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COMMUNICATION

Impact of Non-Solvents on the Structural Features and Enzymatic Digestibility of Cellulose Regenerated from an Ionic Liquid

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The choice of non-solvent has a dramatic influence on the morphology/crystallinity of regenerated cellulose obtained following ionic liquid (IL) dissolution. This, in turn, greatly impacts the enzymatic digestibility of the cellulose. The use of ethanol (in contrast to water) provides a high surface-area, amorphous regenerated material ideal for hydrolysis—ethanol is also more facile to separable from the IL than water during the recovery/recycling process for the IL.

Cellulose, the most abundant polymer in nature, is composed of glucose units linked by β-1,4-glycosidic bonds with the resulting linear polymeric chains having both inter- and intrachain hydrogen bonding in the crystalline polymer.[1,2] Although cellulose provides a potential renewable glucose platform for biofuels and chemicals, cellulose is resistant to hydrolysis due to its high crystallinity and the difficulty of isolating it from the other biomass components. These factors, and its insolubility in most conventional solvents, have made cellulose utilization quite challenging. In recent years, however, ionic liquids (ILs) have been shown to be exceptional solvents for cellulose and thus hold great promise as biomass pretreatment media.[3]

ILs are salts which melt at low temperature, often below room temperature. Some ILs are able to dissolve not only cellulose, but also lignocellulosic biomass and it has been demonstrated that the cellulose recalcitrance to enzymatic hydrolysis is greatly mitigated by the IL dissolution of the biomass followed by recovery as a solid (regeneration) through the addition of a non-solvent.[4-16] The process variables for such IL-based processing of biomass have been extensively investigated including variations in biomass type and particle size, as well as incubation temperature and time.[17-19] Several different non-solvents have reportedly been used—including water, acetone, methanol and ethanol—to regenerate the dissolved cellulose or biomass. Limited attention has been paid, however, to the effects of the non-solvents on the structural properties of the regenerated solid cellulose.

The dissolution of cellulose or biomass within different ILs varies widely with the structure of the ions. Imidazolium-based ILs, such as 1-ethyl-3-methylimidazolium acetate [C_2mim][OAc] and 1-butyl-3-methylimidazolium chloride [C_4mim][Cl], have been the most frequently used for cellulose dissolution and biomass pretreatment. Amongst the well-studied ILs, [C_2mim][OAc] in particular has been identified as a promising IL because it can dissolve not only cellulose, but also lignin. In addition, the IL is a liquid at ambient temperature, has a relatively low viscosity and can be recycled in the process.[19-21] [C_2mim][OAc] was therefore selected for the present study which explored the impact of different non-solvents for cellulose on the properties of the cellulose regenerated from a cellulose-IL mixture (i.e., C/IL) with 5 wt% cellulose.

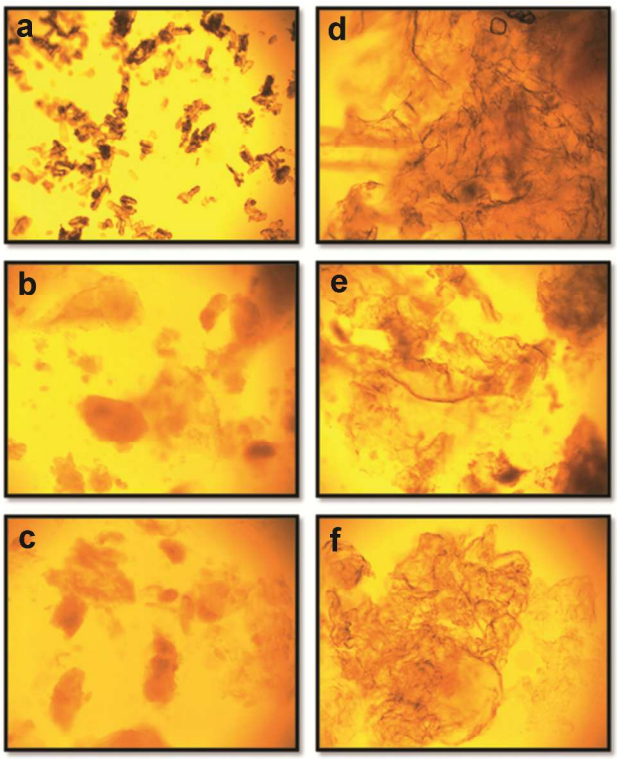

Fig. 1 Microscope images of regenerated cellulose (10x): (a) untreated cellulose (MCC), (b) C/IL-W, (c) C/IL-W/A, (d) C/IL-E, (e) C/IL-E/A and (f) C/IL-IPA.

When acetone (A) was used as the non-solvent, no cellulose precipitated from the C/IL mixture. Upon standing after mixing, the C/IL-A solution instead separated into two liquid phases. The dissolved cellulose was retained in the lower IL-rich phase. Thus, pure acetone is not an effective non-solvent for the C/IL mixture used. In contrast, a precipitate immediately formed when water (W), ethanol (E), isopropanol (IPA), W/A or E/A was used as

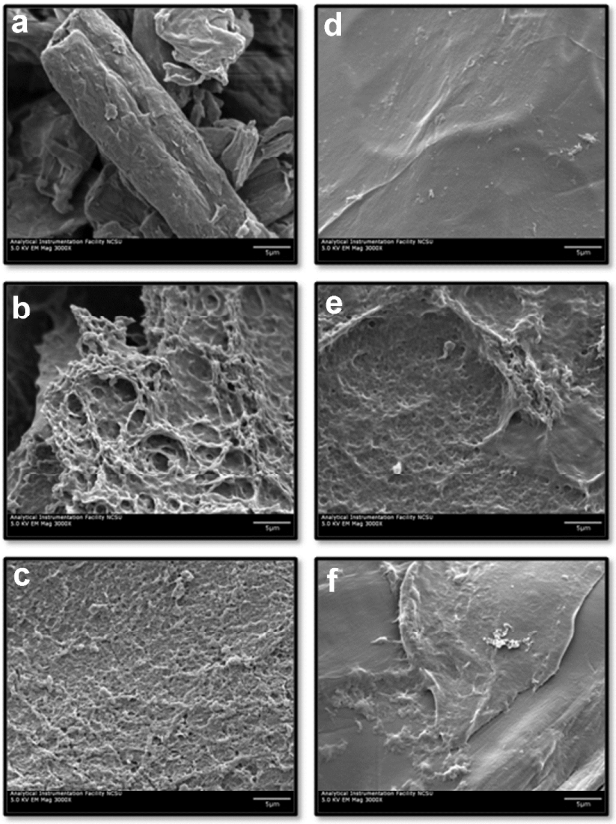

Fig. 2 SEM images of regenerated cellulose (spacer bar is 5 μm): (a) untreated cellulose (MCC), (b) C/IL-W, (c) C/IL-W/A, (d) C/IL-E, (e) C/IL-E/A and (f) C/IL-IPA.

non-solvents. For the water-containing non-solvents (W or W/A), the amount of non-solvent used to regenerate the dissolved cellulose was approximately twice the C/IL mixture volume.[22] Regeneration from water resulted in large clusters/particles, whereas the alcohols (E or IPA) resulted in a spongy solid (Fig. 1). Acetone mixed with either water or ethanol was able to regenerate the dissolved cellulose—when mixed with water (W/A) or ethanol (E/A), particles or a spongy solid resulted, respectively. More solvent (about four times the C/IL mixture volume) was needed to make the C/IL-solvent mixtures stir when alcohols (E, IPA or E/A) were used due to the gelation of the solutions prior to the precipitation of the solid cellulose.

The regeneration process and the properties of the regenerated solids are directly related to the competing interactions between the dissolved cellulose, the IL and the non-solvent. These interactions are dependent on the solvent properties including the general polarity of the solvent molecules and their ability to form hydrogen bonds with cellulose. The Kamlet-Taft parameters α, β and π* and the E_T^N polarity scale have been used to describe these solvent properties. The parameters for [C_2mim][OAc] and non-solvents used are summarized in Table 1.[23-26] The α and β values, respectively, indicate the hydrogen bond donor (HBD) and acceptor (HBA) ability of a given solvent, while the π* value measures the residual polarity of the solvent after the hydrogen-bonding effects have been removed.[24] A large β value (high HBA ability), such as 0.95 for [C_2mim][OAc], is reported to be required to effectively dissolve cellulose.[23,26] Water has high E_T^N

Table 1 E_T^N and Kamlet-Taft parameters of [C_2mim][OAc] and the non-solvents used[22-25]

	T_b (°C)	E_T^N	α	β	π*
[C_2mim][OAc]		0.590	0.40	0.95	1.09
water	100	1.000	1.17	0.47	1.09
ethanol	78	0.654	0.86	0.75	0.54
isopropanol	83	0.546	0.76	0.84	0.48
acetone	56	0.355	0.08	0.43	0.71

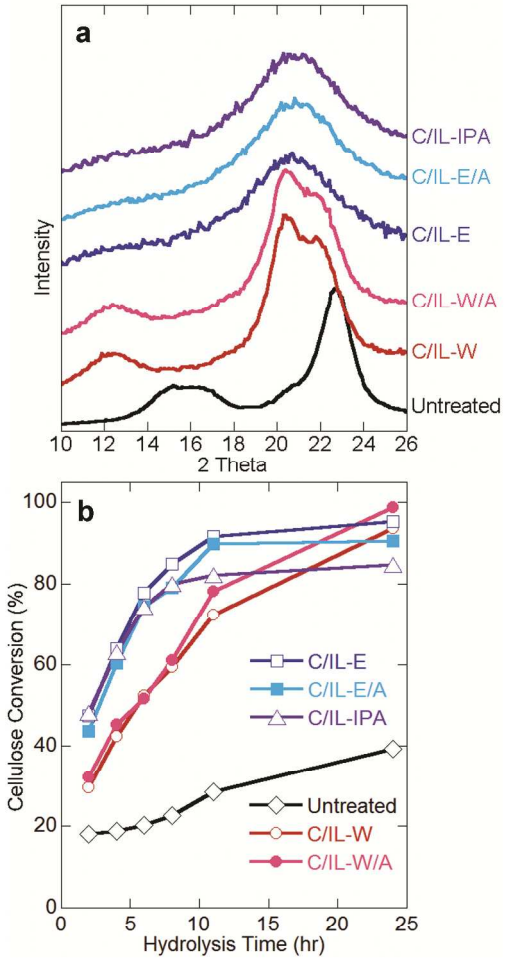

Fig. 3 (a) XRD data and (b) cellulose conversion (%) to D-glucose from the enzymatic hydrolysis for the original (untreated) and regenerated cellulose.

and α values (high polarity and high HBD ability, respectively) and thus strongly interacts with the IL ions, thereby readily displacing the interactions between the IL and cellulose, resulting in the rapid precipitation of the cellulose from the C/IL mixture. The alcohols (E and IPA) also have high E_T^N and α values, although lower than for water (Table 1). Thus, these solvents also displace the IL-cellulose interactions, but more slowly than for water, resulting in gel formation prior to cellulose precipitation. This difference in solvent-ion-biopolymer interactions results in differences in how the biopolymer segments interacts with other biopolymer segments as the ions and solvent molecules are stripped away during the precipitation process…and therefore in differences in the resulting biopolymer crystallinity and morphology. Acetone, in contrast, has smaller E_T^N and α values (Table 1). Thus, the interactions between acetone and the IL are

weaker, reducing the strength of the cellulose-IL interactions, and the addition of acetone does not precipitate the cellulose from the C/IL mixture.

SEM images (Fig. 2) indicate that untreated microcrystalline cellulose (MCC) powder has a compact structure. The regenerated cellulose particles were larger in size, but had a less compact structure. The structural differences between the W- or W/A-regenerated cellulose samples and the samples obtained from the alcohols (E, IPA or E/A) were significant. Cellulose is polymorphic with multiple known crystalline phases.[27-33] Fig. 3a indicates that the untreated cellulose (MCC) has the typical XRD powder pattern of the cellulose-I crystal structure with a broad peak centered at 16° and a sharp peak at 22.7°.[32,33] In contrast, the cellulose regenerated with W or W/A was transformed into the cellulose-II crystal structure as indicated by the peaks at 12.3°, 20.4° and 21.9°.[33] Use of the alcohols (E, IPA and E/A), however, resulted in amorphous cellulose as evidenced by the lack of peaks attributable to either the cellulose-I or -II crystal structures.

As has been widely reported,[4-16] the dissolution and regeneration of cellulose significantly increases the enzymatic digestibility of the cellulose (Fig. 3b). But the cellulose regenerated with the alcohols (E, IPA or E/A) (i.e., amorphous cellulose) showed a significantly higher hydrolysis rate than the cellulose obtained from water (W or W/A) (i.e., cellulose-II) as the non-solvent. In particular, the ethanol-regenerated cellulose had both a rapid hydrolysis rate, as well as a high glucose yield with close to complete conversion in 12 h. It is also noteworthy that the lower boiling point of ethanol makes it much less energy intensive to separate from the IL during the recovery/reuse of the materials once the regeneration step is complete. Thus, ethanol may be a preferential solvent choice for cellulose regeneration instead of the current prevalent use of water for this processing step.

Conclusions

The reported results indicate that the cellulose (dissolved in an IL) regenerated from water is highly crystalline with the cellulose-II crystal structure. In contrast, regeneration with ethanol or isopropanol results in amorphous cellulose. This, along with the need for a much less energy intensive distillation step to separate the non-solvent from the IL for recycling of the materials, suggests that ethanol may be a preferable non-solvent to water for the cellulose regeneration step.

Experimental

Microcrystalline cellulose (MCC) powder (particle size 20 μm, Sigma product number 310697)—referred to as untreated cellulose—was used as the starting material. [C_2mim][OAc] (Sigma Cat. No 51053) was purchased from Sigma-Aldrich and used as-received. Cellulase, NS 22086 (175 FPU ml^{-1}), was obtained from Novozymes North America (FPU stands for filter paper unit). The solvents (acetone, ethanol and isopropanol) were purchased in high purity from Sigma-Aldrich and used as-received.

[C_2mim][OAc] was heated in a round bottom flask at 100 °C for 20 min to remove any moisture in the IL. Cellulose (untreated MCC) was mixed with the IL (5 wt% cellulose) and incubated at 100 °C for 2 h under N_2 while stirring with a magnetic stirrer. The dissolved cellulose turned into a gel. The cellulose-IL (C/IL) gel was divided into 6 parts (about ~20 g each) and kept at 50 °C before regeneration.

Deionized (DI) water (W), acetone (A), ethanol (E), isopropanol (IPA), water-acetone (1/1, v/v) (W/A) and ethanol-acetone (1/1, v/v) (E/A) were used to regenerate the dissolved cellulose by adding the preheated solvents (at 50 °C) to the C/IL gel under rapid mechanical stirring. After stirred 30 min at room temperature, the regenerated cellulose flocs were collected by vacuum filtration and thoroughly washed with the same non-solvent, then washed 5 times with DI water. The collected solids are referred to as C/IL-W, C/IL-A, C/IL-E, C/IL-IPA, C/IL-W/A and C/IL-E/A, respectively. Small amounts of the regenerated cellulose samples were frozen and then vacuum dried at room temperature for characterization, while the remainder was stored in sealed containers at 4 °C for enzymatic hydrolysis and SEM analysis.[34]

A Hitachi S-3200N scanning electron microscope was used to examine the untreated and regenerated (never dried after the regeneration) cellulose. Untreated cellulose was suspended in DI water at room temperature overnight before the SEM measurements. The samples were mounted on SEM stubs with carbon tape, vacuum dried and then coated with a thin layer of Au/Pd. All images were obtained at acceleration voltages of 5 kV and magnifications from 100x to 8000x.

For the powder XRD data, the samples were air-dried, ground and sieved with a 0.15 mm screen were pressed on a sample holder. The XRD measurements were carried out using a Rigaku SmartLab X-ray diffractometer with a Cu target X-ray tube operated at 40 kV and 44 mA as the source. The diffraction patterns were taken in a 2θ range between 10° and 30° using steps of 0.1°.

For the enzymatic hydrolysis, untreated cellulose (MCC) was soaked in DI water at 90 °C for 2 h and then cooled down to room temperature before the enzymatic hydrolysis for a more direct comparison with the regenerated cellulose. Untreated and regenerated cellulose samples (0.25 g of each) and penicillin (50 μl at 1 mg ml^{-1}) (to prevent bacterial contamination) were mixed with citrate buffer (pH 5.0, 50 mM) in a 50 ml flask and the final total solid (TS) loading was controlled at 2.5%. The enzyme was added to the mixture at 7.5 FPU g^{-1} cellulose. Enzymatic hydrolysis was carried out at 50 °C and 200 rpm in a Thermo Scientific MaxQ Mini 4450 Shaker (Dubuque, IA). At a predetermined hydrolysis time, 0.5 ml of the hydrolysate was taken out of the hydrolysis flask, mixed with 0.025 ml H_2SO_4 (8%, w/w) to stop the enzymatic hydrolysis and then centrifuged. The supernatant properly diluted with DI water was mixed with fucose (0.1 mg ml^{-1}) as an internal standard and then filtered with a 0.2-μm membrane. A Dionex ICS-5000 (Dionex, Sunnyvale, CA) was used for the analysis of the monosaccharide concentrations. The monosaccharides were separated using a CarboPac® PA10 column (Dionex, Sunnyvale, CA) at 18 °C with DI water as the eluent at a flow rate of 0.9 ml min^{-1}. The cellulose conversion was calculated as the percentage of produced glucose from the enzymatic hydrolysis relative to the cellulose content.

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Notes and references

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34 Drying results in additional changes to the structure/morphology of the cellulose which are not directly reflective of the materials obtained from the regeneration process.

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Table of Contents Entry

The choice of non-solvent used to precipitate cellulose after dissolution in an ionic liquid strongly influence the resulting cellulose structure.