Bioresource Technology 205 (2016) 34–39

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Thermo-chemical pretreatment and enzymatic hydrolysis for enhancing saccharification of catalpa sawdust

Shuguang Jin [a], Guangming Zhang [b], Panyue Zhang [a,*], Fan Li [a], Shiyang Fan [a], Juan Li [a]

[a] Beijing Key Lab for Source Control Technology of Water Pollution, Beijing Forestry University, Beijing 100083, China
[b] School of Environment and Resource, Renmin University of China, Beijing 100872, China

HIGHLIGHTS

- Catalpa sawdust structures by four thermo-chemical pretreatments were compared.
- Solubilization of cellulose, hemicellulose and lignin were examined.
- Optimal factors were 160 °C, 3 min and 1.75% (w/v) $Ca(OH)_2$ for reducing sugar yield.
- Thermo-$Ca(OH)_2$ pretreatment is a promising method for catalpa sawdust enzymolysis.

ARTICLE INFO

Article history:
Received 3 December 2015
Received in revised form 9 January 2016
Accepted 11 January 2016
Available online 14 January 2016

Keywords:
Catalpa sawdust
Thermo-chemical pretreatment
$Ca(OH)_2$
Enzymatic hydrolysis
Lignocellulosic biomass

ABSTRACT

To improve the reducing sugar production from catalpa sawdust, thermo-chemical pretreatments were examined and the chemicals used including NaOH, $Ca(OH)_2$, H_2SO_4, and HCl. The hemicellulose solubilization and cellulose crystallinity index (CrI) were significantly increased after thermo-alkaline pretreatments, and the thermo-$Ca(OH)_2$ pretreatment showed the best improvement for reducing sugar production comparing to other three pretreatments. The conditions of thermo-$Ca(OH)_2$ pretreatment and enzymatic hydrolysis were systematically optimized. Under the optimal conditions, the reducing sugar yield increased by 1185.7% comparing to the control. This study indicates that the thermo-Ca $(OH)_2$ pretreatment is ideal for the saccharification of catalpa sawdust and that catalpa sawdust is a promising raw material for biofuel.

1. Introduction

The use of fossil fuels has caused serious environmental damages and energy crisis. One way to solve these problems is to develop sustainable biofuels, e.g. cellulosic ethanol, biodiesel, biogas, etc. The biofuel can be produced from a variety of lignocellulosic biomass, including wastes from industry, forestry, agriculture, and municipal solids. Forestry wastes are a category of abundant materials with high content of cellulose. According to statistics, more than a third of a log is turned into wastes in furniture factories (Liu and Shen, 2007). There are 37 million cubic meter forestry wastes produced from forestry production every year in China (Xin et al., 2014). Catalpa wood is mothproof and easy to be dried with low shrinkage, so it has a broad use in construct and furniture industry and the catalpa sawdust from wood processing is huge (Dong et al., 2013). Therefore, it will be of great value to utilize the catalpa sawdust for biofuel production.

Enzymatic hydrolysis of lignocellulosic biomass is a crucial step for biofuel conversion (Krishania et al., 2013). Due to the tight structure of cellulose, hemicellulose and lignin in lignocellulosic biomass, effective enzymatic hydrolysis is difficult without pretreatment. Different pretreatment methods, including physical, chemical and biological methods, have been developed for efficient enzymatic hydrolysis and biofuel production (Krishania et al., 2013). Among these pretreatment methods, thermo-chemical pretreatment method is considered to be one of the best because of its high efficiency (Monlau et al., 2012). Rice hull and rice straw were pretreated by thermo-NaOH (30 °C), and the enzymatic hydrolysis efficiency was significantly enhanced (Cabrera et al., 2014). *Eucalyptus* was pretreated by $Ca(OH)_2$-hydrothermal-milling treatment and the highest glucose yield of 90% was achieved at 170 °C (Ishiguro and Endo, 2015). Acid pretreatment of lignocellulosic biomass commonly used sulfuric acid (H_2SO_4) as the pretreatment chemical. Cotton ginning trash was pretreated by thermo-H_2SO_4

* Corresponding author at: College of Environmental Science and Engineering, Beijing Forestry University, Qinghua East Road 35, Haidian District, Beijing 100083, China. Tel.: +86 15001255497; fax: +86 10 62336900.
E-mail address: panyue_zhang@bjfu.edu.cn (P. Zhang).

http://dx.doi.org/10.1016/j.biortech.2016.01.019
0960-8524/

and a maximum enzymatic glucose conversion of 89% was achieved under optimal conditions (temperature of 180 °C, sulfuric acid concentration of 0.8%, time of 12 min) (McIntosh et al., 2014). Hydrochloric acid (HCl) was also a promising chemical for biomass pretreatment. Thermo-chemical pretreatment of wheat straw at 190 °C with HCl solution showed a total sugar recovery of 86.6% (Ertas et al., 2014).

The objectives of this study were to compare the effects of four kinds of thermo-chemical pretreatments (NaOH, $Ca(OH)_2$, H_2SO_4, and HCl) on the structural features (chemical composition and crystallinity) of catalpa sawdust; to compare the impacts of the thermo-chemical pretreatments on the reducing sugar yield; and to optimize the operating conditions of the best pretreatment method and enzymatic hydrolysis.

2. Methods

2.1. Raw materials

Catalpa sawdust was collected from a furniture factory located in Beijing, China. The sawdust was air-dried for two weeks and screened to obtain different particle size samples with sieve shakers. The screened sawdust was stored in plastic bags and kept at room temperature.

The chemicals, NaOH, $Ca(OH)_2$, H_2SO_4, and HCl (chemical pure) were purchased from Beijing Chemical Industry Group Co., China.

The commercial enzyme was purchased from Sigma–Aldrich Co. LLC, which was generated by *Aspergillus niger* (powder, ⩾0.3 units/mg solid).

2.2. Themo-chemical pretreatment

Four kinds of chemicals including NaOH, $Ca(OH)_2$, H_2SO_4, and HCl were used for the thermo-chemical pretreatment of catalpa sawdust. The thermo-chemical pretreatment was carried out in Teflon-lined stainless steel autoclaves. The catalpa sawdust of 10.0 g was immersed in 200 ml alkali or acid solution with a dosage of 5% (w/v). The suspension was heated and maintained at 100 °C for 1 h. After pretreatment, the samples were cooled to room temperature. The solid residues were recovered by filtration with a vacuum pump and thoroughly washed with distilled water to a neutral pH. Then the solid residues were dried at 105 ± 3 °C for 4 h. The dried solid samples were weighed and kept at 4 °C for further enzymatic hydrolysis, and analysis of chemical composition and crystallinity. Pretreatment chemical, with which the highest reducing sugar in hydrolysate was generated, was selected for subsequent condition optimization study.

2.3. Enzymatic hydrolysis

The pretreated catalpa sawdust of 0.5 g was immersed in citrate buffer (0.05 mol/L, pH = 4.8) and then the cellulase was added at 30 FPU per gram sample. Enzymatic hydrolysis was conducted in a shaking incubator at 50 °C and 180 r/min for 48 h. The samples were taken at certain time interval for the determination of reducing sugar.

2.4. Optimization of thermo-chemical pretreatment and enzymatic hydrolysis

$Ca(OH)_2$ was selected for the optimization of catalpa sawdust pretreatment. For the thermo-chemical pretreatment optimization, particle size, solid content, $Ca(OH)_2$ dosage, pretreatment time and temperature were examined. For the enzymatic hydrolysis, enzyme loading and hydrolysis time were selected to be optimized.

2.5. Analysis methods

The chemical composition of raw and pretreated catalpa sawdust was analyzed by a fiber analyzer (A200i, Ankom, USA). The reducing sugar concentration was measured by DNS method (Miller, 1959). The crystallinity of catalpa sawdust was examined by an X-ray diffractometer (D8 Advance, Bruker, Germany), and the samples were scanned in a 2θ range from 5° to 40° with a step of 0.2°. The crystallinity index (CrI) was calculated by Eq. (1) (Segal et al., 1959):

$$\mathrm{CrI} = 100 \times \left(\frac{I_{002} - I_{amorphous}}{I_{002}} \right) \quad (1)$$

where I_{002} is the diffraction intensity of crystalline structure ($2\theta = 22.6°$), and $I_{amorphous}$ is the diffraction intensity of amorphous fraction ($2\theta = 18.0°$).

3. Results and discussion

3.1. Chemical composition of catalpa sawdust

The cellulose content of catalpa sawdust and other lignocellulosic biomass is shown in Table 1. The catalpa sawdust in this study showed a very high content of cellulose (51.2%), which was higher than many kinds of frequently-used biofuel materials. The high cellulose content in catalpa sawdust is beneficial to biofuel production, since the biomass containing more cellulose can produce more fermentable sugars during enzymatic hydrolysis (Jung et al., 2013).

3.2. Weight loss and composition change after thermo-chemical pretreatment

One goal of pretreatment is to remove lignin and hemicellulose from lignocellulosic biomass because they are stubborn barriers to efficient enzymatic hydrolysis. Therefore, it is necessary to analyze the composition change of samples before and after pretreatment. The weight loss and change of cellulose, hemicellulose and lignin after thermo-chemical pretreatment were analyzed (see Table 2). The weight of catalpa sawdust decreased in different degrees after four kinds of thermo-chemical pretreatments. The weight loss after thermo-NaOH pretreatment was the highest (14.5%), and that of all other three pretreatments was below 6%.

The varying degree of weight loss was attributed to the different pretreatment methods. The weight loss was caused by solubilization of cellulose, hemicellulose and lignin from raw material. For solubilization of these components, the thermo-alkaline pretreatments were stronger than the thermo-acid pretreatments (Table 2). The weight loss of catalpa sawdust was mainly attributed to the solubilization of hemicelluloses. 45.9% and 33.4% hemicellulose was solubilized after the thermo-NaOH and thermo-$Ca(OH)_2$ pretreatments, respectively. The hemicellulose was more easily solubilized in other researches too. The corn straw pretreated by NaOH lost 33.5–88.2% hemicellulose (Chen et al., 2009; Varga et al., 2002). The removal of hemicellulose enhanced the enzymatic hydrolysis of lignocellulosic biomass due to the structure change of lignocellulosic biomass (Himmel et al., 2007). However, hemicellulose is also a kind of easy hydrolysable polymers as the resource for fermentation sugars (Hendriks and Zeeman, 2009). The microbes can utilize these fermentation sugars to produce biofuels. Too much loss of hemicellulose may reduce fermentation sugar