



Improving enzymatic saccharification of eucalyptus with a pretreatment process using $MgCl_2$



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ABSTRACT

Pretreatment using an acid salt is a novel technology for biomass conversion, which not only improves the following enzymatic efficiency but also decreases the equipment corrosion. In the present work, $MgCl_2$ was used as a pretreatment for enzymatic saccharification. After pretreatment, the solid substrate (SS) and pretreatment liquor (PL) were characterized, and the SS was enzymatically hydrolyzed to evaluate the conversion yield of cellulose (CYC). The results showed that the pretreatment using $MgCl_2$ had a strong degradation effect on hemicellulose, whose removal rate was nearly 100% in certain cases. After pretreatment, the efficiency of the enzymatic SS saccharification increased, such that the CYC was as high as 99.5%. The degradation kinetics of hemicellulose and cellulose both followed first-order homogeneous reactions, and the reaction activation energies E_a were 46.63 kJ/mol and 82.26 kJ/mol, respectively. The suitable pretreatment conditions were as follows: 0.2 mol/L $MgCl_2$ at 210 °C for 20 min.

1. Introduction

With the energy crisis and environmental pollution accelerating, research and development of efficient and sustainable clean energy, such as bioethanol, has received increasing attention (Mabee and Saddler, 2010; Alvira et al., 2010). Lignocellulose (mainly consisting of cellulose, hemicellulose and lignin) is the most abundant carbohydrate resource in the world, and is also a renewable resource that can be used in a biorefinery as a substitute for petroleum and other fossil fuels. However, because of its particular characteristics such as dense structure, high lignin content and resistance to enzymes, a pretreatment process is normally conducted prior to enzymatic saccharification. These methods include the use of acids, alkalis, hydrothermal processing, sulfites, etc (Mosier et al., 2005; Kang et al., 2013a).

Many pretreatment methods have been developed and researched for bioethanol production. Presently, the dilute acid method is commonly used as a pretreatment prior to enzymatic hydrolysis because it destroys the lignocellulosic structure and increases the enzymatic hydrolysis efficiency. The hemicellulose in the raw material is nearly

completely degraded into the monosaccharide (Ruiz et al., 2013; Wyman et al., 2009). However, the dilute acid method has some limitations, such as equipment corrosion, environmental pollution, etc. that restrict its utilization. Previous research found that the hemicellulose removal rate and the enzymatic hydrolysis efficiency could be enhanced by pretreatment with inorganic salt (Wang et al., 2009; Chen et al., 2014). Compared with an inorganic acid, inorganic salts, such as chloride salt, have lower equipment corrosion and a relatively milder effect on the raw material. The pretreatment using inorganic salts could provide a weak acid condition and was mainly caused hemicellulose degradation, which was obviously different from that using bases (Liu et al., 2009; Bi et al., 2016; Carvalho et al., 2016). The chloride salts can be divided into several types: alkaline metal chloride (NaCl and KCl), alkaline-earth metal chloride ($MgCl_2$ and $CaCl_2$), and transition metal chloride ($CuCl_2$, $FeCl_2$ and $FeCl_3$) (Yu et al., 2011). Liu and Wyman (2006) treated xylose and xylotriose using KCl, NaCl, $CaCl_2$, $MgCl_2$ and $FeCl_3$. The results showed that the degradation of these two types of sugars could be accelerated by these inorganic salts, in the order of $FeCl_3 > MgCl_2 > CaCl_2 > KCl > NaCl$. The degradation of

Abbreviations: CYC, conversion yield of cellulose; PL, pretreatment liquor; SS, solid substrate

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xylotriase was greater than that of xylose under the same conditions.

Kang et al. (2013a) pretreated miscanthus using several inorganic chlorides including NaCl, KCl, CaCl₂, ZnCl₂ and FeCl₃. These inorganic salts had strong degradation effects on the hemicellulose, in the order of: FeCl₃ > ZnCl₂ > CaCl₂ > KCl > NaCl. When the miscanthus was treated with 0.5% FeCl₃ for 15 min at 200 °C, 100% of the xylose was removed and an enzymatic hydrolysis conversion rate of 71.6% was obtained. Liu and Wyman (2006) pretreated corncob with FeCl₃ at 140–200 °C. More than 90% of the xylose was removed from the raw material, and the cellulose was well retained in the solid substrate. Unlike acid pretreatment, the inorganic salts provide a weakly acidic condition for pretreatment; therefore, the hemicellulose is more easily hydrolyzed than the cellulose and lignin. The authors concluded that the inorganic salt mainly has a degradation effect on the hemicellulose, which differs from pretreatment using an alkali or acid. Until now, research on the use of inorganic salts as pretreatments for biomass conversion focused on several salts, such as FeCl₃ and ZnCl₂ etc., excluding MgCl₂ (Chen et al., 2015; Wei and Wu, 2017). Because the Mg²⁺ ion can absorb or chelate onto the solid substrate, it has a certain promoter action on the cellulase activity and is beneficial for enzymatic hydrolysis (Mandels and Reese, 1957). Moreover, unlike the FeCl₂, FeCl₃, ZnCl₂ etc., the MgCl₂ has little effect for human health and is commonly prepared from seawater and salt lake, which was commonly used as a protective agent for the carbohydrate (cellulose and hemicellulose) in the pulping and bleaching. Therefore, MgCl₂ is a potential inorganic salt for used in pretreatment for biomass conversion.

In the present research, the eucalyptus chips were pretreated with MgCl₂ to explore the degradation of cellulose and hemicellulose, and the obtained SS was hydrolyzed with enzymes to calculate the CYC. Then the severity parameter was introduced to estimate the comprehensive influence of the pretreatment factors. Finally, the reaction kinetics of the cellulose and hemicellulose were discussed, respectively.

2. Materials and methods

2.1. Materials

Eucalypt chips provided by the Jingui Paper Co. Ltd. (Guangxi, China) were milled and screened to obtain a fraction of 40–60 meshes. The fraction was stored in a polyethylene container and kept at 4 °C before using. For the components of eucalypt chips, the NREL LAP method was used, and the results were as follows: glucan 47.86%, xylan 15.50%, arabinan 1.03%, lignin 29.15% and ash 0.58% (Sluiter et al., 2011).

Cellulase (Celluclast 1.5 L, 50.97 FPU/mL) and β-glucosidase (Novozyme 188, 1290.69 CBU/mL) were obtained from Novozymes (Beijing China). All of the other reagents were of analytical grade and purchased from Sinopharm Chemical Reagent Co. (Shanghai, China).

2.2. Pretreatment using MgCl₂

For the pretreatment process, 5 g of eucalyptus sample was placed into a steel reactor (effective volume 45 mL), with a solid/liquid ratio of 1/6. The MgCl₂ salt concentration, pretreatment time and pretreatment temperature were controlled in the range of 0–0.5 mol/L, 5–60 min and 140–220 °C, respectively. The pretreatment process was conducted in an oil bath. After treatment, the reactor was instantly placed into cool water to terminate the reaction. The solid residue from the reactor was transferred into a 50 mL volumetric flask and filtered under vacuum. The filtrate defined as the pretreatment liquor (PL), was analyzed to quantify the sugars and degradation products. The filter residue designated as the solid substrate (SS), was washed with deionized water until the SS was neutral. The washed SS was analyzed to quantify the cellulose, hemicellulose and lignin contents, and then used for enzymatic hydrolysis. Diluted HCl (1 mol/L) was added to the reactor to adjust the pH of the pretreatment process.

2.3. Severity parameter

In the pretreatment process, the pretreatment conditions including time, temperature and MgCl₂ concentration potentially have inter-related effects on the results. To combine the effects of these conditions, a modified severity parameter was applied in the present work, which was defined as follows:

$$M_0 = t \times C^n \times \exp \left[\frac{T_r - T_b}{14.75} \right]$$

Where M_0 is the modified severity parameter; t is the reaction time, min; C is the MgCl₂ concentration, wt. %; T_r is the reaction temperature, °C; T_b is the base temperature, 100 °C; and n is an arbitrary constant (0.849 for acidic medium) (Chum et al., 1990; Silverstein et al., 2007).

2.4. Characteristics of the raw material and the SS

A 40–60 mesh fraction of crushed eucalyptus chips was used as the raw material for analyzing the components, whereas the SS was analyzed directly. The ash content was determined according to TAPPI method T211 OM02. A two-step acid hydrolysis method (concentrated acid hydrolysis and dilute acid hydrolysis) was used to determine the cellulose and hemicellulose contents (Sluiter et al., 2011). According to the SS yield, as well as the contents of cellulose and hemicellulose in the raw material and the SS, the cellulose and hemicellulose losses were calculated.

2.5. Enzymatic hydrolysis

10 g of the SS was placed into a beaker flask and diluted with a buffer solution (sodium acetate and acetic acid, pH 4.8) to a concentration of 2.5% (all based on the oven dried SS, o.d.), then 30 FPU/g of cellulase and 37.5 CBU/g of β-glucosidase were added to the beaker flask. As a note, the high dosage of the cellulase and β-glucosidase was to eliminate the enzyme influence on the CYC. The beaker flask was sealed and put into a shaker for 48 h at 50 °C, with a rotational speed of 150 rpm. After hydrolysis, the beaker flask was heated for 5 min at 90 °C in a water bath to deactivate the enzymes.

2.6. Detection of monosaccharide and sugar degradation compounds

The xylose, glucose, lower molecular weight organic acids and sugar degradation compounds (furfural and 5-HMF) in the PL were detected directly by high performance liquid chromatography (HPLC). For the monosaccharide in enzymatic hydrolysate, the hydrolysate was centrifuged at 10,000 rpm for 10 min and filtrated with a membrane filter (0.22 μm) prior to HPLC detection. The HPLC (Agilent 1260) equipped with a Bio-Rad Aminex HPX-87H (300 × 7.8 mm) column was used, and detecting conditions were as follows: column temperature 55 °C, differential detector, mobile phase H₂SO₄ (0.05 mol/L) with a flow rate of 0.6 mL/min, and a sampling volume of 10 μL. The CYC was calculated as follows:

$$\text{CYC} = 0.9 * (\text{glucose in enzymatic hydrolyzate}) / (\text{cellulose in the SS})$$

3. Results and discussion

3.1. Effect of pretreatment time

To clearly evaluate the effect of the pretreatment time, the raw materials were pretreated under the same conditions, and the pretreatment time was changed. After pretreatment, the SS yield and the components of the PL were determined and calculated, and then the SS was enzymatically hydrolyzed to calculate the CYC. The results are

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