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

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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.

Keywords:

Ionic liquid; Microwave, Lignin; Cellulose; Cellulase; Bioconversion:

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 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 ¹H- and ¹³C-NMR. No peaks attributable to impurities were detected in the ¹H- and ¹³C-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) H₂SO₄ 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 $110 \text{ L g}^{-1} \text{ cm}^{-1}$ [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 $21,500 \times g$ 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 microwave pretreatment in the IL. This might be attributable to that a partially hydrolyzed (hemi)cellulose was lost during washing of the IL. However, it was suggested that microwave irradiation did not excessively degrade the specific macromolecules in kenaf powder under the examined conditions.

3.2. Enzymatic hydrolysis of lignocellulosic biomass after microwave pretreatment in IL

To examine the effects of microwave heating during pretreatment in ILs on subsequent enzymatic hydrolysis, kenaf powder pretreated for 0–20 min was subjected to enzymatic hydrolysis for 48 h. The cellulose saccharification percentage reached

saturation values within 48 h, irrespective of the solvent (buffer or ILs) and pretreatment time (Fig. 3). Except for the cases involving phosphate buffer (Fig. 3A), the cellulose saccharification percentage increased with an increase in microwave pretreatment time (Fig. 3B-D). Thus, microwave irradiation enhances the efficacy of lignocellulose pretreatment in cholinium ILs.

A set of control experiments was also conducted using conventional heating. In the experiment where phosphate buffer was used instead of IL, the cellulose saccharification percentage was approximately 20% after pretreatment for up to 20 min, regardless of whether the samples were pretreated by heating or microwave irradiation (Fig. 4A). The cellulose saccharification percentage remained at approximately 20% throughout the pretreatment time for up to 20 min, even for kenaf powder thermally pretreated in ChFor, ChOAc, and ChPro (open keys in Fig. 4B-D). In contrast, for kenaf powder pretreated by microwave irradiation in these ILs, the cellulose saccharification percentage significantly increased with an increase in pretreatment time (closed keys in Fig. 4B-D). Specifically, almost 90% of cellulose was converted into glucose when kenaf powder was pretreated in ChOAc and ChPro for 20 min. Moreover, the microwave-assisted pretreatment also enhanced the hemicellulose saccharification percentage (Fig. 5). Here, the glucose yield and xylose yield on original biomass basis were also calculated considering the biomass recovery (Fig. 1) and the compositional change (Fig. 2) (see Fig S1 and S2, respectively), indicating that the microwave-assisted pretreatment enhanced the yields. Thus, compared with the conventional pretreatment method using cholinium IL and heating, pretreatment with a combination of cholinium IL and microwave irradiation can significantly enhance the enzymatic hydrolysis of kenaf powder.

3.3. Comparison between microwave and conventional heating for biomass pretreatment in ILs

We previously demonstrated that the cellulose saccharification percentage in kenaf powder reached almost 90% when the biomass was pretreated using [Ch][CA] ILs under conventional heating at 110°C for 16 h [18]. However, the present study revealed that microwave pretreatment in ILs could not only achieve the same levels of cellulose saccharification but also drastically reduce the pretreatment time to 20 min. This enhanced efficiency by microwave pretreatment in ILs may be attributable to the following: 1) Microwave heating is characterized by an internal heating process owing to the direct absorption of energy by polar molecules. ILs can efficiently absorb microwave radiation because they only consist of anions and cations [24,25]. Thus, ILs with high polarity could be easily heated by microwave irradiation, and the resultant internal heating may be responsible for the more effective breakdown of the H-bond network between cellulose microfibrils. 2) Microwave enhanced the diffusion of IL molecules and collision frequency between anions and cations of IL and the wood macromolecules. This may facilitate the inter- and intramolecular hydrogen bonding interactions between cellulose microfibrils and anions of IL, resulting in more rapid pretreatment. There was no positive effect of microwave pretreatment in phosphate buffer on the cellulose saccharification percentage, indicating that microwave irradiation itself had no sufficient pretreatment effect to enhance enzymatic hydrolysis under the examined conditions (closed keys in Fig. 4A). Therefore, the combination of microwave irradiation and ILs synergistically enhanced the cellulose saccharification percentage of kenaf powder.

With regard to energy saving, Wang et al. [14] compared the energy requirement for microwave heating and conventional heating during biomass treatment in ILs. They approximately estimated that the energy required in microwave treatment in ILs was much less than that required in conventional heating. The energy required in microwave pretreatment was also calculated under the conditions employed in the present study. By multiplying the power (100 W) with pretreatment time (20 min), the energy required for pretreatment with microwave heating is determined to be 1.2×10^5 J. On the other hand, the energy required for pretreatment with conventional heating at 110°C for 16 h is determined to be 1.1×10^7 J [14]. Thus, it was suggested that the combination of microwave irradiation and ILs could save energy for pretreatment as well as enhance pretreatment efficiency for cellulose saccharification.

For large-scale application of ILs to the biomass pretreatment process, development of efficient and energy-saving IL recovery methods is also important. Evaporation or ion-exclusion chromatography has been generally used to recycle ILs; however, these methods are costly and complex for large-scale application. Therefore, further research on IL recovery methods is necessary for realization of the IL-based biomass pretreatment process.

4. Conclusions

Microwave pretreatment in ILs was more effective than conventional thermal pretreatment in ILs for enzymatic hydrolysis of kenaf powders. Approximately 40%–70% improvement in the cellulose saccharification percentage was obtained with microwave pretreatment at 110°C in ChFor, ChOAc, and ChPro for 20 min compared with that obtained with thermal pretreatment at 110°C in these ILs.

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Figure captions

Fig. 1 Percentages of kenaf powder recovered after pretreatment in ILs for prescribed times. Open circles: thermal pretreatment in IL; closed circles: microwave pretreatment in IL. (A) Phosphate buffer, (B) ChFor, (C) ChOAc, and (D) ChPro.

Fig. 2 Content of cellulose (bottom of stacked bars), hemicellulose (middle of stacked bars), lignin, and others (top of stacked bars) in kenaf powder pretreated in ILs for prescribed times. Open bars: thermal pretreatment in IL; closed bars: microwave pretreatment in IL. (A) Phosphate buffer, (B) ChFor, (C) ChOAc, and (D) ChPro.

Fig. 3 Time courses of cellulose saccharification percentage during enzymatic hydrolysis of kenaf powder pretreated in IL with microwave irradiation. Circles: pretreated for 0 min; triangles: pretreated for 5 min; squares: pretreated for 10 min; diamonds: pretreated for 15 min; inverse triangles: pretreated for 20 min. (A) Phosphate buffer, (B) ChFor, (C) ChOAc, and (D) ChPro.

Fig. 4 Cellulose saccharification percentages of kenaf powder pretreated in IL for prescribed times. The percentages were obtained 48 h after enzymatic hydrolysis reaction. Open circles: thermal pretreatment in IL; closed circles: microwave pretreatment in IL. (A) Phosphate buffer, (B) ChFor, (C) ChOAc, and (D) ChPro.

Fig. 5 Hemiellulose saccharification percentages of kenaf powder pretreated in IL for prescribed times. The percentages were obtained 48 h after enzymatic hydrolysis reaction. Open circles: thermal pretreatment in IL; closed circles: microwave pretreatment in IL. (A) Phosphate buffer, (B) ChFor, (C) ChOAc, and (D) ChPro.

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