Kinetic Properties of Invertase Immobilized on Cellulose Beads

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In the present work kinetics of inversion of saccharose were studied within the input concentrations of saccharose from 28 to 1533 mmol dm^{-3} using invertase conjugated to bead cellulose. Parameters in the kinetic equation reliably predicting experimental results were determined over the studied concentration range. Only small changes of catalytic activity of invertase were observed when immobilization was carried out by the method described.

In the recent years increasing effort at industrial application of immobilized enzymes has been observed. Though immobilization of enzyme often contributes to a more advantageous economy of the production process, the investigation of kinetic properties of enzyme is often complicated by the immobilization technique. In mathematical modeling of reactors with immobilized enzymes one should take into consideration the influence of mass transfer, axial and radial dispersion, distribution of enzyme molecules in the particles of carrier support, deactivation, *etc.* On account of the complexity of this problem, a separate analysis of individual efforts is required. In studying the internal kinetics of immobilized enzyme typical simplification is for example binding of substrate to the outer surface of a support only. Thus, the influence of internal pore diffusion can be omitted. This enables to evaluate changes that occur in enzymatic kinetic parameters due to immobilization of enzyme and facilitates the investigation of its properties in a porous carrier support.

Gels are often employed for the immobilization of enzymes. Their disadvantage is a low mechanical resistance, which causes that in a fixed bed reactor a deformation of particles due to compaction of bed occurs. Apart from deterioration of flowing properties in the reactor, this phenomenon can have an unfavourable influence on the catalytic properties of biocatalyst [1—3].

Deformation of particles in a fixed bed resulting from elasticity can also occur in the case of employment of bead cellulose as a support of enzyme. In one of our previous works we used bead cellulose for immobilization of invertase and we studied its properties in both batch and fixed-bed reactors [4]. The work reported here presents results of the study of the kinetics of invertase immobilized on the outer surface [4] of bead cellulose in reactors operating in differential and fixed-bed mode. In addition, the purpose of this study was to test the influence of compaction of bed on the reaction rate.

THEORETICAL

Mathematical Model of a Fixed-Bed Reactor with Invertase Immobilized in Particles

The mass balance equation for the substrate in the main flow of fluid in a tubular reactor can be written as

$$\varepsilon\frac{\partial c_s}{\partial t} = D_z\frac{\partial^2 c_s}{\partial z^2} + D_r\frac{1\partial}{r\partial r}\left(r\frac{\partial c_s}{\partial r}\right) - w\frac{\partial c_s}{\partial z} - k_s a(c_s - c_{so}) \qquad (1)$$

where c_s and c_{so} are the substrate concentration in the bulk fluid flow and the concentration on the external surface of biocatalyst particle, respectively. After neglecting axial and radial dispersion, one obtains for the steady-state conditions

$$w\frac{\partial c_s}{\partial z} + k_s a(c_s - c_{so}) = 0 \qquad (2)$$

The mass balance for the substrate in the surroundings of biocatalyst particle is given by

$$k_s a(c_s - c_{so}) = R \qquad (3)$$

In the case of hydrolysis of saccharose catalyzed by invertase, the rate of reaction can be expressed

by the following kinetic equation incorporating the competitive product and substrate inhibitions along with the influence of water concentration on the rate of hydrolysis [5]

$$R = -\frac{dc_s}{dt} = \frac{V_{max} c_{so}}{K_m\left(1 + c_{po}/K_{ip}\right) + c_{so}\left(1 + c_{so}/K_{is}\right)} \cdot \frac{W}{W_o} \qquad (4)$$

where the overall water concentration can be calculated from [6]

$$W = 55.33 - 0.01186\, c_{sz} \qquad (5)$$

where c_{sz} (mmol dm^{-3}) is the initial concentration of saccharose and W_0 is equal to 55.33.

The meaning of other symbols is explained in the list of symbols.

In order to calculate the rate of reaction from eqn (*4*), we need to know the concentration of both product and substrate on the particle surface. One of the possible ways is the simultaneous solution of differential mass balance equation for product and substrate. For our case we employed a more simple method. Under the assumption that in steady-state operation the sum of molecular flows of product and substrate in the surroundings of particle is equal to zero, the solution of eqn (*3*) and of analogical equation for the product gives the following relation

$$c_{po} = \frac{k_s}{k_p}(c_s - c_{so}) + c_{sz} - c_s \qquad (6)$$

The values of coefficients of the external mass transfer k_s and k_p were calculated from the correlation [7]

$$\varepsilon J_d = \frac{0.765}{Re^{0.82}} + \frac{0.365}{Re^{0.386}} \qquad (7)$$

Eqn (*2*) was solved numerically by the Runge—Kutta method of the 4th order with the initial condition $c_s = c_{sz}$ at $z = 0$. Since c_{so} cannot be solved explicitly, the rate of enzyme reaction was calculated by an iterative solution of eqns (*3*) and (*4*) using eqn (*6*).

EXPERIMENTAL

Invertase (E.C. 3.2.1.26, β-fructofuranosidase) grade V: practical (obtained from Sigma, St. Louis, USA) was immobilized by covalent binding into a cellulose activated by 2,4,6-trichlorotriazine by using a procedure presented in our previous work [4].

The equipment consisted of a reservoir (stirred three-necked flask), peristaltic pump, and differential reactor. The whole device was immersed in a temperature-controlled bath ensuring isothermal conditions (25 °C) (Fig. 1). The biocatalyst bed was separated from inert glass beads which ensured a homogeneous distribution, by a sinter. The reactor was composed of two parts which enabled a rapid handling of the immobilized biocatalyst without disturbing the bed of inert glass beads. The length of the bioreactor (with the inner diameter 55 mm) was 120 mm and the bed height of biocatalyst was 5 mm. Flowing of the mixture through the reactor was secured by a peristaltic pump. Superficial velocity in the reactor was 25 mm min^{-1}. Sampling ports on the bioreactor enabled taking of samples in equidistant time intervals of 5 min. The overall concentration of saccharose was calculated from the overall volume of liquid in the system. This value was determined in the following way: the system was perfectly washed by a pure buffer solution and 100 cm^3 of glucose solution with the concentration c_{S1}= 20 mmol dm^{-3} was added. After starting the pump and stirring, concentration of glucose in the reservoir was determined.

The overall volume of the system then gives

$$V = \frac{c_o \cdot 100}{c_1} \cdot \text{cm}^3$$

The kinetics of soluble invertase were studied by measuring the initial rates of hydrolysis of saccharose in 50 mM acetate buffer, pH of 4.65, at 25 °C. The concentration of released glucose in the reaction media has been determined by the glucose oxidase test (Oxochrome glucose Bio-La-Test, Lachema, Brno).

The initial rate of hydrolysis of saccharose by immobilized invertase was estimated in a system with

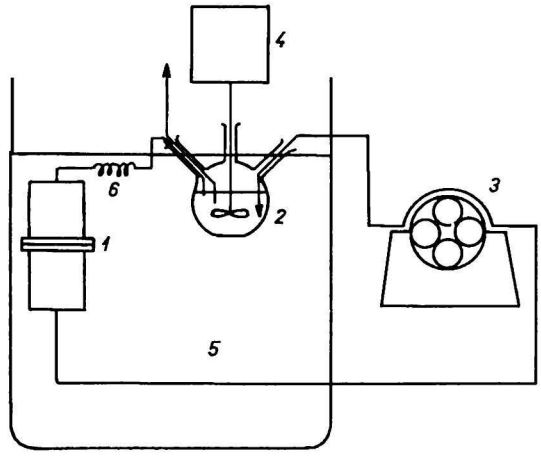

Fig. 1. Scheme of the laboratory device with a differential reactor for the kinetic study of immobilized enzymes. *1.* Differential reactor; *2.* reservoir; *3.* peristaltic pump; *4.* stirrer motor; *5.* temperature-controlled base (25 °C); *6.* heat-exchanger.

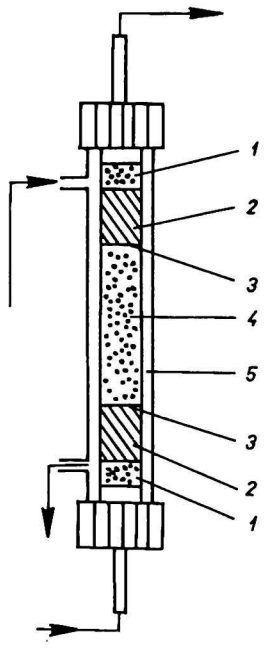

Fig. 2. Packed-bed reactor with enzyme immobilized in a fixed bed. *1*. Piston; *2*. bed of glass particles (average mean diameter of particles 0.4 mm, bed height 30 mm); *3*. stainless steel wire cloth; *4*. bed of particles with immobilized enzyme (average mean diameter of particles 0.358 mm, bed height 210 mm); *5*. temperature-controlled water (25 °C), inner diameter of column 15 mm.

a differential reactor (Fig. 1) where reaction conditions were equal to those reported for a free enzyme. Initial rates of hydrolysis were determined by linear regression from the experimental results obtained. Values of V_{max}, K_m, K_{is} in eqn (*4*) were computed by nonlinear regression using the modified equation, which was obtained for conditions at the beginning of reaction, when the concentration of product is zero and the term expressing the product inhibition can

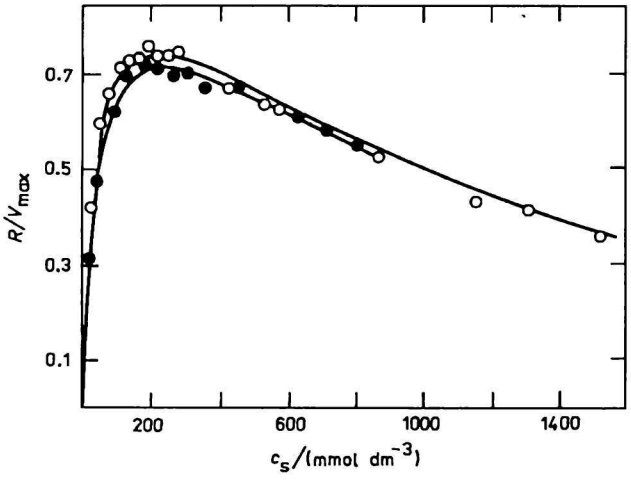

Fig. 3. Estimation of kinetic properties of the free invertase (○) and immobilized invertase in a differential reactor (●).

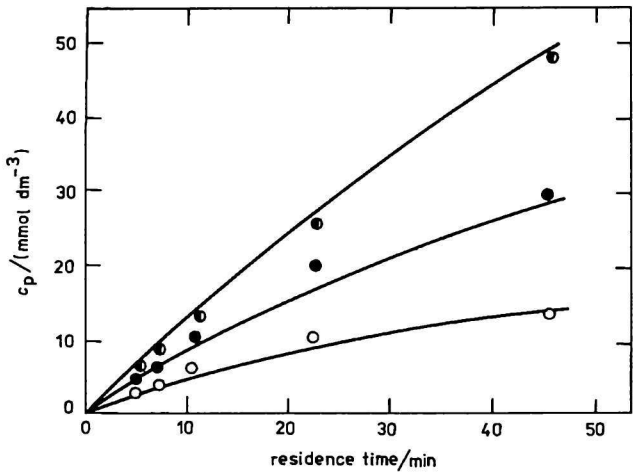

Fig. 4. Application of the given mathematical model to a fixed-bed reactor with immobilized invertase. Input concentrations of saccharose c_i/(mmol dm^{-3}): ○ 20, ● 50, ◐ 200. Curves represent simulated courses. Optimized values of parameters were following: V_{max} = 1.74 mmol dm^{-3} min^{-1} and K_{ip} = 60.70 mmol dm^{-3}.

be neglected

$$R = \frac{V_{max} c_{sz}}{K_m + c_{sz}\left(1 + c_{sz}/K_{is}\right)} \cdot \frac{W}{W_o} \qquad (8)$$

Properties of invertase immobilized on cellulose bead were studied in a glass column equipped with a jacket through which constant temperature water was circulated. Details of packing are shown in Fig. 2.

RESULTS AND DISCUSSION

The data for initial rate of the hydrolysis of saccharose catalyzed by soluble invertase were measured within the input concentrations of saccharose from 28 to 1533 mmol dm^{-3}. Experimental data depicted in Fig. 3 reveal that the initial rate increases gradually until the saccharose concentration reaches about 255 mmol dm^{-3}, after which its inhibition effect is manifested. By nonlinear regression the following parameters in eqn (*8*) were determined: V_{max} = 2.4 mmol dm^{-3} min^{-1}, K_m = 36.4 mmol dm^{-3}, K_{is} = 1783 mmol dm^{-3}. These data fit well values available in literature [5] for the same type of invertase. On the basis of measurements of the initial rate of reaction in a differential reactor with invertase attached to bead cellulose the following parameters of the kinetic equation were obtained: V_{max} = 1.69 mmol dm^{-3} min^{-1}, K_m = 48.5 mmol dm^{-3}, K_{is} = 1752 mmol dm^{-3}. As it is evident from the values K_m and K_{is} corresponding to free and immobilized enzymes as well as from the results depicted in Fig. 3 the catalytic properties of invertase show only little changes during immobilization by the method used.

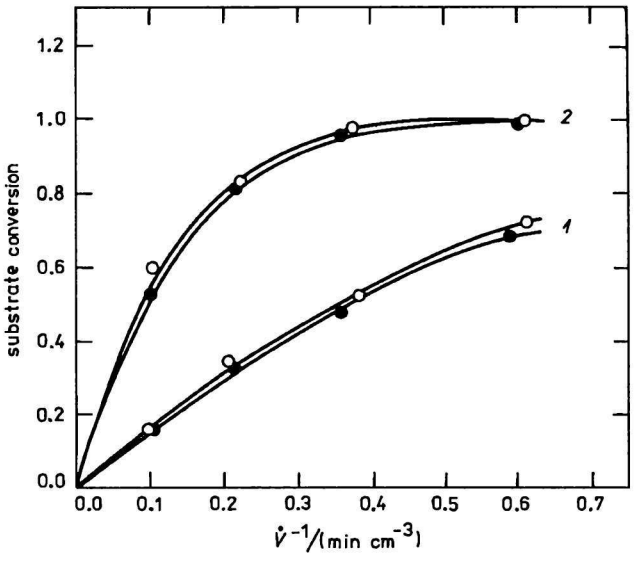

Fig. 5. Influence of bed compaction. Experimental data (○ before compaction, ● after compaction to 84.6 % of the initial height). Initial concentration of substrate: *1.* 250 mmol dm^{-3}, *2.* 50 mmol dm^{-3}.

Values of parameters for the immobilized invertase were used for modeling of kinetics in a fixed-bed reactor. With regard to the achieved higher conversion of substrate, also product inhibition was necessary to be included into the kinetic model. Values of parameters V_{max} and K_{ip} were optimized by the simplex method using experimental data given in Fig. 4. Predictions of the mathematical reactor model agree with experimental data within the correlation coefficient 0.994. Furthermore, a 15 % compaction of the original bed height of particles revealed that the deformation of bead cellulose particles does not play such a role as with gel particles (Fig. 5) because the conversion of substrate showed only a 2 % change.

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SYMBOLS

a	specific surface of particles
c_s, c_{so}	concentration of substrate in the main fluid flow and on the surface of particles, respectively
c_S	input substrate concentration
c_p	product concentration
d_p	particle diameter
D_r, D_z	coefficient of radial and/or axial dispersion
J_d	mass transfer factor
k_s	external mass transfer coefficient
K_m, K_{ls}, K_{ip}	Michaelis constant, constant of substrate inhibition, constant of product inhibition, respectively
Re	Reynolds number ($wd_p\rho/\mu$)
R	rate of reaction
r	inner radius of the reactor — radial coordinate
t	time
w	flow rate
z	axial coordinate
V_{max}	maximum reaction rate
W	total water concentration
ε	bed voidage
μ	viscosity
ρ	density

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