

# Sulfonic Acid Group Functionalized Ionic Liquid Catalyzed Hydrolysis of Cellulose in Water: Structure Activity Relationships

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**Abstract** Catalytic activities of eight sulfonic acid group functionalized ionic liquids in water were compared for the hydrolysis of Sigmacell cellulose (DP ~ 450) in the 150-180 °C temperature range by measuring total reducing sugar (TRS) and glucose produced. The catalytic activity of acidic ionic liquids with different cation types decreases in the order imidazolium > pyridinium > triethanol ammonium cation. Among the sulfonic acid group functionalized imidazolium ionic liquids, the catalysts which contain a single imidazolium ion and a flexible linker between sulfonic acid group and the imidazolium ionic liquid core structure are the most active catalysts.

**Keywords:** cellulose, hydrolysis, ionic liquid catalyst, structure-activity relationship

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## 1. Introduction

Efficient hydrolysis of polysaccharides in lignocellulosic biomass to fermentable monosaccharides is a challenging step and the primary obstacle for the large scale production of cellulosic ethanol from abundant lignocellulosic biomass [1,2,3,4]. Enzyme technologies are currently being tested in more than a dozen of cellulosic ethanol pilot plants in the US and other parts of the world; yet these operations are confronting major challenges in bringing the production cost competitive with gasoline [5]. This is due to factors like high enzyme cost of current cellulase preparations, inability to recycle the enzyme, and energy costs associated with high pressure, high temperature pretreatment step [6,7,8]. The alternative method of saccharification using dilute aqueous sulfuric acid at high temperature and pressure is the older method used in the cellulosic ethanol plants in the 1940's. In fact acid saccharification was replaced by enzyme methods developed in the last two decades due to certain disadvantages of acid hydrolysis. Some of the main disadvantages include poor sugar yields, resulting in low ethanol yield, formation of fermentation inhibitors, and the high energy cost associated with operating at temperatures above 250 °C at high pressures [9,10]. Even though the direct aqueous acid saccharification gives comparatively low sugar yields, several research groups have taken a renewed interest in recent times in reviewing this classical method due to its simplicity and lower cost compared to enzymatic saccharification, which nevertheless requires an energy intense pretreatment [11,10,12].

Ionic liquids are well known [13,14] for their ability to dissolve cellulose and our interest in the search for efficient catalytic methods for saccharification of cellulose has led us to develop -SO<sub>3</sub>H group functionalized Brønsted acidic ionic liquids as solvents as well as catalysts for the degradation of cellulose and cellulosic biomass [15,16,17]. Later we found that these sulfuric acid derivatives with ionic liquid characteristics can be used as catalysts in aqueous phase as well [18]. For example, a dilute aq. solution of acidic ionic liquid 1-(1-propylsulfonic)-3-methylimidazolium chloride was shown to be a better catalyst than aq. sulfuric acid of the same H<sup>+</sup> ion concentration for the degradation of cellulose at moderate temperatures and pressures [18]. In the cellulose hydrolysis experiments using aq. solutions of 1-(1-propylsulfonic)-3-methylimidazolium chloride and sulfuric acid of the same acid strength, these catalysts have been shown to produce total reducing sugar (TRS) yields of 29.5 and 22.0 % respectively [18].

This enhanced catalytic activity of the 1-(1-propylsulfonic)-3-methylimidazolium chloride when compared to sulfuric acid can be explained as a result of an interaction or binding of the ionic liquid catalyst on the cellulose surface, which facilitates the approach of -SO<sub>3</sub>H group for the hydrolysis of the glycosidic link. Furthermore, this observation can be seen as an important lead for the development of an ionic liquid based cellulase mimic type catalyst for depolymerization of cellulose. We hypothesized that this binding ability may depend on the nature of ionic liquid core structure of the catalyst. As far as we are aware the effects of structural variations of any type of ionic liquid core structures, anions, and the separation between -SO<sub>3</sub>H group and cationic core in the

Brønsted acidic catalysts in the hydrolysis of cellulose in aqueous phase is not known. Therefore, in an attempt to develop a recyclable, simple enzyme mimic type acid catalyst and as an extension of our earlier work [15,16,17,18,19] on sulfonic acid substituted imidazolium

ionic liquid catalysts, we have studied a series of sulfonic acid group functionalized Brønsted acidic ionic liquid catalysts shown in Figure 1 for the hydrolysis of cellulose in water at moderate temperatures and pressures.

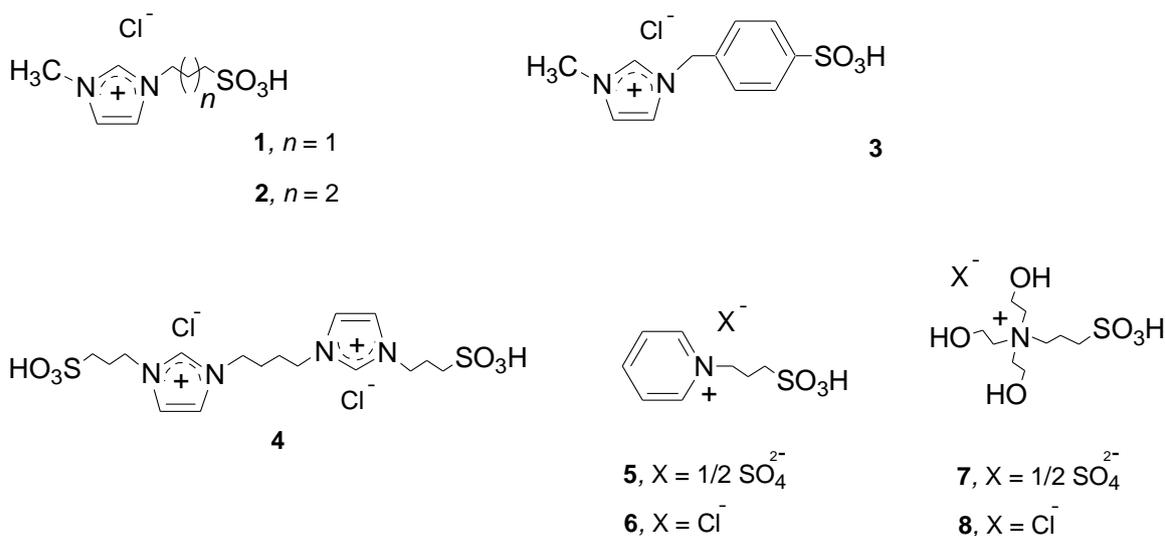


Figure 1. Sulfonic acid group functionalized Brønsted acidic ionic liquid catalysts (1-8)

## 2. Experimental

### 2.1. Materials and Instrumentation

Sigmacell cellulose - type 101 (DP ~ 450, from cotton linters), chemicals for the synthesis of sulfonic acid group functionalized ionic liquid catalysts (1-8) were purchased from Aldrich Chemical Co. USA.  $^1\text{H}$  NMR Spectra were recorded in  $\text{DMSO}-d_6$  on a Varian Mercury plus spectrometer operating at 400 MHz and chemical shifts are given in  $\delta$  (ppm) downfield from TMS ( $\delta = 0.00$ ).  $^{13}\text{C}$  NMR were recorded in the same spectrometer operating at 100 MHz; chemical shifts were measured relative to  $\delta$  ( $\text{CD}_3$ ) $_2\text{SO}$  and converted to  $\delta$  (TMS) using  $\delta$  ( $\text{CD}_3$ ) $_2\text{SO} = 39.52$ . Cellulose hydrolysis experiments were carried out in 25 mL stainless steel solvothermal reaction kettles with Teflon inner sleeves, purchased from Lonsino Medical Products Co. Ltd., Jingsu, China. These reaction kettles were heated in a preheated Cole-Palmer WU-52402-91 microprocessor controlled convection oven with  $\pm 1$  °C accuracy. Total reducing sugars (TRS, total of glucose and glucose oligomers with reducing groups) and glucose concentrations in aqueous solutions were determined using a Carey 50 UV-Vis spectrophotometer and 1 cm quartz cells.

### 2.2. Synthesis of Sulfonic Acid Group Functionalized Ionic Liquid Catalysts (1-8)

#### Synthesis of Ionic Liquid Catalysts 1, 2, 3, 5, and 6

Catalysts 1, 2, 3, 5, and 6 were prepared following literature procedures [15,20]. The structures and purity of the samples were confirmed by  $^1\text{H}$ ,  $^{13}\text{C}$  NMR and acid group determination by titration with standardized aqueous sodium hydroxide, using phenolphthalein as the indicator.

#### Synthesis of Ionic Liquid Catalyst 4

A mixture of 1,1'-(1,4-butanediyl)-bis-imidazole (3.80 g, 20.0 mmol) [21] and 1,3-propanesultone (4.89 g, 40.0 mmol) was heated in a closed round bottom flask at 110 °C for 14 h in an oil bath to give imidazolium salt as a white solid mass. The product was cooled in an ice bath at 0 °C, cold concentrated hydrochloric acid (4.06 g, 40.0 mmol) was slowly added, closed reaction flask was allowed to warm to room temperature in 2 h, and then heated at 90 °C for 14 h in an oil bath. The resulting liquid product was washed with *t*-butyl methyl ether (2x10mL) and dried under vacuum overnight to give sulfonic acid group functionalized ionic liquid catalyst 4 as a colorless viscous oil, 9.64, 96% yield. Titration of a sample with standardized 0.05 M aqueous sodium hydroxide using phenolphthalein as the indicator showed that product is > 99% pure.

$^1\text{H}$  NMR  $\delta$  1.76 (4H, m), 2.07 (4H, m), 2.47 (4H, m), 4.20 (4H, m), 4.27 (4H, t,  $J = 6.8\text{Hz}$ ), 4.76 (2H, bs), 7.79 (4H, s), 9.26 (2H, s)

$^{13}\text{C}$  NMR  $\delta$  26.3, 28.8, 47.9, 48.2, 60.4, 122.9, 136.8

#### Synthesis of Ionic Liquid Catalyst 7

A mixture of triethanolamine (2.98 g, 20.0 mmol) and 1,3-propanesultone (2.44 g, 20.0 mmol) was heated in a closed round bottom flask at 110 °C for 14 h in an oil bath to give triethanolammonium salt as a colorless solid mass. The product was cooled in an ice bath to 0 °C, cold concentrated sulfuric acid (1.00 g, 10.0 mmol) was slowly added, closed reaction flask was allowed to warm to room temperature in 2 h, and then heated at 90 °C for 14 h in an oil bath. The resulting product was washed with *t*-butyl methyl ether (2x10mL) and dried under vacuum overnight to give catalyst 7 as a pale yellow viscous oil, 5.81 g, 91% yield. Titration of a sample with standardized 0.05 M aqueous sodium hydroxide using phenolphthalein as the indicator showed that product is > 99% pure.

$^1\text{H}$  NMR  $\delta$  2.58 (2H, m), 3.27-3.35 (10H, m), 3.63 (6H, m.), 4.78(3H, bs), 7.75(1H, bs)

$^{13}\text{C}$  NMR  $\delta$  22.1, 28.8, 46.9, 52.6, 60.4

### Synthesis of Ionic Liquid Catalyst 8

A mixture of triethanolamine (2.98 g, 20.0 mmol) and 1,3-propanesultone (2.44 g, 20.0 mmol) was heated in a closed round bottom flask at 110 °C for 14 h in an oil bath to give triethanolammonium salt as a colorless solid mass. The product was cooled in an ice bath to 0 °C, cold concentrated hydrochloric acid (2.03 g, 20.0 mmol) was slowly added, closed reaction flask was allowed to warm to room temperature in 2 h, and then heated at 90 °C for 14 h in an oil bath. The resulting liquid product was washed with *t*-butyl methyl ether (2x10mL) and dried under vacuum overnight to give catalyst **8** as a pale yellow viscous oil, 5.66 g, 92% yield. Titration of a sample with standardized 0.05 M aqueous sodium hydroxide using phenolphthalein as the indicator showed that product is > 99% pure.

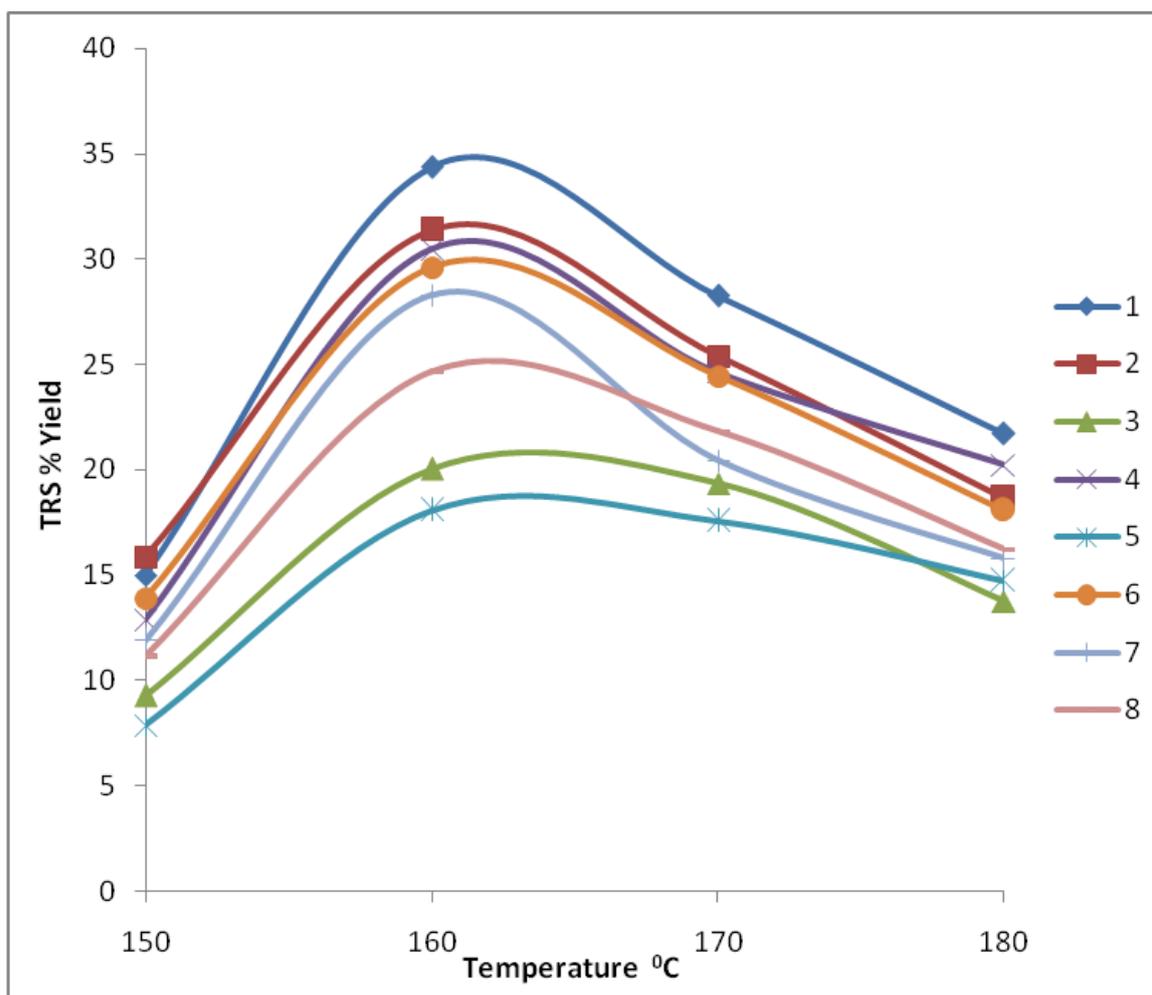
<sup>1</sup>H NMR δ 2.50 (2H, m), 3.27-3.35 (10H, m), 3.73 (6H, m.), 4.43(3H, bs), 9.00(1H, bs)

<sup>13</sup>C NMR δ 22.9, 31.6, 45.7, 50.8, 60.9

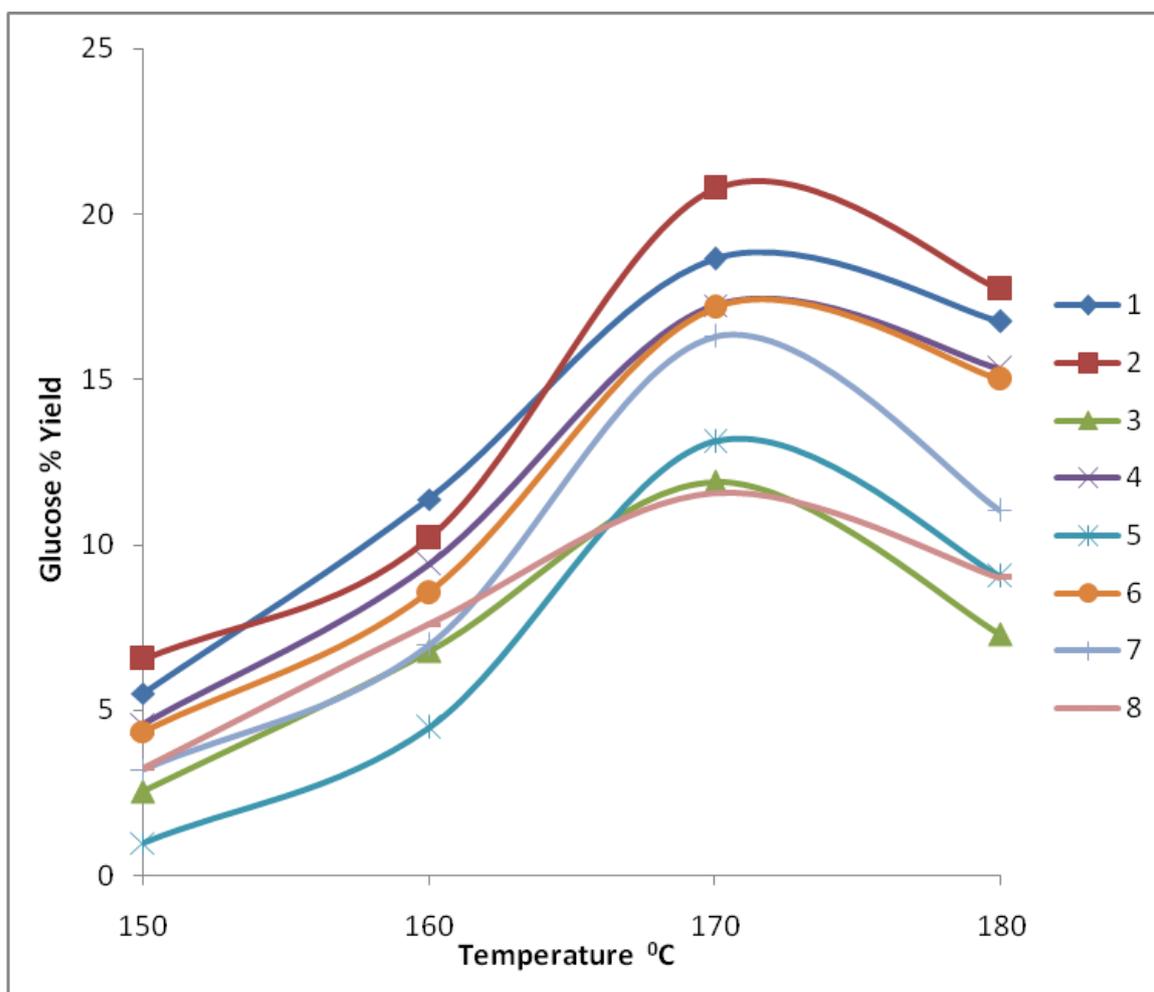
### 2.3. General Experimental Procedure for Hydrolysis of Cellulose Samples in Aqueous Brønsted Acidic Ionic Liquid Catalyst Solutions

Stock solutions of the Brønsted acidic ionic liquid catalyst solutions were prepared by dissolving appropriate

amounts of these acids in deionized water to give acid concentration of 0.0321 mol H<sup>+</sup>/L in each solution. The accuracy of the concentration was checked by titration with standardized aq. NaOH solution using phenolphthalein as the indicator. Sigmacell cellulose-type 101 (DP ~ 450) (0.030 g, 0.185 mmol of glucose unit of cellulose) was suspended in 2.00 mL of aqueous acid solution in a 25 mL high pressure stainless steel reaction kettle with Teflon inner sleeve. The reaction kettle was firmly closed and heated in a thermostated oven maintained at the desired temperature for 3.0 h. Then reaction kettle was removed from the oven and immediately cooled under running cold water to quench the reaction. The contents were transferred into a centrifuge tube and diluted to 10.0 mL with deionized water, neutralized by drop wise addition of 0.5 M aq. NaOH, and centrifuged at 3500 rpm for 6 min. to precipitate the solids before total reducing sugar (TRS) determination using 3,4-dinitrosalicylic acid (DNS) method [22]. The glucose formed was measured using glucose oxidase/peroxidase enzymatic assay [23]. A series of experiments were carried out in duplicate in the 150-180 °C temperature range to study the variations in TRS and glucose yields at different temperatures. The plots of changes in percent yields of total reducing sugar (TRS) and glucose produced at different temperatures are shown in Figure 2 and Figure 3 respectively.



**Figure 2.** The changes in % yields of total reducing sugar (TRS) produced during the hydrolysis of Sigmacell cellulose (DP ~ 450) in aqueous solutions of sulfonic acid group functionalized Brønsted acidic ionic liquid catalysts 1-8 at different temperatures. All acid solutions are 0.0321 mol H<sup>+</sup>/L, reaction time: 3.0 h, 0.030 g of Sigmacell cellulose in 2.00 mL of aq. acid was used in all experiments. Averages of duplicate experiments



**Figure 3.** The changes in % yields of glucose produced during the hydrolysis of Sigmacell cellulose (DP ~ 450) in aqueous solutions of sulfonic acid group functionalized Brønsted acidic ionic liquid catalysts 1-8 at different temperatures. All acid solutions are 0.0321 mol H<sup>+</sup>/L, reaction time: 3.0 h, 0.030 g of Sigmacell cellulose in 2.00 mL of aq. acid was used in all experiments. Averages of duplicate experiments

## 2.4. Analysis of Hydrolyzate

### TRS Assay

A 1.00 mL portion of the clear hydrolyzate solution from the centrifuge tube was transferred into a vial and 2.50 mL of deionized water was added. To this, was added 0.50 mL of DNS reagent [22] and the mixture was incubated in a water bath maintained at 90 °C for 5 min. The reagent blank sample was prepared with 3.50 mL of deionized water and 0.50 mL of DNS reagent and heated similar to the samples. Then the absorbance was measured at 540 nm, against the reagent blank, and TRS concentrations in solutions were calculated by employing a standard curve prepared using glucose.

### Glucose Assay

A 0.20 mL portion of the clear hydrolyzate solution from the centrifuge tube was transferred into a vial, and diluted with 1.80 mL deionized water. At zero time, reaction was started by adding 2.00 mL of glucose oxidase-peroxidase assay reagent [23] to the vial and mixing thoroughly, and the vial was incubated in a water bath at 37 °C for 30 min. Then reaction was quenched by adding 2.00 mL of 6 M HCl to give a pink solution. The reagent blank was prepared by mixing 2.00 mL of deionized water and 2.00 mL of assay reagent, and was treated similarly. Then the absorbance was immediately measured at 540 nm against the reagent blank and glucose concentration in

the solution was calculated by employing a standard curve prepared using glucose.

## 3. Results and Discussion

### 3.1. Synthesis and Characterization of Brønsted Acidic Ionic Liquid Catalysts (1-8)

Ionic liquid catalysts **1**, **2**, **3**, **5**, and **6** (Figure 1) were prepared following literature procedures [15,20]. The synthesized samples showed <sup>1</sup>H, <sup>13</sup>C NMR spectroscopy data in agreement with the published data of these compounds [15,20]. The synthesized samples were confirmed as > 99% pure by titration with standardized aqueous sodium hydroxide, using phenolphthalein as the indicator.

The dicationic ionic liquid catalyst **4** was prepared by condensation of the known 1,1'-(1,4-butanediyl)-bis-imidazole [21] with two equivalents of 1,3-propanesultone and then acidification of the resulting salt with an equivalent amount of concentrated hydrochloric acid. The product **4** showed two low field peaks in the <sup>1</sup>H NMR spectrum at  $\delta$  7.79 (4H, s) and 9.26 (2H, s) for imidazolium ring protons, characteristic of the imidazolium chloride moieties [21]. The <sup>13</sup>C NMR spectrum of **4** showed seven peaks indicating the symmetry of the molecule and the two low field peaks at  $\delta$

122.9, 136.8 could be assigned to C-4,5 and C-2 in the imidazolium rings [21]. The triethanolammonium ionic liquid catalysts **7** and **8** were prepared by condensation of triethanolamine and 1,3-propanesultone to give triethanolammonium salt and then acidification with equivalent amounts of conc. H<sub>2</sub>SO<sub>4</sub> and HCl respectively. The <sup>13</sup>C NMR spectra of compounds **7** and **8** showed five peaks each and the purity of the samples were further confirmed by titration with standardized sodium hydroxide solution.

### 3.2. Comparison of the Activities of Brønsted Acidic Ionic Liquid Catalysts (1-8)

In this study, eight Brønsted acidic ionic liquid catalysts (**1-8**) shown in Figure 1 were compared for the hydrolysis of Sigmacell cellulose type 101 (DP ~ 450) samples. The acidic ionic liquid catalysts used are thermally stable in the temperature range used in this study [24]. The average TRS and glucose yields produced in a series of experiments conducted in eight acid media at 150-180 °C temperature range are shown in Figure 2 and Figure 3 respectively. These results show that catalytic activities of sulfonic acid group functionalized acidic ionic liquids depends on the ionic liquid core structure and the anion in the catalyst. For all the Brønsted acidic ionic liquid catalysts studied the highest TRS yields are observed for samples heated at 160 °C for 3h, whereas the highest glucose yields are seen for samples heated at 170 °C for 3h. The catalysts 1-(1-propylsulfonic)-3-methylimidazolium chloride (**1**) and 1-(1-butylsulfonic)-3-methylimidazolium chloride (**2**), produced the highest acid TRS yields of 34.4 and 31.4% respectively. The same two catalysts **1** and **2** produced the highest glucose yields as well; 18.6 and 20.8 % respectively at 160 °C. The lowest TRS yield is produced from pyridinium ionic liquid catalyst *N*-propylsulfonic pyridinium sulfate (**5**).

In the comparison of monocationic ionic liquid catalysts with Cl<sup>-</sup> counter ion (**1**, **2**, **6**, and **8**), the activity of the Brønsted acidic ionic liquid catalysts generally decreases in the order of imidazolium > pyridinium > triethanolammonium. The dicationic imidazolium ionic liquid catalyst **4** showed the third highest TRS and glucose yields of 30.5 and 17.2% respectively. Interestingly two binding domains on the catalyst failed to enhance the catalytic activity, when compared to catalysts **1**, and **2**. Even though imidazolium cation containing ionic liquids **1** and **2** are the most active ones, another imidazolium ionic liquid catalyst **3** with arylsulfonic acid function showed very poor catalytic activity. This catalyst (**3**) showed the highest TRS and glucose yields of 20.0 and 11.9 % respectively. This observation suggests the importance of a flexible linker to connect the -SO<sub>3</sub>H group to the imidazolium cation for a favorable approach towards the glycosidic links in cellulose.

## 4. Conclusion

We have shown that catalytic activity of the sulfonic acid group functionalized acidic ionic liquids depends on the ionic liquid core structure and the anion in the catalyst. From the series of Brønsted acidic ionic liquid catalysts studied catalysts **1** and **2** produced the highest TRS yields

of 34.4 and 31.4% respectively at 160 °C, and the same two catalysts produced the highest glucose yields of 18.6 and 20.8 % respectively at 170 °C. The results indicate that structure - activity studies can be used as a tool in designing of an efficient small molecule catalyst system that can hydrolyze cellulose in the aqueous phase at moderate temperatures and pressure.

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## Statement of Competing Interests

The authors have no competing interests (financial or others) that may have influenced this study or the conclusions drawn from this study.

## References

- [1] Geddes, C.C., Nieves, I.U., and Ingram, L.O., "Advances in ethanol production". *Current Opinion Biotechnol.*, 22(3), 312-319, 2011.
- [2] Huang, R., Su, R., Qi, W., and He, Z., "Bioconversion of Lignocellulose into Bioethanol: Process Intensification and Mechanism Research". *Bioenerg. Res.*, 1, 1-21, 2011.
- [3] Zhu, J.Y. and Pan, X.J., "Woody biomass pretreatment for cellulosic ethanol production: Technology and energy consumption evaluation". *Bioresource Technol.*, 101(13), 4992-5002, 2010.
- [4] Brethauer, S. and Wyman, C.E., "Review: Continuous hydrolysis and fermentation for cellulosic ethanol production". *Bioresource Technol.*, 101(13), 4862-4874, 2010.
- [5] Zhang, P.F., Zhang, Q., Pei, Z.J., and Wang, D.H., "Cost estimates of cellulosic ethanol production: A review". *J. Manufact. Sci. Eng. Transact. ASME*, 135(2), article: 12005, 2013.
- [6] Sukumaran, R.K., Singhania, R.R., Mathew, G.M., and Pandey, A., "Cellulase production using biomass feed stock and its application in lignocellulose saccharification for bio-ethanol production". *Renewable Energ.*, 34(2), 421-424, 2009.
- [7] Alvira, P., Tomás-Pejaó E., Ballesteros, M., and Negro, M.J., "Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis: A review". *Bioresource Technol.*, 101(13), 4851-4861, 2010.
- [8] Zhu, J.Y., Pan, X., and Zalesny Jr, R.S., "Pretreatment of woody biomass for biofuel production: Energy efficiency, technologies, and recalcitrance". *Appl. Microbiol. Biotechnol.*, 87(3), 847-857, 2010.
- [9] Hu, F. and Ragauskas, A., "Pretreatment and Lignocellulosic Chemistry". *Bioenerg. Res.*, 5(4), 1043-1066, 2012.
- [10] Lenihan, P., Orozco, A., O'Neill, E. Ahmad, M.N.M., Rooney, D.W., and Walker, G.M., "Dilute acid hydrolysis of lignocellulosic biomass". *Chem. Eng. J.*, 156(2), 395-403, 2010.
- [11] Gurgel, L.V.A., Marabezi, K., Zambom, M.D., and Curvelo, A.A.D.S., "Dilute acid hydrolysis of sugar cane bagasse at high temperatures: A Kinetic study of cellulose saccharification and glucose decomposition. Part I: Sulfuric acid as the catalyst". *Ind. Eng. Chem. Res.*, 51(3), 1173-1185, 2012.
- [12] Taherzadeh, M.J., and Karimi, K., "Acid-based hydrolysis processes for ethanol from lignocellulosic materials: A review". *BioResources*, 2(3), 472-499, 2007.
- [13] Wang, H., Gurau, G., and Rogers, R.D., "Ionic liquid processing of cellulose". *Chem. Soc. Rev.*, 41(4), 1519-1537, 2012.
- [14] Mäki-Arvela, P., Anugwom I., Virtanen, P., Sjöholm, R., and Mikkola J.P., "Dissolution of lignocellulosic materials and its constituents using ionic liquids-A review". *Indust. Crop. Product.*, 32(3), 175-201, 2010.

- [15] Amarasekara, A.S., and Owereh, O.S., "Hydrolysis and decomposition of cellulose in Brønsted acidic ionic liquids under mild conditions". *Ind. Eng. Chem. Res.*, 48(22), 10152-10155, 2009.
- [16] Amarasekara, A.S., and Owereh, O.S., "Synthesis of a sulfonic acid functionalized acidic ionic liquid modified silica catalyst and applications in the hydrolysis of cellulose". *Catal. Commun.*, 11(13), 1072-1075, 2010.
- [17] Amarasekara, A.S., and Shanbhag, P., "Degradation of Untreated Switchgrass Biomass into Reducing Sugars in 1-(Alkylsulfonic)-3-Methylimidazolium Brønsted Acidic Ionic Liquid Medium Under Mild Conditions". *Bioenerg. Res.*, 6(2), 719-724, 2013.
- [18] Amarasekara, A.S., and Wiredu, B., "Degradation of cellulose in dilute aqueous solutions of acidic ionic liquid 1-(1-propylsulfonic)-3-methylimidazolium chloride, and p-toluenesulfonic acid at moderate temperatures and pressures". *Ind. Eng. Chem. Res.*, 50(21), 12276-12280, 2011.
- [19] Amarasekara, A.S., and Wiredu B., "Brønsted Acidic Ionic Liquid 1-(1-Propylsulfonic)-3-methylimidazolium-Chloride Catalyzed Hydrolysis of D-Cellobiose in Aqueous Medium". *Int. J. Carbohydr. Chem.*, 2012, 6-9, 2012.
- [20] Amarasekara, A.S., and Wiredu, B., "Single reactor conversion of corn stover biomass to C5–C20 furanic biocrude oil using sulfonic acid functionalized Brønsted acidic ionic liquid catalysts". *Biomass Conversion Biorefin.*, 1, 1-7, 2013.
- [21] Amarasekara, A.S., and Shanbhag, P., "Synthesis and characterization of polymeric ionic liquid poly(imidazolium chloride-1,3-diylbutane-1,4-diyl)". *Polym. Bull.*, 67(4), 623-629, 2011.
- [22] Breuil, C., and Saddler, J.N., "Comparison of the 3,5-dinitrosalicylic acid and Nelson-Somogyi methods of assaying for reducing sugars and determining cellulase activity". *Enzym. Microb. Technol.*, 7(7), 327-332, 1985.
- [23] Bergmeyer, H.U., Bernt, E., ed. *Methods of Enzymatic Analysis*. ed. H.U. Bergmeyer, Academic Press: New York. pp 1205-1212, 1974.
- [24] Amarasekara, A.S., and Owereh, O.S., "Thermal properties of sulfonic acid group functionalized Brønsted acidic ionic liquids". *J. Therm. Anal. Calorim.*, 103(3), 1027-1030, 2011.