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Easily measurable pH as an indicator of aqueous cholinium ionic liquid pretreatment effectiveness of lignocellulose

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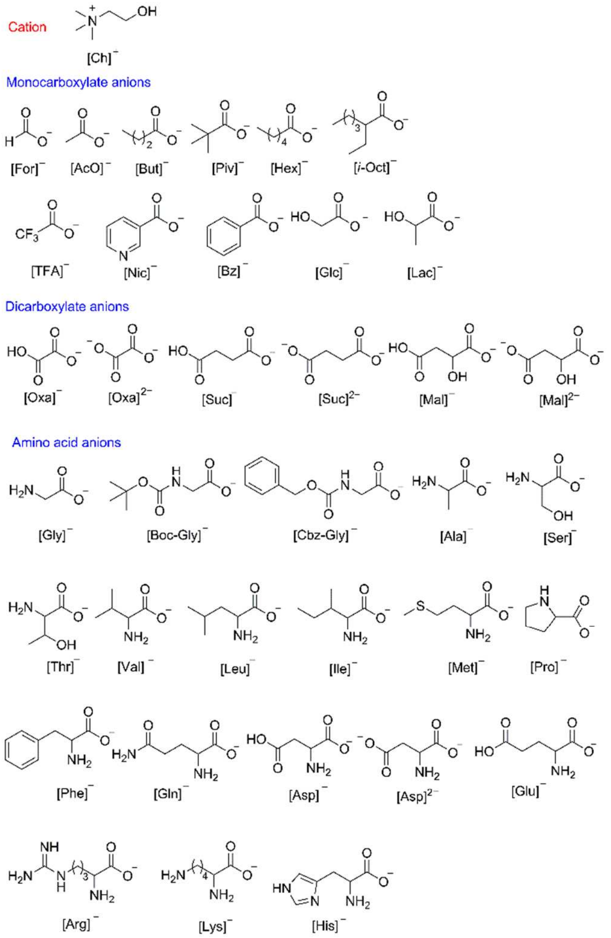
Abstract: Aqueous ionic liquid (IL) solutions have received more interest as promising alternatives to neat ILs for lignocellulose pretreatment recently, due to many advantages. In this work, 39 aqueous cholinium IL solutions were used to pretreat rice straw for improving the subsequent enzymatic saccharification. It was found that there were strong correlations between pH of these aqueous ILs and their delignification capacities, between pH and the polysaccharide digestibility, and between pH and the reducing sugar yields. Lignin of >55% was removed from rice straw after pretreatment with aqueous ILs of > pH 11.0, resulting the high digestibility (88-96% for cellulose and 65-89% for xylan) in the enzymatic saccharification. Establishing such a correlation between pH of aqueous ILs and their pretreatment effectiveness may be useful for the rational design and selection of ILs for lignocellulosic biomass pretreatment.

Keywords: Aqueous ionic liquids; biorefinery; delignification; enzymatic saccharification; lignocellulosic biomass; pretreatment

Introduction

Lignocellulosic biomass composed of cellulose, hemicellulose and lignin is a promising and renewable feedstock for the production of biofuels and chemicals.[1, 2] However, lignocellulosic biomass is very inert to chemical and biological degradation, because of the nature such as high lignification degrees and high cellulose crystallinity as well as the complex hetero-matrix structure. Pretreatment is generally necessary to destroy the inert structure of the biomass for improving the subsequent degradation.[3] Deconstruction of lignocellulosic biomass using ionic liquids (ILs) has attracted considerable interest over the past decade.[4, 5] In spite of significant advances, IL pretreatment of lignocellulosic biomass has many problems such as high cost and high viscosity of these ionic solvents. The addition of water into ILs could reduce their viscosity significantly; as a result, it would not only allow for use of high biomass loading, but also make handling (*e.g.* filtering) easier. Besides, the pretreatment process would be more cost-effective and environmentally friendly due to the replacement of significant quantities of expensive ILs with cheaper and greener water. Unfortunately, water usually exerted a significantly negative effect on IL processing of cellulose and lignocellulose.[6, 7] Recently, several groups reported the successful use of aqueous imidazolium IL solutions for lignocellulose pretreatment by increasing pretreatment severity.[8-10] For example, Zhang et al reported a pretreatment process based on aqueous IL solutions and acid-catalysis at 130 °C, and cellulose digestibility of 94-100% was obtained after the pretreated sugarcane bagasse was hydrolyzed for 72 h.[10]

Establishment of a correlation between easily measurable solvent properties of ILs and their pretreatment effectiveness is useful to advance the rational design and selection of ILs for biomass pretreatment.[11] Previously, Dordick and coworkers suggested that the Kamlet–Taft β parameter was an excellent predictor of IL pretreatment efficacy.[6] Zhao and coworkers reported that the pretreatment efficiencies of ILs could be loosely correlated to the Kamlet–Taft β values of the anions; however, the pretreatment efficacies of aqueous IL solutions could not be simply governed by any single IL property.[12] Recently, our group found that there existed a relationship between the delignification capacity of aqueous cholinium amino acid IL solution and its basicity.[13] Although the Hammett function has been demonstrated to be suitable for characterization of the basicity of the ILs and their aqueous solutions,[13, 14] this method is complex and steps are tediously long. As compared the Hammett function, pH appears to be a simpler characterization method for the acidity and basicity of aqueous solutions. In this work, we studied rice straw pretreatment with aqueous solutions of various cholinium ILs (Scheme 1) and subsequent enzymatic saccharification. It is noteworthy that these ILs are totally composed of renewable biomaterials and have a low environmental impact.[15, 16] The delignification capacities, the polysaccharide digestibility and the reducing sugar yields were correlated with pH of aqueous IL solutions.



Scheme 1 Structures of cholinium ILs

Experimental

Materials

Rice straw obtained locally was mechanically powdered with particle size of 150-200 μm. Cellulase/xylanase from *Trichoderma reesei* (Unit definition: 1 unit will liberate 1.0 μmol of glucose from Sigmacell cellulose Type 20 in 1 h at pH 5.0 and 37 °C) and choline hydroxide aqueous solution (46 wt%) were bought from Sigma-Aldrich (USA) and used as received. L-Amino acids were obtained from Yuanju Biotech. Co. (Shanghai, China). 50% IL aqueous solutions were prepared as described by us recently.[17] Briefly, acids (including various carboxylic acids and amino acids) of equal mole were directly added to 46 wt% choline hydroxide aqueous solution and incubated at room temperature for 2 h. After the addition of a certain amount of water, 50 wt% IL aqueous solutions were afforded.

Pretreatment of rice straw with aqueous IL solutions

Rice straw pretreatment was carried out as described recently.[13] Briefly, 300 mg rice straw samples were incubated under N_2 and 90 °C in 6 g 50 wt% IL solutions and stirring. After 6 h, the suspension was diluted by distilled water of equal volume and centrifuged (17,000 g, 15 min). With the corresponding aqueous IL solution as the control, the supernatant was decanted to a vial for the determination of lignin content at 280 nm with the extinction coefficient of 2.19 L g^{-1} cm^{-1} (Shimadzu UV 2550, Japan) that was obtained based on the slope of linear calibration curve of Kraft lignin.[18] The residues were washed with distilled water (approximately 200 mL) until the supernatant was colorless. Then, the residues were lyophilized and stored in a sealed bag at -20 °C prior to use. Lignin extractability was defined as the ratio of the extracted lignin amount during IL pretreatment to the total lignin amount in the native rice straw. All the experiments have been conducted in duplicate, and the error ranges are less than 5%.

Compositional analysis of rice straw residues

Cellulose, xylan, and lignin contents of rice straw samples were determined according to the standard analytical procedure of the National Renewable Energy Laboratory.[19] Briefly, the samples were treated successively with 72% sulfuric acid at 30 °C for 1 h and 4% acid at 121 °C for 1 h. The sugar contents were determined by HPLC (Waters 515) equipped with a Bio-Rad Aminex HPX-87H column and a refractive index detector (Waters 2410). The mobile phase consisted of 5 mM aqueous H_2SO_4 solution with a flow rate of 0.5 mL min^{-1}. The column temperature was 65 °C. The cellulose and xylan contents were calculated from glucose and xylose contents multiplied by conversion factors of 0.90 and 0.88, respectively. The content of acid-insoluble lignin was determined gravimetrically by using filtering crucibles. The content of acid-soluble lignin was measured spectrophotometrically at 320 nm using the extinction coefficient of 30 L g^{-1} cm^{-1}.[18]

Enzymatic hydrolysis of rice straw residues

Enzymatic hydrolysis was conducted at 50 °C and 200 rpm in 7 mL citrate buffer (50 mM, pH 4.8) containing 20 mg biomass and 35 U mL^{-1} cellulase. Aliquots (200 μL) were withdrawn at specified time intervals, and boiled for 5 min to quench the enzymatic reaction. After centrifugation (18,000 g, 10 min), the glucose and xylose concentrations were measured by HPLC. The HPLC spectrum of the reaction mixture in the enzymatic hydrolysis was shown in Fig. S1 (available as supplementary data). All reactions were performed in duplicate, and the error ranges are less than 5% in all cases.

Cellulose and xylan digestibility were calculated as follows:

$$\text{Polysaccharide digestibility (\%)} = \frac{\text{Released sugar amount} \times \text{conversion factor}}{\text{Polysaccharide amount in the residues hydrolyzed}} \times 100$$

, where the conversion factors are 0.90 and 0.88 for the calculation of cellulose and xylan digestibility, respectively.

Glucose and xylose yields were calculated as follows:

$$\text{Sugar yield (\%)} = \frac{\text{Released sugar amount}}{\text{Theoretic sugar amount in native biomass}} \times 100$$

Results and Discussion

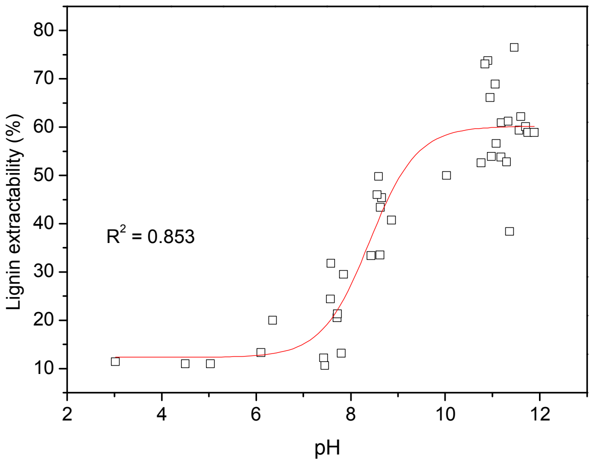


Fig. 1 Correlation between pH of aqueous IL solution and lignin extractability

Thirty-six cholinium ILs were synthesized through simple and green neutralization reactions between choline hydroxide and mono-, dicarboxylic acids and amino acids, with water as the only byproduct. The aqueous solutions of 36 ILs and 3 binary mixtures were used for rice straw pretreatment. The lignin extractability, the recovery and composition of the residues and outcomes of enzymatic digestion of polysaccharides were shown in Table S1 (available as supplementary data), and the time courses of enzymatic hydrolysis of rice straw before and after aqueous IL solution pretreatment were shown in Figs. S2-6 (available as supplementary data). It could be found that pH values of 50% cholinium IL solutions ranged from 3.0 to 12.0 (Fig. 1). Except for 5 ILs, these ILs seemed to be weak bases. In addition, aqueous solutions of most amino acid-based ILs had pH of 11.0-12.0. The delignification abilities of aqueous ILs were attempted to correlate with their pH using a DoseReps fit on OriginPro 8.5. As shown in Fig. 1, there was a clear relationship between lignin extractabilities and pH of IL solutions ($R^2 = 0.85$), especially at pH < 9.0. The delignification capacities of aqueous ILs were comparable, with the range of 11-20%, at pH < 7.0. And the delignification capacities increased sharply with the increment of pH when pH is > 7.0. The results were not surprising, since lignin is a base-soluble biopolymer. It is noteworthy that there is a significant change in the lignin extractabilities (38-77%) at pH 11.0-12.0, indicating that in addition to pH of aqueous IL, the steric hindrance of the IL anion may exert a significant effect on the delignification capacity.

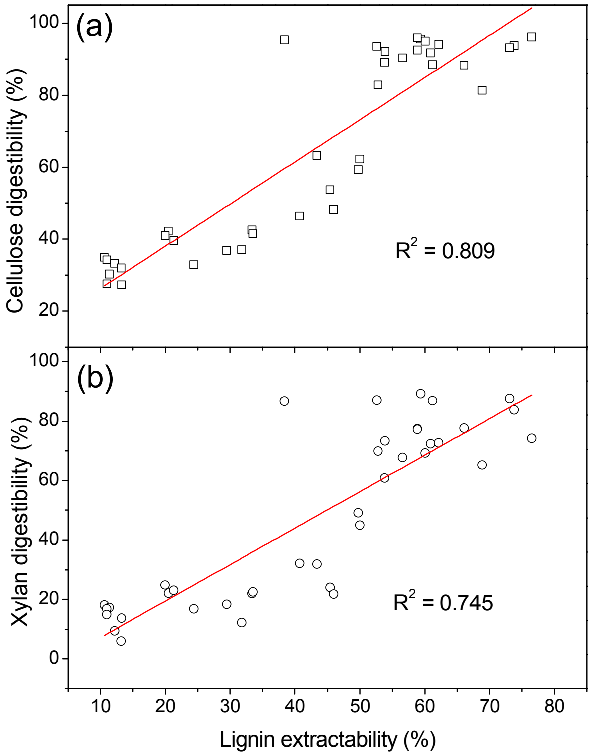


Fig. 2 Correlations between lignin extractability and polysaccharide digestibility

Fig. 2 shows that there are positive linear correlations between lignin extractability and polysaccharide digestibility. Lignin has been known as one of the significant contributors to the recalcitrance of lignocellulosic biomass, since it can limit enzymes access to polysaccharides as a physical barrier or/and by non-productive adsorption.[20, 21]

Recently, we reported that both surface area and pore volume of rice straw would increase significantly with the increment of its delignification degree.[13] Therefore, removal of lignin would result in the increase in the polysaccharide accessibility to enzymes, which may account for the improved enzymatic digestion of polysaccharides. Besides, xylan removal during pretreatment would also make a contribution to the improvements in the subsequent enzymatic saccharification (Fig. S7, available as supplementary data), since xylan is a thermo-, acid-, and base-sensitive biopolymer.[22] In addition to the ability to remove lignin and xylan, the ability of ILs to reduce the cellulose crystalline index (CrI) has been reported to play an important role in the effective pretreatment of lignocellulosic biomass.[23] Table S2 (available as supplementary data) shows that as compared to that of the native, the cellulose CrI of rice straw increases after cholinium IL pretreatment in the absence of water, which is in good agreement with our previous results.[24] It may be attributed to the ability of cholinium ILs to selectively dissolve lignin and xylan; therefore, pretreatment with these ILs would selectively remove amorphous components from rice straw, resulting in neat increases in cellulose CrI of the lignocellulosic materials. In this work, the crystalline structure of cellulose present in rice straw was presumed to change slightly during aqueous cholinium IL pretreatment, because water has a significant negative impact on the dissolution of cellulose in ILs.[6, 7]

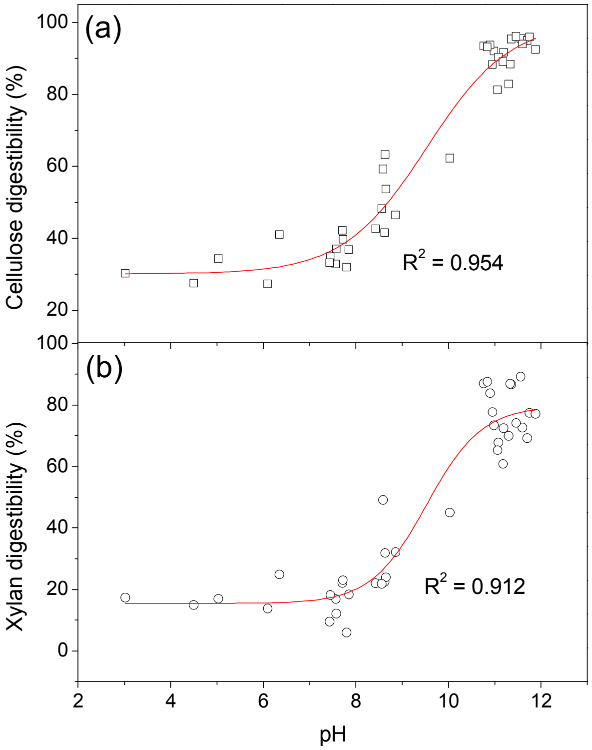


Fig. 3 Correlations between pH of IL solution and polysaccharide digestibility

Based on the above results (Figs. 1 and 2), the polysaccharide digestibility of rice straw pretreated by aqueous IL solutions was correlated to pH of these aqueous IL solutions (Fig. 3). As shown in Fig. 3, pH of aqueous solution of a given IL exhibited clear correlations with the digestibility of cellulose and xylan (R^2 = 0.95 and 0.91, respectively). When pH was less than 8.0, the digestibility of polysaccharides including cellulose and xylan almost kept constant, which may be ascribed to the low lignin and xylan extractabilities (Fig. 1). However, the digestibility of polysaccharides increased markedly with increasing pH when pH > 8.0. And high polysaccharide digestibility was obtained after pretreatment with aqueous IL solutions of pH 11.0-12.0. The results suggest that pH of aqueous IL solution is a good indicator of its efficacy for lignocellulosic biomass pretreatment.

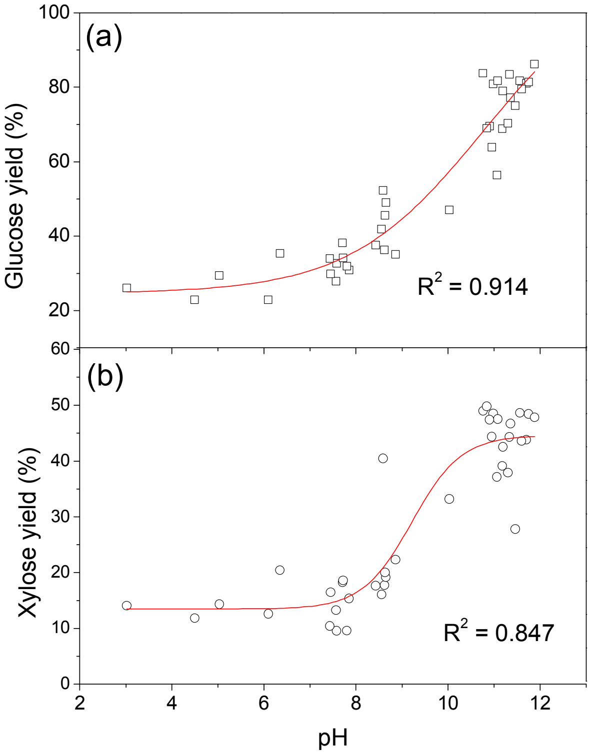


Fig. 4 Correlations between IL solution pH and reducing sugar yield

We also examined the relationships between the reducing sugar yields and pH of aqueous IL solution (Fig. 4). A clear relationship between the glucose yields and pH of aqueous IL solution (R^2 = 0.91) was observed, following a classic dose-response curve. Similarly, the xylose yields also showed a correlation with pH of aqueous IL solution (R^2 = 0.84). As compared to the correlations of pH and polysaccharide digestibility, the correlations between pH and reducing sugar yield appeared to be relatively weaker, which might be attributed to the influence of the losses of polysaccharides because the polysaccharide losses as well as the polysaccharide digestibility was considered in the calculation of the reducing sugar yields.

Conclusions

In summary, readily measurable pH has been demonstrated to be an excellent indicator of aqueous cholinium IL pretreatment efficacy for lignocellulosic biomass. Higher pH value of aqueous IL, more effective rice straw pretreatment will be. Establishment of such a correlation between the readily measurable pH value and the pretreatment efficacy may be useful for the rational design and selection of ILs for lignocellulosic biomass pretreatment. The extensive lignin removal and partial xylan removal from rice straw during pretreatment with aqueous cholinium IL solutions may be responsible for the improved enzymatic saccharification.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at

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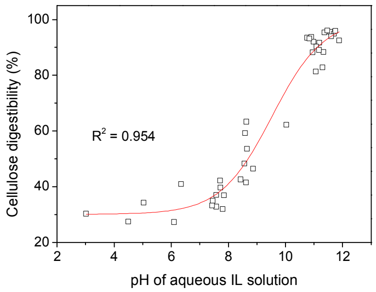
Graphical abstract



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