Biochemical Engineering Journal 90 (2014) 90–95

Contents lists available at ScienceDirect

Biochemical Engineering Journal

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

Short communication

Microwave pretreatment of lignocellulosic material in cholinium ionic liquid for efficient enzymatic saccharification

Kazuaki Ninomiya [a], Takashi Yamauchi [b], Chiaki Ogino [c], Nobuaki Shimizu [a,*], Kenji Takahashi [b,**]

[a] Institute of Nature and Environmental Technology, Kanazawa University, Kakuma-machi, Kanazawa 920-1192, Japan
[b] Division of Material Engineering, Graduate School of Natural Science and Technology, Kanazawa University, Kakuma-machi, Kanazawa 920-1192, Japan
[c] Department of Chemical Science and Engineering, Graduate School of Engineering, Kobe University, 1-1 Rokkodaicho, Nada-ku, Kobe 657-8501, Japan

ARTICLE INFO

Article history:
Received 12 November 2013
Received in revised form 20 May 2014
Accepted 24 May 2014
Available online 2 June 2014

Keywords:
Ionic liquid
Microwave, Lignin
Cellulose
Cellulase
Bioconversion

ABSTRACT

We demonstrated that the enzymatic hydrolysis of cellulose after microwave pretreatment of lignocellulosic material in ionic liquids (ILs) is drastically enhanced compared with that after conventional thermal pretreatment in ILs. Three types of cholinium ILs, choline formate (ChFor), choline acetate (ChOAc), and choline propionate (ChPro), were examined. The cellulose saccharification percentage was approximately 20% for kenaf powders pretreated in ChFor, ChOAc, and ChPro by conventional heating at 110 °C for 20 min. In contrast, approximately 60–90% of cellulose was hydrolyzed to glucose after microwave pretreatment in the same ILs at 110 °C for 20 min.

© 2014 Elsevier B.V. All rights reserved.

1. Introduction

Lignocellulosic materials have gained attention as renewable sources of fermentable sugars for bioconversion into biofuel and platform chemicals, which are converted into value-added products [1]. These bioconversion processes generally consist of the following steps: (i) pretreatment of lignocellulose to enhance the subsequent enzymatic saccharification of cellulose and hemicellulose, (ii) enzymatic hydrolysis of cellulose and hemicellulose to fermentable sugars, and (iii) microbial fermentation of the sugars to biofuel and platform chemicals. However, the crystalline and rigid structures of cellulose and lignin prevent hydrolytic enzymes from accessing the polysaccharides [2]. Therefore, the pretreatment of lignocellulosic biomass is an important unit operation for disrupting the hydrogen bonds in crystalline cellulose and the covalent cross-linkages in the lignin structure to ensure efficient enzymatic hydrolysis of lignocellulose.

Ionic liquids (ILs), which are generally defined as organic salts that melt below 100 °C, have recently received a great deal of attention because they are designer fluids, thermally stable, nonvolatile, and capable of dissolving polar and nonpolar organic, inorganic, and polymeric compounds under mild conditions [3,4]. After it was first reported that ILs are capable of dissolving cellulose [5], it has been demonstrated that cellulose re-precipitated after dissolution in ILs exhibits much higher enzymatic hydrolysis owing to its decreased crystallinity [6]. Moreover, after Fort et al. [7] demonstrated that ILs can dissolve biomass, several groups have applied this IL-assisted method of cellulose pretreatment to various lignocellulosic materials [8–10]. However, in these reports, the dissolution of lignocellulosic biomass in ILs was performed by conventional heating at approximately 110 °C.

Recently, microwave technology has been recognized as a tool for the organic synthesis and processing of polymers [11,12]. Microwave heating is based on an internal heating process based on the direct absorption of energy by polar molecules, which differs from conventional heating that is based on heat transfer. With regard to applications of microwave heating to biomass dissolution in IL, Swatloski et al. [5] revealed that microwaves could increase the solubility of cellulose in IL. Moreover, previous studies have demonstrated that microwave irradiation enhances the dissolution of lignocellulosic materials in IL and subsequent fractionation of cellulose and lignin [13,14]. On the other hand, with regard to its application to IL-assisted biomass pretreatment for subsequent enzymatic hydrolysis, it has been recently demonstrated that

* Corresponding author. Tel.: +81 76 234 4807; fax: +81 76 234 4829.
** Corresponding author. Tel.: +81 76 234 4828; fax: +81 76 234 4829.
E-mail addresses: nshimizu@staff.kanazawa-u.ac.jp (N. Shimizu), ktkenji@staff.kanazawa-u.ac.jp (K. Takahashi).

http://dx.doi.org/10.1016/j.bej.2014.05.013
1369-703X/© 2014 Elsevier B.V. All rights reserved.

microwave irradiation can facilitate the pretreatment of cellulose in IL [15,16]. However, to the best of our knowledge, there has been no report on microwave pretreatment of lignocellulosic materials in IL.

Therefore, in this study, we demonstrated that the pretreatment of lignocellulosic biomass could be enhanced by microwave heating, compared with conventional heating at 110 °C. In particular, we employed the "wholly bio-derived ILs" using cholinium cation combined with carboxylic acid-based anions ([Ch][CA] ILs; [17]), which have been previously demonstrated to be capable of pretreating lignocellulosic materials under conventional heating [18,19]. To validate the methodology of microwave pretreatment in cholinium ILs for the enzymatic hydrolysis of lignocellulose, enzymatic hydrolysis data were compared with data obtained using the existing method of thermal pretreatment in cholinium ILs.

2. Materials and methods

2.1. Lignocellulosic material and reagents

Kenaf core fiber was supplied as a lignocellulosic material by Mitani Sangyo Co., Ltd. (Tokyo, Japan). The fiber was in the form of ground powder, with a size of approximately 1.0 mm. As cholinium ILs, choline formate (ChFor), choline acetate (ChOAc), and choline propionate (ChPro) were prepared by a one-pot neutralization method as described previously [20], with minor modifications [18,19]. In brief, an equimolar amount of carboxylic 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 (RT) for 6 h. Water and methanol were removed *in vacuo* using a rotary evaporator at 40 °C for 1 h and then at 90 °C for 2 h. The resultant residue was dried under vacuum at RT for 16 h. The water contents for the prepared ILs were determined to be below 0.5 wt% by Karl-Fischer titration (Mettler Toledo, DL31). Chemical structures of the prepared ILs were confirmed by ^{1}H and ^{13}C NMR. No peaks attributable to impurities were detected in the ^{1}H and ^{13}C NMR spectra. 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. Microwave pretreatment for enzymatic hydrolysis

Pretreatment for enzymatic hydrolysis was conducted by dissolving kenaf powder in ILs with continuous microwave irradiation, followed by regeneration and separation of the pretreated kenaf from IL. For the dissolution step, 0.25 g of kenaf powder was added to a test tube containing 5 g of IL. The kenaf/IL mixture in the test tube was irradiated with an output power of 100 W at a frequency of 2450 ± 50 MHz using a microwave reactor (green motif Ic, IDX Co., Ltd., Sano, Japan) maintained at 110 °C for 0–20 min. As a control pretreatment, the kenaf/IL mixture in the test tube was heated at 110 °C in a dry oven with magnetic stirring (1200 rpm) for 0–20 min. Another round of control pretreatments was performed at 90 °C using 50 mM phosphate buffer (pH = 5.0) instead of ILs.

For recovering the dissolved kenaf from IL, the kenaf/IL mixture was diluted with 45 mL of deionized water in a 50-mL tube, resulting in the precipitation of kenaf. After stirring, the 50-mL tube was centrifuged (8000 × *g*) for 10 min at 25 °C, and the supernatant were removed. The washing procedure was repeated five times to remove IL. The recovered kenaf was then dried in an oven at 90 °C for 24 h, gravimetrically measured, and ground into powder using a homogenizer (Fastprep® FP100A, MP Biomedicals LLC., Solon, OH). The resultant kenaf powder was subjected to enzymatic hydrolysis and other analyses described below. The pretreatment and analyses were performed in duplicate.

2.3. Compositional analysis

Lignin and cellulose contents of kenaf powder were determined using TAPPI methods [21,22], with minor modifications. In brief, 0.1 g of kenaf powder was mixed with 2 mL of a 72% (v/v) H_2SO_4 aqueous solution at RT for 2 h. The mixture was then 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 then filtered, following which the amount of acid-insoluble lignin was determined by gravimetrically measuring the residue on the filter after drying at 100 °C for 12 h. The amount of acid-soluble lignin was determined by UV absorbance of the filtrate at 205 nm and the absorption coefficient of 110 L g^{-1} cm^{-1} [21]. The sum of both acid-insoluble and acid-soluble lignin was regarded as lignin. The amount of glucose and xylose in the hydrolysate was determined by HPLC equipped with an RI detector (Shimadzu Co., Kyoto, Japan) using a CARBOSep CHO-682 column (Tokyo Chemical Industry Co., Ltd., Tokyo, Japan). The operating conditions were a temperature of 85 °C with a water mobile phase and a flow rate of 0.4 mL/min. The amount of cellulose and hemicellulose was calculated from glucose and xylose contents multiplied by anhydro correction factors of 162/180 and 132/150, respectively.

2.4. Enzymatic hydrolysis

Enzymatic hydrolysis of kenaf powder was conducted in 30-mL vials at 50 °C using a rotary shaker at 130 rpm. The reaction mixture consisted of 0.03 g of kenaf powder and 5 mL of cellulase solution (62 FPU/mL) in 50 mM phosphate buffer (pH = 5.0) with 1% toluene to prevent contamination. Samples were collected after 0 and 48 h and then 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 and xylose concentration of the supernatant was determined using the HPLC as described above. The cellulose saccharification was evaluated as the percentage of cellulose hydrolyzed into glucose to cellulose in kenaf recovered from the pretreatment. The hemicellulose saccharification was also evaluated as the percentage of hemicellulose hydrolyzed into xylose to hemicellulose in kenaf recovered from the pretreatment.

3. Results and discussion

3.1. Recovery and component of lignocellulosic biomass after microwave pretreatment in IL

As pretreatment for enzymatic hydrolysis, kenaf powder was dissolved in ILs with continuous microwave irradiation for 0–20 min, following which it was recovered as a precipitate by adding antisolvents (water). The percentage of recovered kenaf powder was approximately 80% of the untreated originals, regardless of the solvent (buffer or ILs), external energy (heat or microwave), and pretreatment time (Fig. 1). This indicates that a 10–20% loss of kenaf powder is inevitable and intrinsic during the pretreatment and washing steps [23].

To examine the composition of kenaf powder pretreated by microwave irradiation in ILs, cellulose and lignin contents were determined. Untreated kenaf powder contained approximately 35% cellulose, 30% lignin, and 35% hemicellulose (Fig. 2). The composition did not change significantly after pretreatment for up to 20 min regardless of whether the samples were pretreated by heating or microwave irradiation, when phosphate buffer was used instead of IL (Fig. 2A). On the other hand, slight decrease was observed in the percentage of cellulose and hemicellulose with the prolonged

Download English Version:

https://daneshyari.com/en/article/3025

Download Persian Version:

https://daneshyari.com/article/3025

Daneshyari.com