



Effects of ferric chloride pretreatment and surfactants on the sugar production from sugarcane bagasse

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ABSTRACT

An efficient pretreatment with various concentrations of FeCl₃ (0.005–0.2 mol/L) was developed to extract hemicellulose in sugarcane bagasse and enhance the enzymatic hydrolysis of cellulose in pretreated solids. It was found that 0.025 mol/L FeCl₃ pretreated substrate yielded a high glucose yield of 80.1% during enzymatic hydrolysis. Then the characterization of raw material and pretreated solids was carried out to better understand how hemicellulose removal affected subsequent enzymatic hydrolysis. In addition, Tween 80 and Bovine Serum Albumin (BSA) were added to promote enzymatic hydrolysis of pretreated substrate. Together with that obtained from pretreatment, the highest glucose yield reached 97.7% with addition of Tween 80, meanwhile, a reduction of 50% loading of enzyme yielded the same level of glucose. However, the increased yields with additives decreased gradually as the hydrolysis time was extended. Furthermore, the enhancement mechanisms of Tween 80 and BSA were determined.

1. Introduction

With the increasing depletion of fossil fuels and growing concerns about environment, interest in the search for sustainable fuels derived from lignocellulosic biomass has been increasing recently. Among the lignocellulosic biomass, agricultural and agro-industrial residues are targeted for extensive research because of abundance, easy accessibility, and low-cost (Manfredi et al., 2018). Sugarcane bagasse, solid residue after extraction of sugarcane juice, is one of the major agricultural residues in southern China (Batalha et al., 2015). Generally, most of them were used for producing paper pulp or burning, which were unsustainable and not friendly to the environment. The composition of cellulose, hemicellulose, and lignin in sugarcane bagasse could be used to produce biofuels, bio-chemicals, and bio-materials (Zhang et al., 2013). However, the highly heterogeneous material is resistant to be broken down into fermentable sugars. Hence, a pretreatment is needed to destroy the complex structure and improve the enzymatic hydrolysis of sugarcane bagasse (da Silva Martins et al., 2015).

Various pretreatment methods have been reported for efficient lignocellulosic biomass conversion to fermentable sugars, such as acidic pretreatment, alkali pretreatment, organosolv pretreatment, and ionic liquid pretreatment (Sun et al., 2016; Li et al., 2010). Among them,

acidic pretreatment is favored by its advantages, such as low cost, easy recovery, and yielding digestible pretreated solids. And acidic pretreatment using Lewis acids had attracted more and more attention. Recently, Kamireddy et al. (2013) investigated the effect of FeCl₃, CuCl₂, and AlCl₃ on the pretreatment and enzymatic hydrolysis of corn stover, suggesting that their enzymatic digestibility were higher than that pretreated with H₂SO₄ at the same reaction condition. The influence of five different inorganic salts (NaCl, KCl, CaCl₂, ZnCl₂, FeCl₃) on the pretreatment of *Miscanthus* straw was compared, the results indicated that FeCl₃ pretreatment presented the best influence on enzymatic digestibility (71.6%), which acting as a Lewis acid (metal cations), removed 100% of xylan and facilitated the breakdown of the complexes between enzyme and lignin by forming lignin-metal complex, thus improving the enzymatic hydrolysis (Kang et al., 2013). However, NaCl, KCl, CaCl₂ were monovalent alkali metal chlorides, their ability to form lignin-metal complexes is weak, which could not greatly enhance the degradation of hemicellulose and enzymatic hydrolysis (Moodley and Kana, 2017). Chen et al. (2014a) reported that sugarcane bagasse pretreated with 0.1 mol/L FeCl₃ yielded the enzymatic digestibility of 100%. As a good capability of electron acceptor, Fe(III) could coordinate to the oxygen atoms of carbohydrates without protons loss from the hydroxyl groups in the ligand, enhancing the

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removal of hemicellulose during FeCl_3 pretreatment (Wang et al., 2013).

Wang et al. (2013) reported the combination of fungal and FeCl_3 treatment on the enzymatic hydrolysis of poplar wood, yielding 95.4% of reducing sugars with enzyme loading of 45 FPU cellulase and 15 IU β -glucosidase per g substrate. The high loading of cellulase significantly boosted the cost of process, presenting a key barrier to economically viable lignocellulosic biofuels (Mackenzie and Francis, 2014). The cost associated with enzymatic hydrolysis could be reduced by increasing hydrolysis yields, recycling enzyme, or using low enzyme loading. And a productive strategy by addition of additives to increase the hydrolysis yields with existing enzymes was proposed. These additives included surfactants like Tween, non-catalytic proteins such as BSA, and PEG-based polymers. 21 kinds of surfactants were evaluated by Agrawal et al. (2017) to determine the impact on saccharification of pretreated biomass, suggesting that surfactant supplementation was an effective strategy to achieve higher yield of saccharification. Jin et al. (2016) found that saccharification yield of Common reed (*P. australis*) pretreated by steam explosion and dilute acid increased by 88% and 20%, respectively with 1% dosage of Tween 80. Li et al. (2015) investigated the influence of additives (BSA, PEG 6000, and Tween 80) on enzymatic hydrolysis of bamboo shoot and mature bamboo fractions, indicating that additives showed great potential for reducing cellulase dosage. The addition of additives had been proven to enhance the enzymatic hydrolysis of pretreated biomass by reducing unproductive enzyme binding, increasing cellulase activity and stability, or intensifying positive interactions between substrate and enzyme (Rocha-Martin et al., 2017; Yang and Wyman, 2006; Kumar et al., 2012). Although, these studies suggested that surfactants improved the enzymatic hydrolysis, more efforts are still required to understand the mechanism of action of surfactants for improved saccharification especially on diversity of available substrate and also with different enzyme loadings and hydrolysis time.

Though previous literatures reported the positive influence of additives on the enzymatic hydrolysis of lignocellulosic biomass, systematic studies accounting for the influence of additives with decreased enzyme loading, hydrolysis time, and their potential mechanisms were scarce. In this study, an efficient pretreatment of sugarcane bagasse was proposed using FeCl_3 (0.025–0.2 mol/L) at 160 °C with 10 min. The influence of FeCl_3 concentration on the pretreated substrates, pretreatment liquor, and enzymatic hydrolysis was investigated. The modifications of pretreated sugarcane bagasse were determined by X-ray diffraction (XRD), scanning electron microscope (SEM), and thermogravimetric (TG) analysis. Furthermore, the influence of BSA and Tween 80 on the enzymatic hydrolysis of FeCl_3 pretreated solids with various enzyme loading was investigated. In addition, the enzymatic hydrolysis of different substrates (pretreated solid and microcrystalline cellulose) in the presence of additives was compared to understand the acting mechanisms, gaining knowledge to apply and improve enzymatic hydrolysis.

2. Material and methods

2.1. Material

The sugarcane (*Saccharum L.*, Yue 00-236) grown in wengyuan, Shaoguan, China (24° 18' 29" N, 114° 0' 42" E) and harvested in 2015. After extracting the juice and washing, sugarcane bagasse were provided by maoyuan sugar mill located in Shaoguan, China. Then, they were milled to pass through a 20-mesh size screen (< 0.85 mm) using a MiniMill (MF10, IKA, Guangzhou, China), and stored for further study. The raw material were air-dried (9.2% of moisture) and the chemical composition was determined to be 40.5 ± 1.1% glucan, 22.3 ± 0.6% xylan, 3.1 ± 0.2% arabinan, 1.4 ± 0.2% galactan, 21.7 ± 0.8% acid insoluble lignin (AIL), and 2.8 ± 0.3% acid soluble lignin (ASL) using the methods provided by the National Renewable Energy Laboratory

(NREL), which is operated by the Midwest Research Institute for the Department Of Energy (Golden, Co, USA) (Sluiter et al., 2008).

Cellulase (Novozyme Cellic® CTec2, Tianjin, China) was measured to have a cellulolytic activity of 120 FPU/mL according to the DNS method described by Ghose (1987).

2.2. Pretreatment and enzymatic hydrolysis

The pretreatment of sugarcane bagasse was tested in a 1 L cylindrical stainless steel reaction vessel (Parr 4566, Moline, Illinois, USA). 35 g dry biomass was added in 350 mL water or FeCl_3 solutions (0.005, 0.01, 0.025, 0.05, 0.1, 0.15, 0.2 mol/L), and the reaction temperature and time were 160 °C and 10 min based on our previous study (Zhang et al., 2017). It took about 30 min to reach the desired temperature (160 °C). Reaction time of 10 min was recorded when the temperature was 160 °C. At the end of reaction, the reactor was immediately removed from the heater and cooled down by iced water. And it took about 20 min to decrease the temperature to below 40 °C. Then, the supernatant was removed by filtration. After that, the solid was washed with water to remove FeCl_3 .

Following pretreatment, enzymatic hydrolysis was run at 2% (w/v, solid/liquid) for 72 h with an enzyme loading of 20 FPU/g dry substrate at 50 °C with shaking at 150 rpm (Zhang et al., 2017). When the hydrolysis time reached 6, 12, 24, 48, and 72 h, 500 μ L of liquid were taken from the reaction mixture and centrifuged for 5 min at 10,000 rpm to detect the released glucose. To investigate the influence of additives on enzymatic hydrolysis, Tween® 80 or BSA was added at a loading of 150 mg/g dry pretreated biomass (Alftren and Hobley, 2014). Before the addition of cellulase, the additives were incubated with substrate for 30 min for complete interaction between substrate and additives. When cellulase was added into the mixture, time started to be recorded.

2.3. Analytical methods

An ion chromatography (IC) system (DIONEX ICS-3000, USA) with a Dionex™ CarboPac™ PA20 column and an integrated amperometric detector was used to determine and quantify the amounts of sugars obtained from the compositional analysis of raw material and pretreated solids, from the pretreatment liquor, and from the enzymatic hydrolysis of these materials. The eluate was NaOH/H₂O at a flow rate of 0.5 mL/min. For analysis of the inhibitors (5-hydroxymethylfurfural (HMF), furfural, and acetic acid) in pretreatment liquor, High Performance Liquid Chromatography (HPLC) system (Waters e2695, Massachusetts, USA) equipped with a XBridge Amide column (Waters) was used at 35 °C. The mobile phase was acetonitrile and phosphoric acid-sodium dihydrogen phosphate buffer solution (v/v = 1/9) at a flow rate of 0.6 mL/min (Zhang et al., 2017). The glucose yield and the increased yield of glucose by additives were calculated according to the following equations, respectively:

$$\text{Glucose Yield (\%)} = \frac{\text{Glucose produced in enzymatic hydrolysis}}{\text{Glucan amount in raw material} * 1.11} \times 100\%$$

$$\begin{aligned} \text{Increased Yield (\%)} &= \frac{\text{Glucose yield with surfactant} - \text{glucose yield without surfactant}}{\text{Glucose yield without surfactant}} \\ &\times 100\% \end{aligned}$$

2.4. Crystallinity analysis

The crystallinity of raw material and pretreated solids was determined by XRD using a Bruker D8-ADVANCE (Karlsruhe, Germany) with Ni-filtered and Cu radiation ($k = 0.15418$ nm). The scattering angles (2θ) were ranged from 5° to 60° in steps of 0.04° at time intervals of 0.2 s. The Segal method was used to determine the crystalline index

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