VERSITA

Chemical Papers 65 (6) 792–797 (2011)
DOI: 10.2478/s11696-011-0066-6

ORIGINAL PAPER

Enzymatic saccharification of cellulose in aqueous–ionic liquid 1-ethyl-3-methylimidazolium dimethylphosphate–DMSO media

[a]Yu C. He, [a,b]Cui L. Ma*, [a]Dong Q. Xia, [a]Liang Ding, [c]Liang Z. Li, [c]Mu Xian

[a] *College of Pharmaceutical and Life Sciences, Changzhou University, Changzhou 213164, China*

[b] *Changzhou Kangpu Pharmaceutical Co., Ltd. Changzhou 213172, China*

[c] *Qingdao Institute of Bioenergy and Bioprocess Technology, Chinese Academy of Sciences, Qingdao, 266071, China*

Received 4 April 2011; Accepted 20 May 2011

Ionic liquid (IL) 1-ethyl-3-methylimidazolium dimethylphosphate ([Emim]DMP) was chosen as an environment-friendly solvent to enzymatically hydrolyze cellulose in situ. Under optimal reaction condition, 80.2 % of cellulose (10 mg mL^{-1}) were converted to glucose in aqueous–IL–DMSO (φ_r = 74 : 25 : 1) media at 55 °C in 18 h. Finally, fermentability of the recovered hydrolyzates was evaluated using *Saccharomyces cerevisiae* which is able to ferment hydrolyzates efficiently, the ethanol production was 0.44 g g^{-1} of glucose within 24 h of the process. Such information is vital for the saccharification of more complex cellulose materials and for the fermentation of hydrolyzates into biofuel.

Keywords: ionic liquid, 1-ethyl-3-methylimidazolium dimethylphosphate, cellulose, enzymatic saccharification, DMSO, optimization

Introduction

To make cellulosic materials more susceptible to hydrolysis by cellulases, a pretreatment process reducing the cellulose crystallinity and increasing the cellulose porosity is needed (Chandra et al., 2007; Peterson et al., 2007; Saha et al., 2005). Different pretreatment methods have been used for the pretreatment of cellulosic materials (Dadi et al., 2006; Hendriks & Zeeman, 2009; Moulthrop et al., 2005; Remsing et al., 2008; Yáñez et al., 2006) since regenerated cellulose with amorphous structure provides more surfaces for enzymes to attack on. In recent years, ionic liquid (IL) was found to be a stable and effective solvent for dissolving and pretreating cellulose (Dadi et al., 2006; Heinze et al., 2001; Moulthrop et al., 2005; Zhao et al., 2009). However, many ILs induce different degrees of cellulase inactivation (Zhao et al., 2008). A recovery process of the IL-treated cellulose was required prior to enzymatic saccharification of cellulose, which was a cumbersome, painstaking and time consuming task. To eliminate the recovery of IL-treated cellulose and simplify the entire process for cellulose saccharification, one promising strategy is the enzymatic in situ saccharification of IL-treated cellulose in biocompatible IL–aqueous media. Kamiya et al. (2008) reported enzymatic saccharification of cellulose in aqueous–IL [Emim]DEP (φ_r = 1 : 4) media; over 50 % of the cellulose mass could be converted to glucose in 24 h. However, in the above aqueous–IL systems, efficiency of cellulose hydrolysis into glucose is still not high enough. In-depth studies concerning biocompatibility of ILs and appropriate reaction media for enzymatic in situ saccharication are necessary to make hydrolysis more applicable.

In our study, biocompatible IL [Emim]DMP was used for the in situ saccharification process. Scanning electron microscopy (SEM), Fourier transformed IR (FTIR), and X-ray diffraction (XRD) were applied to establish possible means for the enzymatic hy-

*Corresponding author, e-mail: macuiluan@163.com

drolysis enhancement. After optimization of the reaction parameters, an in situ saccharification process in aqueous–IL [Emim]DMP–DMSO media was started. Moreover, fermentability of glucose recovered from the hydrolyzates was investigated.

Experimental

Avicel PH-101 (cellulose) was purchased from Fluka (Buchs, Switzerland). Cellulase was kindly donated by Genencor (Shanghai, China). The ethanol producing strain *S. cerevisiae* was preserved by our laboratory. All other reagents and chemicals used were also from commercial sources and of reagent grade.

Stock solution of cellulose was prepared by adding cellulose to IL [Emim]DMP for 20 min at 55 ℃. Then, acetate acid-sodium acetate buffer (50 mM, pH 5.0) was added to make a total 1 mL solution; water-insoluble fractions (i.e., regenerated cellulose) were formed after an addition of the aqueous buffer. After dispersing the mixture, enzymatic reaction was initiated by an addition of 3 mg mL^{-1} of cellulase to an aqueous–IL mixture containing 0–100 % (φ_r) of IL. The final concentration of IL-treated cellulose was 10 mg mL^{-1}. The enzymatic saccharification of cellulose was carried out at 55 ℃ and 160 min^{-1}.

To investigate the effects of organic solvent additives on cellulase activity, enzymatic reaction was initiated by an addition of 3.0 mg mL^{-1} of cellulase to an aqueous–IL mixture containing 10 mg mL^{-1} of IL-treated cellulose, 25 % (φ_r) of IL and 0.5 % (φ_r) of different organic solvents. Enzymatic hydrolysis was run for 1 h in aqueous–IL [Emim]DMP–organic solvent (φ_r = 74.5 : 25 : 0.5) media at 55 ℃ and 160 min^{-1}. To investigate the effects of DMSO on enzymatic hydrolysis of cellulose, enzymatic reaction was initiated by an addition of 3.0 mg mL^{-1} of cellulase to an aqueous–IL–DMSO mixture containing 25 % (φ_r) of IL and 0–25 % (φ_r) of DMSO. Concentration of IL-treated cellulose was 10 mg mL^{-1}. Finally, enzymatic hydrolysis was run for 18 h in a shaker at 55 ℃ and 160 min^{-1}. To investigate the effects of cellulase concentrations on cellulose saccharification, enzymatic reaction was initiated by an addition of 1.0–8.0 mg mL^{-1} of cellulase to an aqueous–IL–DMSO (φ_r = 74 : 25 : 1) mixture containing 10 mg mL^{-1} of IL-treated cellulose. Enzymatic hydrolysis was run for 1–12 h in a shaker at 55 ℃ and 160 min^{-1}.

After enzymatic hydrolysis of cellulose, the mixture of IL and hydrolyzates from enzymatic in situ hydrolysis of cellulose was filtered and the IL and sugars were separated in a column filled with neutral Al_2O_3 according to recently published literature (Xian et al., 2009). Isolated yields of IL [Emim]DMP and glucose in hydrolyzates were 96.8 % and 98.7 %, respectively. Then, the hydrolyzates (recovered sugars) were used in ethanol fermentation immediately without further purification.

Fermentability of the recovered hydrolyzates was evaluated using the ethanol producing strain *S. cerevisiae*. The inoculum was prepared by transferring a loop of cells of *S. cerevisiae* into 20 mL of the YEPD medium (5.0 g L^{-1} of yeast extract, 10.0 g L^{-1} of peptone, and 10.0 g L^{-1} of D-glucose) and its incubation at 30 ℃ for 24 h. The cells were harvested by centrifugation (8000 min^{-1}, 10 min), suspended in sterilized water and used in the following ethanol fermentation; the cells' suspension (0.2 mL) was inoculated into 20 mL of the fermentation medium (glucose in hydrolyzates: 25.0 g L^{-1}, $MgSO_4$: 0.20 g L^{-1}, NH_4Cl: 1.0 g L^{-1}, and KH_2PO_4: 2.0 g L^{-1}, pH 6.0) in a 100 mL flask. The fermentation was carried out at 30 ℃ under semi aerobic conditions. Control experiment was conducted using 25.0 g L^{-1} of D-glucose as the carbon source in the fermentation.

Cellulase activity was determined by the standard filter paper assay and expressed as filter paper units per gram of glucan (FPU) (Dadi et al., 2006). One FPU is defined as the enzyme releasing 1 μmol of glucose equivalents per minute from the filter paper. The released glucose was assayed using a commercial analysis kit containing glucose oxidase (Institute of Biological Products, Shanghai, China).

Biomass concentrations were estimated using a spectrophotometer at 600 nm by measuring optical density of the cultures. Glucose, xylose, galactose, and arabinose were assayed by ion chromatography (ICS-3000, Dionex Corporation, Sunnyvale, CA, USA). The ion moderated partition chromatography sugar column PA10 was employed for assaying these monosaccharide. The samples were eluted with 75 mM NaOH at the flow rate of 1.0 mL min^{-1}. The injection volume was 1 mL, and the observed pressure drop was 18.6 MPa. Ethanol was analyzed by HPLC equipped with a HPX-87H column (Bio-Rad, Shanghai, China) at 65 ℃ eluted with 10 mM sulfuric acid at the flow rate of 0.8 mL min^{-1} and monitored using a RID-10A detector (Shimadzu Co., Japan). Fourier transformed IR (FTIR) was used to assay the IL [Emim]DMP-treated celluloses. Spectra (4000–800 cm^{-1}) were recorded with the resolution of 4 cm^{-1} and 64 scans per sample. About 2.5 mg samples were prepared by mixing with 120.0 mg of spectroscopic grade KBr and were pressed in a standard device using the pressure of 41.4 MPa to produce 13 mm diameter pellets. The background spectrum of pure KBr was subtracted from that of the sample spectrum. Scanning electron microscopy (SEM) (Hitachi S-4800, Japan) was used to assay the IL [Emim]DMP-treated celluloses. The samples were coated with a thin gold layer using a vacuum sputter-coater to improve the conductivity of the samples and thus the quality of the SEM images prior to the analysis. SEM operated at 5 keV was used to image the cellulose samples. X-ray diffraction (XRD) (Bruker D8 ADVANCE, Germany) was used to assay IL [Emim]DMP-treated cellulose. Samples were

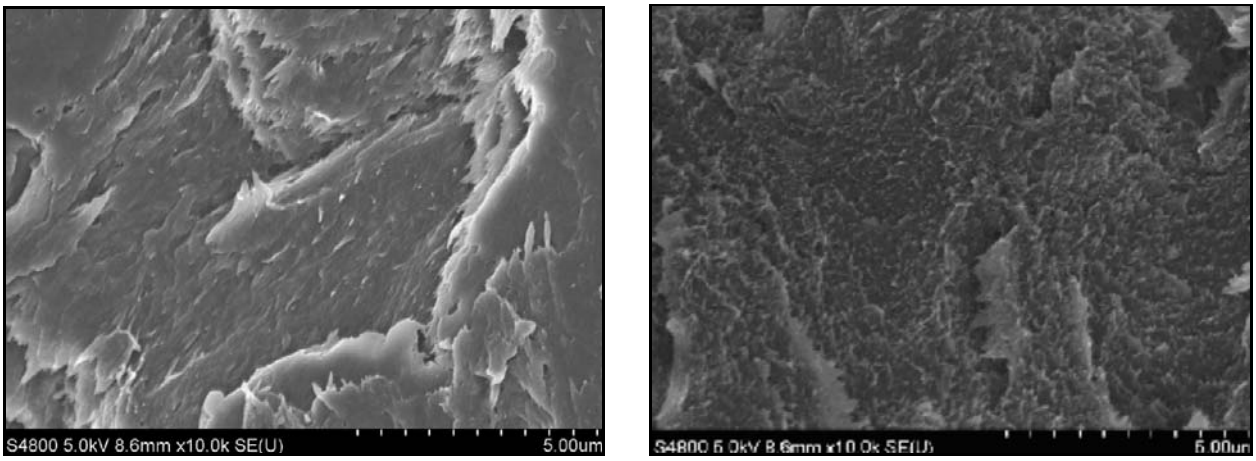

Fig. 1. SEM micrographs of untreated cellulose (A) and IL-treated cellulose (B).

Table 1. Infrared ratios expressed as crystallinity indexes of regenerated cellulose; from FTIR spectra, two infrared ratios, LOI and TCI, were calculated (Zhao et al., 2009): (1) $\alpha_{1437\mathrm{cm}^{-1}}/\alpha_{899\mathrm{cm}^{-1}}$, referred to as the crystallinity index or lateral order index (LOI); (2) $\alpha_{1437\mathrm{cm}^{-1}}/\alpha_{899\mathrm{cm}^{-1}}$, known as the total crystallinity index (TCI)

Cellulose	LOI	TCI
Untreated cellulose	1.16	1.00
Cellulose treated by IL at 55 ℃	1.05	0.96

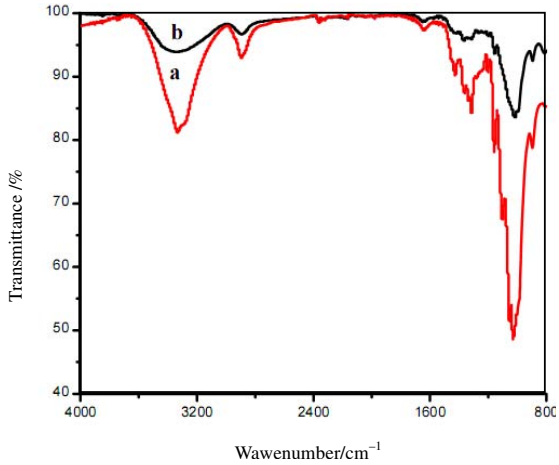

Fig. 2. FTIR spectra of untreated cellulose (a) and IL-treated cellulose (b).

scanned by XRD over the angular range of 5.0–60°, 2θ, in continuous scan mode.

Results and discussion

Pretreatment of cellulose with IL [Emim]DMP and enzymatic saccharification of cellulose in aqueous–IL [Emim]DMP media

Encouraged by recent findings that show ILs containing alkylphosphate anions as capable of dissolving cellulose (Kamiya et al., 2008), the solubility of one kind of IL [Emim]DMP has been proved to be 6.9 % at 55 ℃. To visualize the changes of cellulose structure after its pretreatment with IL [Emim]DMP for 20 min at 55 ℃, the FTIR, XRD, and SEM images of IL-treated cellulose were recorded. As shown in Table 1, LOI of IL-treated cellulose significantly decreased, from 1.16 to 1.05, and TCI of cellulose decreased from 1.00 to 0.96. As shown in Fig. 1, SEM micrographs of IL-treated cellulose are different than that of the untreated one. It is evident that the fibers were partially disrupted. Probably during the regeneration process, the rapid precipitation with water prevented the IL-treated cellulose from restructuring into its original crystalline structure (Mikkola et al., 2007). Based on the FTIR and XRD analysis (Figs. 2 and 3),

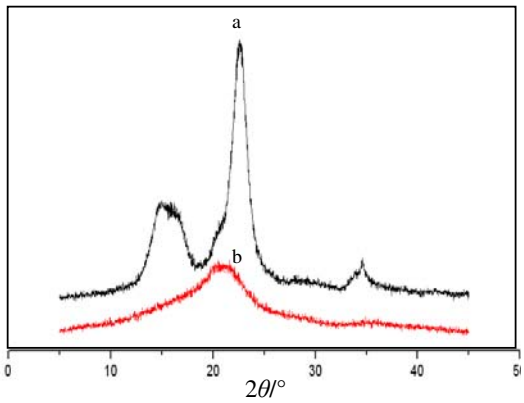

Fig. 3. XRD micrographs of untreated cellulose (a) and IL-treated cellulose (b).

the IL-treated cellulose, lost its crystalline structure and assumed a more amorphous form. Consequently, the IL-treated cellulose with its amorphous structure provided more surfaces for enzymes to attack on.

To demonstrate the efficiency of cellulose conver-

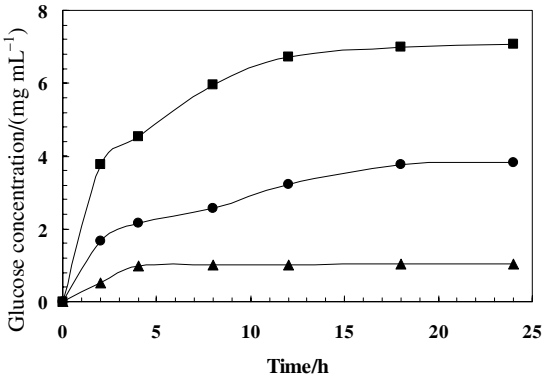

Fig. 4. Time course of the enzymatic hydrolysis of cellulose in different aqueous–IL systems. Conditions: 10 mg mL^{-1} of cellulose, different concentrations of [Emim]DMP, 3.0 mg mL^{-1} of cellulase, hydrolysis run for 24 h at 55 °C and pH 5.0. (●) – in aqueous media, untreated cellulose, (■) – in IL–aqueous media ($\varphi_r = 1:3$), IL-treated cellulose, (▲) – in IL–aqueous media ($\varphi_r = 1:1$), IL-treated cellulose.

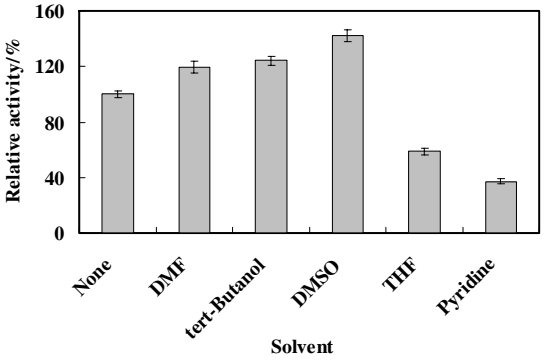

Fig. 5. Effect of organic solvent on cellulase activity. Conditions: 10 mg mL^{-1} of IL-treated cellulose, 25 % (φ_r) of [Emim]DMP, 0.5 % (φ_r) of organic solvent, 3.0 mg mL^{-1} of cellulase, hydrolysis run for 1 h at 55 °C and pH 5.0. All experiments were performed in triplicate. Error bars show the standard deviation of triplicate measurements.

sion to glucose in aqueous–IL media, time courses of enzymatic saccharification of IL-dissolved cellulose in different aqueous–IL [Emim]DMP mixtures were monitored (Fig. 4). After the hydrolysis of cellulose for 24 h in aqueous–IL [Emim]DMP ($\varphi_r = 1:3$) media, the reaction solution became clear and 7.06 mg mL^{-1} of glucose were obtained (starting amount of 10 mg mL^{-1}); glucose formation in the aqueous–IL media was approximately two fold higher than that of the aqueous media without IL, indicating that the IL was highly biocompatible with the in situ saccharification process. The above results are similar to those obtained in the aqueous–[Emim]DEP ($\varphi_r = 1:4$) media in a former study (Kamiya et al., 2008). That is, the conversion of cellulose to glucose was preferred at the IL [Emim]DMP to water ratio of 1 : 3 (φ_r). Further increase in the IL concentration would lead to a dramatic decrease in the activity of cellulase and the yield of glucose (data not shown). At the IL [Emim]DMP to water ratio of 1 : 1 (φ_r), 1.04 mg mL^{-1} of glucose was obtained from the hydrolysis of cellulose (starting amount of 10 mg mL^{-1}) after 24 h.

Based on the above data, it can be concluded that the intact structure of cellulose was disrupted by the IL [Emim]DMP pretreatment at 55 °C and resulted in a porous and amorphous regenerated cellulose that greatly enhanced enzymatic hydrolysis in aqueous–IL media. In previous reports (Dadi et al., 2006), higher pretreatment temperature ($\geq$ 100 °C) was required. Moreover, a following cumbersome recovery process for the regeneration of cellulose produced by cellulose pretreatment with ILs prior to enzymatic saccharification was also needed. In our study, the low pretreatment temperature of 55 °C, just around the operative temperature range for cellulase, was employed in the dissolving and pretreatment of cellulose. This pretreatment process eliminated the need for regenerated cellulose recovery and it could be carried out under mild conditions. Also, IL [Emim]DMP could be chosen as a biocompatible IL for enzymatic in situ saccharification of cellulose.

Optimization of enzymatic saccharification of cellulose conditions in aqueous–IL [Emim]DMP–organic solvent media

In order to obtain a high saccharification rate, it was necessary to develop an appropriate reaction system for enzymatic saccharification of cellulose. Organic solvent can enhance the cellulase activity (Lucas et al., 2001) and aqueous–IL–organic solvent media can be effectively used in the saccharification process. In our study, 2-methylpropan-2-ol, DMF, DMSO, pyridine, and THF (φ_r = 0.5 %) were added into the aqueous–IL [Emim]DMP media, respectively. As shown in Fig. 5, DMF, 2-methylpropan-2-ol, and DMSO enhanced the cellulase activity while THF and pyridine resulted in its significant decrease. Among these organic solvents, DMSO (φ_r = 0.5 %) enhanced the cellulase activity 1.4 fold and thus was chosen as an appropriate organic additive.

Furthermore, the effects of different concentrations of DMSO, varied from 0 % to 25 % (φ_r), in the aqueous–IL [Emim]DMP media were investigated. As shown in Fig. 6, DMSO at the concentrations of 0.5–2.0 % (φ_r) enhanced the cellulase activity, however, 4 % (φ_r) of DMSO significantly reduced the cellulase activity. At the addition of DMSO into the enzymatic hydrolysis media at the concentrations of 0 %, 0.5 %, 1 %, 2 %, and 4 % (φ_r), conversions of cellulose to glucose obtained in 18 h were 69.3 %, 73.6 %, 80.2 %, 62.4 %, and 44.7 %, respectively. It is obvious that 1 % (φ_r) of DMSO effectively increases the cellulase activity and the yield of glucose in aqueous–IL

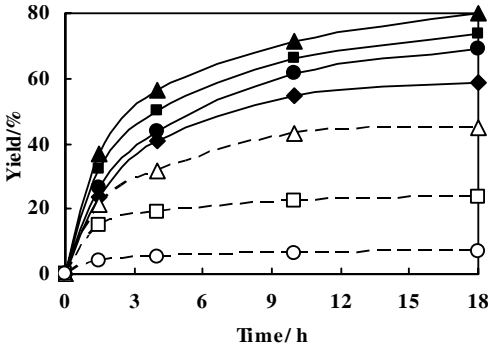

Fig. 6. Time course of enzymatic hydrolysis of cellulose in aqueous–IL–DMSO media with different concentration of DMSO. Conditions: 10 mg mL^{-1} of IL-treated cellulose, 25 % (φ_r) of [Emim]DMP, 0–25 % (φ_r) of DMSO, 3.0 mg mL^{-1} of cellulase, hydrolysis run for 18 h at 55 °C and pH 5.0. (●) – 0 % DMSO; (■) – 0.5 % DMSO; (▲) – 1 % DMSO; (♦) – 2 % DMSO; (△) – 4 % DMSO; (□) – 10 % DMSO; (○) – 25 % DMSO.

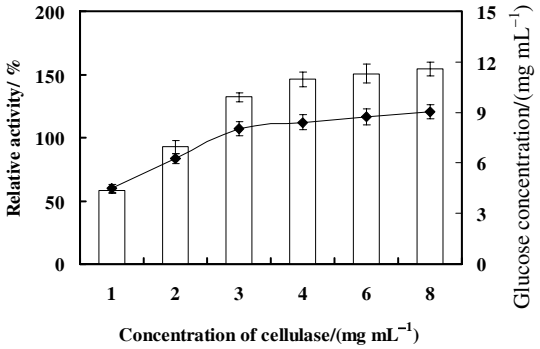

Fig. 7. Effect of cellulase concentration. Conditions: 10 mg mL^{-1} of IL-treated cellulose, 25 % (φ_r) of [Emim]DMP, 1.0–8.0 mg mL^{-1} of cellulase, hydrolysis run at 55 °C and pH 5.0. (♦) – glucose conversion for 18 h; (white bar) – relative activity. All experiments were performed in triplicate. Error bars show the standard deviation of triplicate measurements.

[Emim]DMP–DMSO media (Fig. 3). Therefore, 1 % (φ_r) of DMSO was added into the hydrolysis media during the saccharification of cellulose.

In order to enhance the conversion of cellulose to glucose, the effect of cellulase concentration was also investigated. As shown in Fig. 7, the conversion of cellulose to glucose increased markedly, from 1 mg mL^{-1} to 3 mg mL^{-1} in 18 h, with the cellulase concentration and the corresponding maximal conversion reached 80.2 %. Further increase of cellulase concentration from 3 mg mL^{-1} to 8 mg mL^{-1} did not cause any obvious change in the conversion. Obviously, an optimal enzyme concentration for any enzymatic reaction can be found. Considering the enzyme cost, 3.0 mg mL^{-1} was chosen as the optimal cellulase concentration in this reaction media. Using ion chromatography for assaying hydrolyzates obtained from the enzymatic saccharification of the IL-treated cellulose, the concentrations of glucose, xylose, galactose, and arabinose in hydrolyzates were 8.02 mg mL^{-1}, 1.01 mg mL^{-1}, 0.60 mg mL^{-1}, and 0.21 mg mL^{-1}, respectively.

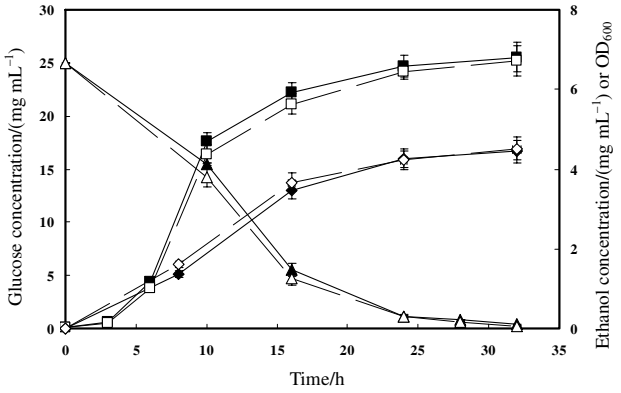

Fig. 8. Time course of recovered hydrolyzates fermentation by *S. cerevisiae*. Fermentation was carried out at 30 °C under semi aerobic conditions. Ethanol fermentation medium: glucose in hydrolyzates (or pure glucose): 25.0 g L^{-1}; $MgSO_4$: 0.20 g L^{-1}; NH_4Cl: 1.0 g L^{-1}; and KH_2PO_4: 2.0 g L^{-1}, pH 6.0. All experiments were performed in triplicate. Error bars show the standard deviation of triplicte measurements. (▲) – Glucose, hydrolyzate; (△) – glucose, control; (■) – OD_{600}, hydrolyzate; (□) – OD_{600}, control; (●) – ethanol, hydrolyzate; (○) – ethanol, control.

Based on the above data, optimal enzymatic hydrolysis conditions were obtained. The aqueous–IL [Emim]DMP–DMSO (φ_r = 74 : 25 : 1) media were used as the reaction media. After being hydrolyzed by 3 mg mL^{-1} of cellulase for 18 h at 55 °C and 160 min^{-1}, the conversion of cellulose to glucose reached 80.2 %. A high effective enzymatic in situ saccharification process with phosphate based IL [Emim]DMP eliminating the need to recover regenerated cellulose, with the conversion of cellulose to glucose of over 80 % (φ_r), has been developed This process can be useful in integrated bioprocesses such as bioethanol production from cellulosic materials.

Fermentability of recovered hydrolyzates from enzymatic hydrolysis of cellulose in aqueous–IL [Emim]DMP–DMSO media

Fermentability of the recovered hydrolyzates containing glucose was evaluated using the ethanol producing strain *S. cerevisiae*. Fermentation profiles are presented in Fig. 8. The initial concentration of 25.0 g L^{-1} to 0.37 g L^{-1} of glucose in the hydrolyzates was consumed within 32 h. After 24 h of fermentation, the ethanol production was 0.44 g g^{-1} of glucose, which represents 86.1 % of the theoretical yield. Fermentation was also conducted with pure glucose as the control sample. These results are similar to those reported in recent works on fermentation of recov-

ered hydrolyzates obtained from aqueous media without ILs (Jeihanipour & Taherzadeh, 2009), indicating that the recovered hydrolyzates obtained from the aqueous–IL [Emim]DMP–DMSO media do not have any negative effects on the cell growth and ethanol production. Although this fermentation process was not optimized, potential application of recovered hydrolyzates was demonstrated in our case.

Conclusions

In our study, IL [Emim]DMP showed favorable solubility with cellulose and biocompatibility with cellulase. Thus, the novel reaction system involving an aqueous–IL [Emim]DMP–DMSO ($\varphi_r = 74 : 25 : 1$) mixture was chosen for enzymatic saccharification of cellulose. After optimization of the reaction parameters, the conversion of cellulose to glucose reached 80.2 %. Furthermore, fermentability of the recovered hydrolyzates was evaluated using *Saccharomyces cerevisiae*. This microbe is able to ferment hydrolyzates to ethanol without negative effects. In summary, our work presents the IL [Emim]DMP as a promising solvent for enzymatic in situ hydrolysis of cellulose in aqueous–IL [Emim]DMP–DMSO media; however, its cost is very high and further study on this subject is needed.

Acknowledgements. This work was financially supported by the Natural Foundation of Guangxi (No. 0991001), the Talent Introduction Foundation of the Changzhou University (No. ZMF10020077), University student's Science & Technology Creation Foundation of the Changzhou University, and the Open Funding Project of the State Key Laboratory of Bioreactor Engineering, East China University of Science and Technology.

References

Chandra, R. P., Bura, R., Mabee, W. E., Berlin, A., Pan, X., & Saddler, J. N. (2007). Substrate pretreatment: The key to effective enzymatic hydrolysis of lignocellulosics? *Advances in Biochemical Engineering/Biotechnology*, *108*, 67–93. DOI: 10.1007/10_2007_064.

Dadi, A. P., Varanasi, S., & Schall, C. A. (2006). Enhancement of cellulose saccharification kinetics using an ionic liquid pretreatment step. *Biotechnology and Bioengineering*, *95*, 904–910. DOI: 10.1002/bit.21047.

Heinze, T., & Liebert, T. (2001). Unconventional methods in cellulose functionalization. *Progress in Polymer Science*, *26*, 1689–1762. DOI: 10.1016/S0079-6700(01)00022-3.

Hendriks, A. T. W. M., & Zeeman, G. (2009). Pretreatments to enhance the digestibility of lignocellulosic biomass. *Bioresource Technology*, *100*, 10–18. DOI: 10.1016/j.biortech.2008.05.027.

Jeihanipour, A., & Taherzadeh, M. J. (2009). Ethanol production from cotton-based waste textiles. *Bioresource Technology*, *100*, 1007–1010. DOI: 10.1016/j.biortech.2008.07.020.

Kamiya, N., Matsushita, Y., Hanaki, M., Nakashima, K., Narita, M., Goto, M., & Takahashi, H. (2008). Enzymatic in situ saccharification of cellulose in aqueous-ionic liquid media. *Biotechnology Letters*, *30*, 1037–1040. DOI: 10.1007/s10529-008-9638-0.

Lucas, R., Robles, A., García, M. T., Alvarez de Cienfuegos, G., & Gálvez, A. (2001). Production, purification, and properties of an endoglucanase produced by the hyphomycete *Chalara* (syn. *Thielaviopsis*) *paradoxa* CH32. *Journal of Agricultural and Food Chemistry*, *49*, 79–85. DOI: 10.1021/jf000916p.

Mikkola, J.-P., Kirilin, A., Tuuf, J.-C., Pranovich, A., Holmbom, B., Kustov, L. M., Murzin, D. Y., & Salmi, T. (2007). Ultrasound enhancement of cellulose processing in ionic liquids: from dissolution towards functionalization. *Green Chemistry*, *9*, 1229–1237. DOI: 10.1039/b708533h.

Moulthrop, J. S., Swatloski, R. P., Moyna, G., & Rogers, R. D. (2005). High-resolution ^{13}C NMR studies of cellulose and cellulose oligomers in ionic liquid solutions. *Chemical Communications*, *12*, 1557–1559. DOI: 10.1039/b417745b.

Petersson, A., Thomsen, M. H., Hauggaard-Nielsen, H., & Thomsen, A.-B. (2007). Potential bioethanol and biogas production using lignocellulosic biomass from winter rye, oilseed rape and faba bean. *Biomass and Bioenergy*, *31*, 812–819. DOI: 10.1016/j.biombioe.2007.06.001.

Remsing, R. C., Hernandez, G., Swatloski, R. P., Massefski, W. W., Rogers, R. D., & Moyna, G. (2008). Solvation of carbohydrates in *N,N'*-dialkylimidazolium ionic liquids: A multinuclear NMR spectroscopy study. *The Journal of Physical Chemistry B*, *112*, 11071–11078. DOI: 10.1021/jp8042895.

Saha, B. C., Iten, L. B., Cotta, M. A., & Wu, Y. V. (2005). Dilute acid pretreatment, enzymatic saccharification and fermentation of wheat straw to ethanol. *Process Biochemistry*, *40*, 3693–3700. DOI: 10.1016/j.procbio.2005.04.006.

Xian, M., Li, L., He, Y., Tan, W., Li, Q., & Yang, F. (2009). Chinese Patent No. 200910093300.8. Shanghai, China: China Patent & Trademark Office.

Yáñez, R., Alonso, J. L., & Parajó, J. C. (2006). Enzymatic saccharification of hydrogen peroxide-treated solids from hydrothermal processing of rice husks. *Process Biochemistry*, *41*, 1244–1252. DOI: 10.1016/j.procbio.2005.12.020.

Zhao, H., Baker, G. A., Song, Z., Olujabo, O., Crittle, T., & Peters, D. (2008). Designing enzyme-compatible ionic liquids that can dissolve carbohydrates. *Green Chemistry*, *10*, 696–705. DOI: 10.1039/B801489B.

Zhao, H., Jones, C. L., Baker, G. A., Xia, S., Olujabo, O., & Person, V. N. (2009). Regenerating cellulose from ionic liquids for an accelerated enzymatic hydrolysis. *Journal of Biotechnology*, *139*, 47–54. DOI: 10.1016/j.jbiotec.2008.08.009.