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

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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 isoctane/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 synths.

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 prospect1. 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 chemo-catalytic 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 disadvantages5. Among the biological production methods, asymmetric epoxidation by mono-oxidase and peroxidase, enantioselective hydrolysis by epoxide hydrolase (EH), and kinetic resolution by lipase have been considered as alternatives due to their excellent enantioselectivity and regioselectivity1,3,4.

Enantioselective 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 nematocidi5, the opposite enantiomer (R)-SO can be used for the synthesis of the NK-1 receptor antagonists (+)-CP-99,994, aralkylamine calcimimetic (R)-(+)NPS-R-568, and the nucleoside analogs with antiviral activity5. Some biocatalysis approaches used for producing chiral SO include asymmetric epoxidation of styrene using styrene monooxygenase7 and resolution of racemic styrene oxide (rac-SO) using EH8. Recombinant Escherichia coli cells harboring the styrene monooxygenase genes from Pseudomonas sp. VLB1209, Paraglaciecola agarilytica NO27, Marinobacterium litorale DSM 235457, Pseudomonas sp. LQ268 and Rhodococcus opacus 1CP9 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)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 spectrum1. To develop the needs of high performance enzymes, recent efforts have been focused on the searching, cloning, expression, purification, biologic characteristics, catalytic mechanism10–13, modification of enantioselectivity and activity, resolution of protein structure and homology modeling of various new EHS14-16. Some of EHS exhibit modest to excellent enantioselectivities toward rac-SO, e.g., Aspergillus usamii17, Sphingomonas sp. HXN-20017, Novosphingobium aromaticivorans17, Agrobacterium radiobacter EH18, Rhodotolula glutinis19, Aspergillus niger EH20. 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. aromaticivorans17. 34.3% of (S)-SO with 98.2% ee from rac-SO (1 M) was obtained using the cell-free extract of engineered E. coli, expressing the recombiant A. usamii EH in a biphasic system21. 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% ee20. 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% yield19. 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% ee8.

However, thus far, there are only a few reported examples of using EH to prepare (R)-SO21-23. 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 rerio23. Bacillus megaterium ECU1001 EH has been cloned and applied into resolution of (R)-SO (5 mM) to provide (S)-EH with 53% ee22. Unfortunately, the enantioselectivities of these EHS are not satisfactory for producing (R)-SO. In previous report, the gene coding
for the *Agromyces mediterranus* EHase was cloned and heterologously expressed in *E. coli*. 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 construction of recombinant *E. coli* cells (BL21/pET28a) expressing the EH gene was (GenBank accession nos. JX467176) was reported previously. The recombinant *E. coli* was grown at 37°C in Luria-Bertani (LB) medium (1 L 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. 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 obtained 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 ul) were taken at different time points, extracted immediately with 1 ml ethyl acetate. After centrifugation, the ethyl acetate layer was dried over Na₂SO₄ 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 as the amount of SO that catalyzed the reduction of 1 μmol SO at 30°C. The enantiomeric excess (ee) was calculated as the amount of SO that catalyzed the reduction of 1 μmol SO at 30°C.

**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 an obvious decrease in enantioselectivity (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.
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 sensitive 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

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

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

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. Interestingly, Tween-80, showed an obvious activation effect on EH, Triton X-100 and Tween-20 also showed a similar improvement effect, while PEG 600 had nearly no effect 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 surfactants 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 dimethylsulfoxide (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 logP values below 2.0 are considered extremely toxic, while those with values greater than 4.0 are biocompatible. In addition, those with logP 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 enantiomeric resolution 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....

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

| Detergent [Reagents] | Initial hydrolysis rate [mol/min/mg] | Time [h] | ee [%] | Yield [%] |
|----------------------|------------------------------------|----------|--------|-----------|
| Control              | 11.7 ± 1.5                         | 2.6      | ≥ 99   | 39.8 ± 0.9 |
| Triton-X-100         | 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.**

| Organic solvent     | Log P | Initial hydrolysis rate [μmol/min/g] | Time [h] | Yield [%] | ee [%] |
|---------------------|-------|------------------------------------|----------|-----------|--------|
| None\(^a\)          | –     | 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   |
| isoctane            | 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-Decene            | 5.70  | 7.0 ± 0.6                          | 4.4      | 40.5 ± 0.7 | 90.4   |
| Dodecane            | 6.8   | 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 re-
action, 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 obvi-
ous 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](image)

(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

![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)](image)

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

Table 3. Comparison of rac-SO resolution between AmEH and other EHs
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. 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/isoctane 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, 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. Enantipure (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 enantioselective 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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