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DESIGN OF LOW-COST IONIC LIQUIDS FOR LIGONCELLULOSIC BIOMASS PRETREATMENT

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ABSTRACT

The cost of ionic liquids (IL) is one of the main impediments to IL utilization in the cellulosic biorefinery, especially in the pretreatment step. In this study, a number of ionic liquids were synthesized with the goal of optimizing solvent cost and stability whilst demonstrating promising processing potential. To achieve this, inexpensive feedstocks such as sulfuric acid and simple amines were combined into a range of protic ionic liquids containing the hydrogen sulfate $[HSO_4]^-$ anion. The performance of these ionic liquids was compared to a benchmark system containing the IL 1-ethyl-3-methylimidazolium acetate $[C_2C_1im][OAc]$. The highest saccharification yields were observed for the triethylammonium hydrogen sulfate IL, which was 75% as effective as the benchmark system. Techno-economic modeling revealed that this promising and yet to be optimized yield was achieved at a fraction of the processing cost. This study demonstrates that some ILs can compete with the cheapest pretreatment chemicals, such as ammonia, in terms of effectiveness and process cost, removing IL cost as a barrier to the economic viability of IL-based biorefineries.

INTRODUCTION

The success of lignocellulosic biorefineries depends upon efficiently pretreating the feedstock in order to render its components cellulose, hemicellulose and lignin amenable to further processing.[1-3] Ionic liquids (ILs) have proven to be promising pretreatment solvents.[4, 5] $[C_2C_1im][OAc]$, also known as [Emim][Ac], is one of the most intensively studied candidates for use in cellulosic bio-refineries. Its advantages include an effectiveness that is independent of biomass type,[6-8] moderate reaction conditions in terms of time and temperature and high operating equipment compatibility. Additionally, $[C_2C_1im][OAc]$ has been shown to be both environmentally benign and compatible with organisms used for downstream conversion.[9, 10] However, recent studies of its stability under

process conditions have raised concerns about the suitability of this ionic liquid for biomass pretreatment,[11] particularly in light of the high current cost of this and other ILs that have been suggested for biorefinery applications.[12]

One proven way to reduce this cost for multi-ton IL production is through the use of protic ILs (ILs with a protonated amine for a cation), as in the BASF BASIL process and indeed all large-scale IL industrial processes to date.[13] This class of ILs is synthesized through simple neutralization of an organic amine with a mineral acid, to yield an IL that does not require purification. Hence protic ILs will be far less expensive than traditional dialkylimidazolium-based salts. For example, we recently estimated that bulk production of triethylammonium hydrogen sulfate $[HNEt_3][HSO_4]$ will be as low as \$1.24 kg^{-1}.[14] The model also showed that any protic hydrogen sulfate IL will have its cost dominated by the choice of amine, due to the extremely low cost of bulk sulfuric acid and the simple synthesis. We also showed that due to the large reduction in synthetic steps (from 30 steps to 7) compared to $[C_2C_1im][OAc]$, the synthesis of protic ILs similar to $[HNEt_3][HSO_4]$ is bound to have lower environmental impact due to a reduction in waste by-products, solvent losses, energy usage and CO_2 generation.

We report here a study that was designed to identify low-cost ILs that would efficiently pretreat lignocellulose. ILs were designed with the goal that they should 1) enhance enzymatic hydrolysis, 2) work under hydrous conditions (20% w/w water), 3) be thermally stable at processing conditions and 4) be cost effective. To assess this, we present enzymatic hydrolysis data, mass, lignin and glucan recovery data and crystallinity indices for switchgrass biomass treated with the chosen ionic liquids. We did not seek to optimize the processing conditions but to do a comparative study on a range of cost effective ILs in order to understand changes in performance based on structural changes and to benchmark this group of ILs against the widely used $[C_2C_1im][OAc]$. We also investigated IL peak decomposition temperatures and conducted a preliminary techno-economic analysis.

IL design rationale: The ILs were designed for this study on the basis that (i) ILs based on the hydrogen sulfate anion have proven to be effective delignifiers, (ii) protic cations in $[HSO_4]^-$ ILs appear to work as well as their non-protic analogues (iii) protic ILs can be produced more cheaply due to cheaper starting materials and ease of synthesis[15], and (iv) many alkylamines are produced in large quantities at low cost today; ethyl- and ethanolamines are particularly attractive in this regard. The range of ionic liquids was selected so that the effect of number of alkyl chains could be investigated; therefore mono-, di-, and triethylammonium hydrogen sulfate were synthesized. Three further ILs had an OH group added to each alkyl chain, in order to study the effects of hydrogen bonding potential in the IL-biomass mixture. Finally, to investigate any steric effects, diisopropyl ammonium hydrogen sulfate was synthesized. **Table 1** illustrates the structures of the ILs used. All of the ILs were tested as mixtures in 20% water and compared with untreated biomass and the effect of the benchmark ionic liquid $[C_2C_1im][OAc]$, also containing 20% water.

MATERIALS AND METHODS

Detailed experimental procedures are given in the Supplementary Information. 1-ethyl-3-methylimidazolium acetate, $[C_2C_1im][OAc]$, was purchased from Sigma-Aldrich while all other IL were synthesized according to procedures described in the ESI, similar to techniques described elsewhere.[15] Switchgrass *(Panicum virgatum*) was provided by Dr Daniel Putnam, University of California at Davis. The air-dried biomass was milled by a Thomas-Wiley Mini Mill fitted with a 40-mesh screen (Model 3383-L10 Arthur H. Thomas Co., Philadelphia, PA, USA) and sieved to the nominal sizes of 40-60 mesh (250-400 μm). All feedstocks were further dried in a vacuum oven at 40 °C overnight prior to pretreatment to eliminate the variability of moisture content. All pretreatments were conducted in the presence of 20% water (w/w IL) and data compared to $[C_2C_1im][OAc]$:$[H_2O]$ (4:1 w/w) by a methodology described elsewhere.[16] The carbohydrate composition of biomass and residual biomass after hydrolysis was determined with a modified quantitative saccharification (QS) procedure. [17] The standard NREL biomass protocol was used to measure lignin and ash [18]. All enzymatic hydrolysis experiments were conducted with 20 mg protein (Novozymes Cellic® CTec 2) per gram of glucan. The XRD experiments were performed on a PANalytical Empyrean X-ray diffractometer equipped with a PIXcel^{3D} detector and operated at 45 kV and 40 kA using Cu Kα radiation (λ= 1.5418 Å). All spectra were subjected to baseline correction using PeakFit1 4.12 software (Systat Software Inc., Chicago, IL) assuming Gaussian distribution function as the shape of the resolved peaks and Saviszky-Golay smoothing [19]. The crystallinity index (CrI) was determined by Segal method [20]. Thermogravimetric analysis (TGA) was performed using a Mettler Toledo model TGA/DSC 1.

RESULTS AND DISCUSSION

Pretreated biomass characterization: IL performance was evaluated by measuring the glucose yields after enzymatic saccharification of these solids. The mass recovery, cellulose crystallinity, the lignin and glucan recoveries and ash contents of the pretreated, recovered substrates were investigated in an attempt to explain the differences in IL performance.

Suitability of pulp for enzymatic hydrolysis: A high glucose hydrolysis yield is an important marker of lignocellulose pretreatment quality. The glucose is regarded as the main product of the biorefinery and used in the fermentation to biofuels and other chemicals. Hence we compared the performance of the hydrogen sulfate ILs by carrying out an enzymatic saccharification assay on the recovered solid and benchmarked these yields against the $[C_2C_1im][OAc]$:H_2O system.

Development of saccharification yields over time is shown in **Figure 1. Figure 2** highlights the yields after 48 hours. At this point, all ILs performed better than the untreated biomass. The 4:1 $[C_2C_1im][OAc]$:$[H_2O]$ gave a yield of 59.7% (+/- 1.1). Treatment with the diethyl-, triethyl- and diisopropylammonium ILs resulted in the highest saccharification yields. The best hydrogen sulfate ionic liquid was triethylammonium hydrogen sulfate, which resulted in a glucose yield of 45.0% (+/- 6.6). This represents a 75% efficacy compared to $[C_2C_1im][OAc]$:$[H_2O]$. Diethylammonium hydrogen sulfate also compared favorably at 67% of the $[C_2C_1im][OAc]$:$[H_2O]$ mixture. The

diisopropylammonium IL was 60% as effective. The monoethylammonium and all three ethanolammonium ILs performed less well, with the best IL in this group only achieving 50% as effective a pretreatment as the $[C_2C_1im][OAc]$:$[H_2O]$.

It thus appears that adding -OH groups to the cation reduces hydrolysis yield. It also appears that increasing the number of alkyl chains improved enzymatic hydrolysis yield. There was a step change between the mono- and disubstituted ammonium cations in both the simple and alcohol substituted cases, and then a more gradual increase from two to three alkyl chains. The ILs with disubstituted cations (diethyl-, diisopropyl- and diethanolammonium) were the most similar in performance. The performance of the diisopropylammonium IL indicates that steric effects do not play a part in hydrolysis efficacy, at least for cations with the short alkyl chain lengths (n=2-3).

Mass recovery: The mass recovered after pretreatment, washing and drying from the $[C_2C_1im][OAc]$:H_2O mixture was 79%. Recoveries for the hydrogen sulfate ILs compared to the benchmark system was similar or lower with a range of 57-78% mass recovered **(Figure 3).** No discernable trend was observed with varying alkyl chain number, or the addition of -OH groups to the IL cation. This suggests that other factors such as cellulose crystallinity or composition of the recovered solid may determine the saccharification yield.

Crystallinity: The crystallinity index of biomass treated with $[C_2C_1im][OAc]$ has been well documented to decrease, with the fibrillar structure of cellulose being broken up and the cellulose I structure converted to cellulose II. This phenomenon was not observed with biomass treated with the ammonium hydrogen sulfate ILs, where the crystallinity index increased slightly compared to untreated switchgrass (**Table 2**). An increase in crystallinity index is often indicative of hemicellulose and/or lignin removal and hydrogen sulfate ionic liquids have been shown to be effective delignification agents in other studies. [3, 15] Hence we investigated the composition of the recovered solid in more detail.

Glucan and lignin recovery: Lignin and glucan contents were measured using the acid hydrolysis method. They were then compared to the glucan and lignin in the untreated switchgrass to calculate the glucan and lignin recoveries (**Figure 4).** The amount of glucan found in the pretreated material was more or less the same for all ILs under the conditions applied. However, a reduced amount of lignin compared to the original biomass was observed for the diethyl-, triethyl- and diisopropylammonium ILs. These ILs also achieved the best enzymatic hydrolysis yields among the hydrogen sulfate ILs. This suggests that the ability to remove lignin (delignification) is an important trait of effective hydrogen sulfate ILs, while this is not necessary for $[C_2C_1im][OAc]$.

Surprisingly, for some ILs, lignin recoveries were substantially higher than the original lignin content. This was observed for monoethyl- and monoethanolammonium hydrogen sulfate, with lignin recoveries of 200 and 150%, respectively. These ILs were also responsible for the lowest saccharification yields. We hypothesize that this is due to pseudolignin formation, which was observed previously for acidic solvents such as hydrogen sulfate based ILs.[21-23] Pseudolignin is a yet-to-be-defined substance or mixture of compounds that incorporates products of hemicellulose but is

detected as lignin by the acid hydrolysis method. It appears that pseudolignin formation is detrimental to saccharification.

Ash and sulfur content: During compositional analysis, a significant variability of the ash content in the recovered solid was observed (**Figure 5**). Monoethylammonium IL and all the ethanolammonium ILs resulted in ash contents higher than in untreated switchgrass (2.9%). More ash was present when the cation had fewer alkyl chains.

We further investigated the nature of the ash by measuring the sulfur content in the recovered biomass. Sulfur is indicative of the presence of the hydrogen sulfate anion. It is the only source for sulfur in the system, as biomass typically does not contain measurable quantities of sulfur. As hypothesized, some of the material recovered after hydrogen sulfate IL treatment was found to contain sulfur (**Figure 5**), while the $[C_2C_1im][OAc]$ treated biomass showed no sulfur content.

The quantity of sulfur and the ash content appeared to be correlated. The sulfur content was higher for samples that had higher ash contents. Lower quantities were noted for the alkylammonium hydrogen sulfate ILs. The sulfur levels in biomass treated with trisubsitituted triethylammonium hydrogen sulfate and the bulkier disubstituted diisopropylammonium hydrogen sulfate were below the detection limit, corresponding to <0.3 w/w [%]. High sulfur and ash contents also seemed to coincide with excess recovery of lignin/pseudolignin formation (**Figure 4** and **5**).

Incorporation of sulfur could be caused by incomplete removal of IL, but the biomass washing procedure was quite exhaustive, so we hypothesize that the sulfur may be bound chemically. We are currently investigating the mechanism for the sulfur absorption into the pretreated solid. This may also confirm the reason for the significant differences in sulfur contents. For example, the differences could be due to the cation size (smaller ILs having more sulfur per unit volume) or it could be a kinetic effect.

In summary, it was found that lower ash and sulfur content correlated with reduction in lignin content and better glucose yield upon enzymatic saccharification.

IL thermal stability: An important requirement of ILs employed in industrial processes is that they must be recoverable for repeated use. This requires them to be structurally stable under processing conditions. For ILs based on organic cations such as the ones investigated in this study, typical degradation processes are the loss of alkyl chains from the cation (dealkylation) followed by a number of other reactions as the temperature approaches the range where C-C and C-H bonds break. The benchmark IL $[C_2C_1im][OAc]$ has been shown to be problematic in this respect.[11]

In order to investigate the thermal stabilities of the ILs applied in this study and compare them with $[C_2C_1im][OAc]$, TGA analysis was carried out. The values resulting from the TGA analysis, T_{PEAK} and T_{ONSET}, are shown in **Table 3**. It shows that T_{PEAK} was 322, 301 and 290 °C for the mono-, di- and triethylammonium ILs, respectively. These thermal stabilities appear to trend with electron density on the nitrogen of the ammonium group. There was also a trend in decreasing thermal stability with increasing hydroxyethyl chain number, with T_{PEAK} of 306, 294, 294 °C for the mono-,

di- and triethanolammonium ILs, respectively. The diisopropylammonium cation showed the lowest T_{PEAK} at 280 °C, which could be due to a higher prevalence of Hoffmann dealkylation for the branched alkyl chains as compared to the substitution reaction that is observed for linear alkyl substituents.[24]

Overall, TGA analysis suggests that decomposition of all hydrogen sulfate ILs occurs at substantially higher temperatures than that of $[C_2C_1im][OAc]$, for which a T_{ONSET} of 215 °C has been measured.[11] Similarly, the protic acetate ILs that were recently shown to delignify biomass also suffer from very low stabilities in the TGA.[25] This is likely due to incomplete protonation of the amines by acetic acid rather than loss of alkyl chains. This 'volatility' will require protic acetate ILs to be contained during treatment. In contrast, the high stability of the protic ammonium hydrogen sulfate complexes will avoid occurrence of hazardous vapors and explosions and structural damage to the cation.

Despite this, it should be noted that long term stabilities for ionic liquids are considerably lower than T_{ONSET} and T_{PEAK} values would suggest,[11, 26, 27] and hence biomass processing temperatures should also be lower. For example, dealkylation reactions have been observed for $[C_2C_1im][OAc]$ at temperatures as low as 120°C.

We also would like to highlight that the chemical nature of the degradation products should be considered. It is noteworthy that the likely "impurities" generated by dealkylation of tri- and dialkylammonium hydrogen sulfate ILs will be less substituted ammonium cations. For example the dealkylation product of triethylammonium hydrogen sulfate will be diethylammonium ethyl sulfate (Scheme 1). The anion can then hydrolyze to ethanol and HSO_4. Since di- and monoalkylated ammonium ILs still performed well in this study, the reduction in performance due to the presence of dealkylation products is likely to be slight. In summary, the alkylammonium hydrogen sulfate ILs exhibit high thermal stabilities that are much greater than the benchmark system. This suggests that long-term use in a continuous process will be feasible.

Technoeconomic analysis: In order to demonstrate the effect of a lowered ionic liquid price on the economics of ethanol production, we made use of a previously published process model.[12] This model was originally used to study the effect of IL price, recycling, and loading on the economics of ethanol production. It includes the unit operations needed to produce ethanol from corn stover and assumes ionic liquid pretreatment based on a generalized protocol. It solves the material and energy balances associated with ethanol production, and uses the results of these balances to perform a cost estimation. The cost estimation is then used to calculate other economic parameters such as the capital cost, the operating cost, the net present value of the investment, etc. The IL price applied here was taken from our recent techno-economic model that estimated the cost of bulk production of two protic hydrogen sulfate ionic liquids.[14] Given the $[HNEt_3][HSO_4]$ sulfate cost of \$1.24 kg^{-1}, the process model was used to calculate the effect of lowering the IL price from \$50 kg^{-1} (a price often associated with ILs used for pretreatment) to \$1.25 kg^{-1}on the minimum ethanol selling price (MESP). The MESP is the price of the biofuel that would make the net present value of the ethanol facility equal to zero over its 25-year lifetime. The financial assumptions (discount rate, debt-equity split, loan interest, etc.) were left unchanged compared to the previous study.

The results of the analysis are illustrated in **Figure 6**, which shows the effects on the MESP for a range of IL recycle rates and IL/biomass ratios at IL cost of \$50 $kg^{-1}$and \$1.25 kg^{-1}. At high IL prices, *e.g.* at an IL purchasing price of \$50 kg^{-1}, the MESP was >\$6 gal^{-1}, even when assuming a very high IL recycle rate of 99.6%, and 1:1 IL/biomass loading, versus \$3.22 gal^{-1} for an IL cost of \$1.25 kg^{-1}. This is close to the MESP that has been calculated for dilute acid pretreatment of corn stover, \$2.15 gal^{-1}.[28]

Looking at the cost structure more closely it becomes clear that the IL price impacted the MESP so much because of the fraction of the annual operating cost devoted to purchasing make up IL for replacing the non-recovered IL. For example, at an IL purchasing price of \$50 kg^{-1}, 64% of the raw material cost were due to the ionic liquid (the remainder of the raw material cost being the lignocellulosic feedstock), whereas an IL price of \$1.25 kg^{-1} reduced the IL contribution to the raw material cost to 4.2%. This is the main reason for addressing IL price as a primary design criterion. Fortunately, this study shows that IL cost can be mitigated by careful design of the IL.

A note on IL toxicity: Toxicity is an important consideration before commercialization of a process employing chemicals. The toxicity of the newly investigated ILs is not yet known.[29] However, due to their rather simple construction from an acid and a base, we expect a similar toxicity as the parent amine and acid.

Amine toxicity depends on chain length (longer alkyl chains increase toxicity), so this can be minimized by choosing shorter chain alkylamines such as we have done here. We'd like to point out that triethylamine does not bioaccumulate and the highest toxicity potential of triethylamine is through inhalation, which is vastly reduced when bound in an ionic complex. The high decomposition temperatures (above 280°C) demonstrate that the amines are not volatilized in a wide temperature window, while this is likely to be an issue for protic ILs made from amines and weak bases such as acetic acid.

The hydrogen sulfate anion should not be toxic in dilute form. Regarding corrosiveness, the hydrogen sulfate salts are acidic (pK_a of the HSO_4^- anion in dilute solution is ~ 2), however, they are significantly less so than concentrated sulfuric acid and other strong acids. Corrosion could be a concern for process safety and equipment compatibility and needs to be considered in more detailed impact assessments.

CONCLUSIONS

We have designed and synthesized a series of new protic ionic liquids based upon the [HSO_4] anion and examined their effectiveness as pretreatment solvents for switchgrass. The new ILs were examined for their ability to enhance enzymatic saccharification yields, their stability and their potential for remaining in the biomass product streams. We demonstrated that good yields can be achieved with some of these investigated ILs. The most effective solvent was approximately 75% as efficient at increasing cellulose digestibility as the benchmark IL [C_2C_1im][OAc], at a fraction of the proposed bulk production cost and better thermal stability, while leaving low levels of residual IL in the cellulose pulp.

The mode of enhanced saccharification yield for the alkylammonium ILs was shown to be through lignin removal, but not decrystallization, of cellulose. This demonstrates that high enzymatic hydrolysis kinetics can be achieved in the absence of cellulose decrystallization. This also indicates that the cellulose pulps isolated this way may be used for applications where highly crystalline cellulose would be desired.

The process that is associated with the use of these hydrous ammonium hydrogen sulfate ILs also greatly simplifies biomass processing – the recovery of the biomass pulps by filtration or centrifugation without the need for antisolvent precipitation offers an inherent processing benefit as it reduces organic solvent and water usage.

In addition, the high thermal stability of the ILs employed will reduce solvent degradation and the associated waste. Therefore, with room for optimization, and a low cost synthesis route established, this study demonstrates that IL cost is no longer an impediment to its use in the biorefinery concept.

Scheme 1: Dealkylation of triethylammonium hydrogen sulfate

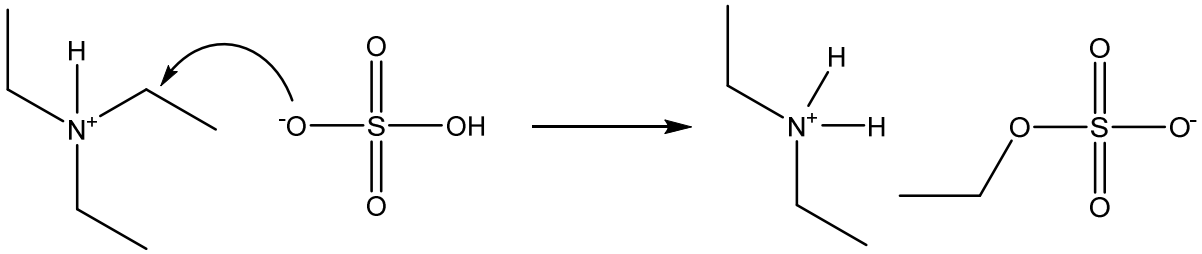


Table 1. Chemical structure of designed ionic liquid cations, with an $[HSO]_4^-$ anion.

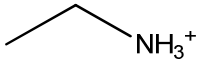
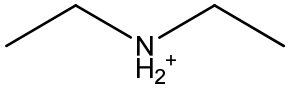
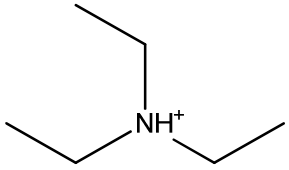
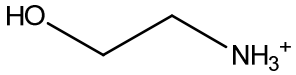
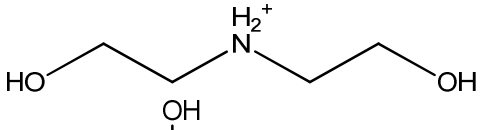
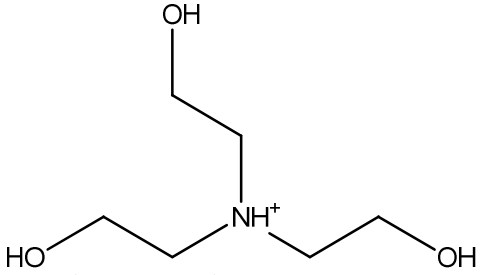
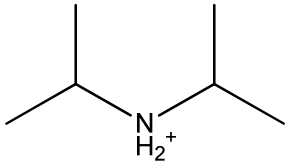
Cation name	Cation structure
monoethylammonium	
diethylammonium	
triethylammonium	
monoethanolammonium	
diethanolammonium	
triethanolammonium	
diisopropylammonium	

Table 2 Crystallinty index of recovered biomass after treatment with ionic liquids

Pretreatment solvent	Crystallinity Index
Untreated switchgrass	0.31
$[C_2C_1im][OAc]$	0.08
Monoethylammonium	0.42
Diethylammonium	0.38
Triethylammonium	0.41
Monoethanolammonium	0.36
Diethanolammonium	0.35
Triethanolammonium	0.31
Diisopropylammonium	0.33

Table 3. Thermal stabilities of IL, denoting the onset of decomposition T_{ONSET} and the peak decomposition temperature, $T_{PEAK.}$

	T_{ONSET} /[°C]	T_{PEAK} /[°C]
Monoethylammonium	294	322
Diethylammonium	281	301
Triethylammonium	277	290
Monoethanolammonium	299	306
Diethanolammonium	289	294
Triethanolammonium	278	294
Diisopropylammonium	269	280

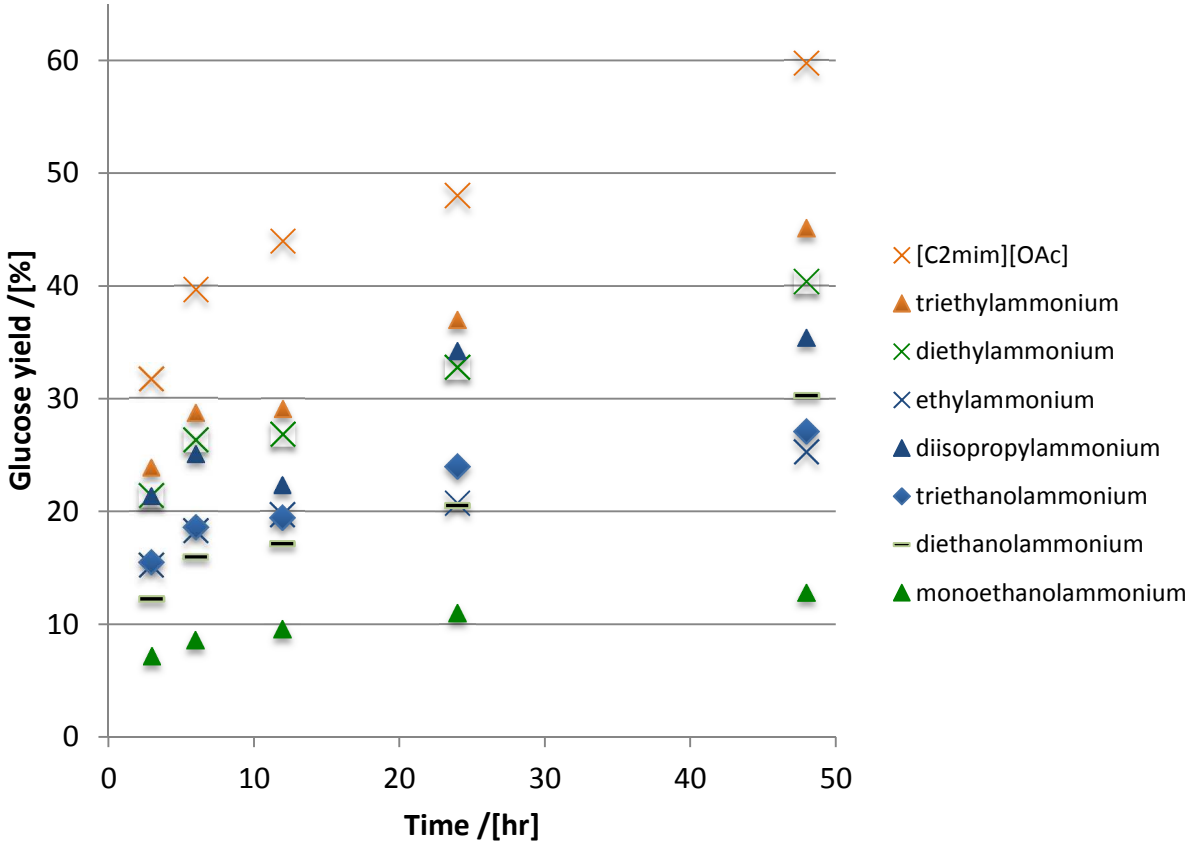


Figure 1. Enzymatic hydrolysis kinetics up to 48 hours. Glucose yield was the amount of glucose released into the hydrolysate relative to the glucan that was present in the recovered pretreated biomass.

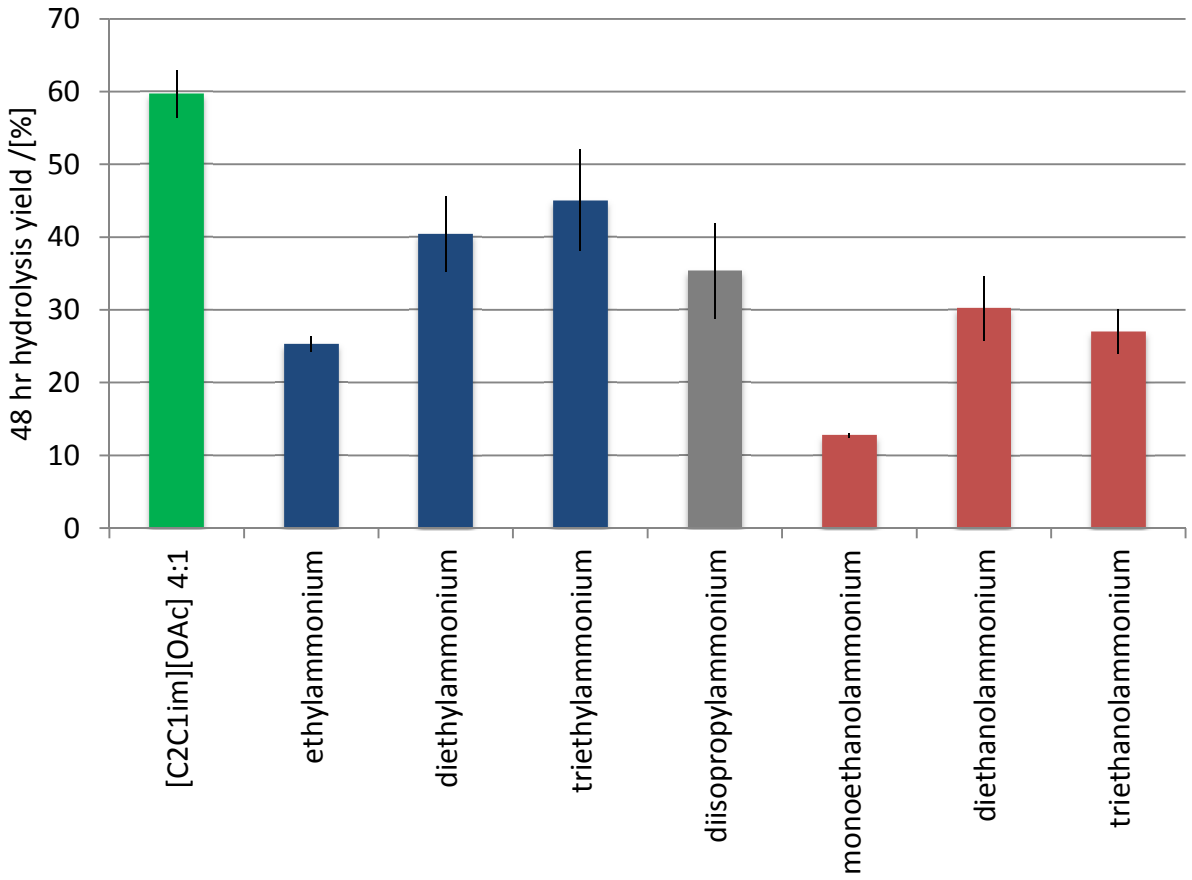


Figure 2. Glucose yield after 48 hour enzymatic hydrolysis (relative to glucan content in the pretreated biomas

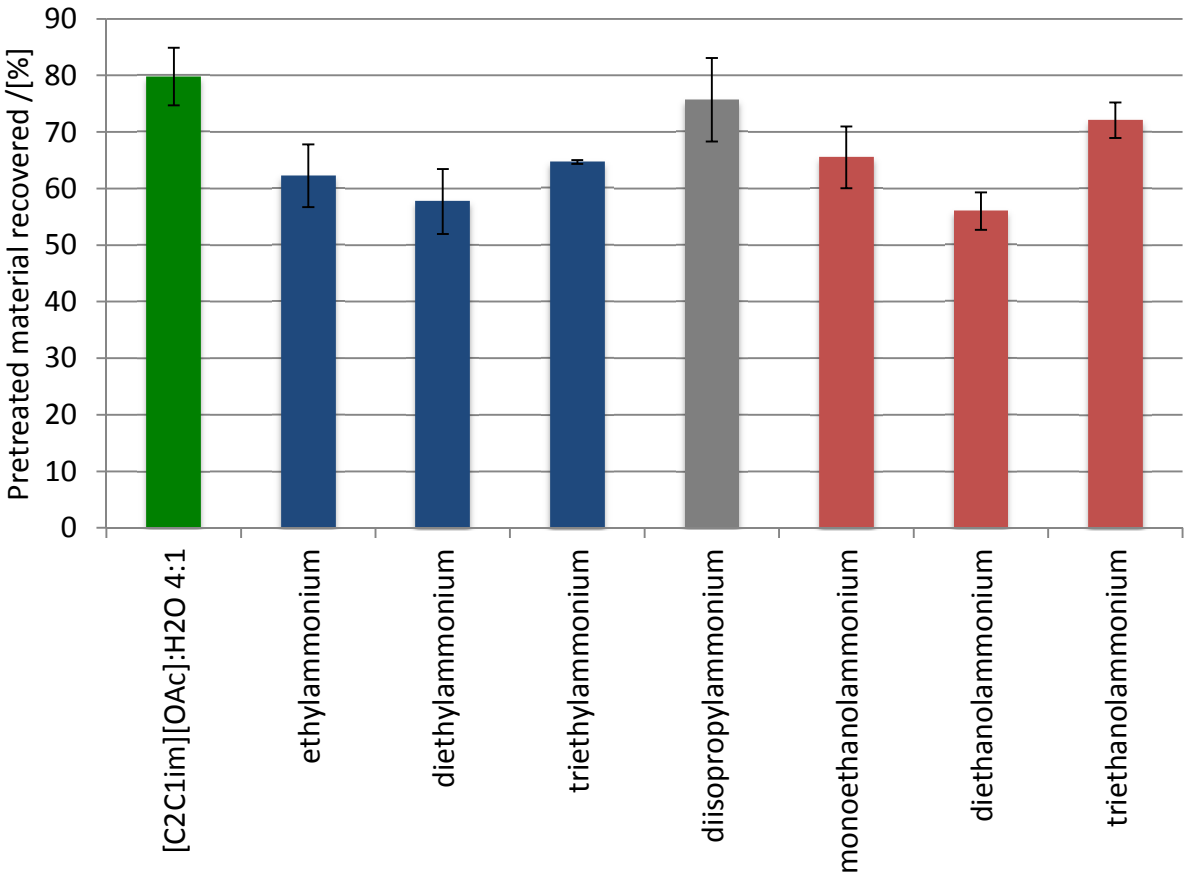


Figure 3. Percentage material recovered as solid post-pretreatment

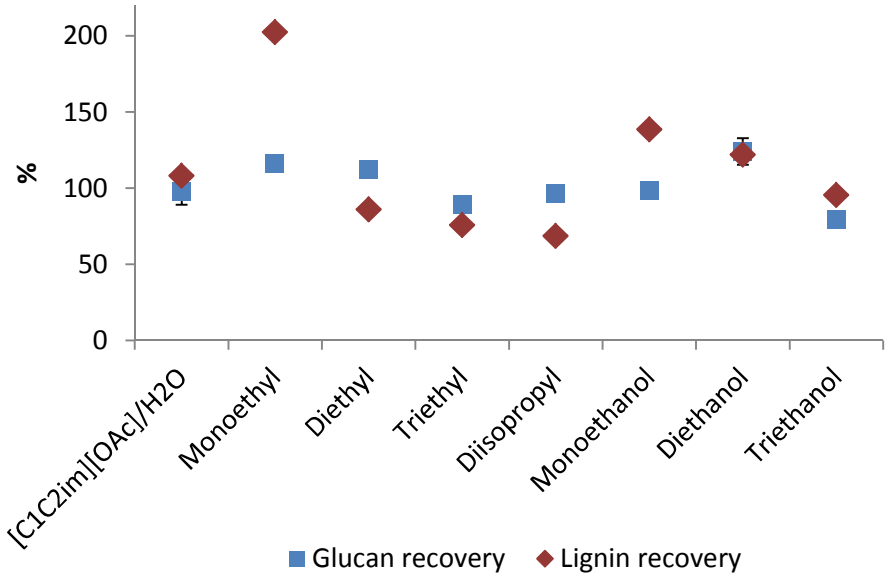


Figure 4 Glucan and lignin recovery in pretreated material prior to enzymatic hydrolysis.

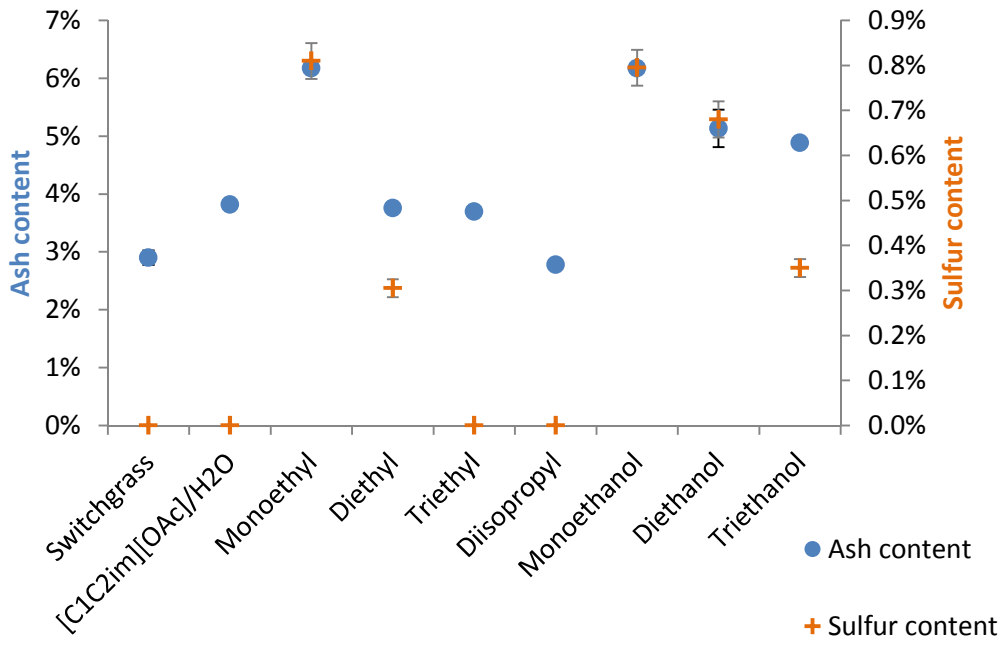


Figure 5. Ash and sulfur content in pretreated material w/w [%]

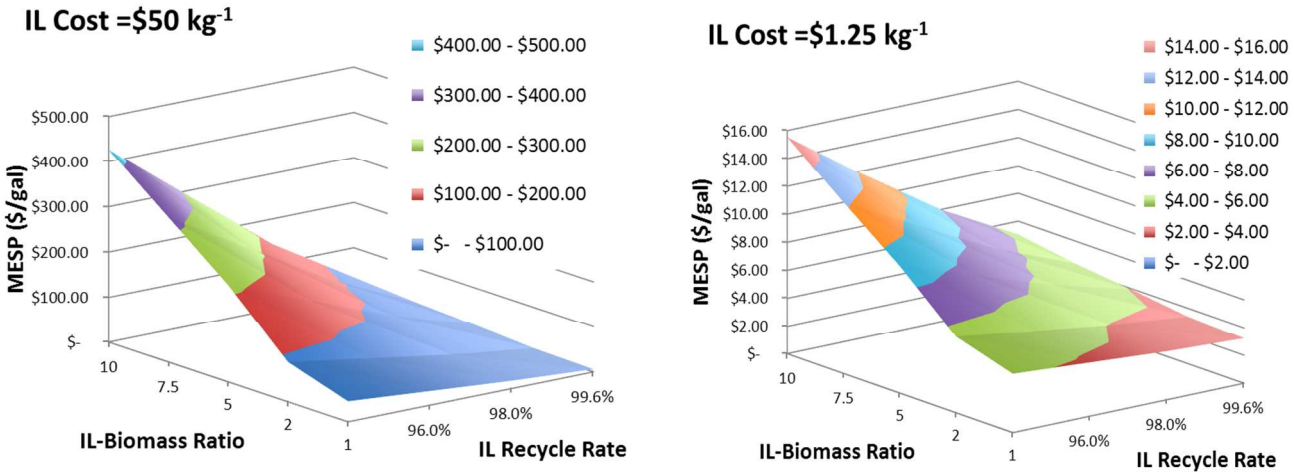


Figure 6. Influence of IL-biomass ratio and IL recycle rate on MESP when the IL price is $50/kg and $1.25/kg on MESP

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DESIGN OF LOW-COST IONIC LIQUIDS FOR LIGONCELLULOSIC BIOMASS PRETREATMENT

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Electronic Supplemental Information

MATERIALS AND METHODS

All chemicals used were reagent grade and purchased from Sigma-Aldrich (St. Louis, MO), unless otherwise noted. 1-ethyl-3-methylimidazolium acetate, [C_2C_1im][OAc], was purchased from Sigma-Aldrich and used as received. Switchgrass *(Panicum virgatum*) was provided by Dr Daniel Putnam, University of California at Davis. The air-dried biomass was milled by a Thomas-Wiley Mini Mill fitted with a 40-mesh screen (Model 3383-L10 Arthur H. Thomas Co., Philadelphia, PA, USA) and sieved to the nominal sizes of 40-60 mesh (250-400 μm). All feedstocks were further dried in a vacuum oven at 40 °C overnight prior to pretreatment to eliminate the variability of moisture content.

IL synthesis: The synthesis of the protic ionic liquids was achieved by combining an acid and a base in stoichiometrically equal amounts, similar to techniques described elsewhere.[17] Each amine/sulfuric acid adduct was prepared by the dropwise addition of H_2SO_4 (95%) in water (3 ml of water per every 1 ml of H_2SO_4) to a solution of the amine in water (1 ml of water per every 1 ml of amine). The purity of the starting materials was considered when calculating the quantities to be combined. The mixtures were stirred at room temperature for several hours. Water was then removed *in vacuo* at 50 ºC for 48 h. We have endeavored to control the acid:base ratio at 1:1 by careful dosing of sulfuric acid and amine based on the purity stated by the manufacturer. However, since purity of both acid and base was <100%, we cannot guarantee that the mixtures used in this study had the exact compositions we aimed for (up to 4% deviation possible, within the limits of elemental analysis).

Pretreatment: All pretreatments were conducted in the presence of 20% water (w/w IL) and data compared to [C_2C_1im][OAc]:[H_2O] (4:1 w/w) by a methodology described elsewhere.[18] Briefly,

10 % (w/w) switchgrass in IL:H_2O (4:1 w/w), 20g total reaction mass, was loaded in a Syrris globe reactor at 120 °C for 180 min, unless otherwise noted. The solution was allowed to cool to 50 °C, and then soaked in 3 parts (w/w) ethanol to retain lignin in solution and precipitate dissolved biomass, if necessary. Further to this, the solution was filtered and the solids washed twice with 3 parts (w/w) deionized water to remove residual IL and the recovered biomass was then lyophilized.

Carbohydrate and lignin assays: The carbohydrate composition of biomass and residual biomass after hydrolysis was determined with a modified quantitative saccharification (QS) procedure [19]. In the modified QS, secondary hydrolysis was conducted in the presence of 1 % (w/w) sulfuric acid at 121 °C for 1 h to more accurately determine the quantities of sugars susceptible to acid degradation (e.g., xylan). After $CaCO_3$ neutralization and centrifugation, monomeric sugars in the supernatant were measured with an Agilent HPLC equipped with a Bio-Rad Aminex HPX-87P column (Richmond, CA) at a rate of 0.6 mL of deionized water per min at 60 °C. Glucose yield after hydrolysis was calculated as follows, where glucan in recovered biomass was converted to glucose:

$$Glucose\ Yield\ [\%] = \frac{mass\ glucose\ in\ hydrolysate}{mass\ glucose\ in\ recovered\ biomass} \times 100 \qquad (1)$$

The standard NREL biomass protocol was used to measure lignin and ash [20]. Briefly, solids remaining after two-stage acid hydrolysis were held at 105 °C overnight. The mass of the dried solids corresponds to the amount of acid-insoluble lignin and ash in the sample. The mass of the ash-only fraction was then determined by heating the solids to 575 °C for 24 h. The acid-soluble lignin content of the sample was determined by measuring the UV absorption of the acid hydrolysis supernatant at 320 nm wavelength and an absorptivity of 25. Total lignin was calculated as the sum of acid soluble and acid insoluble lignin.

The acid soluble lignin (ASL) on an extractives free basis was calculated as follows:

$$\%ASL = \frac{UV_{Abs} \times Volume_{filtrate} \times Dilution}{\varepsilon \times ODW_{sample} \times Pathlength} \times 100$$

UVabs = average UV-Vis absorbance for the sample at appropriate wavelength

ε = Absorptivity of biomass at specific wavelength

ODW_{sample}=weight of sample in milligrams

Pathlength=pathlength of UV-Vis cell in cm

All carbohydrate and lignin assays were conducted in triplicate.

Enzymatic hydrolysis assay: The pretreated samples were diluted to 10 g glucan per liter in a 50 mM sodium citrate buffer (pH 4.8) supplemented with 0.1 % (w/v) NaN_3, to prevent growth of microorganisms. All enzymatic hydrolysis experiments were conducted in triplicate. Pretreated samples were completely suspended in a rotary shaker at 250 rpm at 50 °C. The enzyme loadings were 20 mg protein (Novozymes Cellic® CTec 2) per gram of glucan. Eight hundred microliters of well-mixed hydrolysate were removed, followed by immediate centrifugation at 13,000 rpm

for 5 min. Exactly 500 μL of the supernatant was transferred to another micro-centrifuge tube and kept at room temperature for 30 min, to allow the conversion of all cellobiose to glucose. The supernatant was then acidified by adding 30 μL of 10 % (w/w) sulfuric acid, followed by freezing overnight. The frozen samples were thawed, mixed well, and then centrifuged at 13,000 rpm for 5 min, to remove any precipitated solid sediments. The soluble glucose in the enzymatic hydrolysate was measured by HPLC equipped with a Bio-Rad Aminex HPX-87H column at a rate of 0.6 mL of 0.1 % (v/v) sulfuric acid per min at 60 °C. After all remaining hydrolysate was decanted, the pellets were re-suspended in 20 mL of water and centrifuged to remove residual soluble sugars from the pellets. The sugar content of the washed pellets was determined by modified QS as described above. Enzymatic glucan digestibility after 48 h was calculated using the ratio of soluble glucose yield in the supernatant after enzymatic hydrolysis to the sum of this soluble glucose and the soluble glucose obtained from the pellet.

X-ray diffraction assay: The XRD experiments were performed on a PANalytical Empyrean X-ray diffractometer equipped with a PIXcel^{3D} detector and operated at 45 kV and 40 kA using Cu Kα radiation (λ= 1.5418 Å). The patterns were collected in the 2θ range from 5 to 55° with the step size of 0.026° and the exposure time of 300 seconds. A reflection-transmission spinner was used as a sample holder and the spinning rate was set at 8 rpm throughout the experiment. All spectra were subjected to baseline correction using PeakFit1 4.12 software (Systat Software Inc., Chicago, IL) assuming Gaussian distribution function as the shape of the resolved peaks and Saviszky-Golay smoothing [21]. The crystallinity index (CrI) was determined by Segal method [22].

TGA assay: Thermogravimetric analysis (TGA) was performed using a Mettler Toledo model TGA/DSC 1. 1-3 mg of each specimen was placed in a 40 μL platinum crucible, and heated under argon from 30-105 °C at 5 °C min^{-1} and the temperature held for 5 minutes. A 90-105 °C cycle with a five minute hold was repeated 5 times to remove moisture and the temperature was then increased to 350 °C at a heating rate of 10 °C min^{-1}. T_{ONSET} was determined by the standard method. Briefly this was calculated by tangentially extrapolating the pretransition mass loss curve and determining its intercept with the tangent of the point of steepest slope in the transition region. T_{PEAK} was calculated by the peak of the first derivative of the TGA weight loss curve.