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ENZYMES IMMOBILIZED ON A MAGNETIC SUPPORT

PRELIMINARY STUDY OF A FLUIDIZED BED ENZYME REACTOR

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Summary

Enzymes were immobilized on a magnetic support by a cocross-linking method with an inactive protein. Active porous particles obtained were used in a fluidized bed reactor. Outlet product concentration was measured as a function of flow rate and inlet substrate concentration. No enzyme leakage was observed and a circular magnet surrounding the column was shown to be an efficient device for the retention of fine particles.

A large number of physical and chemical procedures for enzyme insolubilization have been performed and have recently been reviewed [1—3]. In practice, the immobilized enzyme is suited for repetitive or continuous use of its catalytic function, and may be used for multiple analytical and preparative applications.

For these purposes, continuous stirred tank reactors and packed beds were the most commonly used devices [4—5]. In this field, Robinson et al. [7] recently described immobilization of α-chymotrypsin and β-galactosidase on a modified cellulose with magnetic properties. They reported preliminary results for a continuous stirred tank reactor. New methods for binding enzyme molecules into a water insoluble matrix were recently described [6]. About thirty enzymes were efficiently immobilized, and enzyme-porous particles obtained by these methods were packed into a column and the kinetic

Abbreviation: BAEE, *N*-benzoyl-L-arginine ethyl ester.

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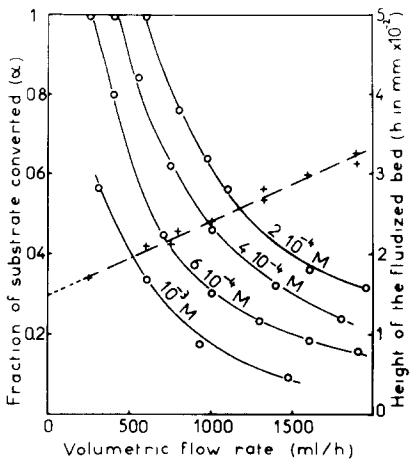

Fig. 1. Fraction of substrate converted (○—○) and height of the fluidized bed (+ — — +) vs volumetric flow rate. Molarities of inlet substrate concentrations are indicated on the curves.

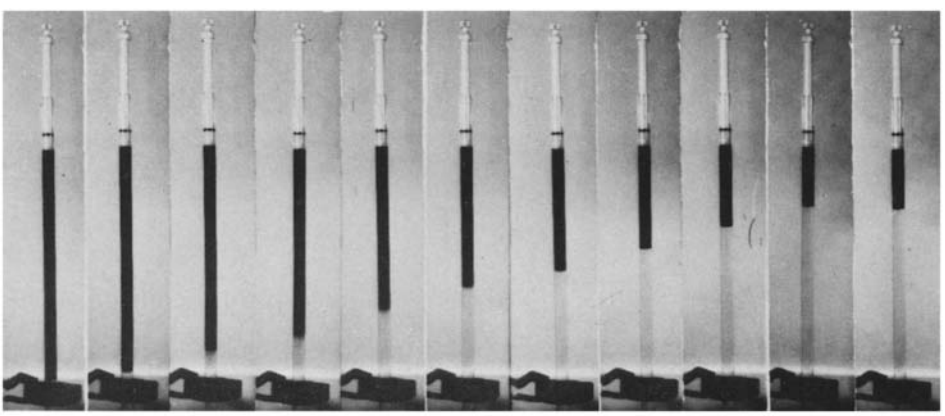

Fig. 2. Photos of the fluidized bed taken at different flow rate values, increasing from 200 ml · h^{-1} to 2500 ml · h^{-1} (from left to right).

behaviour of such a reactor was studied for both stationary state and transient conditions [4]. This paper describes the preparation of enzyme immobilized on a magnetic support and the preliminary results obtained when using these particles in a fluidized bed reactor.

Preparation of the immobilized enzymes: the method used is a cocross-linking between the enzyme and an inactive protein (human plasma albumin) with glutaraldehyde. Magnetic iron oxide particles (100—200 μm in diameter and purchased from La Radiotechnique, Evreux-France) are added as an inert charge before polymerization occurs. The procedure for the production of papain (EC 3.4.4.10) active particles is given as an example: 30 g of magnetic

iron oxide are impregnated with 25 ml of 0.02 M phosphate buffer, pH 6.8, containing 8% plasma albumin, 0.4% glutaraldehyde and 100 mg crystalline papain (Miles-Yeda Ltd). A thick suspension was obtained which was frozen at −30 °C for several hours, then slowly warmed overnight in a refrigerator at 4 °C. The proteinic copolymer formed was thoroughly rinsed, then freeze–dried, ground and sieved to obtain a given range of particle sizes (170–250 microns in diameter).

Measurement of enzyme activities: hydrolysis of *N*-benzoyl-L-arginine ethyl ester (BAEE) was followed by the absorbance increase at 254 nm, using a thermostated double beam spectrophotometer. For the reactor experiments a continuous flow cell (1-cm light path) was used.

Free enzyme: 10 mg of papain (twice crystallized, purified by affinity chromatography and purchased from Miles-Yeda Ltd) were dissolved in 10 ml 10^{-3} M HCl to avoid proteolysis. BAEE at various concentrations (10^{-3} to 10^{-4} M) was dissolved in pH 6.0, phosphate citrate buffer (0.05 M of each and 10^{-3} M in both cysteine and EDTA). The activity of 50-μl samples of papain solution was measured at various BAEE concentrations. For the free enzyme a maximal activity of 19 I.U. (μmoles BAEE $\cdot$ min^{-1} $\cdot$ mg^{-1} at pH 6.0 and 20 °C) and a $K_m = 5 \cdot 10^{-3}$ M were found.

Bound enzyme: in a thermostated beaker, fitted with a little turbine impeller, 100 mg samples of enzyme particles were suspended in 100 ml phosphate citrate buffer (0.05 M, pH 6.0 and 10^{-3} M in both cysteine and EDTA), containing BAEE at various concentrations. 254 nm absorbance of the solution (pumped through a sintered glass disk) was continuously recorded by using the flow-through spectrophotometric cell. The total circulating volume was 5 ml. For the bound papain a maximal activity of 0.05 I.U. (μmoles BAEE $\cdot$ min^{-1} per mg of support at pH 6.0 and 20 °C) and an apparent Michaelis constant $K'_m = 1.2 \cdot 10^{-2}$ M were found. Identical values were found for proteinic copolymers (bearing papain activity) prepared without magnetic particles.

Fluidized bed reactor: 10 g of active porous particles suspended in phosphate citrate buffer were poured in a thermostated chromatographic column (Whatman P.C. 1050: 10 mm internal diameter, 50 cm length). In order to retain particles, the top of the column was surrounded by a circular magnet. Substrate solutions at various concentrations ($2 \cdot 10^{-4}$ M to 10^{-3} M) were made to flow through the column by a peristaltic pump (Desaga A.G. Heidelberg). 254 nm absorbance of the outlet solution was continuously recorded. Precise determination of flow rates was made by collection of the column effluent. Water circulation thermostated the whole system at 20 ± 0.05 °C. The fraction of substrate converted (α) as a function of the flow rate is presented in Fig. 1. α (dimensionless) is defined as:

$$\alpha = \frac{S_1 - S_0}{S_1}$$

where S_1 and S_0 are, respectively, the inlet and outlet substrate concentrations. In the experimental conditions selected here, the conversion ratio remains equal to 100% for small flow rates, and decreases with increasing flow rates, the shape of the experimental curve results from various phenomena: related variations of the reaction volume and of the residence time; variation of the concentration of the enzyme particles in the reaction volume; variations in the movement of the particles (with diffusion and backmix effects).

The height of the fluidized bed varied linearly with flow rate (Fig. 1) and the minimum fluidizing velocity [8] was found to be about 250 $ml \cdot h^{-1}$. Photos of the fluidized bed (without the thermostated jacket) were taken at different flow rates (Fig. 2). A carry over of fine particles occurred at high flow rates but was efficiently stopped by the magnet. The absence of iron and enzyme leakage was checked in the outlet solution. Further work along this line is in progress, including long term assays, kinetic modeling and applications.

References

1 Gryskiewicz, J. (1971) Folia Biol. 19, 119—150
2 Silman, M.I. and Katchalski, E. (1966) Annu. Rev. Biochem. 35, 873—908
3 Melrose, G.J. (1971) Rev. Pure Appl. Chem. 21, 83—119
4 Gellf, G., Kernevez, J -P., Thomas, D. and Broun, G. (1973) Biotechnol. Bioeng. In the press
5 Wingard, L.B. (1972) Adv. Biochem. Eng. 2, 1—48
6 Broun, G , Thomas, D., Gellf, G., Domurado, D., Berjonneau, A.M. and Guillon, C. (1973) Biotechnol. Bioeng. 15, 359—375
7 Robinson, P.J., Dunnill, P. and Lilly, M.D. (1973) Biotechnol. Bioeng. 15, 603—606
8 Denbigh, K.G and Turner, J.C.R. (1971) Chemical Reactor Theory, pp 155—163, Cambridge University Press