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Investigation of the Effects of Ionic Liquid 1-Butyl-3-methylimidazolium Acetate Pretreatment and Enzymatic Hydrolysis of *Typha capensis*

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ABSTRACT: Pretreatment of *Typha capensis* (TC) with ionic liquid (IL) 1-butyl-3-methylimidazolium acetate [BMIM(OAc)] shows that the structural integrity-like degree of polymerization, polydispersity index, and lignin were altered, providing easy access to enzymes, and improved its digestibility to fermentable sugars. Hydrolysis of pretreated samples by a pre-optimized mixture of cellulose enzymes achieved an optimal reducing sugar yield (RSY) of 82.4 g/100 g after 6 h of pretreatment incubation. Because the costs of ILs are high, pre-hydrolysis and recycling are used to improve the economics. Pre-hydrolysis steps using sodium hydroxide followed by IL treatment were found to be better than sulfuric acid pre-hydrolysis followed by IL. Pre-hydrolysis treatment with alkali reduced pretreatment time from 6 h to 15 min, and total solid (TS) was increased from 5 to 10% without significant reduction in the glucose and RSYs. During solvent recycling, about 10% of initial lignin at each cycle was being accumulated into the liquid stream containing the IL, with minimal traces of carbohydrates. Treatment of the recycled IL at 10th and 15th cycles enabled recovery of about 93% of the IL-soluble lignin released into the liquid stream and improved the effectiveness of pretreatment. The investigation reveals the possibility of improving IL pretreatment outcome by increasing the TS through NaOH pre-hydrolysis. With the possible recycling of the IL up to 15 cycles, the overall costs of using this procedure are considerably reduced.

1. INTRODUCTION

Biomass has great prospects for bioethanol production as a transportation fuel to replace or substitute wherever possible the present dominating non-renewable fossil fuels. However, current production of bioethanol relies on food-based materials, such as corn, sugarcane, and cassava, and this can affect food security. Therefore, using cellulosic biomass as a feedstock for ethanol production is a viable option given its ubiquitous nature. However, the major obstacle to effective use of these promising resources is its recalcitrance nature to hydrolysis; hence, pretreatment is required. Pretreatment refers to the process of making the cellulose in the residue more accessible to further chemical or biological treatment.[1] Several pretreatment methods have been advanced, basically classified as biological, physical, chemical, and physicochemical depending upon the forces or energy consumed in the process.[2] Detailed reviews on this subject have been provided elsewhere.[2−5] Pretreatment of cellulosic biomass in a cost-effective manner is a major challenge of cellulose to ethanol technology development; efforts are toward reducing the formation of inhibitor and sugar degradation, limiting the expenditure of energy, chemical, water, and waste production.[6]

One of the new technologies that hold promise in lignocellulosic pretreatment is the use of a class of materials composed of ions that are liquids at relatively low temperatures (below 100 °C) called ionic liquids (ILs). They are thermally stable for a wide range of temperatures (150−400 °C).[7] ILs have been found to effectively dissolve cellulosic biomass, making the carbohydrates accessible to enzymatic hydrolysis to release monomers for fermentation. Solubilized cellulose can be recovered by rapid precipitation with some anti-solvents. The impacts of IL pretreatment include reduction of cellulose crystallinity and delignification or restructuring of lignin, which leads to enhanced digestibility of cellulose by enzymes into fermentable sugars.

Desirable characteristics attributable to some ILs are premised on their recyclability, non-volatility, low toxicity, and low flammability.[8] However, despite the several advantages of IL pretreatment of lignocellulosics, they are currently far from being used on a large scale because of their high cost.[9] Parameters widely studied by researchers include pretreatment temperature, residence time, particle size, substrate, and IL chemistry.[10−15] Commonly reported optimum conditions requires that IL must be used in vast excess to the biomass.[9]

However, if IL lignocellulosic pretreatment should proceed beyond laboratory scale, there is need for more studies on increasing the total solids (TS) as well as reusing the IL, which could improve the process economics. Shill et al. described the process of recovery of 1-ethyl-methylimidazolium acetate [EMIM(OAc)] for recycling using an aqueous kosmotropic

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salt solution.[16] They recommended removal of lignin from recycled IL not only as a strategy to prolong the lifetime of IL but also to harness lignin as a high-value product. Lignin could be precipitated using a solution of water and organic solvents, such as ethanol[17] or acetone.[14] The cellulose, lignin, and the IL components can thus be separated, and the latter can be reused.

To optimize the organosolv pretreatment processes, combinative pretreatments involving a pre-hydrolysis step have been described in the literature. This approach enhances the dissolution of lignin for ethanol organosolv treatments.[17,18] Nguyen et al. reported a pre-hydrolysis step using aqueous ammonia solution followed by a treatment with EMIM(OAc) to improve cellulose recovery and enzymatic glucose conversion.[12]

Typha capensis (TC) is a fast growing and highly prolific invasive grass found throughout the world as a common pest that grows in wetlands.[19] TC has special features that enable it to cope with both extremes of water and drought.[19] The dry matter density of TC varies with soil type, water depth, and availability of nutrients. Yield estimates for TC range from 6 to 20 tons of dry matter/ha.[20] These properties make TC a good substrate that can be used for bioethanol production.

In the present study, the pretreatment of TC using 1-butyl-3-methylimidazolium acetate [BMIM(OAc)] was studied. The possibility of increasing TS through a pre-hydrolysis step using NaOH or sulfuric acid at mild conditions was assessed. The study also evaluated the possibility of recycling IL while preserving the pretreatment quality.

2. EXPERIMENTAL SECTION

2.1. Materials. The TC sample was obtained from the canals in the Asian Institute of Technology (AIT). Laboratory Analytical Procedures (LAPs) developed by the National Renewable Energy Laboratory (NREL) were used for sample preparation.[21] Cut samples were dried to 6% moisture content at 45 °C and milled to ≤2 mm. Polymix Hammer mill PX-MFC 90D Dispersing and Mixing Technology by Kinematica with a maximum of 6000 rpm was used to mill the samples, operated at 4500 rpm and 2 mm screen.

Chemicals used, including BMIM(OAc) (product number 39952) as the IL, cellulase enzyme powder from *Trichoderma longibrachiatum* (product number C9748), and cellobiase enzyme in liquid form from *Aspergillus niger* (product number C6105), were all Sigma-Aldrich products.

2.2. Composition Analysis. To analyze the composition of treated and untreated samples, a two-step acid hydrolysis method based on a LAP described by Sluiter et al. was used to fractionate biomass into quantifiable acid-soluble [mainly monosaccharide and uronoic acid, analyzed by high-performance liquid chromatography (HPLC)] and acid-insoluble (mainly lignin, obtained as a residue and quantified on the basis of initial dry weight differences) components.[22] Soluble lignin was analyzed using ultraviolet−visible (UV−vis) spectroscopy.

2.3. Enzyme Activity Determination, Characterization, and Hydrolysis of Pretreated TC. Initial experiments were performed to determine the activities of the enzymes. The filter paper assay for saccharifying cellulase (FPU assay) and cellobiase assay were used to determine the activities of the cellulase and cellobiase using procedures as described by Ghose.[23]

The activity of cellulase enzyme [filter paper unit (FPU)] was found to be 1.01 units/mg of cellulase powder, and cellobiase unit (CBU) activity was found to be 278.01 units/mL of cellobiase liquid.

The best combination of 25 FPU of cellulase (C9748) and 70 units/mL of cellobiase (C6105) per gram of substrate for a period of 48 h was used in all experiments. Pretreated TC under different conditions was hydrolyzed enzymatically in a 40 mL Erlenmeyer flask using the determined optimal cellulase and cellobiase combination loadings in pH 5 acetic acid buffer at 2% solid (w/v) and incubated at 50 °C in air bath shaking incubator at 150 rpm. To prevent microorganism growth during hydrolysis, sodium azide was added in the slurry at 0.3% loading (w/v). After the 48 h optimal incubation residence time, 0.4 mL of sample was drawn and placed in Eppendorf tubes. To arrest enzymatic activity, the samples in the Eppendorf tube were placed in boiling water at 97 °C for 5 min and cooled and 0.2 mL of sample was drawn and diluted with 1 mL of distilled water, placed in Eppendorf tubes, and kept in the refrigerator at −4 °C until further tests.

2.4. Pretreatment, Precipitation, Anti-solvent Comparison, and Recycle. To optimize the incubation time, 5.0 g of BMIM(OAc) was mixed with 0.26 g of dry TC in a 40 mL beaker covered with aluminum foil and incubated without stirring in an oven at 110 °C for a range of 0.5−24 h.

Incubated mixtures of TC and IL were put into a water bath at room temperature to cool to about 40 °C for about 10 min. A total of 10 mL of distilled anti-solvent (water or ethanol) was added and briskly stirred in the beaker with a tuning fork. The cellulose-rich residue precipitated was separated by filtration through Whatman filter paper no. 2 using a Buchner funnel under reduced pressure. This stage was repeated 3 times, giving a total of about 40 mL of filtrate solution of anti-solvent and IL. The filtrate was retained for recovery and recycle of the IL. During recycle trials, aliquots of the filtrate at each cycle of pretreatment were preserved for sugars and lignin accumulation analysis. The precipitate was further washed with excess anti-solvent until the filtrate became colorless, when almost all IL and soluble materials have been removed from the residue. The residue was then dried, first at room temperature (22−37 °C) for 12 h and then in an oven at 40 °C for about 12 h. The dried, regenerated cellulose-rich TC was packed in sealable cellophane bags labeled and kept in a drawer at room temperature until required. To compare between water and ethanol as regenerating anti-solvents, two sets of experiments, one precipitated with water and the second precipitated with ethanol, were performed initially for 4 cycles and dried, pretreated samples were obtained and labeled appropriately.

IL was recovered from the preserved anti-solvent IL solution by evaporating the anti-solvent at a reduced pressure using a table top Buchi Rotavapor R-144 and Buchi water bath B-480, operated at 28 rpm and 50 °C for about 81 min. Recovered IL was reused up to 20 cycles. Recyclability of BMIM(OAc) was studied using the optimum anti-solvent and incubation residence time determined in previous experiments.

2.5. Combinative Alkali and Dilute Acid Treatment with IL. Alkali and acid treatments, each followed by IL treatment, were tested to see which gave better results. First, alkaline treatment was performed by mixing 5 g of TC in 50 mL of 1% (w/v) NaOH in a 250 mL beaker. The beaker was covered with aluminum foil and incubated in a water bath at 50 °C for 12 h.[24] After incubation, 150 mL of distilled water was added to wash the samples. The process was repeated several times until wash solution became colorless. The pulp was finally separated by filtration using Buchner filtering arrangement at a reduced pressure. Washed pulp was oven-dried overnight at 40 °C, followed by treatment with BMIM(OAc) at different incubation residence times from 5 to 0.25 h and 5−14% TS. Likewise, for dilute acid, mild condition treatment with dilute sulfuric acid at 0.273% (w/w) acid concentration, 10% TS, autoclaved at 121 °C for 45 min of reaction time, was conducted. Washed pulp was dried followed by IL treatment in the same manner as mentioned above.

2.6. Analytical Methods. Total reducing sugar of the hydrolysates consisting of both cellulose and hemicellulose sugars was tested using the 3,5-dinitrosalicyclic acid method by Miller, with slight modifications, as described by Nigam and Ayyani.[25,26] Values of reducing sugar yields (RSYs) were calculated in grams per 100 g of pretreated substrate using the relation

$$\mathrm{RSY} = \{(\mathrm{R1} \times V)/\mathrm{SL}\} \times 100$$

where R1 is the RSY concentration in mg/mL, V is the volume of liquid in aqueous solution of hydrolysate in milliliters, SL is the weight

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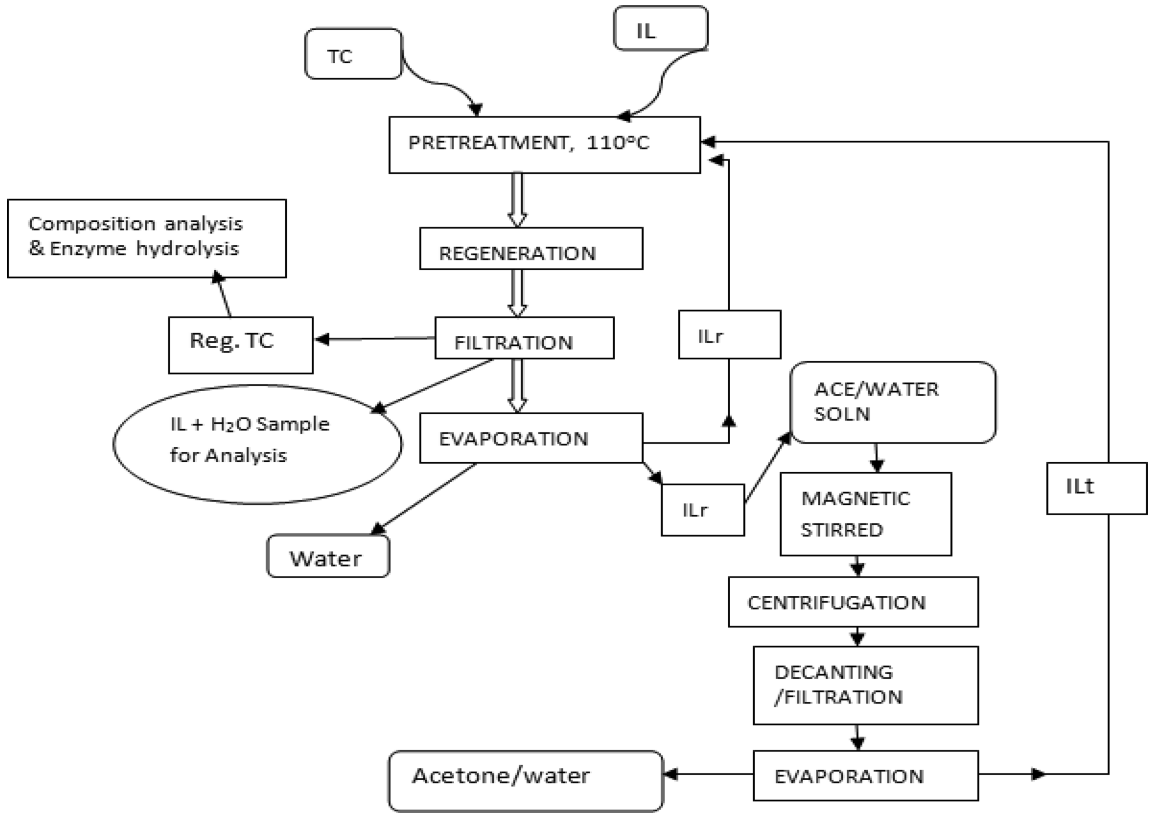

Figure 1. Flowchart of the IL recycle process used in this study. ILr, ionic liquid recycled (this goes through treatment after some cycles); ILt, treated ionic liquid for reuse.

of pretreated substrates in the hydrolysate in milligrams on a dry basis, and 100 is the conversion factor to obtain results in grams per 100 g.

Ready-to-use (RTU) glucose oxidase peroxidase from Biomerieux was used to test the glucose concentration (main cellulose sugar component) in the hydrolysates. Values were calculated in grams per 100 g of pretreated substrates using the relation

$$\text{Glc} = \{(\text{G1} \times V)/\text{SL}\} \times 100$$

where Glc is the glucose in grams per 100 g, G1 is the glucose concentration in mg/mL, V is the volume of hydrolysates in milliliters, SL is the TS hydrolyzed, and 100 is the conversion factor to obtain results in grams per 100 g.

A Dionex ICS-3000 system was used to separate and quantify sugars and uronic acid. The system consists of a SP gradient pump, an autosampler (AS), an electrochemical detector (ED) with a gold working electrode, an Ag/AgCl reference electrode, and Chromeleon, version 6.8 (Dionex Corp., Sunnyvale, CA). Stationary phase used was a Carbopac PA20 (3 × 150 mm) Dionex column with a guard column (3 × 50 mm, Dionex), while the mobile phase had water, 250 mM NaOAc, and 1 M NaOH/20 mM NaOH. Monomers and uronic acids were separated using isocratic conditions and linear gradient elution. All eluents were degassed before use by flushing helium for 20 min with 250 mM NaOH solution and re-equilibrated for 10 min with starting conditions. Samples were injected through 25 L full loop, and separations were affected at a temperature of 35 °C and a discharge rate of 0.4 mL/min. Pulse sequence for pulsed amperometric detection consisted of +100 mV potential (0−200 ms), +100 mV integration (200−400 ms), −2000 mV (410−420 ms), +600 mV (430 ms), and −100 mV (440−500 ms).

Molecular weight (MW) analysis was carried out using the size-exclusion chromatography (SEC) method. Pretreated and control raw material samples were extracted for holocellulose followed by cellulose extraction using the procedure by the Technical Association of Pulp and Paper Industry (TAPPI), with slight modifications.[27] Carbanilation of cellulose was then carried out using pyridine and phenyl isocyanate reacted at 65 °C and agitated at 300 rpm for about 68 h until cellulose was fully dissolved, to eliminate unreacted phenyl isocyanate, and cooled. The mixture was washed 3 times in 100 mL of methanol/water (7:3) and 2 times in water; cellulose tricarbanilates were purified by centrifugation at 4000 rpm for 40 min at each stage of washing. The SEC method was used to analyze the cellulose tricarbanilates for MW and molecular weight distribution using a Hewlett-Packard 1090 series HPLC system consisting of an in-line filter and an autosampler, an UV detector, and three columns of Styragel HR1, HR3, and HR4 (Waters, Inc., Milford, MA) linked in series. The eluent was tetrahydrofuran (THF), flowing at 0.8 mL/min at room temperature. Cellulose tricarbanilate samples (1 mg/mL) were dissolved in THF and solution-filtered with a 0.45 μm filter. A total of 20 μL of filtered samples was injected into the SEC column system, detected with an UV detector at 236 nm. Standard narrow polystyrene samples were used to calibrate the system, and data were received with Agilent ChemStation Revision A.10.10 and analyzed with Agilent GPC Addon Revision A.02.02 software. The number-average molecular weight (M_n), weight-average molecular weight (M_w), and polydispersity index (PDI) were calculated by gel permeation chromatography (GPC) software. The degree of polymerization (DP) value was obtained by dividing M_w and M_n of the cellulose tricarbanilates by the M_w of the anhydrous glucose tricarbanilate derivative (519 Da).

Cross-polarization/magic angle spinning (CP/MAS) ^{13}C solid-state nuclear magnetic resonance (NMR) analysis was carried out for treated and untreated TC samples by putting ground samples in a 4 mm diameter zirconium oxide rotors fixed with Kel-F caps. A Bruker Avance 400 MHz spectrometer operating at a ^{13}C frequency of 100.59 MHz was used, and all spectra were acquired at room temperature using Bruker's MAS probe.

UV spectrophotometry was used to estimate the lignin in the liquid fraction of each cycle of pretreatment using the procedure outlined by Shill et al., with slight modifications to comply with pretreatment conditions in this study.[16]

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Table 1. Compositions of Solid and Soluble Fractions for Untreated and Treated TC at Different Cycles of IL Reuse[a]

	solid fraction								liquid state		enzymatic hydrolysis	
cycle	KL	% delign	Rha	Ara	Gal	Glc	Xyl	Man	ILSL	ILL	RSY	Glc
0	25.66		0.36	2.40	1.53	38.03	9.97	0.67			40.18	25.12
1	22.42	12.63	0.11	2.97	1.43	37.89	8.85	0.57	3.03		84.57	58.23
2	23.05	10.17	0.12	1.77	0.88	36.94	9.31	0.42	6.95		82.61	57.20
3	23.08	10.05	0.15	1.90	1.01	35.38	9.40	0.48	10.83		81.12	55.95
4	23.73	7.52	0.11	1.54	0.81	29.56	7.70	0.37	13.33		78.52	42.06
5	23.78	7.33	0.17	2.05	1.07	37.59	10.26	0.51	16.94		76.61	38.78
6	23.12	9.90	0.07	1.36	0.69	26.90	6.71	0.24	20.86		77.00	37.33
10[b]	23.44	8.65	0.15	1.87	1.03	36.67	9.13	0.38	28.09	26.32	62.12	32.51
11	22.98	10.44	0.17	1.87	0.97	37.30	9.68	0.47	4.06		65.32	35.12
15[b]	22.62	11.85	0.07	1.36	0.69	26.90	6.71	0.24	10.32	9.52	62.03	32.82
16	23.12	9.90	0.09	1.42	0.78	33.77	8.10	0.38	3.78		64.53	33.53
20[b]	23.63	7.91	0.10	1.51	0.77	31.81	7.49	0.29	11.38	10.58	60.12	30.02

[a]% delign = percentage of delignification attained at each cycle. All other data are in grams per 100 g of dry weight of TC. cycle 0, untreated TC; KL, Klason lignin; Rha, rhamnose; Ara, arabinose; Gal, galactose; Glc, glucose; Xyl, xylose; Man, manose; ILSL, IL soluble lignin accumulated in the recycled IL; ILL, recovered IL lignin. [b]After IL washing (IL lignin removal). Data are mean values of two or three replications with maximum standard deviations (SD) of ±3.86; outliers were removed from the replications.

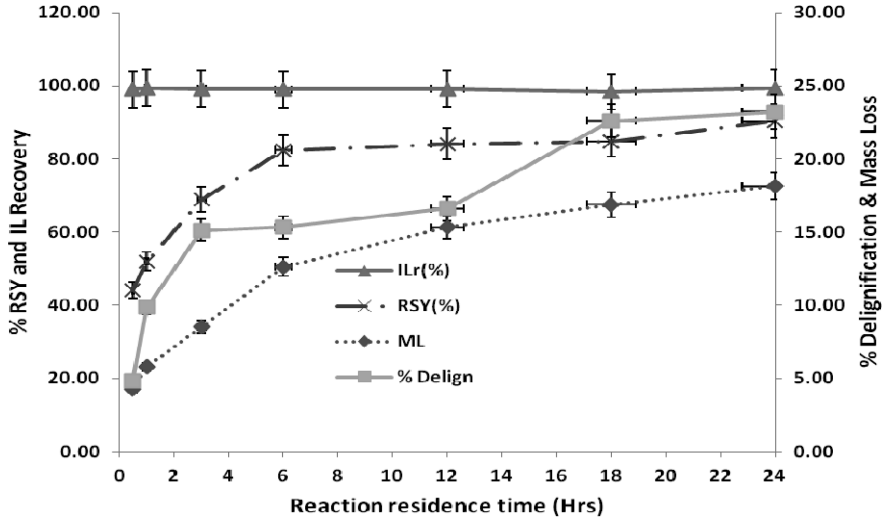

Figure 2. Effect of the incubation residence time on TC regeneration, mass loss, BMIM(OAc) recovery, delignification, and RSY after enzymatic hydrolysis. ML, mass loss as a percentage of the initial mass of raw sample; ILr, recovered BMIM(OAc) for reuse after TC regeneration as a percentage of the initial mass of BMIM(OAc) used; % delign, mass of lignin removed as a percentage of the initial lignin in the raw sample; and RSY, reducing sugar yield as a percent of the pretreated substrate hydrolyzed.

For the determination of the sugars released into the liquid stream during pretreatment, samples were first hydrolyzed by mixing 0.1 mL of 72% H_2SO_4 and 1.0 mL of distilled water with 0.1 mL samples, followed by an autoclave at 121 °C for 1 h. Hydrolyzed samples were filtered using 0.45 μm polytetrafluoroethylene (PTFE) syringe membranes to separate high-molecular-mass components.[16] Dionex HPLC previously described was used to determine the monosaccharides in the filtrate.

To recover and reduce the accumulation of lignin in the recycled IL, the IL was obtained after the 10th, 15th, and 20th cycles were treated with an acetone/water solution (1:1, v/v), which precipitated lignin. A total of 100 mL of an acetone/water solution was poured into a 250 mL beaker containing the recovered IL. The beaker was sealed with parafilm; the mixture was magnetically stirred at 200 rpm at room temperature for 1 h; and the IL soluble lignin was precipitated by centrifugation at 2000 rpm for 20 min.[14,17] The solid was separated from the liquid by decanting through filter paper, while the solids remained in the centrifuge tube. Precipitated lignin in the centrifuge tube was dried overnight at 40 °C, while the IL was recovered from the liquid stream by evaporating acetone/water using a rotavapor at a reduced pressure (see the process flowchart in Figure 1).

2.7. Statistical Analysis. All experiments in this study were carried out in duplicate or triplicate, and analytical errors were calculated on the basis of these replications. SPSS software was used to analyze the statistical significance of RSYs using the *t* test method at 95% confidence intervals.

3. RESULTS AND DISCUSSION

3.1. Chemical Composition of TC. The main structural composition in the TC samples was 38.03% cellulose, 14.93% hemicelluloses, and 25.66% lignin (Table 1). About 15% of the biomass is composed of hemicellose sugars, mainly xylose, which constitutes 66% of the hemicelluloses. In many biomasses that have been investigated, cellulose is generally the largest fraction, at about 40−50% by weight, with hemicelluloses at about 20−40%; thus, values from TC in our investigation are within the range and comparable to other lignocellulosic biomasses.[28,29]

3.2. Study of TC Treatment Using BMIM(OAc). *3.2.1. One Stage IL Pretreatment.* To determine the

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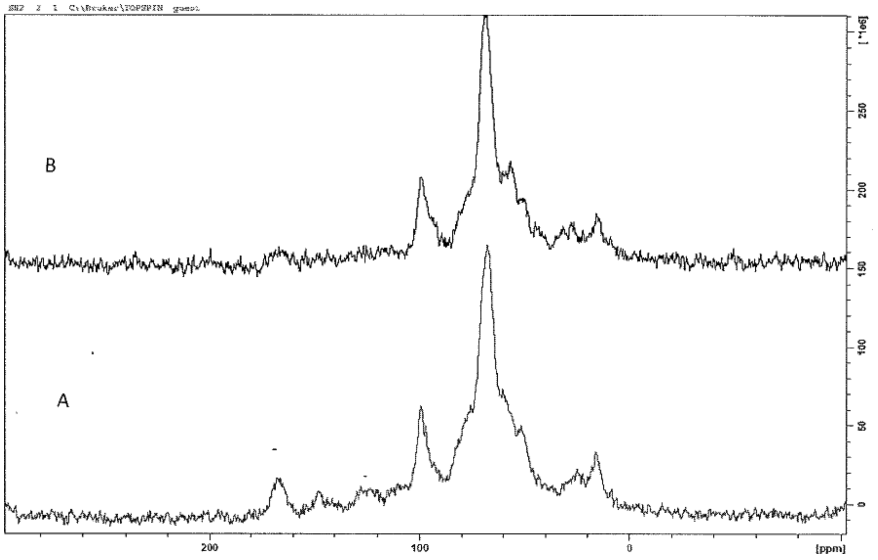

Figure 3. CP/MAS spectra of (A) untreated TC and (B) BMIM(OAc)-treated TC.

effectiveness of IL pretreatment on TC, a solid/IL ratio of 5% (w/w) and a temperature of 110 °C were used throughout the study based on reported data.[10−15] Because a wide range of IL pretreatment incubation residence times ranging from 15 min[15] to 24 h[12] have been reported, pretreatment incubation residence time was initially optimized.

The effect of the incubation residence time of TC in BMIM(OAc) from 0 to 24 h on mass loss, mass recovery, delignification yield, and RSY after enzymatic hydrolysis is given in Figure 2. Results of investigations show that there is no measurable difference in IL recovered for the different incubation times. This testifies to the stability of ILs at temperatures up to 150 °C, irrespective of the time subjected to such temperatures.[7]

On the other hand, increases in the delignification yield accompanied with increases in the mass loss with the duration of the treatment were observed. Sun et al. described degradation of biopolymers with a longer cooking time.[14] Thus, mass loss can be attributed to decomposition and release into the liquid stream of non-structural materials (extractives), part of lignin, hemicelluloses, and to a less extent, cellulose components from the TC. Studies have shown that decomposition of hemicelluloses leads to the formation of both acetic and carboxylic acids.[30] This could provide acidic conditions in the reaction mixture of IL and biomass during the pretreatment. In addition, during TC regeneration, some dissolved carbohydrate oligomers and monomers produced during the reaction as degradation byproducts may become soluble in the anti-solvent (water) used to regenerate TC.[31]

The RSY increased with an increasing pretreatment reaction residence time from 44.1 g/100 g of pretreated substrate at 30 min to a maximum of 90.3 g/100 g of substrate at 24 h (Figure 2). It appears from Figure 2 that, in the conditions of the pretreatment, the optimum duration was 6 h, for which the corresponding values of regenerated TC and IL recovered were 84.42 and 99.00% of initial weights, respectively. Pretreatments for further analysis were conducted using this trade-off time.

3.2.2. Effects of BMIM(OAc) Pretreatment on the Composition and Structural Integrity of TC. Pretreated TC, isolated after 6 h of treatment, was analyzed to obtain a more complete picture of the changes occurring during the pretreatment. On the basis of SEC and GPC analyses, structural parameters, such as DP, PDI, and Klason lignin (KL), were established to be 2295, 25.6%, and 4.3, respectively, for raw TC and 1887, 21.8%, and 2.8, respectively, for BMIM(OAc) pretreated samples. This indicates disruption of the initial structure, and therefore, enhanced digestibility during enzymatic hydrolysis was possible. Disruption of the intact structure of lignocellulosics by IL {1-ethyl-methylimidazolium diethyl phosphate [EMIM(DEP)]} pretreatment resulting in a porous amorphous structure and enhanced enzymatic hydrolysis has been reported earlier.[32]

Panels A and B of Figure 3 show the solid-state CP/MAS ^{13}C carried out for treated and untreated TC with signals assigned on the basis of data from the literature.[17,33,34] The region between 65 and 90 ppm is composed of peaks associated with *O*-alkyl carbons.[34] In this region, intense peaks are observed at 68 ppm, which are due to C-6 and C-4 carbons of amorphous cellulose, and the shoulder between 72 and 80 ppm indicates overlapping signals because of C-2, C-3, and C-5 carbons of all polysaccharides. The presence of these peaks in both spectra (treated and untreated TC) indicates that these compounds were preserved following pretreatment. Resonances associated with di-*O*-alkyl carbons are between 90 and 120 ppm[33,34] in this region. The resonances between 92 and 105 ppm observed in both spectra are assigned to and indicate the presence of aromatic carbon (C-1) of cellulose and C-1 of hemicelluloses and starch. Between 120 and 160 ppm is the aromatic−olefinic region, and peaks observed between 148 and 153 ppm are associated with oxygen-substituted aromatic carbons in lignin. The relatively low intensity of this peak in Figure 3B (treated TC) implies partial removal of lignin following pretreatment, leading to improved access by enzymes during hydrolysis. In the carboxyl region between 160 and 180 ppm, peaks at 174 ppm are assigned to acetate groups of glucuronic acid in hemicelluloses, carboxyl groups of cutin and lipids, and amide carbons of proteins. The low intensity of this peak observed in the spectrum in Figure 3B compared to that in Figure 3A implies substantial removal of these compounds during pretreatment, which also contributes to the mass loss of pretreated TC.

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Table 2. Effect of the Combination of Stepwise Alkali (NaOH) or Dilute H_2SO_4 and BMIM(OAc) Pretreatment on RSYs (y, Treated; n, Not Treated)

		NaOH + BMIM(OAc)			dilute H_2SO_4 + BMIM(OAc)		
			BMIM(OAc)			BMIM(OAc)	
sample number	t (h)	NaOH	TS (%)	RSY[a] (g/100 g)	H_2SO_4	TS (%)	RSY (g/100 g)
1	n	y	n	48.17 ± 3.59	y	n	29.35 ± 0.26
2	6	n	5	82.39 ± 0 0.72	n	5	82.39 ± 0.32
3	5	y	5	88.61 ± 1.09	y	5	84.78 ± 1.68
4	4	y	5	88.35 ± 1.82	y	5	83.58 ± 0.07
5	3	y	5	87.6 ± 1.6	y	5	80.89 ± 1.07
6	2	y	5	86.27 ± 0.91	y	5	75.17 ± 2.7
7	1	y	5	86.16 ± 1.07	y	5	69.67 ± 1.44
8	0.5	y	5	79.89 ± 0.07	y	5	64.84 ± 0.85
9	0.25	y	5	79.17 ± 1.12	y	5	59.81 ± 2.75
10	0.25	y	6.5	78.67 ± 2.53			
11	0.25	y	8	78.23 ± 1.99			
12	0.25	y	10	77.95 ± 0.00			
13	0.25	y	12	72.33 ± 0.58			
14	0.25	y	14	71.74 ± 1.05			

[a]RSY values are mean values of two replications ± SD.

3.3. Effects of Pre-hydrolysis on TC Treatment with BMIM(OAc). For a combination of pretreatment procedures, dilute sulfuric acid pretreatment (121 °C and 45 min) and NaOH treatment [1% (w/v), 50 °C, 12 h],[24] followed by BMIM(OAc), were carried out (Table 2). For dilute sulfuric acid alone (control), a value of 29.35 g/100 g of RSY was obtained after enzymatic hydrolysis. A maximum value of RSY for combined sulfuric acid and IL treatment was 78.78 g/100 g at 5% TS and 5 h of treatment time. This value declined with a decrease in the treatment time to a minimum value of 59.81 g/100 g at 15 min and 5% TS.

Concerning alkali pre-hydrolysis, a control experiment with NaOH treatment alone yielded 48.17 g/100 g of reducing sugar following enzymatic hydrolysis, while the optimum value of RSY after enzymatic hydrolysis of the BMIM(OAc)-treated sample at 6 h of treatment time and 5% TS was 82.39 g/100 g. The second stage of pretreatment by BMIM(OAc), using previously NaOH-treated samples, was tried for a treatment time range of 0.25−5 h and a TS range of 5−14% at the IL treatment stage. The maximum RSY of 88.59 g/100 g was realized at 5% TS and 5 h of pretreatment time. Reducing sugar gradually declined with an increase in TS and a decreasing treatment time to a minimum value of 71.74 g/100 g at 15 min of treatment time and 14% TS. A value of 77.95 g/100 g of RSY achieved at 15 min of treatment time and 10% TS is not statistically different from the optimum value at a 95% confidence interval. Thus, pretreatment time at the IL stage can be reduced from 6 h to 15 min, while TS was increased from 5 to 10% by a combination of alkali with IL treatments.

Overall, combinative NaOH with IL was found to be more effective in terms of dissolution power than dilute sulfuric acid. The lignin content of untreated TC and NaOH- and H_2SO_4-treated samples was established to be 25.66, 22.73, and 29.4%, respectively . The reduction in the lignin content and modification of its structure by NaOH treatment could enable a better permeation by IL to further break the hydrogen bonds with a shorter incubation time, as previously observed.[24,32,35]

3.4. BMIM(OAc) Recycling. In view of the obvious need for reuse of IL based on its cost, BMIM(OAc) recyclability was studied with respect to the effectiveness of pretreatment measured by the regenerated TC, IL recovery, and released sugars after enzymatic hydrolysis of treated samples at each cycle. The effect of reuse of BMIM(OAc) was tested for up to 20 cycles. To understand the effect of IL treatment on the effectiveness of TC pretreatment, two parallel experiments were conducted: (i) reuse with IL treatment at cycles 10, 15, and 20 by washing recovered IL with an acetone/water solution to precipitate and recover lignin and (ii) reuse without IL treatment. In both cases, the study reveals that the quantity of IL recovery and regenerated TC were not affected by the number of cycles. Between 90 and 99% of starting material is recoverable at each cycle for up to 20 cycles that were tried. Also, in both cases, best results of fermentable sugar recovery following enzymatic hydrolysis occurred at the first cycle of pretreatment; thereafter, RSY and Glc gradually reduced.

For assays with IL treatment at cycles 10, 15, and 20, reducing sugar and glucose yields reduced from maximum values of 84.57 and 58.23 g/100 g of substrate at the first cycle (original form of IL) to the minimum values of 60.12 and 30.02 g/100 g of substrate at the 20th cycle, respectively (Table 1). Analysis of IL at each cycle reveals that only traces of glucose and galactose were detected. On the other hand, averages of 3.0 g/100 g of lignin were released into the recycled IL at each cycle and are being accumulated. This represents about 10% of initial lignin in the biomass. Treatment of IL by precipitating lignin using an acetone/water solution at the 10th and 15th cycles improved the pretreatment outcome of TC using recycled IL (see Table 1). As a result of the IL treatment, (1) about 93% of accumulated lignin in the recycled IL at cycles 10, 15, and 20 were recovered, (2) the minimum RSY value of 60.1% occurred at the 20th cycle compared to a value of 52.8% at the 20th cycle for continuous recycle of IL without treating the IL, and (3) values of RSY and Glc after IL treatment at cycles 11 and 16 reveal that IL treatment improved pretreatment effectiveness of IL. On the basis of the improvement at cycles 11 and 16, IL treatment is recommended after every five cycles and it may be possible to reuse IL up to 15 times without significant reduction in pretreatment efficiency.

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For reuse of IL without treatment of IL, reducing sugar and glucose yields reduced from 84.64 and 57.45 g/100 g at the first cycle to 52.81 and 29.13 g/100 g at the 20th cycle, respectively (detailed data not shown). Although analysis of sugars released into the liquid streams and lignin accumulation were not performed for this set of recycling, the trend of lignin accumulation and near absence of monomeric sugars in the recycled IL observed in alternate experiments are likely to be the same.

Thus, while the weight of recovered IL is not affected by the number of cycles, its quality in terms of pretreatment effectiveness and dissolution ability gradually reduces with increasing cycle reuse. A previous study on the recyclability of IL by Li et al.[32] reported that EMIM(DEP) could be recycled up to 5 times with RSYs no less than 52%.

4. CONCLUSION

Pretreatment of TC with BMIM(OAc) was examined, and the enzymatic hydrolysis of IL pretreated TC was assessed (with a RSY of 82.4% after 6 h of pretreatment incubation). Because the costs of ILs are high, pre-hydrolysis and solvent recycling were evaluated to improve the economics. Pre-hydrolysis before IL treatment using sodium hydroxide was found to be better than using dilute sulfuric acid. Pre-hydrolysis treatment with alkali reduced pretreatment time from 6 h to 15 min, and TS was increased from 5 to 10% without a significant reduction in the glucose and RSYs. During IL recycling, about 10% of initial lignin at each cycle was being accumulated in the liquid stream. Treatment of the recycled IL using an acetone/water solution enabled recovery of about 93% of the IL soluble lignin released into the liquid stream and improved the effectiveness of pretreatment.

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Notes

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NOMENCLATURE

IL = ionic liquid
TC = *Typha capensis*
RSY = reducing sugar yield
TS = total solid
DP = degree of polymerization
PDI = polydispersity index
BMIM(OAc) = 1-butyl-3-methylimidazolium acetate
KL = Klasson lignin

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