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Enhanced enzymatic hydrolysis of eucalyptus by synergy of zinc chloride hydrate pretreatment and bovine serum albumin

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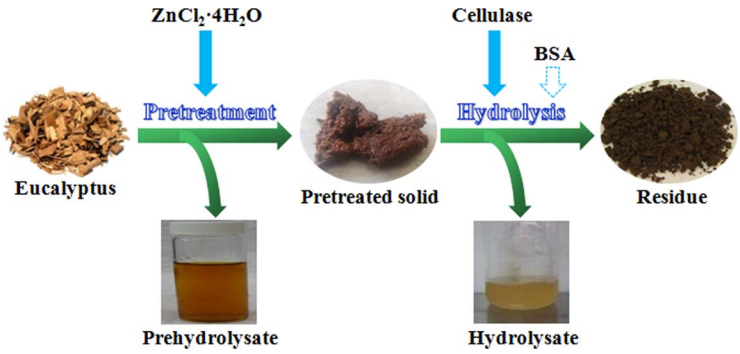
GRAPHICAL ABSTRACT



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ABSTRACT

Enhancement of eucalyptus enzymatic saccharification by synergy of $ZnCl_2$ hydrate pretreatment and bovine serum albumin (BSA) was investigated in this study. The result showed that the $ZnCl_2$ hydrate pretreatment could not only selectively extract up to ~100% of the hemicellulose from eucalyptus, but also convert portion of high crystalline cellulose I into low crystalline cellulose II, which both beneficial for enhancing subsequent pretreated solids enzymatic saccharification. The addition of BSA into enzymatic hydrolysis step could significantly promote the glucose release from pretreated solids, especially, under the low enzyme loading. Furthermore, the material balance indicated that the highest glucose yield of this study was 35.5 g/100 g raw material, which representing 90.3% of glucose in raw eucalyptus, combined with the xylose yield, 13.9 g/100 g eucalyptus, it can be concluded that $ZnCl_2$ hydrate pretreatment offered the potential to co-produce xylose and glucose from eucalyptus.

1. Introduction

Due to a lack of resource sustainability of fossil fuels as well as negative environmental effects from emissions, more and more research efforts focus on the lignocelluloses conversion into various durable fuels and chemicals (Climent et al., 2014; Zhou et al., 2011). In fact, lignocelluloses are one of the most abundant and low cost materials in the world, and have been considered as the ideal feedstock to produce bio-based ethanol or chemicals (Xu et al., 2016; Wei and Wu, 2016). However, the intrinsic three-dimensional cell-wall structure of lignocelluloses composed of cellulose microfibril aggregates linked with a lignin and hemicellulose matrix makes it difficult for using this material to produce bio-ethanol or chemicals directly (Seo et al., 2011). For this reason, an efficient pretreatment technology is necessary to break down

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the polymeric structures of lignocelluloses and enhance the accessibility of enzyme to solid substrate during enzymatic hydrolysis step.

A large number of pretreatment approaches including dilute acid pretreatment, alkaline pretreatment, hydrothermal pretreatment, ionic liquid, and organosolv pretreatment combined with various severe conditions such as high temperature, pressure, and dosage of chemicals have been studied in recent years (Wei et al., 2012; Qing et al., 2017; Zhuang et al., 2016; Yuan et al., 2017; Kamoldeen et al., 2017). These pretreatment categories do have the effect for enhancing lignocelluloses conversion to fermentable sugars, but they have their own disadvantages. For example, it is difficult to separate and recover the degradation products of lignin and hemicellulose during alkaline pretreatment. Dilute acid pretreatment requires corrosion-resistance equipment. Hydrothermal pretreatment, has the capacity to remove most of hemicellulose, but the insoluble solids resulting from pretreatment are not very good for subsequent enzymatic hydrolysis because of the high residual of lignin. Organosolv pretreatment needs to consume a lot of organic solvent and is not economically feasible. $ZnCl_2$ hydrate, which has a water to salt molar ratio equal or less the coordination number of the cation, was proved to be a reaction medium can effectively reduce the reaction temperature for cellulose processing in previous study (Lu and Shen, 2011; Wilcox et al., 2015). Besides, $ZnCl_2$ hydrate also makes cellulose less crystallinity and weaker intermolecular or intra-molecular hydrogen bond network via the interaction between the Zn^{2+} and hydroxyl groups in cellulose (Sen et al., 2016; Xiong et al., 2016), which were both beneficial for cellulase accessibility. To our knowledge, $ZnCl_2$ hydrate as a medium for lignocelluloses pretreatment has never been reported in previous studies.

In recent years, several researches indicated that the addition of additives such as non-catalytic proteins (BSA), non-ionic surfactants (Tween 80), and polymers (polyethylene glycol, PEG) into the enzymatic hydrolysis step could effectively increase the conversion of lignocelluloses to fermentable sugars (Eriksson et al., 2002; Li et al., 2016a; Monschein et al., 2014). Li et al. (2015) reported that the glucose and xylose yields in enzymatic hydrolysate were obviously enhanced by the addition of various additives such as BSA, PEG 6000, and Tween 80 into aqueous ammonia pretreated bamboo. Zhang et al. (2017) reported that with the addition of BSA, or Tween 80 into $FeCl_3$-pretreated sugarcane bagasse, the glucose yields after 72 h hydrolysis increased by 9.0, and 5.0%, respectively. Several mechanisms have been employed to explain the influence of additives on enzymatic hydrolysis performance (Yang and Wyman, 2006; Li et al., 2016b). Non-catalytic proteins generally having a great attraction for lignin and lignin containing substrate, and could effectively reduce unproductive cellulase adsorption to lignin, which finally resulting more enzymes available for hydrolysis. Polymers and non-ionic surfactants can promote enzymatic hydrolysis of lignocelluloses probably by increasing cellulase activity and stability, changing the substrate structure to make it more accessible to enzymes.

In this study, an effective lignocelluloses pretreatment technology was proposed by using $ZnCl_2$ hydrate, and successfully used for eucalyptus pretreatment at low temperature. The influence of $ZnCl_2$ hydrate on the composition of various pretreated solids, liquids, as well as subsequent enzymatic hydrolysate were investigated. Besides, Analytical techniques such as FT-IR and XRD were used for analysis the changes of functional groups and crystallinity of eucalyptus after $ZnCl_2$ hydrate pretreatment, respectively. In addition, the influence of additive and enzyme loadings on optimal pretreated solid enzymatic hydrolysis were also studied and compared. This work aims to provide information on novel and effective lignocelluloses pretreatment technology by using $ZnCl_2$ hydrate, and give insight into the mutual behavior of the $ZnCl_2$ hydrate and the additive on eucalyptus enzymatic saccharification.

2. Materials and methods

2.1. Materials

Eucalyptus used in this study was obtained from a pulp mill in Guangzhou, China, and milled to less than 1 mm before further use. The contents of cellulose, hemicellulose, and lignin in non-pretreated and pretreated eucalyptus samples were determined based on US National Renewable Energy Laboratory Analytical Procedure (Sluiter et al., 2008).

All the chemicals and reagents used in this research were purchased from Sigma-Aldrich, except for the cellulase, which was supplied by Novozyme, China, and was measured to have an activity of 120 FPU/mL (DNS method). All purchased chemicals and reagents were used as received without further purification.

2.2. Pretreatment

Experiments for eucalyptus $ZnCl_2$ hydrate pretreatment were performed in a 100 mL glass vial. In brief, a certain amount of anhydrous $ZnCl_2$ was firstly dissolved into deionized water to obtain $ZnCl_2$ hydrate ($ZnCl_2{\cdot}RH_2O$, with R = 4.0). Then the above $ZnCl_2$ solution (60 mL) was mixed with a mass of eucalyptus (6.0 g), and heated to the designed temperature within 10–15 min. The temperature was controlled with ± 1 °C of the set value via an oil bath and the agitation speed was fixed at 300 rpm. Since the boiling point of the $ZnCl_2$ hydrate is higher than the reaction temperature used in this study, there was no pressure build up. This was why the reaction can be conducted in a simple glass vial. At the end of the reaction, the vial was rapidly removed from the oil bath and cooled down in ice bath. The mixtures were separated by centrifuge, and the supernatants were analyzed by ion chromatography (IC) and high performance liquid chromatography (HPLC) to determine the concentrations of sugars and sugar-derived products, respectively. The insoluble solid fraction were collected, thoroughly washed with excess deionized water, and oven-dried for next analysis or hydrolysis.

2.3. Enzymatic hydrolysis

Enzymatic hydrolysis of eucalyptus raw material and various pretreated solids were carried out in an acetate buffer solution (0.05 mol/L, pH 4.8) with 2% dry matter (w/v) at 50 °C for 6, 12, 24, 48, and 72 h at the enzyme dosage of 20 FPU/g dry substrate. The speed of the shaker was set at 200 rpm. Besides, two drops of acetic ether were also added to avoid microorganisms. When the enzymatic hydrolysis reached the prescribed time, the mixtures were separated by centrifugation, and the supernatants were collected and analyzed by IC to fix the released glucose, the enzymatic hydrolysis residues were dried and stored for next analysis. Similarly, the enzymatic hydrolysis of optimal pretreated solid by synergy of BSA was also carried out as the above system with the cellulase loading of 5, 10, and 20 FPU/g dry substrate for 24, 48, and 72 h, respectively. The BSA dosage for above hydrolysis experiments were 100 mg/g dry substrate.

2.4. Analytical methods

Sugar products, such as glucose and xylose, were quantified by a IC system with an electrochemical detector and a CarboPac PA20 column at 30 °C eluted by water/200 mmol L^{-1} NaOH gradient at a flow rate of 0.5 ml min^{-1} (Wei et al., 2017). Inhibitors including HMF, furfural, and acetic acid were analyzed by a HPLC system which equipped with an UV-detector and a XBridge C_{18} column at 35 °C. The mobile phase was an acetonitrile: phosphoric acid-sodium dihydrogen phosphate buffer solution (pH 2.6) = 1:9 (v/v) with a flow rate of 0.6 ml min^{-1} (Wei and Wu, 2017). Oligosaccharides, such as gluco-oligomers and xylo-oligomers in the prehydrolysate, were back-calculated after a secondary hydrolysis into monomer sugars with 4% sulfuric acid (Wei et al.,