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Improving *Gluconobacter oxydans* performance in the *in situ* removal of the inhibitor for asymmetric resolution of racemic 1-phenyl-1,2-ethanediol

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HIGHLIGHTS

- We found an efficient method to produce (*S*)-1-pheny-1,2-ethanediol from its racemate.
- The oxidative product, mandelic acid, is the main inhibitor for the reaction.
- *In situ* removal of inhibitor by resin D301 is used to overcome this inhibition.
- The adsorptive method allows the yield of pure (*S*)-PED increased by four times.
- Our result gives a higher space–time yield than that from the previous reports.

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ABSTRACT

Gluconobacter oxydans DSM2003 was used to catalyze the oxidation of racemic 1-phenyl-1,2-ethanediol (PED) for the production of (*S*)-enantiomer. The oxidative product mandelic acid produced strong inhibition to this reaction and largely reduced the activity of biocatalyst, which was the key problem in the reaction. In order to overcome this bottleneck, an anion exchange resin was selected and introduced as adsorbent for the *in situ* removal of the inhibitor from the reaction system. This method increased the substrate concentration from 12 to 60 g/L and the yield of (*S*)-PED by approximately five times from 4.9 g/L, on the premise that the enantiomeric excess (ee) value of (*S*)-PED remained above 96% and the reaction time was no more than 20 h. Moreover, the final space–time yield was over 1.2 g/L/h, which was higher than that reported from previous studies.

1. Introduction

Optically pure 1,2-diols are important precursors and intermediates for various synthetic applications. Among these compounds, optically pure 1-phenyl-1,2-ethanediol (PED) is a valuable and versatile chiral building block for the synthesis of pharmaceuticals, agrochemicals, pheromones and liquid crystals. Many efforts have already been devoted to the preparation of such compound using different methods. However, the chemical synthesis is quite complicated and often requires costly chemical reagents (Iwasaki et al., 1999; Hasegawa et al., 1990; Su et al., 2004). Therefore the bioconversion of the optically pure PED is more attractive.

Several bioconversion methods have already been reported. Some of these methods include the asymmetry resolution by lipase-catalyzed transesterification to produce (*S*)-PED (Bosetti et al., 1992), the selective oxidation of the racemate catalyzed by glycerin dehydrogenase to produce (*S*)-PED (Liese et al., 1996), the stereospecific dihydroxylation of styrene catalyzed by naphthalene dioxygenase to produce (*R*)-PED (Lee and Gibson, 1996), the asymmetric synthesis of (*R*)-PED from styrene oxide catalyzed by two enzymes mixture of the *Solanum tuberosum* and *Agrobacterium radiobacter* AD1 epoxide hydrolases (Cao et al., 2006), and the enantio-conversions catalyzed by the whole cell of the microbe. Whole cell-catalyzed conversions have been used in the production of optically pure PED to avoid the coenzyme addition or additional coenzyme regeneration system. For example, *Candida parapsilosis* IFO0708 (Hasegawa et al., 1990) and *Candida parapsilosis* CCTCC M203011 (Nie et al., 2004) could translate the (*R*)-PED in the racemate to the (*S*)-PED. *Bacillus* sp. ECU0015 catalyzes the hydrolysis of phenyl-1,2-ethanediol cyclic carbonates to produce (*S*)-PED (Chang et al., 2010).

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Some developments have been used in the bioconversion reactions to improve the efficiency and yield in the production of optically pure PED. For example, an aqueous two-phase system is used in the *C. parapsilosis* CCTCC M20301-catalyzed bioconversion to produce (*S*)-PED, which could increase the yield of (*S*)-PED to 27 g/L after 60 h (Xu et al., 2010), thus the space–time yield is 0.45 g/L/h. A "two-in-one" resin-based *in situ* product removal strategy is used to improve the production efficiency of (*S*)-PED in the *C. parapsilosis* CCTCC M20301-catalyzed bioconversion (Hu et al., 2010a). This method has increased the yield of (*S*)-PED to 46 g/L, which is the highest so far reported, but the whole reaction needs approximately 90 h, which leads a low space–time yield (0.51 g/L/h). Despite a lot of efforts have been devoted to the production of optically pure PED, the quicker and more effective bioconversions are still needed.

Gluconobacter oxydans (*G. oxydans*) is famous for its capacity to incompletely and specifically oxidize polyol substrates. Therefore, this microbe has been used to produce stereoselective or enantioselective chemicals, such as (keto-) D-gluconic acid, 6-(2-hydroxyethyl)-amino-6-deoxy-α-L-sorbofuranose, β-hydroxyisobutyric acid, and D-α-hydroxyl acid (Gupta et al., 2001; De Muynck et al., 2007; Deppenmeier et al., 2002; Herrmann and Sahm, 2005; Gao and Wei, 2006).

In our previous studies, the biooxidation of diols to the corresponding hydroxyl acids was determined by exploiting the membrane-bound alcohol dehydrogenase activity of whole cells of *G. oxydans*, which was very effective in the dehydrogenation of primary alcohols and diols (Wei et al., 2009a,b, 2010). It was reported that *G. oxydans* DSM2003 was able to oxidize 1, 2-propanediol D-(−)-lactic acid (Su et al., 2004). In addition, (*R*)-2-hydroxybutyric acid could be produced from 1,2-butanediol catalyzed by *G. oxydans* DSM2003 (Gao et al., 2012).

In the present study, the medium-free cells of *G. oxydans* were used to produce (*S*)-PED because they could specifically oxidize the (*R*)-PED to (*R*)-mandelic acid. The biocatalysis process was optimized and the adsorbent resins were introduced in the reaction system for the *in situ* separation of mandelic acid which engendered serious inhibition on the biocatalysts to enhance the final (*S*)-PED yield.

2. Methods

2.1. Chemicals

(*R*)/(*S*)-1-Phenyl-1,2-ethanodiol and (*R*)/(*S*)-mandelic acid as standard samples were purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA). (*R,S*)-PED as substrate was purchased from Yueyang Yetop Fine Chemical Co., Ltd. (Yueyang, China). The resins used were purchased from Shanghai Huazhen Science and Technology Co., Ltd. (Shanghai, China). Detailed properties of these resins were shown in Table 1. All other chemicals were of analytical grade and commercially available.

2.2. Microorganisms and culture conditions

The *G. oxydans* DSM 2003 strain used in this study was screened and preserved by our lab. The strain was grown in a liquid medium containing: 80 g/L sorbitol, 20 g/L yeast extract, 1 g/L KH_2PO_4, 0.5 g/L $MgSO_4{\cdot}7H_2O$, 0.1 g/L glutamine. All media were sterilized by autoclaving at 115 °C for 20 min. The seed cultures were inoculated from a sorbitol broth agar plate and incubated in an orbital shaker (200 rpm, 30 °C) until the late exponential growth phase (about 22 to 24 h). A 10% (v/v) inoculum of these cells was added to the liquid medium cultures and incubated at 30 °C for approximately 22 h to 24 h.

2.3. Biotransformation procedure

Unless stated otherwise, the reaction mixture without resins in a 10 mL system comprised 0.1 mol/L potassium phosphate buffer (pH 6.0), a certain concentration of (*R,S*)-PED, and 100 g/L wet cells; whereas the mixture with resins in a 10 mL system comprised deionized water, 100 g/L wet cells, a certain concentration of (*R,S*)-PED, and corresponding resins. The Erlenmeyer flasks (50 mL) with the reaction mixture were incubated in an orbital shaker (200 rpm, 30 °C) for a certain time. The pH of the reaction mixture was adjusted to 6.0 using the solution of NaOH and HCl according to the changes of the tested pH value of the reaction solution during the entire reaction process.

2.4. Effect of mandelic acid upon deracemization of (R,S)-PED

To determine the effect of the product (mandelic acid) on the bio-oxidation of (*R,S*)-PED catalyzed by *G. oxydans*, different amounts of mandelic acid ranging from 2 to 10 g/L were added to the reaction system, with a substrate concentration of 10 g/L. The conversion results were determined by high-performance liquid chromatography (HPLC).

2.5. Tolerance of cells to mandelic acid

To determine the tolerance of the biocatalyst to mandelic acid, fresh cells were suspended in potassium phosphate buffer containing different concentrations of mandelic acid at 30 °C. After certain period of contact, the cells were separated, washed, used to catalyze a new reaction in the reaction system containing 10 g/L PED and then incubated at 30 °C. The residual activities of the cells were determined through the initial oxidation rates.

2.6. Selection of the resin

The resins were pre-treated as the instructions. To select an appropriate resin, five types of resins were introduced and their adsorption behavior of mandelic acid was measured as follows: 10 mL solution containing 10 g/L mandelic acid was transferred into a 50 mL flask (pH was adjusted to 5.5–6.0). Subsequently, 1 g of resin was added into the flask and shaken at 200 rpm for 1 h at 30 °C. The concentration of residual mandelic acid was analyzed by HPLC.

Three types of resins with high adsorption capacity were chosen, and their adsorption rates were compared. Approximately 1 g of each resin was added to 10 mL of solution containing 20 g/L mandelic acid (pH was adjusted to 5.5–6.0). The concentration

Table 1
Properties of anion exchange resins.

Resins	Resin matrix	Alkalinity	Moisture (%)	Bulk density (g/mL)	Specific density (g/mL)	Particle size (mm)
D301	Styrene resin	Weak	50–60	0.65–0.75	1.03–1.07	0.315–1.25
D315	Acrylic resin	Weak	47–57	0.68–0.80	1.07–1.12	0.315–1.25
D345	Phenol-formaldehyde resin	Weak	45–55	0.65–0.80	1.15–1.25	0.315–1.20
711	Styrene resin	Strong	45–53	0.77–0.87	1.24–1.28	0.3–1.20
717	Styrene resin	Strong	45–53	0.77–0.87	1.24–1.28	0.3–1.20