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# Novel cellulose pretreatment solvent: phosphonium-based amino acid ionic liquid/cosolvent for enhanced enzymatic hydrolysis

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**Abstract:** The potential of halogen-free and imidazolium-free phosphonium-based amino acid ionic liquids (AAILs) has been investigated as new solvents for cellulose pretreatment for the subsequent enzymatic hydrolysis of cellulose. AAILs alone did not dissolve cellulose (Avicel), even at 120°C. However, when polar solvents such as dimethylsulfoxide (DMSO) were added as cosolvents, AAILs became an acceptable solvent for cellulose at 30°C. The solubility of cellulose in tetrabutylphosphonium glycine ([TBP][Gly])/cosolvent reached 15%. The enzymatic hydrolysis of cellulose was dramatically enhanced by pretreatment with AAIL/cosolvent, and the glucose yield reached 100% when the novel AAIL tetrabutylphosphonium *N*,*N*-dimethylglycine ([TBP][DMGly]) was used in combination with DMSO as cosolvent. The enzymatic conversion of cellulose to glucose in 6% and 13% [TBP][DMGly]/DMSO buffer solutions reached 98% and 79%, respectively. The decrease in cellulase activity owing to residual [TBP][DMGly]/DMSO was not significant. Hence, it is possible to conduct the dissolution and enzymatic hydrolysis of cellulose in a one-batch process in a phosphonium-based AAIL/cosolvent system.

**Keywords:** amino acid ionic liquid, Avicel, cellulose, DMSO as cosolvent for ILs, saccharification

## Introduction

Ionic liquids (ILs), which are composed of an anion and a cation, have attracted a lot of attention owing to their strong solubilization ability, adjustable structure, and recyclability. Since 2002, when cellulose was first dissolved in an IL (Swatloski et al. 2002), a growing number of ILs have been tested as solvents for lignocelluloses (Fukaya et al. 2006; Liu et al. 2012; Qu et al. 2013a,b; Abushammala et al. 2015; Roselli et al. 2015). Moreover, enhanced enzymatic hydrolysis of lignocellulosic biomass was found after IL pretreatment. Usually, high dissolving temperatures (≥80°C) were applied to disrupt hydrogen bonds in the highly crystalline cellulose and increase the accessibility for enzymes (Uju et al. 2012). Compared with untreated cellulose, glucose yields were greatly increased to 80%–90% in an IL/cosolvent system at 110°C (Mai et al. 2014) and by combining with peracetic acid pretreatment (Uju et al. 2013) or ultrasound irradiation (Ninomiya et al. 2013).

Many ILs suitable for cellulose dissolution contain an imidazolium ring and/or halogen are not environmentally compatible or they are even toxic (Kumar et al. 2011; Ma et al. 2014). Therefore, the development of eco-friendly and biocompatible ILs is urgently required. Amino acid ionic liquids (AAILs) are not only halogen free but also have lower viscosity than conventional ILs at room temperature (r.t.) (Kagimoto et al. 2006; Jiang et al. 2008). They have the potential to be an ideal solvents for cellulose and are expected to be compatible with a subsequent enzymatic hydrolysis. The application of AAILs for cellulose dissolution is seldom documented. Ohira et al. (2012a) reported that *N*,*N*-diethyl-*N*-(2-methoxyethyl)- *N*-methylammonium alanine ([$N_{221ME}$][Ala]) dissolved 12% of cellulose at 100°C and Ohira et al. (2012b) found that a combination of this IL with dimethylsulfoxide (DMSO) permits cellulose dissolution at r.t. It was reported that *N*-methyl-*N*-(2-methoxyethyl)-pyrrolidin-1-ium 2,6-diaminohexanoate ([$P_{1ME}$][Lys]) dissolves lignin below 60°C, but requires 80°C for cellulose dissolution (Hamada et al. 2013). Liu et al. (2012) prepared a series of r.t.-ILs based on cholinium (Ch) as the cation and amino acids (AAs) as the anion, but the cellulose was scarcely soluble (<5 mg $g^{-1}$) in [Ch][AA].

AAIL-pretreated celluloses have been submitted to further enzymatic hydrolysis, and it is known that their conversion to glucose occurs with a higher conversion ratio than that for untreated cellulose (Liu et al. 2012; Ohira et al.

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2012a). Mizuno et al. (2012) reported that pretreatment by [$N_{221ME}$][Ala] was least effective among the tested ILs in terms of crystallinity decrement and hydrolysis speed of the regenerated cellulose. The complete reaction from AAIL-pretreated cellulose to glucose has not been investigated.

Herein, the potential of phosphonium-based AAILs (Figure 1) as new pretreatment solvents for the enzymatic hydrolysis of cellulose should be evaluated. Three AAILs with TBP as the cation and a natural AA as the anion ([TBP][AA]) are the focus (Kagimoto et al. 2006). Furthermore, a novel [TBP][AA] with *N,N*-dimethylglycine as the anion ([TBP][DMGly]) was synthesized for the first time. It is potentially more stable than [TBP][AA] because the reactive amino group is protected by methyl groups. This may suppress side reactions of cellulose during the dissolution or pretreatment. All four ILs should also be tested with six cosolvents with Avicel as model cellulose. The DPs of the corresponding regenerated celluloses will be reported as well as their susceptibility to enzymatic hydrolysis. The latter step was also conducted without removing the AAILs to evaluate the compatibility between cellulase and AAIL/cosolvent.

## Materials and methods

**Materials:** Microcrystalline cellulose (Avicel PH-101; Sigma-Aldrich Co., St. Louis, MO, USA) was dried under vacuum over phosphorus pentoxide ($P_2O_5$) at r.t. for 24 h. All other chemicals (Wako Pure Chemical Industries, Osaka, Japan) were reagent grade and used without purification. Cellulase (Onozuka RS) was purchased from Yakult Pharmaceutical Industry Co., Ltd., (Tokyo, Japan). The enzymatic activity was 16,000 filter-paper units (FPU) $g^{-1}$. The optimum pH and temperature of the enzyme were determined from the glucose yield based on Avicel PH-101 as substrate; the highest glucose yield was obtained at pH 4.5 and 50°C. These results are consistent with those obtained from the supplier (optimum pH: 4.0–5.0; optimum temperature: 50–60°C). The average protein content (0.237 mg $mg^{-1}$, two measurements) of Onozuka RS was determined according to the Bio-Rad Protein Assay procedure (Bio-Rad, Hercules, CA, USA) with bovine gamma globulin as the standard.

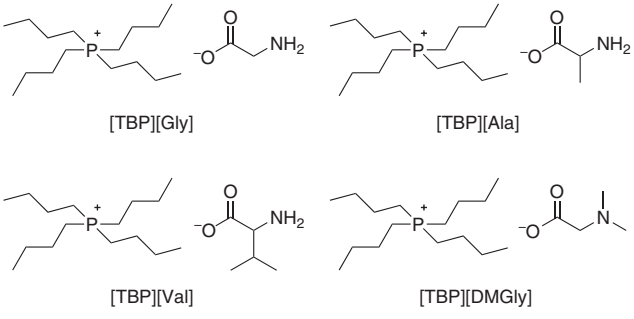


**Figure 1:** Structures of amino acid ionic liquids (AAIL).
TBP, tetrabutylphosphonium; Gly, glycine; Ala, alanine; Val, valine; DMGly, *N,N*-dimethylglycine.

**Typical procedure for the preparation of [TBP][AA]:** To a stirred solution of the AA (36.3 mmol) in water (11 ml), an aqueous solution of [TBP][OH] (40%, 8.29 g, 30 mmol) was added dropwise at r.t. After gentle mixing for 10 min, water was removed by a rotary evaporator at 50°C. The crude IL was washed with acetonitrile and methanol (3:2, v/v) to remove excess AA. Finally, the product was dried under vacuum for 24 h at 70°C (Kagimoto et al. 2006). For the synthesis of [TBP][DMGly], [TBP][OH], and *N,N*-DMGly ethyl ester were stirred at 70°C for 2 h and the excess ester was removed by extraction with ethyl acetate. The structure of [TBP][AA] (Figure 1) was confirmed by $^1$H NMR spectra (Bruker AVANCE II 400 FT-NMR (400 MHz) spectrometer). Chemical shifts and coupling constants are reported in δ values (ppm) and Hz, respectively. [TBP][Gly]: (DMSO-$d_6$) δ 0.82 (12H, t, $J$ = 7.2 Hz, -$CH_2C\underline{H}_3$×4), 1.27–1.39 (16H, m, -$C\underline{H}_2C\underline{H}_2$-×4), 2.10 (8H, m, $PCH_2$-×4), 2.53 (2H, s, -$CH_2COO$). [TBP][Ala]: (DMSO-$d_6$) δ 0.82 (12H, t, $J$ = 7.2 Hz, -$CH_2C\underline{H}_3$×4), 0.88 [3H, d, $J$=6.8 Hz, -CH($C\underline{H}_3$)-], 1.27–1.39 (16H, m, -$C\underline{H}_2C\underline{H}_2$-×4), 2.06–2.13 (8H, m, $PCH_2$-×4), 2.68 [1H, q, $J$=6.8 Hz, -$C\underline{H}(CH_3)$-]. [TBP][Val]: (DMSO-$d_6$) δ 0.56 [3H, d, $J$=6.8 Hz, -CH($C\underline{H}_3$)$_2$], 0.69 [3H, d, $J$=6.8 Hz, -CH($C\underline{H}_3$)$_2$], 0.82 (12H, t, $J$=7.2 Hz, -$CH_2C\underline{H}_3$×4), 1.27–1.39 (16H, m, -$C\underline{H}_2C\underline{H}_2$-×4), 1.78 [1H, m, -$C\underline{H}(CH_3)_2$], 2.10 (8H, m, $PCH_2$-×4), 2.48 (1H, s, -CHCOO). [TBP][DMGly]: ($CDCl_3$) δ 0.87 (12H, t, $J$=6.9 Hz, -$CH_2C\underline{H}_3$×4), 1.38–1.44 (16H, m, -$C\underline{H}_2C\underline{H}_2$-×4), 2.25 (8H, m, $PCH_2$-×4), 2.34 (6H, s, $NCH_3$×2), 2.95 (2H, s, -$CH_2COO$).

**Solubility of cellulose in AAIL/cosolvent:** Cellulose (0.01 g) was added to 1 g AAIL/cosolvent and magnetically stirred at 30°C. When the resulting solution became transparent, another 0.01 g cellulose was added. This procedure was repeated until the solution became hazy or the viscosity of the solution became very high for stirring. The solubility was calculated based on the total amount of added cellulose until the solution became transparent within 24 h.

**DP measurements:** Cellulose (0.1–0.3 g) was mixed with AAIL/cosolvent (1.0 g per 1.0 g) and stirred magnetically under argon at 30°C until complete dissolution. The solution was then poured into 100 ml distilled water for cellulose regeneration. After vacuum filtration, the residual AAIL/cosolvent was washed with 100 ml distilled water three times. The regenerated cellulose was dried under vacuum over $P_2O_5$ at r.t. for 24 h. The viscosimetric DP determination was performed in copper (II) ethylenediamine solution (Tao et al. 2016). Each sample was measured in triplicate and the DPs in Table 1 are the averages of three runs.

**Enzymatic hydrolysis:** Cellulose (18 mg) was mixed with AAIL/cosolvent (90 mg per 90 mg) and magnetically stirred until the solution became clear. Then, acetate buffer (0.1 mol $l^{-1}$, pH 4.5, 2.8 ml) was added with vigorous stirring for 30 min. Regenerated cellulose was triturated by spatula. The suspension was centrifuged at 4000 rpm for 20 min and the regenerated cellulose was then washed with distilled water (2.4 ml) three times to remove residual IL and cosolvent. This washing procedure was omitted when the compatibility of cellulose and the IL was examined. Finally, acetate buffer (0.1 mol $l^{-1}$, pH 4.5, 2.4 ml) was added to adjust the total volume of the enzymatic solution to 2.8 ml. The pH was adjusted to 4.5 with a small amount of additional acetic acid if necessary.

Enzymatic hydrolysis was conducted at 50°C and 200 rpm by adding 2.11 mg cellulase (protein content: 0.5 mg) (Ohira et al. 2012a). At a given time interval, 50 µl of the reaction solution was withdrawn and the reaction was quenched by heating at 105°C for 5 min. Before

**Table 1:** Dissolution and regeneration of cellulose (Avicel PH101, DP=208) in ILs with cosolvents.

| No. | IL | Cosolvent | Cellulose (wt%) | Temp. (°C) | Time (h) | Solubility[a] | Yield (%) | DP (σ) |
|---|---|---|---|---|---|---|---|---|
| 1 | [TBP][Gly] | – | 2 | 120 | 5 | – | – | – |
| 2 | [TBP][Gly] | DMSO | 15 | 30 | 5 | + | 99.4 | 197 (0.1) |
| 3 | [TBP][Gly] | NMI | 15 | 30 | 1.5 | + | 99.0 | 196 (0.2) |
| 4 | [TBP][Gly] | Pyridine | 12 | 30 | 24 | + | 99.1 | 204 (0.2) |
| 5 | [TBP][Gly] | NMP | 12 | 30 | 24 | + | 98.0 | 203 (0.2) |
| 6 | [TBP][Gly] | DMAc | 8 | 30 | 4 | + | 97.6 | 200 (0.1) |
| 7 | [TBP][Gly] | DMF | 8 | 30 | 1 | + | 97.3 | 194 (0.3) |
| 8 | [TBP][Ala] | – | 2 | 120 | 2 | – | – | – |
| 9 | [TBP][Ala] | DMSO | 5 | 30 | 0.5 | + | 96.8 | 198 (0.2) |
| 10 | [TBP][Ala] | NMI | 5 | 30 | 1 | + | 95.7 | 197 (0.2) |
| 11 | [TBP][Ala] | Pyridine | 5 | 30 | 24 | + | 96.2 | 205 (0.2) |
| 12 | [TBP][Ala] | NMP | 5 | 30 | 24 | + | 96.0 | 199 (0.1) |
| 13 | [TBP][Ala] | DMAc | 5 | 30 | 2 | + | 95.3 | 199 (0.3) |
| 14 | [TBP][Ala] | DMF | 5 | 30 | 1 | + | 97.5 | 205 (0.1) |
| 15 | [TBP][Val] | – | 2 | 120 | 2 | – | – | – |
| 16 | [TBP][Val] | DMSO | 5 | 30 | 1 | + | 98.0 | 202 (0.4) |
| 17 | [TBP][DMGly] | – | 2 | 120 | 1 | – | – | – |
| 18 | [TBP][DMGly] | DMSO | 10 | 30 | 2.5 | + | 97.5 | 205 (0.2) |
| 19 | [TBP][DMGly] | DMF | 10 | 30 | 24 | + | 96.3 | 199 (0.2) |
| 20 | [TBP][DMGly] | Pyridine | 5 | 30→50 | 24h+5min | + | 98.5 | 200 (0.1) |
| 21 | [TBP][DMGly] | NMP | 5 | 30→50 | 24h+5min | + | 99.4 | 201 (0.2) |

[a]+, sample was completely dissolved and the solution was very clear; –, sample was not dissolved. σ, standard deviation of the three measurements. DMAc, *N,N*-dimethylacetamide; DMF, *N,N*-dimethylformamide; NMI, *N*-methylimidazole; NMP, *N*-methyl-2-pyrrolidone; DMSO, dimethylsulfoxide; TBP, tetrabutylphosphonium; Gly, glycine; Ala, alanine; Val, valine; DMGly, *N,N*-dimethylglycine.

analysis by HPLC, all the samples were filtered by a syringe filter (pore size 0.2 µm, Advanced Microdevices Pvt., Ltd., Ambala, India). Each experiment was conducted in triplicate.

**Quantification of glucose by HPLC:** The detection system comprised a PU-980 "intelligent" HPLC pump (JASCO, Tokyo, Japan), an RI-2031 Plus RI detector (JASCO), and an Asahipak NH2P-40 3E column (3.0 mm ID×250 mm l) (Shodex, Tokyo, Japan). The flow rate of the eluent ($CH_3CN/H_2O$, 65:35, v/v) was 0.32 ml $min^{-1}$. The retention time (Rt) of glucose was 10.08 min (±0.39%). Identification of peaks was established by comparison of the Rt with that of a known standard compound. Calibration of peaks was performed via a linear relationship between the peak area and the corresponding concentration of the standard solution. The conversion to glucose (Y) was calculated: $Y(\%)=(C_{glc}\times162/180)/C_{cell}\times100$, where $C_{glc}$ and $C_{cell}$ are the concentrations of glucose and cellulose, respectively, in mg $ml^{-1}$.

# Results and discussion

## Phosphonium-based AAILs as cellulose solvents in the presence of a cosolvent

The potential of TBP-based AAILs, including [TBP][Gly], [TBP][Ala], [TBP][Val], and [TBP][DMGly], as cellulose solvents was evaluated with and without a cosolvent, as shown in Table 1. The cosolvents were aprotic polar organic solvents, including a neutral solvent DMSO, basic solvents (pyridine and *N*-methylimidazole, NMI), amide-related solvents (*N,N*-dimethylformamide, DMF; *N,N*-dimethylacetamide, DMAc), and *N*-methyl-2-pyrrolidone (NMP).

Herein, three [TBP][AA] were selected from the 20 [TBP][AA] containing natural AA, because of their low viscosity, as it is one of the requirements for effective cellulose dissolving. The reported viscosities of [TBP][Gly], [TBP][Ala], and [TBP][Val] at 25°C are 415, 344, and 423 cP, respectively, which are lower than those of other [TBP][AA] (Kagimoto et al. 2006). The potentially less reactive novel [TBP][DMGly] was synthesized and also tested. However, without cosolvent, all of the AAILs failed to dissolve cellulose (Avicel), even at 120°C (Table 1, entries 1, 8, 15, and 17). Therefore, the above-mentioned aprotic polar organic solvents served as cosolvents because they increased the solubilization efficiency of 1-allyl-3-methylimidazolium chloride ([Amim]Cl) for cellulose dissolution (Tao et al. 2016).

[TBP][Gly], which contains the simplest AA, was the most effective AAIL solvent in combination with a cosolvent (entries 2–7). [TBP][Gly]/DMSO and [TBP][Gly]/NMI dissolved 15% cellulose, whereas [TBP][Gly]/pyridine and [TBP][Gly]/NMP dissolved 12% cellulose. DMAc and DMF were also effective, and 8% cellulose was dissolved in [TBP][Gly]/DMAc and [TBP][Gly]/DMF.

[TBP][Ala] has the lowest viscosity, but its solubilization efficiency was not as high even with a cosolvent. All combinations of [TBP][Ala] with the six cosolvents resulted in 5% cellulose dissolution at 30°C (entries 9–14). DMSO exhibits the highest solubilization efficiency with the shortest dissolution time. The combination of [TBP][Val] with DMSO was also investigated, but also resulted in 5% cellulose dissolution (entry 16).

The potential of the novel IL [TBP][DMGly] as a cellulose solvent is listed with the entries 18–21. [TBP][DMGly]/DMSO dissolved 10% cellulose in 2.5 h, whereas [TBP][DMGly]/DMF required 24 h to dissolve the same amount. Pyridine and NMP were less effective: 5% of cellulose was not completely dissolved in [TBP][DMGly]/pyridine or [TBP][DMGly]/NMP at 30°C within 24 h and required additional heating at 50°C for 5 min to complete the dissolution. Again, DMSO was most effective. The solubilization efficiency of [TBP][DMGly] was sufficiently high in the presence of a cosolvent, although [TBP][DMGly] was less effective than [TBP][Gly].

The DP of regenerated celluloses from [TBP][AA] ranged from 194 to 205, showing a slight decrease compared to the original cellulose (DP=208). These results indicate that no significant degradation occurred during the dissolution process. Tao et al. (2016) showed that polar organic cosolvents not only increase cellulose solubility in [Amim]Cl but also exhibit protective effects preventing DP decrement.

[TBP][Gly] displayed the highest solubilizing efficiency in combination with a cosolvent. It has been reported that the effectiveness for cellulose dissolution of an IL is estimated from the Kamlet-Taft parameters (α: hydrogen bond acidity, β: hydrogen bond basicity, and π*: dipolarity) (Fukaya et al. 2008; Brandt et al. 2010). Particularly, a high β-value is desirable for effective cellulose dissolution. The value of β is generally affected by the anion in ILs. Therefore, the solubilization efficiency of [TBP][AA] should depend on the nature of the AA. The β-values of [TBP][Gly], [TBP][Ala], and [TBP][Val] are 1.11, 1.03, and 0.78, respectively (Spange et al. 2001). Thus, the highest solubilization ability of [TBP][Gly] in the presence of a cosolvent can be reasonably explained by its highest β-value. The length of the alkyl chain in the IL anion may also affect the dissolution owing to its steric effect (Zhao et al. 2013). Anions with a longer alkyl chain have a negative effect on the interaction between the anion and the hydroxy protons of cellulose. Accordingly, the highest dissolution efficiency of [TBP][Gly] can also be explained by the absence of alkyl side chain in Gly.

The key role of cosolvents is obvious with the ILs in focus, and DMSO turned to be the most effective one. Much research work has already been dedicated to this topic (Gericke et al. 2011; Rinaldi 2011; Ohira et al. 2012b; Andanson et al. 2014). One of the theories is that cosolvents decrease the viscosity of ILs and improve the mobility of free ions in ILs, and contribute in this way to the interaction between IL and cellulose. The anions in ILs disrupt the hydrogen-bonding network of cellulose (Xu et al. 2013) and DMSO has exceptionally high efficiency for cellulose swelling, which facilitates dissolution (Fidale et al. 2008). In combination with TBP-based AAILs, cosolvents also decrease the viscosity.

## Effect of [TBP][Gly]/DMSO ratio on dissolution of cellulose

The effects of the AAIL/cosolvent ratio were examined with [TBP][Gly]/DMSO at 30°C, while proportion of the IL was changed from 0% to 100% (Figure 2). Neat [TBP][Gly] and neat DMSO did not dissolve cellulose at all, however, with increasing IL moiety in the IL/DMSO the dissolution efficiency improved. With the combination [TBP][Gly]/DMSO (50:50), 15% of cellulose was dissolved but with a proportion of IL higher than 70%, cellulose became insoluble again.

The results can be summarized that cosolvents improve dissociation and solvation of AAILs, and that the solvated AAILs plays a key role in dissolving cellulose. Moreover, the viscosity decrement of the solution in an IL/cosolvent system is also important. At 50°C, the viscosity of the solvent systems is relatively low, but nevertheless the solubilization efficiency was worse than that at 30°C

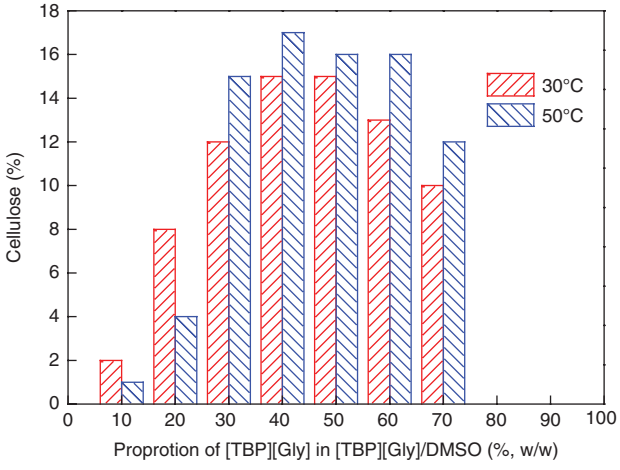


**Figure 2:** Solubility of cellulose in [TBP][Gly]/DMSO with different mass ratios at 30°C and 50°C.
DMSO, dimethylsulfoxide; TBP, tetrabutylphosphonium; Gly, glycine.

when the proportion of [TBP][Gly] was lower than 30%. No doubt, further studies are necessary to understand this observation.

## Effect of AAIL/cosolvent pretreatment on enzymatic hydrolysis

Cellulose was dissolved in phosphonium-based AAILs/DMSO, and acetate buffer was then added to regenerate cellulose. The regenerated cellulose was hydrolyzed by cellulase for 24 h in acetate buffer at 50°C after removing the residual AAIL and DMSO by washing, as shown in Figure 3a and Table 2. For comparison, the original cellulose and cellulose pretreated by [Amim]Cl/DMSO were also subjected to enzymatic hydrolysis.

The initial hydrolysis rate (glucose yield after 1 h) was significantly increased by all the IL pretreatments (Table 2). Clearly, the hydrogen bonding in the supramolecular structure of cellulose is altered by dissolution in the AAIL/DMSO system and thus the cellulose became more accessible for cellulase (Auxenfans et al. 2012; Cui et al. 2014; Ebner et al. 2014).

Cellulose pretreated by AAILs/DMSO displays a higher initial hydrolysis rate than that pretreated by [Amim]Cl/DMSO. Significantly, almost complete conversion of cellulose to glucose was achieved by a [TBP][DMGly]/DMSO pretreatment. The efficiency of cellulase is correlated with the residual amount of ILs associated with regenerated cellulose. Imidazolium- and halogen-based ILs were reported to decrease the cellulase activity (Bose et al. 2012; Li et al. 2013). Even trace amounts of these ILs may worsen significantly the cellulase activity (Zhao et al. 2009) and this makes an extensive removal necessary for the residual IL before enzymatic conversion (Datta et al. 2010; Shi et al. 2013). This seems to be not the case in the experiments in the present paper, where only a simple regeneration and washing processes was applied. The finding that the enzymatic efficiency was highest with [TBP][DMGly]/DMSO may be due to the compatibility of [TBP][DMGly] with cellulase.

Figure 3b shows the enzymatic hydrolysis of regenerated cellulose in 6% AAIL/cosolvent buffer solutions. When compared with the above data obtained after removing the AAILs/cosolvent by washing, negative effects on the initial hydrolysis rate were observed (Table 2). However, the final conversion to glucose was not affected very much. During 24 h, the conversions rate were 98% and 89% in 6% [TBP][DMGly]/DMSO and 6% [TBP][Gly]/DMSO, respectively. Accordingly, it is possible to conduct the dissolution and enzymatic hydrolysis of cellulose in one-batch process with phosphonium-based AAILs. The cosolvents also affect the activity of cellulase significantly. In 6% [TBP][Gly]/pyridine, the glucose yield was 78% in 24 h, i.e. ca. 10% lower

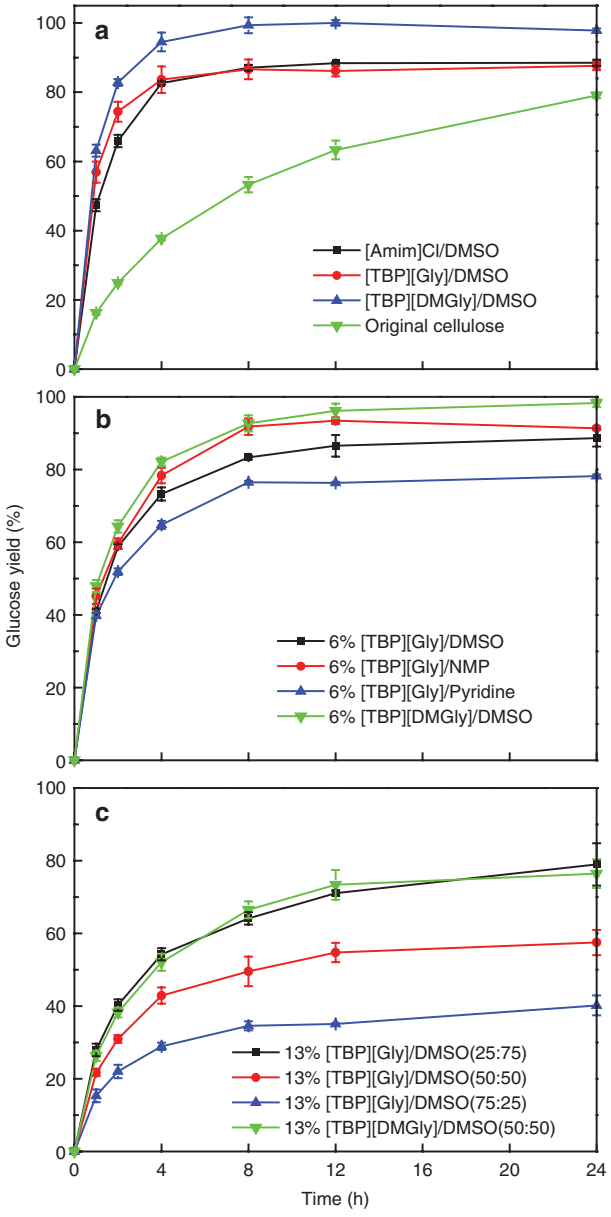


**Figure 3:** Enzymatic hydrolysis of cellulose in acetate buffer (0.1 mol $l^{-1}$, pH 4.5) or in AAIL/cosolvent buffer solution at 50°C. (a) Hydrolysis of original cellulose and regenerated cellulose pretreated by IL/DMSO (50:50) in acetate buffer. (b) Hydrolysis of regenerated cellulose pretreated by AAIL/cosolvent (50:50) in 6% AAIL/cosolvent (50:50) buffer solution. (c) Hydrolysis of regenerated cellulose in 13% AAIL/cosolvent buffer solution; pretreated by [TBP][Gly]/DMSO (25:75), (50:50), (75:25), and [TBP][DMGly]/DMSO (50:50). The error bars show the standard deviations of triplicate experiments. [Amim]Cl, 1-allyl-3-methylimidazolium chloride; DMSO, dimethylsulfoxide; TBP, tetrabutylphosphonium; Gly, glycine; DMGly, *N,N*-dimethylglycine; NMP, *N*-methyl-2-pyrrolidone; AAIL, amino acid ionic liquid.

**Table 2:** Initial conversion rate of cellulose to glucose.

| No | Pretreatment | Details of enzymatic hydrolysis[a] | Initial conversion rate (% $h^{-1}$) |
|---|---|---|---|
| 1 | – | buffer | 16.3 |
| 2 | [Amim]Cl/DMSO (50:50) | buffer | 47.3 |
| 3 | [TBP][Gly]/DMSO (50:50) | buffer | 56.9 |
| 4 | [TBP][DMGly]/DMSO (50:50) | buffer | 63.1 |
| 5 | [TBP][Gly]/DMSO (50:50) | 6% [TBP][Gly]/DMSO (50:50) in buffer | 41.1 |
| 6 | [TBP][Gly]/NMP (50:50) | 6% [TBP][Gly]/NMP (50:50) in buffer | 45.1 |
| 7 | [TBP][Gly]/pyridine (50:50) | 6% [TBP][Gly]/pyridine (50:50) in buffer | 39.8 |
| 8 | [TBP][DMGly]/DMSO (50:50) | 6% [TBP][DMGly]/DMSO (50:50) in buffer | 48.0 |
| 9 | [TBP][Gly]/DMSO (25:75) | 13% [TBP][Gly]/DMSO (25:75) in buffer | 28.0 |
| 10 | [TBP][Gly]/DMSO (50:50) | 13% [TBP][Gly]/DMSO (50:50) in buffer | 21.7 |
| 11 | [TBP][Gly]/DMSO (75:25) | 13% [TBP][Gly]/DMSO (75:25) in buffer | 15.3 |
| 12 | [TBP][DMGly]/DMSO (50:50) | 13% [TBP][DMGly]/DMSO (50:50) in buffer | 26.4 |

[a]Enzymatic hydrolysis was conducted in acetate buffer or in AAIL/cosolvent buffer solution at pH 4.5 and 50°C.
[Amim]Cl, 1-allyl-3-methylimidazolium chloride; DMSO, dimethylsulfoxide; TBP, tetrabutylphosphonium; Gly, glycine; NMP, *N*-methyl-2-pyrrolidone; DMGly, *N,N*-dimethylglycine.

than in the case of 6% [TBP][Gly]/DMSO or 6% [TBP][Gly]/NMP. Some organic solvents are also reported to decrease the activity of β-glucosidase (Rather et al. 2012).

The effects of the AAIL/cosolvent ratios on the hydrolysis of regenerated cellulose from AAILs were also examined (Figure 3c), while the ratio of [TBP][Gly] to DMSO was especially relevant. Both the final glucose yield and the initial glucose production rate decreased drastically at elevated mass ratios of the IL. When the IL/DMSO ratio was 25:75, 79% glucose yield was obtained in 13% IL/DMSO buffer solution, whereas the yield was 40% in 13% IL/DMSO (75:25). The higher compatibility of [TBP][DMGly] with cellulase was further proved by the experimental results according to which the glucose yield was 76% in 13% [TBP][DMGly]/DMSO (50:50), whereas the yield was 58% in 13% [TBP][Gly]/DMSO (50:50).

## Conclusions

TBP-based AAILs are effective solvents for cellulose pretreatment in the context of the subsequent enzymatic hydrolysis of cellulose. In particular, a novel AAIL, [TBP][DMGly], was the most effective in the presence of DMSO leading to nearly 100% conversion of cellulose to glucose. The biocompatibility of [TBP][DMGly] with cellulase was higher than that in the case of the other ILs. In this system, it was possible to dissolve and hydrolyze cellulose in one-batch process. The ratio of [TBP][Gly] to DMSO greatly affects the dissolution efficiency and enzymatic hydrolysis of cellulose and this ratio can be easily adjusted according to the requirements.

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