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High-pressure homogenization pretreatment of four different lignocellulosic biomass for enhancing enzymatic digestibility

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HIGHLIGHTS

- HPH pretreatment enhanced enzymatic digestibility of lignocellulosic biomass.
- HPH expanded accessible surface area of biomass for enzymolysis.
- HPH destroyed structure of lignocellulosic biomass.
- Grass clipping with HPH pretreatment is a promising biomass for enzymolysis.

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ABSTRACT

Grass clipping, corn straw, catalpa sawdust and pine sawdust were pretreated with high-pressure homogenization (HPH) to enhance the enzymatic digestibility. With a working pressure of 10 MPa, all the four lignocellulosic biomass were significantly changed, such as decrease in particle size, structure destruction, and crystallinity change. Results showed that lignocellulosic biomass pretreated with HPH yielded more reducing sugar, which was suitable for subsequent biofuel production. After 48-h enzymatic hydrolysis, the maximum reducing sugar yield of 229.42 mg/g was achieved for grass clipping. For corn straw, the enzymatic hydrolysis efficiency increased by 68.37% at most. However, reducing sugar yield of catalpa sawdust and pine sawdust was relatively lower. Low lignin content and crystallinity might make grass clipping the most suitable material for HPH pretreatment, thus leading to high hydrolysis efficiency. HPH pretreatment could increase biofuel output in a mild condition without adding any chemicals.

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1. Introduction

Due to the growing scarcity of fossil fuels and serious damage of natural environment caused by burning fossil fuels, research on utilizing renewable lignocellulosic biomass as raw fuel materials is becoming more and more important (Kwietniewska and Tys, 2014; Wieczorek et al., 2014). Biofuel production from lignocellulosic biomass is one of the most promising directions (Zhao et al., 2014a). Lignocellulosic biomass, which is composed of cellulose, hemicellulose and lignin, can be classified into 3 categories: crop residues, broadleaf wood and coniferous wood (Galbe and Zacchi, 2007).

In general, the raw lignocellulosic biomass without any pretreatment is difficult to be used for efficient biofuel production. Due to different native structures and chemical compositions, the lignocellulosic materials should be pretreated with suitable methods. Choosing the suitable raw materials and corresponding pretreatment methods is necessary for effective production of biofuels. A large number of biomass pretreatment methods have been developed for different raw materials, such as comminution, irradiation, steam explosion, liquid hot water, alkaline pretreatment, acid pretreatment, ionic liquids pretreatment, enzymatic pretreatment and combined pretreatment (Mosier et al., 2005; Zhao et al., 2014b).

In this paper, mass conversion of grass clipping, corn straw, catalpa sawdust and pine sawdust was examined, which are typical agriculture and forestry wastes in China. These lignocellulosic materials belong to different species, having different fiber structures and physical properties. The potential of grass, corn straw and pine wood bioconversion have been proved

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(Oldenburg et al., 2011), and some pretreatment methods were applied to these feedstocks. Yu et al. (2014) pretreated lawn grass by four mild methods, and soaking aqueous ammonia was found to be the most effective pretreatment for improving the bio-methane yield. Corn straw is an important and abundant renewable feedstock. A number of pretreatment methods, including physical, chemical, physicochemical and biological treatment, have been utilized to pretreat corn straw. Guo et al. (2013) reported that steam explosion and sodium hydroxide followed by *Aspergillus oryzae* fermentation significantly increased glucose production of corn straw. Pine wood is considered as a potential source of biofuel, but the recalcitrant structure formed by rigid lignocellulose impedes its efficient utilization. Alkaline pretreatment with NaOH was used to improve bio-methane production of different part of pine wood, and it was found that the highest amount of bio-methane of 213 ml/(gVS) was produced from needle leaves (Salehian and Karim, 2013). Alkaline pretreatment of softwood pine showed that pretreatment at a high temperature for a short retention time was effective (Salehian et al., 2013). As a kind of hardwood, catalpa wood is generally used for making furniture. Catalpa sawdust as an abundant by-product of furniture production has the potential for biofuel production like other hardwood.

High-pressure homogenization (HPH) is widely used in food, chemical, pharmaceutical and biological processes (Chung et al., 2014; Toro-Funes et al., 2014; Xu et al., 2014; Zhai et al., 2014), which has the similar principle with steam explosion and ammonia explosion (Chen et al., 2010). The raw materials are firstly forced by a very high pressure and then the pressure reduces instantly and quickly. The blasting effect causes a combination of huge pressure drop, highly focused turbulent eddies and strong shearing forces to make the raw materials be crushed (Ye and Harte, 2014; Zhang et al., 2012). Since HPH can avoid or significantly reduce chemical usage, it shows a promising potential in more broad applications, for example, HPH pretreatment was used to disintegrate sewage sludge for improving sludge biodegradation (Lan et al., 2013; Zhang et al., 2012). However, this method was seldom tested for lignocellulosic biomass pretreatment. Chen et al. (2010) reported the HPH combined with alkaline treatment on sugarcane bagasse, which exhibited a significant increase of enzymatic digestibility of 95.5%.

The objective of this work was to pretreat the four kinds of lignocellulosic materials with HPH, and analyze the characteristics of pretreated lignocellulosic biomass and enzymatic hydrolysis efficiency of those pretreated materials.

2. Methods

2.1. Materials

Grass clipping of *Festuca arundinacea* (a type of lawn grass) was mowed from Beijing Forestry University campus; corn straw was harvested in a farm located in Inner Mongolia; catalpa sawdust and pine sawdust were collected from a furniture factory located in Beijing. The raw materials were milled by a laboratory hammer mill (DF-25S, Dade, China) and screened by an 80 meshes sieve shaker.

2.2. HPH pretreatment

The samples were prepared as a 0.5% suspended solution in 2000 ml tap water. A high pressure homogenizer (JJ-30, Shengtong, China) was used for sample pretreatment. The samples were poured into the entrance hopper of homogenizer and then the working pressure was adjusted to 10 MPa. After pretreatment, the samples were recovered by filtration with a vacuum pump. The samples were then processed by freeze-drying for 48 h and stored in a freezer.

2.3. Enzymatic hydrolysis

Enzyme used for hydrolysis was generated by *Aspergillus niger* (powder, $\geqslant$0.3 units/mg solid), purchased from Sigma–Aldrich Co. LLC. Enzymatic hydrolysis of samples was conducted in a shaking incubator at 50 °C and 180 rpm for 48 h. The reaction system contained 0.5 g sample and 20 ml citrate buffer (0.05 mol/L, pH = 4.8), and enzyme loading for 1 g sample was 30 FPU cellulase. The samples were taken at certain time interval for the determination of reducing sugar.

2.4. Analysis methods

The composition of raw materials was analyzed by a fiber analyzer (A200i, Ankom, USA) (Vogel et al., 1999). Particle size distribution was measured by particle size analyzer (Mastersizer 2000, Malvern, UK). The disperser was water, and stirring speed was 180 rpm. Structural change of samples was examined by scanning electron microscopy (S-3400 N II, Hitachi, Japan). All samples were sputter-coated with gold before scanning, and the acceleration voltage was 10 kV.

The moisture was calculated by Eq. (1) (Sluiter et al., 2008):

$$\text{Moisture} = 100 \times \frac{\text{Weight}_{\text{pre-dried}} - \text{Weight}_{\text{dried}}}{\text{Weight}_{\text{pre-dried}}} \tag{1}$$

where $\text{Weight}_{\text{pre-dried}}$ is the weight of sample before drying, and $\text{Weight}_{\text{dried}}$ is the weight of sample after drying at 105 ± 3 °C for a minimum of four hours.

Reducing sugar concentration was measured by 3,5-dinitrosalicyclic acid method (Miller, 1959). Crystallinity of samples was determined by X-ray diffractometer (D8 Advance, Bruker, Germany). Specimens were scanned at 6s/step from $2\theta = 5°$ to $40°$ with a step of 0.2°. Crystallinity index (CrI) was calculated by Eq. (2):

$$\text{CrI} = 100 \times \frac{I_{0.02} - I_{\text{amorphous}}}{I_{0.02}} \tag{2}$$

where $I_{0.02}$ is the diffraction intensity of crystalline structure ($2\theta = 22.6°$), and $I_{\text{amorphous}}$ is the diffraction intensity of amorphous fraction ($2\theta = 18.0°$).

3. Results and discussion

3.1. Particle size distribution and SEM observation

The sudden expansion caused by HPH can obviously destroy the plant cell structure (Chen et al., 2010; Dumay et al., 2013; Yusaf and Al-Juboori, 2014). Although all samples were screened by 80 meshes sieve shaker, they showed different initial particle size, because they have different chemical compositions, crystallinities, structures, and so on. The average particle size of the four kinds of samples was summarized in Fig. 1. The average particle size of samples decreased after HPH pretreatment, and the decrease degree varied for different samples. The grass clipping had the smallest initial average particle size of 153.077 μm and also the least reduction in average particle size of 2.142 μm. The initial average particle size of pine sawdust was the biggest; however, the reduction was the most obvious (from 200.353 to 160.813 μm). After HPH pretreatment, four kinds of samples suffered different degrees of destruction, and the final average particle size was almost around 155.00 μm. Stronger damage to the lignocellulosic materials was difficult to occur under a homogenization pressure of 10 MPa.

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