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Use of an ionic liquid to improve asymmetric reduction of 4′-methoxyacetophenone catalyzed by immobilized *Rhodotorula* sp. AS2.2241 cells

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ABSTRACT

Rhodotorula sp. AS2.2241, a newly isolated strain, was used as biocatalyst for asymmetric reduction of 4′-methoxyacetophenone (MOAP) to enantiopure (*S*)-1-(4-methoxyphenyl)ethanol {(*S*)-MOPE}. Despite the improved efficiency of the reaction with immobilized cells compared to free cells, the inhibition of the reaction by substrate and product in monophasic aqueous system proved to be big problem. For high efficient biotransformation, several water-immiscible ionic liquids (ILs) were employed as green solvents to construct ionic liquid-involving biphasic systems. Of the six ILs tested, $C_4MIM{\cdot}PF_6$ exhibited the best biocompatibility with the cells, and consequently the biocatalytic reduction proceeded with the fastest initial reaction rate and the highest maximum substrate conversion in the $C_4MIM{\cdot}PF_6$-based biphasic system. To better understand the bioreduction conducted in the $C_4MIM{\cdot}PF_6$-based biphasic system, various variables that influenced the performance of the reaction were examined. The optimal buffer pH, reaction temperature, volume ratio of buffer to $C_4MIM{\cdot}PF_6$ and substrate concentration were 7.5, 25 °C, 4/1 and 40 mM, respectively. Under the optimal conditions, the initial reaction rate, maximum substrate conversion and product *e.e.* were 1.6 μmol/h, 95.5% and >99%, respectively. Additionally, the cells still remained above 90% of their original activity in the $C_4MIM{\cdot}PF_6$-based biphasic system, which was much higher than that in the monophasic buffer system (about 25% of their original activity), after being repeatedly used for 8 batches (50 h per batch), indicating that $C_4MIM{\cdot}PF_6$ markedly enhanced the operational stability of the cells.

1. Introduction

Enantiomerically pure chiral alcohols are important building blocks for the synthesis of pharmaceuticals, pesticides, pheromones, flavors, fragrances and advanced materials [1–3]. Among them, enantiopure (*S*)-1-(4-methoxyphenyl)ethanol {(*S*)-MOPE} is a key chiral synthon for the preparation of cycloalkyl [*b*] indoles, which can be used for the treatment of general allergic response [4,5]. Enantiopure chiral alcohols can be synthesized mainly through asymmetric reduction of prochiral ketones using either chemical or biological methods. For economic, environmental and social reasons, biocatalytic methods have recently gained much attention [2,6–8]. Whole cells rather than isolated enzymes were used preferentially to avoid enzyme purification and cofactor addition or the requirement for an associate system for cofactor regeneration, since such reactions often require stoichiometric amount of nicotinamide cofactors.

There have been several reports on the biocatalytic asymmetric reduction of 4′-methoxyacetophenone (MOAP) to (*S*)-MOPE with plant cell cultures [9,10], microbial cells [11–14] and ketoreductases [15] as the biocatalysts, but the maximum substrate conversion achieved was disappointingly low (≤62.3%). Most of the reported biocatalysts afforded relatively low product *e.e.* (≤77%) in aqueous systems, except for *Fusarium caucasicum* 18791 (product *e.e.*:>99%, yield: 17%) and *Aspergillus niveus* 12276 (product *e.e.*:>99%, yield: 15%) which gave very low yield in spite of the high product *e.e.* Recently, Yang et al. described the biocatalytic reduction of MOAP in a monophasic aqueous system catalyzed by free *Rhodotorula* sp. AS2.2241 cells [14], which gave better results (product *e.e.*:>99%, yield: 50%) than *F. caucasicum* 18791 or *A. niveus* 12276. However, the yield (50%) with free *Rhodotorula* sp. AS2.2241 cells was still relatively low, possibly resulting from the inhibition of the reaction by substrate and product and the toxicity of substrate to the cells. To achieve both high yield and product *e.e.*, immobilization of *Rhodotorula* sp. AS2.2241 cells and biphasic systems consisting of an aqueous phase and a second water-immiscible ionic liquid phase was tried in the present work (Scheme 1).

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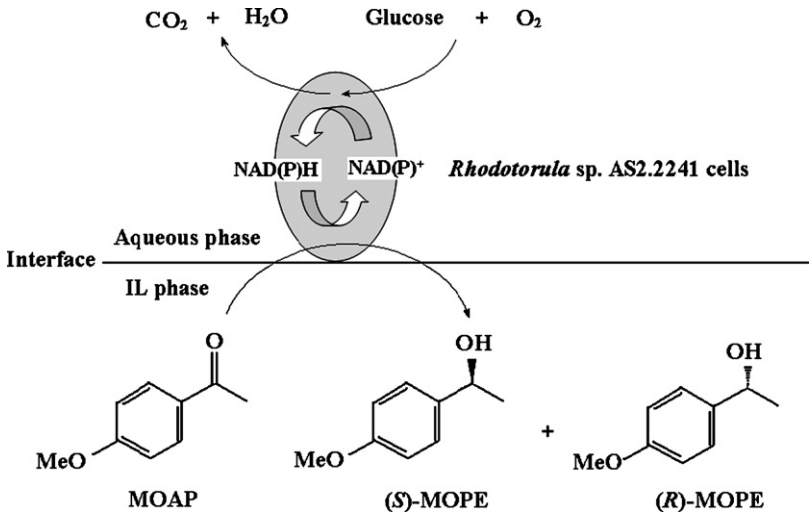

Scheme 1. The biocatalytic asymmetric reduction of MOAP catalyzed by *Rhodotorula* sp. AS2.2241 cells.

2. Materials and methods

2.1. Biological and chemical materials

The yeast strain, *Rhodotorula* sp. AS2.2241, was from the State Key Laboratory of Bioreactor Engineering, East China University of Science and Technology.

MOAP (>97% purity) and *n*-nonane (>99% purity) were purchased from Aldrich-Fluka (USA). (*R*)-MOPE and (*S*)-MOPE (>98% purity) were from Alfa Aesar (China). The ILs 1-butyl-3-methylimidazolium hexafluorophosphate $C_4MIM{\cdot}PF_6$, 1-hexyl-3-methylimidzolum hexafluorophosphate ($C_6MIM{\cdot}PF_6$), 1-ethyl-3-methylimidzolum bis(trifluoromethanesulfonyl)imide ($C_2MIM{\cdot}Tf_2N$) and 1-butyl-3-methylimidzolum bis(trifluoromethanesulfonyl)imide ($C_4MIM{\cdot}Tf_2N$) were purchased from Lanzhou Institute of Chemical Physics (China) and were all of over 99% purity. 1-Pentyl-3-methylimidzolum hexafluorophosphate ($C_5MIM{\cdot}PF_6$) and 1-heptyl-3-methylimidzolum hexafluorophosphate ($C_7MIM{\cdot}PF_6$) were kindly donated by Dr. Xue-Hui Li (Department of Chemical Engineering, South China University of Technology, China) and were both of over 96% purity. All other chemicals were obtained from commercial sources and were of analytical grade.

2.2. Cultivation of Rhodotorula sp. AS2.2241

Rhodotorula sp. AS2.2241 cells were cultivated in the medium containing 1.5% (w/v) glucose, 0.5% (w/v) yeast extract, 0.5% (w/v) peptone, 0.05% (w/v) KH_2PO_4, 0.05% (w/v) K_2HPO_4, 0.1% (w/v) NaCl and 0.05% (w/v) $MgSO_4{\cdot}7H_2O$. The medium was autoclaved at 121 °C for 20 min. A preculture was prepared by inoculation of 100 ml of the complex medium with fresh cells from an agar plate culture. Incubation was performed in a 300 ml Erlenmeyer shaking-flask, which was shaken at 150 r/min and 30 °C. After 20 h incubation the cells were in the exponential growth phase and were collected by centrifugation (1150 × *g*, 15 min), washed twice with distilled water, and separated from the aqueous medium by centrifugation to give a cell wet mass (cwm) of 5–6 g per 300 ml batch. The ratio 'cell wet mass'/'cell dry mass' of around 4.0 was determined by lyophilization of samples of wet cells. The wet cells were immobilized on calcium alginate as described below.

2.3. Immobilization of Rhodotorula sp. AS2.2241 cells

A homogenous cell/sodium alginate suspension was prepared at 25 °C by adding 10 g of a suspension containing fresh cells (5 g wet cells in 5 g water) into 30 ml of a homogeneous aqueous sodium alginate solution (2%, w/v), which was prepared by dissolving sodium alginate in deionized water, heating (80 °C) and stirring vigorously. The suspension was added dropwise by an injector to a gently stirred $CaCl_2$ solution (2%, w/v), where the calcium alginate pearls with a load of 13.2% (w/w) of *Rhodotorula* sp. AS2.2241 cells (based on cwm) precipitated. The pinhole size of injector and the drop rate were adjusted in such a way that the diameter of the pearls was about 1 mm. The pearls were kept in the $CaCl_2$ solution for another 1 h at 25 °C and collected by filtration, washed with water and re-suspended in an aqueous solution of 20% (w/v) glucose, 0.9% (w/v) NaCl and 0.05% (w/v) $CaCl_2$ at 4 °C for 24 h. They were stored at 4 °C for later use.

2.4. General procedure for the asymmetric reduction of MOAP with immobilized Rhodotorula sp. AS2.2241 cells

In a typical experiment, 2.5 ml of the aqueous Tris–HCl buffer (50 mM) monophasic system or the IL/Tris–HCl buffer (50 mM) biphasic system containing a fixed amount of MOAP and 20% (w/v) glucose was pre-incubated in a 10-ml Erlenmeyer flask capped with a septum for 30 min at 180 r/min and the appropriate temperature. The reaction was initiated by adding 0.32 g/ml immobilized *Rhodotorula* sp. AS2.2241 cells to the reaction system. The bottom IL phase acts as a reservoir of substrate and product, while the upper aqueous phase provides an agreeable environment for the immobilized cells.

Aliquots (10 μl) were withdrawn at specified time intervals from the aqueous phase for the buffer monophasic system or from the IL phase for the IL/buffer biphasic system. Then the samples were extracted three times with isopropyl ether (30 μl) containing 5.6 mM *n*-nonane (as an internal standard) prior to GC analysis. Details of the substrate concentration, buffer pH, reaction temperature and volume ratio of buffer to IL are specified for each case. The substrate concentration throughout the paper refers to substrate concentration present in the IL phase for IL/buffer biphasic system unless specified.

2.5. Viability assay

Some beads were withdrawn at specified time intervals from the aqueous phase and then added to 0.1 M trisodium citrate to dissolve the beads. The microbial cell suspension was diluted and dyed with 0.1% Methylene Blue for 5 min. Microscopic pictures were taken and analyzed for blue dead cells and colourless viable ones.

2.6. Determination of partition coefficients

The partition coefficients ($K_{IL/aq}$) were determined by dissolving 10 mM, 20 mM and 40 mM MOAP or MOPE, respectively, in IL/buffer biphasic system with shaking (180 r/min) for 48 h at 25 °C. The concentration of MOAP or MOPE in the ionic liquid phase and the aqueous phase was then analyzed by GC. The concentration of MOAP or MOPE in each phase varied linearly with the total amount of the respective chemical added to the two-phase system. Then the slope was calculated and used for the quantification of the partition coefficients for MOAP and MOPE between the IL phase and the aqueous phase.

2.7. Operational stability of immobilized Rhodotorula sp. AS2.2241 cells

The operational stability of immobilized *Rhodotorula* sp. AS2.2241 cells was examined in $C_4MIM{\cdot}PF_6$/buffer biphasic system and Tris–HCl buffer system. Aliquots of the immobilized cells were added into the corresponding mixture {Tris–HCl buffer system: 5 mM MOAP, 2.5 ml Tris–HCl buffer (50 mM, pH 8.0) containing 20% (w/v) glucose; $C_4MIM{\cdot}PF_6$/buffer biphasic system: 40 mM MOAP, 0.5 ml $C_4MIM{\cdot}PF_6$, 2.0 ml Tris–HCl buffer (50 mM, pH 7.5) containing 20% (w/v) glucose}. The reduction reactions were conducted at 25 °C and 180 r/min. Upon the completion of the reaction, the immobilized cells were filtered off and washed twice with fresh water, and then added to a fresh batch of substrate solution. The reduction activity of the cells was assayed in each batch. The relative activity of the immobilized cells in the first batch was defined as 100%.

2.8. Analytical procedure

The reaction mixtures were assayed by a Shimadzu GC 2010 model with a flame ionization detector and a chiral column (20% permethylated β-cyclodextrin 30 m × 0.25 mm × 0.25 μm) from Hewlett Packard (USA). The split ratio was 100:1. Both the injector and the detector were kept at 250 °C. The column temperature was held at 140 °C for 10 min and then increased to 145 °C at a rate of 1 °C/min, and then kept constant for 4 min. The carrier gas was nitrogen at 3.0 ml/min. The retention times for *n*-nonane, MOAP, (*R*)-MOPE and (*S*)-MOPE were 2.00, 17.00, 17.70 and 18.20 min, respectively. The initial reaction rate, the substrate conversion and the enantiomeric excess of (*S*)-MOPE were calculated from the GC analysis results. Experiments showed that no side reaction took place and the product yield was equal to the substrate conversion. All reported data are averages of experiments performed at least in duplicate and the average error for the results is less than 2.0%.

3. Results and discussion

It has been reported that immobilization of cells can improve the maximum substrate conversion and the product *e.e.* of bioreductions catalyzed by whole cells [7,15,16]. Therefore, we conducted the asymmetric reduction of MOAP with immobilized *Rhodotorula* sp. AS2.2241 cells in aqueous buffer system and an enhancement in the maximum substrate conversion was observed (from 50% to around 60%) with the same initial substrate concentration (10 mM). The probable reason for this is that the immobilization changes the distribution of the substrate and product and makes the cells more stable. The low efficiency of the asymmetric reduction of MOAP in the case of higher substrate conversion even with immobilized cells suggested the severe inhibition of the reaction by substrate and product and the existence of the reversed reaction which preferentially converts (*S*)-MOPE to MOAP.

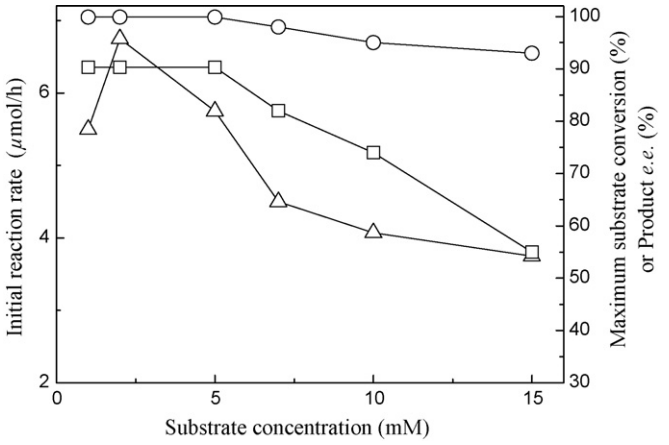

Fig. 1. Effect of substrate concentration on asymmetric reduction of MOAP catalyzed by immobilized *Rhodotorula* sp. AS2.2241 cells in aqueous buffer system. Reaction condition: various concentration of MOAP, 2.5 ml Tris–HCl buffer (50 mM, pH 8.0) containing 20% (w/v) glucose, 0.32 g/ml immobilized cells, 25 °C, 180 r/min. Symbols: (○) product *e.e.*; (□) maximum substrate conversion; (△) initial reaction rate.

Subsequently, the effect of substrate concentration on the asymmetric reduction of MOAP with immobilized *Rhodotorula* sp. AS2.2241 cells in the monophasic aqueous buffer was investigated. As shown in Fig. 1, the initial reaction rate markedly increased with increasing substrate concentration up to 2.0 mM, and further increase in substrate concentration gave rise to a sharp drop in the initial rate, demonstrating that there exists a pronounced inhibition of the reaction by substrate on the reaction performed in aqueous buffer system even under the very low substrate concentration used in this experiment. The maximum substrate conversion and the

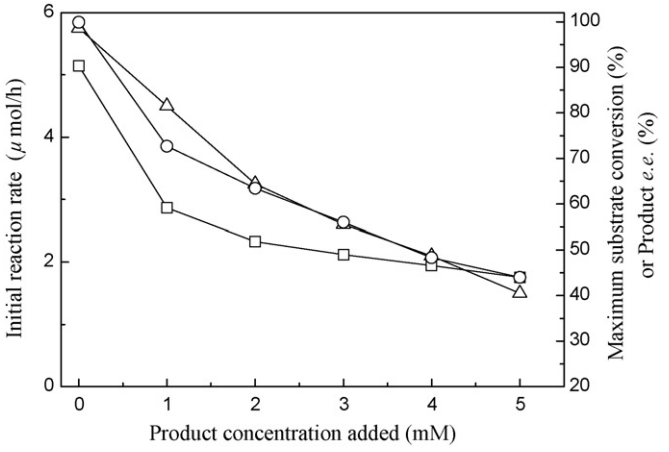

Fig. 2. Effect of product added to reaction system on asymmetric reduction of MOAP catalyzed by immobilized *Rhodotorula* sp. AS2.2241 cells in aqueous buffer system. Reaction condition: 5 mM MOAP, 2.5 ml Tris–HCl buffer (50 mM, pH 8.0) containing various concentration of product and 20% (w/v) glucose, 0.32 g/ml immobilized cells, 25 °C, 180 r/min. Symbols: (○) product *e.e.*; (□) maximum substrate conversion; (△) initial reaction rate. Product *e.e.*: the *e.e.* value of the formed product, not including the product added.

Table 1
Effect of various water-immiscible IL on asymmetric reduction of MOAP catalyzed by immobilized *Rhodotorula* sp. AS2.2241 cells

Entries	Media	V_0 (μmol/h)	C^a (%)	*e.e.* (%)
1	$C_4MIM{\cdot}PF_6$/buffer	1.19	69.5	>99
2	$C_5MIM{\cdot}PF_6$/buffer	0.92	55.1	>99
3	$C_6MIM{\cdot}PF_6$/buffer	0.67	51.6	>99
4	$C_7MIM{\cdot}PF_6$/buffer	0.53	38.2	>99
5	$C_2MIM{\cdot}Tf_2N$/buffer	1.12	66.4	>99
6	$C_4MIM{\cdot}Tf_2N$/buffer	1.04	58.3	>99

Reduction condition: 10 mM MOAP, 0.5 ml various water-immiscible IL, 2.0 ml Tris–HCl buffer (50 mM, pH 7.5) containing 20% (w/v) glucose, 0.32 g/ml immobilized beads, 25 °C, 180 r/min.
[a] The maximum substrate conversion.

product *e.e.* kept at around 90% and 99%, respectively, with increasing substrate concentration up to 5 mM, beyond which further rise in substrate concentration led to a clear decrease in the maximum substrate conversion and the product *e.e.* On the other hand, serious inhibition of the reaction by product was also observed for the bioreduction conducted in aqueous monophasic system. As can be seen in Fig. 2, the initial reaction rate, maximum substrate conversion and product *e.e.* decreased obviously with increasing concentration of the product (*S*)-MOPE added into the reaction system.

Biphasic systems consisting of an aqueous phase and a water-immiscible organic phase are often used to avoid the inhibition of the reaction by substrate and product that occur in aqueous system. The microbial cells stay in aqueous phase, and the substrate and product mainly remain in organic phase, thus allowing easy isolation of product and reuse of catalyst [7]. Unfortunately, the operational stability of the yeast cells was poor [17]. Furthermore, the use of conventional organic solvents in such processes is problematic because they are often toxic to the cells, sometimes explosive and usually environmentally harmful [18,19]. The volatile nature of such solvents is also a serious threat to the operator, particularly when they are employed on a large scale. Hence, there is currently an increasing need for green solvents such as supercritical fluids and ionic liquids as alternatives to traditional organic solvents.

Ionic liquids are a promising new class of solvents for biotransformations. Many kinds of ILs have proven to be biocompatible with microbial cells, including baker's yeast, *Escherichia coli* and *Geotrichum candidum*, in highly efficient whole-cell biocatalytic processes in IL-containing systems [20–24]. Cull et al. [25] firstly successfully used IL ($C_4MIM{\cdot}PF_6$) instead of toluene in a biphasic system for hydrolysis of 1,3-dicyanobenzene catalyzed by *Rhodococcus* R312 cells. The IL acted as a reservoir for the poorly water-soluble substrate and product, thereby decreasing the inhibition of the reaction by substrate and product observed in a monophasic aqueous system. To our knowledge, only one attempt has been made to carry out the biocatalytic asymmetric reduction of MOAP in IL-based biphasic systems [26], where only baker's yeast cells were employed as the biocatalysts, which unfortunately exhibited no reduction activity towards MOAP. As the effects of ILs on a reaction mediated by different microbes have been found to vary widely [27], six water-immiscible ILs available were tested in the present study for their potential as a second solvent phase for the asymmetric reduction of MOAP catalyzed by immobilized *Rhodotorula* sp. AS2.2241 cells. We initially focused on the influence of the cations and anions of these ILs on the asymmetric reduction of MOAP catalyzed by immobilized *Rhodotorula* sp. AS2.2241 cells (Table 1). It was noted that *Rhodotorula* sp. AS2.2241 cells could effectively catalyze the asymmetric reduction of MOAP in the $C_4MIM{\cdot}PF_6$-based reaction system, while the

Table 2
Partition coefficients for MOAP and (*S*)-MOPE

Media	Partition coefficients between the two phases	
	MOAP	(*S*)-MOPE
$C_4MIM{\cdot}PF_6$/buffer	45.9	9.2
$C_5MIM{\cdot}PF_6$/buffer	44.5	9.4
$C_6MIM{\cdot}PF_6$/buffer	43.7	9.6
$C_7MIM{\cdot}PF_6$/buffer	42.1	9.8
$C_2MIM{\cdot}Tf_2N$/buffer	36.8	8.9
$C_4MIM{\cdot}Tf_2N$/buffer	31.3	6.4

baker's yeast cells could not catalyze the reduction of MOAP in system containing the same ionic liquid [26]. For the biphasic systems involving PF_6^--based ILs ($C_nMIM{\cdot}PF_6$, n = 4–7) (Table 1, entries 1–4), both the initial reaction rate and the maximum substrate conversion clearly decreased with the elongation of the alkyl chain (i.e. increasing n value), possibly resulting from the increase of IL's viscosity with increasing n value to some extent [28]. Besides, both the slightly reduced partition coefficients of MOAP between IL and buffer (Table 2) and the lowered biocompatibility of IL with *Rhodotorula* sp. AS2.2241 cells (Fig. 3) with increasing n value could partially explain for this observation. As can be seen in Fig. 3, the cell viability clearly decreased in the presence of substrate compared to in the absence of substrate, suggesting that MOAP manifests substantial toxicity to *Rhodotorula* sp. AS2.2241 cells. So higher partition coefficient of MOAP between IL and buffer could effectively eliminate the substrate toxicity to the cells. In the case of Tf_2N^--based ILs ($C_2MIM{\cdot}Tf_2N$ and $C_4MIM{\cdot}Tf_2N$), the change profiles of the initial reaction rate and the maximum substrate conversion with the elongation of the alkyl chain are similar to those observed with PF_6^--based ILs. The partition coefficients of MOAP and (*S*)-MOPE in $C_2MIM{\cdot}Tf_2N$/buffer biphasic system are higher than the corresponding values in $C_4MIM{\cdot}Tf_2N$/buffer biphasic system (Table 2), which is in good accordance with the observation that the cell viability is higher in $C_2MIM{\cdot}Tf_2N$-containing system than in $C_4MIM{\cdot}Tf_2N$-containing system with the substrate (Fig. 3). The results might account for the observations that the maximum substrate conversion and the initial reaction rate were a little higher in $C_2MIM{\cdot}Tf_2N$/buffer biphasic system than those in $C_4MIM{\cdot}Tf_2N$/buffer biphasic system, as indicated in Table 1. Additionally, the biocatalytic reduction proceeded more slowly in the biphasic system containing $C_4MIM{\cdot}Tf_2N$ than $C_4MIM{\cdot}PF_6$, showing

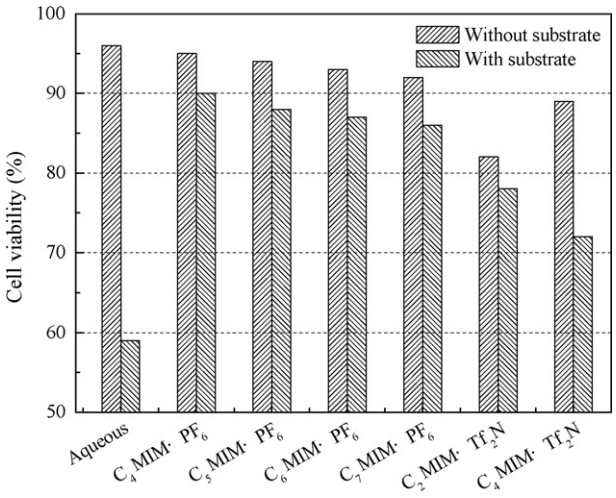

Fig. 3. Cell viability of *Rhodotorula* sp. AS2.2241 after exposure for 24 h to biphasic systems consisting buffer and 20% (w/v) (v/v) ILs compared to pure buffer system without substrate MOAP and with substrate MOAP (10 mM).

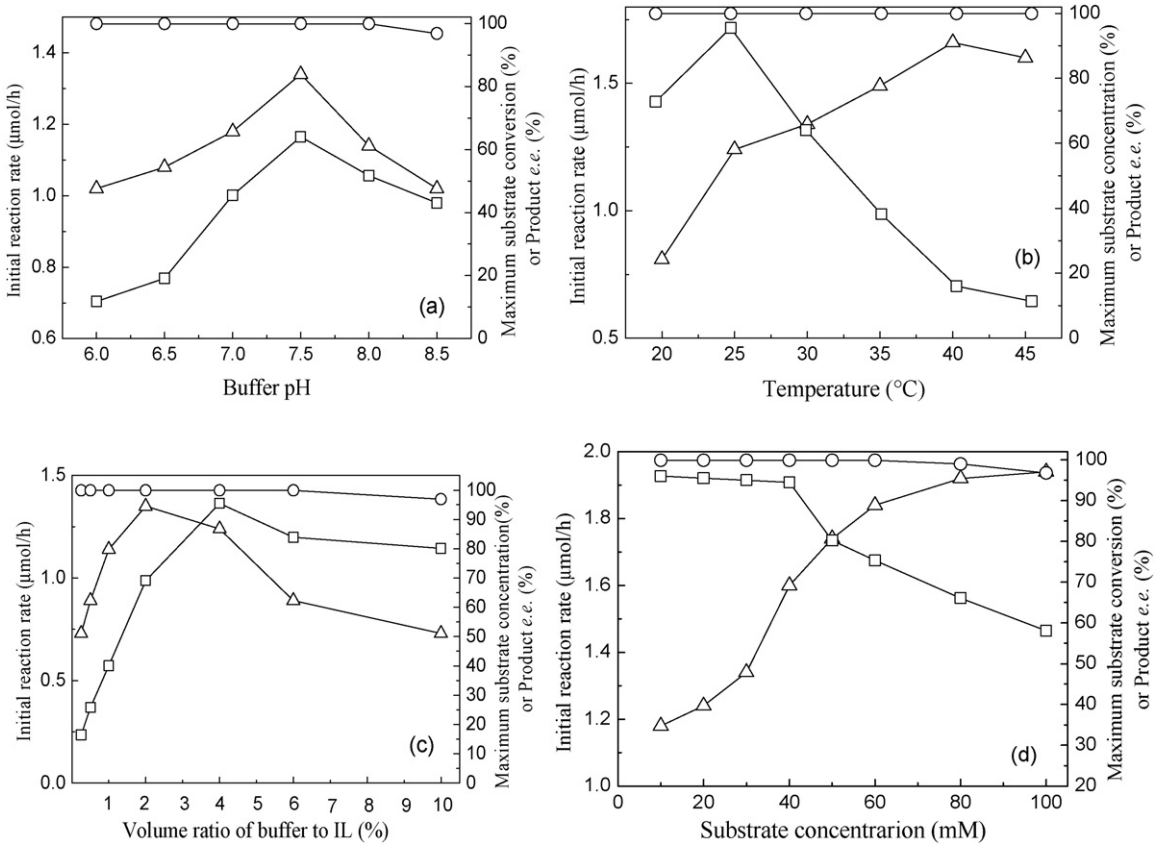

Fig. 4. The asymmetric reduction of MOAP mediated by immobilized *Rhodotorula* sp. AS2.2241 cells in the $C_4MIM{\cdot}PF_6$/buffer biphasic system. (a) Effect of buffer pH {$C_4MIM{\cdot}PF_6$/buffer volume ratio: 1/4 (v/v); 30 °C; 20 mM MOAP}. (b) Effect of reaction temperature {$C_4MIM{\cdot}PF_6$/buffer volume ratio: 1/4 (v/v); buffer pH 7.5; 20 mM MOAP}. (c) Effect of volume ratio of aqueous phase to ionic liquid phase {buffer pH 7.5; 25 °C; 20 mM MOAP}. (d) Effect of substrate concentration {$C_4MIM{\cdot}PF_6$/buffer volume ratio: 1/4 (v/v); buffer pH 7.5; 25 °C}. Symbols: (○) product *e.e.*; (□) maximum substrate conversion; (△) initial reaction rate.

that the anion of IL has significant effect on the reaction. Of the six ILs tested, $C_4MIM{\cdot}PF_6$ gave the highest maximum substrate conversion and the fastest initial rate, and was consequently chosen as the second phase in IL/buffer biphasic system for subsequent investigation.

It is well known that buffer pH plays an important role in bioreductions [28,29]. Fig. 4a illustrates the significant effect of buffer pH on the reaction in the $C_4MIM{\cdot}PF_6$/buffer biphasic system. The reaction accelerated and the maximum substrate conversion increased with increasing buffer pH from pH 6.0 to 7.5. Further increase in buffer pH led to poor reaction rate and maximum substrate conversion. It is clear that pH 7.5 is the optimal buffer pH for the reaction. From Fig. 4b, a rise in reaction temperature clearly boosted the initial reaction rate up to 40 °C, and further rise in temperature led to a sharp drop in initial reaction rate. The maximum substrate conversion clearly decreased when the temperature was above 25 °C. This might be due to the inactivation of the cells after being incubated for a long time at a higher temperature. Obviously, 25 °C is the optimum temperature for the reaction. It has been reported that the effect of volume ratio of two phase on biocatalytic reactions varies widely and unpredictably [17,30,31]. As shown in Fig. 4c, the volume ratio of the aqueous buffer phase to the IL phase (V_{aq}/V_{IL}, ml/ml) substantially affected the initial reaction rate and the maximum substrate conversion, but had slight effect on the product *e.e.* Enzymes and active cells are commonly inactivated by direct contact with the interface between the aqueous and non-aqueous phases [11] and so the obvious enhancement in the initial reaction rate and the maximum substrate conversion with the increase of V_{aq}/V_{IL} up to 4/1 can be easily understood because as the V_{aq}/V_{IL} value increases it becomes less likely that the cells will contact the IL. Further rise in the V_{aq}/V_{IL} ratio led to a decline in the initial reaction rate, possibly owing to the lower substrate concentration in the aqueous phase. So it is clear that the optimum V_{aq}/V_{IL} is 4/1. As can be seen in Fig. 4d, the initial reaction rate increased with increasing substrate concentration, while the product *e.e.* showed no clear variation. When substrate concentration was above 40 mM, the increase in substrate concentration led to a marked drop in the maximum substrate conversion, possibly owing to the inhibition of the reaction by product. Obviously, the optimal substrate concentration in the $C_4MIM{\cdot}PF_6$/buffer system was 40 mM.

Fig. 5a,b depicts the time-course profiles of the biocatalytic reduction of MOAP in the aqueous monophasic system and in the $C_4MIM{\cdot}PF_6$-containing biphasic system under the optimal conditions for each medium. The initial reaction rate was clearly lower in the $C_4MIM{\cdot}PF_6$/buffer biphasic system than in the aqueous monophasic system (1.6 μmol/h vs. 5.7 μmol/h). This might be attributable to the much lower substrate concentration in the aqueous phase and the severe mass transfer limitation with the IL-containing biphasic system. As evident in Fig. 5, the reaction rate decreased sharply with reaction time in the aqueous monophasic system possibly owing to the pronounced inhibition of the reaction by product while the reaction rate decreased relatively slowly with reaction time in the $C_4MIM{\cdot}PF_6$-containing biphasic system probably due to the *in situ* extraction of the formed product into the IL phase. Therefore, the reaction time required to reach the chemical equilibrium in the $C_4MIM{\cdot}PF_6$-containing biphasic system was nearly the same as that observed in the

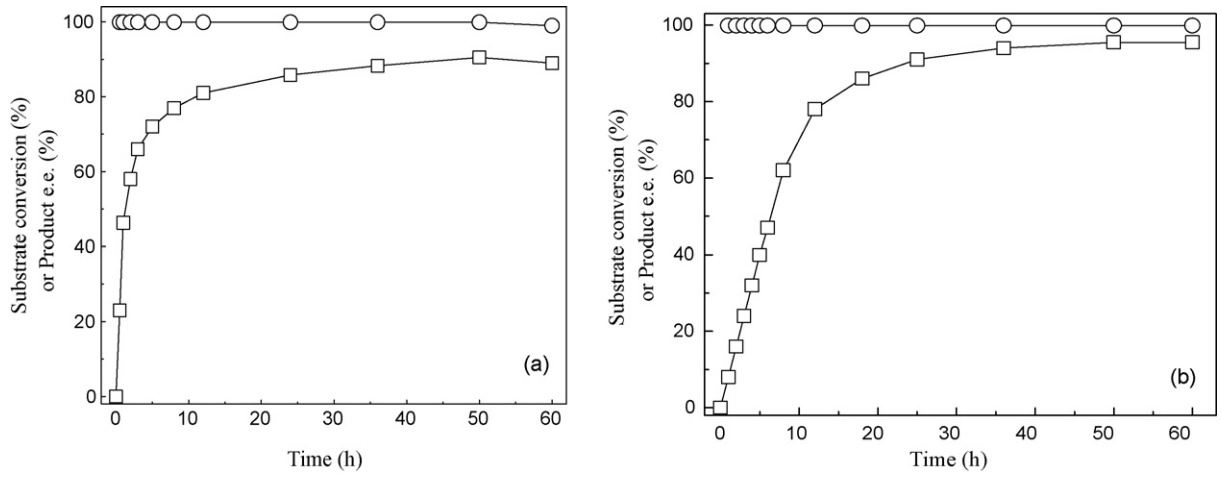

Fig. 5. Time-course profiles of the asymmetric reduction of MOAP catalyzed by immobilized *Rhodotorula* sp. AS2.2241 cells in the aqueous monophasic system (a) {5 mM (12.5 μmol) MOAP, 2.5 ml Tris–HCl buffer (50 mM, pH 8.0) containing 20% (w/v) glucose, 0.32 g/ml immobilized cells, 25 °C, 180 r/min} and in the $C_4MIM{\cdot}PF_6$/buffer biphasic system (b) {40 mM (20 μmol) MOAP, 0.5 ml $C_4MIM{\cdot}PF_6$, 2.0 ml Tris–HCl buffer (50 mM, pH 7.5) containing 20% (w/v) glucose, 0.32 g/ml immobilized cells, 25 °C, 180 r/min}. Symbols: (○) product *e.e.*; (□) substrate conversion.

aqueous monophasic system (around 50 h). However, more product (40 mM × 0.5 ml × 95.5% = 19.1 μmol) could be formed in the $C_4MIM{\cdot}PF_6$/buffer biphasic system than in the aqueous monophasic system (5 mM × 2.5 ml × 90.5% = 11.3 μmol) within 50 h. The product *e.e.* kept around 99% constantly in both reaction systems.

As can be seen in Fig. 6, the operational stability of the immobilized cells was significantly enhanced in the $C_4MIM{\cdot}PF_6$-containing biphasic system as compared to that in the aqueous monophasic system. The immobilized cells still remained above 90% of their initial activity after being used repeatedly for 8 batches (50 h per batch) in the $C_4MIM{\cdot}PF_6$/buffer biphasic system. In contrast, the relative activity of the immobilized cells was only 25% after being re-used for the same period of time in the aqueous monophasic system. The excellent solvent properties of the IL $C_4MIM{\cdot}PF_6$ for the toxic substrate and product (Table 2) and the good biocompatibility of $C_4MIM{\cdot}PF_6$ (Fig. 3) could partly account for these observations. The interactions between the IL and the carrier (calcium alginate) [32] used for the immobilization of *Rhodotorula* sp. AS2.2241 cells may also contribute to the good operational stability of the immobilized cells in $C_4MIM{\cdot}PF_6$-containing biphasic system.

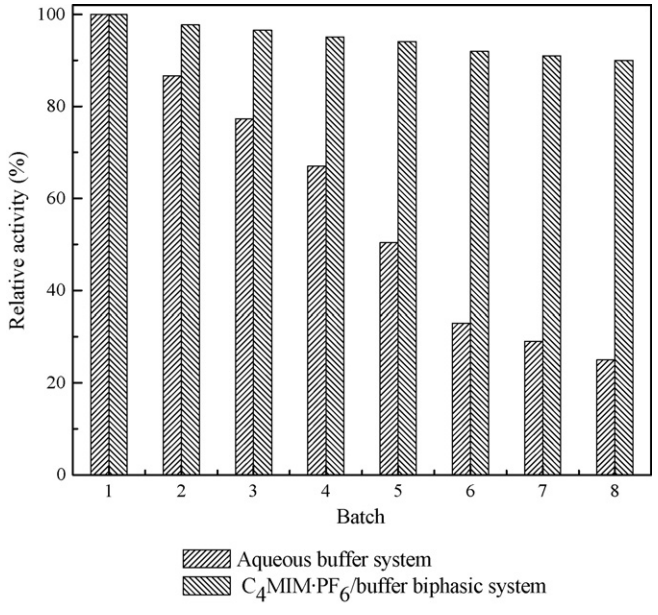

Fig. 6. Operational stability of immobilized *Rhodotorula* sp. AS2.2241 cells. Reaction condition: aqueous buffer monophasic system: 5 mM MOAP, 2.5 ml Tris–HCl buffer (50 mM, pH 8.0) containing 20% (w/v) glucose, 0.32 g/ml immobilized cells, 25 °C, 180 r/min, 50 h per batch; $C_4MIM{\cdot}PF_6$/buffer biphasic system: 40 mM MOAP, 0.5 ml $C_4MIM{\cdot}PF_6$, 2.0 ml Tris–HCl buffer (50 mM, pH 7.5) containing 20% (w/v) glucose, 0.32 g/ml immobilized cells, 25 °C, 180 r/min, 50 h per batch. The relative activity of the immobilized cells in the first batch was defined as 100%.

4. Conclusions

The water-immiscible IL $C_4MIM{\cdot}PF_6$ can markedly enhance the efficiency of MOAP reduction to enantiopure (*S*)-MOPE mediated by immobilized *Rhodotorula* sp. AS2.2241 cells and the stability of the cells due to the excellent solvent properties of $C_4MIM{\cdot}PF_6$ for MOAP and its good biocompatibility with *Rhodotorula* sp. AS2.2241. Thus, the asymmetric synthesis of enantiopure (*S*)-MOPE catalyzed by immobilized *Rhodotorula* sp. AS2.2241 cells in the presence of the IL $C_4MIM{\cdot}PF_6$ appears to be very promising and competitive.

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