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Significantly enhanced enzymatic hydrolysis of rice straw via a high-performance two-stage deep eutectic solvents synergistic pretreatment

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HIGHLIGHTS

- Enhanced enzymatic digestion of biomass after two DESs treatment in specific order.
- A good balance of components loss achieved by the synergistic action of the two DESs.
- Water presence facilitated the sugar recovery by tuning the severity.
- Significant xylan removal and degradation during CO participated processes.
- A simple and mild pretreatment process was established with a glucose yield of 90.2%.

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ABSTRACT

A two-stage deep eutectic solvents (DESs) treatment was shown to be an effective method for improving the utilization of certain DESs, and the specific order of pretreatment, such as malic acid/proline (MP) or choline chloride/oxalic acid (CO) during the first stage and choline chloride/urea (CU) during the second stage, resulted in better performance for enhancing the sugar yield due to the synergistic effect of the two DESs on biomass fractionation. Moreover, the presence of water during these processes could balance the loss of components by tuning the pretreatment severity, thus ensuring higher sugar yields. By eliminating the washing step after the first stage treatment, enhanced cellulose recovery and glucose yield were achieved for the CO-CU pretreatment in the presence of 5% water, and a simpler process was established with a glucose yield of 90.2% after a 3-h treatment at 100 °C.

1. Introduction

Lignocellulosic biomass is considered as a promising, renewable and low-cost energy source for reducing the dependence of humans on conventional fossil fuels (Binod et al., 2010; Procentese et al., 2016). However, lignocellulosic biomass is highly recalcitrant to chemical and biological degradation due to its complex heteromatrix structure in which the cellulose is highly crystalline, the hemicellulose circulates around the cellulose, and the lignin occupies the remaining space and prevents exposure of the polysaccharides to water, other chemicals or biocatalysts (Zhao et al., 2012). Thus, to enhance the conversion of lignocellulosic biomass to fermentable sugars or other high-value chemicals, pretreatment is always required (Agbor et al., 2011; Alvira et al., 2010). In the past decade, ionic liquids (ILs) have received considerable attention and have been extensively exploited for biomass pretreatment due to their excellent ability to destroy the crystalline structure of cellulose or remove lignin or hemicellulose, which significantly increases polysaccharide accessibility to enzymes and improves the subsequent enzymatic hydrolysis (Tadesse and Luque, 2011). However, the shortcomings of ILs, such as toxicity, poor biodegradability and high cost, restrict their use in industrial applications, even though efforts have been made to obtain new ILs that are greener and cheaper than the traditional ones (Hou et al., 2012; Pinkert et al., 2011). Recently, deep eutectic solvents (DESs), a class of interesting solvents, have been used as a sustainable alternative to ILs with similar properties but lower cost and high atom economy (Abbott et al., 2004; Zhang et al., 2012). Moreover, some DESs have been shown to be biocompatible towards biocatalysts, such as enzymes, and show promising potential for biocatalysis and biotransformation (Sheldon, 2016; Vigier et al., 2015). Furthermore, some DESs have also displayed the unique ability to dissolve biomass components and pretreat

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biomass, which is an application that is still in its infancy (Kumar et al., 2016; Procentese et al., 2015; Xu et al., 2016). Since Francisco et al., tested the solubility of cellulose, lignin and starch in DESs based on natural materials (Francisco et al., 2012), this novel solvent system has attracted increasing attention in the field of biomass pretreatment. The results from recent studies suggest that certain DESs have an excellent lignin extraction ability to enhance cellulose enzymatic hydrolysis, particularly for the acidic DESs, such as choline chloride (ChCl)/lactic acid. However, for DES-based biomass pretreatment, the number of DESs with excellent pretreatment capacity is limited, and no effort has been exerted to develop better uses of the mild DESs. Generally, organic-acid-based DESs, such as ChCl/lactic acid (Alvarez-Vasco et al., 2016; Kumar et al., 2016; Zhang et al., 2016) and ChCl/formic acid (Xu et al., 2016), perform well with acid/ChCl molar ratios of 2:1 or more for the processing of corncob, rice straw and wood. However, other DESs, such as weakly basic ChCl/urea (CU) and neutral ChCl/glycerol pretreatments, produce only moderate sugar yields (Procentese et al., 2015) or even similar saccharification efficiencies with the untreated sources (Xu et al., 2016). The combination between two methods was reported to achieve a higher cellulose content in the recovered solid fraction and a higher reducing sugar yield than those when employing single method processes (Chen et al., 2012; Qing et al., 2014), and pretreatments combining two methods, or two-stage chemical pretreatments, that always use dilute acid in the first stage have been shown capable of creating synergistic effects for high cellulose enzymatic degradability (Auxenfans et al., 2014; Kim et al., 2011). For example, the saccharification rate was 2-fold higher than that of the single pretreatment observed for the combination pretreatment with ILs and dilute acid in which the dilute acid selectively extracted hemicelluloses and the IL removed lignin (Auxenfans et al., 2014). Therefore, a combination pretreatment is highly likely to serve as a feasible strategy to exploit and reinforce the utilization of the defective DESs in biomass pretreatment.

Thus, in this work, three typical DESs, namely, CU, malic acid/proline (MP) and ChCl/oxalic acid (CO), were selected for rice straw pretreatment. According to the specific properties of these DESs, two-DESs combination pretreatments were used to explore the enhanced use of each DES and to ensure the maximal improvement in biomass fractionation, cellulose recovery and saccharification. In addition, to better understand the pretreatment mechanism, the composition change and hemicellulose degradation were thoroughly investigated. Finally, based on the combination pretreatment, a facile and efficient method was established to fully exploit DESs with different properties to achieve excellent cellulose recovery and glucose yield.

2. Materials and methods

2.1. Reagents

Cellulase/xylanase from *Trichoderma reesei*, xylan from bench wood and Kraft lignin were purchased from Sigma-Aldrich (St. Louis, MO) and were used as received. Microcrystalline cellulose (MCC) was purchased from FMC (Philadelphia, PA). Rice straw, obtained locally, was mechanically powdered to particle sizes of 250–400 μm. The other chemicals used were of the highest purity commercially available.

2.2. DES preparation

ChCl, oxalic acid, malic acid and proline were dried under vacuum at 80 °C for 5 h before use. Three DESs were selected, CU, CO, and MP, in molar ratios of 1:2, 2:1 and 1:3, respectively. The mixture was heated and stirred at a certain temperature in a closed flask until a homogenous colourless solution was formed. The prepared DESs were then stored in a vacuum drying oven before use.

2.3. Solubility of lignin, xylan and MCC in the DESs or DES-water

The solubility of pure lignin, xylan and MCC in the DESs or DES-water was determined according to our previous report with few modifications (Liu et al., 2012). First, 10 mg of the sample was added to a glass vial containing 4 g of each solvent at 120 °C with stirring, and its solubility was visually checked. If the sample was soluble, then another 5 or 10 mg of the sample was added into the solution after it became homogeneous, until the solvent could not dissolve any more material within 24 h. Subsequently, the dissolved cellulose and xylan were regenerated by adding water or ethanol to the mixture, respectively. Then, the mixture was separated by centrifugation, and the supernatant was collected for further sugar and furfural or 5-hydroxymethyl furfural (HMF) detection. The sugars were monitored using high performance liquid chromatography (HPLC) (Waters 2515) equipped with a Bio-Rad Aminex HPX-87H column and a refractive index detector (Waters 2410). The mobile phase was a 5 mmol L^{-1} sulfuric acid aqueous solution, the flow rate was 0.5 mL min^{-1}, and the column and detector temperatures were 65 °C and 50 °C, respectively. Furfural and HMF in the supernatant were detected using HPLC (Shimadzu LC-20AT) equipped with a C-18 column (Agilent, Eclipse XDB-C18) and a diode- array detector (DAD) at 280 nm (Shimadzu, SPD-M20). The mobile phase was acetonitrile/water (15/85, v/v), and the flow rate was 1 mL min^{-1}. The furfural and HMF yields were calculated as follows:

$$\text{Furfural yield }(\%) = \text{Furfural amount in supernatant }(\text{g}) \times 1.375/\text{Total xylan dissolved in the DES or DES-water mixture }(\text{g}) \times 100$$

(where 1.375 is the ratio of the molecular weight of $C_5H_8O_4$ to $C_5H_4O_2$ (Chen et al., 2012)).

$$\text{HMF yield }(\%) = \text{HMF amount in supernatant} \times 1.286/\text{Total MCC dissolved in DES or DES-water mixture} \times 100$$

(where 1.286 is the ratio of the molecular weight of $C_6H_{10}O_5$ to $C_6H_6O_3$ (Chen et al., 2012)).

2.4. Pretreatment of rice straw with the DES

Rice straw pretreatment was conducted as described in our previous work with slight modifications (Hou et al., 2012). For a single pretreatment process, rice straw samples were incubated in DES systems with a biomass loading of 5 wt%, stirred and refluxed at 120 °C for a specific time. Then, to avoid precipitation of the extracted hemicellulose and to facilitate the separation of the highly viscous pretreatment mixture, the mixture was diluted in hot water (not ethanol) and centrifuged (17,000*g*, 15 min). The supernatant was collected and used for sugar, furfural and HMF analysis by HPLC, and the residues were washed with distilled water until the pH of the supernatant was neutral. Then, the residues were lyophilized and stored in a sealed bag at −20 °C prior to use. With respect to the two-stage combination pretreatment, 300 mg of rice straw was first mixed with 6 g of either DES or DES-water and then stirred and refluxed at 120 °C for half of the total pretreatment time. After being washed with distilled water until the pH of the supernatant was neutral, the residues were lyophilized and then subjected to the second stage of pretreatment in which the residues were treated with the same conditions as those