an effective solvent for lignocellulosic biomass pretreatment at higher temperature.[4,38] When the IL content was varied in the range of 5−100%, it was found that higher IL content favored delignification. For example, 26.9% lignin was removed after pretreatment by 5% [Ch][Lys]−water mixture, while the lignin extractability of 45.0% was obtained with 80% [Ch][Lys]−water mixture. The composition of the biomass changed significantly after pretreatment, which was tracked by FTIR analysis (Figure S4, Supporting Information). For example, the cellulose contents increased markedly from 39.5% to 52.5−61.9%, and acid-insoluble lignin contents decreased from 22.7% to 14.5−21.6% due to delignification. It could be demonstrated by the great decreases in the intensity of the peaks at 1732 cm^{-1} (carbonyl C═O stretching in hemicellulose),[32] 1513 cm^{-1} (lignin aromatic skeletal vibrations)[30], and 1249 cm^{-1} (C−O stretching in lignin and hemicellulose)[34] in FTIR spectra after pretreatment. It was also found that losses of polysaccharides increased at high IL contents. For example, xylan losses increased remarkably from 6% to 21% with increasing [Ch][Lys] content from 5% to 100%, while the similar trend was observed for cellulose losses (3−10%). Hence, control of water content may be a facile and effective way to regulate pretreatment severity to avoid significant losses of polysaccharides, especially alkali-sensitive xylan, which is in agreement with others' results.[14,15]

Interestingly, the presence of water in the IL did not greatly exert a negative effect on pretreatment effectiveness, although the delignification capacity of the IL decreased. The enzymatic hydrolysis of sugar cane bagasse was enhanced significantly after pretreatment by IL−water mixtures as compared to that of the native biomass. For example, after pretreatment by ≥50% IL−water mixtures, the cellulose digestibility reached 89−92% in the enzymatic hydrolysis (Figure S5, Supporting Information), and glucose yields of >80% were obtained (Table 3). However, the maximum xylose yield was obtained with 20% IL−water mixture, and further increase in IL content led to the decline in xylose yields. The possible reason is that high IL contents (≥50%) led to more xylan losses, while the xylan digestibility (59−62%) was kept constant. Hence, 50% [Ch][Lys]−water mixture was used for the following studies because of its high pretreatment efficiency for large size sugar cane bagasse.

Scale-Up Pretreatment and ILs Reusability. Encouraged by the above-described results, we evaluated the scale-up pretreatment of sugar cane bagasse without comminution or size reduction. In order to put into a 100 mL flask, sugar cane bagasse was cut into 60 mm length (Figure 3). After

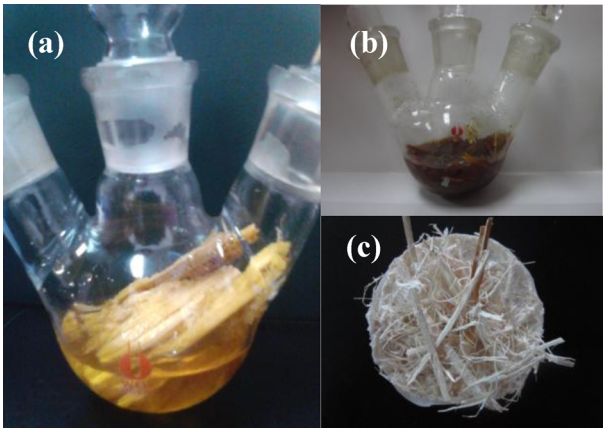

Figure 3. Photographs of the pretreatment process. (a) Before pretreatment, (b) after pretreatment, and (c) the dried residues after pretreatment.

pretreatment, the IL−water mixture became dark, and the residues including the hard rind became softer and whiter, likely due to removal of lignin. The quantitative analysis in the subsequent studies was based on the composition of 100 mesh sugar cane bagasse powder because of the heterogeneity of the components in the residues (including the rind and pith). The pretreatment effectiveness of [Ch][Lys]−water mixture on a

larger scale was demonstrated by the significantly enhanced enzymatic hydrolysis of the residues (Figure 4). For example,

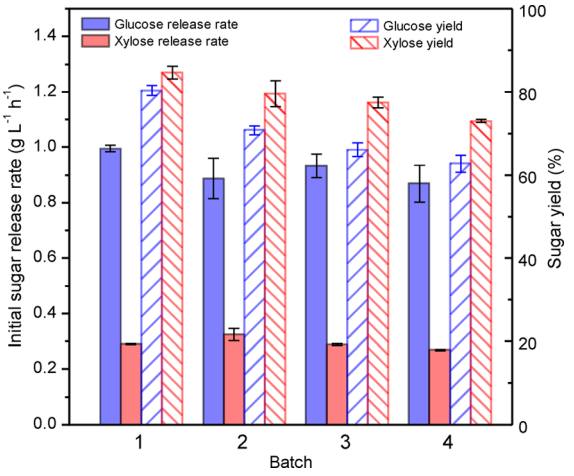

Figure 4. Reuse of [Ch][Lys] for sugar cane bagasse pretreatment. Pretreatment process: 2.5 g samples of sugar cane bagasse (including the rind and pith) with the size of around 60 mm × 30 mm × 8 mm were incubated in 50 g of 50% [Ch][Lys]−water mixture under N_2 with magnetic stirring at 90 °C, 6 h/batch. Reaction conditions: 20 mg pretreated residues, 17.5 U mL^{-1} cellulase, 7 mL citric acid buffer (50 mM, pH 4.8), 50 °C, 200 rpm.

good initial sugar release rates (1.0 mg mL^{-1} h^{-1} for glucose and 0.3 mg mL^{-1} h^{-1} for xylose) and high sugar yields (80% for glucose and 84% for xylose) were obtained in the enzymatic hydrolysis of sugar cane bagasse after pretreatment by 50% [Ch][Lys]−water mixture at 90 °C for 6 h. Although the glucose yield is comparable to those by the nonrenewable imidazolium ILs aqueous solutions,[14,15,32,39] the pretreatment conditions are much milder, and the xylose yield is much higher in this work than in previous reports. For example, Brandt et al. reported the sugar yields of approximately 80% for glucose and 30% for xylose after *Miscanthus* pulp was pretreated by 80% [Bmim][$MeSO_4$]− and 80% [Bmim][HSO_4]−water mixtures at 120 °C for 8 h.[14] It is worth noting that the xylose yield is much higher in the enzymatic hydrolysis of the hetero sugar cane bagasse biomass (including the rind and pith) than those in the enzymatic hydrolysis of sugar cane bagasse pith residues (84% vs 45−53%), which may be attributed to the higher xylan content in the rind (16.8% vs 12.1% in the pith). Also, low xylan loss in the dense rind might contribute to the high xylose yield.

To assess the reusability of the IL for sugar cane bagasse pretreatment, the [Ch][Lys]−water mixture was recovered and was directly reused for three batches without separation of lignin (Figure 4). It was found that the initial sugar release rates changed slightly after 50% [Ch][Lys]−water mixture was reused three times, but the glucose and xylose yields decreased significantly to 62.7% and 73.0%, respectively. This could be accounted for by the decline in delignification capacity of IL−water mixture (from 36.4% to 28.6%) during successive cycles of reuse due to progressive accumulation of lignin in the solvent. The removal of lignin by organic solvents[40] from IL−water mixture may improve the pretreatment effectiveness during reuse.

Pretreatment by [Ch][Lys] Prepared with Different Methods. Although ILs have a lot of promising physicochemical properties and have proved to be effective solvents for lignocellulosic biomass pretreatment,[7,41] the preparation of ILs, especially the purification, is usually time consuming and arduous. In addition, the atom economy of the preparation of ILs appears not to be as good as that of the low-transition temperature mixtures that have emerged recently.[42] Although the synthesis of [Ch][AA] ILs is simple and green, low reaction temperatures (0−4 °C) and long reaction times (48 h) are needed, and their purification is arduous.[16] Therefore, an atom-economic and facile preparation method in which the same equivalent of lysine was directly added to commercially available 46 wt % choline hydroxide aqueous solution and incubated at room temperature for 2−24 h was proposed, and after the addition of a certain amount of water, 50% IL aqueous solutions without additional purification steps were directly used for sugar cane bagasse pretreatment (Table 4). As shown in Table 4, the three IL−water mixtures displayed comparable delignification capacity (36.1−38.3%), which can be explained rationally by the same IL structures (Figure S6, Supporting Information). Also, the initial saccharification rates (1.00−1.06 mg mL^{-1} h^{-1} for glucose release and 0.30−0.33 mg mL^{-1} h^{-1} for xylose release) and sugar yields (80.3−84.6% for glucose and 81.0−85.3% for xylose) were comparable in the enzymatic hydrolysis.

Contaminations in the solvent can influence the dissolution capacity of an IL, thus resulting in a negative effect on pretreatment.[12] Therefore, the effect of the reactant contaminations in the mixtures on sugar cane bagasse pretreatment was studied (Table 4). The IL−water mixtures with and

Table 4. Effect of [Ch][Lys] Prepared with Different Methods on Pretreatment and Subsequent Enzymatic Hydrolysis

IL synthesis			pretreatment[d]		enzymatic hydrolysis of residues[e]			
					initial saccharification rate (mg mL^{-1} h^{-1})		sugar yield (%)	
ratio[a]	time (h)	temperature	lignin extracted (%)	residues recovery (%)	glucose	xylose	glucose	xylose
1:1[b]	48	0−4 °C	36.4	59.4	1.00	0.30	80.3	84.6
1:1	24	rt[c]	36.1	62.4	1.04	0.30	80.6	81.0
1:1	2	rt	38.8	63.7	1.16	0.33	84.6	85.3
1.1:1	2	rt	37.5	63.3	1.11	0.28	84.6	74.1
1:1.1	2	rt	35.7	62.8	1.12	0.29	79.2	77.6

[a]The molar ratio of choline hydroxide to lysine. [b]IL was prepared as described by us recently.[19] [c]Room temperature. [d]A total of 2.5 g samples of sugar cane bagasse (including the rind and pith) with the size of around 60 mm × 30 mm × 8 mm was incubated in 50 g of 50% [Ch][Lys]−water mixture under N_2 with magnetic stirring at 90 °C for 6 h. [e]Reaction conditions: 20 mg pretreated residues, 17.5 U mL^{-1} cellulase, 7 mL citric acid buffer (50 mM, pH 4.8), 50 °C, 200 rpm.

dx.doi.org/10.1021/sc300172v | *ACS Sustainable Chem. Eng.* 2013, 1, 519−526

without the reactant impurities displayed comparable delignification capacity (35.7−37.5% vs 38.8%), and the initial saccharification rates and sugar yields were satisfactory in the enzymatic hydrolysis after pretreatment by these mixtures. The slightly low xylose yields (74.1−77.6%) might be attributed to the heterogeneity of raw materials. This indicates the reactant impurities in the solvent have a marginal effect on the pretreatment effectiveness.

In summary, novel [Ch][AA] ILs−water mixtures have proved to be effective solvents for sugar cane bagasse pretreatment. After pretreatment, the enzymatic hydrolysis of sugar cane bagasse was enhanced significantly, which could be attributed to selective removal of lignin instead of deconstruction of the cellulose crystalline structure. Interestingly, a size-independent sugar cane bagasse pretreatment process has been successfully developed, which can remarkably reduce the costs due to being free of energy-extensive size reduction. More importantly, [Ch][AA] ILs-mediated pretreatment is highly tolerant toward moisture, thus eliminating energy-extensive desiccation of ILs and sugar cane bagasse. The pretreatment process established in this work is promising due to easy handling, mild conditions, low energy consumption, and environmental friendliness, etc., and its large scale application potential has been further reinforced by the studies of successful scale-up process and simple and atom-economic ILs preparation. Further studies, such as extraction of lignin from ILs−water mixtures, are needed for comprehensive utilization of the constituents of sugar cane bagasse.

■ ASSOCIATED CONTENT

Supporting Information

Time courses of enzymatic hydrolysis (Figures S1, S3, and S5), XRD (Figure S2) and FTIR spectra (Figure S4) of the native and pretreated sugar cane bagasse and NMR spectra (Figure S6) of the IL [Ch][Lys]. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This study was supported by the Research Funds for Zhujiang Nova Program (2012J2200011), National Natural Science Foundation of China (20906032 and 21072065), and Fundamental Research Funds for the Central Universities, SCUT (2012ZP0009).

■ REFERENCES

(1) Hamelinck, C. N.; Hooijdonk, G.; Faaij, A. P. C. Ethanol from lignocellulosic biomass: Techno-economic performance in short-, middle- and long-term. *Biomass Bioenergy* **2005**, *28* (4), 384−410.

(2) Tong, X.; Ma, Y.; Li, Y. Biomass into chemicals: Conversion of sugars to furan derivatives by catalytic processes. *Appl. Catal., A* **2010**, *385* (1), 1−13.

(3) Pandey, A.; Soccol, C. R.; Nigam, P.; Soccol, V. T. Biotechnological potential of agro-industrial residues. I: Sugarcane bagasse. *Bioresour. Technol.* **2000**, *74* (1), 69−80.

(4) Vallejos, M. E.; Zambon, M. D.; Area, M. C.; da Silva Curvelo, A. A. Low liquid−solid ratio (LSR) hot water pretreatment of sugarcane bagasse. *Green Chem.* **2012**, *14*, 1982−1989.

(5) Yang, B.; Wyman, C. E. Pretreatment: The key to unlocking low-cost cellulosic ethanol. *Biofuels Bioprod. Bioref.* **2008**, *2* (1), 26−40.

(6) Alvira, P.; Tomas-Pejo, E.; Ballesteros, M.; Negro, M. J. Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis: A review. *Bioresour. Technol.* **2010**, *101* (13), 4851−4861.

(7) Mora-Pale, M.; Meli, L.; Doherty, T. V.; Linhardt, R. J.; Dordick, J. S. Room temperature ionic liquids as emerging solvents for the pretreatment of lignocellulosic biomass. *Biotechnol. Bioeng.* **2011**, *108* (6), 1229−1245.

(8) Muhammad, N.; Man, Z.; Bustam, M. A.; Mutalib, M. I. A.; Wilfred, C. D.; Rafiq, S. Dissolution and delignification of bamboo biomass using amino acid-based ionic liquid. *Appl. Biochem. Biotechnol.* **2011**, *165* (3−4), 998−1009.

(9) Petkovic, M.; Seddon, K. R.; Rebelo, L. P. N.; Silva Pereira, C. Ionic liquids: A pathway to environmental acceptability. *Chem. Soc. Rev.* **2011**, *40* (3), 1383−1403.

(10) Coleman, D.; Gathergood, N. Biodegradation studies of ionic liquids. *Chem. Soc. Rev.* **2010**, *39* (2), 600−637.

(11) Doherty, T. V.; Mora-Pale, M.; Foley, S. E.; Linhardt, R. J.; Dordick, J. S. Ionic liquid solvent properties as predictors of lignocellulose pretreatment efficacy. *Green Chem.* **2010**, *12* (11), 1967−1975.

(12) Pinkert, A.; Marsh, K. N.; Pang, S.; Staiger, M. P. Ionic liquids and their interaction with cellulose. *Chem. Rev.* **2009**, *109* (12), 6712−6728.

(13) Swatloski, R. P.; Spear, S. K.; Holbrey, J. D.; Rogers, R. D. Dissolution of cellulose with ionic liquids. *J. Am. Chem. Soc.* **2002**, *124* (18), 4974−4975.

(14) Brandt, A.; Ray, M. J.; To, T. Q.; Leak, D. J.; Murphy, R. J.; Welton, T. Ionic liquid pretreatment of lignocellulosic biomass with ionic liquid−water mixtures. *Green Chem.* **2011**, *13* (9), 2489−2499.

(15) Fu, D.; Mazza, G. Aqueous ionic liquid pretreatment of straw. *Bioresour. Technol.* **2011**, *102* (13), 7008−7011.

(16) Liu, Q. P.; Hou, X. D.; Li, N.; Zong, M. H. Ionic liquids from renewable biomaterials: Synthesis, characterization and application in the pretreatment of biomass. *Green Chem.* **2012**, *14* (2), 304−307.

(17) Hou, X. D.; Smith, T. J.; Li, N.; Zong, M. H. Novel renewable ionic liquids as highly effective solvents for pretreatment of rice straw biomass by selective removal of lignin. *Biotechnol. Bioeng.* **2012**, *109* (10), 2484−2493.

(18) Hou, X. D.; Li, N.; Zong, M. H., Renewable bio ionic liquids-water mixtures-mediated selective removal of lignin from rice straw: Visualization of changes in composition and cell wall structure. *Biotechnol. Bioeng.* **2013**, http://dx.doi.org/10.1002/bit.24862.

(19) Hou, X. D.; Li, N.; Zong, M. H., Significantly enhancing enzymatic hydrolysis of rice straw after pretreatment using renewable ionic liquid−water mixtures. *Bioresour. Technol.* **2013**, http://dx.doi.org/10.1016/j.biortech.2013.02.118.

(20) Ohira, K.; Abe, Y.; Kawatsura, M.; Suzuki, K.; Mizuno, M.; Amano, Y.; Itoh, T. Design of cellulose dissolving ionic liquids inspired by nature. *ChemSusChem* **2012**, *5* (2), 388−391.

(21) Ohira, K.; Yoshida, K.; Hayase, S.; Itoh, T. Amino acid ionic liquid as an efficient cosolvent of dimethyl sulfoxide to realize cellulose dissolution at room temperature. *Chem. Lett.* **2012**, *41* (9), 987−989.

(22) Hayashi, T. Xyloglucans in the primary cell wall. *Annu. Rev. Plant Biol.* **1989**, *40* (1), 139−168.

(23) Chang, V. S.; Holtzapple, M. T. Fundamental factors affecting biomass enzymatic reactivity. *Appl. Biochem. Biotechnol.* **2000**, *84* (1), 5−37.

(24) Ryu, D. D. Y.; Lee, S. B.; Tassinari, T.; Macy, C. Effect of compression milling on cellulose structure and on enzymatic hydrolysis kinetics. *Biotechnol. Bioeng.* **2004**, *24* (5), 1047−1067.

(25) Bahcegul, E.; Apaydin, S.; Haykir, N. I.; Tatli, E.; Bakir, U. Different ionic liquids favor different lignocellulosic biomass particle

sizes during pretreatment to function efficiently. *Green Chem.* **2012**, *14*, 1896−1903.
(26) Rivers, D. B.; Emert, G. H. Factors affecting the enzymatic hydrolysis of bagasse and rice straw. *Biol. Wastes* **1988**, *26* (2), 85−95.
(27) Sluiter, A.; Hames, B.; Ruiz, R.; Scarlata, C.; Sluiter, J.; Templeton, D.; Crocker, D. *Determination of Structural Carbohydrates and Lignin in Biomass*; NREL/TP-510−42618; National Renewable Energy Laboratory: Golden, CO, 2008.
(28) Teeäär, R.; Serimaa, R.; Paakkarl, T. Crystallinity of cellulose, as determined by CP/MAS NMR and XRD methods. *Polym. Bull.* **1987**, *17* (3), 231−237.
(29) Ninomiya, K.; Soda, H.; Ogino, C.; Takahashi, K.; Shimizu, N. Effect of ionic liquid weight ratio on pretreatment of bamboo powder prior to enzymatic saccharification. *Bioresour. Technol.* **2012**, *128*, 188−192.
(30) Schwanninger, M.; Rodrigues, J.; Pereira, H.; Hinterstoisser, B. Effects of short-time vibratory ball milling on the shape of FT-IR spectra of wood and cellulose. *Vib. Spectrosc.* **2004**, *36* (1), 23−40.
(31) Zhao, X. B.; Wang, L.; Liu, D. H. Peracetic acid pretreatment of sugarcane bagasse for enzymatic hydrolysis: A continued work. *J. Chem. Technol. Biotechnol.* **2008**, *83* (6), 950−956.
(32) Zhang, Z.; O'Hara, I. M.; Doherty, W. O. S. Pretreatment of sugarcane bagasse by acid-catalysed process in aqueous ionic liquid solutions. *Bioresour. Technol.* **2012**, *120*, 149−156.
(33) Cetinkol, O. P.; Dibble, D. C.; Cheng, G.; Kent, M. S.; Knierim, B.; Auer, M.; Wemmer, D. E.; Pelton, J. G.; Melnichenko, Y. B.; Ralph, J. Understanding the impact of ionic liquid pretreatment on eucalyptus. *Biofuels* **2010**, *1* (1), 33−46.
(34) Li, C.; Cheng, G.; Balan, V.; Kent, M. S.; Ong, M.; Chundawat, S. P. S.; Sousa, L. d.; Melnichenko, Y. B.; Dale, B. E.; Simmons, B. A.; Singh, S. Influence of physico-chemical changes on enzymatic digestibility of ionic liquid and AFEX pretreated corn stover. *Bioresour. Technol.* **2011**, *102* (13), 6928−6936.
(35) Vidal, B. C.; Dien, B. S.; Ting, K.; Singh, V. Influence of feedstock particle size on lignocellulose conversion: A review. *Appl. Biochem. Biotechnol.* **2011**, *164* (8), 1405−1421.
(36) Fu, D.; Mazza, G.; Tamaki, Y. Lignin extraction from straw by ionic liquids and enzymatic hydrolysis of the cellulosic residues. *J. Agric. Food Chem.* **2010**, *58* (5), 2915−2922.
(37) Lee, S. H.; Doherty, T. V.; Linhardt, R. J.; Dordick, J. S. Ionic liquid-mediated selective extraction of lignin from wood leading to enhanced enzymatic cellulose hydrolysis. *Biotechnol. Bioeng.* **2009**, *102* (5), 1368−1376.
(38) Mosier, N.; Hendrickson, R.; Ho, N.; Sedlak, M.; Ladisch, M. R. Optimization of pH controlled liquid hot water pretreatment of corn stover. *Bioresour. Technol.* **2005**, *96* (18), 1986−1993.
(39) Fu, D.; Mazza, G. Optimization of processing conditions for the pretreatment of wheat straw using aqueous ionic liquid. *Bioresour. Technol.* **2011**, *102* (17), 8003−8010.
(40) Xin, Q.; Pfeiffer, K.; Prausnitz, J. M.; Clark, D. S.; Blanch, H. W. Extraction of lignins from aqueous-ionic liquid mixtures by organic solvents. *Biotechnol. Bioeng.* **2012**, *109* (2), 346−352.
(41) Tadesse, H.; Luque, R. Advances on biomass pretreatment using ionic liquids: An overview. *Energy Environ. Sci.* **2011**, *4* (10), 3913−3929.
(42) Francisco, M.; van den Bruinhorst, A.; Kroon, M. C. New natural and renewable low transition temperature mixtures (LTTMs): Screening as solvents for lignocellulosic biomass processing. *Green Chem.* **2012**, *14* (8), 2153−2157.