ON-LINE WATER REMOVAL DURING ENZYMATIC ASYMMETRIC ESTERIFICATION IN ORGANIC MEDIA

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SUMMARY
Enantioselective esterification of 2-bromopropionic acid with n-butanol using Candida cylindracea lipase was carried out in n-pentane at various initial water contents. Reaction rate as well as enantioselectivity decreased at high water content. A heteroazeotropic distillation method was applicable to remove the excess water continuously and to work at the optimum reaction conditions.

INTRODUCTION
A critical aspect of using enzymes in organic media is the water requirement. Both the catalytic activity (Goldberg and Legoy, 1990; Dordick, 1992) and enantioselectivity of enzymes (Gubicza et al., 1994; Kitaguchi et al., 1990) can be improved by changes in water content of the reaction mixture. Some methods of controlling the water content in organic reaction mixtures are known: addition of salt hydrates appears attractive (Kvittingen et al., 1992; Dudal et al., 1995). To remove water formed, various types of molecular sieves have been used to shift the equilibrium toward synthesis (Akoh et al., 1992; Berger et al., 1992). Pervaporation was first applied by Van der Padt et al. (1993) to remove selectively the water produced during enzymatic synthesis in solvent-free system. Recently, it was reported, that this method has been applicable in organic solvent, too (Kwon et al., 1995). Commercial scale-up of these methods has not yet been reported so far.

A new, simple method was developed for continuous removing the water produced during enzymatic esterification. Using n-pentane or a mixture of n-pentane and other organic solvents of higher boiling point, the excess water was removed by heteroazeotropic distillation. Enantioselective esterification of 2-bromopropionic acid was chosen as test reaction.
MATERIALS AND METHODS

Lipase from *Candida cylindracea* (E.C. 3.1.1.3.), 690 units/mg solid was from Sigma Chemical Co. It was used in powdered form without pretreatment. All the chemicals used were of highest purity commercially available. Prior to use, they were dried with 4A molecular sieve (Linde).

The reactions were carried out at the optimized reaction parameters (Gubicza et al., 1994) in 50 ml n-pentane, at 1:6 (mol/mol) acid:alcohol molar ratio with 0.25 g lipase in closed vials with shaking. Experiments with 20-fold volumes were performed in a mixed glass reactor, equipped with stillhead for heteroazeotropic distillation. The reaction took place at 35 °C, at the boiling point of the water/n-pentane binary system. After separation water was removed and n-pentane was recirculated in the reactor. By controlling the heating of the reactor, the amount of the water content in the reaction mixture could be held constant.

The synthetic activity of the lipase was characterized by determining the total ester yield, using gas chromatograph with an FFAP fused silica capillar column (25 m x 0.32 mm). In order to characterize the enantioselectivity, the quotient of the initial reaction rate of the (R)- and (S)-esters was determined on chiral LIPODEX E fused silica capillar column (25 m x 0.32 mm). Water content of the reaction mixture (% w/w) was measured by an automatic Karl-Fischer titrator.

RESULTS AND DISCUSSION

Water is essential in organic solvents as well, to maintain the native, catalytically active conformation of the enzyme. The initial water content of the reaction mixture was adjusted by adding water to the dried solvent. In the first set of experiments the water content continuously increased from the initial value by the water produced during the esterification. By increasing the initial water content, the ester yield raised up to 0.2-0.3 % water content, then significantly decreased (Table 1). Similar experiments at controlled water content were performed using heteroazeotropic distillation. In this case, the optimum of water content was displaced to a higher value (0.5 %).

The low conversions measured at reduced water content can be explained by the fact that in this case the water essential for the activity of the lipase is missing. After a period with optimal conditions of 0.2-0.3 % initial water content, the ester yield decreased, due to the water formed, favouring the hydrolysis. The optimum level of water could be achieved by continuous removal of water. At 0.5 % constant water content in the reaction mixture the ester yield showed maximum value.