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Production of (*R*)-styrene oxide by recombinant whole-cell biocatalyst in aqueous and biphasic system

Feng Xue*, **Jian Gao**

School of Marine and Bioengineering, Yancheng Institute of Technology, Yancheng 224051, P.R. China
Corresponding author: e-mail: coldsun_xf@163.com

The enantioselective resolution of racemic styrene oxide (*rac*-SO) to (*R*)-SO by whole cells of a recombinant *Escherichia coli* expressing epoxide hydrolase (EH) activity in aqueous and biphasic system were studied. Some parameters that may alter this bio-resolution, such as the concentration of recombinant cell, substrate and product were evaluated. The effect of the addition of different additives on the course of *rac*-SO biotransformation was also investigated. The results showed that the yield and the enantiomeric excess (*ee*) of (*R*)-SO were dependent on these variables. When the kinetic resolution was conducted with 350 mM of *rac*-SO, enantiopure (*R*)-SO with high (≥99%) *ee* was obtained with a yield of 38.2% yield at 12.2 h in the presence of 10% (v/v) Tween 80. An isooctane/aqueous system was developed to overcome the adverse factors in the aqueous phase, resulting in an improvement of yield from 38.2% to 42.9%. The results will provide a useful guidance for further application of this enzyme in the biocatalytic production of chiral synthons.

Keywords: Kinetic resolution, Epoxide hydrolase, Enantioselectivity, (*R*)-styrene oxide.

INTRODUCTION

Enantiopure epoxides are important intermediates for the synthesis of chiral compounds and drugs, which have broad market application prospect[1]. Kinetic resolutions of racemate or organocatalytic asymmetric epoxidation of olefins have already been developed for the synthesis of optically active epoxides. However, many of these chemocatalytic methods suffer from significant limitations including the rather expensive and toxic metal catalysts or limited substrate spectrum. At present, biocatalysis has been considered as a promising green method for overcoming these disadvantages[2]. Among the biological production methods, asymmetric epoxidation by monooxygenase and peroxidase, enantioselective hydrolysis by epoxide hydrolase (EH), and kinetic resolution by lipase have been considered as alternatives due to their excellent enantioselectivity and regioselectivity[1, 3, 4].

Enantiopure styrene oxide (SO) is an important building block for the preparation of different types of chiral compounds. For instance, (*S*)-SO is an intermediate for the synthesis of anticancer agent Levamisole, anti-HIV agent (-)-hyperolactone C, and nematocide[5], the opposite enantiomer (*R*)-SO can be used for the synthesis of the NK-1 receptor antagonists (+)-CP-99,994, arylalkylamine calcimimetic (*R*)-(+)-NPS-R-568, and the nucleoside analogs with antiviral activity[6]. Some biocatalysis approaches used for producing chiral SO include asymmetric epoxidation of styrene using styrene monooxygenase[7] and resolution of racemic styrene oxide (*rac*-SO) using EH[8]. Recombinant *Escherichia coli* cells harboring the styrene monooxygenase genes from *Pseudomonas* sp. VLB120[9], *Paraglaciecola agarilytica* NO2[7], *Marinobacterium litorale* DSM 23545[7], *Pseudomonas* sp. LQ26[7] and *Rhodococcus opacus* 1CP[10] have been cultivated and used in the epoxidation of the styrene. Most of the styrene monooxygenases can yield (*S*)-SO with excellent enantiomeric excess (>99% *ee*)[7, 9]. However, low substrate concentration and cofactor dependent limit the application of styrene monooxygenase. Nowadays, the use of EHs for the preparation of chiral SO is one of the most fascinating research areas in biochemical engineering, since EHs have several advantages as biocatalysts such as cofactor independent, ubiquitous in nature and broad substrate spectrum[1]. To develop the needs of high performance enzymes, recent efforts have been focused on the searching, cloning, expression, purification, biologic characteristics, catalytic mechanism[11–13], modification of enantioselectivity and activity, resolution of protein structure and homology modeling of various new EHs[14–16]. Some of EHs exhibit modest to excellent enantioseletivities toward *rac*-SO, e.g., *Aspergillus usamii*[5], *Sphingomonas* sp. HXN-200[8], *Novosphingobium aromaticivorans*[17], *Agrobacterium radiobacter* EH[18], *Rhodotorula glutinis*[19], *Aspergillus niger* EH[20]. Enantiopure (*S*)-SO could be obtained from its racemates (17 mM) with an optical purity of 99% *ee* and 11.7% yield using purified EH from *N. aromaticivorans*[17]. 34.3% of (*S*)-SO with 98.2% *ee* from *rac*-SO (1 M) was obtained by using the cell-free extract of engineered *E. coli*, expressing the recombinant *A. usamii* EH in a biphasic system[5]. A preparative-scale (120 g/L) kinetic resolution of *rac*-SO using immobilized EH from *A. niger* was performed in a batch reactor and (*S*)-SO was both obtained with about 50% yield and 99% *ee*[20]. From a practical point of view, the use of the *E. coli* cells as catalyst is of economic advantage over the use of the cell-free extracts or purified enzyme. The recombinant cells expressing EH from *R. glutinis* were used for the hydrolysis of *rac*-SO (526 mM) to give (*S*)-SO in 98% *ee* and 36% yield[19]. The recombinant cells containing *Sphingomonas* sp. HXN-200 EH gene has been applied into resolution of *rac*-SO (1000 mM) to provide (*S*)-SO with 36.4% isolated yield and more than 99% *ee*[8].

However, thus far, there are only a few reported examples of using EH to prepare (*R*)-SO[21–23]. Enantiopure (*R*)-SO could be obtained from its racemates (100 mM) with an optical purity of 99% *ee* using the recombinant *E. coli* expressing the soluble EH gene of *Danio rerio*[21]. *Bacillus megaterium* ECU1001 EH has been cloned and applied into resolution of (*R,S*)-SO (5 mM) to provide (*S*)-ECH with 53% *ee*[22]. Unfortunately, the enantioselectivities of these EHs are not satisfactory for producing (*R*)-SO. In previous report, the gene coding

for the *Agromyces mediolanus* EHase was cloned and heterologously expressed in *E. coli*[24]. The enzymatic properties of expressed recombinant EH were characterized, displaying high selective towards (*R*)-epichlorohydrin, in the resolution of *rac*-epichlorohydrin. The interesting report prompted us to study the enantioselective resolution of *rac*-SO. Herein, we report the results of the study of the enzymatic transformations and the preparation of the valuable (*R*)-SO.

EXPERIMENTAL SECTION

Chemicals

rac-SO, (*R*)-SO, (*S*)-SO, 1-Phenyl-1,2-ethanediol, 1-chlorohexane were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). All other chemicals used were analytical reagent grade and are commercially available.

Strains and growth condition

The consruction of recombinant *E. coli* cells (BL21/pET28a) expressing the EH gene was (GenBank accession nos. JX467176) was reported previously[24]. The recombinant *E. coli* was grown at 37°C in Luria-Bertani (LB) medium (1L LB-medium contains 10.0 g tryptone, 5.0 g yeast extract, and 10.0 g NaCl, pH 7.0) containing kanamycin (50 μg mL^{-1}) until the optical density reached 0.8 at 600 nm. Then, cells were induced by addition of 0.1 mM isopropyl-β-D-thiogalactopyranoside (IPTG) and growth was carried out at 28°C for extra 10 h. Then, the cells were harvested, washed twice with 100 mM sodium phosphate buffer (pH 8.0) and stored at –20°C.

Analytical methods

The enantiomeric excess (*ee*) and yield of (*R*)-SO were analyzed by an GC-14C (Japan) gas chromatography, equipped with a Chiraldex G-TA column (30 m length, 0.25 mm ID and 0.12 μm film thickness) fitted with a FID detector[16]. The temperatures of the injector and flame ionization detector were 220°C. The column oven temperature was maintained at 110°C for 10 min. The retention times of (*S*)-SO and (*R*)-SO were 5.4 and 6.0 min, respectively. The quantitative data corresponding to (*R*)- or (*S*)-SO was calculated by comparing the peak area with 1-chlorohexane and then determined the concentrations of chiral SO by comparison with standard curve.

Enantioselective resolution of *rac*-SO by the recombinant *E. coli* in aqueous buffer

The reaction was conducted at 30°C in 10 ml 100 mM sodium phosphate buffer (pH 8.0) in 50 ml screw-cap bottles. Cell density was varied in the range of 10 and 70 mg/ml , and *rac*-SO was in the range of 20 and 100 mM. The biotransformation was performed in the water bath shaker (200 rpm) at the reaction temperature. Samples (400 μl) were taken at different time points, extracted immediately with 1 ml ethyl acetate. After centrifugation, the ethyl acetate layer was dried over Na_2SO_4 and analyzed by a chiral GC.

To measure the effect of diol concentration on the kinetic resolution of *rac*-SO in aqueous buffer, reaction mixture containing various concentrations of racemic phenyl-1,2-ethanediol (0–300 mM), 40 mM *rac*-SO with a wet cell (40 g/L) in 100 mM sodium phosphate buffer (pH 8.0) at 30°C and 200 rpm. The depletion of *rac*-SO was determined by chiral GC as described above. The reaction solution without adding racemic phenyl-1,2-ethanediol was used as control.

Enzyme assay

EH activity was assayed as described above, and then the appropriate amount of *rac*-SO was added and the mixed system was carried out in an reactor at 30°C for the appropriate time. The methods of sample extraction and analysis are the same to the above. One unit (U) is defined as the amount EH that catalyzed the reduction of 1 μmol SO at 30°C. The enantiomeric excess (*ee*) was calculated by the peak area of the two enantiomers [$ee(\%) = (R\text{-}S)/(R+S)\times100$]. Yield was derived from the ratio of the remaining epoxide concentration to the initial *rac*-SO concentration.

Effect of surfactants and cosolvents on the enantioselective resolution of *rac*-SO in aqueous buffer

To investigate the effects of cosolvents and surfactants on the asymmetric resolution of *rac*-SO, various cosolvents and surfactants were added to the reaction system. After the given amount of freshly prepared *E. coli* cells and *rac*-SO were added, the reaction mixtures were shaken at 30°C and 200 rpm. The batch kinetic resolutions were monitored by periodical withdrawing of sample aliquots from the reaction mixture.

Effects of organic solvents on the enantioselective resolution of *rac*-SO in two-phase system

Batch kinetic resolution experiments were carried out in a 50-ml screw-capped vial. To 8 ml cell suspension (0.04 g/ml) in 100 mM sodium phosphate buffer (pH 8.0), was added 2 ml of organic reagent, and the mixture solution was preincubated for 3 min at 30°C. After the *rac*-SO was added, the reaction started and the mixtures were shaken at 30°C and 200 rpm. The progress of reaction was followed by the samples periodically withdrawn from the reaction flask, analyzed by GC.

RESULTS AND DISCUSSION

Effect of initial substrate concentration in aqueous system

To investigate the effect of initial substrate concentrations on the hydrolysis rate and enantioselectivity, enantioselective resolution by the recombinant *E. coli* was performed at various *rac*-SO concentrations ranging from 20 to 100 mM. Reactions were conducted at temperature 30°C and pH 8.0. As shown in Figure 1A, enantiopure (*R*)-SO with more than 99% *ee* was obtained for the substrate concentrations up to 100 mM in the enantioselective resolution of *rac*-SO by using the resting cells (40 g/L). However, the yield decreased from 43.3% to 36.7%, and the reaction time required for 99% *ee* increased with an increase in the initial substrate concentrations. Increasing the racemic SO concentration to 150 mM resulted in a obvious decrease in eantioselectivity (91.6% *ee*) by using the recombinant whole cells (40 g/L), but the reaction can be continued beyond 150 mM *rac*-SO with holding the enantioselectivity as the amount of the recombinant *E. coli* increased.

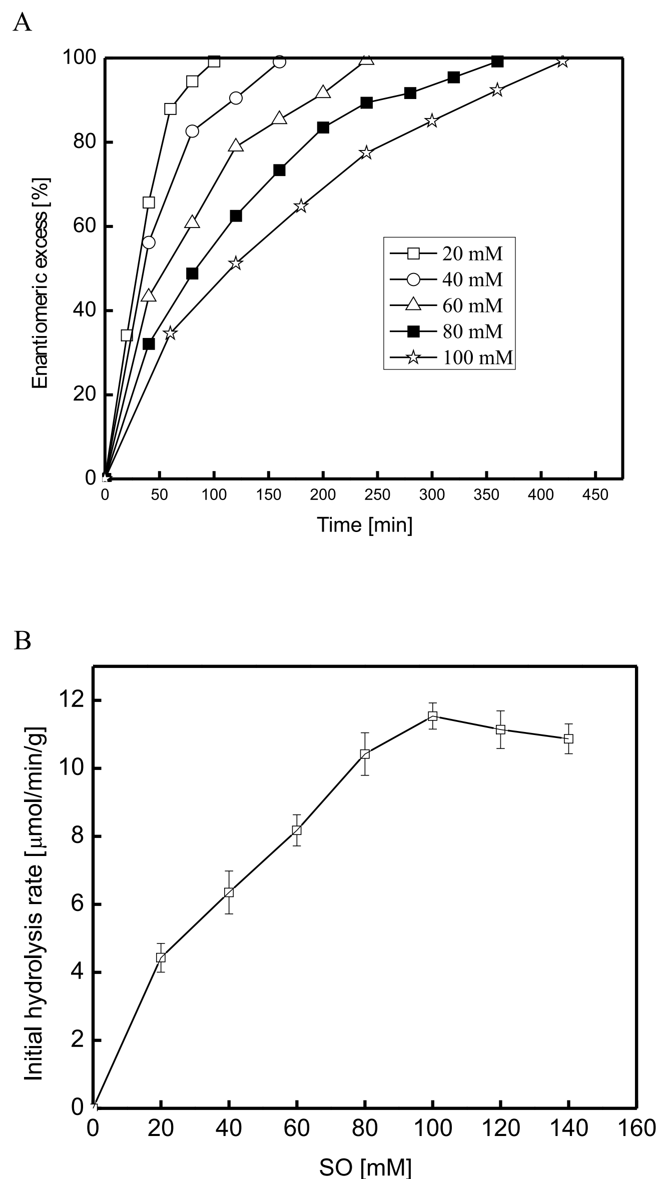

Figure 1. Effect of initial substrate concentrations on *ee* (A) and initial rate (B) of enantioselective hydrolysis of *rac*-SO by recombinant *E. coli*

Another experiments were carried out to evaluate the influences of *rac*-SO concentration on the reaction rate. The results shown in Figure 1B indicate that the reaction becomes saturated at *rac*-SO concentrations above 100 mM. At the same time, a slight substrate inhibition was observed.

Effect of cell concentration in aqueous system

The effect of varying the recombinant cell concentrations on the reaction time and yield was investigated to maximize the yield and minimize the reaction time. Cell concentration was changed in the range of 10 mg/ml to 70 mg/ml, while temperature and *rac*-SO concentration were fixed at 30°C and 40 mM, respectively. As shown in Figure 2, the reaction times required to reach 99% *ee* decreased when the cell concentration was increased, while the yield maximum exists at a specific cell concentration. The yield of (*R*)-SO was above 35% (theoretical, 50%) in all case, with a maximum value of 40.7% at around 40 mg/ml. The data showed that the resistance of the mass transfer of *rac*-SO in aqueous media plays an important role in resolution reaction[25].

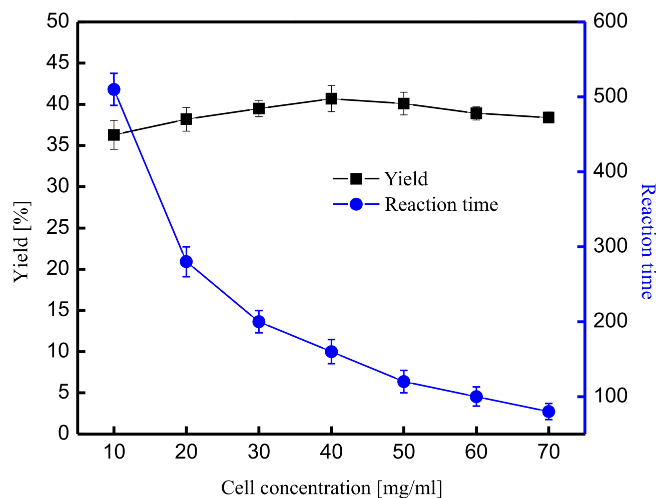

Figure 2. Effect of cell concentration on the yield of (*R*)-enantiomer at 99% *ee* and the process time required to reach 99% *ee*

Therefore, the reaction time could be readily decreased with a relatively low reduction in the yield by increasing cell concentration.

Effect of diol inhibition to EH activity

EHs are generally known to be sentitive to product inhibition[5, 26]. To investigate this effect, racemic phenyl-1,2-ethanediol at various concentrations was added to the cell suspension in 100 mM sodium phosphate buffer. The mixture was then used for enantioselective hydrolysis of 40 mM *rac*-SO at 30°C and pH 8.0. As shown in Figure 3, the reaction rate decreased with the increase of initial phenyl-1,2-ethanediol concentration. But there was no obvious inhibitory effect of the phenyl-1,2-ethanediol on the hydrolysis rate when the concentration of phenyl-1,2-ethanediol was lower than 100 mM. There was only 18% hydrolysis activity by

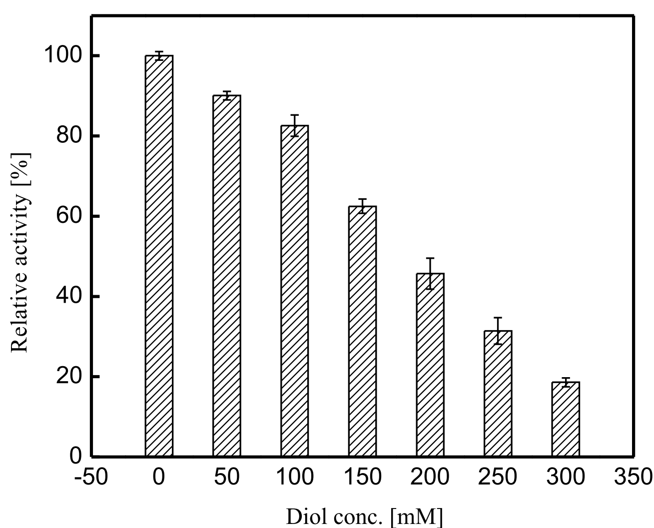

Figure 3. Effect of the concentration of diol on the activity of recombinant EH. Reaction mixture containing various concentrations of racemic phenyl-1,2-ethanediol (0–300 mM), 40 g/L wet cell, and 40 mM *rac*-SO in 100 mM sodium phosphate buffer (pH 8.0) was incubated at 30°C and 200 rpm for 30 min.. The relative activity was calculated based on the recombinant EH activity without adding racemic phenyl-1,2-ethanediol

increasing phenyl-1,2-ethanediol concentration to 300 mM. It was necessary to remove phenyl-1,2-ethanediol from the enzyme-containing aqueous phase for reducing the product inhibition.

Effect of surfactants and cosolvents in aqueous system

The stimulatory effect of the detergents on the enantioselective hydrolysis of the racemic epoxides prompted further investigation of the effect of adding detergents on the initial hydrolysis rate and enantioselectivity of the EH-catalyzed resolution of *rac*-SO. Biocatalyst stability plays an important role in performing a successful enzymatic hydrolysis at high substrate concentration[27]. As is known to all, the surfactant is used to stabilize the enzyme activity[28–30]. Surfactants are also known to increase cell well permeability[31], and hence make the enzyme more available for substrates. In order to investigate the stimulatory effect of the recombinant whole-cell biocatalyst on enantioselective resolutions of surfactants, the kinetic resolution of 40 mM *rac*-SO was carried out in the presence of 10% (v/v) surfactant. Interesting, Tween-80, showed a obvious activation effect on EH, Triton X-100 and Tween-20 also showed a similar improvement effect, while PEG 600 had nearly no effected on EH activity.

With the addition of organic cosolvent, the activity of EH can be inhibited, as well as the chemical stability and solubility of epoxide substrates can be improved, thereby enhancing the volumetric productivity[26]. Results of the effects of various cosolvents on the activity of recombinant *E. coli* at the concentration of 5% (v/v) are presented in Table 1. The EH maintained its activity when dimethylsufoxide (DMSO) were used as a cosolvent in the instance of batch resolution of *rac*-SO, while the enantioselectivities were not improved or lowered in aqueous buffer. The addition of ethanol and isopropanol as a cosolvent was inhibitory to recombinant EH of *A. mediolanus*.

Effect of various organic solvents in biphasic system

The effects of several organic solvents on the activity of the recombinant EH in *E. coli* were investigated. The important criterion that must be considered when selecting an organic solvent is its biocompatibility. According to Laane et al., those solvents with log*P* values below 2.0 are considered extremely toxic, while those with values greater than 4.0 are biocompatible. In addition, those with log*P* between 2.0 and 4.0 influence its biocompatibility to an uncertain degree[32]. In our experiments, nine kinds organic solvents were examined to determine their effects on the resolution of *rac*-SO to (*R*)-SO catalyzed by whole cells from a recombinant *E. coli* system in the biphasic system. According to the literature, the enzyme activity can be influenced not only by the properties, but also by the hydrophobicity of the organic solvent. So it was not directly proportional to the degree of hydrophobicity of the organic solvent[33, 34]. Based on the Table 2, due to the excellent solvent property of *n*-isooctane for styrene oxide and relatively good biocompatibility with EH in the *n*-isooctane /buffer biphasic system (1:4, v/v), the initial hydrolysis rate was clearly faster, the product *ee* and yield value were much higher. In addition, it is a relatively good solvent for *rac*-SO but a poor one for 1-phenyl-1,2-ethanediol, which permits an easy separation of the enantiopure SO. The selection of a phase volume ratio for biphasic whole-cell biotransformation was considered after investigating the influences of this factor on the EH activity and enantioselectivity (data not shown). An organic-aqueous two-phase system composed of sodium phosphate buffer (100 mM, pH 8.0) and isooctane (v/v, 7:3) was determined to be used for the enantiospecific hydrolysis of *rac*-SO.

Kinetic resolution of *rac*-SO at high substrate concentration

In order to further explore the potential application of the EH, higher *rac*-SO concentration reaction was

Table 1. Effect of surfactants and cosolvents on initial hydrolysis rate and yield of (*R*)-SO

Detergent [Reagents]	Initial hydrolysis rate [mol/min/g]	Time [h]	*ee* [%]	Yield [%]
Control	11.7 ±1.5	2.6	≥99	39.8 ±0.9
Triton-X100	13.7 ±2.8	2.4	≥99	40.4 ±1.4
Tween 80	15.1 ±1.9	2.2	≥99	43.4 ±2.3
Tween 20	14.1 ±3.2	2.3	≥99	41.6 ±1.2
PEG 600	11.2 ±2.4	2.8	≥99	38.4 ±1.6
DMSO	11.0 ±1.6	2.9	≥99	36.5 ±1.7
Ethanol	9.2 ±2.7	3.0	71.5	48.2 ±1.9
Isopropanol	8.8 ±2.5	3.2	65.4	37.8 +1.8

Table 2. Effect of various organic solvents on asymmetric hydrolysis of *rac*-SO catalyzed by *A. mediolanus* EH.[a]

Organic solvent	Log *P*	Initial hydrolysis rate [μmol/min/g]	Time [h]	Yield[c] [%]	*ee* [%]
None[b]	–	11.5 ±0.7	2.6	39.2 ±0.6	≥99
Ethyl acetate	0.64	2.3 ±0.9	5.8	46.7 ±0.5	15.8
Butyl acetate	1.7	7.8 ±1.4	4.2	44.3 ±0.9	81.6
Cyclohexane	2.5	7.9 ±0.7	4.0	41.5 ±0.7	98.2
n-Hexane	3.50	8.7 ±0.5	3.8	41.3 ±1.2	≥99
n-heptane	4.0	8.6 ±1.3	3.9	41.8 ±0.3	≥99
Isooctane	4.5	9.4 ±1.1	3.0	43.2 ±0.5	≥99
dodecanol	5.0	7.3 ±0.8	4.2	40.1 ±0.4	98.4
n-Decane	5.70	7.0 ±0.6	4.4	40.5 ±0.7	90.4
Dodecane	6.6	6.3 ±0.9	4.8	42.5 ±0.6	86.3

[a] Hydrolysis reactions were performed in solvent system containing 20%(v/v) organic solvents to evaluate the biocompatibility of the organic solvents.

[b] Enantioselective hydrolysis in aqueous buffer in the absence of any organic solvent.

[c] Yield of the remaining enantiopure SO.

employed. The reaction was carried out at 350 mM *rac*-SO under the conditions of pH 8.0, temperature 30°C, and cell concentration of 0.1 g/ml, in the presence of 10% (v/v) Tween 80. As shown in Figure 4, chiral (*R*)-SO with an *ee*≥99% was obtained in 12.2 h. The yield was about 38.2%. The time-course of the batch kinetic resolution is shown in Figure 4. Following a 12.2 h reaction, 134 mM enantiopure (*R*)-SO was obtained with 99% *ee* and the yield reached 38.2%. An increasing substrate concentration (up to 400 mM) caused a obvious decrease in enantioselectivity. After 16 h, 35.3% of

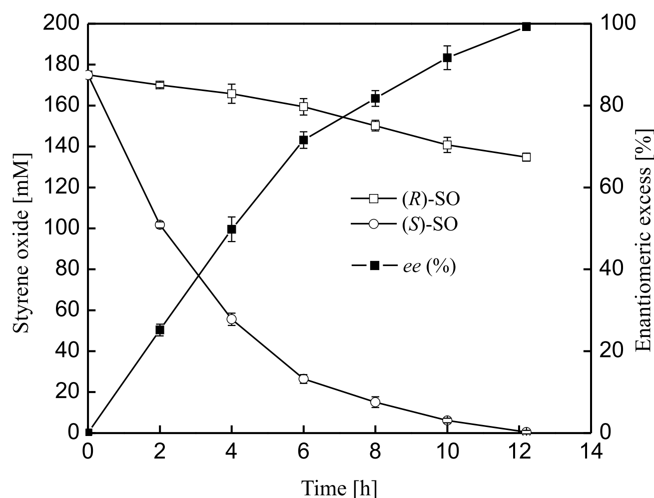

Figure 4. Batch kinetic resolution of 350 mM *rac*-SO by the recombinant *E. coli* expressing the EH gene in the presence of 10%(v/v) Tween 80

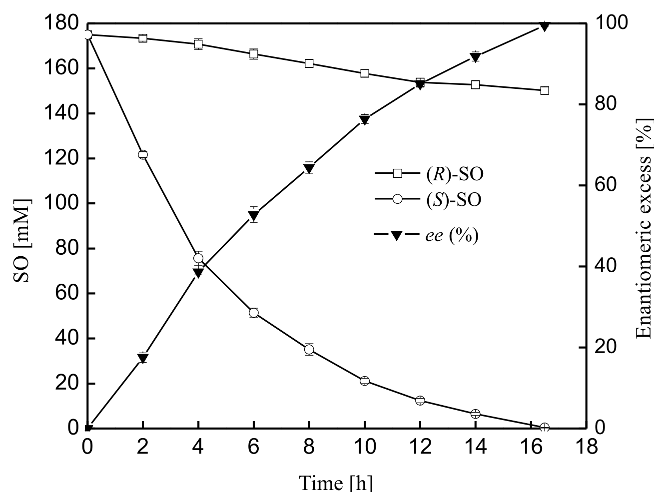

Figure 5. Enantioselective hydrolysis of 350 mM *rac*-SO with wet cell in 100 mM sodium phosphate buffer (pH 8.0) and in buffer/*n*-isooctane (7:3)

(*R*)-SO was obtained in 91.7% *ee*. If the concentration of *rac*-SO reached 500 mM, the final *ee* of (*R*)-SO would be significant decrease, even if the reaction time was prolonged to over 24 h and more cells was added. This result may be mainly attributed to the substrate and product diol inhibition.

In order to reduce the spontaneous hydrolysis rate and substrate inhibition, a two-liquid phase system containing isooctane and buffer (3:7, v/v) was applied for the bioconversion. Hydrolysis of 350 mM *rac*-SO was performed with 3 ml isooctane and 7 ml buffer

Table 3. Comparison of rac-SO resolution between AmEH and other EHs

Epoxide hydrolase source	Reaction system	Catalyst form	Catalyst concentration [g/L]	Conc. [mM]	Time	*ee* [%]/abs. conf.	Final yield [%][c]	Reference
Aspergillus usamii	biphasic	recombinant *E. coli* cell-free extract	20	1000	2 h	98.2/(*S*)	34.3	5
Novosphingobium . aromaticivorans	aqueous	recombinant *E. coli* purified enzyme	n.a.	17	20 min	99/(*S*)	11.7	17
Sphingomonas sp. HXN-200	biphasic	recombinant *E. coli* cell	0.5	200	150 min	99.1/(*S*)	41.6	35
Rhodotorula glutinis	aqueous	recombinant *Pichia pastori* whole-cell	21[a]	526	16 h	98/(*S*)	36	36
Mugil cephalus	aqueous	recombinant *E. coli* whole-cell	20[a]	20	60 min	99/(*S*)	15.4	37
Aspergillus niger	aqueous	Immobilized enzyme	n.a.	1000	n.a.	99/(*S*)	50	38
microsomal EH Danio rerio	aqueous	recombinant *E. coli* whole-cell	40[a]	40	30 min	99/(*S*)	23.5	39
soluble epoxide hydrolase Danio rerio	aqueous	recombinant *E. coli* purified enzyme	n.a.	20	80 min	99/(*R*)	34.8	21
Bacillus megaterium ECU1001	aqueous	recombinant *E. coli* purified enzyme	n.a.	5	30 min	53/(*R*)	43	22
Agromyces mediolanus	aqueous	recombinant *E. coli* whole-cell	100[b]	350	12.2 h	99/(*R*)	38.2	This study
Agromyces mediolanus	biphasic	recombinant *E. coli* whole-cell	100[b]	350	16.5 h	99/(*R*)	42.9	This study

[n.a.] not available
[a] dry cell weight
[b] wet cell weight
[c] Final yield of the remaining enantiopure SO.

containing (0.1g/ml wet cell). As shown in Figure 5, the catalytic rate for (*R*)-SO and (*S*)-SO were slightly slower in biphasic system than in a single aqueous buffer. This may be due to isooctane has toxic effects on biological cells and enzymes, which result in decreased biological activities. Another reason was due to the decreased autohydrolysis rates[35].

Compared with a single-phase system, the organic solvent/buffer two-phase system not only effectively inhibited the spontaneous hydrolysis of *rac*-SO, but also significantly improved the yield of product. Finally (*R*)-SO was formed in ≥99% *ee* and 42.9% yield. In addition, the substrate concentration would be achieved 400 mM with *ee* (96.2%) of (*R*)-SO in buffer/isooctane two-phase systems. In comparison with the aqueous monophasic system, a higher *ee* and yield of (*R*)-SO was obtained. However, a very low *ee* of (*R*)-SO was obtained when the initial concentration of SO was 500 mM, even if the reaction time is extended to 28 h. The possible cause for above result is that the 1-phenyl-1,2-ethanediol was mostly dissolved in the water phase, thus product inhibition occurred even in biphasic system.

Many EHs have so far been reported for the hydrolysis of *rac*-SO. Most of them demonstrated (*R*)-enantioselectivity in the hydrolysis giving (*S*)-SO in high *ee* and yield, and only a few could preferentially hydrolyze (*S*)--SO, retaining the useful (*R*)-SO (Table 3). Therefore, compared with the existing reports[21, 22], the EH from *A. mediolanus* for the hydrolysis of SO with higher (*S*)-enantioselectivity and yield than any other known native EHs. Through enzymatic engineering, including directional evolution, immobilization, or combination of these technologies, enzyme activity, thermostability, or enantioselectivity can be further improved.

CONCLUSION

The enantioselective hydrolysis of *rac*-SO was investigated by using the recombinant *E. coli* cell containing *A. mediolanus* EH. This study showed that the recombinant cell biocatalyst, product and substrate concentrations were sensitive factors with respect to both hydrolysis rate and enzyme enantioselectivity. The addition of 10% Tween-80 obviously increased the EH activity and the yield of (*R*)-SO. Isooctane was selected as the optimum organic phase solvent. Enantiopure (*R*)-SO with 38.2% yield and enantiopurity as high as ≥99% *ee* was obtained *via* enantioselective resolution of 350 mM *rac*-SO by using single cell biocatalyst in the aqueous phase. An organic-aqueous biphasic system composed of 3:7 (v/v) isooctane and sodium phosphate buffer (100 mM, pH 8.0) can be used for the enantioslective hydrolysis of (*R*)-SO affording 42.9% yield and 99% *ee* for a substrate concentration of 350 mM. This study demonstrated that (*R*)-SO could be prepared using the easily available and low-cost whole-cell biocatalysts.

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