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Enhancing Asymmetric Reduction of 3-Chloropropiophenone with Immobilized *Acetobacter* sp. CCTCC M209061 Cells by Using Deep Eutectic Solvents as Cosolvents

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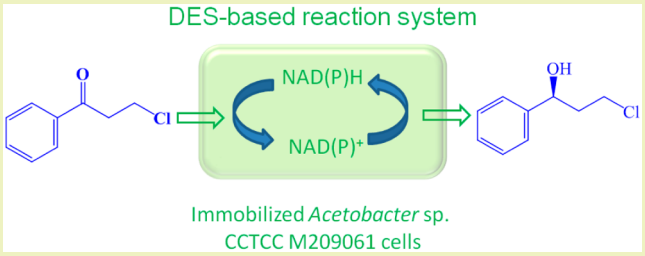
Supporting Information

ABSTRACT: Use of deep eutectic solvents (DESs) to improve biocatalytic asymmetric reduction of 3-chloropropiophenone to (*S*)-3-chloro-1-phenylpropanol catalyzed by whole-cell of *Acetobacter* sp. CCTCC M209061 was successfully performed. The cells immobilized on PVA-sodium sulfate exhibited markedly enhanced stability. Diverse DESs, as cosolvents, manifested significantly different influences on the reaction. Among them, the DES choline chloride/urea ([ChCl][U]) showed the best biocompatibility and moderately increased the cell member permeability, as demonstrated by MAR and flow cytometry assays, and consequently gave the best results. For the bioreduction conducted in the [ChCl][U]-containing system, the optimum [ChCl][U] content, substrate concentration, glucose concentration, pH and temperature were 5% (v/v), 10.0 mmol/L, 60 mmol/L, 5.5 and 30 °C, respectively. Under the optimized conditions, the obtained yield and product *e.e.* were 82.3% and above 99.0% at a reaction time of 6 h, respectively, and the productivity was 1.37 mmol/L/h. The efficient whole-cell biocatalytic process proved to be feasible on a 500 mL preparative scale. Moreover, the combination of water-immiscible ionic liquid $C_4MIM{\cdot}PF_6$ with [ChCl][U] in a biphasic system further enhanced substrate concentration (16.0 mmol/L), product yield (93.3%) and productivity (1.87 mmol/L/h) significantly, showing to be very promising for biocatalytic synthesis of (*S*)-3-chloro-1-phenylpropanol with immobilized *Acetobacter* sp. CCTCC M209061 cells.



KEYWORDS: *Biocatalysis, immobilized Acetobacter sp. CCTCC M209061, deep eutectic solvent, reduction, 3-chloropropiophenone, sustainable chemistry*

INTRODUCTION

Chiral alcohols containing additional functional groups are very important intermediates in synthesis of chiral pharmaceuticals, agrochemicals, flavors and functional materials.[1,2] For example, (*S*)-3-chloro-1-phenylpropanol ((*S*)-CPL) is an essential part for preparing antidepressants (*S*)-fluoxetine, nisoxetine and (*R*)-tomoxetine that show tremendous application potential.[3,4] Currently, enantiopure chiral alcohols can be obtained by either chemical catalysis or biocatalysis. Due to the higher efficiency, higher enantioselectivity, milder reaction condition and so on, biocatalysis has gained much more attention than chemical catalysis.[5] Meanwhile, whole-cell biocatalysts, rather than isolated enzymes, are more favorable for bioreductions, because they are more convenient and stable sources of enzymes and need no expensive coenzyme addition, thereby reducing costs significantly.[6] In addition, immobilized cells offer advantages over free cells owing to their better separability, recyclability and stability.[7]

To date, some researchers have made several attempts to transform 3-chloropropiophenone (CPE) to CPL. Janeczko reported the production of optically active (*S*)-CPL by several yeast strains.[8] However, in this case, the observed yield of (*S*)-CPL and product *e.e.* were very low. Yang reported the use of preheated immobilized *Saccharomyces cerevisiae* CGMCC 2266 cells and *Candida utilis* cells to produce (*S*)-CPL with 80.0% conversion and 99.0% *e.e.*, but a long reaction time was recorded (48 h).[9,10] To our knowledge, few studies have been reported about the bacterial whole cell-mediated reduction of CPE. Lately, a new bacterial strain, *Acetobacter* sp. CCTCC M209061, isolated from Chinese kefir grains by our group, was used as an efficient biocatalyst for highly enantioselective anti-Prelog reduction of prochiral ketones.[11]

Over the last several years, deep eutectic solvents (DESs) have been considered as another promising alternative to conventional ionic liquids (ILs) as green solvents.[12,13] DESs are eutectic mixtures composed of two or three cheap and safe

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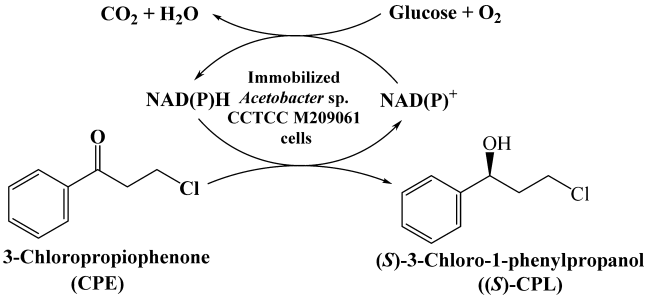
components that could associate with each other through hydrogen bond interactions.[14] These solvents not only share the similar characteristics with ILs such as low vapor pressure, nonflammability, thermal stability, but also have some special virtues including relatively low cost, good sustainability, biocompatibility and biodegradability.[15,16] Most importantly, the formation of these solvents is very simple and needs no further purification steps. Up to now, there appears to be some researches related to the applications of DESs as cosolvents in biocatalytic reactions, especially in enzyme-catalyzed reactions.[17−19] However, few accounts were published about whole-cell biocatalysis in a DES-containing system.[20]

In the present study, we attempted for the first time to use various DESs as cosolvents in aqueous system to improve the biocatalytic asymmetric reduction of CPE with immobilized *Acetobacter* sp. CCTCC M209061 cells (Scheme 1). The biocompatibility of these DESs with the cells and their effects on the cell member integrity were investigated systematically as well as several influential factors on the reaction. Furthermore, the combination of water-immiscible ILs with DESs in a biphasic system was explored to further improve the reaction efficiency.

Scheme 1. Asymmetric Reduction of CPE with Immobilized *Acetobacter* sp. CCTCC M209601 Cells in DES-Containing System



EXPERIMENTAL SECTION

Materials. *Acetobacter* sp. CCTCC M209061 was isolated from Chinese kefir grains by our research group and conserved in our laboratory.[11] 3-Chloropropiophenone (96% purity) and (*R*)-3-chloro-1-phenylpropanol (98% purity) were purchased from TCI (Japan). (*S*)-3-Chloro-1-phenylpropanol (98% purity) was bought from J&K Scientific. The five ILs used in this work were purchased from Lanzhou Institute of Chemical Physics (China). All other chemicals were from commercial sources and were of analytical grade.

Cultivation and Immobilization of *Acetobacter* sp. CCTCC M209061 Cells. *Acetobacter* sp. CCTCC M209061 was cultivated according to our previous described methods.[21] Details about cultivation and immobilization are provided in the Supporting Information.

Preparation of DESs. Quaternary ammonium salts (choline chloride) and hydrogen bond donors (e.g., oxalic acid, malonic acid, glycerol, ethylene glycol, imidazole and urea) were mixed at the mole ratio of 1:2; and tetrabutylammonium bromide and imidazole were mixed at a molar ratio of 3:7.[20] Then the mixtures were heated at 100 °C for 2 h until a transparent homogeneous liquid was formed.

General Procedure for Asymmetric Reduction of CPE. In a typical experiment, the reduction was carried out by adding free (0.05 g/mL) or immobilized (0.80 g/mL) *Acetobacter* sp. CCTCC M209061 cells into 5.0 mL of DMSO (3%, w/v)-containing aqueous TEA-HCl buffer (100 mmol/L, pH 5.0, as the control) or DES-containing buffer system containing a predetermined amount of cosubstrate glucose and CPE in a 25 mL septum-capped Erlenmeyer flask preincubated in a shaking incubator at 30 °C and 180 rpm. Aliquots (50 μL) were withdrawn at specified time intervals. The product and the residual substrate were extracted with acetic ether (50 μL) containing 5.0 mmol/L *n*-tetradecane (internal standard) prior to gas chromatography (GC) analysis. For the bioreduction performed in the DES-containing aqueous buffer/water-immiscible IL biphasic system, the volume ratio of DES-containing aqueous buffer to IL was 4:1. Details about DES content, cosubstrate concentration, substrate concentration, buffer pH and reaction temperature were specified for each case. For the preparative scale (500 mL) of (*S*)-CPL, the immobilized cells and the substrate were added in proportion to a typical experiment.

Cell Metabolic Activity Retention (MAR) Measurement. The cell metabolic activity retention was measured by determining the glucose consumption of the cells after being exposed to DESs or ILs for a certain time. Details of cell MAR measurement are provided in the Supporting Information.

Cell Membrane Permeability Measurement. In a typical experiment, 5 mL of different DESs (5%, v/v)-containing systems or aqueous TEA-HCl buffer (100 mmol/L, pH 5.0) containing 0.05 g/mL *Acetobacter* sp. CCTCC M209061 cells were incubated for 24 h in a 25 mL Erlenmeyer flask capped with a septum at 30 °C and 180 rpm. For the cell membrane integrity measurement, *Acetobacter* sp. CCTCC M209061 cells were harvested and added to sterile normal saline to wash the cells. The cell suspension was diluted to be 10^6 cfu/mL and dyed with propidium iodide (final concentration 50 μg/mL) at 4 °C in the dark, and then was subject to cell membrane integrity measurement using flow cytometry (FCM). The FCM assay was conducted with a BD FACS Verse Coulter. The fluorescent emission was excited at 488 nm, and recorded at 550−600 nm. Data from FCM were analyzed using BD FACSuite software.

Operational Stability of Biocatalysts. 0.80 g/mL immobilized cells was added to the TEA-HCl buffer system (100 mmol/L, pH 5.0) with [ChCl][U] as cosolvent or not containing 70 mmol/L glucose and 10.0 mmol/L CPE. Then, the reaction was carried out at 30 °C and 180 rpm and was repeated over five batches (6 h per batch). For each batch, the immobilized cells were recovered by filtering, washed three times with distilled water, and then added again to a fresh batch of reaction medium to start a new batch of the reaction. The relative activity of the cells employed for the first batch was defined as 100%.

GC Analysis. This reaction was analyzed by a Shimadzu GC2010 model with a flame ionization detector and a HP-chiral column (20% permethylated β-cyclodextrin, 30 m × 0.25 mm × 0.25 μm, USA) with a split ratio of 30:1. The injector and the detector were both kept at 250 °C. The column temperature was held at 140 °C constant for 30 min. Nitrogen was used as the carrier gas at a flow rate of 1.56 mL/min. The retention-times for *n*-tetradecane, CPE and (*S*)-CPL were 4.9, 16.1 and 27.5 min, respectively. The average error for this determination was less than 1.0%. All reported data are averages of experiments performed at least in duplicate.

RESULTS AND DISCUSSION

It is well-known that immobilization exhibited tremendous influence on initial reaction rate, product yield and product *e.e.* of a biocatalytic reduction.[22,23] In our previous study, the comparison of the asymmetric reduction of CPE using immobilized and free *Acetobacter* sp. CCTCC M209061 cells showed that although immobilized cells afforded a lower initial reaction rate than the free cells (1.63 mmol/h vs 3.85 mmol/h), nearly the same yield (82.9% vs 83.9%) and product *e.e.* (above 99.0%) were obtained and the stability of immobilized cells was much better. Consequently, the immobilized cells were used in this work.

Effects of Various DESs on the Bioreduction. Initially, we conducted the reaction in various DES-containing cosolvent systems to evaluate the effects of various DESs on the bioreduction. Among the examined seven DESs, the six DESs were made from choline chloride as the quaternary ammonium

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salts and another one was tetrabutylammonium bromide-based DES (Figure 1).

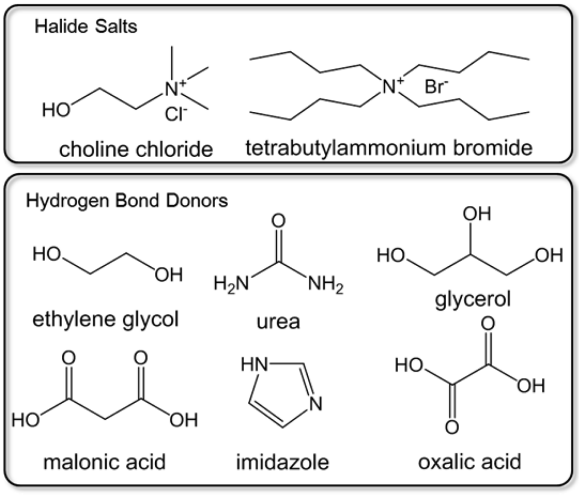

Figure 1. Structures of halide salts and hydrogen bond donors used in the preparation of deep eutectic solvents.

As depicted in Table 1, the DESs structure exerted a significant influence on the catalyst performance. When the hydrogen bond donors were glycerol, ethylene glycol and urea, bioreduction smoothly proceeded and the product *e.e.* was slightly affected compared with the control. However, no products were detected with the hydrogen bond donors being oxalic acid, malonic acid and imidazole, indicating that the carbonyl reductase required for the redox reaction was seriously inactivated resulted from these components. Of the tested DESs, the best result was obtained in the [ChCl][U]-containing system, in which the reaction time required for the equilibrium reaction was shortened dramatically (6 vs 10 h, Figure S1, Supporting Information) and the productivity was improved from 0.34 to 0.57 mmol/L/h. Thus, [ChCl][U] was selected as the most suitable cosolvent for the reaction system, due to the fastest initial reaction rate, the highest product yield and productivity compared with those afforded in a neat aqueous reaction system.

Table 1. Effects of Various DESs on the Biocatalytic Asymmetric Reduction of CPE with Immobilized *Acetobacter* sp. CCTCC M209061 Cells[a]

cosolvent	initial reaction rate (mmol/h)	yield[b] (%)	reaction time (h)	product *e.e.* (%)	productivity (mmol/L/h)
control	1.63	85.2	10.0	>99.0	0.34
[ChCl][OA]	n.r.	n.r.	n.r.		
[ChCl][MA]	n.r.	n.r.	n.r.		
[ChCl][Gly]	1.57	82.2	10.0	>99.0	0.33
[ChCl][EG]	1.47	79.9	10.0	>99.0	0.32
[ChCl][IM]	n.r.	n.r.	n.r.		
[ChCl][U]	2.39	86.0	6.0	>99.0	0.57
[Bu_4NBr][IM]	n.r.	n.r.	n.r.		

[a]Reaction conditions: 5.0 mL TEA-HCl buffer (100 mmol/L, pH 5.0, 5% v/v DES), 4.0 mmol/L CPE, 0.80 g/mL immobilized cells, 70 mmol/L glucose, 30 °C, 180 rpm. [b]isolated yield.

To further understand the effect of [ChCl][U] on the reaction, a comparison of the catalyst performance was carried out by the addition of [ChCl][U] and its components separately. As displayed in Table 2, both the initial reaction rate and the product yield were lower than those detected in aqueous monophasic system when choline chloride or urea was added individually. However, the reaction efficiency could be remarkably improved by the addition of [ChCl][U] as the initial reaction rate and the productivity increased by around 1.5- and 1.7-fold, respectively.

Table 2. Effects of [ChCl][U] and the Related Compositions on the Asymmetric Reduction of CPE with Immobilized *Acetobacter* sp. CCTCC M209061 Cells[a]

additives	initial reaction rate (mmol/h)	yield[b] (%)	reaction time (h)	product *e.e.* (%)	productivity (mmol/L/h)
control	1.63	85.2	10.0	>99.0	0.34
[ChCl][U][c]	2.39	86.0	6.0	>99.0	0.57
ChCl[d]	1.59	83.7	10.0	>99.0	0.33
U[d]	1.61	84.5	10.0	>99.0	0.34

[a]Reaction conditions: 5.0 mL TEA-HCl buffer (100 mmol/L, pH 5.0, 5% v/v [ChCl][U]), 4.0 mmol/L CPE, 0.80 g/mL immobilized cells, 70 mmol/L glucose, 30 °C, 180 rpm. [b]isolated yield. [c]5% v/v [ChCl][U]. [d]ChCl and U were added according to the content of 5% v/v [ChCl][U].

Biocompatibility of Various DESs. To gain a deeper insight into the effect of various DESs on the biocatalytic reduction of CPE, the biocompatibility of the used DESs with *Acetobacter* sp. CCTCC M209061 was assessed by directly measuring the sugar metabolic activity retention (MAR) of the microbial cell.[24] Figure 2 clearly shows that the MARs measured in different DESs-containing aqueous systems with substrate were lower than those observed without substrate, indicating that CPE was toxic to the cells to some extent. Besides, the cells in the aqueous buffer had the higher MAR than those in DES-containing systems, which could be attributed to the toxicity of DESs to the microorganisms.[15]

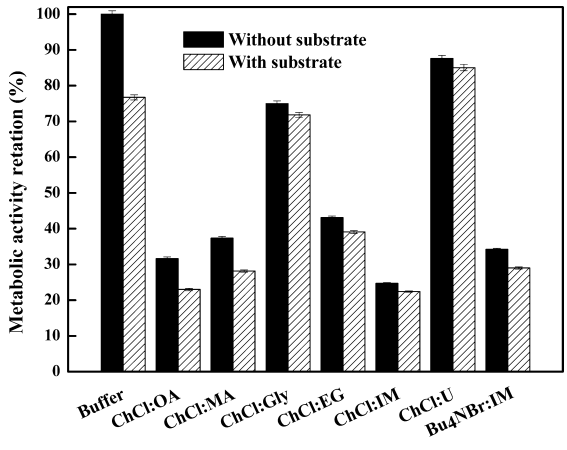

Figure 2. Effect of various DESs on the glucose metabolic activity retention of immobilized *Acetobacter* sp. CCTCC M209061 cells Reaction conditions: 5.0 mL TEA-HCl buffer (100 mmol/L, pH 5.0), 5% (v/v) different DES, 4.0 mmol/L CPE, 0.80 g/mL immobilized cells, 70 mmol/L glucose, 30 °C, 180 rpm.

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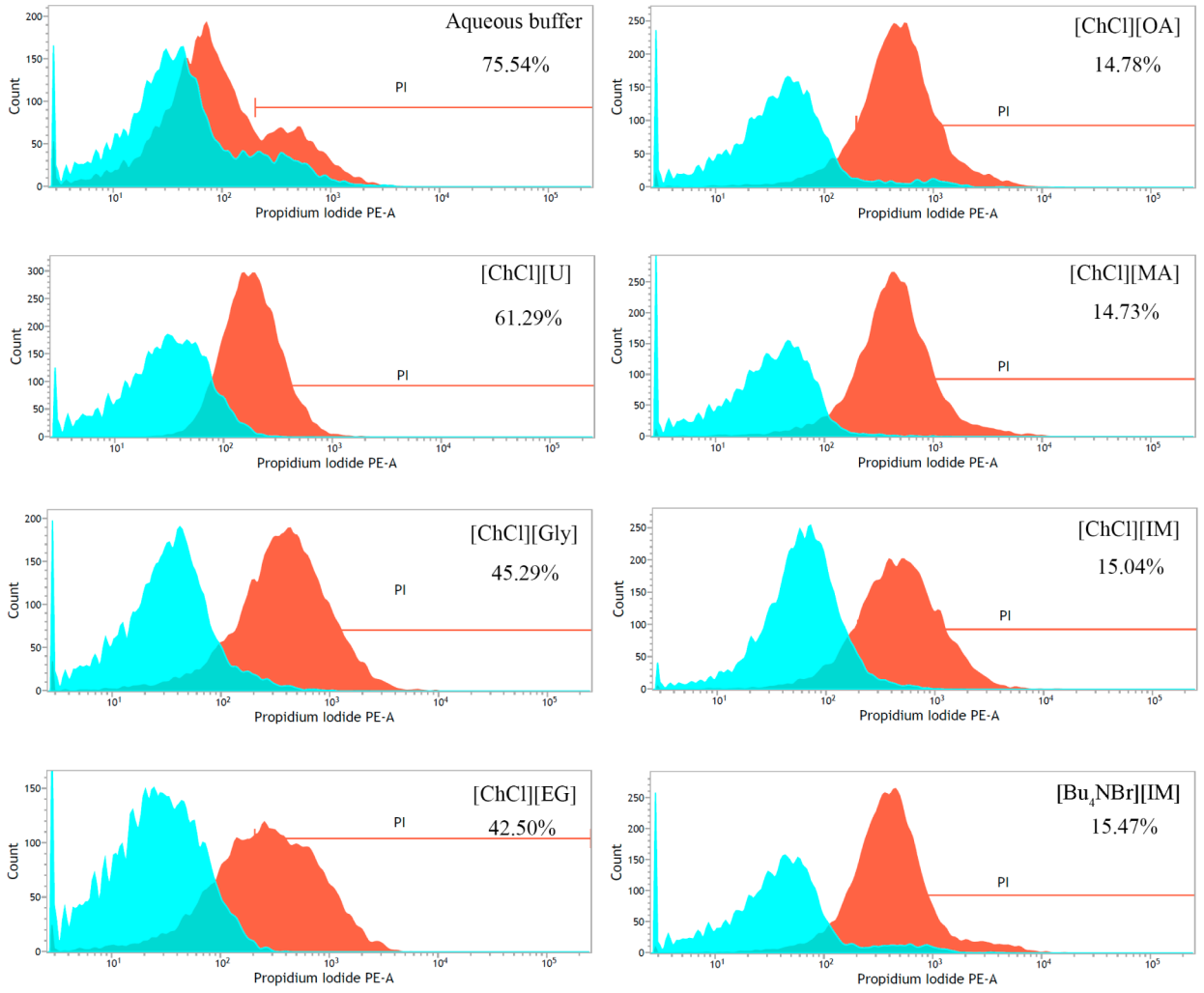

Figure 3. Membrane integrity of *Acetobacter* sp. CCTCC M209061 cells affected by various DESs.

Among all the tested DESs, [ChCl][U] exhibited the best biocompatibility with the cells as shown by the highest MAR (87.6%). For the DESs with imidazole as hydrogen bond donors, the MAR of the cells with [Bu_4NBr][IM] was inferior to that with [ChCl][IM] regardless of the presence of substrate. Relative studies on the underlying influence mechanism of DES to the biocatalyst are now underway in our laboratory.

Effect of Various DESs on Cell Membrane Permeability. The results discussed above clearly demonstrated that various DESs manifested significantly different influences on the bioreaction, which might be associated with the cell membrane permeability caused by the DESs. In general, the increase in the cell membrane permeability could be favorable for the mass transfer of the substrate and the product, but it also led to the cell death caused by the contact with toxic substrate. Hence, it was necessary to investigate the influence of various DESs on the cell membrane permeability.

It is known that flow cytometer (FCM) with propidium iodide (PI) as cell fluorescein dye was a particularly simple and accurate method for measuring the membrane integrity.[25] Figure 3 shows that the cell membrane integrity of *Acetobacter* sp. CCTCC M209061 cells appeared to be reduced by the addition of DESs used. The cells maintained a relative higher cell membrane integrity in the presence of [ChCl][U], which could reduce the product inhibition and thus accounted for the best performance obtained in that reaction system. However, the DESs with hydrogen bond donors being oxalic acid, malonic acid and imidazole exerted detrimental effect on the cell membrane integrity, which coincided with the poor biocompatibility of these DESs. This could well explain the poor biocatalytic efficiency observed in these cosolvents system containing the three components (Table 1). Obviously, a slight increase in membrane permeability was conducive to the catalytic efficiency of the cells.

Effects of Key Variables on the Biocatalytic Reaction. It was generally accepted that adding a suitable concentration of ILs was able to appropriately increase the permeability of cell membranes of microorganisms without affecting their physical and catalytic activities.[26,27] So, it was of great interest to evaluate the effect of [ChCl][U] contents on the biocatalytic asymmetric reduction of CPE with immobilized *Acetobacter* sp. CCTCC M209061 cells. As shown in Figure 4, the initial reaction rate increased significantly as the [ChCl][U] content increased from 1% to 5% (v/v) and reached its maximum value of 2.39 mmol/h at 5%, while the product *e.e.* constantly remained above 99.0%. A further increase of [ChCl][U] content would bring about a substantial reduction in the initial reaction rate and the product yield. The reasons could be that the cell membrane integrity decreased seriously at high

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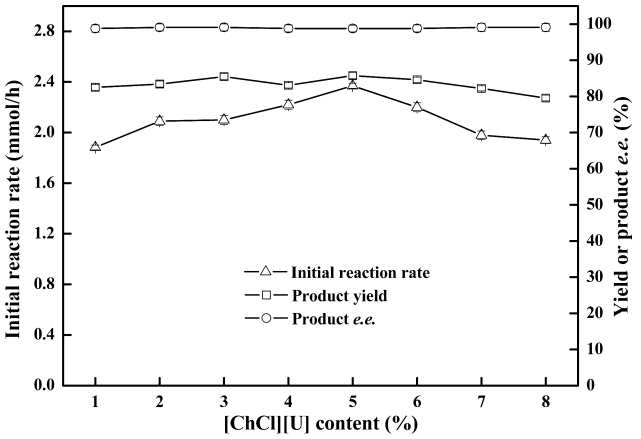

Figure 4. Effects of [ChCl][U] contents on the biocatalytic asymmetric reduction of CPE Reaction conditions: 5.0 mL TEA-HCl buffer (100 mmol/L, pH 5.0, different content of [ChCl][U], v/v), 4.0 mmol/L CPE, 0.80 g/mL immobilized cells, 70 mmol/L glucose, 30 °C, 180 rpm.

concentration of [ChCl][U], thus raising the negative effect. To get deeper insight into the influence of [ChCl][U] content, the effect of its contents on the pH of the reaction systems was also examined. Unexpectedly, the pH varied little with the variation of [ChCl][U] content (5.06 to 5.15). In all, addition of 5% [ChCl][U] was advantageous to the bioreduction of CPE.

Coenzyme recycling was one of the most important issues influencing the biocatalytic reduction reactions.[28] For *Acetobacter* sp. CCTCC M209061 cell-based bioreduction, glucose has been found to be the favorable cosubstrate for coenzyme regeneration in the cells.[29] Figure S2 (Supporting Information) shows that the preferable concentration of glucose was 60 mmol/L. Figure S3 (Supporting Information) illustrates that the initial reaction rate grew rapidly while the maximum yield decreased slowly and the product *e.e.* remained nearly above 99.0% with the substrate concentration increasing from 4.0 to 10.0 mmol/L. However, further increase of the substrate concentration led to a decline in the initial reaction rate and the maximum yield, suggesting an obvious inhibition effect of the substrate and the product. Taking all the factors into account, the best substrate concentration in [ChCl][U]-containing aqueous systems was 10.0 mmol/L, which was 3 times higher than that observed in aqueous monophasic system. The buffer pH showed a tremendous influence on the bioreduction (Figure S4, Supporting Information), and the most suitable buffer pH was found to be 5.5. Also, reaction temperature showed significant influence on the bioreduction and 30 °C was demonstrated to be the optimal temperature (Figure S5, Supporting Information).

Under the optimized conditions described above, the initial reaction rate, maximum yield of (*S*)-CPL and product *e.e.* observed in the [ChCl][U]-containing system was 3.08 mmol/h, 82.3% and >99.0%, respectively. Obviously, the substrate concentration and initial reaction rate were significantly enhanced by adding the DES [ChCl][U] into the aqueous reaction system (10.0 vs 4.0 mmol/L; 3.08 vs 1.63 mmol/h). Additionally, the reaction time required to obtain the maximum yield was shorten (from 10 to 6 h), and the productivity was greatly improved (from 0.34 to 1.37 mmol/L/h) with addition of [ChCl][U]. Therefore, the reaction efficiency of the biocatalytic reduction of CPE in the presence of [ChCl][U] were clearly superior to that in [ChCl][U]-free aqueous system. To our knowledge, the results presented here were better than those reported previously in other similar studies.[8,30]

Operational Stability of the Immobilized Cells in [ChCl][U]-based Reaction System. To estimate the reusability of the immobilized cells, the reuse of the cells was investigated in the [ChCl][U]-containing reaction system under the optimized reaction conditions. The immobilized cells showed superior retention of activity in DES-based system compared to that in aqueous buffer (Figure 5). After five

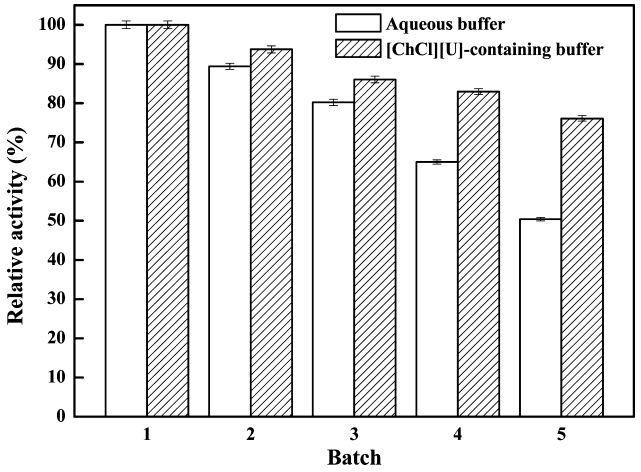

Figure 5. Operational stability of immobilized cells in [ChCl][U]-containing system Reaction conditions: 5.0 mL TEA-HCl buffer (100 mmol/L, pH 5.5, 5% (v/v) [ChCl][U]), 3.0 mmol/L and 10.0 mmol/L CPE for aqueous buffer and [ChCl][U]-containing system respectively, 0.80 g/mL immobilized cells, 60 mmol/L glucose, 30 °C, 180 rpm.

batches (6 h per batch), the biocatalyst retained nearly 80.0% of its initial activity in system with DES while the corresponding value in that without addition of DES was 50.4%. Furthermore, the product *e.e.* constantly kept above 99.0% in each batch. Obviously, the immobilized cells manifested good operational stability in the [ChCl][U]-containing system.

Preparative Scale Bioreduction. To show the feasibility of the biocatalytic reduction of CPE catalyzed by immobilized *Acetobacter* sp. CCTCC M209061 cells in [ChCl][U]-containing system, the bioreduction reaction was also carried out on a larger (500 mL) scale under the optimal reaction conditions. The biocatalytic reaction was monitored by GC analysis, and the product was extracted from the reaction mixture with acetic ether upon the completion of the reaction. In spite of being lower than that obtained on the 5.0 mL scale (82.3%), the isolated yield of (*S*)-CPL (around 82.0%) was much higher than that reported previously,[8] and the product *e.e.* was still more than 99%. Furthermore, no emulsification of the DES-based cosolvent system was observed on a preparative scale, so the immobilized cells and the product could be separated readily from the reaction system. Hence the whole-cell biocatalytic process of CPE reduction into (*S*)-CPL in the presence of [ChCl][U] is promising.

However, it was noted that the appropriate substrate concentration in the [ChCl][U]- containing cosolvent system was not high yet (around 10.0 mmol/L) for the large-scale industrial application, owing to the toxic and inhibitory effects of substrate and/or product on the biocatalytic reaction. Therefore, the substrate concentration and the yield need to be

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Table 3. Comparison of the Biocatalytic Reduction of CPE in Various Reaction Media

reaction media	substrate concentration (mmol/L)	initial reaction rate (mmol/h)	yield[a] (%)	reaction time (h)	product *e.e.* (%)	productivity (mmol/L/h)
aqueous buffer	4.0	1.63	85.2	10.0	>99.0	0.34
[ChCl][U]-containing buffer[b]	10.0	3.08	82.3	6.0	>99.0	1.37
$C_4MIM{\cdot}PF_6$/buffer[c]	16.0	1.50	89.9	12.0	>99.0	1.20
$C_4MIM{\cdot}PF_6$/[ChCl][U]-containing buffer[d]	16.0	2.82	93.3	8.0	>99.0	1.87

[a]Isolated yield. [b]Reaction conditions: 5.0 mL TEA-HCl buffer (100 mmol/L, pH 5.5, 5% v/v [ChCl][U]), 10.0 mmol/L CPE, 0.80 g/mL immobilized cells, 60 mmol/L glucose, 30 °C, 180 rpm. [c]Reaction conditions: 5.0 mL $C_4MIM{\cdot}PF_6$/TEA-HCl buffer (100 mmol/L, pH 4.5)(volume ratio: 1:4), 16.0 mmol/L CPE, 0.80 g/mL immobilized cells, 70 mmol/L glucose, 30 °C, 180 rpm. [d]Reaction conditions: 5.0 mL $C_4MIM{\cdot}PF_6$/TEA-HCl buffer (100 mmol/L, pH 4.5, 5% v/v [ChCl][U])(volume ratio: 1:4), 16.0 mmol/L CPE, 0.80 g/mL immobilized cells, 70 mmol/L glucose, 30 °C, 180 rpm.

further improved. The combination of water-miscible DES with water-immiscible IL to form a biocompatible biphasic system could relieve these negative influences, and was subsequently examined for the bioreduction.

Combining DESs with Water-Immiscible ILs as Cosolvents for the Bioreduction. In many cases,[31−33] where there existed the inhibition of substrate and/or product toward biocatalytic reaction, a biphasic system containing water-immiscible IL was generally a preferable option to improve the biocatalytic process. However, it also suffered from some drawbacks such as relatively slow initial reaction rate and long reaction time. As for the biocatalytic reduction of CPE catalyzed by *Acetobacter* sp. CCTCC M209061 cells, the pronounced toxic and inhibitory effect of substrate was observed in a monophasic system, as indicated in Figures 2 and S3 (Supporting Information). On the other hand, the addition of the water-miscible DES [ChCl][U] into an aqueous system was able to accelerate the biocatalytic reaction, as demonstrated by the results shown in Table 2. Thus, it is of great interest to combine water-miscible [ChCl][U] DES with water-immiscible ILs to significantly improve the bioreduction of CPE.

Of the assessed five water-immiscible ILs, $C_4MIM{\cdot}PF_6$ manifested the best biocompatibility with *Acetobacter* sp. CCTCC M209061 (Figure S6, Supporting Information) and was selected as a second phase of the biphasic system. As evident in Table 3, the introduction of $C_4MIM{\cdot}PF_6$ to form the biphasic system significantly enhanced substrate concentration (from 10.0 to 16.0 mmol/L) and productivity (1.37 to 1.87 mmol/L/h) compared with the [ChCl][U]-containing monophasic system, but lowered the reaction rate. In comparison with the aqueous/$C_4MIM{\cdot}PF_6$ biphasic system, the [ChCl][U]-containing aqueous/$C_4MIM{\cdot}PF_6$ biphasic system afforded a greatly faster reactivity (2.82 vs 1.50 mmol/h), a relatively shorter reaction time (8 vs 12 h) and a remarkably higher productivity (1.87 vs 1.20 mmol/L/h) as well as a moderately higher yield (93.3% vs 89.9%). So, it was concluded that the combination of [ChCl][U] with $C_4MIM{\cdot}PF_6$ could further improve the biocatalytic reduction of CPE with immobilized *Acetobacter* sp. CCTCC M209061 cells.

CONCLUSIONS

The biocatalytic asymmetric reduction of CPE to (*S*)-CPL cells was successfully conducted with high yield and product *e.e.* in DES-containing aqueous system catalyzed by immobilized *Acetobacter* sp. CCTCC M209061 cells. Different DESs exhibited varied influence on the bioreduction. Of all the tested DESs, [ChCl][U] was the most suitable cosolvent for this reaction, which not only showed the best biocompatibility with *Acetobacter sp.* CCTCC M209061 cells but also moderately increased cell membrane permeability. The optimal substrate concentration was highly improved with addition of [ChCl][U], about 3 times more than that observed in aqueous buffer. And the reaction time required to reach equilibrium was also shortened. In addition, the preparation of (*S*)-CPL on a large scale was feasible in the [ChCl][U]-containing system, in which the biocatalyst exhibited much higher operational stability. Furthermore, the combination of water-miscible [ChCl][U] and water-immiscible $C_4MIM{\cdot}PF_6$ to form a biphasic system further improved substrate concentration and productivity significantly. Therefore, using DESs as cosolvents in biotransformation is very promising and of great interest for sustainable chemistry.

ASSOCIATED CONTENT

Supporting Information

Additional materials and methods, abbreviations, time course of biocatalytic reduction of CPE with and without [ChCl][U], effects of key variables on the biocatalytic reaction and effect of various ILs on the MAR of the cells. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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