Wang et al. Bioresources and Bioprocessing (2015) 2:6
DOI 10.1186/s40643-015-0037-9

Bioresources and Bioprocessing
a SpringerOpen Journal

RESEARCH Open Access

Biocatalytic synthesis of ethyl (*R*)-2-hydroxy-4-phenylbutyrate with a newly isolated *Rhodotorula mucilaginosa* CCZU-G5 in an aqueous/organic biphasic system

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Abstract

Background: Optically active ethyl (*R*)-2-hydroxy-4-phenylbutyrate [(*R*)-HPBE] is an important chiral building block for the synthesis of angiotensin-converting enzyme (ACE) inhibitors. It is reported that microbial or enzymatic reduction of ethyl 2-oxo-4-phenyl-butyrate (OPBE) is an attractive way to produce optically active (*R*)-HPBE.

Results: The asymmetric reduction of OPBE to synthesize optically active (*R*)-HPBE with a newly isolated *Rhodotorula mucilaginosa* CCZU-G5 as catalyst was investigated in an aqueous/organic solvent biphasic system. *R. mucilaginosa* CCZU-G5 showed a good tolerance (the metabolic activity retention >80%) in the biphasic system composed of aqueous buffer and organic solvent with a log *P* value over 4.6. Isooctane was found to be the most suitable organic phase solvent. In the biphasic system, the volumetric phase ratio, OPBE concentration, cell concentration, reaction temperature, and buffer pH were optimized. Under the optimum conditions (volumetric phase ratio: 1/1, OPBE concentration: 100 mM, cell concentration: 0.075 g/mL, pH 7.5, 35°C), the final yield and the optical purity of (*R*)-HPBE reached 98.3% and >99.0% enantiomeric excess (*ee*), respectively, after 12 h of reaction.

Conclusions: All the results suggested that the OPBE-reducing enzymes in a newly isolated *R. mucilaginosa* cells possess highly stable and excellent stereoselectivity by establishing an aqueous/organic biphasic system.

Keywords: *Rhodotorula mucilaginosa* CCZU-G5; Biphasic system; Isooctane; Ethyl (*R*)-2-hydroxy-4-phenylbutyrate

Background

Optically active ethyl (*R*)-2-hydroxy-4-phenylbutyrate [(*R*)-HPBE] is an important chiral building block for the synthesis of angiotensin-converting enzyme (ACE) inhibitors such as benazepril, enalapril, and lisinopril [1]. In general, ACE inhibitors prevent the conversion of the precursor decapeptide angiotensin I to the powerful vasoconstrictor substance angiotensin II and have been demonstrated to be potent antihypertensive drugs [2]. In recent years, various chemical and biological approaches for (*R*)-HPBE preparation have been reported, mainly in two ways: kinetic resolution and synthesis. However, chemical synthesis usually involves multiple steps and stringent reaction conditions [3], and resolution methods are limited by theoretical maximum yield of only 50% [4].

Microbial or enzymatic reduction of ethyl 2-oxo-4-phenyl-butyrate (OPBE) is an attractive way to produce optically active (*R*)-HPBE, since OPBE can be easily synthesized and is relatively cheap. Several biocatalysts have been used in the synthesis of (*R*)-HPBE, including the hydrolysis and transesterification catalyzed by lipase [5] and the reduction of OPBE catalyzed by isolated dehydrogenase [6] and whole cells [7,8]. Since the reduction reaction requires stoichiometric amounts of nicotinamide cofactors, whole cells rather than isolated enzymes were used preferentially to avoid enzyme purification and cofactor addition [9].

In the past decade, however, only a few microorganisms have been reported as efficient biocatalysts in the reduction of OPBE to (*R*)-HPBE. Chadha et al. reported

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that the enantioselective reduction of OPBE to (*R*)-HPBE could be achieved by using cell-free aqueous extracts of the callus of *Daucus carota* (wild carrot) with a high yield (90%) and enantiomeric excess (*ee*) (99%) [10]. However, their process required a high cell/substrate ratio of 100:1, a large amount of cells, and a long reaction time of 10 days. Dao et al. and Lacerda et al. reported the successful reduction of OPBE with *Pichia angusta* and *Saccharomyces cerevisiae*, respectively, to give (*R*)-HPBE with a moderate enantioselectivity (81% *ee*) [7,11]. Recently, Chen et al. described the successful preparation of (*R*)-HPBE with favorable *ee* (99%) and yield (92%) by using *Candida boidinii* CIOC21 [12]. However, the relatively low concentrations of the substrate (around 4.1 g/L) and product (around 3.8 g/L) in their process would restrict its application in large-scale production. In addition, Zhang et al. used *Candida krusei* SW2026 to produce (*R*)-HPBE from 20 g/L of OPBE with excellent *ee* (97.4%) and a moderate yield (82%) [8].

Recently, we have isolated a new yeast strain *Rhodotorula mucilaginosa* CCZU-G5 from vineyard soil samples and used it in preparing (*R*)-HPBE with high *ee* and yield. However, a severe substrate inhibition was observed when the tested OPBE concentration in an aqueous single-phase system was high due to the high hydrophobicity of the substrate and its toxicity to the cells, and the highest substrate concentration that the bacterium could transform was only 50 mM. The aqueous/organic solvent biphasic system is a good alternative to resolve the aforementioned problems occurred in the aqueous system. The organic solvent phase in the biphasic system acts as a substrate reservoir and prevents the cells in the aqueous phase from being damaged by high substrate concentration. This biphasic system has attracted great attention over the past few decades, and several successful examples have been reported [13-15].

In this study, resting cells of *R. mucilaginosa* CCZU-G5 were used as biocatalysts for asymmetric reduction of OPBE in an aqueous/organic solvent biphasic system. Various parameters such as substrate concentration, cell concentration, reaction temperature, and pH were investigated and optimized to improve the yield and optical purity (*ee*) of (*R*)-HPBE. Compared to the monophasic aqueous system, the asymmetric reduction of OPBE in the aqueous/organic solvent biphasic system gave excellent *ee* and a much higher yield due to reduced substrate and product inhibition. To our best knowledge, this is the first study using *R. mucilaginosa* cells for high-yield and high-purity production of (*R*)-HPBE.

Methods

Chemicals

(*R*)-HPBE was purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA) OPBE was supplied by Wujin Fine Chemical Factory Co., Ltd. (Changzhou, Jiangsu, China). All other reagents and solvents were commercially available, and were of analytical grade purity.

Microbial strain and cultivation conditions

The yeast *R. mucilaginosa* CCZU-G5 was isolated from vineyard soil samples and preserved in China General Microbiological Cultures Collection Center (CGMCC 6328). It was grown in the following medium: glucose 20 g/L, yeast extract 10 g/L, peptone 15 g/L, $(NH_4)_2SO_4$ 1 g/L, $MgSO_4{\cdot}7H_2O$ 1 g/L, pH 7.0. The strain was incubated aerobically at 30°C and 180 rpm in 500-mL Erlenmeyer flasks with 70 mL sterilized medium. After 72 h of growth, the cells were harvested by centrifugation (8,000×*g* for 10 min) at 4°C, washed twice with 0.85% (*w*/*v*) NaCl, and then stored at 4°C for further use.

Bioconversion in monophasic aqueous system

The reduction of OPBE in the aqueous system was conducted in a 50-mL Erlenmeyer flask capped with a septum. Two grams of wet cells was suspended in 20.0 mL phosphate-buffered saline (PBS) (0.1 M, pH 7.0) with 1 g glucose and 0.4 mmol OPBE. The cell suspensions were subsequently incubated in a rotary incubator at 35°C and 180 rpm. The mixture was centrifuged to remove the cells at different time intervals, and the supernatant was extracted three times with ethyl acetate and dried over anhydrous Na_2SO_4 for gas chromatography (GC) analysis.

Tolerance assay of *R. mucilaginosa* CCZU-G5 in biphasic systems

Four grams of harvested cells was suspended in 100 mL PBS (0.1 M, pH 7.0) to give a final concentration of 0.04 g/mL. Ten milliliters of the cell suspension was added to 10 mL of each of the different organic solvents in a 50-mL Erlenmeyer flask capped with a septum. The cell suspension (0.04 g/mL) in PBS without any organic solvent was used as a control. All cell suspensions were subsequently incubated in a rotary incubator at 30°C and 180 rpm for 24 h. The cell suspensions were then centrifuged (8,000×*g* for 10 min) at 4°C. Then the cells were transferred to 10 mL of 20 g/L glucose and incubated at 30°C and 180 rpm. After 4 h, the suspensions were centrifuged, and the supernatants were analyzed using a spectrophotometer to determine the concentration of glucose through DNS method to obtain the amount of consumed glucose [16]. The metabolic activity retention is defined as the ratio of the amount of glucose consumed by the cells pretreated in the biphasic system to that consumed by the cells pretreated in PBS.

Bioconversion in biphasic systems

Experiments examining the asymmetric reduction of OPBE in biphasic systems were performed in 50-mL

Erlenmeyer flasks. A certain amount of wet yeast cells were suspended in 0.1 M PBS and mixed with 10 mL organic solvent containing OPBE to form the aqueous/organic biphasic system (shown in Scheme 1). The flasks were incubated in a rotary shaker at 180 rpm and a specified temperature. Due to their high hydrophobicity, the substrate OPBE and the product (*R*)-HPBE were primarily partitioned in the organic phase, while the cells were mainly suspended in the aqueous phase. Unless otherwise specified, the concentration of the substrate and product were calculated based on the volume of the whole biphasic system. Six hundred-microliter aliquots of the sample were withdrawn from the organic phase at different time intervals: 100 μL aliquots were mixed with 100 μL dodecane as the internal standard for GC analysis, and 500 μL aliquots were used for high-performance liquid chromatography (HPLC) analysis.

Analytical methods

The concentrations of OPBE and HPBE were determined with a gas chromatography. (GC-950, Shanghai Haixin Chromatographic Instrument Co., Ltd., Shanghai, China) equipped with a flame ionization detector and a SE-30 capillary column (30 m, i.d. 0.5 mm). The (*R*)-HPBE and (*S*)-HPBE were analyzed using an Agilent 1260 HPLC system (Sta. Clara, CA, USA) equipped with a Chiralcel OD-H column (4.6 mm × 250 mm, 5 μm, Diacel, Hyogo, Japan) using *n*-hexane:isopropanol (95:5, *v*/*v*) as eluent at a flow rate of 1.0 mL/min. The detection was performed at 225 nm.

The *ee* value was calculated as follows:

$$ee = \frac{[R]-[S]}{[R]+[S]} \times 100\%$$

where [*R*] and [*S*] are the peak areas of (*R*)-HPBE and (*S*)-HPBE, respectively. The yield of (*R*)-HPBE was calculated from the final molar concentration of the product, C_p, and the initial molar concentration of the substrate, C_s as follows:

$$\text{Yield} = \frac{c_p}{c_s} \times 100\%$$

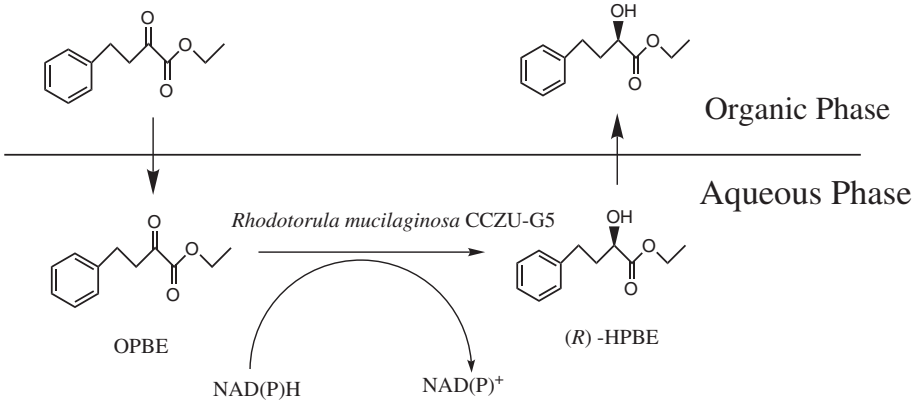
Results and discussion

Tolerance of *R. mucilaginosa* CCZU-G5 in different biphasic systems

The metabolic activity of cells could be affected by the organic solvent in a biphasic system [16,17]. It has been reported that solvents with a higher log *P* are more hydrophobic and thus more advantageous for enzymatic reactions [8]. In this work, 11 organic solvents with different log *P* values ranging from 0.68 to 5.6 were studied and compared for their toxicity to *R. mucilaginosa* CCZU-G5. As the toxicity indicator, cell viability or metabolic activity retention (*R* %) in the various biphasic systems was evaluated and the results were shown in Table 1. In general, there was a positive correlation between activity retention and the log *P* value of the organic solvent. Satisfactory metabolic activity retention over 80% was attained when the log *P* value of the solvent was over 4.6. The maximal metabolic activity retention reached 97.8% in the water/n-decane system. The metabolic activity retention was low when the log *P* value of the solvent was lower than 3.2. For example, it was just 14.7% in the water/ethyl acetate biphasic system because the cell membrane would be destroyed by the polar organic solvent with a low log *P* value [18].

Selection of organic solvents

The asymmetric reduction of OPBE to (*R*)-HPBE with resting cells of *R. mucilaginosa* CCZU-G5 in an aqueous medium has been optimized in our previous work (20 mM OPBE, yield 76.3%, *ee* 99.2%, productivity 6.8 g/L/day). The aqueous systems supported a desirable *ee* value, but the highest substrate concentration that could be transformed



Scheme 1 Mechanism of *R. mucilaginosa* CCZU-G5 for asymmetric reduction of OPBE to (*R*)-HPBE biphasic system.

Table 1 Tolerance and asymmetric reduction of OPBE to (*R*)-HPBE with *R. mucilaginosa* CCZU-G5 in different organic/aqueous biphasic systems

Organic solvent	Log *P*	Metabolic activity retention (%)[a]	Yield (%)[b]	*ee* (%)[b]
EtOAC	0.68	14.5 ± 0.6	11.3 ± 0.7	16.5
Butyl acetate	1.7	17.1 ± 0.7	14.3 ± 1.3	18.4
Benzene	2.0	17.5 ± 0.6	36.1 ± 1.1	45.5
Toluene	2.5	22.3 ± 0.7	44.1 ± 1.0	49.4
Cyclohexane	3.2	50.4 ± 1.3	53.1 ± 1.5	68.9
n-Hexane	3.5	67.2 ± 0.9	69.3 ± 0.5	>99.0
n-Heptane	4.0	70.5 ± 0.8	89.3 ± 0.4	>99.0
n-Octane	4.5	78.8 ± 0.7	90.3 ± 0.4	>99.0
Isooctane	4.6	80.1 ± 0.6	98.1 ± 0.3	>99.0
n-Hydride	5.1	92.2 ± 0.4	63.1 ± 0.3	>99.0
n-Decane	5.6	98.0 ± 0.4	52.8 ± 0.9	>99.0

[a]Metabolic activity retention (*R*) of *R. mucilaginosa* CCZU-G5: 0.4 g wet cells suspended in 10 mL PBS buffer (0.1 M, pH 7.0) was mixed with 10 mL of different organic solvents incubated at 30°C and 180 rpm for 24 h. The cell suspension in PBS without any organic solvent was used as a control. Then the harvested cells were transferred to 10 mL of 20 g/L glucose and incubated at 30°C and 180 rpm for 4 h. [b]Reaction conditions: 2.0 g wet cells suspended in 10 mL PBS buffer (0.1 M, pH 7.0) containing 1.0 g glucose was mixed with 10 mL of an organic solvent containing 2 mmol OPBE and incubated at 30°C and 180 rpm for 24 h.

with 0.1 g/mL cell was merely 50 mM. To enhance the substrate concentration and overcome the substrate-tolerance obstacle [14,19], the reaction was explored in the aqueous/organic biphasic system. With the addition of organic solvents, the solubility of the substrate could be enhanced. Besides, the hydrophilic microbial cells (in aqueous phase) could be separated from the hydrophobic substrate and the product in the organic phase. The selection of the organic solvent as substrate and product carrier in an aqueous/organic solvent biphasic system is based mainly on its biocompatibility towards the biocatalyst and adequate solubility of both substrate and product [20]. Therefore, the influence of organic solvents on the catalytic activity and enantioselectivity was studied in 11 different biphasic systems with different log *P* values ranging from 0.68 to 5.6. In general, there was a positive correlation between the product yield and the log *P* value of organic solvent (Table 1), with the exceptions of *n*-hydride and *n*-decane, which have log *P* values of over 5 but gave a relatively low product yield. The maximal yield of 98.1% was achieved in the aqueous/isooctane biphasic system. The yield decreased significantly in the organic solvents with lower log *P* values such as butyl acetate and EtOAC, which gave a poor yield of 11.3%. The *ee* values of (*R*)-HPBE were higher than 99.0% when the log *P* values of the organic solvents were more than 3.2. Considering the yield and the *ee* value, the aqueous/isooctane biphasic system was selected for further study.

Effects of phase volume ratio

The volumetric phase ratio influences the phase interfacial area between two phases, which, in turn, affects biotransformation in the biphasic systems [21]. The effects of the volume ratio of the aqueous phase to the organic phase (V_{aq}/V_{org}) on the asymmetric reduction of OPBE were studied in the aqueous/isooctane biphasic system. A fixed volume (10 mL) of isooctane was mixed with different volumes (2 to 20 mL) of the aqueous phase containing 2 g cells to form biphasic aqueous/isooctane system. As seen in Figure 1, the product yield increased when the phase ratio (V_{aq}/V_{org}) went up from 0.2 to 0.8 due to the increased availability of substrate molecules in aqueous phase. With a phase ratio of 0.8 to 1.2, the maximum yield of (*R*)-HPBE (98.1%) was achieved. However, the yield decreased when the phase volume ratio was higher than 1.2 probably because of the increased sensitivity to the substrate toxicity when the cells were diluted. Although the yield was highly dependent on the phase volume ratio, excellent optical purity of the product (*ee* >99.0%) was observed in the aqueous/isooctane biphasic system, regardless of the phase ratio.

Effects of temperature

It is well known that the reaction temperature is an important factor affecting the catalytic characteristics such as the activity, enantioselectivity, and stability of a biocatalyst [22]. As shown in Figure 2, the product yield increased gradually when the temperature rose from 25°C to 35°C. However, at higher temperatures, the yield decreased significantly with increasing the temperature, indicating thermal deactivation of the cells at higher temperatures. The *ee* value was not significantly affected by temperature and remained above 99.0% at all temperatures studied.

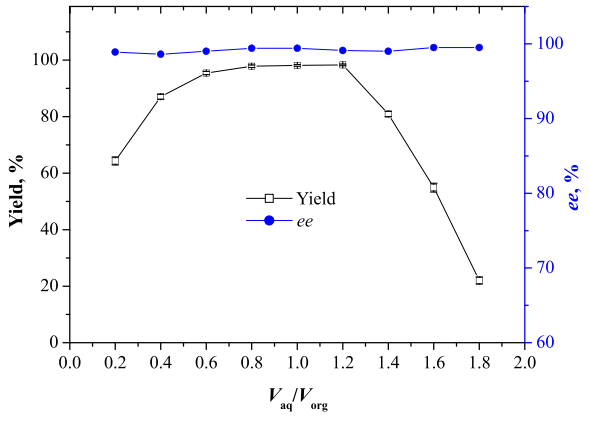


Figure 1 Effects of phase volume ratio on asymmetric reduction of OPBE to (*R*)-HPBE in aqueous/isooctane biphasic system. Reaction conditions: 2.0 g wet cells suspended in a certain volume of PBS (0.1 M, pH 7.0) containing 1.0 g glucose and mixed with 10 mL isooctane containing 2 mmol OPBE and incubated at 30°C and 180 rpm for 24 h.

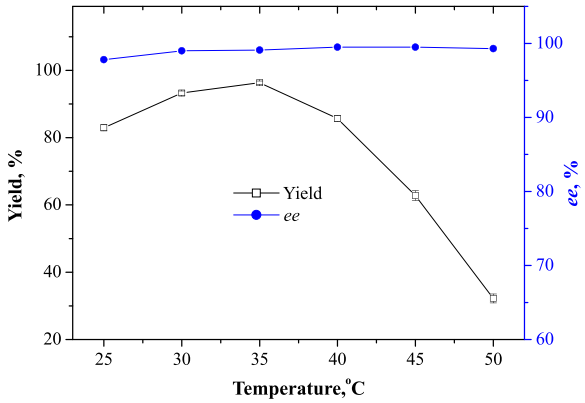


Figure 2 Effects of temperature on asymmetric reduction of OPBE to (*R*)-HPBE in aqueous/isooctane biphasic system. Reaction conditions: 2.0 g wet cells in 10 mL PBS (0.1 M, pH 7.0) containing 1.0 g glucose, mixed with 10 mL isooctane containing 2 mmol OPBE, and incubated at various temperatures and 180 rpm for 24 h.

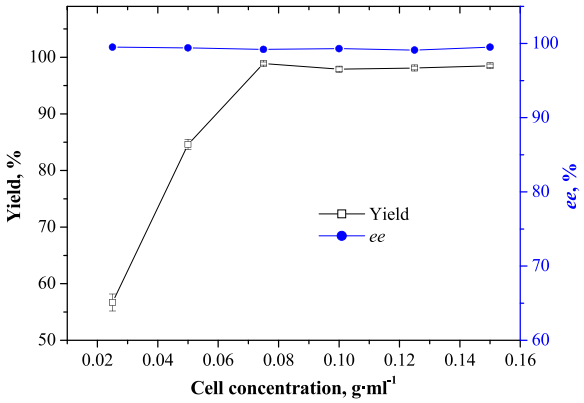


Figure 4 Effects of cell concentration on asymmetric reduction of OPBE to (*R*)-HPBE in aqueous/isooctane biphasic system. Reaction conditions: 2.0 g wet cells in 10 mL PBS (0.1 M, pH 7.5) containing 1.0 g glucose, mixed with 10 mL isooctane containing 2 mmol OPBE, and incubated at 35°C and 180 rpm for 24 h.

Effects of pH

The reaction pH plays a crucial role in bioreduction and influences not only enzymatic enantioselectivity and activity but also the regeneration of coenzymes [23-25]. In addition, variations in the pH may also alter the ionic state of substrates and the enzymes involved in the reactions [26]. Figure 3 shows the effects of aqueous medium pH (over the range of 5.5 to 8.0) on the asymmetric reduction of OPBE to (*R*)-HPBE. The highest product yield was obtained at pH 7.5. The product yield decreased dramatically at lower or higher pH values. It is clear that pH could exert a tremendous influence on the reaction rate and yield in the aqueous/isooctane system. However, the *ee* value was not significantly affected by the pH and remained above 99.0% for all pH values studied. Based on the results, pH 7.5 was the optimum for the reaction.

Effects of cell concentration

The concentration of biocatalyst is an important factor in enzymatic reactions because it affects both enantioselectivity and reaction rate [19]. In order to investigate the effects of cell concentration on OPBE reduction in aqueous/isooctane system, the *ee* and yield of the product were determined at different cell concentrations. As shown in Figure 4, the cell concentration in the aqueous phase had no effect on the *ee* value but had a marked

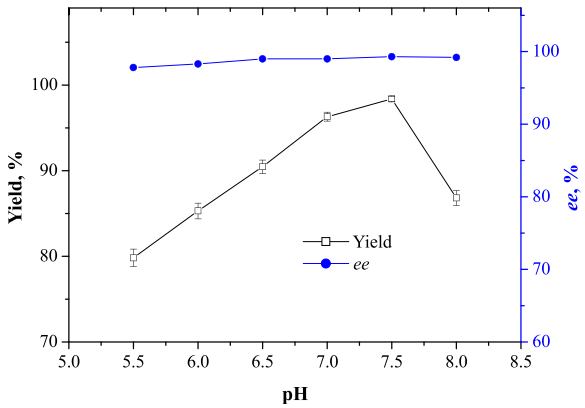


Figure 3 Effects of pH on the asymmetric reduction of OPBE to (*R*)-HPBE in aqueous/isooctane biphasic system. Reaction conditions: 2.0 g wet cells in 10 mL PBS containing 1.0 g glucose, mixed with 10 mL isooctane containing 2 mmol OPBE, and incubated at 35°C and 180 rpm for 24 h.

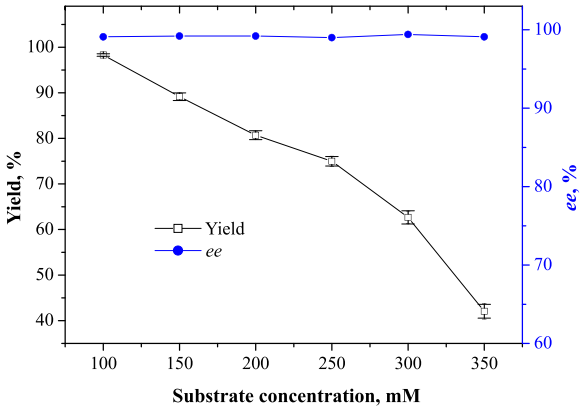


Figure 5 Effects of substrate concentration on asymmetric reduction of OPBE to (*R*)-HPBE in aqueous/isooctane biphasic system. Reaction conditions: 1.5 g wet cells and 1.0 g glucose in 10 mL PBS (0.1 M, pH 7.5), mixed with 10 mL isooctane containing different amounts of OPBE, 35°C, 180 rpm, 24 h. The OPBE concentration is based on the total liquid volume in the system.

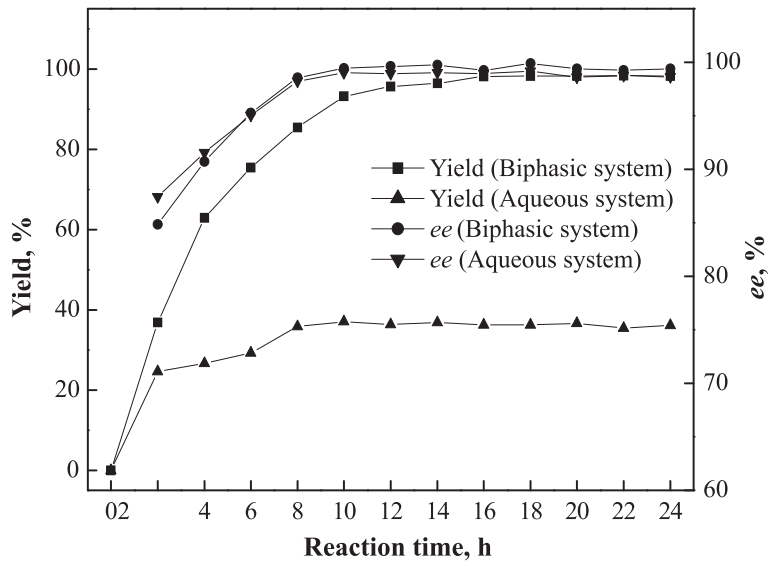


Figure 6 Time courses of the asymmetric reduction of OPBE to (*R*)-HPBE. Time courses of the asymmetric reduction of OPBE to (*R*)-HPBE in a monophasic aqueous system and in the aqueous/isooctane biphasic system. Reaction conditions: 1.5 g wet cells suspended in 10 mL PBS (0.1 M, pH 7.5) containing 1.0 g glucose mixed with 10 mL isooctane containing 2 mmol OPBE incubated at 35°C and 180 rpm. The monophasic aqueous system was 20 mL PBS (0.1 M, pH 7.0) containing 1.0 g glucose and 0.4 mmol OPBE.

effect on the reaction rate and thus yield, which increased to approximately 98% with increasing the cell concentration to 0.075 g/mL. Further increasing of the cell concentration had no effect on the yield.

Effects of substrate concentration

The substrate concentration in a reaction medium affects not only the reaction rate but also the enantioselectivity of the reaction [27]. Therefore, it is of great importance to investigate the effect of substrate concentration on the OPBE asymmetric reduction in the aqueous/isooctane biphasic system. Experiments with different initial substrate concentrations from 100 to 350 mM were conducted to investigate the effects of the substrate concentration on the asymmetric reduction of OPBE to (*R*)-HPBE under the optimal conditions obtained above. With the OPBE concentration increasing from 100 to 300 mM, the productivity increased from 10.2 to 20.3 g/L/day, although the yield reduced from 98% to 65% (Figure 5). When the substrate concentration was 350 mM, both yield and productivity decreased to 42% and 15.3 g/L/day, respectively, indicating that a high OPBE concentration (>300 mM) would significantly inhibit the catalytic activity of cells even in the biphasic system. However, no significant influence on the enantioselectivity was observed at high concentrations of both substrate and product, and the *ee* value of product was consistently above 99.0%.

Comparison of the aqueous/organic solvent biphasic system with the monophasic aqueous system

The reaction in the aqueous/isooctane biphasic system was compared with that in the monophasic aqueous

Table 2 Comparison between *R. mucilaginosa* CCZU-G5 and other reported microorganisms

Strain	Reaction media	OPBE conc	Reaction time (h)	*ee* (%)	Yield (conversion) (%)	References
R. mucilaginosa CCZU-G5	Aqueous phase	20 mM	8	99.2 (*R*)	76.3	This study
	Aqueous/isooctane	100 mM[a]	12	>99.0 (*R*)	98.3	This study
		200 mM[a]			>80	
Saccharomyces cerevisiae	Water/benzene	400 mM	48	87.5 (*R*)	(41.9)	Shi et al., 2009 [28]
Candida krusei SW2026	Water/dibutyl phthalate	100 mM	16	97.4 (*R*)	82	Zhang et al., 2009 [8]
	Aqueous phase	10 mM	14	99.7 (*R*)	95.1	
Candida boidinii CIOC21	Aqueous phase	20 mM	12	99.0 (*R*)	92	Chen et al., 2009 [12]
Pichia angusta	Aqueous phase	0.14% in volume	24	81 (*R*)	(100)	Lacerda et al., 2006 [7]

[a]Based on the total volume of the whole biphasic system.

system. As shown in Figure 6, the reaction in the monophasic aqueous system with 100 mM OPBE stopped after 8 h with a low yield (36.1%) and low productivity (11.4 g/L/day), which can be attributed to the substrate and product inhibition. In contrast, the reaction reached a high yield of 98.3% and a high productivity of 20.4 g/L/day after 12 h in the aqueous/isooctane biphasic system. Apparently, using isooctane as the substrate carrier eliminated the substrate inhibition and enhanced the reaction rate in the biphasic system. In addition, the *in situ* extraction of the product from the aqueous phase to the organic phase also decreased the product inhibition and allowed the reaction to continue until almost all substrate had been converted to the product, thus resulting in a high yield (98.3%) in the aqueous/isooctane biphasic system. Furthermore, the optical purity of the product has not changed (>99% *ee*) by introducing isooctane as the organic phase. It is noted that the reaction rate in the aqueous/isooctane biphasic system could be limited by mass transfer between the two phases [27], which can be increased by increasing the interfacial area between the two phases. Overall, the aqueous/isooctane biphasic system is advantageous for the asymmetric reduction of OPBE catalyzed by the microbial cells.

To date, only a few microorganisms have been described as efficient biocatalysts in the reduction of OPBE to (*R*)-HPBE. Table 2 compares the performances of the reported strains and *R. mucilaginosa* CCZU-G5 as biocatalysts in the preparation of (*R*)-HPBE from OPBE. Although excellent yield and *ee* value can be obtained with *C. krusei* SW2026 and *C. boidinii* CIOC21 in aqueous phase systems [8,12], the low substrate loading restricted their industrial applications. To increase the substrate concentration, Zhang et al. employed a water/dibutyl phthalate biphasic system [8]. Although the concentration of OPBE increased ninefold, the product yield decreased from 95.2% to 82%. To the best of our knowledge, only Shi et al. reduced OPBE at as high as 0.4 M with *S. cerevisiae* in water/benzene biphasic system [28]. However, the conversion of OPBE and *ee* of (*R*)-HPBE were only 41.9% and 87.5%, respectively. Moreover, the reaction time was very long. When the reduction of OPBE (100 mM) was carried out with *R. mucilaginosa* CCZU-G5 in aqueous/isooctane biphasic system, the shortest reaction time, excellent *ee* and product yield were achieved in the present study. Even if the concentration of OPBE was increased to 200 mM, the yield was still over 80%, and the *ee* was not affected. Compared with other strains listed in Table 2, *R. mucilaginosa* CCZU-G5 is a more competitive and promising biocatalyst for asymmetric reduction of OPBE to (*R*)-HPBE.

Conclusions

In this study, an aqueous/isooctane biphasic system was successfully established for asymmetric reduction of OPBE to (*R*)-HPBE with a newly isolated strain *R. mucilaginosa* CCZU-G5. Several factors such as volume ratio of the aqueous phase to the organic phase, reaction temperature, reaction pH, cell concentration, and substrate concentration significantly influenced the reaction rate and product yield. However, the optical purity of the product was not significantly affected and maintained at high levels of >99%. Under optimum reaction conditions (35°C, pH 7.5, 0.075 g/mL of cells, 100 mM OPBE, 1:1 of volume phase ratio), *R. mucilaginosa* CCZU-G5 exhibited excellent catalytic capability, giving product an excellent yield (98.3%) and *ee* (>99%).

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

L-QW designed the study and drafted the manuscript. Z-QW revised the manuscript. QQ provided the experimental guidance. S-TY provided the experimental guidance and manuscript revision. J-JM and L-JW conducted the experiments and drafted the manuscript. All authors read and approved the final manuscript.

Acknowledgements

This work was supported by National Natural Science Foundation of China (No. 21102011) and the Science and Technology Supporting Project of Changzhou (No. CE20145041).

Received: 15 December 2014 Accepted: 21 January 2015
Published online: 18 February 2015

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