Bioresource Technology 128 (2013) 188–192

Contents lists available at SciVerse ScienceDirect

Bioresource Technology

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

Effect of ionic liquid weight ratio on pretreatment of bamboo powder prior to enzymatic saccharification

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HIGHLIGHTS

- Biomass was pretreated with different ionic liquid (IL)/biomass ratios (0–10 g/g).
- Cholinium IL (less expensive and less toxic than conventional IL) was used.
- An IL/biomass ratio of 3 g/g was critical for sufficient biomass pretreatment.
- At an IL/biomass ratio of 3 g/g, biomass was not liquid but solid.
- The solid-state biomass pretreatment using IL can reduce cost and waste water.

ARTICLE INFO

Article history:
Received 10 July 2012
Received in revised form 20 October 2012
Available online 31 October 2012

Keywords:
Cholinium
Lignocellulose
Cellulose
Enzymatic hydrolysis
Ionic liquid/biomass ratio

ABSTRACT

The pretreatment efficiency of weight ratios ranging from 0 to 10 of the ionic liquid, cholinum IL, to bamboo powder was investigated. An IL/biomass ratio of 3 g/g was critical to obtain a cellulose saccharification ratio of 80%. At this ratio, the treated bamboo powder remained as a solid. The solid-state pretreatment required a minimum amount of cholinium IL, which could reduce the cost of IL-assisted pretreatment and reduce the amount of wastewater generated in the process.

1. Introduction

The biorefinery of lignocellulosic materials, such as waste woods and agricultural residues, into ethanol and other valuable metabolites generally consists pretreatment to enhance the subsequent enzymatic saccharification of cellulose and hemicellulose; enzymatic hydrolysis of cellulose and hemicellulose to fermentable sugars, and microbial fermentation of these sugars to ethanol or other metabolites (Adsul et al., 2011). Among these steps, pretreatment is an important unit operation because it greatly affects the efficiency and methodology used during the subsequent saccharification and fermentation processes.

Ionic liquids (ILs) are generally defined as organic salts that melt below 100 °C. They are thermally stable, non-volatile, and can dissolve polar and non-polar organic, inorganic, and polymeric compounds such as cellulose under mild conditions (Olivier-Bourbigou et al., 2010; Swatloski et al., 2002). Precipitation of dissolved cellulose produces a polymer with much higher enzymatic hydrolysis efficiency owing to its decreased crystallinity (Dadi et al., 2006). ILs can also dissolve lignocellulosic biomass (Fort et al., 2007; Kilpeläinen et al., 2007; Lee et al., 2009; Li et al., 2009).

Almost all of the ILs used for lignocellulose pretreatment contained imidazolium cations (Sun et al., 2010); however, these ILs are quite expensive. To reduce the cost of IL-assisted pretreatment of lignocellulosic materials, studies have investigated the reusability of ILs (Lee et al., 2009; Li et al., 2009; Nguyen et al., 2010; Shill et al., 2011; Wu et al., 2011) and the minimum IL amount required for lignocellulosic pretreatment (Wu et al., 2011).

A new-generation ILs has been synthesized by combining cholinium cations with amino acid-based ([Ch][AA] ILs) (Hu et al., 2007) or carboxylic acid-based anions ([Ch][CA] ILs) (Fukaya et al., 2007). These completely bio-derived cholinium ILs are less expensive than imidazolium ILs (Plechkova and Seddon, 2008).

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0960-8524/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.biortech.2012.10.097

Since cholinium ILs such as [Ch][AA] ILs and [Ch][CA] ILs can be used to pretreat lignocellulosic materials to enhance their subsequent enzymatic hydrolysis (Liu et al., 2012; Hou et al., 2012), the current study investigated the minimum amount of cholinium IL required, with the aim of further reducing the cost of IL-assisted pretreatment. In addition to investigating enzymatic hydrolysis, the crystallinity of cellulose in lignocellulose after pretreatment with different amounts of ILs was examined.

2. Methods

2.1. Lignocellulosic material and reagents

Ground bamboo powder (particle size approximately 1 mm) was used as the lignocellulosic material. Choline acetate (ChOAc) was used as a model cholinium IL since it has pretreatment abilities similar to those of imidazolium ILs. ChOAc was prepared using the one-pot neutralization method described by Yu et al. (2008) with minor modifications. Briefly, an equimolar amount of acetic acid was added dropwise to a choline hydroxide solution (45 wt.%) in methanol (Sigma–Aldrich, St. Louis, MO, USA) with cooling. The mixture was stirred at room temperature for 6 h. Water and methanol were removed *in vacuo* using a rotary evaporator at 40 °C for 1 h and at 90 °C for 2 h. The resulting residue was dried under vacuum at room temperature for 16 h. The water content of ChOAc was below 0.5 wt.% according to Karl-Fischer titration (Mettler Toledo, DL31). The chemical structure of the choline acetate was confirmed by ^{1}H- and ^{13}C-NMR. In the ^{1}H- and ^{13}C-NMR spectrum, there were no peaks attributed to impurities (data not shown). 1-ethyl-3-methylimidazolium acetate (EmimOAc) was purchased from Kanto Chemical Co. Inc. (Tokyo, Japan). Cellulase from *Trichoderma viride* [Meicelase, 6200 filter paper units (FPU) per gram] was kindly donated by Meiji Seika Pharma Co. Ltd. (Tokyo, Japan). All other chemicals were obtained from commercial sources and were of reagent grade.

2.2. Pretreatment for enzymatic hydrolysis

Bamboo powder (0.5 g) was mixed with 0–1.5 g of IL in plastic wrap or dissolved in 5 g of IL in a 30 mL vial. The resulting bamboo/IL mixture was heated in a dry oven at 110 °C without stirring, except for the IL/biomass ratio of 10, where the mixture was heated at 110 °C with magnetic stirring at 1200 rpm. (During the pretreatment process, the change in weight of IL/biomass sample was negligible.) After heating for 180 min, the bamboo/IL mixture was suspended with 45 mL of deionized water in a 50 mL tube. After stirring, the tube was centrifuged (8000*g*) for 10 min at 25 °C and the supernatant was removed. The washing procedure was repeated five times to remove the IL. The recovered bamboo was dried in an oven at 90 °C for 24 h, weighed gravimetrically, and ground into powder using a homogenizer (Fastprep® FP100A, MP Biomedicals LLC., Solon, OH, USA).

2.3. Composition analysis

Lignin and cellulose contents of the bamboo powder were determined by TAPPI methods (TAPPI, 1991, 2002) with minor modifications. Briefly, 0.1 g of bamboo powder was mixed with 2 mL of 72% (v/v) H_2SO_4 aqueous solution for 2 h at room temperature. The mixture was transferred to a 200 mL Erlenmeyer flask, diluted with 75 mL of water, and autoclaved at 121 °C for 15 min. The dilute acid hydrolysate was filtered and the amount of acid-insoluble lignin was determined gravimetrically by measuring the residue on the filter after drying at 100 °C for 12 h. The amount of acid-soluble lignin was determined from the UV absorbance of the filtrate at 205 nm using an absorption coefficient of 110 L g^{-1} cm^{-1} (TAPPI, 2002). The sum of both acid-insoluble and acid-soluble lignin was regarded as lignin. The amounts of glucose and hemicellulose in the hydrolysate were determined with a high-performance liquid chromatograph (HPLC) equipped with a refractive index (RI) detector (Shimadzu Co., Kyoto, Japan) using a CARBOSep CHO-682 column (Tokyo Chemical Industry Co., Ltd, Tokyo, Japan). The operating conditions were 85 °C with a water mobile phase at a flow rate of 0.4 mL/min. The amounts of cellulose and hemicellulose were calculated from the glucose and xylose contents multiplied by anhydrous correction factors of 162/180 and 132/150, respectively.

2.4. Enzymatic hydrolysis

Enzymatic hydrolysis of the bamboo powder was done in 30 mL vials at 50 °C using a rotary shaker at 130 rpm. The reaction mixture consisted of 0.03 g of bamboo powder and 5 mL of cellulase solution (62 U/mL) in 50 mM phosphate buffer (pH 5.0) with 1% toluene to prevent microbial growth. Samples were collected after 0 and 48 h and heated at 90 °C for 5 min to inactivate the enzyme. After centrifugation of the heated sample at 21,500*g* for 1 min, the glucose concentration of the supernatant was determined as described above. The cellulose saccharification ratio was calculated as the percentage of cellulose hydrolyzed into glucose to cellulose in bamboo recovered after pretreatment.

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2.5. X-ray diffractometry

For powder X-ray diffractometry (PXRD), the bamboo sample was scanned using a horizontal X-ray diffractometer equipped with Cu Kα radiation (Ultima IV, Rigaku Corporation, Tokyo, Japan) over the 2θ range of 5–40° with a scan step of 0.05°. The acceleration voltage and current were 40 kV and 30 mA, respectively. The background intensity without bamboo powder was subtracted from the sample intensity. The crystallinity index (CrI) was calculated from the PXRD data according to the peak height method (Segal et al., 1959) using the following equation

$$\mathrm{CrI} = \frac{I_{002} - I_{\mathrm{am}}}{I_{002}} \times 100$$

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

2.6. Fourier transform infrared spectrometry

For fourier transform infrared (FTIR) spectrometry, 10 mg of the bamboo sample was mixed with 50 mg of spectroscopic grade KBr, ground, and pressed to produce transparent pellets using a laboratory mill and press (QM-1 and QP-1, Chromato Science, Osaka, Japan). Analysis was conducted using an FTIR spectrometer (Nexus 470, Thermo Fisher cientific K.K., Yokohama, Japan) over the wave number range of 4000–400 cm^{-1}, with a resolution of 2 cm^{-1} and 32 scans per sample. The background spectrum of pure KBr was