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Enhancement of eucalypt chips' enzymolysis efficiency by a combination method of alkali impregnation and refining pretreatment

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HIGHLIGHTS

- $Mg(OH)_2$ impregnation could reduce the refining energy consumption significantly.
- The specific surface area could be increased obviously by the M + R process.
- $Mg(OH)_2$ impregnation has a positive effect on enzymolysis efficiency.

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ABSTRACT

A combination process of alkali impregnation and refining was used as a pretreatment to improve the production of fermentable sugar. The surface structures and crystallinities of wood samples were characterized to explain the relationships between the pretreatment action and enzymatic efficiency. After refining, the reducing sugar contents in hydrolyzates were analyzed by UV–Vis and HPLC. The results showed that the enzymatic efficiency could be improved by the combined pretreatment, due to the increment of specific surface area and the release of more free hydroxyls. Comparing to the sodium hydroxide and deionized water, the impregnation with magnesium hydroxide had low refining energy consumption and high yield of reducing sugar (glucose and xylose) in enzymolysis process, where about 560 kWh/t of the energy was saved in refining, and the yield of the reducing sugar was as high as 91.53%. And the enzymolysis could be improved by a certain amount of magnesium ions.

1. Introduction

Lignocellulosic materials, naturally from photosynthesis, are a type of renewable resource with abundant sources, which can be used to produce high value-added chemical products (Nigam and Singh, 2011; Mielenz, 2001). The hardwood is a common lignocellulose resource, which has a rapid growth rate and many advantages in biorefinery industry comparing to the softwood (Isenberg, 1980). As one of the three main hardwoods, eucalypt planted in many regions all over the world is a potential feedstock for bioethanol production (Zhu and Pan, 2010).

Enzymolysis is a common method to obtain the fermentable sugars, and the enzymatic efficiency of lignocellulosic biomass can be enhanced by adding a pretreatment stage before the biomass conversion (Ibrahim et al., 2011; McIntosh and Vancov, 2010). However, the economic factors should be considered for the industrial application. So it is necessary to explore an efficient, low cost and environmentally friendly pretreatment technology for the bioethanol production (Sills and Gossett, 2011; Zhao et al., 2008).

So far, the mechanical refining process has been commonly applied in the pulping and papermaking industry to produce high yield pulps, such as TMP (Thermo-Mechanical Pulp), CTMP (Chemi-Thermo-Mechanical Pulp) and APMP (Alkali Peroxide Mechanical Pulp) (Mohini et al., 2002). In the production of the mechanical pulps, the refining energy consumption is extremely high, which could come up to 1800–2880 MJ/t (Zhu and Pan, 2010; Schell and Harwood, 1994). Normally, a chemical treatment is used prior to the refining process to reduce the energy consumption. Based on chemical species, the chemical treatment can be classified into acidic, alkaline and neutral method, where the alkaline method is used most widely in the mechanical pulping. Similarly, a chemical impregnation is also needed in the biomass pretreatment, when the refiner is used. Considering its pH buffering property and safe handling, magnesium hydroxide is a prefer choice for chemical impregnation of lignocellulosic materials. In the mechanical and chemical pulping, it was reported that the refining energy could be significantly reduced when the $Mg(OH)_2$

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and H_2O_2 were used as a chemical treatment (Griffiths and Abbot, 1994; Harrison et al., 2008), and the $Mg(OH)_2$ had a perfect protective effect on carbohydrates because of the existence of Mg^{2+} (Pang et al., 2012; Yang et al., 2012).

For the points of energy saving and efficient utilization of raw material, alkaline impregnation with refining pretreatment was employed to enhance the bioconversion efficiency of the eucalypt chips. The $Mg(OH)_2$ was applied in impregnation process, which could reduce the generation of inhibitor, prevent equipment from damaging and set suitable conditions for fermenting. In this case, the effects of alkalis including magnesium hydroxide and sodium hydroxide on the refining energy reduction and enzymatic efficiency were investigated.

2. Methods

2.1. Materials

Eucalypt chips provided by the Jingui Paper Co. Ltd. (Guangxi, China), had been chipped and screened, with the size of 30–50 mm in length and 10–20 mm in width.

Cellulase was obtained from Sigma–Aldrich (Shanghai, China), with the activity of 79.16 FPU/g. Other reagents were analytic purity grade and obtained from the Sinopharm Chemical Reagent Co.

2.2. Pretreatment of eucalypt chips

2.2.1. Mechanical compression

Eucalypt chips were washed and soaked with deionized water for 12 h, then compressed using an extruder equipped with a 6″-MSD (Modular Screw Device, Andritz, Austria). The compressed solids were sealed in a plastic bag and kept in a cool room under 4 °C.

2.2.2. Alkaline impregnation and refining

2.0 kg of the extruded samples were impregnated with 3% of sodium hydroxide (S + R) or 3% of magnesium hydroxide (M + R) respectively (all based on the oven dried raw material), then diluted with deionized water and kept the concentration of the samples for 20%. The diluted samples were stored under 50 °C for 1 h. For comparison, a blank sample (Blank) impregnated with deionized water was conducted under the same conditions.

After impregnation, the wood samples were directly refined for 5 passes using a KRK refiner (KRK No. 2500-II, KUMAGAI RIKI KOGYO Co., Ltd. Tokyo, Japan), with the plate-gaps from 0.6 mm to 2.0 mm.

The specific energy of refining was calculated by the followed formula:

$$\text{Specific energy consumption}(\text{kWh/t}) = (E_1 - E_0)/m$$

where E_1 was the energy input of the refiner with stock feeding (kWh), E_0 was the energy consumption without stock feeding (kWh), m was the dry-mass of the wood chips (t), and the energy accuracy was 0.007 kWh.

2.3. Characteristics of the wood samples

Wood samples, collected from compression stage and refining pretreatment, were characterized by means of SEM, specific surface area analyzer and X-ray diffractometer.

2.3.1. Specimen preparation

For SEM observation and the specific surface area analysis, the wood samples were gradually dehydrated by a set of alcohol solutions (30%, 50%, 70%, 95% and 100%, v/v) and dried in a vacuum oven at 55 °C for overnight (Westermarck et al., 1998). For the preparation of crystallinity analysis, the wood samples were dried by a vacuum oven.

2.3.2. Determination

The dried wood samples were coated with carbon-palladium in the presence of argon gas. The Hitachi S-4700 Scanning Electron Microscope was used for observing the microstructure of the samples at a voltage of 20 kV.

The Specific Surface Area Analyzer (ASAP2020, Micromeritics Instrument Corporation, Georgia, USA) was used for the measurement of specific surface area, according to the Brunauer–Emmett–Teller (BET) method (Germain et al., 2008).

Prior to crystallinity analysis, samples were ground into R 80–120 mesh, and measured using a horizontal X-ray diffractometer (D8-FOCUS, Bruker Company, Ettlingen, Germany). The crystallinity (CrI) was calculated as the following formula:

$$\text{CrI} = (I_{002} - I_{\text{am}}) \times 100/I_{002}$$

where I_{002} was the maximum intensity of the (002) lattice diffraction, I_{am} was the minimum intensity between the (101) and (002) lattice diffraction.

The contents of α-cellulose, holo-cellulose, klason–lignin, benzene–ethanol extractives and ash in the raw material and the pretreated solids were measured according to the TAPPI methods (Pettersen, 1984).

2.4. Enzymolysis and determination of the enzymatic hydrolyzates

The raw material and pretreated solid samples were used as the enzymatic substrates. Before enzymolysis, the substrate was diluted with acetate buffer (0.1 M sodium acetate and 0.1 M acetic acid, pH = 4.8) to keep the concentration for 2.5% (w/w). A commercial cellulase with a dosage of 30 FPU/g (Sigma, C1794) was added into the diluted substrate. The mixture was placed into a cultivation shaker (200 rpm), where the reaction temperature was kept at 50 °C and continued for 48 h. After enzymolysis, enzymatic slurry was centrifuged at 10,000 rpm for 10 min to obtain hydrolyzates. The reducing sugar content in the hydrolyzate was detected by a DNS method, which had been described in more detail previously (Pala et al., 2001).

The calibration curve was illustrated as the following formula,

$$Y = 0.987x - 0.0356, \quad R^2 = 0.9999$$

where x was the glucose concentration (mg/ml), y was the absorbance of the glucose at the wavelength of 540 nm.

The composition of the reducing sugar in the enzymatic hydrolyzates was analyzed using a high performance liquid chromatography (HPLC 1200, Germany) equipped with an Aminex HPX-87H column (300 mm × 7.8 mm ID, Bio Rad, USA). The column temperature was set at 55 °C. 10 μl of liquid were eluted at a rate of 0.6 ml/min with sulfuric acid (0.005 M).

The concentration of Mg^{2+} in the enzymatic hydrolyzates was measured using an inductively coupled plasma-optical emission spectroscopy (ICP-OES, Optima 7000DV, Perkin Elmer, USA). The liquid samples were mixed with 5% of nitric acid to break down organisms and eluted at a rate of 1.5 ml/min.

3. Results and discussion

3.1. Evaluation the effect of alkaline impregnation and refining

To evaluate the influence of mechanical and chemi-mechanical pretreatments on refining energy saving and polysaccharides