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THE INFLUENCE OF ULTRASOUND ON THE REACTION OF IMMOBILIZED ENZYMES

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The effect of ultrasonic waves in the MHz range on the activity of immobilized α-amylase and glucoamylase was investigated. Measurements of the catalytic efficiency were carried out in a flow cell, in dependence on the concentration, the flow rate, and the molecular weight of the substrate, as well as on the size of the carrier particles (porous polystyrene). The results show that the ultrasonic effect might be explained by the promotion of the diffusion processes predominately within the Nernst diffusion layer of the carrier beads.

1. Introduction

Ultrasonic effects on enzymes, hormones and other biological macromolecules are well described in the literature [1, 5]. An enhancement of the catalytic activity of enzymes in solution, however, has not yet been established in any case.

On the other hand, such ultrasonic effects could be demonstrated at immobilized enzymes [2], i.e. enzymes which have been confined to insoluble carriers either by chemical or physical attachment or entrapment [3].

Frequently the immobilization is accompanied by a loss of catalytic efficiency. An additional diffusion limitation in the vicinity of the active molecules, due to the presence of the carrier network, and an unstirred liquid layer on the surface of the support are the reasons for this phenomenon [4,8].

The present paper reports on experimental investigations to improve the catalytic efficiency of enzymes immobilized on a porous polystyrene carrier by means of ultrasonic waves.

2. Diffusion controlled enzyme-carrier systems

The mass transport during the heterogeneous catalysis may be classified into external and internal transportation. The former is limited to the substrate as well as to the product molecules by an unstirred layer, the so-called Nernst diffusion layer (Fig. 1). The mass transport rate per unit area of the substrate may be denoted by the equation [8]

$$\varphi = \frac{D(s - s')}{\delta}, \tag{1}$$

where D is the diffusion coefficient of the substrate in the unstirred layer; δ is the thickness of this layer; s and s' are the substrate concentrations in the

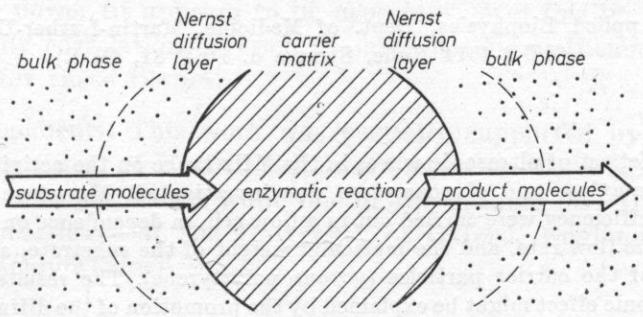

Fig. 1. Schematic representation of the mass transport during the heterogeneous catalysis

well mixed bulk phase and at the surface of the polymer matrix, respectively. The concentration profile across the carrier is illustrated in Fig. 2. The mass transport rate φ, which decisively influences the total rate of the catalytic reaction, may be accelerated by an increase in the concentration gradient $(s - s')/\delta$ within the Nernst diffusion layer. The thickness δ is dependent on the hydro-

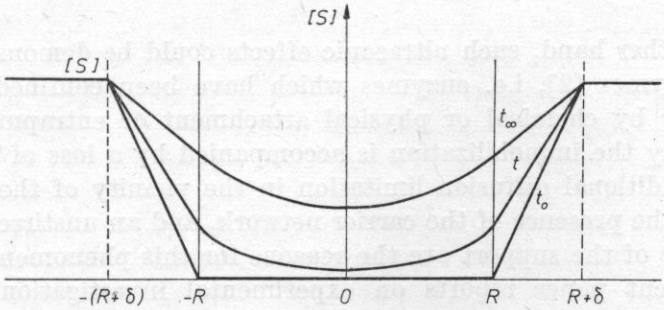

Fig. 2. Substrate concentration profile across the carrier matrix and the adjacent liquid range (after [4])

dynamic streaming around the carrier beads and cannot be reduced indefinitely in this way. In a well stirred reactor δ is of the order of magnitude of 100 μm. By means of ultrasonic waves, however, δ can be reduced to the thickness of the acoustic boundary layer

$$\delta_{\text{acoust.}} = \sqrt{\frac{2\nu}{\omega}}; \qquad \omega = 2\pi f, \tag{2}$$

where ν is the kinematic viscosity and f is the sound frequency. E.g., $\delta_{\text{acoust.}}$ amounts to 0.1 μm in aqueous solutions at 5 MHz.

The substrate concentration s' on the surface of the porous support is dependent on the rate of the internal mass transport (pore diffusion). Generally, a sound effect on this part of the diffusion may be neglected, since the diameter of the pores is small compared with the thickness of the acoustic boundary layer.

3. Material and method

The enzymes used were α-amylase (Novo A/S, Denmark) and glucoamylase (Merck, Darmstadt) with Zulkowsky starch (Merck, Darmstadt) and maltose (VEB Laborchemie, Apolda) as substrates. The enzymes were bound to porous polystyrene (type Y58, VEB Chemiekombinat Bitterfeld), used in two fractions (0.15-0.20 mm — charge 1; 0.63-0.80 mm — charge 2). The biochemical preparation and analysis procedures are described elsewhere [6].

The measuring cell (Fig. 3) has a cylindrical shape with a volume of 1 ml and is faced by two 30 μm thick plastic foils. The cell is placed in a thermo-

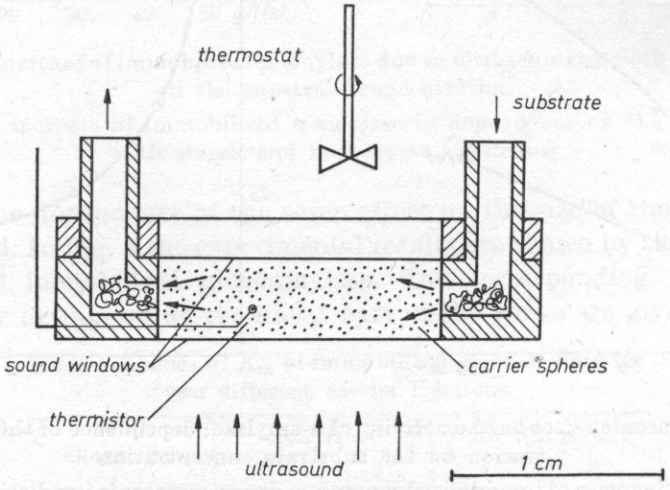

Fig. 3. Schematic representation of the reaction cell

stat-equipped vessel at a distance of about 2 cm from the transducer. The experiments were carried out in a continuous, non-focused soundfield at a frequency of 7 MHz. The maximum sound intensity, measured by a radiation force float, was 10^3 W/m^2.

4. Results and discussion

Immobilized α-amylase and starch as substrate were examined to demonstrate the effect of the ultrasound irradiation on the enzymatic reaction. The reaction is clearly accelerated in the presence of the soundfield, as represented in Fig. 4. The same result can be shown by the Lineweaver-Burk plot. The change of the Michaelis-Menton constant can be interpreted by the promotion of the substrate diffusion into the carrier matrix. The maximum reaction rate is changed, too.

A further indication of the sonic influence on the diffusion processes is the dependence of the activity increase in the sound field on the flow rate in the cell and on the substrate concentration, respectively (Figs. 5 and 6). An acceleration of the flow reduces the thickness of the unstirred layer around the carrier beads due to the hydrodynamic mechanisms, and consequently the sonic effect decreases. If the substrate concentration s is raised the concentration s' changes in the same measure. This procedure can be continued until

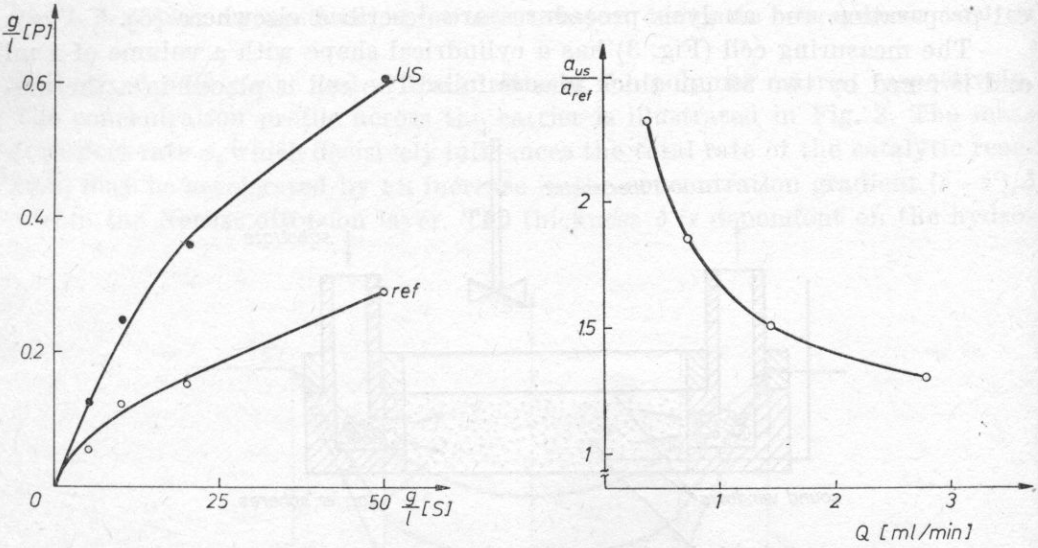

Fig. 4. The ultrasonic effect on the activity of α-amylase; dependence of the product concentration on the substrate concentration

Fig. 5. Activity increase of immobilized α-amylase due to ultrasonic irradiation in dependence on the substrate flow rate

the reaction is no longer controlled by the film diffusion. Obviously, the sonic effect diminishes correspondingly.

If α-amylase is replaced by glucoamylase two substrates can be used and the sound influence can be studied dependending on the molecular size. In Fig. 7 the activity increase is plotted versus the sound intensity for starch (MW $\approx$ $\approx$ 15.000) and maltose (MW 348), respectively as substrates. The effect is proportional to the sound intensity and, as expected from the results obtained hitherto, the effect is much smaller for the substrate with the lower molecular weight.

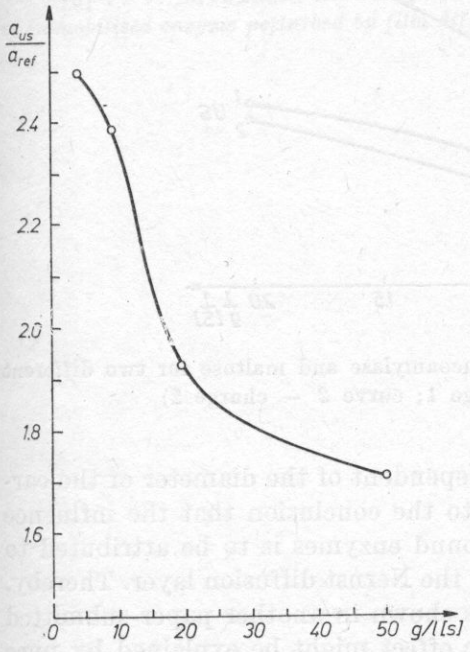

Fig. 6. Activity increase of immobilized α-amylase due to ultrasonic irradiation in dependence on the substrate concentration

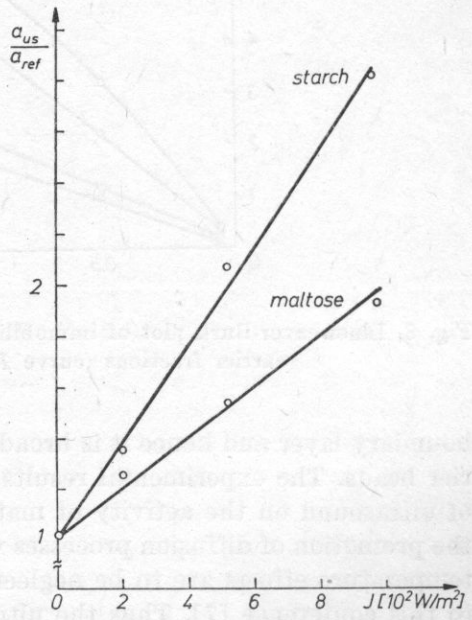

Fig. 7. Activity increase of immobilized α-amylase in dependence on the sound intensity with starch and maltose as substrates

Finally the dependence of the sonic effect on the size of the carrier beads were examined. In Fig. 8 the experimental results are shown by the Lineweaver-Burk plot of immobilized glucoamylase. The corresponding values of K_m determined by extrapolating the linear part of the curve are given in Table 1.

Table 1. Values of K_m of immobilized glucoamylase for two different carrier fractions

charge no	Reference		With ultrasound	
	1	2	1	2
K_m [g/l]	40.6	49.1	11.3	9.2

The reference value for the charge with the smaller beads is lower than that for the bigger ones. Under ultrasound the two values agree within the limits of the experimental conditions. This is an indication that in the presence of an ultrasonic field the mass transport is determined by the thickness of the acoustical

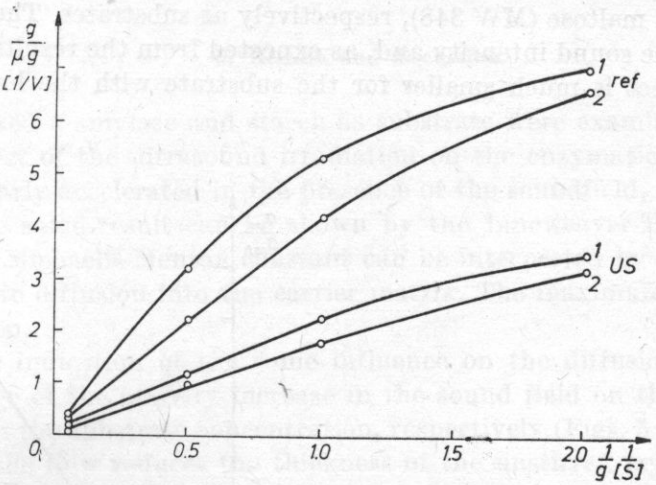

Fig. 8. Lineweaver-Burk plot of immobilized glucoamylase and maltose for two different carrier fractions (curve *1*: charge 1; curve *2* — charge 2)

boundary layer and hence it is broadly independent of the diameter of the carrier beads. The experimental results lead to the conclusion that the influence of ultrasound on the activity of matrix bound enzymes is to be attributed to the promotion of diffusion processes within the Nernst diffusion layer. Thereby, temperature effects are to be neglected, as shown in another paper submitted to this conference [7]. Thus the ultrasonic effect might be explained by pure mechanical, non-cavitational interactions like acoustic streaming or microstreaming.

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