# Efficient asymmetric hydrolysis of styrene oxide by Mung bean epoxide hydrolases in a supercritical CO$_2$/buffer biphasic system

Bin Wang\*, Qinting He, Li Shu, Fengli Yang, Guobin Liang, Weiqiao Liu

School of Chemistry & Environmental Engineering, Jiangsu University of Technology, Changzhou, 213001, China

A B S T R A C T

The asymmetric hydrolysis of styrene oxide (SO) by Mung bean epoxide hydrolases in a supercritical carbon dioxide (scCO$_2$)/buffer biphasic system was investigated. With the addition of 0.80 M sodium bicarbonate, the yield of (R)-1-phenyl-1,2-ethanediol ((R)-PED) improved from 20.8% to 47.1%. Compared to the case of the aqueous monophasic system, the enantiomer excess (*ee*) of (R)-PED improved from 81.8% to 91.5%. Under the optimized conditions for the enzymatic reaction, i.e., at a pressure, temperature, SO concentration, and water content of 12 MPa, 40 °C, 80 mM, and 6.0% (v/v), respectively, an (R)-PED yield of 49.2% and *ee* of 94.1% were achieved. As the water content had a significant effect on the (R)-PED yield and *ee*, a stepwise reaction method was adopted. When the initial water content was 8.0% (v/v) and 1% (v/v) water was added at intervals of 1 h, the (R)-PED yield and *ee* reached 49.1% and 97.2%, respectively.

## 1. Introduction

Enantiopure epoxides and vicinaldiols are versatile chiral building blocks used for the preparation of drugs, pesticides, and fine chemicals such as β-3-adrenergic receptor agonists, anti-obesity drugs, anticancer agents, and *N*-methyl-D-aspartate receptor antagonists [1,2]. (S)- or (R)-1-phenyl-1,2-ethanediol (PED) is a valuable and versatile chiral building block used for the synthesis of pharmaceuticals, agrochemicals, pheromones, liquid crystals, etc [3,4]. Thus, much attention has been devoted to the preparation of enantiopure PED through chemical or biological approaches. Among the biocatalytic routes used, asymmetric hydrolysis of racemic epoxides by epoxide hydrolases (EHs) is promising because highly enantiopure compoundss can be obtained with environmental safety and high economic benefits [5]. EHs have been found in many organisms such as plants, insects, bacteria, yeast, fungi, and animals [6–8]. Owing to its low cost and ready availability, the EH from Mung bean is regarded as a very attractive biocatalyst for synthesis, and it can efficiently catalyze the asymmetric hydrolysis of styrene oxide (SO) to (R)-PED in aqueous systems [9,10]. Two novel EHs from Mung bean were discovered for the first time, and they showed high enantioselectivity for the asymmetric hydrolysis of SO [11]. However, the poor solubility of the substrate and non-enzymatic hydrolysis in aqueous systems resulted in low enantiomeric purity and volumetric productivity of the desired chiral product. In order to overcome these limitations, biphasic systems were examined. In a

hexane/buffer biphasic system, SO and Mung bean EHs were dissolved in the organic solvent and buffer, respectively [12]. Although the hexane/buffer biphasic system inhibited the spontaneous hydrolysis of SO to some extent, hexane was toxic to the biocatalyst. To improve the EH activity in a biphasic system, an alternative solvent, an ionic liquid (IL), was utilized in place of the organic solvent. The C$_4$MIM•PF$_6$/buffer biphasic system not only dissolved the substrate in high concentrations but also effectively inhibited non-enzymatic hydrolysis [13]. However, the high cost of ILs restricts its large-scale application.

In recent years, supercritical CO$_2$ (scCO$_2$) has become more attractive because it is readily available, inexpensive, nontoxic, and environmentally benign and facilitates post-reaction separation [14,15]; thus, scCO$_2$ is a suitable reaction medium for enzymatic reactions. However, a disadvantage of the scCO$_2$/buffer biphasic system is the generation of carbonic acid in the aqueous phase, which leads to a decrease in pH [16]. This low pH is an important drawback because most enzymes are only active within a narrow range of pH values, which are generally close to neutral. To overcome this limitation, an additive was used with the scCO$_2$ system; sodium bicarbonate proved to be efficient in controlling the pH within a narrow range [17].

In the present study, Mung bean EHs were efficiently utilized in the asymmetric hydrolysis of SO to (R)-PED in an scCO$_2$/buffer biphasic system for the first time. The optimum conditions for this biocatalytic reaction were determined, and the stepwise reaction method was investigated to increase the yield and *ee* of the product.

---

\* Corresponding author.
  *E-mail address:* wangbin@jsut.edu.cn (B. Wang).
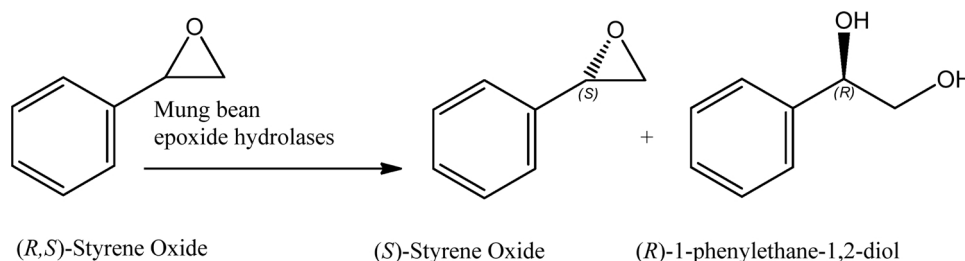
Scheme 1. Asymmetric hydrolysis of (R,S)-styrene oxide.

## 2. Experimental

### 2.1. Biological and chemical materials

Mung beans were purchased from a local supermarket. (R,S)-Styrene oxide (98% purity) and (R)- and (S)-1-phenyl-1,2-ethanediol (98% purity) were obtained from Meryer Chemical Technology Co., Ltd., China. Carbon dioxide was supplied as compressed gas (99.99%). All other chemicals were purchased from Sinopharm Chemical Reagent Co., Ltd., China and were of analytical grade.

### 2.2. Partial purification of EHs from Mung bean

Partial purification of EHs from Mung bean was performed according to previously reported methods [13]. After being soaked fully in distilled water (1 L), Mung beans (100 g) were dehusked and ground to a fine paste, and 500 mL Tris-HCl buffer (50 mM, pH 7.4) containing 1 mM EDTA was added to this paste. The mixture was stirred at 0 °C for 1 h and subsequently centrifuged (10,000 rpm) for 15 min at 4 °C, after which the sediment was discarded. $(NH_4)_2SO_4$ (272 g) was slowly added to the supernatant at 4 °C; after 30 min, the mixture was centrifuged (10,000 rpm) for 15 min at 4 °C, and the sediment was discarded. $(NH_4)_2SO_4$ (511 g) was slowly added to the supernatant; after 30 min, the mixture was centrifuged (10,000 rpm) for 15 min at 4 °C, and the yellow sediment was collected as crude EH. The solid yellow pellet was dissolved in 50 mL fresh Tris–HCl buffer (100 mM, pH 7.0) and partially purified via ultrafiltration. The protein, with a molecular weight of 20–50 kDa, was collected and further lyophilized to obtain a pale yellow powder.

### 2.3. Assay of Mung bean EH activity

The EH powder was added to 2 mL phosphate buffer (100 mM, pH 7.0) containing SO (10 mM). The reaction was incubated at 35 °C and 220 rpm for 10 min and 4 mL n-hexane was then added to extract the residual substrate. The liberated PED was analyzed by high-performance liquid chromatography (HPLC). One unit of enzyme activity is defined as the amount of enzyme that produced 1 μmol of PED per min under the above conditions. The activity of the enzyme powder was 172.3 U/g.

### 2.4. Analytical methods

The conversion of SO was detected by an HPLC equipped with a ZORBAX Eclipse XDB-C 18 (4.6 mm × 250 mm, 5-μm silica gel) column and ultraviolet (UV) detector set at 220 nm. The mobile phase was composed of water/methanol (70/30, %v/v) with a flow rate of 0.5 mL/min at 30 °C. The enantiomeric excess (ee) of the product was assayed using an HPLC equipped with a Chiralpack IB (Daicel, 4.6 mm × 250 mm) column and UV detector set at 220 nm. The mobile phase was composed of n-hexane/isopropanol (95/5, %v/v) with a flow rate of 0.4 mL/min at 30 °C.

The ee values for (R)-PED and (S)-SO were calculated as $ee_P = \frac{c_{PR} - c_{PS}}{c_{PR} + c_{PS}} \times 100\%$ and $ee_S = \frac{c_S - c_R}{c_S + c_R} \times 100\%$ respectively. The

conversion of c was calculated according to the changes in the number of substrates. $c_{PR}$ and $c_{PS}$ are the concentration s of (R)- and (S)-PED, and $c_R$ and $c_S$ are the concentration of (R)- and (S)-SO, respectively. The enantioselectivity was determined using the following equation: $E = \frac{\ln[1-c(1+ee_p)]}{\ln[1-c(1-ee_p)]}$.

### 2.5. General procedure for the asymmetric hydrolysis of SO using Mung bean EHs

Asymmetric hydrolysis was carried out in a 100-mL high-pressure reactor. In a typical experiment, 5%–18% (v/v) Tris-HCl buffer (100 mM, pH 7.0), 100 mg EHs powder, 0.4–1.0 M sodium bicarbonate (based on the volume of the buffer), and 10–120 mM (R,S)-SO (based on the total volume of the reactor) were added. Subsequently, $CO_2$ was injected to achieve the desired pressure (8.0–16.0 MPa). The reactor was kept at 31–50 °C and stirred at 200 rpm. Aliquots (20 μl) were withdrawn at various intervals from the $scCO_2$ and aqueous phases using a high-pressure sampler, and $scCO_2$ was supplied to regain the original pressure, which decreased slightly after sampling. In the stepwise reaction, 3% (v/v) buffer, 30 mg EH, and 9.6 g (R,S)-SO were added to the reactor, and 1% (v/v) buffer was then added at intervals of 1 h to the total volume of 6% (v/v) during hydrolysis.

## 3. Results and discussion

### 3.1. Asymmetric hydrolysis of SO by Mung bean EHs

Initially, the EH powder was evaluated for asymmetric hydrolysis of SO in the buffer system (Scheme 1) and 49.1% yield and 81.8% ee were obtained (Table 1). However, when the substrate was transferred from the buffer solution to the $scCO_2$/buffer biphasic system, only 20.8% yield was obtained. This indicates that the EHs were partially denatured in the $scCO_2$/buffer biphasic system due to the formation of carbonic acid and lower pH [16]. Although the activity of the EHs was lost in the $scCO_2$/buffer system, the ee value of the product improved to 90.8%, which indicates that the biphasic system inhibited the non-enzymatic

**Table 1**
Asymmetric hydrolysis of SO by Mung bean EHs.

| Solvents | Additives[c] | $ee_s$ (%) | $ee_p$ (%) | E | Yield (%) |
|---|---|---|---|---|---|
| Buffer[a] | No | 96.1 | 81.8 | 38.9 | 49.1 |
| ScCO₂/buffer[b] | No | 25.3 | 90.8 | 26.6 | 20.8 |
| ScCO₂/buffer | 0.4 M | 57.1 | 91.2 | 38.5 | 36.8 |
| ScCO₂/buffer | 0.6 M | 81.5 | 91.5 | 56.9 | 45.1 |
| ScCO₂/buffer | 0.8 M | 88.6 | 91.5 | 66.4 | 47.1 |
| ScCO₂/buffer | 0.9 M | 88.9 | 91.1 | 62.5 | 47.0 |
| ScCO₂/buffer | 1.0 M | 88.2 | 90.7 | 59.7 | 47.0 |

[a] Conditions: 10 mM SO, 100 mg EHs, 100 mL buffer (pH 7.0, 100 mM), 35 °C, 3 h.
[b] Conditions: 10 mM SO, 100 mg EHs, 12 mL buffer (pH 7.0, 100 mM), 9 MPa, 35 °C, 3 h.
[c] Additives was sodium bicarbonate, the concentrations of sodium bicarbonate were calculated according to the volume of buffer.