한국생물공학회 정기총회 및 연구논문발표회 발표논문

# Production of enantiopure epoxides by yeast epoxide hydrolase using a two-phase membrane bioreactor

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영문 abstract

Large-scale resolution of epoxides by the yeast *Rhodotorula glutinis* was demonstrated in an aqueous/organic two-phase cascade membrane bioreactor. Due to the chemical instability and low solubility of epoxides in aqueous phases, an organic solvent was introduced into the reaction mixture in order to enhance resolution of epoxide. A cascade hollow-fiber membrane bioreactor was used *(i)* to minimize the toxicity of organic solvents towards the epoxide hydrolase of *Rhodotorula glutinis*, and *(ii)* to remove inhibitory amounts of formed diol from the yeast cell containing aqueous phase. Dodecane was selected as a suitable solvent and 1,2-epoxyhexane as a model substrate. By use of this membrane bioreactor, highly concentrated (0.9 M in dodecane) enantiopure (>98% ee) (S)-1,2-epoxyhexane (6.5 g, 30% yield) was obtained from its racemic mixture.

## 서론

Enantiopure epoxides are versatile chiral synthons in organic synthesis. They are valuable chiral building blocks in the preparation of more complex enantiopure bioactive compounds such as pharmaceuticals and agrochemicals. Various chemical and biological production methods have been reviewed.[1] In biological production routes, enantioselective hydrolysis of racemic epoxides by use of epoxide hydrolases might be commercially feasible because with this method it is possible to obtain enantiopure epoxides with high enantiomeric purities from relatively cheap and readily available racemic epoxides.[2] Microbial kinetic resolution of epoxides has generally been carried out in aqueous buffer

systems with as a drawback that most epoxides are also chemically hydrolyzed. The instability of epoxides in aqueous phases can result in a remarkable decrease in yield of residual enantiopure epoxide and lead to some loss of enantiomeric purity for the product diol after hydrolytic kinetic resolution.[3] Furthermore, epoxides can be dissolved in aqueous media at low concentrations only. The chemical hydrolysis and/or low solubility of epoxides in aqueous phases may limit application of these kinetic resolution processes in an industrial scale. Instability and/or low solubility of epoxides in aqueous phases may be overcome by the use of organic solvents.[4] Our attention was directed to employ an hollow-fiber membrane bioreator to protect the biocatalysts from the toxicity of organic solvents.[5]

In the present study, we describe the application of an aqueous/organic two-phase hollow-fiber membrane bioreactor for preparative-scale resolution of epoxides. For this study, 1,2-epoxyhexane was chosen as a model substrate and cells of the yeast *Rhodotorula glutinis* CIMW 147 were used as a biocatalyst.

## 재료 및 방법

The yeast *Rhodotorula glutinis* CIMW 147 was cultivated in a mineral medium as previously described.[5]

The hollow-fiber membrane, Bio Nephross Allegro (COBE Nephross, The Netherlands), was made of regenerated cellulose, Hemophan®, with a diffusive surface area of 1.05 $m^2$ and the housing material was composed of acrylonitrile-butadiene-styrene copolymers (ABS). The hollow fiber module consisted of a microporous hydrophilic ultrafiltration membrane (6500 membranes per module, diameter 200 $\mu$m, thickness of membrane 6.5 $\mu$m). Two identical membranes were employed in cascade for hydrolysis and subsequent diol extraction, respectively. For enantioselective hydrolysis, cell suspensions (7 g dry weight) in 100 mM potassium phosphate buffer (pH 8.0, 160 ml), placed in 35 °C water bath were recirculated through the shell sides of each hollow fiber membrane module at the rate of 60 ml/min by a Masterflex pump. The substrate, racemic 1,2-epoxyhexane dissolved in dodecane, was passed through the lumen side of the first membrane in counter current direction. The rate of the substrate flow was 30 ml/min and the total volume of this stream was 75 ml. For diol extraction, 100 mM potassium phosphate buffer (pH 8.0, 2.4 l) was circulated through the lumen side of the second membrane with the rate of 60

ml/min. Chemically inert Viton (Cole-parmer, USA) was used for connecting tubings. The membrane bioreactor could be reused several times without significant damage. The membrane bioreactor was pre-operated for 3 h without cells to obtain a partition equilibrium of the epoxide substrate between the organic and aqueous phase.

## 결과 및 고찰

*R. glutinis* showed relatively high activities when hydrophobic aliphatic and alicyclic alkanes were used as a solvent. However, hydrophilic solvents such as ethylacetate, diethyl ether, and chlorinated hydrocarbons were strongly inhibitory to epoxide hydrolase of *R. glutinis*. Dodecane was selected to be applied in the hydrolytic kinetic epoxide resolutions although it is still inhibitory to epoxide hydrolysis. To minimize this effect, a hollow-fiber membrane bioreactor was devised.

In Fig. 1(A), enantioselective hydrolysis of 1,2-epoxyhexane at an initial amount of 226 mmol in 48 ml dodecane, resulted after 14 h in enantiopure (>98% ee) (S)-1,2-epoxyhexane (6.5 g, 65 mmol, 30% yield in dodecane phase). The initial rate of hydrolysis and E value were 40 nmol/min, mg dw and 23, respectively. (R)-1,2-Hexanediol was produced preferentially and accumulated up to 110 mmol with 64% ee and 49% yield as shown in Fig. 1(B). 1,2-Epoxyhexane was also present in the aqueous phase including both cell suspension and diol extraction buffer whereas no 1,2-hexanediol was detected in dodecane.

The use of organic solvents in the kinetic resolution process under the less harmful environment was realized by the use of a membrane bioreactor. Moreover, by using a two-phase membrane bioreactor, kinetic resolution of racemic epoxides can be performed even at very high initial concentrations. By this new approach, enantiopure epoxide can be easily prepared on a large-scale. In particular, the method is useful for large-scale resolution of chemically unstable and/or water-insoluble epoxides. In addition, by using two membranes in cascade configuration, the formed diols can be easily separated from the epoxides. In this way, production of enantiopure diols will be facilitated as well. The relation between cell density, membrane area, buffer volume and flow rate is necessary for further scale-up of this method. Optimization experiments for determination of this relation are under investigation now. The demonstrated application of a membrane bioreactor will be a useful method for large-scale

hydrolytic kinetic resolution of epoxides and for possible other bioconversions in organic media.

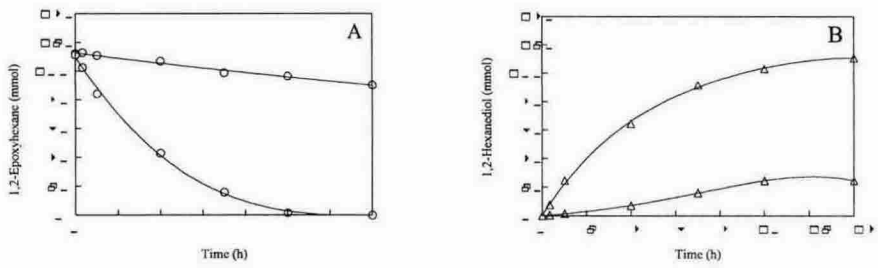


**Fig. 1** Enantioselective hydrolysis of 1,2-epoxyhexane in a cascade two-phase membrane bioreactor. Time course of epoxide hydrolysis (**A**) and 1,2-hexanediol production (**B**) by glucose-grown cells of *Rhodotorula glutinis*. Epoxide and diol during the pre-run period are not shown.
The total amounts of (R)-1,2-Epoxyhexane (○), (S)-1,2-epoxyhexane (●), (S)-1,2-hexanediol (▲) and (R)-1,2-hexanediol (△) in dodecane, cell suspension, and diol extraction buffer are shown.

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