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Chemocatalytic Hydrolysis of Cellulose at 37 °C, 1 Atm.

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The metal salt - Brönsted acidic ionic liquid system composed of $ZnCl_2.1.74H_2O$-1-(1-propylsulfonic)-3-methylimidazolium chloride can directly hydrolyze untreated cellulose in 78% total reducing sugar and 19% glucose yield at 37 °C, 1 Atm. in 4.0 days. The new chemical catalyst is used at a temperature lower than typical cellulase enzyme operating conditions.

Cellulose is the most abundant biopolymer on earth and the resourceful utilization of cellulose is a paramount factor in a sustainable carbon based future. The hydrolysis of this polysaccharide is a formidable challenge due to its complex molecular architecture with stiff polymeric chains and close packing via strong inter and intra-molecular hydrogen bonds[1]. The current technology for this process is high temperature-pressure aq. sulfuric acid pretreatment followed by the use of cellulase enzymes for the hydrolysis[1,2]. The alternative method of mineral acid catalyzed hydrolysis gives poor sugar yields and requires even higher temperature-pressure conditions[3]. While these operations are highly energy consuming and expensive, there is an urgent need for a more efficient catalytic method for harvesting the full potential of the most abundant renewable carbohydrate polymer.

Since the 2002 report on the use of 1-butyl-3-methylimidazolium salts, which are room temperature ionic liquids (ILs) for the dissolution of cellulose, considerable efforts have been devoted to improve the solubility of cellulose in ILs for processing of cellulosic biomass[4,5,6]. Then, in 2007 Zhao et al. reported the first use of ILs as a solvent to hydrolyze cellulose[7]. Where they attained a 77% total reducing sugar (TRS) yield after 9 h at 100 °C by using conc. H_2SO_4 as the acid catalyst for cellulose dissolved in 1-butyl-3-methylimidazolium chloride[7]. In a follow up work our research group introduced the incorporation of the $-SO_3H$ acid catalyst function to IL, thus eliminating the use of a separate acid and considerably improving the efficiency of cellulose hydrolysis[8,9,10]. Where we first reported that Brönsted acidic ionic liquid (BAIL) 1-(1-propylsulfonic)-3-methylimidazolium chloride (PSMIMCl) can be used as an acid catalyst as well as the solvent; yielding 62% TRS after 1.5 h, at 70 °C, 1 Atm[8]. Later we found that these sulfuric acid derivatives with IL characteristics can be used as catalysts in aqueous phase as well[9,11]. For example a dilute aqueous solution of PSMIMCl was shown to be a better catalyst than aq. H_2SO_4 of the same H^+ concentration for the degradation of cellulose at 150-160 °C at 0.38-0.52 MPa[9]. This enhanced catalytic activity of the PSMIMCl when compared to H_2SO_4 can be explained as a result of an interaction or binding of the BAIL on the cellulose surface, which facilitates the approach of $-SO_3H$ group for hydrolysis of the glycosidic link[12,13]. This observation can be seen as an important lead for the development of a BAIL based cellulase mimic type catalyst for cellulose hydrolysis.

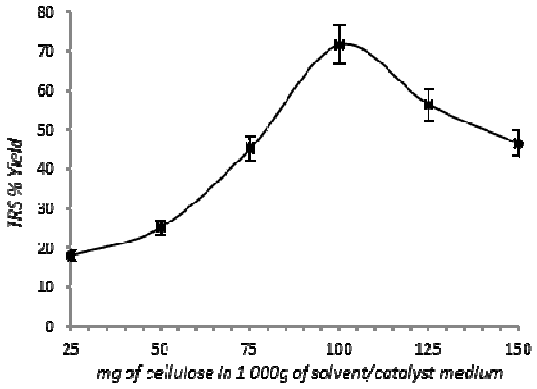

Fig. 1. The total reducing sugar (TRS) % yields produced during the hydrolysis of Sigmacell cellulose - type 101 (DP ~ 450, from cotton linters) in 30% (w/w) BAIL in $ZnCl_2.1.74H_2O$ at 37 °C, 4.0 d.

Then there are new directions, such as the incorporation of metal ions like Mn^{2+}, Fe^{3+}, Cu^{2+}, and Zn^{2+} as Lewis acid co-catalysts to the Brönsted acid system, shown to improve the cellulose pretreatment and acid hydrolysis efficiencies[14,15,16]. In addition, we have recently found that addition of a catalytic amount of Mn^{2+}, Fe^{3+} or Co^{2+} chloride salt can be used to improve the sugar yields during the acid catalyzed saccharification of corn stover in water at 140-170 °C[17]. As

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these new discoveries point out the advantages of mixing Lewis and Brönsted acid catalysts for cellulose hydrolysis, we hypothesized that catalytic activity of the BAILs could also be further enhanced by incorporating -OH chelating metal ions and anions like Cl^- to the catalyst. Consequently we have studied the cellulose hydrolysis in a number of metal chloride-BAIL systems to test this hypothesis. Where $ZnCl_2$ based systems showed much promise and here we report the first catalyst that can hydrolyze untreated cellulose under conditions closer to ambient temperature.

We have examined the cellulose hydrolysis by measuring the TRS and glucose yields in mediums containing variable amounts of PSMIMCl in $ZnCl_2$. These mixtures contained controlled amount of water as well, since water attack at the glycosidic link is the key step in the hydrolysis. Our earlier work using neat BAIL have shown that ~ 2.0 equivalents of water per glucose unit is sufficient for cellulose hydrolysis[8]. As well as preliminary tests have revealed that anhydrous $ZnCl_2$ can be converted to a viscous, colorless, clear liquid of hydrated $ZnCl_2$ by the addition of 1.74 equivalents of water at 23 °C. This $ZnCl_2.1.74H_2O$ was used throughout the study. In the initial stages a mixture of 30% (w/w) BAIL in $ZnCl_2.1.74H_2O$ was used to find the optimum weight of cellulose that can be hydrolyzed in a gram of solvent/catalyst medium. During these experiments TRS produced from hydrolysis of 25-150 mg cellulose in 1.000g of solvent/catalyst at 37 °C were measured using 3,5-dinitrosalicylic acid (DNS) method[10] and the results are shown in figure 1. Which revealed that 100 mg of cellulose in 1.000g of 30% (w/w) BAIL-$ZnCl_2.1.74H_2O$ produces the highest TRS % yield; thus 100 mg of cellulose per 1.000 g of catalyst/solvent were used in the next set of experiments.

Next we have investigated the effects of reaction time and the composition of the BAIL-$ZnCl_2$ on TRS and glucose yields. The cellulose samples were mixed in 0-40% BAIL in $ZnCl_2.1.74H_2O$ and incubated at 37.0±0.1 °C for 1-8 days. After the specified period the samples were diluted with water, neutralized (NaOH), unreacted cellulose and precipitated zinc salts were removed by centrifugation (1700*g*) to give clear sugar solutions; in which TRS and glucose were measured[10] (ESI). The average TRS and glucose % yields produced in 0-40% BAIL in $ZnCl_2.1.74H_2O$ medium for 0-8 days at 37±0.1 °C are presented in the 3D plots 2A and 2B respectively.

This optimization analysis shows that 30% (w/w) BAIL in $ZnCl_2.1.74H_2O$ produced the highest TRS% yield of 78% after 4.0 d at 37±0.1 °C. Moreover, a reduction in TRS yields are observed in samples of more than 4.0 d, and this may be due to the decomposition of sugars in the highly acidic medium[18]. The highest glucose yield of 19% was also achieved in reactions carried out in the same 30% (w/w) BAIL in $ZnCl_2.1.74H_2O$ at 4.0 d. Additionally, the glucose yield also decreased after 4.0 d as TRS (figure 2B). With the aim to check the cellulose hydrolysis in only $ZnCl_2.1.74H_2O$ medium we have prepared a mixture containing 10% (w/w) cellulose in $ZnCl_2.1.74H_2O$ and incubated at 37 °C for 4 days. However no reducing sugars were detected. Similar experiment with 10% (w/w) cellulose in BAIL also failed to produce any reducing sugars. The experiments carried out in $ZnCl_2$-BAIL mixtures with water contents higher than 1.74 H_2O per mol of $ZnCl_2$ also failed to produce sugars.

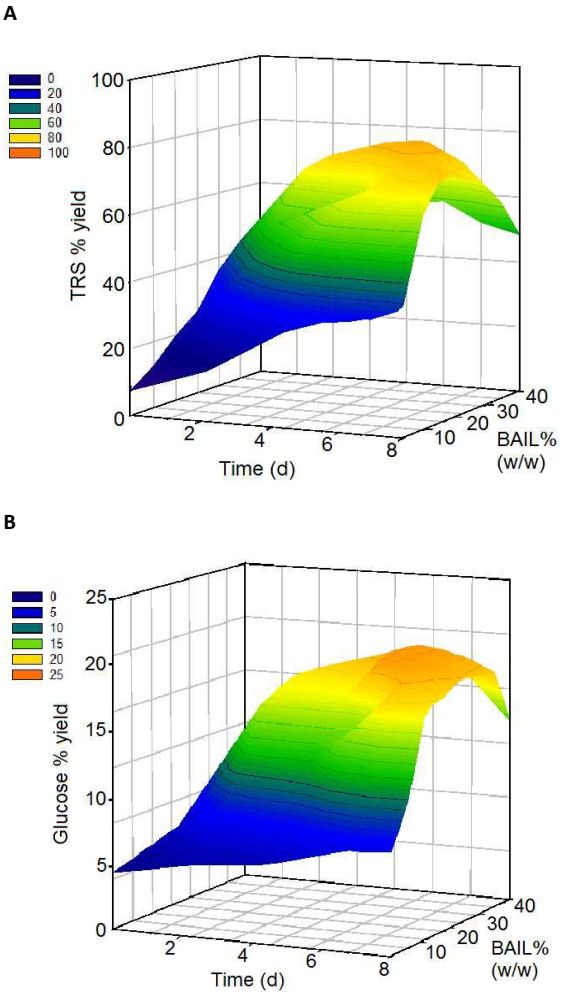


Fig. 2. (**A**) Total reducing sugar (TRS) and (**B**) glucose % yields produced during the cellulose hydrolysis using 0-40% BAIL in $ZnCl_2.1.74H_2O$ for 0-8 d, 37±0.1 °C. 100 mg Sigmacell cellulose - type 101 (DP ~ 450, from cotton linters) was used. Averages of duplicate experiments.

Furthermore, we have carried out an experiment at 50 °C, for 4 days to check if the glucose yield improves at a higher temperature. Unfortunately at 50 °C, glucose yield slightly decreased to 16% from earlier 19%; nevertheless TRS yield remained the same. Even though the new catalyst system gives a good TRS yield, it produces only a low glucose yield. This may be due to well-known[19] dehydration of glucose into 5-hydroxymethylfurfural and subsequent decomposition and polymerization under highly acidic reaction conditions.

In order to compare the new catalyst system with cellulose hydrolysis using the standard enzymatic hydrolysis under NREL/TP-5100-63351 protocol[20] we have carried out an additional experiment. Cellulase from *Trichoderma reesei* ATCC 26921, which catalyzes the breakdown of cellulose into glucose, cellobiose, and higher glucose polymers was used as the enzyme cocktail. The TRS and glucose in the hydrolyzate were measured using DNS method and glucose oxidase-peroxidase assay as in the BAIL in $ZnCl_2.1.74H_2O$ catalyst system experiments. The results of this

experiment showing the changes in TRS and glucose yields with time are shown as figure 3. The results of the standard NREL protocol enzymatic hydrolysis experiment carried out at 50 °C shows higher TRS and glucose yields than the new catalyst system presented in this work. However, the new BAIL in $ZnCl_2.1.74H_2O$ catalyst works at 37 °C, and does not require a pre-treatment of cellulose before the hydrolysis.

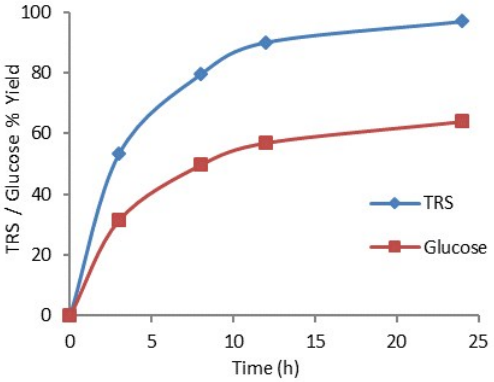
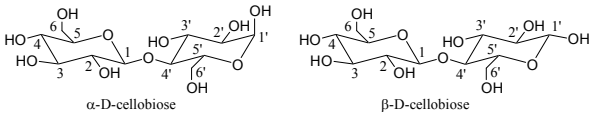


Fig. 3. The changes in TRS and glucose yields with time for Sigmacell cellulose - type 101 (DP ~ 450, from cotton linters) hydrolyzed according to NREL/TP-5100-63351 protocol[20] using cellulase enzyme from *Trichoderma reesei* ATCC 26921 at 50 °C, pH = 5.0, sodium citrate buffer

In an attempt to explain the unprecedented catalytic activity in the $ZnCl_2$–BAIL system we have studied the interaction of cellulose model compound cellobiose with $ZnCl_2$ and $ZnCl_2$–BAIL using NMR spectroscopy. The initial attempts to record ^1H and ^{13}C NMR of cellobiose in $ZnCl_2.1.74D_2O$ at 23 °C was not successful due to high viscosity of the medium. However, dilution of the $ZnCl_2$ up to $ZnCl_2.4D_2O$ allowed recording good ^{13}C spectra after ~ 15K scans (figure 4). Though, ^1H NMR still produced broad unresolved peaks. With this tool we have studied the ^{13}C spectra of cellobiose in D_2O[21], $ZnCl_2.4D_2O$ and in three different $ZnCl_2.4D_2O$ + BAIL mediums. The ^{13}C spectrum in D_2O was used as a reference and the chemical shifts changes ($\Delta\delta$) were calculated for each carbon in α/β–D-cellobiose (figure 4) for the solvent change from D_2O to $ZnCl_2.4D_2O$ and these $\Delta\delta$ values are shown in figure 5[22, 23]. Which shows that changing the solvent to $ZnCl_2.4D_2O$ results in significant chemical shift changes in a number of carbons, suggesting a strong interaction between cellobiose and $ZnCl_2$. The largest decreases in chemical shifts of -0.549, -0.696 and -0.851 ppm were observed for C6, C6'-α and C6'-β respectively. The major increases in δ of 0.66, 0.495, 0.513 and 0.359 ppm were observed for C5, C1'-α, C1'-β and C5'-β. This clearly indicates that Zn^{2+} cations and/or Cl^- anions interacts or binds with a specific region of cellobiose. The highest chemical shift changes occur in C6, additionally all C6 peaks showed peak broadening as well.



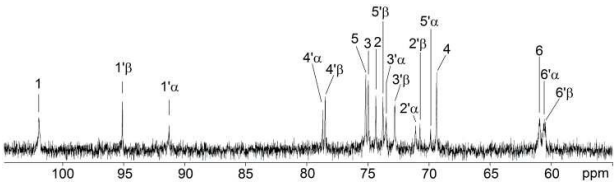


Fig. 4. ^{13}C-NMR spectrum of α/β D-cellobiose in $ZnCl_2.4D_2O$ (50 mg/0.6 mL) at 23 °C.

These effects are most probably due to chelation with the C6 hydroxyl groups. In the next stage we have recorded three additional ^{13}C spectra after adding increasing amounts of BAIL into the same NMR tube to provide cellobiose : BAIL mole ratios 1:0.06, 1:0.48 and 1:1.14 respectively. The $\Delta\delta$ values in these three mediums are also shown in figure 5. The BAIL modified $ZnCl_2$ medium $\Delta\delta$ curves are very close to the first $\Delta\delta$ curve obtained without BAIL. This may be due to very small or negligible effect by the addition of the BAIL on the interaction/binding of $ZnCl_2$ with carbohydrate. Therefore, the NMR data suggests that $ZnCl_2$ plays an important role by interacting with carbohydrate and probably changing the conformation of the polysaccharide, facilitating the BAIL catalyzed hydrolysis.

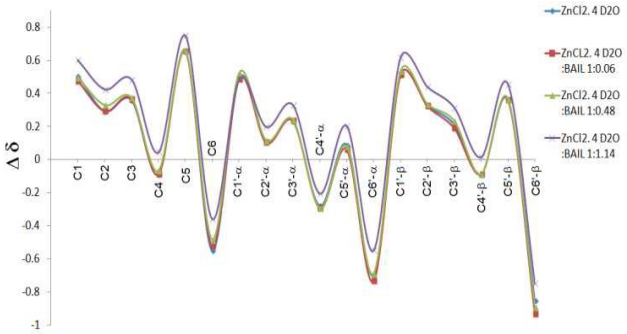


Fig. 5. ^{13}C NMR chemical shift changes ($\Delta\delta$) of D-cellobiose due to different solvent mediums at 23 °C. Chemical shifts changes are measured relative to D-cellobiose in D_2O

Furthermore, we have calculated the activation energy (E_a) for the hydrolysis of cellulose in 30% (w/w) BAIL in $ZnCl_2.1.74H_2O$ at 25-37 °C by using the first order rate constants as 121±2 $kJmole^{-1}$ (ESI). The generally accepted E_a for H_2SO_4 catalyzed cellulose hydrolysis in water is 170-185 $kJmole^{-1}$ [24,25]. Therefore the low E_a in the new catalyst further supports the synergistic effect of BAIL and hydrated $ZnCl_2$.

The mechanism of hydrolysis may involve the transfer of acidic proton of the BAILs -SO_3H group to glycosidic oxygen of cellulose, resulting protonation of the oxygen. The co-catalyst $ZnCl_2$ may facilitate the protonation by interaction with the polysaccharide and changing the conformation, as evident from the ^{13}C NMR study of the model compound. Next, the attack of water at glycosidic carbon can cleave the C-O bond, completing the hydrolysis. As further studies of the present approach we are proposing to study the recycling of the catalysts after separation from sugars by using a combination of precipitation of Zn salts and ion exchange chromatography methods. In addition we are currently working on improving the glucose yield.

Conclusions

The high catalytic activity observed in this study may be due to interaction of $ZnCl_2$ with cellulose, disrupting the H-bonding network and similar interactions of carbohydrates with Mn^{2+} [26]. and Fe^{3+} [27] are reported in the literature. As far as we are aware the mildest conditions reported for a chemocatalytic cellulose hydrolysis is 70 °C, 1 Atm, for 1.5 h, in neat PSMIMCl[8]. Therefore the present result is a significant improvement and the first example of a cellulose hydrolysis by a chemically catalyzed system in conditions close to room temperature.

Acknowledgments
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Graphical Abstract

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