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Effect of constituents molar ratios of deep eutectic solvents on rice straw fractionation efficiency and the micro-mechanism investigation

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ABSTRACT

Effective obtaining fermentable sugars and other platform chemicals from rice straw mediated by choline chloride- oxalic acid dihydrate (CO) and the corresponding micro-mechanism investigation were conducted in this work. Choosing a suitable constituents molar ratio of CO appeared to be a feasible method to achieve an appropriate pretreatment severity for a good biomass fractionation. Pretreatment by using CO with high oxalic acid dihydrate to choline chloride molar ratio could afford easily digestible cellulose-rich materials (CMRs, > 80% of enzymatic digestion) and lignin-rich materials (LRMs) of high purity (> 82%) due to extensive hemicellulose removal (> 93%) and delignification (around 70%). Cell wall microstructure characterizations based on confocal laser scanning microscopy (CLSM) and transmission electron microscopy (TEM) verified the stronger xylan and lignin removal ability of CO with higher oxalic acid dihydrate content. Moreover, the considerable removal of xylan confirmed by CLSM was beneficial to delignification and the subsequent cellulose enzymatic hydrolysis. Furthermore, ultra-structural changes of lignin distribution in cell walls based on TEM indicated the occurrence of partial delignification, preferentially in cell corner (CC) and compound middle lumen (CML) rather than in the secondary cell walls. These findings provides a new insight into the detailed mechanism of acidic DESs mediated biomass deconstruction.

1. Introduction

Lignocellulosic biomass from agriculture wastes such as rice straw is a promising feed stock for economic and sustainable production of liquid biofuels and derivative chemicals in large scale (Procentese et al., 2016). Cellulose, hemicellulose and lignin are the three main components of the lignocellulosic biomass, and could be converted to the corresponding chemicals (Gavrilescu, 2014). However, the inherent complex structure of biomass makes the main components recalcitrant to remove or degrade (Zhao et al., 2012). Consequently, efficient fractionation of the lignocellulosic biomass for comprehensive utilization of its main constituents avoiding lots of waste of resources is an challenge (Agbor et al., 2011). Since the pioneering work by Rogers et al. (Swatloski et al., 2002), ionic liquids (ILs) have been extensively explored in the field of biomass processing for their many advantages over the traditional acid-, base- or organic solvent- based techniques (Ninomiya et al., 2017; Tadesse and Luque, 2011; Xu et al., 2017). And efforts have been made on renewable ILs exploration for selective delignification of biomass (An et al., 2015; Hou et al., 2012). However, the high cost and environmentally unfriendly process of common ILs preparation restricted their future large-scale application. Recently, deep eutectic solvents (DESs) have emerged as alternatives to ILs in biomass deconstruction due to their similar merits and cheaper and greener comparing with traditional ILs (Vigier et al., 2015). And some DESs were successfully used to dissolve biomass components and pretreat various biomass (Fang et al., 2017; Kumar et al., 2016; Procentese et al., 2015; Zulkefli et al., 2017), and results from more recent studies suggested that DESs like choline chloride- oxalic acid dihydrate (CO), displayed a good performance in pretreating biomass (Liu et al., 2017; Zhang et al., 2016). More specifically, CO extracted 98.5% of lignin from corncob (Zhang et al., 2016), and microwave-assisted CO treatment of wood was confirmed to be an efficient approach to break the lignin-carbohydrate complexes (LCCs) linkage in wood and extract lignin with high purity (Liu et al., 2017). Actually, CO (1:1) has been reported to be a good catalyst and solvent for conversion of xylose or xylan into furfural with high yields (Zhang et al., 2014). Besides, in our earlier study (Hou et al., 2017), this acidic DES exhibited more excellent hemicellulose removal and conversion performance than delignification from rice straw, thus a clear relationship between xylan removal and cellulose digestibility was further established in our more recent work (Hou et al., 2018). Other acidic DESs such as choline chloride (ChCl) − double hydrogen bond donors (formic acid and acetic

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acid) pretreatment of rice straw at 130 °C also proved to be able to remove more hemicelluloses (60.1%) than lignin (43.6%) (Xing et al., 2018). However, the detailed effect of CO constituents ratios on biomass fractionation efficiency particularly for the conversion of hemicellulose and the recovery of lignin is limited. In addition, previous studies used transmission electron microscopy (TEM) or confocal laser scanning microscopy (CLSM) coupled with immunolabeling techniques to understand the mechanism of dilute acid pretreatment (Brunecky et al., 2009), hydrothermal pretreatment (DeMartini et al., 2011; Ma et al., 2015) and ionic liquids pretreatment (Hou et al., 2013b) based on the changes of xylan or lignin distribution in cell wall during the processes. Nevertheless, to the best of our knowledge, little information is available about DESs pretreatment mechanism investigation at cellular and ultra-structural level except for the delignification mechanism study based on chemical structure analysis (Liu et al., 2017).

In present work, CO of different constituents ratios were applied for rice straw pretreatment to evaluate their effect on biomass fractionation and the subsequent enzymatic hydrolysis of cellulose. Furthermore, xylan and lignin distributions in cell wall after treatment by using CO of different constituents ratios were compared by TEM and CLSM combined with immunolabeling technique to gain a better understanding of DES pretreatment mechanism at cellular level.

2. Materials and methods

2.1. Reagents

Cellulase/xylanase from *Trichoderma reesei* ATCC 26921 (Lot # C8546), Xylan from beechwood and Kraft lignin were purchased from Sigma-Aldrich (St. Louis, MO, USA) and used as received. LM11 antibody (PlantProbes, Leeds, UK) and anti-rat IgG Alexa Fluor 488 (Invitrogen, Carlsbad, CA) were used as received. Choline chloride (98%) and oxalic acid dihydrate were purchased from Aladdin Biochemical Technology Co., Ltd. (Shanghai, China). Rice straw, obtained locally, was mechanically powdered to particle sizes of 250–600 μm, and it contains cellulose (34.2 ± 0.5%), xylan (19.6 ± 0.2%), anabinan (3.1 ± 0.2%), acid insoluble lignin (AIL, 20.4 ± 0.3%), acid soluble lignin (ASL,1.51 ± 0.04%), acetyl groups (2.8 ± 0.2%), protein (2.9 ± 0.2%), ash (10.7 ± 0.4%) and moisture (4.6 ± 0.1%). Other chemicals were of the highest purity commercially available.

2.2. DESs preparation

Choline chloride (ChCl) and oxalic acid dihydrate were mixed in different molar ratios, and the mixture was heated and stirred at 80 °C in a closed flask until the homogenous colourless solution was formed. Then, the mixture was cooled down to room temperature to store in a sealed vial before use.

2.3. Solubility of pure lignin and xylan in CO of different constituents molar ratios

The solubility of pure lignin and xylan in CO of different constituents molar ratios were determined according to previous report with few modifications (Liu et al., 2012). First, 10 mg sample was added to a glass vial containing 4 g CO at 90 °C with stirring. If it was soluble, another 5 or 10 mg sample was added into the solution after it became homogeneous until the solvent could not dissolve any more material within 24 h. All data recorded were the average values of duplicates with standard derivates.

2.4. Rice straw pretreatment and fractionation using CO of different constituents molar ratios

Rice straw pretreatment was conducted as described previously (Hou et al., 2017), and the procedures were depicted in Fig. 1. Briefly, the rice straw was mixed with CO (ChCl: oxalic acid dihydrate molar ratios were 2:1, 1:2 and 1:2) in a biomass loading of 5 wt%, and the mixture was stirred and refluxed at 90 °C for 3 h or 120 °C for 1 h. Then, ethanol was added into the pretreatment mixture to regenerate polysaccharides keeping most of the extracted lignin remaining in the liquids, then the mixture was thoroughly separated by centrifugation. The solid fraction of the pretreatment mixture was washed with ethanol and then water, then lyophilized and recovered as cellulose-rich materials (CRMs) prior to compositional analysis and enzymatic hydrolysis. The washings was condensed by evaporation and combined with the liquid fraction of the pretreatment mixture for recovery of extracted lignin. Two volumes of water was added into the solution to regenerate lignin, followed by stirring at 4 °C for 4 h. Then the precipitate was collected by centrifugation, and then washed with water until the washings was neutral and lyophilized as lignin-rich materials (LRMs). The remaining supernatant together with condensed washings was adjusted to the pH value of 6.8 and precipitated with three volume of 96% ethanol under continuous stirring (da Costa Lopes et al., 2013), then the precipitate was obtained as hemicellulose-rich materials (HRMs) after centrifugating, washing with 96% ethanol and drying at 70 °C in oven. The remaining supernatant combined with the condensed washings of HRMs was collected and used for xylose and furfural determination as well as DES recovery. All experiments were made at least in duplicates, and all data (fraction recovery yields) were shown as average values with standard deviations.

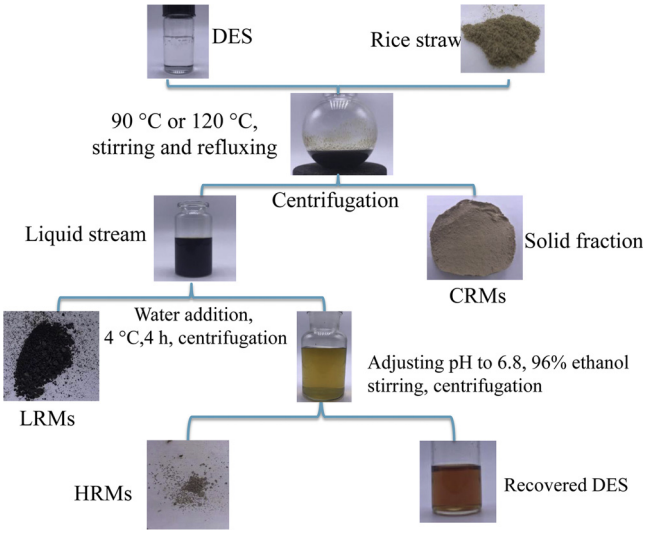

Fig. 1. The rice straw fractionation procedures.

2.5. Composition analysis of CRMs, LRMs and the liquid stream

The cellulose, xylan, and lignin contents of the solid samples were determined by standard NREL analytical procedure (Sluiter et al., 2008). Xylose and furfural in the liquid stream were also detected by HPLC. Sugars were monitored using HPLC (Waters 2515) equipped with a Bio-Rad Aminex HPX-87H column and a refractive index detector (Waters 2414). 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 was detected using an HPLC (Shimadzu LC-20AT) equipped with a C-18 column (Agilent, Eclipse XDB-C18) and a DAD detector 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}. All composition analysis experiments were conducted at least in duplicates, and all data were shown as average values (standard deviations less than 5%). The related values were calculated as follows: