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Enhancement of enzymatic in situ saccharification of cellulose in aqueous-ionic liquid media by ultrasonic intensification

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ABSTRACT

A new approach for in situ enzymatic saccharification of cellulose in ionic liquids (ILs)-aqueous media is presented in which ultrasonic pretreatment was used to enhance the conversion of cellulose. For this purpose, the solubility of cellulose and the activity of cellulase were investigated in six alkylphosphate ILs. 1-Methyl-3-methylimidazolium dimethylphosphate ([Mmim][DMP]) giving favorable solubility and biocompatibility was selected to establish aqueous-ILs system for enzymatic in situ saccharification of cellulose. After further optimization of reaction parameters concerning cellulase concentration, temperature and IL concentration, higher conversion (95.48%) of cellulose was obtained in the media of aqueous-[Mmim][DMP] by conducting the pretreatment of cellulose with ultrasonic heating, whereas the conversion of cellulose untreated was 42.77%. Scanning electronic microscopy (SEM) and viscosity analysis indicated that IL-treated cellulose under ultrasonic condition was subjected to depolymerization, which led to more efficient saccharification. The findings of this study would have great implications for developing a continuous process for transformation of biomass such as straw cellulose to ethanol or other hydrocarbons.

1. Introduction

Cellulose is the most abundant carbohydrate component of biomass. Along with the diminishing of fossil fuel resources and global heating warnings caused by greenhouse gas emissions, the efficient utilization of cellulosic biomass is gaining increasing attention (Kilpeläinen et al., 2007). However, the high crystallinity of cellulose makes it difficult to hydrolysis into individual glucose subunits (Dadi, Varanasi, & Schall, 2006; Finkenstadt & Millane, 1998). Therefore, the cellulose pretreatment process has been extensively promoted as one of the solutions to the energy crisis. Hydrolysis of cellulose is generally catalyzed by mineral acids. Unfortunately, the chemical saccharification of cellulose using inorganic acids produces abundant by-products that may increase the environmental load (Schäfer et al., 2007). During the past decades, considerable attention has been paid to developing novel solvents for dissolution of cellulose, such as *N*-methyl-morpholine-*N*-oxide, LiCl/*N*,*N*-dimethylacetamide, ammonium fluorides/dimethylsulfoxide (Gericke, Schlufter, Liebert, Heinze, & Budtova, 2009; Kuo & Lee, 2009). Nevertheless, these solvents possess several undesired properties (e.g., high toxicity, volatility and high costs), limiting their commercial application. In order to overcome these economic and environmental disadvantages mentioned above, scientists have embarked on the research to find efficient solvents for cellulose (Hermanutz, Gähr, Uerdingen, Meister, & Kosan, 2008).

Ionic liquids (ILs) are environmentally friendly molten salts, most of which have the virtues of excellent solvency, low melting point, nonvolatility and designability (Ohno & Fukaya, 2009). The physical and chemical properties of ILs can be adjusted to adapt to different reactions by the choice of cations, anions and substituents (Yang & Pan, 2005). Due to these unique qualities, IL is taken as a promising alternative to traditional solvents, especially as a new reaction medium for biocatalysis (Rantwijk & Sheldon, 2007). Enzymatic hydrolysis of cellulose in ILs has become a research hotspot in recent years (Watanabe, 2010), but being hampered by the incompatibility of ILs with cellulase. It has been demonstrated that many ILs would induce inactivation and unfolding of the cellulase (Turner, Spear, Huddleston, Holbrey, & Rogers, 2003). One strategy to solve this problem is to pretreat the cellulose with ILs and then subject to enzymatic hydrolysis. Previous work in this lab has shown that wheat straw recovered from the IL 1-ethyl-3-methylimidazolium diethylphosphate ([Emim][DEP]) was enzymatic-hydrolyzed more easily than the untreated substrates (Li et al., 2009). However, it is a painstaking and time consuming task to regenerate cellulose hydrolytes from ILs. A potential approach to overcome the above drawback is to in situ hydrolyze dissolved cellulose in ILs, which requires to develop ILs compatible with both cellulose solubility and cellulase activity. Recently, Kamiya et al. (2008) reported that

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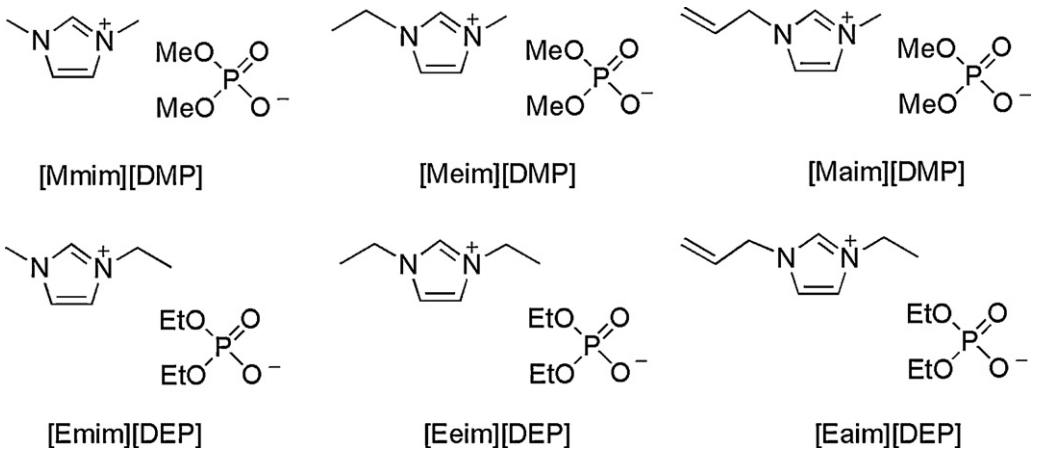

Fig. 1. Chemical structure of six ILs: [Mmim][DMP] (1-methyl-3-methylimidazolium dimethylphosphate); [Meim][DMP] (1-methyl-3-ethylimizolium dimethylphosphate); [Maim][DMP] (1-methyl-3-allylimidazolium dimethylphosphate); [Emim][DEP] (1-ethyl-3-methylimidazolium diethylphosphate); [Eeim][DEP] (1-ethyl-3-ethylimizolium diethylphosphate); [Eaim][DEP] (1-ethyl-3-allylimizolium diethylphosphate).

by adjusting the ratio of [Emim][DEP] to water, the enzymatic in situ saccharification of cellulose in aqueous-ILs media was made possible. Over 50% of the cellulose could be converted to glucose in 24 h, indicating that [Emim][DEP] shows good compatibility with cellulase. But in aqueous-[Emim][DEP] system, the efficiency of cellulose hydrolysis into glucose is still not high enough. In-depth studies concerning biocompatibility of ILs and process intensification of in situ enzymatic saccharification would be necessary to make the hydrolysis more applicable.

Here, we screened ILs with good performance in both dissolution and in situ enzymatic hydrolysis of cellulose. After systematic optimization of the reaction parameters, an in situ saccharification process in aqueous-ILs media was established. Especially, the significant effects of ultrasound pretreatment on the enzymatic hydrolysis were investigated in detail. Scanning electron microscope (SEM) and viscosity analysis were applied to elucidate the possible reasons for the enhancement of enzymatic hydrolysis.

2. Material and methods

2.1. Reagents

Microcrystalline cellulose was purchased from Sigma-Fluka Chemical Co. Cellulase from *Trichoderma reesei* was obtained from Beijing Solarbio Science & Technology Co., Ltd. (China). All other reagents were of analytical grade and dried or distilled before use.

2.2. Synthesis of ILs

Six imidazolium-based ILs (Fig. 1) were synthesized as follows: equal molar amounts of alkylimidazole and trialkylphosphate were added to a round-bottom flask and stirred vigorously at 150 °C for 15 h (Nie, Li, Sun, Meng, & Wang, 2006). The reactions were carried out under nitrogen atmosphere to prevent the uptake of water from the air. After cooling to room temperature, the mixture was washed with ether for three times to remove the unreacted starting material. Then the volatile residues were evaporated from the crude product at 80 °C for 6 h with a rotary evaporator. All of the ILs were dried under vacuum at 80 °C for 24 h before use.

2.3. Dissolution of cellulose in ILs

The solubility of cellulose in each IL was measured by a standardized procedure (Mazza, Catana, Vaca-Garcia, & Cecutti, 2009). A weighed amount of IL was added to clear glass vials equipped with a magnetic stirrer and placed into a heating oil bath. Small precise amounts of microcrystalline cellulose (about 10 mg) were added discretely into the vials. The mixture was stirred for dissolution until the solution was just within the boundary of transparency. Experiments were conducted at different heating temperatures (from 40 °C to 90 °C increased by 10 °C every time) and the solubility of cellulose was calculated by the following formula:

$$\text{Solubility} = \frac{\text{Weight of dissolved cellulose}}{\text{Weight of ILs}} 100\%$$

2.4. Ultrasonic pretreatment experiments

Five milligram of microcrystalline celluloses was dissolved in 0.5 ml of six different ILs to equal concentration. Then the cellulose solutions were pretreated with the help of an ultrasonic generator (KQ-300VED, Kunshan Sonication Co., China) at a frequency of 45 kHz. The sonication power was 100 W and the sonication time was 30 min. The temperature was maintained at 60 °C during the ultrasonic heating experiments. Cellulose solutions treated at 60 °C by conventional heating for 30 min served as the control group.

2.5. Enzymatic in situ saccharification of cellulose in aqueous-ILs media

Sodium acetate buffer (pH 4.8) was added to the cellulose solutions prepared by ultrasonic pretreatment, resulting in a heterogeneous suspension. The final reaction system was a total 0.5 ml solution containing various concentrations of ILs, namely 20%, 50% and 100% (v/v). Enzymatic reaction was initiated by addition of cellulase to the aqueous-ILs mixture. The enzymatic solution was vortex mixed and then the enzymatic hydrolysis was carried out in a constant temperature incubator shaker at 160 rpm. The efficiency of enzymatic saccharifcation was evaluated by quantifying the glucose released after 24 h. Then optimization experiments were designed to investigate the effect of cellulase concentration, temperature and ILs concentration on the enzymatic hydrolysis.

2.6. Analysis methods

Glucose released by cellulose hydrolysis was quantified using an SBA-40C Biological Sensing Analyzer (Biology Institute of Shandong Academy of Sciences, China). One milligram per milliliter glucose solution was used as a standard for the measurement.

Table 1
Solubility (wt%) of microcrystalline cellulose in six ILs at different temperature.

ILs	40 °C	50 °C	60 °C	70 °C	80 °C	90 °C
[Mmim][DMP]	4.65	5.30	7.36	8.75	9.84	10.64
[Meim][DMP]	5.09	6.43	7.15	9.42	12.96	16.18
[Maim][DMP]	0.56	1.80	3.00	6.24	7.86	9.06
[Emim][DEP]	3.84	5.11	6.20	8.96	9.60	11.48
[Eeim][DEP]	–[a]	–	1.17	2.08	5.86	9.13
[Eaim][DEP]	–	–	2.04	4.46	5.25	6.25

[a] Means not detected.

The viscosity of cellulose solutions in ILs was measured following the method of Evlampieva with some modifications (Evlampieva, Vitz, Schubert, & Ryumtsev, 2009). Microcrystalline cellulose was added to ILs and dissolved by conventional heating or ultrasonic heating pretreatment mentioned above, respectively. After cooling to room temperature, pyridine was added dropwise into the solution, resulting to the final stable mixture. An ubbelohde viscometer was used to perform the viscosity measurement. The viscosity of these concentration-gradient solutions was measured at 30 °C.

A microscopic evaluation of morphological changes occurring during ultrasound pretreatment of microcrystalline cellulose was studied using a Hitachi S-4800 FE Scanning Electron Microscopy, where samples were coated with a thin layer of gold in an automatic sputter coater before observation. The accelerating voltage was 5 kV and images of the samples were acquired at 5000 magnification.

3. Results and discussion

3.1. Solubility of cellulose in ILs

The mechanism of cellulose dissolution in ILs has been studied in detail by Remsing, Swatloski, Rogers, and Moyna (2006). The dissolution of cellulose in 1-butyl-3-methylimidazolium chloride ([Bmim]Cl) involved hydrogen-bonding between the carbohydrate hydroxyl protons and the chloride ions of IL in a 1:1 stoichiometry. ILs dissolve cellulose by weakening the inter- and intra-molecular hydrogen bonds of the cellulose chains with its hydrogen bond acceptability, especially from anion. ILs that can dissolve cellulose always consist of anions such as Cl^-, $HCOO^-$, CH_3COO^-. Carboxylate salts such as 1-allyl-3-methylimidazolium formate can dissolve 10 wt% of cellulose at 60 °C and 20 wt% at 85 °C, but they were defective in poor thermal stability and tedious synthesis procedure (Fukaya, Sugimoto, & Ohno, 2006).

In contrast, alkylphosphate type ILs can be easily synthesized through one-step method and persist perfect thermal stability. All the six ILs synthesized above were liquids at room temperature with lower viscosity than the corresponding halogen type ILs. Owing to the strong hydrogen bond acceptability of the phosphate anion, alkylphosphate type ILs showed considerable solubility of cellulose at different temperature compared to chloride-based ILs which could only dissolve cellulose above 70 °C (Fukaya et al., 2006; Zhao et al., 2008). As listed in Table 1, all the six ILs dissolved cellulose over 70 °C, while only [Eeim][DEP] and [Eaim][DEP] showed no significant solubility at temperatures below 60 °C. As a whole, [Mmim][DMP] and [Meim][DMP] performed better than other ILs, having solubility of 4.5–7.5% within the range of 40–60 °C which was just around the operative temperature for cellulase.

3.2. Evaluation of biocompatibility of different ILs

High melting point, strong polarity and cumbersome synthesis process have hindered the application of in situ cellulose enzymatic hydrolysis in chloride- and carboxylate-type ILs (Turner et al., 2003; Zhao et al., 2009). As for the alkylphosphate type ILs, initial research which received beneficial results of in situ enzymatic saccharification was proceeded using [Emim][DEP] (Kamiya et al., 2008). The results were consistent with the rule that the effect of ions on enzyme activity and stability usually follows the Hofmeister series, that is to say, anions exert their adverse effect on enzyme as follows: $F^- < PO_4^{3-} < SO_4^{2-} < CH_3COO^- < Cl^- < Br^- < I^- < SCN^-$ (Zhao, 2005).

Based on previous results, we performed enzymatic saccharification using alkylphosphate type of ILs-aqueous system to investigate the impact that ILs brought to cellulase. The biocompatibility of six ILs with cellulase was evaluated by glucose released during the reaction. Table 2 revealed the amounts of glucose released at the three given concentrations of ILs. For all of the six ILs, 20% was the best concentration for enzymatic saccharification. Especially, [Mmim][DMP] showed the best performance at this concentration, the conversion could reach 39.68% after 24 h, almost three-fold higher than that of the control (11.05%). At two other concentrations (i.e., 50%, 100%), no activity of cellulase was detected.

The cellulase activity in 20% ILs descended in the following sequence: [Mmim][DMP] > [Meim][DMP] > [Maim][DMP] > [Eeim][DEP] > [Eaim][DEP] > [Emim][DEP], indicating that ILs with the dimethylphosphate anion were more compatible with cellulase than diethylphosphate ones. According to the theory of Hofmeister series, the effects of $[DMP]^-$ on the solubility of proteins is greater than $[DEP]^-$ due to its smaller side chain. Cellulase in the $[DMP]^-$ solutions persists much more stable secondary and tertiary structure, and thus exhibits a more efficient catalytic activity.

3.3. Effect of ultrasonic heating treatment on enzymatic hydrolysis of cellulose in ILs

Former research has demonstrated that ultrasound pretreatment could bring about enhancement of cellulose dissolution in ILs (Mikkola et al., 2007). Cellulose dissolved in ILs was pretreated by ultrasound to investigate its effect on enzymatic saccharification of cellulose. As the results showed in Table 2, ultrasonic treatment exhibited a significant improvement on conversion of cellulose to glucose. In contrast to cellulose with conventional heating pretreatment, the conversion of cellulose pretreated by ultrasonic heating increased notably, especially when ILs concentration was 20%. Activities were detected in all of the three dimethylphosphate type ILs at the concentration of 50%, whereas activity was only detected in [Eeim][DEP] among diethylphosphate ILs at this concentration. None but [Mmim][DMP] showed tiny compatibility with cellulase when ILs concentration reached 100%. The conversion of cellulose treated with ultrasound reached 53.15% in [Mmim][DMP] after 24 h, while that were 16.09% for control and 39.68% for cellulose treated with conventional heating, suggesting that ultrasonic pretreatment contributed to saccharification assuredly. In addition, ultrasonic heating pretreatment also enhanced cellulose hydrol-

Table 2
The conversion of cellulose to glucose in ILs after 24 h with conventional heating pretreatment (CHP) and ultrasonic heating pretreatment (UHP).

ILs concentration	20%		50%		100%	
	CHP	UHP	CHP	UHP	CHP	UHP
[Mmim][DMP]	39.68%	53.18%	–[a]	1.00%	–	0.30%
[Meim][DMP]	35.86%	48.14%	–	0.70%	–	–
[Maim][DMP]	26.32%	34.36%	–	0.30%	–	–
[Emim][DEP]	1.64%	2.18%	–	–	–	–
[Eeim][DEP]	12.55%	18.55%	–	0.10%	–	–
[Eaim][DEP]	9.55%	11.00%	–	–	–	–

[a] Means not detected. The conversion of cellulose to glucose during enzymatic hydrolysis in aqueous media with CHP and UHP were 11.05% and 16.09%, respectively.

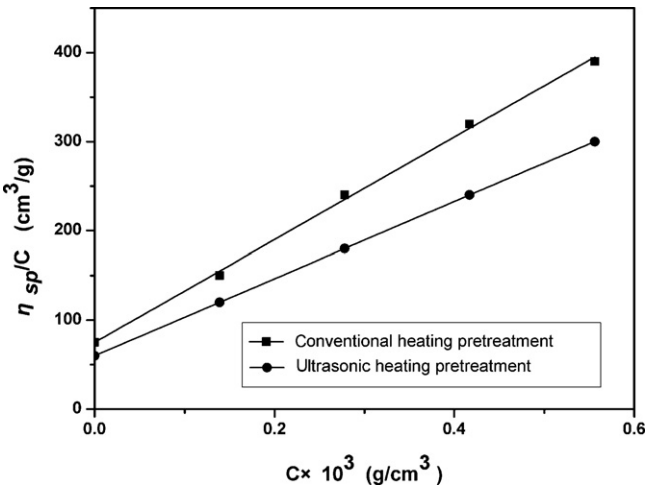

Fig. 2. Viscosity of cellulose solution in [Mmim][DMP]-pyridine mixture ([Mmim][DMP]: pyridine = 1: 8, v/v) with conventional and ultrasonic heating pretreatment respectively.

ysis in aqueous media. The conversion of cellulose to glucose was raised from 11.05% (cellulose pretreated with conventional heating in aqueous system) to 16.09%. Zhang, Fu, and Liang (2008) have reported similar findings that ultrasonic wave-assisted alkali pretreatment improved the enzymatic hydrolysis rate of lignocellulosic substrates.

To explain why ultrasonic heating enhanced enzymatic hydrolysis of cellulose, the viscosities of cellulose/ILs pretreated by conventional and ultrasonic heating were analyzed according to the methods mentioned in the experimental part. Fig. 2 illustrated the η_{sp}/c–c flow curves of cellulose/[Mmim][DMP] solutions at different concentrations (c is the concentration of cellulose and η_{sp} is the specific viscosity). The intrinsic viscosity $[\eta]$ ($[\eta] = \lim_{c\to 0}\eta_{sp}/c$) was deduced from the classical way of double extrapolation to zero concentration. It is obvious $[\eta]$ of solution prepared with ultrasound treatment (60 cm^3/g) was much lower than that of solution prepared under conventional heating condition (75 cm^3/g). According to the Mark–Houwink equation:

$$[\eta] = KM^{\alpha}$$

K and α are the parameters depending on the particular polymer-solvent system; M is the mean molecular weight of a polymer.

The molecular weight of cellulose was positively correlated with $[\eta]$. It indicated that cellulose dissolved by ultrasonic heating displayed a lower molecular weight than that dissolved by conventional heating. More disruption must occur during ultrasonic treatment, resulting in the lessening in crystalline and degree of polymerization (DP).

SEM analyses were also employed to investigate the changes in the structure of cellulose regenerated from [Mmim][DMP] under conventional and ultrasonic heating pretreatment conditions, respectively. SEM graphs suggested that in original microcrystalline cellulose, the major component present was ordered and condensed fibrils (Fig. 3a). After being regenerated from ILs (Fig. 3b), the surface became rough and swollen. When the cellulose was subjected to ultrasonic pretreatment, the structure of cellulose regenerated further got even loosen and less compact (Fig. 3c), illustrating the disruption of linkages in cellulose to a certain extent. More accessible external and internal surface area of cellulose was attainable as binding site for cellulase, leading to the enhancement of enzymatic saccharification (Singh, Simmons, & Vogel, 2009).

3.4. Optimization of reaction conditions for enzymatic hydrolysis of cellulose

Based on the preliminary findings obtained above, [Mmim][DMP] which showed good performance in both solubility and biocompatibility was selected for the next optimization experiments under ultrasonic conditions.

3.4.1. Effect of cellulase concentration on enzymatic saccharification of cellulose

The cellulase concentration was an important factor for enzymatic saccharification of cellulose. In order to enhance the conversion of cellulose to glucose, the effect of cellulase concentration was investigated in detail. As shown in Fig. 4a, the conversion of cellulose after 24 h was enhanced markedly along with the increase of cellulase concentration from 1 mg/ml to 8 mg/ml, and the corresponding maximal conversion reached 82.77%. Further increase of cellulase concentration from 8 mg/ml to 12 mg/ml did not bring obvious changes to the hydrolysis efficiency. Previous literature illustrated that despite the enzyme concentration increased, no more glucose released during the enzymatic hydrolysis of cellulose in [Bmim]Cl (Turner et al., 2003). According to the study of Zhao et al. (2009), 5.0 mg/ml cellulase loading did not bring out better results, but even slower reaction rate compared to 3.0 mg/ml cellulase loading. Considering the cost of the enzyme, we chose 8 mg/ml as the optimal cellulase concentration.

3.4.2. Effect of temperature on enzymatic saccharification of cellulose

The catalytic activities of cellulase were severely diminished at high temperature (Andreaus, Azevedob, & Cavaco-Paulo, 1999), whereas low temperature is not conducive to the solubility of cellulose in aqueous-ILs system. Thus, we chose a range from 40 °C to 60 °C to investigate the effect of temperature on hydrolysis of cellulose. As suggested in Fig. 4b, the reaction temperature appeared to obviously affect the conversion of cellulose to glucose. Like most enzyme-catalyzed reactions, the rate of cellulose hydrolysis increased as the temperature was raised. After 24 h, approximately

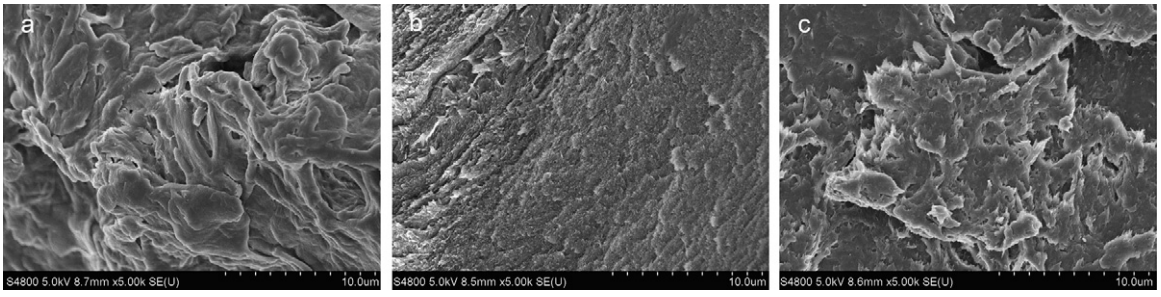

Fig. 3. SEM micrographs of cellulose: (a) untreated cellulose; (b) regenerated cellulose with conventional heating pretreatment; and (c) regenerated cellulose with ultrasound heating pretreatment.

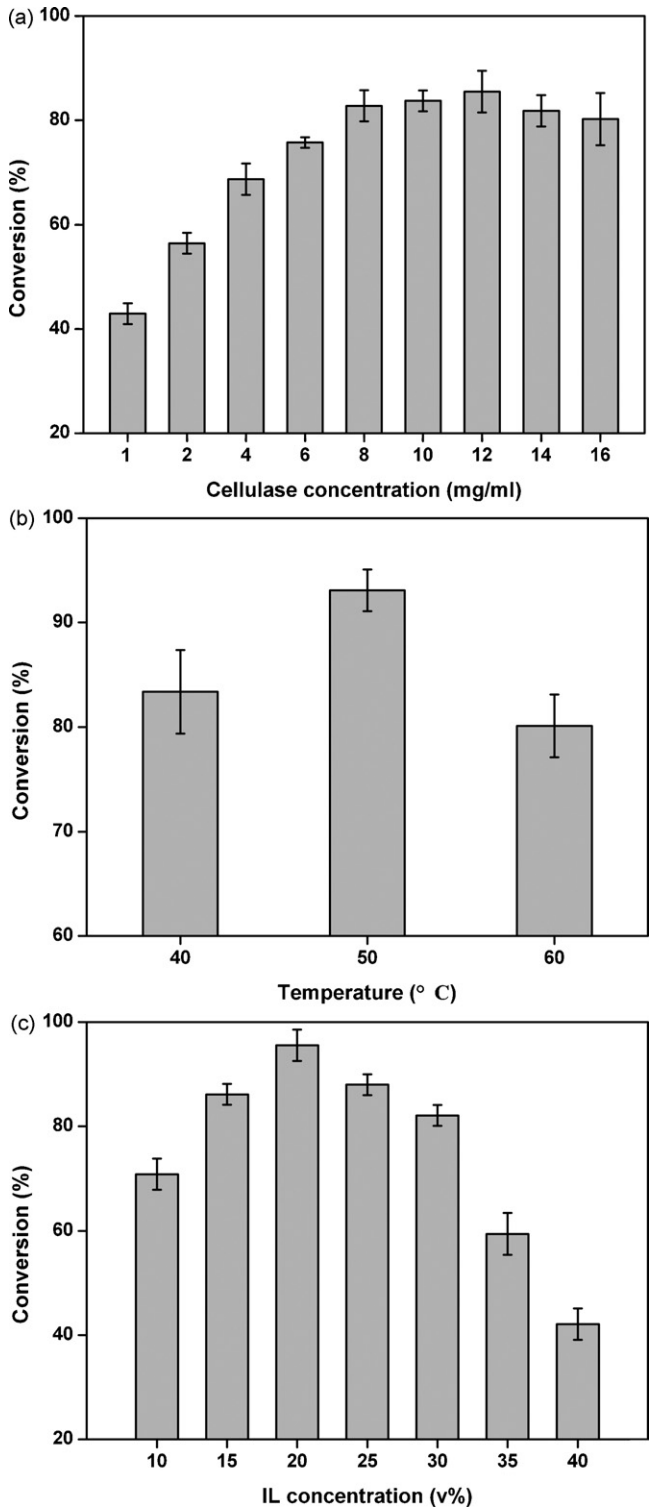

Fig. 4. Effect of reaction parameters on the saccharification of cellulose: (a) cellulase concentration (10 mg/ml cellulose, 60 °C, 20% [Mmim][DMP]); (b) temperature (10 mg/ml cellulose, 8 mg/ml cellulase, 20% [Mmim][DMP]); and (c) ILs concentration (10 mg/ml cellulose, 8 mg/ml cellulase, 50 °C).

93.09% of the cellulose was converted to glucose at 50 °C compared to 83.36% at 40 °C. However, when the temperature was further raised to 60 °C, only 80.09% of the cellulose was converted. Enzymes were sensitive to temperature and the thermal stability of cellulase significantly decreased at temperature above 50 °C (Eriksson, Börjesson, & Tjerneld, 2002). Thus, the reaction rate reached a maximum at the optimal temperature, whereas high temperature would inactivate enzymes.

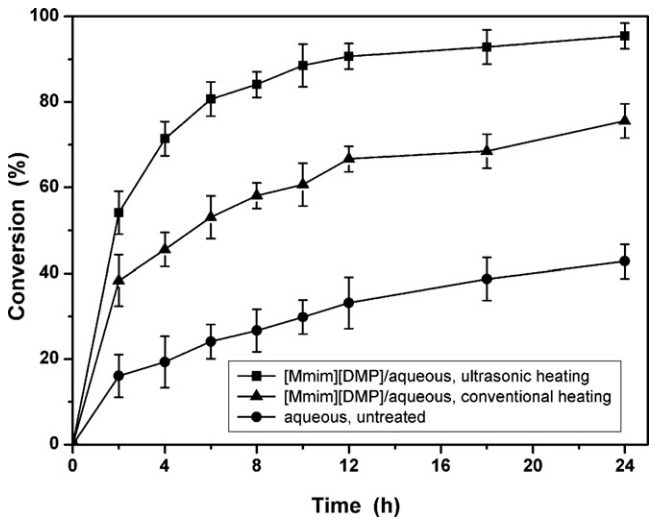

Fig. 5. Time course of enzymatic saccharification of cellulose. Reaction conditions: 10 mg/ml cellulose, 8 mg/ml cellulase, 20% [Mmim][DMP], 50 °C.

3.4.3. *Effect of ILs concentration on enzymatic saccharification of cellulose*

According to the preliminary experiment results, the enzymatic saccharification of cellulose was the most efficient when ILs were 20% of the total volume. Intensive study was carried out to find the optimal concentration of [Mmim][DMP] around 20%. Fig. 4c showed the conversion of cellulose at different ILs concentrations after a 24-h reaction. When the proportion of ILs was within 20%, glucose formation was enhanced along with the ILs concentration. The conversion of cellulose raised from 70.82% to 95.55% by increasing [Mmim][DMP] concentration from 10% to 20%. One possible reason was that ILs pretreatment of cellulose generated more adsorption sites for the cellulase. On the other hand, any increase of ILs concentration above 20% caused a sharp reduction in the conversion of cellulose. When ILs concentration was 40%, the conversion was only 42.09%. This might be explained by the adverse effect of high ILs concentration on cellulase. The above results were similar to the aqueous-[Emim][DEP] system in a former study (Kamiya et al., 2008), that is, the conversion of cellulose to glucose was preferable at an IL to water ratio of 1:4 (v/v). Any further increase in the concentration of ILs would lead to decreasing of conversion dramatically.

3.5. *Time course of in situ enzymatic saccharification of cellulose*

Finally, to demonstrate the enhancement of cellulose conversion based on the optimal conditions obtained above, the time course of enzyme reactions proceeded in aqueous-[Mmim][DMP] mixture (pretreated with ultrasonic heating or conventional heating) and aqueous media, respectively. All of the reactions were heterogeneous solid-liquid system which is the classic characteristic of enzymatic hydrolysis of cellulose (Gan, Allen, & Taylor, 2003). The glucose released during the enzymatic hydrolysis was monitored continuously (Fig. 5). Cellulose pretreated with ultrasonic heating and hydrolyzed in aqueous-ILs media exhibited the best conversion at each predetermined time. After 24 h, the reaction solution became clear and the conversion of cellulose reached 95.48%, compared to 75.57% of cellulose pretreated with conventional heating and 42.77% of the untreated cellulose. Much higher conversion efficiency was achieved under ultrasonic conditions in

our study than former reports (Jones & Vasudevan, 2010; Kamiya et al., 2008).

Moreover, it is worth mentioning that the recovery of glucose and ILs from the in situ reaction system using neutral alumina column chromatography is being carried out in our laboratory. Encouraging results have been obtained and will be reported later. All these findings will provide a good basis for reducing the cost and promote the integrated utilization of cellulose.

4. Conclusions

Six imidazolium-based alkylphosphate type ILs were investigated in this study, and all of them could dissolve cellulose. Especially, [Mmim][DMP] showed favorable solubility and biocompatibility simultaneously. Thus, a novel aqueous-[Mmim][DMP] reaction system was established for in situ enzymatic saccharification of cellulose. After optimizing the reaction parameters, the conversion of cellulose with ultrasonic heating pretreatment increased by 52.71% compared to that of cellulose untreated. The results of viscosity and SEM analysis showed that pretreatment with ultrasonic heating in ILs decreased the crystallization and degree of polymerization of cellulose, which might contribute to increased rate of enzymatic hydrolysis of cellulose. Overall, our work offered a new reaction system aqueous-[Mmim][DMP] which was qualified to be perfect candidate as alternative media for in situ enzymatic hydrolysis of cellulose. Additionally, this study highlighted the role of ultrasound pretreatment in enhancing the conversion of cellulose during the enzymatic reaction. Direct in situ enzymatic hydrolysis of cellulose using aqueous-ILs system would finally lead to efficient utilization of the cellulosic biomass.

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