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Preliminary exploration on pretreatment with metal chlorides and enzymatic hydrolysis of bagasse

*Liheng Chen, Rong Chen, Shiyu Fu**

State Key Laboratory of Pulp and Paper Engineering, South China University of Technology, Guangzhou 510640, China

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ABSTRACT

Converting biomass to fermentable sugar is the critical step in the biomass refinery. Moreover, pretreatment of biomass plays an important role in improving the conversion of biomass to sugar. In this study, sugarcane bagasse was pretreated by metal chloride Lewis acids (0.1 mol L^{-3} $CrCl_3$, $FeCl_3$, $FeCl_2$, $ZnCl_2$ and $AlCl_3$ solution) for cellulase hydrolysis. The effects of pretreatments on the yield, chemical components, and sequential cellulase hydrolysis of pretreated bagasse were investigated. The results indicated that metal chlorides with different pKa values could efficiently remove the hemicellulose in bagasse during pretreatment. Furthermore, an inhibition factor (IF) quantitatively reflecting difficulty of cellulase hydrolysis was proposed. The low IF means the facile cellulase hydrolysis. The IF of Fe (III)-pretreated bagasse could decrease to 1.35. In this case, the enzymatic digestibility of bagasse approached to 100%.

1. Introduction

To pursue alternatives to fossil energies is an indispensable trend, as the fossil energies suffer from the limited source and deteriorating environment [1,2]. Biofuels such as bio-alcohols, regarded as environmentally friendly alternatives, have been exploited extensively [3]. Lignocellulosic bioconversion is a competitive way to produce bioethanol because of its mild operating condition and the absence of by-products [4]. However, lignocellulose, feedstock of the process, has a rigid matrix structure comprised of cellulose, hemicellulose and lignin [5]. The presence of lignin and hemicellulose makes lignocellulosic bioconversion, especially the enzymatic hydrolysis of cellulose, hard to be implemented [6,7]. Generally, the factors that have been identified to affect the hydrolysis of cellulose include porosity (accessible surface area) of the waste materials, cellulose fiber crystallinity, and lignin and hemicellulose content [8]. The removal of lignin and hemicellulose from biomass, reduction of cellulose crystallinity and augment of porosity in pretreatment processes can significantly improve the enzymatic hydrolysis of biomass. Hence, pretreatment prior to enzymatic hydrolysis is imperative for lignocellulosic feedstock. Variety of biomass pretreatments have been reported to promote the lignocellulosic bioconversion and cut down the total costs. The pretreatment is usually performed by steam explosion, liquid hot water, dilute acid, inorganic salt, lime and ammonia [9]. Each pretreatment method has its own advantages and disadvantages

* *Corresponding author*. Tel.: +86 20 87113940; fax: +86 20 22236078.
E-mail address: shyfu@scut.edu.cn (S. Fu).
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for specific lignocellulosic feedstock [9]. In all cases, pretreatment, which could effectively remove hemicellulose in biomass, is crucial to enzymatic hydrolysis of lignocellulose. However, a complicated factor is that different biomass applied to the same pretreatment process will present different patterns of hemicellulose or lignin removal and of enzymatic digestibility, even under the same conditions [10].

In this study, sugarcane bagasse was pretreated by metal chloride Lewis acids for cellulase hydrolysis. With regard to the feedstock, sugarcane bagasse is a typical agricultural residue, derived from sugarcane after juice extraction [11]. In order to make full use of bagasse, it is essential to pursue a promising route such as bioconversion [12]. For pretreatment methods, metal chlorides have been applied to some biomass species, such as corn stover and waste fiber, which were reported to have a great effect on the sequent enzymatic hydrolysis of biomass [13–15]. Metal chlorides are particularly attractive as lignocellulose pretreatment agents, because they are less corrosive than inorganic acids and can be recycled [13]. Moreover, these metal chlorides were performed on the conversion of bagasse to sugar could not only expand the use of bagasse, but explore the suitability of this pretreatment process.

Since high efficiency and economic feasibility of pretreatment is vital for large scale commercialization of biofuels and value added bio-chemicals, the kinetics of pretreatment for lignocellulosic bioconversion need to study to get an optimal condition. However, little information about the kinetics of enzymatic hydrolysis of lignocellulose pretreated by metal chlorides was reported except the effects of metal chlorides on biomass pretreatment [16]. In the recent years, the Michaelis–Menten equation has been widely used to study kinetics of enzymes performed on various substrates [17,18]. Nevertheless, it is not suitable for cellulase hydrolysis of lignocellulosic biomass, owing to the complexity of the enzymatic hydrolysis of biomass which is heterogeneous insoluble substrates [17]. Herein, we would investigate the effects of metal chloride Lewis acids pretreatment on the bagasse enzymatic hydrolysis, as well as explore the kinetics for further understanding the mechanism of these pretreatment.

2. Materials and methods

2.1. Materials

The feedstock, sugarcane bagasse, was obtained from Guangxi Guitang Group, Guangxi province, China. The sugarcane (Saccharum spp. hybrids) used in this sugar mill was cultivated in Guigang (Guangxi province, China) and harvested at an age of (10–11) months by harvester. The sugarcane was pressed through rollers to abstract the juice. The solid residues, known as bagasse, were air-dried and used in this study after depithing. They were milled by micro plant grinding machine (FZ102, Tianjin Taisite Instrument Co. China), collected through 10-mesh screen and then air-dried for further use. The mass fractions of glucan, xylan, lignin and ash in air-dried sugarcane bagasse were (36.02 ± 0.31) %, (19.41 ± 0.07) %, (24.20 ± 1.60) % and (0.55 ± 0.03) %, respectively. Chemicals used in this paper were of analytical reagent grade. All experiments were performed in duplicate under the same conditions, and average values were reported.

2.2. Pretreatment

Pretreatment was performed in a high pressure reactor (4530 series, Parr Co., US) with a total volume of 1 L. The bagasse of 30 g dry weight was loaded in the reactor, and mixed with the metal chloride solution. The total volume of liquid was 300 cm^3. The initial concentration of metal chloride ($CrCl_3$, $FeCl_2$, $FeCl_3$, $ZnCl_2$ and $AlCl_3$) for the pretreatment was 0.1 mol L^{-1}. In addition, dilute sulfuric acid pretreatment was carried out in this study as a control method to compare with metal chloride pretreatment. The sulfuric acid (about 0.4 g) was added until the pH value equaled to solution of ferric chloride pretreatment. After complete agitation, the pH value of solution was detected by DELTA 320 pH Meter. The reactants were initially at room temperature, and then heated to 170 °C for 30 min. The reactor was immediately removed from the heating jacket and allowed to cool down when the pretreatment was finished. The pretreated bagasse was then separated by filtration. The liquid part of pretreatment was analyzed by pH meter, ion chromatography (IC) and high-pressure liquid chromatography (HPLC) to determine pH values, the concentration of glucose, xylose, 5-hydroxymethylfurfural (HMF) and furfural, respectively. The solid portion rinsed with deionic water was used for the cellulase hydrolysis. Meanwhile, composites in the pretreated solid were analyzed.

2.3. Enzymatic hydrolysis

Pretreated bagasse of 1 g dry weight was hydrolyzed in the 100 mL flask by cellulase (Celluclast 1.5 L) with 20 Filter Paper Unit (FPU) per 1 g substrate and β-glucosidase (Novozyme 188) with 25 cellobiase units (CBU) per 1 g substrate. The cellulase hydrolysis of reaction mixture (50 cm^3) was carried out in a HAc/NaAc buffer (0.05 mol L^{-1}, pH = 4.8) on a rotary shaker (50 °C, 2.5 Hz). Aliquots of 0.1 cm^3 were taken at different time points, kept in boiling water for 1 min to inactivate the cellulase, and then centrifuged to remove water-insoluble solids. The supernatant of samples was analyzed by the glucose oxidase–peroxidase method (GOPM) for glucose content [19]. The cellulose enzymatic digestibility (CED) of pretreated bagasse was described as follows:

$$\mathrm{CED} = \frac{0.9 \times \text{ Glucose Mass}}{\text{Substrate Mass} \times \text{Mass Fractrion of Glucan}} \times 100\% \quad (1)$$

2.4. Analysis methods

The components of treated fibers were determined according to the National Renewable Energy Laboratory (NREL, Golden, CO) analytical methods for biomass [20]. All liquid fractions for analysis by IC were diluted appropriately with the ultrapure water and then filtered by a 0.22 μm filter. The concentration of glucose and xylose were determined by IC system (Dionex ICS-3000) with a CarboPac PA20 column at 30 °C. The quantification of HMF and furfural in the pretreatment