Bioresource Technology 182 (2015) 245–250

Contents lists available at ScienceDirect

Bioresource Technology

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

Chemical characteristics and enzymatic saccharification of lignocellulosic biomass treated using high-temperature saturated steam: Comparison of softwood and hardwood

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HIGHLIGHTS

- High temperature saturated steam treatment of cedar and beech was evaluated.
- Steam treatment followed by milling treatment improved enzymatic saccharification.
- Saccharification rate of steam-treated beech with milling treatment was 94%.
- Necessity of milling treatment after steam treatment is dependent on wood species.

ARTICLE INFO

Article history:
Received 20 November 2014
Received in revised form 1 February 2015
Accepted 2 February 2015
Available online 11 February 2015

Keywords:
High-temperature saturated steam
Chemical characteristics
Enzymatic saccharification
Softwood
Hardwood

ABSTRACT

This study investigated the effect of high-temperature saturated steam treatments on the chemical characteristics and enzymatic saccharification of softwood and hardwood. The weight loss and chemical modification of cedar and beech wood pieces treated at 25, 35, and 45 atm for 5 min were determined. Fourier transform infrared and X-ray diffraction analyses indicated that solubilization and removal of hemicellulose and lignin occurred by the steam treatment. The milling treatment of steam-treated wood enhanced its enzymatic saccharification. Maximum enzymatic saccharification (i.e., 94% saccharification rate of cellulose) was obtained using steam-treated beech at 35 atm for 5 min followed by milling treatment for 1 min. However, the necessity of the milling treatment for efficient enzymatic saccharification is dependent on the wood species.

1. Introduction

Primary energy self-sufficiency in Japan is only 4%, and this value is much lower than other developed countries. Even when nuclear power generation is considered as a domestically produced energy, the rate slightly increases to 17%, implying that Japan is hugely dependent on imported energy (approximately 80%). Therefore, the use of renewable energy sources such as biomass has been considered as an alternative to fossil fuels in Japan.

For the significant production of domestic biofuels, the development of a method to convert lignocellulosic biomass such as wood, bamboo, and agricultural waste (such as rice straw) into ethanol and useful materials is desirable because it would not compete with food production. However, because cellulosic components (i.e., substrates for ethanol production) are strongly covered with lignin in the lignocellulosic biomass, pretreatment (delignification) is necessary for efficient enzymatic saccharification followed by alcohol fermentation to increase the accessibility of cellulose to cellulases (Karp et al., 2013). Various pretreatment methods have been developed and applied to target the conversion of biomass to biofuel including: acid treatment (Torget et al., 1988), alkaline treatment (Ucar, 1990), ammonia fiber expansion (Dale et al., 1996), organosolv treatment (Bonn et al., 1987), liquid hot water treatment (Sreenath et al., 1999), microwave irradiation (Ooshima et al., 1984), superheated steam treatment (Bahrin et al., 2012), and steam explosion (Ramos et al., 1992).

Steam explosion, introduced as a biomass pretreatment process by Mason (1926), has become one of the simplest and environmentally friendly techniques and is a novel pretreatment method with a high efficiency at breaking lignin structures to release cellulose and hemicellulose from lignocellulosic biomass. It has attracted significant attention by researchers in the field of both bioethanol and biomethane production (Bungay, 1982; Hooper and Li, 1996; Lipinsky, 1981; Sanchez and Cardona, 2008). Steam explosion involves exposing the biomass sample to saturated steam under

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http://dx.doi.org/10.1016/j.biortech.2015.02.005
0960-8524/

pressure, which penetrates the cell wall by diffusion and achieves mechanical separation by the sudden pressure release, creating a shear force around the surrounding structure and resulting in the mechanical breakdown of lignocellulosic biomass cell walls. For the sudden steam explosion process, the deflation time should be short to achieve the full effect of steam explosion for destroying the lignocellulosic complex composed mainly of cellulose and lignin, increasing the accessibility of cellulose component to cellulase attack (Hendriks and Zeeman, 2009). From that point of view, the technical features of the steam explosion device itself limits the scaling-up application because deflation time is basically determined by the intrinsic structure of the equipment. In the scaling-up application the traditional valve blow mode operating equipment with a large valve requires a long deflation time, which in turn decreases the shearing effect on the biomass (Yu et al., 2012). Therefore, it is necessary to examine whether a high-temperature saturated steam treatment without the sudden release of steam pressure can provide an efficient pretreatment effect of biomass or not using various wood species. Furthermore, it is more desirable and practical if the large-scale process uses the high-temperature saturated steam treatment.

In this investigation, the feasibility of enzymatic saccharification of lignocellulosic biomass using a high-temperature saturated steam treatment was examined. The weight loss, chemical characteristics, and enzymatic saccharification of lignocellulosic biomass treated using high-temperature saturated steam with and without milling treatments were examined using softwood and hardwood biomass. Furthermore, the pretreatment effects of high-temperature saturated steam treatment on softwood and hardwood biomass were compared using cedar and beech, respectively.

2. Methods

2.1. Lignocellulosic biomass samples

Softwood biomass, cedar (*Cryptomeria japonica*), and hardwood biomass, beech (*Fagus japonica*), were cut into pieces approximately 10-cm-long, 3-cm-wide, and 1.5-cm-thick, and then treated by high-temperature saturated steam with and without milling as described below.

2.2. High temperature saturated steam treatment

The wood pieces were treated in a batch steam treatment apparatus (steam explosion apparatus NK-2L; Japan Chemical Engineering and Machinery Co. Ltd., Osaka, Japan) (Asada et al., 2012). The apparatus consisted of a steam generator, a pressurized digester, a receiver, and a condenser with a silencing action. The digester was insulated to maintain a constant temperature. The capacity of the digester was 2 L, the highest pressure was 6.7 MPa, and the highest temperature was 281 °C. Three wood pieces were introduced into the digester and exposed to high-temperature saturated steam at a pressure of 25 atm (225 °C), 35 atm (243 °C), and 45 atm (258 °C), and steaming time of 5 min. The prescribed temperature was reached in a few seconds. After saturated steam exposure, a ball valve at the top of the reactor was slowly opened to bring the digester to atmospheric pressure without applying shear force at a reduction speed of approximately 10 atm/min, and then the steam-treated wood pieces were collected from the digester for measuring the weight loss of steam-treated wood correctly.

2.3. Milling treatment

The steam-treated wood pieces were ground by a crusher mill (Wonder Crush Mill D3V-10, Osaka Chemical Co. Ltd., Osaka, Japan) at 25,000 rpm for 1 min. The particle sizes after milling were approximately 0.01–1 mm.

2.4. Fourier transform infrared (FTIR) analysis

Changes in the functional groups of the pretreated wood were recorded by FTIR spectrometry (FT/IR-670 Plus; JASCO, Japan). First, the samples were ground and dried at 105 °C. The sample (1.5 mg) was mixed with 200 mg potassium bromide (KBr). The role of KBr was to hold the fiber flour during the test. Transparent pellets were prepared from the blend and analyzed from 500 to 4000 cm^{-1}.

2.5. X-ray diffraction (XRD) analysis

The XRD patterns of pretreated wood were obtained by the X-ray diffractometer (RINT2000; RIGAKU, Japan). First, the samples were ground and dried at 105 °C. The sample was scanned in the range of 5–35° (2θ) with a scanning speed of 2° min^{-1} at 40 kV and 30 mA under 25 °C. The crystallinity index (CrI) of the sample was estimated according to the method proposed by Isogai and Usuda (1989).

$$\mathrm{CrI}\ (\%) = (I - I_B)/I \quad (1)$$

where I and I_B are the peak intensity at $2\theta = 16°$ and that of slope line at the same peak, respectively.

2.6. Extraction and component analysis

The amounts of the components, i.e., water soluble material, acetone soluble lignin, acid soluble lignin, acid insoluble material (a high-molecular weight lignin and ash), cellulose, and hemicellulose, in the steam-treated wood pieces with milling treatment were measured by the following extraction and separation procedure. One gram of each sample (in triplicate) was extracted with 60 mL of distilled water (DW) for 24 h at room temperature with stirring at 500 rpm. The solid and liquid materials were separated by filtration (ADVANTEC No. 131), and the filtrate (i.e., water soluble material) was recovered from the liquid, and then concentrated, dried, and weighed. The monomeric sugars, organic acids, 5-hydroxymethylfurfural (5-HMF), and furfural concentrations in the water-soluble material were determined by high performance liquid chromatography (HPLC) (LC-20AT HPLC system; Shimadzu, Kyoto, Japan) using an ion-exchange column (Aminex HPX-87H; Bio-Rad, Hercules, CA, USA: 300 × 7.8 mm; mobile phase, 5 mM H_2SO_4; temperature, 65 °C; flow rate, 0.6 mL/min; and injection volume, 10 μL) (Davis et al., 2006). The solid precipitate was extracted with 30 mL of acetone at room temperature for 24 h with stirring at 500 rpm to dissolve the acetone soluble lignin (a low-molecular weight lignin). After filtration (ADVANTEC No. 131), concentration, and drying of the extract, the acetone soluble lignin was weighed. The residue from the acetone extraction consisted of acid soluble lignin, acid insoluble material, cellulose, and hemicellulose. Thereafter, 0.2 g of this residue was added with 3 mL of 72%(w/w) sulfuric acid and kept at room temperature for 4 h. After 4 h, the mixture was transferred to a 100 mL conical flask and diluted with 75 mL DW, and then autoclaved for 1 h at 121 °C. After the sulfuric acid insoluble residue was washed with hot water, it was oven dried at 105 °C to the constant weight, and its mass was recorded (acid insoluble material). Acid soluble lignin in the hydrolyzed liquid was determined by UV spectrophotometry at 205 nm (Sluiter et al., 2012). Furthermore, the residue from the acetone extraction was hydrolyzed with 10 mL of 72%(w/w) sulfuric acid at 30 °C for 60 min. Then, the reaction mixture was diluted to 4%(w/w) sulfuric and autoclaved at 121 °C for 60 min. The amount of cellulose was determined on the basis of the monomer