Biophysical Chemistry 7 (1977) 229–234

CONSECUTIVE IMMOBILIZED ENZYMATIC REACTIONS WITH AND WITHOUT ENZYME DENATURATION

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Received 17 March 1977
Revised manuscript received 24 June 1977

Consecutive biochemical reactions in an immobilized enzyme particle under the effects of internal and external diffusional resistances are analyzed. A rigorous nonlinear reaction kinetics is employed and the steady state effectiveness factor with negligible enzyme denaturation compared with the previous prediction by the first-order kinetics. It is found that the difference between them is rather substantial under most circumstances. The cases with significant enzyme denaturation are also investigated by using an unsteady state model. The substrate concentration responses to variation of the physical and kinetic parameters reveal many interesting characteristics of the reaction system.

1. Introduction

Because of their unique activities and specificities toward substrate, enzymes are important catalysts for inducing biochemical reactions and are widely used in continuous conversion of substrates in food processing industries. In many industrial applications, enzymatic reactions are generally carried out in homogeneous systems where the soluble enzymes are mixed homogeneously with the substrate solution. To isolate the final products, the soluble enzymes are generally denatured by heating or by hydrolysis, which results in considerable loss of enzymes. The fact that enzymes are expensive and very difficult to isolate points out the importance of immobilizing the enzymes to water-insoluble supports. The immobilized enzymes, which are covalently attached to or physically adsorbed in water-insoluble supports, can be used in suspension or in packed-bed form. The advantages of the use of immobilized enzymes are the stability against denaturing agents and ease of recovery for repeated use for continuous conversion of substrates.

Quite recently, there has been a growing interest in the kinetic studies of immobilized enzymes [1–4]. However, most of the previous investigations were primarily concerned with single immobilized enzymatic reaction only. In fact, a number of enzymatic reactions often follow a consecutive reaction sequence in which the product from one reaction step becomes the substrate for the next reaction [5,6]. Some examples of consecutive enzymatic reactions can be found in Wakman [5], Laidler and Bunting [6] and Goldman and Katchalski [7]. Compared to those of single enzymatic reactions, kinetic studies of consecutive enzymatic reactions are still meager. Goldman and Katchalski [7] appear to be the first authors examining biochemical reactions of two-enzyme system. However, they considered simple first-order reaction only which actually can not exhibit the true characteristics of the enzymatic reactions. Lin [8] examined the consecutive enzymatic reactions in a packed-bed reaction by assuming negligible substrate diffusion resistance inside the immobilized enzyme particles. More recently, Krishna and Ramachandran [9] investigated the effect of mass transfer resistance on the consecutive enzymatic reactions. These authors also employed the same first-order kinetics as was done by Gold and Katchalski [7].

As indicated by the previous review, there is a need to investigate the effect of mass transfer resistance on the consecutive enzymatic reactions by using a rigorous reaction kinetics which is normally nonlinear in characteristic rather than linear. In addition, all the

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previous investigations considered steady state reactions only. In the exact sense, the immobilized enzymatic reactions are transient because of inherent enzyme denaturation. Under favorable reaction conditions, such as at relatively low reaction temperatures and/or free from denaturing agents, immobilized enzymes can last several weeks or even longer. For this instances steady state reactions are a good approximation to the true situation. In fact, such favorable reaction conditions may be difficult to maintain in real large-scale industrial reaction systems and the immobilized enzymes can be completely denatured in a few hours. Therefore, an unsteady state reaction becomes important for this case. This problem has not been dealt with before and will be explored in this work.

2. Steady state model

The consecutive immobilized enzymatic reactions can be represented by the following reaction sequence

$$\mathrm{S}_1 \xrightarrow{\text{enzyme 1}} \mathrm{S}_2 \xrightarrow{\text{enzyme 2}} \mathrm{P}, \qquad (1)$$

where S_1 is the initial substrate, S_2 the product of the first reaction and the substrate for the second reaction and P the final product. By assuming that the enzymes are uniformly immobilized in the spherical water-insoluble supports, a material balance for each substrate can be represented by

$$D\left(\frac{\mathrm{d}^2 S_1}{\mathrm{d}r^2} + \frac{2}{r}\frac{\mathrm{d}S_1}{\mathrm{d}r}\right) - \frac{V_1 S_1}{S_1 + k_{\mathrm{m}1}} = 0, \qquad (2)$$

$$D\left(\frac{\mathrm{d}^2 S_2}{\mathrm{d}r^2} + \frac{2}{r}\frac{\mathrm{d}S_2}{\mathrm{d}r}\right) + \frac{V_1 S_1}{S_1 + k_{\mathrm{m}1}} - \frac{V_2 S_2}{S_2 + k_{\mathrm{m}2}} = 0, \qquad (3)$$

and the boundary conditions are given by

$$r = 0; \quad \mathrm{d}S_1/\mathrm{d}r = 0, \quad \mathrm{d}S_2/\mathrm{d}r = 0, \qquad (4)$$

$$r = r_0; \quad D\frac{\mathrm{d}S_1}{\mathrm{d}r} = h_1(S_{10} - S_1), \quad D\frac{\mathrm{d}S_2}{\mathrm{d}r} = h_2(S_{20} - S_2), \qquad (5)$$

where S_1 and S_2 are the concentration of the first and second substrates, D the diffusion coefficient, V_1 and V_2 the maximum reaction rates, $k_{\mathrm{m}1}$ and $k_{\mathrm{m}2}$ the Michaelis constants, h_1 and h_2 the mass transfer coefficients, S_{10} and S_{20} the concentrations of the first and second substrates in the bulk phase and r the radial coordinate. If $S_1 \ll k_{\mathrm{m}1}$ and $S_2 \ll k_{\mathrm{m}2}$, eqs. (2) and (3) reduce to those considered by Krishna and Ramachandran [9]. In fact, the first-order reaction constitutes a special case of the real situation and the majority of the consecutive immobilized enzymatic reaction can only be represented by the general equations, eqs. (2) and (3).

For convenience of computation, eqs. (2) through (5) can be cast into dimensionless forms by using the following dimensionless variables and parameters

$$C_1 = \frac{S_1}{S_{10}}, \quad C_2 = \frac{S_2}{S_{10}}, \quad \phi_1 = \frac{V_1 r_0^2}{D S_{10}}, \quad \phi_2 = \frac{V_2 r_0^2}{D S_{10}},$$

$$R = \frac{r}{r_0}, \quad \mathrm{Sh}_1 = \frac{h_1 r_0}{D}, \quad \mathrm{Sh}_2 = \frac{h_2 r_0}{D}, \quad K_{\mathrm{m}i} = \frac{k_{\mathrm{m}i}}{S_{10}},$$

$$\alpha = \frac{S_{20}}{S_{10}},$$

Eqs. (2) through (5) then become

$$\frac{\mathrm{d}^2 C_1}{\mathrm{d}R^2} + \frac{2}{R}\frac{\mathrm{d}C_1}{\mathrm{d}R} - \frac{\phi_1 C_1}{C_1 + K_{\mathrm{m}1}} = 0, \qquad (6)$$

$$\frac{\mathrm{d}^2 C_2}{\mathrm{d}R^2} + \frac{2}{R}\frac{\mathrm{d}C_2}{\mathrm{d}R} + \frac{\phi_1 C_1}{C_1 + K_{\mathrm{m}1}} - \frac{\phi_2 C_2}{C_2 + K_{\mathrm{m}2}} = 0 \qquad (7)$$

and

$$R = 0; \quad \mathrm{d}C_1/\mathrm{d}R = 0, \quad \mathrm{d}C_2/\mathrm{d}R = 0, \qquad (8)$$

$$R = 1; \quad \mathrm{d}C_1/\mathrm{d}R = \mathrm{Sh}_1(1 - C_1),$$

$$\mathrm{d}C_2/\mathrm{d}R = \mathrm{Sh}_2(\alpha - C_2), \qquad (9)$$

in which ϕ_1 and ϕ_2 are the Thiele moduli and Sh_1 and Sh_2 the modified Sherwood numbers. The modified fied Sherwood numbers represent the relative importance of the internal to the external mass transfer resistances. If they are very large, the external mass transfer resistance is negligible and the boundary conditions at $R = 1$ become $C_1 = 1$ and $C_2 = \alpha$. This situation may occur when the immobilized enzyme particles are suspended in a well-mixed tank reactor. However, in a packed-bed reactor, the external mass transfer resistance becomes significant because of the stagnant liquid film coated around each immobilized enzyme particle.

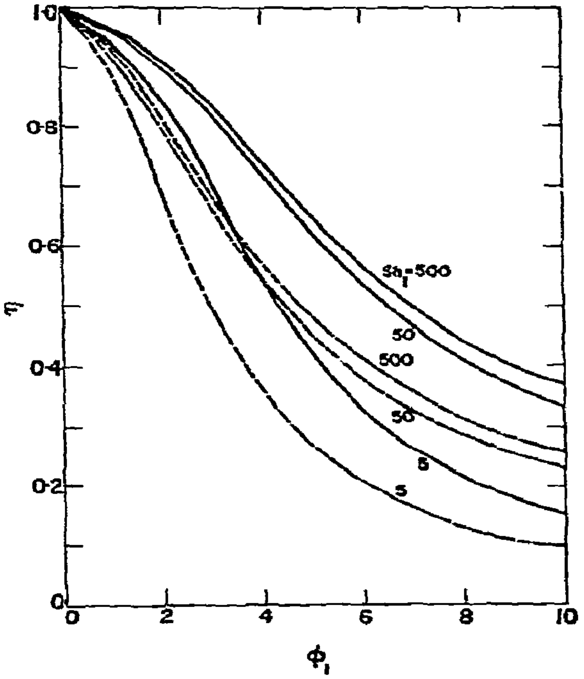

Fig. 1. Comparison of the effectiveness factors predicted by the first-order kinetics (broken lines) and by the nonlinear one (solid lines) with $K_{m1} = K_{m2} = 0.5$.

The effectiveness factor, which is defined as the ratio of true reaction rate to that without mass transfer resistances, can be represented in dimensionless form by

$$\eta = \frac{3(1+K_{m1})}{\phi_1^2}\left(\left.\frac{dC_1}{dR}\right|_{R=1}\right). \tag{10}$$

Although eqs. (6) and (7) are nonlinear, they can be integrated numerically by an iterative Runge–Kutta method [10] on a digital computer.

Fig. 1 shows the comparison of the results obtained using the nonlinear reaction kinetics (solid lines) and those obtained by Krishna and Ramachandran [9] (broken lines). It is apparent that the difference between them is rather substantial and the predictions by the first-order reaction kinetics can be as much as 50% below the present predictions. This indicates that representation of the nonlinear kinetics by the first-order one is not quite acceptable except for the cases with $k_{m1} \gg S_1$ and $k_{m2} \gg S_2$ which may occur at the end of the reaction or when the initial substrate concentrations are very low.

3. Unsteady state model

As mentioned previously, the activity of enzymes, either in soluble or in immobilized form, tends to decrease during the reaction. The above analysis under steady state conditions is valid only if the denaturation time of immobilized enzymes is much longer than the reaction time under consideration. In many industrial applications, this may not be always the case. Hence, to explore the reaction behavior of the system where the denaturation time of immobilized enzymes is relatively short, an unsteady state model is needed.

The enzyme denaturation rate is rather complex function of the PH, temperature, enzyme concentration, substrate concentration and so forth [6]. Under most circumstances, a first-order kinetics is a good approximation [6,11]

$$dE_i/dt = -k_{ci}E_i, \quad i = 1,2, \tag{11}$$

in which E_i represents the concentration of the ith enzyme and k_{ci} the corresponding denaturation rate coefficient.

The substrate balance equations under unsteady state conditions can be obtained by incorporating the unsteady state terms into eqs. (2) and (3)

$$\frac{\partial S_1}{\partial t} = D\left(\frac{\partial^2 S_1}{\partial r^2} + \frac{2}{r}\frac{\partial S_1}{\partial r}\right) - \frac{k_1E_1S_1}{S_1+k_{m1}}, \tag{12}$$

$$\frac{\partial S_2}{\partial t} = D\left(\frac{\partial^2 S_2}{\partial r^2} + \frac{2}{r}\frac{\partial S_2}{\partial r}\right) + \frac{k_1E_1S_1}{S_1+k_{m1}} - \frac{k_2E_2S_2}{S_2+k_{m2}}, \tag{13}$$

where k_1 and k_2 are the turnover numbers for the first and second reactions and E_1 and E_2 the enzyme concentrations as given by eq. (11). The boundary conditions for the above equations are the same as those of eqs. (4) to (6) and the initial condition can be represented by

$$t = 0; \quad S_1 = S_2 = 0. \tag{14}$$

By combining with eq. (11) and casting into dimensionless forms, eqs. (12) to (14) become

$$\frac{\partial C_1}{\partial \tau} = \frac{\partial^2 C_1}{\partial R^2} + \frac{2}{R}\frac{\partial C_1}{\partial R} - \frac{\phi_1^2 C_1 \exp(-\beta_1\tau)}{C_1+K_{m1}}, \tag{15}$$

$$\frac{\partial C_2}{\partial \tau} = \frac{\partial^2 C_2}{\partial R^2} + \frac{2}{R}\frac{\partial C_2}{\partial R}$$

$$+\frac{\phi_1^2 C_1 \exp(-\beta_1 \tau)}{C_1 + K_{m1}} - \frac{\phi_2^2 C_2 \exp(-\beta_2 \tau)}{C_2 + K_{m2}} \qquad (16)$$

and

$$\tau = 0; \quad C_1 = C_2 = 0, \qquad (17)$$

$$R = 0; \quad \partial C_1/\partial R = \partial C_2/\partial R = 0, \qquad (18)$$

$$R = 1; \quad \frac{\partial C_1}{\partial R} = \mathrm{Sh}_1(1 - C_1), \quad \frac{\partial C_2}{\partial R} = \mathrm{Sh}_2(\alpha - C_2), \qquad (19)$$

where τ represents the dimensionless time, tD/r_0^2, β_1 and β_2 the dimensionless denaturation rate coefficients, $k_{e1}r_0^2/D$ and $k_{e2}r_0^2/D$. The nonlinear partial differential equations of eqs. (15) and (16) can be solved numerically by the Crank-Nicolson finite difference method [10] for C_1 and C_2 for different characteristic parameters. Some interesting results are shown in the following figures.

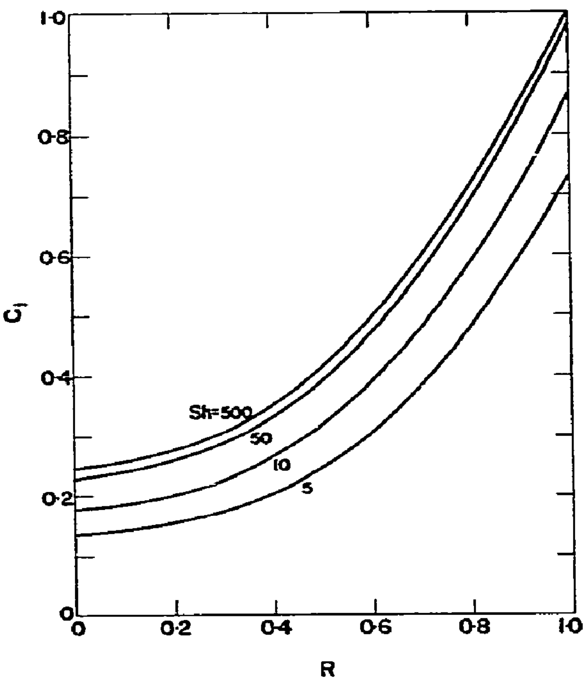

Fig. 2. Effect of the modified Sherwood number on the dimensionless concentration distributions of the first substrate with $\tau = 1.0$, $\beta_1 = \beta_2 = 1$, $\phi_1 = \phi_2 = 5$, $K_{m1} = K_{m2} = 0.5$, $\alpha = 0$ and $\mathrm{Sh}_1 = \mathrm{Sh}_2 = \mathrm{Sh}$.

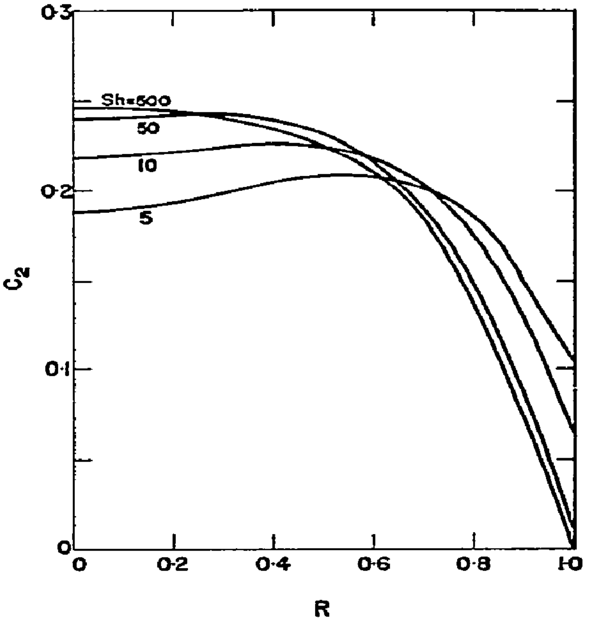

Fig. 3. Effect of the modified Sherwood number on the dimensionless concentration distributions of the second substrate with the same parameters as used in fig. 2.

Figs. 2 and 3, respectively, show the effect of the modified Sherwood number on the dimensionless concentration distributions of the first and second substrates. In these and the following figures, the modified Sherwood numbers are assumed to be the same for both reactions, i.e. $\mathrm{Sh}_1 = \mathrm{Sh}_2$. It is seen that the first substrate concentration increases steadily with increasing modified Sherwood number. As the modified Sherwood number increases, the external mass transfer resistance decreases in importance and this tends to facilitate the diffusion of the substrate into the immobilized enzyme particle. As can be seen in fig. 2, the dimensionless substrate concentration at the particle surface is very close to one for $\mathrm{Sh} > 50$ implying that at this high modified Sherwood number, the external mass transfer resistance is negligible. This situation will be realized in a well-stirred tank reactor where the stagnant liquid film outside the particle is reduced to minimum.

Fig. 3 demonstrates the dimensionless concentration distributions of the second substrate pertaining to the same kinetic parameters of fig. 2. It is of interest to note that the central substrate concentration at low modified Sherwood number is lower than that at a high one while the situation at the surface is reversed.

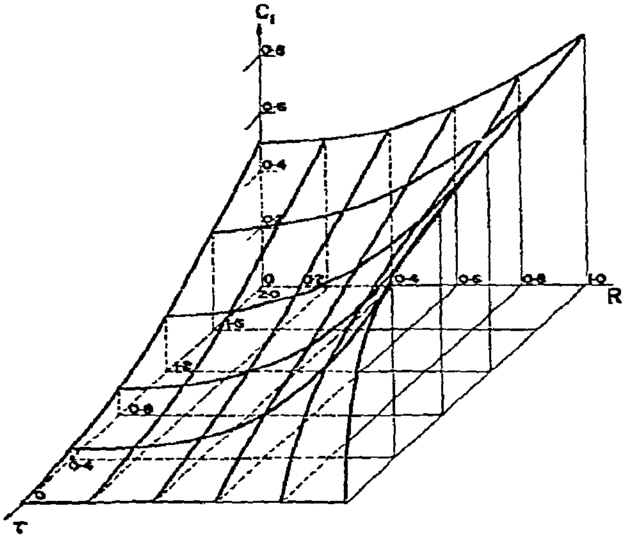

Fig. 4. Transient responses of the dimensionless concentration of the first substrate with the same parameters as used in fig. 2.

At high modified Sherwood number, the concentration of the first substrate inside the particle is high as shown in fig. 2 because of reduced external mass transfer resistance, hence more second substrate is generated. However, at the particle surface, the second substrate concentration must become closer to that in the bulk liquid phase, which is assumed to be zero here, as the modified Sherwood number increases. This is the reason the second substrate concentration at the surface increases with decreasing modified Sherwood number.

The dimensionless concentration of the first substrate against the dimensionless radial coordinate and the dimensionless time is displayed in fig. 4 for a fixed Thiele modulus and modified Sherwood number. The dimensionless substrate concentration increases more rapidly with increasing dimensionless time at the surface than at the particle center. This is anticipated because the step change of substrate concentration at the surface takes some time to reach the particle center. The substrate concentration profile increases steadily with increasing dimensionless time and tends to asymptotically approach one at very large τ. This is due to the fact that after very long reaction time, the immobilized enzymes will be completely denatured and the whole immobilized enzyme particle is full of both substrates.

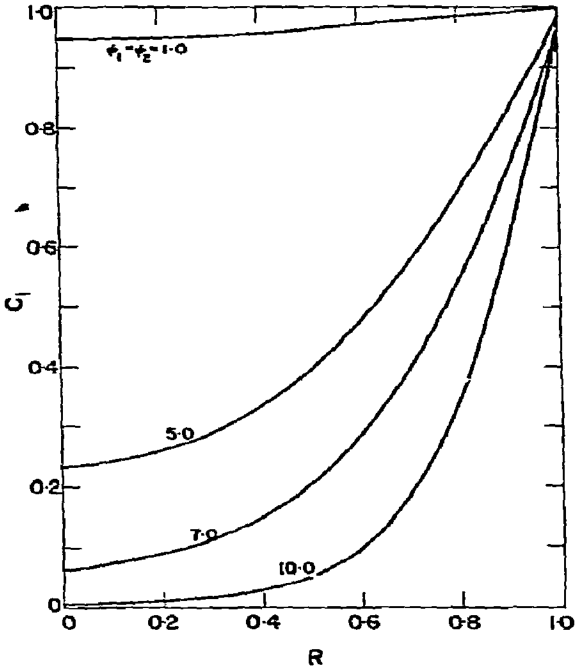

Fig. 5. Effect of the Thiele modulus on the dimensionless concentration distributions of the first substrate with $Sh_1 = Sh_2 = 50$, $K_{m1} = K_{m2} = 0.5$, $\beta_1 = \beta_2 = 1$, $\tau = 1$ and $\alpha = 0$.

The effect of the Thiele modulus on both substrate concentration distributions are shown in figs. 5 and 6 for a particular dimensionless time of 1.0. Fig. 5 displays that the concentration distribution of the first substrate increases with decreasing Thiele modulus because of reduced reaction rates. At a low Thiele modulus of 1.0, the substrate concentration is rather high implying low substrate conversion. It is also observed that at this particular dimensionless time, the substrate concentration decreases so rapidly for Sh > 10 that it is completely consumed before it reaches the particle center. The radius where the substrate concentration approaches zero is generally called the critical radius. For a fixed Thiele modulus, the critical radius, if existing, will decrease with increasing dimensionless reaction time because of decreasing enzyme activity.

The dimensionless concentration distribution of the second substrate in fig. 6 is fairly low at $\phi = 1.0$ when compared to others at higher ϕ. This is due to low conversion of the first substrate as mentioned

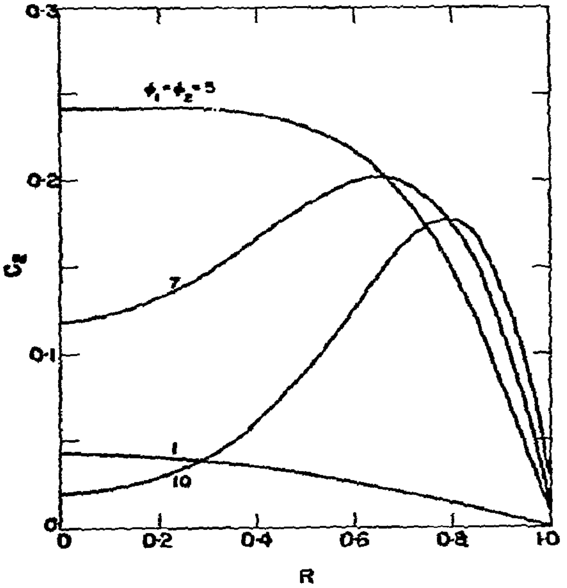

Fig. 6. Effect of the Thiele modulus on the dimensionless concentration distributions of the second substrate with the same parameters as used in fig. 5.

previously. However, it is not very clear why the central substrate concentration at $\phi = 5.0$ is higher than that at $\phi = 7.0$ which in turn is higher than that at $\phi = 10.0$. It might be due to high conversion of the second substrate to final product at high Thiele modulus. The reason the substrate concentration profiles for $\phi = 7.0$ and 10.0 reach a maximum is not very clear either.

References

[1] R.G. Carbonnel and M.D. Kostin, AIChE J. 18 (1972) 1.
[2] B. Atkinson, *Biochemical* Reactors (Pion Press, London, 1974).
[3] S.H. Lin, Biotechnol. Bioeng. 17 (1975) 1237.
[4] T. Kobayshi and K.J. Laidler, *Biotechnol.* Bioeng. 16 (1974) 77.
[5] S.A. Wakman and W.C. Davison, Enzymes (Williams and Wilkins, Baltimore, 1926).
[6] K.J. Laidler and P.S. Bunting, The chemical kinetics of enzyme action (Oxford University Press, London, 1973).
[7] R. Goldman and E. Katchalski, T. Theor. Biol. 32 (1971) 243.
[8] S.H. Lin, Comp. Biol. Med. 2 (1972) 329.
[9] R. Krishna and P.A. Ramachandran, J. Appl. Chem. Biotechnol. 25 (1975) 623.
[10] L. Lapidus, Digital computation for chemical engineers (McGraw-Hill, New York, 1962).
[11] S.P. O'Neill, Biotechnol. Bioeng. 14 (1972) 201.