Ionic liquid/ultrasound pretreatment and *in situ* enzymatic saccharification of bagasse using biocompatible cholinium ionic liquid

Kazuaki Ninomiya^a, Asami Kohori^b, Mai Tatsumi^b, Koji Osawa^b, Takatsugu Endo^b,
Ryohei Kakuchi^b, Chiaki Ogino^c, Nobuaki Shimizu^a, and Kenji Takahashi^{b,*}

^aInstitute of Nature and Environmental Technology,

Kanazawa University, Kakuma-machi, Kanazawa 920-1192, Japan

^bFaculty of Natural System, Institute of Science and Engineering, Kanazawa University,
Kakuma-machi, Kanazawa 920-1192, Japan

^cDepartment of Chemical Science and Engineering, Graduate School of Engineering,
Kobe University, 1-1 Rokkodaicho, Nada-ku, Kobe 657-8501, Japan

* Corresponding author.

Tel.: +81 76 234 4828; fax: +81 76 234 4829

E-mail address: ktkenji@staff.kanazawa-u.ac.jp (K. Takahashi)

Abstract

Choline acetate (ChOAc), a cholinium ionic liquid (IL), showed almost the same bagasse pretreatment capability as 1-ethyl-3-methylimidazolium acetate (EmimOAc), a conventional imidazolium IL used for biomass pretreatment. Moreover, ChOAc showed less of an inhibitory effect on cellulase than EmimOAc. Thus, ChOAc was used for IL/ultrasound-assisted pretreatment and *in situ* enzymatic saccharification, where IL was not washed out from the pretreated bagasse but diluted with the addition of a buffer solution. When *in situ* saccharification was performed for 48 h in the presence of 10% ChOAc, the cellulose and hemicellulose saccharification percentages were 80% and 72%, respectively. When ChOAc was increased to 20%, the saccharification percentages were 72% and 53%, respectively. However, the values were just 28% and 2%, respectively, in case of 20% EmimOAc. A glucose/xylose solution free from IL and ChOAc aqueous solution without these sugars could be recovered separately by electrodialysis of the hydrolysate of *in situ* saccharification.

Keywords:

Cholinium, Ionic liquid, Pretreatment, *in-situ* saccharification, Lignocellulose.

1. Introduction

Ionic liquids (ILs), generally defined as organic salts that melt below 100°C, have received much attention of late, because they are thermally stable, nonvolatile, and capable of dissolving various polymeric compounds even under mild conditions. A previous study demonstrated that ILs can dissolve cellulose (Swatloski, et al., 2002), and that cellulose re-precipitated after being dissolved in ILs exhibits a much greater efficiency of enzymatic hydrolysis due to its decreased crystallinity (Dadi, et al., 2006). Moreover, several groups have extended this IL-assisted pretreatment method to various lignocellulosic biomasses (Brandt, et al., 2013). It has been demonstrated that the IL-assisted pretreatment methods are more effective than the conventional methods using diluted acid or ammonia (Li, et al., 2010; Li, et al., 2011).

In the IL-assisted pretreatment process, the pretreated biomass has to be washed extensively so as to remove the residual IL because the residual IL in pretreated biomass causes inhibition of cellulolytic enzymes and fermentative microorganisms during saccharification and fermentation (Datta, et al., 2010; Ouellet, et al., 2011). However, this extensive washing of pretreated biomass results in large amounts of diluted IL aqueous solution, which leads to a high cost for concentrating IL from its diluted aqueous solution by evaporation and for treating the resultant wastewater. The cost issue can be an obstacle for scale-up of IL-assisted pretreatment processes.

Recently, researchers have suggested a simple process comprising IL-assisted pretreatment and *in situ* enzymatic saccharification without washing out IL from the pretreated biomass and with the addition of a smaller amount of water for diluting IL in order to save costs derived from the extensive washing step (Kamiya, et al., 2008; Yang, et al., 2010; Li, et al., 2010; Feng, et al., 2011; Wang, et al., 2011; Li, et al., 2012; Abels,

et al, 2013; Shi, et al, 2013; Xu, et al., 2014). Among these, some reports further demonstrated the separation of a monomeric sugar solution and an IL aqueous solution after *in situ* enzymatic saccharification (Li, et al., 2010; Feng, et al., 2011; Li, et al., 2012; Abels, et al., 2013; Shi, et al., 2013). To reduce the water use for diluting IL and to achieve the higher sugar yield/concentration in the *in situ* enzymatic saccharification, the following factors are important: 1) high loading of biomass to IL (less IL amount per unit biomass) during the IL pretreatment step, reducing water use per unit biomass; 2) use of biocompatible IL and/or IL-tolerant cellulase enzymes, which reduce water use for diluting IL before enzymatic saccharification because a higher IL concentration is possible. Some *in situ* enzymatic saccharification has even been performed by employing biocompatible IL such as 1-ethyl-3-methylimidazolium diethylphosphate (EmimDEP) (Kamiya, et al., 2008), 1,3-dimethylimidazolium dimethylphosphate (MmimDMP) (Yang, et al., 2010; Li, et al., 2010; Feng, et al., 2011; Abels, et al., 2013), as well as conventional IL such as 1-ethyl-3-methylimidazolium acetate (EmimOAc) (Wang, et al., 2011; Shi, et al., 2013). Others employed the IL tolerant cellulase (Shi, et al., 2013). Moreover, recently, both biocompatible IL [1-ethyl-3-methylimidazolium dimethylphosphate (EmimDMP)] and IL tolerant cellulase were used simultaneously (Xu, et al., 2014). However, to the best of our knowledge, all IL used for *in situ* saccharification was limited to the imidazolium IL.

Completely bio-derived cholinium ILs were reported in 2007 that contain cholinium cations combined with amino acid-based anions (Hu, et al., 2007) or carboxylic acid-based anions (Fukaya, et al., 2007). In previous studies, it was reported that these cholinium ILs could be used for pretreatment to enhance the enzymatic hydrolysis of lignocellulosic material (Liu, et al., 2012; Ninomiya, et al., 2013a,b,c).

Moreover, our previous study demonstrated that choline acetate (ChOAc) was less toxic for fermentative microorganisms than EmimOAc (Ninomiya, et al., 2013b). However, to the best of our knowledge, there is no report on *in situ* enzymatic saccharification using cholinium IL as a candidate for biocompatible IL.

Therefore in the present study, we employ ChOAc as a biocompatible cholinium IL in order to demonstrate the IL pretreatment and *in situ* enzymatic saccharification of lignocellulosic biomass. The data for pretreatment capability, inhibition to commercial cellulase, and performance of the *in situ* saccharification process were then compared with data obtained using the standard imidazolium IL, EmimOAc. Moreover, we have demonstrated electrodialysis-assisted separation of IL and monomeric sugar obtained by *in situ* enzymatic saccharification.

2. Materials and Methods

2.1. Biomass, IL, and cellulase

Bagasse powder (approximately <3 mm) was purchased from Sanwa Cellulosin Co., Ltd. (Yokkaichi, Japan) as the lignocellulosic material. The bagasse powder was ground by a mill and then sieved to obtain a powder of 250–500 μm . Microcrystalline cellulose (Avicel[®] PH-101) was purchased from Sigma–Aldrich (St. Louis, MO, USA). Phosphoric acid swollen cellulose (PASC) was prepared from the microcrystalline cellulose according to the method described previously (Wood, 1988).

Choline acetate (ChOAc) was prepared by a one-pot neutralization method (Yu, et al., 2008) with minor modifications. An equimolar amount of acetic acid was added dropwise to a choline hydroxide solution (45 wt%) in methanol (Sigma–Aldrich) 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 90°C for 2 h. The resultant residue was dried under a vacuum at RT for 16 h. The water content for ChOAc was measured below 0.5 wt% by Karl-Fischer titration (AQ-2200, Hiranuma Sangyo Co., Ltd, Mito, Japan). 1-ethyl-3-methylimidazolium acetate (EmimOAc) was purchased from Kanto Chemical Co., Inc. (Tokyo, Japan).

A commercial cellulase Cellic® CTec2, a complex blend of cellulase, hemicellulose, and β -glucosidase, (batch number VCNI 0008, 106 filter paper units (FPU) per milliliter) was purchased from Novozymes (Franklinton, NC, USA). The FPU of the cellulase was determined by the method reported by NREL (Adney and Baker, 2008). All other chemicals were from commercial sources and of reagent grade.

2.2. IL-assisted pretreatment and *in situ* enzymatic saccharification

Bagasse was pretreated with IL and then hydrolyzed enzymatically without washing out IL from the treated bagasse. For the pretreatment step, 0.25 g of bagasse powder was added to a 15 mL Corning tube containing 5 g of IL (EmimOAc or ChOAc). The biomass/IL mixture in the tube was sonicated, since our previous study revealed that sonication was more effective than conventional heating (Ninomiya, et al., 2013c). The ultrasound was irradiated for 60 min at 24 kHz and an emission power of 35 W using an ultrasonic processor (UP201S with sonotrode S3, Hielscher Ultrasonics GmbH, Teltow, Germany) in a water bath maintained at 25°C. Bagasse powder without the IL pretreatment was used as a negative control sample, when necessary.

For the subsequent step of enzymatic hydrolysis, the biomass/IL mixture was diluted with 45, 20, 11.7, and 7.5 mL of 50 mM phosphate buffer (pH = 5.0) to adjust the IL concentration to 10, 20, 30, and 40 wt%. For the positive control, IL was washed

out from the pretreated biomass. The biomass/IL mixture was diluted with 45 mL of deionized water in a 50-mL tube, which resulted in the precipitation of the biomass. After stirring, the 50-mL tube was centrifuged ($8,000 \times g$) for 10 min at 25°C , and the supernatant was removed. The washing procedure was repeated five times to remove IL. Then, the biomass was re-suspended in 5 mL of the phosphate buffer, where IL concentration was assumed to be 0 wt% in this study. A cellulase solution was added to the biomass suspension at 40 FPU/g-dry bagasse. The enzymatic hydrolysis reaction was conducted in 100-mL vials at 50°C using a reciprocal shaker at 130 rpm.

During the enzymatic reaction, samples were collected after 0, 3, 6, 9, 12, 24, 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 concentrations in the hydrolysate were determined by high-performance liquid chromatography (HPLC), as described below. Cellulose saccharification was evaluated as the percentage of cellulose hydrolyzed into glucose from cellulose in the original bagasse. In the same manner, hemicellulose saccharification was evaluated as the percentage of hemicellulose hydrolyzed into xylose from hemicellulose in the original bagasse.

2.3. Electrodialysis-assisted separation of IL and monosaccharide

An electrodialysis system (DW-Lab, AGC Engineering. Co., Ltd, Chiba, Japan) was used to remove salt, in this case IL, from the *in situ* enzymatic hydrolysate, which contains soluble sugars and IL in a buffer solution. The electrodialysis system comprises a membrane stack, circulation pumps for each of three compartments (desalination tank, concentration tank, and electrolyte tank: working volumes are all 250 mL), and a DC power supply (PMC18-3A, Kikusui electronics Corp., Yokohama, Japan) (Fig. S1). The

membrane stack has 5 pairs of anion-exchange and cation-exchange membranes (SELEMION[®], AGC Engineering. Co., Ltd) with an effective area of 50 × 60 mm. For the desalination tank, the hydrolysate of the *in situ* enzymatic saccharification (containing the soluble sugars and the IL) was added to the solution to be desalinated, where the insoluble saccharification residue (mainly lignin) was in advance removed from the hydrolysate. For the concentration tank, 250 mL of distilled water was added as the initial solution. For the electrolyte tank, 250 mL of Na₂SO₄ aqueous solution (4 wt%) was added as an electrode rinse solution. These three solutions were circulated at a flow rate of 4 L/min, respectively. The applied voltage was set to be 8 V throughout the operation.

During the electrodialysis, samples were collected from the desalination tank. The glucose and xylose concentrations in the desalination tank were determined by HPLC, as described below. IL (ChOAc) concentrations in the desalination tank were also determined by HPLC.

2.4. Compositional analysis of biomass

The cellulose, hemicellulose, and lignin contents of the original bagasse were determined according to the NREL method (Sluiter, et al., 2012) with minor modifications. In brief, 0.1 g of the sample was mixed with 2 mL of a 72% (v/v) H₂SO₄ aqueous solution for 2 h at RT. 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 acid-diluted hydrolysate was filtered, after which the amount of acid-insoluble lignin was gravimetrically determined 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 ultraviolet

(UV) absorbance of the filtrate at 205 nm and an absorption coefficient of 110 L g⁻¹ cm⁻¹. The sum of the acid-insoluble and acid-soluble lignin was regarded as the total lignin amount. The amount of sugars in the hydrolysate was determined by 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 column was run at 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 the glucose and xylose content multiplied by anhydro correction factors of 162/180 and 132/150, respectively.

2.5. Powder X-ray diffractometry (PXRD)

For PXRD, the biomass (bamboo powder, microcrystalline cellulose, or alkali lignin) was scanned using a horizontal X-ray diffractometer equipped with Cu-K α radiation (Ultima IV, Rigaku Corporation, Tokyo, Japan) over the 2 θ range 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 the sample was subtracted from the intensity obtained for respective samples. The crystallinity index (CrI) was calculated from PXRD data according to the peak-height method using the following equation:

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

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

3. Results and discussion

3.1. Pretreatment capability of ChOAc or EmimOAc for enzymatic hydrolysis of bagasse

To examine the pretreatment capability of ChOAc or EmimOAc for subsequent enzymatic hydrolysis of lignocellulosic biomass, bagasse powder was pretreated in each IL and then hydrolyzed by Ctec2 cellulase for 48 h, where the IL was washed out from the pretreated biomass before saccharification to eliminate any inhibitory effect of IL on the cellulolytic enzyme. Figure 1 shows the time profile for the enzymatic reaction of pretreated bagasse up to 48 h. Without IL pretreatment, cellulose and hemicellulose saccharification percentages were 24% and 9% of the theoretical maximum at 48 h. In contrast, with ChOAc pretreatment, cellulose and hemicellulose saccharification percentages increased with an elapsed enzymatic reaction time, reaching 85% and 100% of the theoretical maximum at 48 h. These time profiles are approximately in line with the cases using EmimOAc, although the cellulose saccharification percentage was somewhat less than the case of EmimOAc. These results indicated that ChOAc had a sufficient pretreatment capability for lignocellulosic materials, which was at almost the same level as EmimOAc under the examined pretreatment condition.

To examine the cellulose crystallinity in the bagasse powder pretreated in ChOAc or EmimOAc with ultrasound irradiation, XRD analysis was conducted and the CrI was determined on the basis of the XRD spectra. The CrI values were 61.9%, 49.1%, and 41.7% for the original biomass, biomass pretreated in ChOAc, and biomass pretreated in EmimOAc, respectively (Table S1). These results was roughly consistent with the cellulose saccharification results shown in Fig 1A, suggesting that ChOAc had a substantial pretreatment capability for lignocellulosic biomass

EmimOAc has been frequently thought of as the most effective IL for cellulose dissolution and pretreatment of enzymatic hydrolysis (Brandt, et al., 2013). Pretreatment with ChOAc enhanced the cellulose saccharification to the same degree as pretreatment

with EmimOAc (Fig 1), suggesting that the acetate anion has an important role in the reduction of cellulose crystallinity. In general, anions act as hydrogen bond acceptors that interact with the hydroxyl group of cellulose, thereby weakening the crystalline structure of cellulose (Remsing, et al., 2006), whereas cations interact with lignin through hydrogen bonding and π - π interactions (Janesko, 2011). In contrast, the inhibitory effects of ChOAc on cellulase were significantly lower than that of EmimOAc (Fig. 2), because the cholinium cation was reported to be more biocompatible than the imidazolium cation (Ninomiya, 2013; Petkovic, et al., 2010; Hou, et al., 2013). Therefore, it is suggested that ChOAc is a promising alternative to EmimOAc for the IL pretreatment and for *in situ* saccharification of lignocellulosic biomass.

3.2. Inhibitory effect of ChOAc or EmimOAc on cellulolytic enzyme

To examine the inhibitory effect of ChOAc or EmimOAc on cellulase enzyme, enzymatic saccharification of the PASC as amorphous cellulose was conducted for 24 h using Ctec2 cellulase in the presence of the prescribed concentration of each IL. Figure 2 shows dose-response relationships between PASC saccharification activity and IL concentration. ChOAc shows a significantly smaller inhibitory effect on cellulase compared with EmimOAc. The half maximal (50%) inhibitory concentration (IC_{50}) for Ctec2 cellulase was approximately 32 wt% and 16 wt% for ChOAc and EmimOAc, respectively. Thus, it was found that ChOAc shows less of an inhibitory effect on cellulase compared with EmimOAc under the examined conditions.

In general, cellulase enzymes in the IL aqueous solution lose their activity due to a higher salt concentration and ionic strength. Thus, a more biocompatible IL has been

screened so as to perform the *in situ* enzymatic saccharification effectively. As the biocompatible candidate in imidazolium IL, MmimDMP was often studied with regard to its pretreatment capability of biomass (Yang, et al., 2010; Li, et al., 2010), its inhibitory effect on cellulase (Li, et al., 2010; Engel, et al., 2010; Wahlström, et al., 2010), and its application to *in situ* saccharification processes (Yang, et al., 2010; Li, 2010; Feng, et al., 2011; Abels, et al., 2013). On the other hand, the present study for the first time compared the cholinium IL (ChOAc) and the imidazolium IL (EmimOAc) in terms of the pretreatment capability of biomass (Fig. 1), the inhibitory effects on cellulase (Fig. 2) and the performance of *in situ* enzymatic saccharification (Fig. 3). Some researchers (Wahlström, et al., 2014) demonstrated that MmimDMP has a smaller inhibitory effect on cellulase than EmimOAc using the endoglucanases (such as Cel7B or Cel5A) and microcrystalline cellulose, which were different experimental conditions from the present study. There have been no reports on a direct comparison between ChOAc and MmimDMP in terms of inhibitory effects on cellulase enzymes and *in situ* saccharification performance under the same conditions (type of cellulase and substrate, cellulase loading to biomass, cellulase reaction time, i.e., exposure time to IL, and so on). We are now investigating this point to explore the appropriate IL for *in situ* saccharification. Moreover, it is necessary to employ an IL-tolerant cellulase such as thermophilic cellulase (Datta, et al., 2010; Shi, et al., 2013) as well as biocompatible IL for further reduction of water use for diluting IL prior to *in situ* saccharification.

3.3. IL pretreatment and *in situ* saccharification using ChOAc or EmimOAc

To demonstrate the IL pretreatment and *in situ* enzymatic saccharification of lignocellulosic biomass using ChOAc or EmimOAc, bagasse powder was pretreated in

each IL and then *in situ* hydrolyzed by Ctec2 cellulase for 48 h after diluting to the prescribed IL concentration without washing out the IL from the pretreated biomass. Figure 3 shows the cellulose and hemicellulose saccharification percentage obtained at 48 h of the *in situ* enzymatic saccharification with different IL concentrations. In the cases for ChOAc, the cellulose and hemicellulose saccharification percentages were larger under the examined IL concentrations, ranging from 10 to 40%. When the *in situ* saccharification was conducted in the presence of 10% ChOAc, the cellulose and hemicellulose saccharification percentages were 80% and 72%, respectively. In the case of 20% ChOAc, the cellulose and hemicellulose saccharification percentages reached 72% and 53%, respectively. In contrast, in the case of 20% EmimOAc, the cellulose and hemicellulose saccharification percentages were just 28% and 2%, respectively. Thus, it was found that ChOAc was more suitable for the *in situ* enzymatic saccharification process than EmimOAc.

The biomass-to-sugar conversion process employed in this study has been referred as “IL pretreatment and *in situ* saccharification” or “one-pot IL pretreatment and saccharification.” The present study is the first to demonstrate ChOAc showing both biomass pretreatment capability and biocompatibility for cellulase, and then to apply ChOAc to the *in situ* saccharification of lignocellulosic material. This *in situ* saccharification eliminates the conventional step of washing out IL from the pretreated biomass, which can reduce the amount of wastewater and can prevent a “wash out” of soluble oligosaccharides generated during the biomass pretreatment with IL (typically seen in the case of EmimOAc (Table S1)). However, according to the techno-economic analysis for the overall process of *in situ* saccharification (Konda, et al., 2014), it is needed to satisfy the high biomass loading (33–50 wt%) during the pretreatment step as

well as the low IL cost (\$2.5–0.7/kg) and the high IL recovery (97–99.6%) for ensuring economic sustainability. Our previous study (Ninomiya, et al., 2013a) revealed that ChOAc pretreatment at a biomass loading of 25 wt% (biomass: IL = 1: 3) did not deteriorate pretreatment efficiency (i.e., cellulose saccharification), but the cellulose saccharification percentage decreased to 50–60% when biomass loading was increased to 50 wt% (biomass: IL = 1: 1). Thus, this is required to enhance pretreatment efficiency even when the biomass loading is 50 wt% by a combined use of IL and microwave irradiation. This process will make the *in situ* saccharification process more economical and environmentally friendly due to less IL use and less wastewater discharge.

3.4. Removal of IL from hydrolysate of *in situ* saccharification by electrodialysis

To obtain the monosaccharide solution free from IL after the *in situ* enzymatic saccharification, electrodialysis was performed to remove IL from the hydrolysate of *in situ* saccharification, containing soluble sugars and ChOAc. Figure 4 shows the time courses of glucose, xylose, and ChOAc concentrations in the desalination tank during electrodialysis. The hydrolysate was obtained from the *in situ* enzymatic saccharification at 10 wt% of ChOAc concentration. ChOAc concentration decreased with an elapsed time of electrodialysis, reaching almost zero at 72 min. Glucose and xylose concentrations did not decrease, and rather slightly increased due to the removal of ChOAc from the hydrolysate. In contrast, in the concentration tank of the electrodialyzer, glucose and xylose were not detected even after 120 min of electrodialysis. The recovery percentage of ChOAc was 99% at the 120 min point. Thus, it was found that a monosaccharide solution free from IL and IL aqueous solution without soluble sugars could be obtained through electrodialysis of the hydrolysate of

the *in situ* enzymatic saccharification.

The *in situ* saccharification is free from the washing out of IL from the pretreated biomass, and instead requires the separation of IL from the hydrolysate of the pretreated biomass. To date, to separate IL from the hydrolysate of *in situ* enzymatic (or chemical) saccharification, various principles have been employed such as ion-exclusion chromatography (Li, et al., 2010; Feng, et al., 2011; Sun, et al., 2013), electrodialysis with an ion-exchange membrane (Abels, et al., 2013), liquid–liquid extraction (Li, et al., 2012; Shi, et al., 2013), and an aqueous biphasic system using kosmotropic salts (Sun, et al., 2013). In the present study, the electrodialysis method was selected (Fig. 4), because it does not require any extra reagents such as acids and bases used in the phase separation described above (Li, et al., 2012; Sun, et al., 2013). Moreover, the electrodialysis method itself has already been employed in the salt production industry, although chromatography was unsuitable for scale-up to an industrial level. The present study did not conducted a separation and recovery of cellulase enzyme from the sugar solution. On this point, some researchers have demonstrated recovery of enzyme and IL by means of ultrafiltration and subsequent electrodialysis, respectively, after the the *in situ* saccharification of cellulosic material (Abels, et al., 2013). Now, we are going to develop the ChOAc-assisted *in situ* saccharification of lignocellulosic biomass accompanied with enzyme and IL recovery.

4. Conclusions

ChOAc, which showed a biomass pretreatment capability and a smaller inhibitory effect on cellulase than EmimOAc, was used for IL/ultrasound-assisted pretreatment and *in situ* enzymatic saccharification. The cellulose and hemicellulose saccharification

percentage was 80% and 72%, respectively, when the *in situ* saccharification was performed for 48 h in the presence of 10% ChOAc. The cellulose and hemicellulose saccharification percentage reached 72% and 53%, respectively, even in the case of 20% ChOAc. A glucose/xylose solution free from IL and ChOAc aqueous solution without these sugars could be recovered separately by electrodialysis of the hydrolysate of the *in situ* enzymatic saccharification.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at

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Figure captions

Fig. 1 Time courses of saccharification percentage for cellulose (A) and hemicellulose (B) during enzymatic reaction of bagasse powder pretreated with IL. Prior to the enzymatic reaction, IL was washed out from the pretreated bagasse powder. Closed circles: ChOAc, open circles: EmimOAc, gray circles: control without pretreatment. The error bars indicate the standard deviation from three independent experiments.

Fig. 2 Dose-response relationships between relative cellulase activity and IL concentration. Cellulase activity was evaluated by the amount of glucose generated from PASC for 24 h of enzymatic reaction in the presence of IL at the subscribed concentration, and expressed as a percentage of the value without IL addition. Closed circles: ChOAc, open circles: EmimOAc. The error bars indicate the standard deviation from three independent experiments.

Fig. 3 Saccharification percentages for cellulose (A) and hemicellulose (B) obtained at 48 h of the *in situ* saccharification of bagasse powder at the subscribed IL concentration in the saccharification step. Closed bars: ChOAc, open bars: EmimOAc, gray bars: control without pretreatment. The error bars indicate the standard deviation from three independent experiments.

Fig. 4 Time course of ChOAc, glucose, and xylose concentrations in the desalination tank during electrodialysis of hydrolysate obtained from the *in situ*

saccharification of bagasse powder at 10% ChOAc concentration for
saccharification step. Closed circles: ChOAc, closed triangles: glucose, closed
diamonds: xylose.

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.