Enantioselective hydrolysis of epichlorohydrin using whole *Aspergillus niger* ZJB-09173 cells in organic solvents

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The enantioselective hydrolysis of racemic epichlorohydrin for the production of enantiopure (*S*)-epichlorohydrin using whole cells of *Aspergillus niger* ZJB-09173 in organic solvents was investigated. Cyclohexane was used as the reaction medium based on the excellent enantioselectivity of epoxide hydrolase from *A. niger* ZJB-09173 in cyclohexane. However, cyclohexane had a negative effect on the stability of epoxide hydrolase from *A. niger* ZJB-09173. In the cyclohexane medium, substrate inhibition, rather than product inhibition of catalysis, was observed in the hydrolysis of racemic epichlorohydrin using *A. niger* ZJB-09173. The racemic epichlorohydrin concentration was markedly increased by continuous feeding of substrate without significant decline of the yield. Ultimately, 18.5% of (*S*)-epichlorohydrin with 98% enantiomeric excess from 153.6 mM of racemic epichlorohydrin was obtained by the dry cells of *A. niger* ZJB-09173, which was the highest substrate concentration in the production of enantiopure (*S*)-epichlorohydrin by epoxide hydrolases using an organic solvent medium among the known reports.

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1. Introduction

Enantiopure epichlorohydrin is a versatile, reactivity compound and valuable chiral synthon that can easily react with various compounds such as nucleophiles, eletrophiles and acids (Kasai *et al.* 1998) and can be used to prepare optically active pharmaceuticals (Kasai *et al.* 1992). The chemical synthesis of epichlorohydrin enantiomers requires expensive heavy metals as catalysts, resulting in high production costs and serious environmental pollution (Larrow *et al.* 2003). Furthermore, its requirement for sophisticated equipment also leads to high production costs and more difficult operations (Kim and Choi 2004; Kim *et al.* 2007). Biotechnological routes have been considered for the synthesis of epichlorohydrins using haloalcohol dehalogenase (Assis *et al.* 1998; Spelberg *et al.* 2004) or epoxide hydrolase (Spelberg *et al.* 2002; Thompson and Hammock, 2007). However, the synthesis of chiral epichlorohydrin using haloalcohol dehalogenase requires a nucleophile, limiting its appication (Archelas and Furstoss 2001).

Epoxide hydrolases, which catalyse the conversion of epoxides into their corresponding diols through the addition of a water molecule, have been found in animals (Meijer and Depierre 1988), plants (Blee and Schuber 1992) and microorganisms (Hechtberger *et al.* 1993; Jacobs *et al.* 1991; Liu *et al.* 2011). Epoxide hydrolases from microorganisms, especially those from *Aspergillus niger*, have drawn considerable attention and been widely used in the preparation of chiral epoxides because of their high enantioselectivity (Morisseau *et al.* 1997; Nellaiah *et al.* 1996). The hydrolysis of epoxides catalysed by epoxide hydrolases usually performed in aqueous reaction media (Furstoss *et al.* 2004; Manoj *et al.* 2001; Morisseau *et al.* 1999). However, for some epoxides such as epichlorohydrin, the enzymatic hydrolysis in aqueous system exhibits low enantioselectivity because of spontaneous, non-enantioselective hydrolysis of

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the substrates (Choi *et al.* 1998). To address this issue, organic solvents were used as the reaction media for increasing the enantioselectivity (Choi *et al.* 1999; Lee 2007). Therefore, the enantioselective hydrolysis of racemic epichlorohydrin in organic solvents, using epoxide hydrolase as the biocatalyst, is a potential method of preparing the chiral epichlorohydrin.

Hydrolysis of epoxides using epoxide hydrolase in an organic solvent medium is different from that in an aqueous medium. Organic solvents significantly affect the enzyme activity, enzyme stability and enantioselectivity (Miroliaei and Nemat-Gorgani 2002; Karboune *et al.* 2006; Lee 2007). In addition, substrate and product inhibition, which are common phenomena in the hydrolysis of epoxides by epoxide hydrolases (Jones *et al.* 2005; Kotik and Kyslik 2006), may also be different in organic solvents (Baldascini and Janssen 2005). Recently, Choi (Choi *et al.* 1999) reported on the possible occurrence of significant substrate or product inhibition in hydrolysis of epichlorohydrin by epoxide hydrolase from *A. niger*, which resulted in the low enantiomeric excess (*ee*) at high substrate concentration. So far, the initial concentration of epichlorohydrin was very low in the production of enantiopure (*S*)-epichlorohydrin by epoxide hydrolases using an organic solvent medium.

In this study, we identified the optimal organic solvent for hydrolysis of the racemic epichlorohydrin using *A. niger* ZJB-09173 and investigated the effects of the organic solvent on enzyme stability. Moreover, substrate and product inhibition for the enantioselective hydrolysis of racemic epichlorohydrin were investigated in detail, as well as the possibility of increasing the epichlorohydrin concentration by reducing such inhibition.

2. Materials and methods

2.1 *Materials*

Racemic epichlorohydrin and 1,3-dichloro-2-propanol were purchased from Huadong Medicine Co. (Hangzhou, China). (*S*)- and (*R*)-epichlorohydrins were obtained from Ya Wang Kang-Li Co., Ltd. (Shenzhen, China). The free fatty acid phase (FFAP) capillary column was obtained from the Lanzhou Institute of Chemical Physics (Lanzhou, China), and the Chiraldex BGB-175 capillary column was from BGB Analytic AG Co. (Shanghai, China). All other chemicals were obtained from commercial sources and are of reagent grade.

2.2 *Microorganism and culture conditions*

A. niger ZJB-09173, a strain that has high activity of epoxide hydrolase, was maintained at 4°C on potato dextrose agar (PDA) slants, and monthly transferred. The microorganism was cultured in a 500 mL flask containing 100 mL liquid medium (prepared using 18 gL^{-1} glucose, 10 gL^{-1} soybean meal, 0.4 gL^{-1} KH_2PO_4, 0.8 gL^{-1} K_2HPO_4 and 0.2 gL^{-1} $MgSO_4$) and agitated at 150 rpm at 30°C. After 4 days of culturing, the mycelia were harvested via vacuum filtration.

2.3 *Enzyme assay*

For the hydrolysis of racemic epichlorohydrin, 1 g of *A. niger* ZJB-09173 wet pellets (100 g wet pellets correspond to 18 g dry mass) and 50 μL of racemic epichlorohydrin were added into 10 mL medium. The mixture was incubated at 150 rpm in a thermostatic bath at 30°C for 10 min. A 0.5 mL sample was obtained, and the amount of residual epichlorohydrin was quantified through gas chromatography (GC). One unit of epoxide hydrolase activity was defined as the amount of enzyme needed to catalyse 1 μmol of racemic epichlorohydrin into its corresponding diol per minute.

2.4 *Enantioselectivity of the enzymatic hydrolysis in organic solvents*

Eight organic solvents (*n*-heptane, dimethylophthalate, methol, iso-butanol, ehyl acetate, dichloromethane, cyclohexane and *n*-hexane) were investigated to determine the optimal medium for the enantioselective hydrolysis of epichlorohydrin. The reaction was initiated by adding 1 g of *A. niger* ZJB-09173 wet pellets into 10 mL of each organic solvent containing 64 mM racemic epichlorohydrin. Approximately 0.5 mL of the reaction mixture was obtained after 10 min and 3 h for the determination of enzyme activity and *ee*, respectively.

2.5 *Enzyme stability in the organic solvent*

Exactly 10 mL of the optimal organic solvent (determined in the preceding section) and 1 g wet pellets were combined in a 50 mL conical flask. The mixture was then incubated at 30°C for different times without shaking. Racemic epichlorohydrin (50 μL) was then added into the mixture, and the reaction was sustained for 10 min at 150 rpm. 0.5 mL reaction mixture was then obtained for the determination of enzyme activity, as previously described. The control experiment was conducted in 0.1 M sodium phosphate buffer (pH 8.0).

2.6 *Effect of substrate concentration on the hydrolysis of epichlorohydrin*

The experiment was performed at 30°C in 10 mL reaction mixtures containing 1 g of wet pellets and different amounts

of racemic epichlorohydrin (6.4, 12.8, 25.6, 38.4, 51.2, 64.0, 76.8, 89.6, 102.4 and 128 mM). Approximately 0.5 mL reaction mixture was then removed after 10 min for the determination of the reaction rate through GC analysis and the *ee* of residual (*S*)-epichlorohydrin was estimated every 1 h until it reached the maximum. In addition, the initial reaction rates on the (*S*)- and (*R*)-epichlorohydrin were also tested at different concentrations of optical pure (*S*)- and (*R*)-epichlorohydrin, respectively.

2.7 *Effect of product concentration on the hydrolysis of epichlorohydrin*

The reaction was initiated by adding 1 g wet pellets into 10 mL reaction systems containing 64 mM racemic epichlorohydrin and different concentrations of 3-chloro-1,2-propanediol (12.8 mM to 128 mM). The reaction mixtures were then incubated at 30°C. The initial reaction rate was measured after 10 min and the *ee* of residual (*S*)-epichlorohydrin was estimated every 1 h until it reached the maximum.

2.8 *Production of (S)-epichlorohydrin with the wet cells by re-adding substrate*

The reaction was started by the addition of 2.5 mL epichlorohydrin into 500 mL reaction system containing 50 g wet pellets. Then the experiments were performed as follows: (A) 0.5 mL of epichlorohydrin was added to the reaction mixture every 30 min during the course of the reaction. At 2.5 h of reaction time, the final epichlorohydrin concentration reached 128 mM and the re-addition of epichlorohydrin was stopped. (B) The epichlorohydrin was re-added continuously with the rate of 0.02 mL/min by peristaltic pump until the final epichlorohydrin concentration reached 128 mM at 125 min of reaction time. As the initial total volume of reaction system was 500 mL, the added volume of epichlorohydrin was neglible. The concentrations of each enantiomer of epichlorohydrin were determined at different reaction times through GC analysis.

2.9 *Determination of the optimal water content*

The wet pellets of *A. niger* ZJB-09173 were dried in a vacuum freeze dryer for 2 days. The dry powder (350 mg) was suspended in 50 mL screw-cup bottles containing 10 ml of organic solvent. Various water contents from 0 to 5% (v/v) were added and the reaction was started when 50 μL of the racemic epichlorohydrin was added. The enzyme activity and *ee* were determined as described above.

2.10 *Production of (S)-epichlorohydrin with the dry powder by continuous feeding of substrate*

17.5 g dry powders of *A. niger* ZJB-09173 were suspended in 500 mL reaction system. The reaction was started by adding 2.5 mL of epichlorohydrin and the optimal water content (determined in the preceding section). The epichlorohydrin was re-added continuously with the rate of 0.02 mL/min by peristaltic pump until the final epichlorohydrin concentration reached 153.6 mM at 175 min of reaction time. The concentrations of each enantiomer of epichlorohydrin were determined at different reaction times through GC analysis.

2.11 *Analytical methods*

The wet mycelium was dried at 100°C until a constant weight was obtained, and the dry mass was measured. The *ee* value of the residual epichlorohydrin was calculated from the ratio of the concentrations of the *R* and *S* enantiomers of epichlorohydrin using the following formulas: $ee = 100 \times (S - R)/(S + R)$. The yield of optically pure epichlorohydrin was calculated using the formula, $\text{yield} = 100 \times S/(S_0 + R_0)$, where S_0 and R_0 denote the initial (*S*)- and (*R*)-epichlorohydrin concentrations, respectively. Quantitative analysis of epichlorohydrin concentration was performed using the external standard method. After water was removed from the reaction mixture using anhydrous sodium sulphate, a 1 μL reaction mixture was obtained and analysed using a GC system with a chiral capillary BGB-175 column (0.25 mm ID×30 m) fitted with a flame ionisation detector. The temperatures of the column, injector and detector were 90, 220 and 220°C, respectively.

3. Results and discussion

3.1 *Screening of organic solvents*

Epichlorohydrin can be spontaneously hydrolysed in aqueous buffers without enantioselectivity, resulting in low *ee* and recovery yield (Choi *et al.* 1998). Similar results were obtained in our previous study, wherein the yield of enantiopure (*S*)-epichlorohydrin in 100 mM phosphate buffer (pH 8.0) by the *A. niger* ZJB-09173 cell suspension was less than 10% (data not shown). An organic solvent was therefore used as the medium, in order to prevent the chemical hydrolysis.

The effects of eight organic solvents with different hydrophilic or hydrophobic properties on the enantioselective hydrolysis of racemic epichlorohydrin were investigated (figure 1). The epoxide hydrolase from *A. niger* ZJB-09173

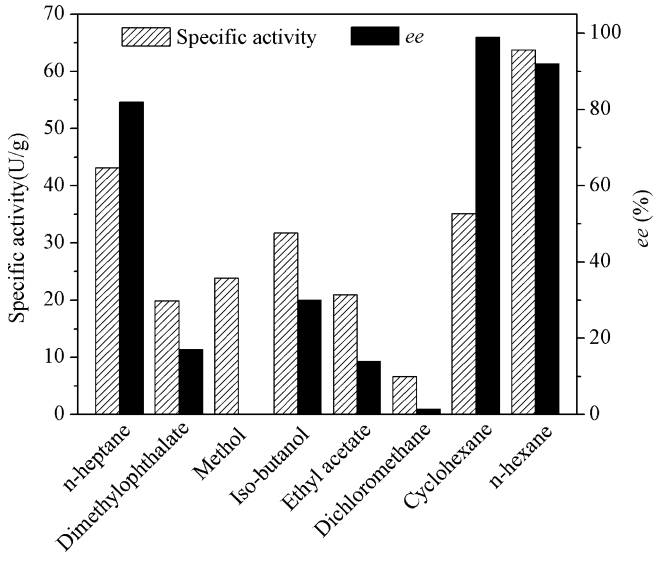

Figure 1. Effect of various organic solvents on specific activity and *ee* in the enantioselective hydrolysis of racemic epichlorohydrin using *A. niger* ZJB-09173 cells.

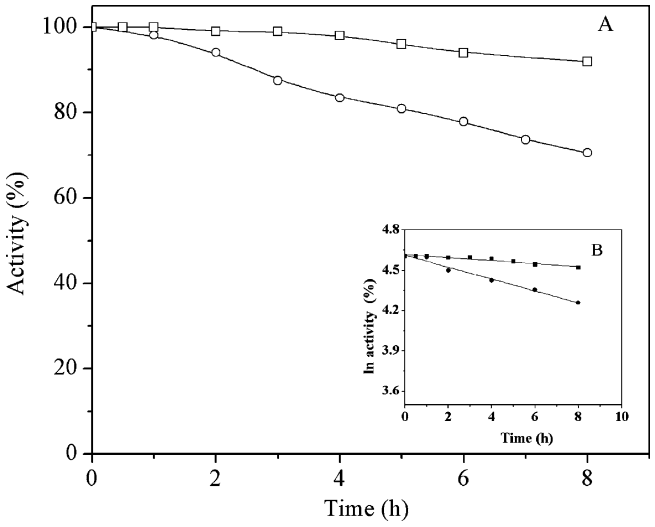

Figure 2. Stability of epoxide hydrolase from *A. niger* ZJB-09173 in the cyclohexane (●, ○) and sodium phosphate buffer (□, ■) reaction media at 30°C.

exhibited high activity and *ee* in hydrophobic solvents such as heptane, cyclohexane and *n*-hexane, but its activity was not directly proportional to the degree of hydrophobicity (*n*-heptane>*n*-hexane>cyclohexane) of the solvents. In addition, the *ee* value when cyclohexane was used as the medium was 99%, while 82% and 92% was obtained in *n*-heptane and *n*-hexane, respectively. As the epoxide hydrolase from *A. niger* ZJB-09173 showed the highest enantioselectivity in cyclohexane, cyclohexane was selected as the optimal reaction medium.

3.2 *Effect of cyclohexane on the enzyme stability*

The organic medium significantly affects the enzyme activity and stability (Karboune *et al.* 2006; Miroliaei and Nemat-Gorgani, 2002). The enzyme activity of epoxide hydrolase from *A. niger* ZJB-09173 in cyclohexane was lower than that in an aqueous system. Thus, the stability of this enzyme in cyclohexane was investigated (figure 2).

Figure 2 shows the significant effect of cyclohexane on epoxide hydrolase stability. Nearly 30% of the enzyme activity in cyclohexane was lost after 8 h incubation at 30°C, whereas only 8% was lost in sodium phosphate buffer (pH 8.0). The half-life values of the enzyme in sodium phosphate buffer and cyclohexane, which were calculated using the plot of ln of the remaining activity versus the incubation time (figure 2B), were 63 and 16 h, respectively. The rate of enzyme inactivation in cyclohexane was nearly three times faster than that in sodium phosphate buffer at 30°C. Several enzymes are much more thermostable in pure organic media than in water because of the increase in their conformational rigidity in organic media (Tsitsimpikou *et al.* 1994; Volkin *et al.* 1991), but the stability of enzymes in a two-liquid-phase system will significantly decrease due to molecular toxicity and interfacial toxicity (Baldascini and Janssen, 2005). In the current study, a possible reason for the decrease in stability of epoxide hydrolase from *A. niger* ZJB09173 in cyclohexane is the toxicity of cyclohexane on cells.

3.3 *Effect of substrate on the hydrolysis of epichlorohydrin*

As far as industrial applicability is concerned, one major restriction for enzymatic reaction may be the low substrate concentration. The influence of substrate concentration on the reaction is mainly reflected in the reaction rate and enantioselectivity. In this experiment, 10 different substrate concentrations, ranging from 6.4 to 128 mM, were investigated (figure 3). The initial hydrolysis rate of racemic epichlorohydrin increased with increasing of substrate concentration, and then started decreasing at substrate concentrations above 75 mM or so, indicating the occurrence of substrate inhibition.

It can be seen that in addition to starting the substrate inhibition, there was a loss of the high enantioselectivity. At 128 mM, the value of *ee* had decreased from 98% to less than 70%, indicating that high concentration of substrate may result in decline of *ee* value (Choi *et al.* 1999). The yield of (*S*)-epichlorohydrin was 16.9%, 17.3%, 17.5% and 17.2% at the substrate concentrations of 25.6, 51.2, 64 and 76.8 mM (data not shown), respectively. In order to explore it further, the enzyme activity on the optical pure (*R*)- and (*S*)-epichlorohydrin was investigated (figure 4). The V_{max} and K_m

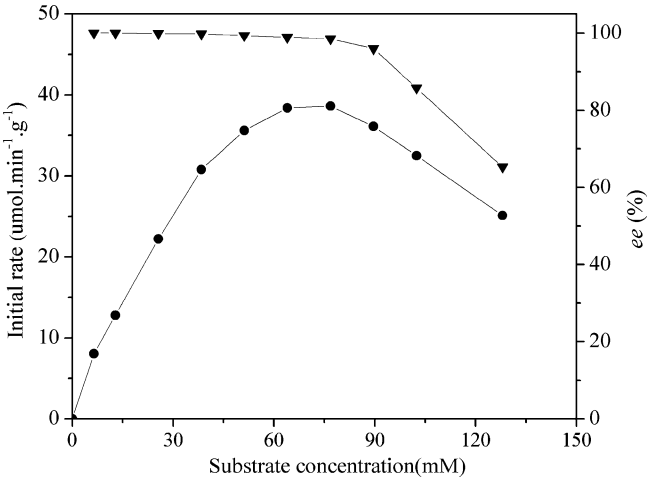

Figure 3. Effect of substrate concentration on the hydrolysis of racemic epichlorohydrin in the cyclohexane reaction medium. Symbols: initial reaction rate (●), *ee* values of (*S*)-epichlorohydrin (▼).

values for the (*R*)- and (*S*)-epichlorohydrin, determined using the Lineweaver-Burk plot, were 57.8 μmol min^{-1} g^{-1} and 18.4 mM, and 36.2 μmol min^{-1} g^{-1} and 37.0 mM, respectively (data not shown). This result demonstrated that the epoxide hydrolase from *A. niger* ZJB-09173 can also hydrolyse the *S* enantiomer, although the hydrolysis of the *R* enantiomer preferentially occurred due to the higher V_{max} and lower K_m. Therefore, after a concentration threshold, the *S* enantiomer probably started to be an inhibitor of the hydrolysis of the *R* enantiomer, and as the *S* enantiomer was also hydrolysed, there was a decrease of the *ee* value of (*S*)-epichlorohydrin.

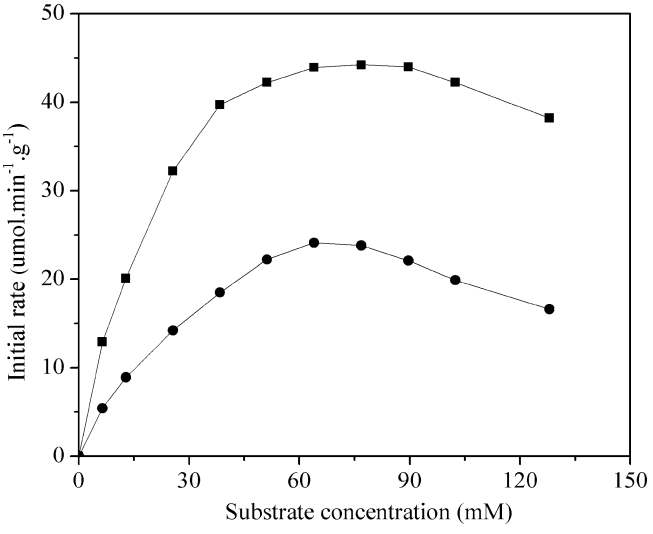

Figure 4. Effect of substrate concentration using (*R*)- (■) or (*S*)-epichlorohydrin (●) on the enzyme activity.

3.4 *Product concentration on hydrolysis of epichlorohydrin*

In the current study, the effect of 3-chloro-1,2-propanediol on the hydrolysis of racemic epichlorohydrin was also investigated to establish whether there was product inhibition. Figure 5 shows that the product concentration had no significant negative effect on the initial reaction rate and *ee*. However, the slight increase in the initial reaction rate when the 3-chloro-1,2-propanediol concentration was 102.4 and 128 mM remains to be explained.

Based on the results above, substrate inhibition, instead of product inhibition, occurred in the hydrolysis of racemic epichlorohydrin by *A. niger* ZJB-09173 in cyclohexane. Although Choi (Choi *et al.* 1999) had reported on the significant inhibition in the hydrolysis of racemic epichlorohydrin by *A. niger* epoxide hydrolase in cyclohexane, he did not determine whether the inhibition was attributed to the substrate or product concentration. Woo (Woo *et al.* 2010) reported the absence of substrate inhibition in the synthesis of (*S*)-epichlorohydrin using epoxide hydrolase from *Novosphingobium aromaticivorans* until the concentration of racemic epichlorohydrin reached 500 mM. However, the reaction occurred in the aqueous system, so that the yield of (*S*)-epichlorohydrin decreased to only 11.9% with substrate concentration increasing to 500 mM.

3.5 *Production of chiral epichlorohydrin with wet cells by re-adding substrate*

In the previous sections, the occurrence of decline of enantioselectivity at high substrate concentrations was shown. Therefore, re-addition of substrate was performed to explore

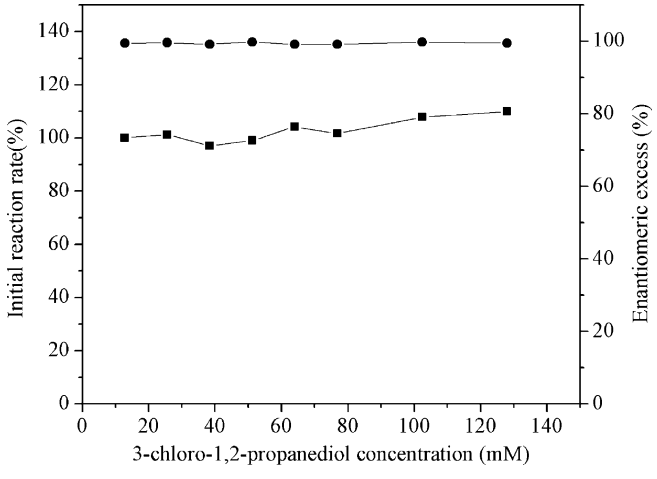

Figure 5. Effect of product concentration on the initial reaction rate (■) and *ee* (●) in the hydrolysis of epichlorohydrin by *A. niger* ZJB-09173 cells.

the possibility of increasing the enantioselectivity at high substrate concentrations.

In experiment A, 0.5 mL (6.4 mmol) of epichlorohydrin was re-added intermittently into the reaction mixture every 30 min (figure 6A). As the specificity of the epoxide hydrolase from *A. niger* ZJB-09173 was not absolute, both *S* and *R* enantiomers of epichlorohydrin were hydrolysed. The hydrolysis of (*S*)-epichlorohydrin was slower than (*R*)-epichlorohydrin due to the higher K_m and lower V_{max} of the (*S*)-epichlorohydrin compared to that of the (*R*)-epichlorohydrin. Therefore, the concentration of (*S*)-epichlorohydrin increased to 43.1 mM but the concentration of (*R*)-epichlorohydrin was only 30.4 mM when the re-addition of substrate was stopped at 2.5 h of reaction time. After 11 h, >98% *ee* value of residual (*S*)-epichlorohydrin from 128 mM racemic epichlorohydrin was obtained, with a yield 12.8%. In experiment B, the racemic epichlorohydrin was added with the rate of 0.02 ml/min (0.256 mmol) via continuous feeding once the reaction was started (figure 6B). Therefore, the concentrations of both (*R*)- and (*S*)-epichlorohydrin in the process of re-addition of substrate were maintained ralative higher than that in experiment A. Continuous feeding appeared to be better than discontinuous, because the final concentration of (*S*)-epichlorohydrin was higher, which means a higher yield.

Table 1. Effect of water content on the enantioselective hydrolysis of epichlorohydrin by the dry powder of *A. niger* ZJB-09173 in cyclohexane

Water content (%,v/v)	Enzyme activity (U/g)	*ee* (%)	Yield (%)
0	0	0	49.5
0.5	10.1	26.5	43.2
1	10.9	60.7	33.9
2	13.5	87.7	22.9
3	16.2	94.6	20.9
4	19.1	>98	19.6
5	24.0	>98	18.5

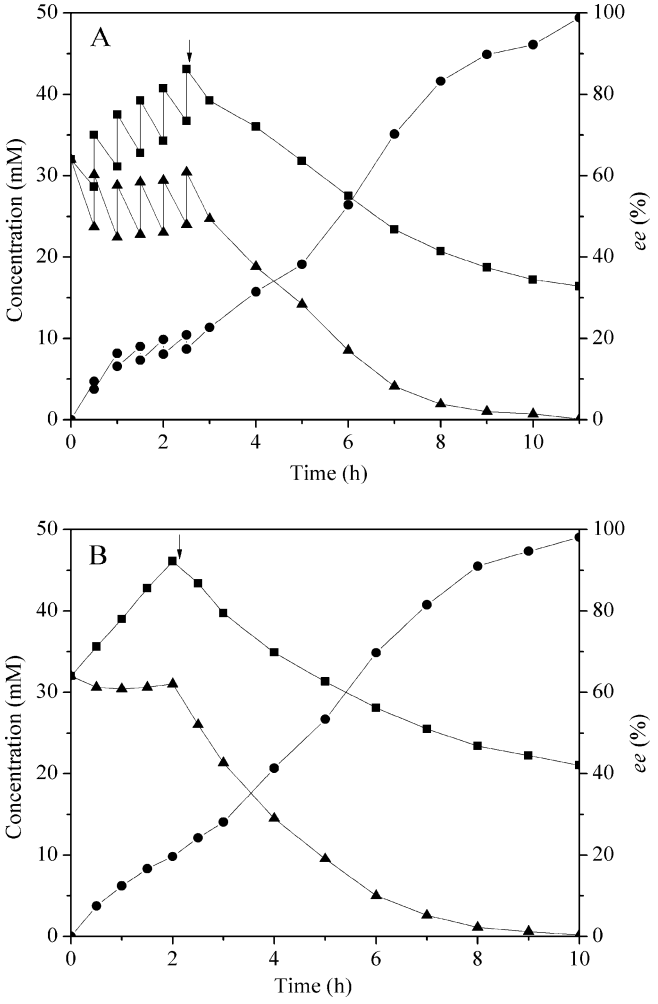

Figure 6. Production of chiral epichlorohydrin with the wet cells of *A. niger* ZJB-09173 by re-adding 0.5 mL epichlorohydrin every 30 min intermittently (Fig.6A) and 0.02 ml epichlorohydrin every minute continuously (Fig.6B). The arrows in the graphs represented that the addition of substrate was stopped at this reaction time. Symbols: (■), concentration of (*S*)-epichlorohydrin; (▲), concentration of (*R*)-epichlorohydrin; (●), *ee* (%).

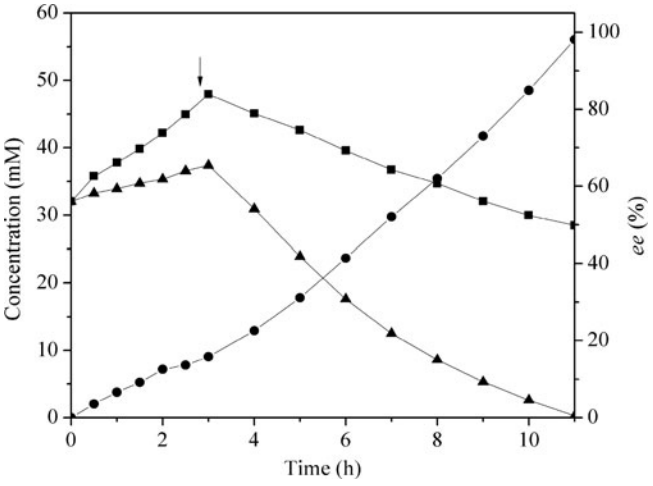

Figure 7. Production of chiral epichlorohydrin with the dry cells of *A. niger* ZJB-09173 by continuous feeding of substrate and water in cyclohexane. The arrow in the graph represented that the addition of substrate was stopped at this reaction time. Symbols: (■), concentration of (*S*)-epichlorohydrin; (▲), concentration of (*R*)-epichlorohydrin; (●), *ee* (%). The initial concentration of epichlorohydrin and water content were 64 mM and 4% (v/v), respectively. 20 µL epichlorohydrin was added every minute by continuous feeding until the final concentration of epichlorohydrin was 153.6 mM at the 175 min of reaction time.

These results demonstrated that the enantioselectivity in the hydrolysis of racemic epichlorohydrin at high substrate concentration using *A. niger* ZJB-09173 can be significantly increased by the re-addition of the substrate. Furthermore, a 16.4% yield of enantiopure (*S*)-epichlorohydrin with *ee*> 98% was obtained from 128 mM racemic epichlorohydrin, which was almost the same as that obtained in the batch resolution at the 64 mM initial epichlorohydrin. Compared with the hydrolysis of racemic epichlorohydrin in an aqueous medium (Woo *et al.* 2010), the yield of optical pure epichlorohydrin in the cyclohexane did not significantly decrease with the increasing of substrate concentration. However, the wet cells tended to get together into lumps with the prolongation of the reaction time in the cyclohexane medium, resulting in decline of the reaction rate.

3.6 *Production of chiral epichlorohydrin with the dry cells by re-adding substrate*

The experiments with wet cells had the inconvenience that although water was necessary as it was a reactant, an excess water content in the organic solvent medium had several negative effects on the reaction including (a) accelarating the spontaneous hydrolysis of epichlorohydrin and (b) making the cell together into lumps. Therefore, the dry cell powder was chosen as catalyst in this section and the optimal water content in the reaction system at 64 mM racemic epichlorohydrin was investigated.

As shown in table 1, the reaction rate and *ee* increased with the increasing of water content. The *ee* reached the maximum at 4% of water content, and more water content would result in reducing of the yield. The yield of (*S*)-epichlorohydrin increased to 19.6% at 4% of water content from 17.5%, which was obtained using the wet cells (equivalent with 8% (v/v) of water content in the reaction system).

Based on the above results, the hydrolysis of epichlorohydrin with the dry cell powder by continuous feeding of substrate was also investigated (figure 7). 3.5 mL (44.8 mmol) of racemic epichlorohydrin was re-added with the flow rate 0.02 mL/min within the first 175 min of reaction. The hydrolysis rate after 8 h by the dry cell powder, compared with the initial rate, was maintained ralative higher than that by the wet cells. A possible explanation of this result was that the dry cells tended to disperse more uniform in the organic medium due to the less water content. After 11 h of reaction, >98% *ee* of (*S*)-epichlorohydrin was obtained with the yield of 18.5% from 153.6 mM racemic epichlorohydrin. Compared with the hydrolysis of epichlorohydrin by the wet cells of *A. niger* ZJB-09173 in cyclohexane, the epichlorohydrin concentration and the yield of (*S*)-epichlorohydrin were both increased by using the dry cell powder as catalyst. This result was probably attributed to the higher enzyme activity and less spontaneous hydrolysis of epichlorohydrin due to less water content. Furthermore, 153.6 mM was the highest substrate concentration in the production of enantiopure (*S*)-epichlorohydrin by epoxide hydrolases using an organic solvent medium among the known reports. We have succeed in providing a strategy to improve the epichlorohydrin concentration in the organic media: relative to those enantioselective hydrolysis of epichlorohydrin by epoxide hydrolases (Choi *et al.* 1999; Lee, 2007), the epichlorohydrin concentration and overall productivity were clearly enhanced.

4. Conclusions

In this work, cyclohexane was chosen as the reaction medium as the epoxide hydrolase from *A. niger* ZJB-09173 exhibited the highest enantioselectivity in cyclohexane. However, cyclohexane had a negative effect on the stability of epoxide hydrolase from *A. niger* ZJB-09173. Both enzyme activity and *ee* value of (*S*)-epichlorohydrin were decreased at high substrate concentration. The *ee* of the residual (*S*)-epichlorohydrin can be increased by continuous feeding of epichlorohydrin at the begining of the reaction. However, the wet cells tended to get together into lumps in the cyclohexane medium with prolongation of reaction time, resulting in decline of the reaction rate. Therefore, dry powder of *A. niger* ZJB-09173 was used and the substrate concentration and the yield of (*S*)-epichlorohydrin were both enhanced. Overall, our study performed a detailed characterisation of the hydrolysis of racemic epichlorohydrin in an organic medium and offered potential method of improving the epichlorohydrin concentration in this system. Further work is being done in our laboratory to increase the stability of *A. niger* ZJB-09173 epoxide hydrolase in cyclohexane.

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