**Abstract**

Epoxide hydrolases (EHs) demonstrating high degree of enantioselectivity or enantioconvergence are useful biocatalysts for the production of optically active epoxides and vicinal diols, which can serve as chiral building blocks for syntheses of biologically active drugs.

EHs can play an important role also in degradations of xenobiotics.

Genes encoding EHs Kau2 and Kau8 were expressed in *E. coli* host strains TOP10 and RE3. Enantioselectivities and regioselectivities of Kau2 and Kau8 in supernatants of desintegrated cells were determined for four substrates: *tert*-butylglycidyl ether, *para*-chlorostyrene oxide, *para*-nitrostyrene oxide, α-methylstyrene oxide.

The highest values of enantioselectivity and regioselectivity were achieved with Kau2 and *para*-nitrostyrene oxide as a substrate. The Kau2 was chosen for further experiments on the basis of these results.

Kau2 was overexpressed in the recombinant strain RE3(pSEKau2). We performed two batch cultures and one fed-batch culture in stirred bioreactor. The highest volumetric activity of 4500 U/l was obtained in the case of fed-batch culture.

Two phase system consisting of polyethylenglycole 6000 and sodium citrate (pH 7.7) was used for Kau2 purification from the supernatant of desintegrated cells. Purification factor 2.6 +/- 0.3 was achieved and specific activity ($A_{spec.}$) of Kau2 equalled 2.85 U/mg of protein.

Two immobilization methods were applied for purified Kau2: 1) crosslinking enzyme aggregates and 2) enzyme covalent binding to epoxy groups of polyacrylamide carriers. Three commercially available carriers were used: Eupergit C, Eupergit C250L, and Sepabeads EC-EP.

In the first case, negative results we obtained and after optimization of the second method the best results were achieved with Eupergit C250L.

Catalytic efficiency of immobilized Kau2 of 37.1 % and $A_{spec.}$ of 4.4 U/g (dry weight).

Yield of activity after immobilization was 3.1 % (ratio carrier : protein = 20 : 1)

(In Czech)