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CONSECUTIVE IMMOBILIZED ENZYMATIC REACTIONS IN CONTINUOUS STIRRED TANK REACTOR SYSTEMS

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The performance characteristics of two-enzyme reaction in a continuous stirred tank reactor (CSTR) are analytical investigated in this work. A model is formulated to describe the substrate concentration variations by taking into account the external and internal diffusion resistances. It is found that the reaction system exhibits the characteristics of reaction control or diffusion control depending on the operating conditions. The single CSTR model is also extended to describe the multiple CSTR system. The latter model enables the prediction of the number of CSTRs in series required to achieve a prescribed substrate conversion.

1. Introduction

Recent advances in immobilized enzyme technology have made considerable progress towards the economical utilization of enzymes in large-scale industrial processes, especially in food processing industries [1–3]. The main advantage of using immobilized enzymes is the recoverability of these enzymes for reuse in continuous flow rector systems.

A study of the previous investigations indicates that much attention has focused on biochemical reactions with single enzymes. In fact, multi-enzyme reactions also occur frequently. For example, hydrolytic conversion of carbohydrates to lower molecular weight products can be efficiently carried out via multi-enzyme reactions. In spite of their importance, multi-enzyme reactions have received far less attention when compared to those with single enzyme. Goldman and Katchalski [4], Hultin [5], Martensson [6], Krishna and Ramachandran [7] and Lin [8,9] were among the investigators examining the theoretical and experimental aspects of these problems. These works mainly investigated the characteristic of immobilized multi-enzyme reactions in a spherical or planar solid support. Only one previous work [8] has considered the immobilized multi-enzyme reactions in a packed-bed reactor.

For optimum design of continuous reactor systems, relevant information regarding the performance characteristics of multi-enzyme reactions in the reactor is very important. The previous work by this author [8] concerning two-enzyme reaction in a packed-bed reactor assumed that the substrate diffusion resistance inside the particle are negligible, which was not quite right, as shown by other investigations [4,7,9]. The purpose of this work is to examine the effects of operating conditions and kinetic parameters on the substrate conversion on immobilized two-enzyme reaction in continuous stirred tank reactor (CSTR) systems. Although the CSTR systems have been generally considered to be less efficient than the packed-bed reactor in terms of substrate conversion, they have many advantages over the latter. These advantages include better control of operating pH and temperature which are essential in most biochemical reactions, easy replacement of immobilized enzymes, low cost and high flexibility because of relative simplicity and being able to handle colloidal and insoluble substrates which otherwise will cause operational difficulties in a packed-bed reactor. In view of this, it may be of practical interest to know the substrate conversion characteristics of immobilized two-enzyme reaction in these reactor systems.

2. Substrate conversion in a single CSTR

The consecutive reactions are assumed to be represented by

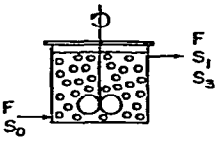
$$S \xrightarrow{\text{enzyme 1}} P_1 \xrightarrow{\text{enzyme 2}} P_2$$

in which S represents the initial substrate, P_1 the product from the first reaction and the substrate for the second one and P_2 the final desired product. The schematic diagram of a single CSTR is shown in fig. 1(a) and the enlarged immobilized enzymatic particle with two substrate distributions shown in fig. 1(b). By assuming that the enzymes are uniformly immobilized in the particle, the steady state material balances for each substrate in the reactor and in the particle are represented by

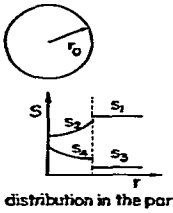
$$FS_0 - FS_1 - \frac{3\alpha V\epsilon}{r_0}\left(D\left.\frac{dS_2}{dr}\right|_{r=r_0}\right) = 0\,, \tag{1}$$

$$-FS_3 - \frac{3\alpha V\epsilon}{r_0}\left(D\left.\frac{dS_4}{dr}\right|_{r=r_0}\right) = 0\,, \tag{2}$$

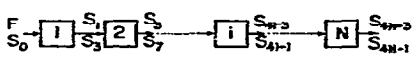
$$D\left(\frac{d^2S_2}{dr^2} + \frac{2}{r}\frac{dS_2}{dr}\right) - \frac{V_2S_2}{S_2 + k_{m2}} = 0\,, \tag{3}$$



(a) Single CSTR system



(b) Substrate distribution in the particle



(c) Multiple CSTR system

Fig. 1. Schematic diagram of reactor configuration.

$$D\left(\frac{d^2S_4}{dr^2} + \frac{2}{r}\frac{dS_4}{dr}\right) + \frac{V_2S_2}{S_2 + k_{m2}} - \frac{V_4S_4}{S_4 + k_{m4}} = 0\,, \tag{4}$$

and the boundary conditions are

$$r = 0; \qquad dS_2/dr = dS_4/dr = 0 \tag{5}$$

$$r = r_0; \quad D\,dS_2/dr = h_2(S_1 - S_2)\,, \tag{6}$$

$$D\,dS_4/dr = h_4(S_3 - S_4)\,. \tag{7}$$

In the above equations, S_1 and S_2 are, respectively, the concentrations of the first substrate in the bulk and solid phases, S_3 and S_4 the corresponding concentrations of the second substrate, F the volumetric flow rate, S_0 the inlet substrate concentration, α the fraction of the reactor volume occupied by the immobilized enzymatic particles, V the volume of reactor, ϵ the porosity of the particle, r_0 the particle radius, D the effective molecular diffusivity, V_2 and V_4 the maximum reaction rates, k_{m2} and k_{m4} the Michaelis constants, h_2 and h_4 the mass transfer coefficients and r the radial coordinate.

By introducing the following dimensionless parameters and variables

$$C_i = S_i/S_0, \quad R = r/r_0, \quad \beta = 3\alpha V\epsilon D/r_0^2F$$

$$\phi_i = (V_i r_0^2/DS_0)^{1/2}, \quad K_{mi} = k_{mi}/S_0, \quad Sh_i = h_i r_0/D$$

Eqs. (1) to (7) can be cast into the following forms

$$1 - C_1 - \beta(dC_2/dR\,|_{R=1}) = 0 \tag{8}$$

$$-C_3 - \beta(dC_4/dR\,|_{R=1}) = 0 \tag{9}$$

$$\frac{d^2C_2}{dR^2} + \frac{2}{R}\frac{dC_2}{dR} - \frac{\phi_2^2C_2}{C_2 + K_{m2}} = 0 \tag{10}$$

$$\frac{d^2C_4}{dR^2} + \frac{2}{R}\frac{dC_4}{dR} + \frac{\phi_2^2C_2}{C_2 + K_{m2}} - \frac{\phi_4^2C_4}{C_4 + K_{m4}} = 0 \tag{11}$$

and

$$R = 0; \qquad dC_2/dR = dC_4/dR = 0 \tag{12}$$

$$R = 1; \qquad dC_2/dR = \mathrm{Sh}_2(C_1 - C_2) \tag{13}$$

$$dC_4/dR = \mathrm{Sh}_4(C_3 - C_4)\,. \tag{14}$$

Although C_1 and C_3 are involved in the boundary conditions, eqs. (13) and (14), they can be eliminated by

using eqs. (8) and (9). Hence,

$$R = 1; \qquad \frac{dC_2}{dR} = \frac{Sh_2}{1+\beta Sh_2}(1-C_2), \qquad (13a)$$

$$\frac{dC_4}{dR} = -\frac{Sh_4}{1+\beta Sh_4} C_4 . \qquad (14a)$$

Eqs. (10) and (11) subject to eqs. (12), (13a) and (14a) are then independent of C_1 and C_3 and can be integrated numerically by an iterative Runge–Kutta method [10] on a digital computer to give the dimensionless concentration distributions of the two substrates.

An overall substrate balance around the reactor gives

$$S_0 = S_1 + S_3 + P, \qquad (15)$$

where P is the concentration of the final product. The percentage substrate conversion can be obtained by rearranging eq. (15)

$$\text{Substrate conversion} = P/S_0 = 1 - (C_1 + C_3), \qquad (16)$$

which can be combined with eqs. (8) and (9) to yield

Substrate conversion

$$= \beta(dC_2/dR|_{R=1}) + \beta(dC_4/dR|_{R=1}) . \qquad (17)$$

Hence, when the substrate concentration gradients at the particle surface are known, the substrate conversion can be readily calculated from eq. (17). For simplicity, it is assumed in the following figures that $Sh_1 = Sh_2 = Sh$, $K_{m1} = K_{m2} = K_m$ and $\phi_1 = \phi_2 = \phi$.

Fig. 2 shows the substrate conversion against the Thiele modulus, ϕ, for different modified Sherwood numbers, Sh. According to the definition, the modified Sherwood number represents the relative magnitude of the internal to the external substrate diffusion resistances. Since the internal substrate diffusion resistance always exists to certain extent for a finite immobilized enzymatic particle, the magnitude of the modified Sherwood number mainly depends on the external substrate diffusion resistance, which in turn depends on the operating conditions of the reactor. If rigorous agitation is provided in the reactor, the external substrate diffusion resistance can be reduced to minimum and the modified Sherwood number may become very large. Under this circumstance, the boundary conditions in eqs. (13) and (14) reduce to $C_1 = C_2$ and $C_3 = C_4$. In real situations, these conditions may be difficult to realize and the modified Sherwood number plays an important role in regulating the substrate diffusions. Bird et al. [11] have shown that the modified Sherwood number is equal to two for a single particle suspended in an infinite, motionless liquid. This constitutes the lower bound for the present case and under normal operating conditions, the modified Sherwood number will be expected to be larger than this figure. It is apparent in fig. 2 that the substrate conversion is significantly enhanced as the modified Sherwood number increases. Especially at high Thiele modulus, the effect of the modified Sherwood number on the substrate conversion is much more pronounced. It is noted that difference in the substrate conversions at different modified Sherwood numbers greater than 50 is much less significant compared to that at a lower Sh. This implies that the external substrate diffusion resistance may be neglected for a modified Sherwood number greater than 50.

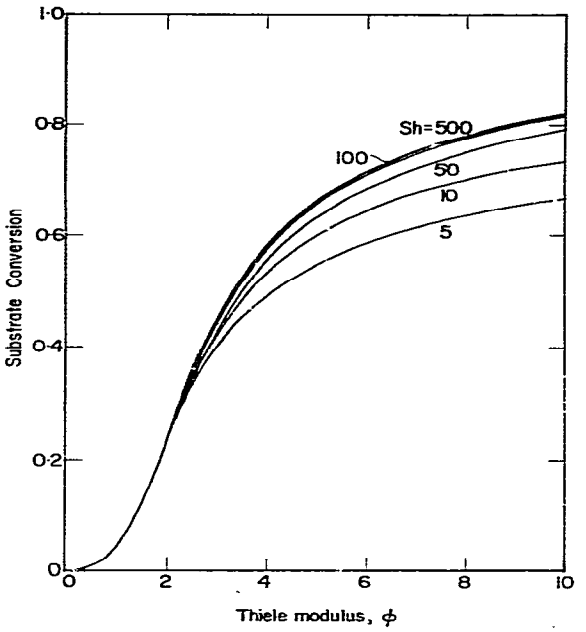


Fig. 2. Effect of the modified Sherwood number on the substrate conversion in a single CSTR with $\beta = 0.6$ and $K_m = 0.5$.

It is also observed in fig. 2 that the effect of the modified Sherwood number on the substrate conversion is only appreciable at a Thiele modulus greater

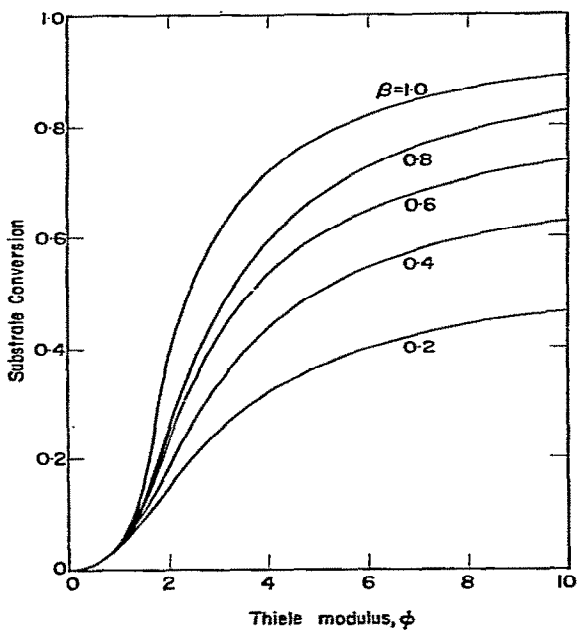


Fig. 3. Effect of the dimensionless parameter, β, on the substrate conversion in a single CSTR with Sh = 10 and $K_m = 0.5$.

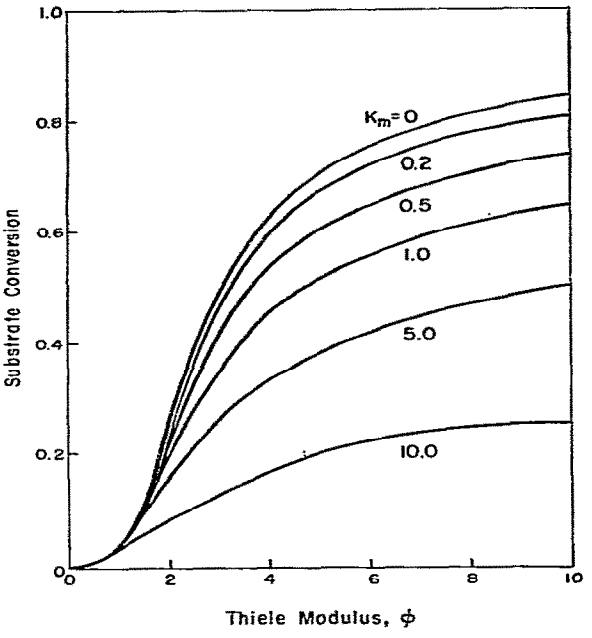


Fig. 4. Effect of the dimensionless Michaelis constant on the substrate conversion in a single CSTR with Sh = 10 and $\beta = 0.6$.

than 3. Below that value, the effect of the modified Sherwood number is very small. In addition, at large Thiele modulus, the substrate conversion curves tend to level off. Since the Thiele modulus includes the maximum reaction rates, the diminishing effect of high Thiele modulus on the substrate conversion signifies that the reaction is diffusion controlled. In other words, the process of substrate diffusion is much slower than the substrate consumption by the enzymatic reactions inside the particle and becomes predominating in determining the overall reaction speed. On the other hand, at low Thiele modulus, the process becomes reaction controlled because of diminishing effect of the modified Sherwood number.

The effect of the dimensionless parameter, β, on the substrate conversion is shown in fig. 3. It seems that the effect of β on the substrate conversion is more pronounced than that of the modified Sherwood number. The substrate conversion curves tend to level off at high Thiele modulus too, implying the predominating effects of the substrate diffusion resistances on the substrate conversion. At low end of the Thiele modulus, however, the characteristics of reaction control appear only at $\phi = 1.0$ or less.

There are two extreme cases representing the nonlinear Michaelis–Menren rate equation used in eqs. (3) and (4). If k_{mi} is much smaller than S_i, the reaction rate becomes a zero order expression. On the other hand, if k_{mi} is much larger than S_i, the reaction rate reduces to a linear one. These two extreme cases have been frequently employed in many of the previous investigations in evaluating the effectiveness factor in an immobilized enzyme particle. For these instances, the governing material balance equation becomes linear and analytical solution can be obtained. For the present work, it might be of interest to see how the Michaelis constant, k_{mi}, affects the substrate conversion. In fig. 4, the substrate conversion is plotted against the Thiele modulus using dimensionless Michaelis constant as parameter. Since the dimensionless substrate concentration is less than one inside the immobilized enzyme particle, the case with $K_m = 10$ perhaps is suffi-

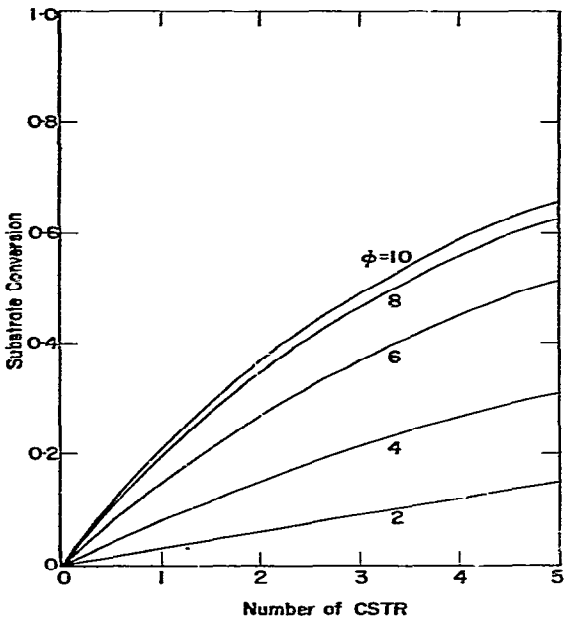


Fig. 5. Effect of the Thiele modulus on the substrate conversion in a five-reactor system with $\beta = 0.5$, Sh = 10 and $K_m = 0.5$.

cient to exhibit the performance characteristics of a first order reaction. On the other hand, the zero order reaction is apparently represented by the curve with $K_m = 0$, which yields the maximum substrate conversion as anticipated. This figure also reveals that as the Thiele modulus is further increased beyond 10, all the substrate conversion curves tend to approach a constant value and the reaction becomes diffusion controlled. The characteristics of reaction control appear at a Thiele modulus less than or equal to one.

3. Substrate conversion in multiple CSTR system

As evidenced in the previous figures, the substrate conversion in a single CSTR is quite low at low Thiele modulus and/or low value of β. For these instances, the substrate conversion may not be able to meet the commercial requirement. In order to enhance the substrate conversion, more reactors are needed. In fact, multiple reactor systems have been reported in use in the literature. Pirt and Callow [12] found that the penicillin production is significantly increased in a two-reactor system. Sykyta et al. [13] recommended a three-reactor system for the streptomycin production. Much better substrate conversion could also be obtained in the production of protease by using a two-reactor system [14].

The mathematical model developed in the previous section can be extended to describe the multiple CSTR systems. The reactor sequence is shown in fig. 1(c). For this case, eqs. (10) and (11) subject to eqs. (12), (13a) and (14a) describe the first CSTR. From the second reactor on, the following equations apply

$$\frac{d^2C_{4i-2}}{dR^2} + \frac{2}{R}\frac{dC_{4i-2}}{dR} - \frac{\phi^2_{4i-2}C_{4i-2}}{C_{4i-2} + K_{m,4i-2}} = 0 \qquad (18)$$

$$\frac{d^2C_{4i}}{dR^2} + \frac{2}{R}\frac{dC_{4i}}{dR} + \frac{\phi^2_{4i-2}C_{4i-2}}{C_{4i-2} + K_{m,4i-2}} - \frac{\phi^2_{4i}C_{4i}}{C_{4i} + K_{m,4i}} \qquad (19)$$

subject to

$$R = 0; \quad \frac{dC_{4i-2}}{dR} = \frac{dC_{4i}}{dR} = 0 \qquad (20)$$

$$R = 1; \quad \frac{dC_{4i-2}}{dR} = \frac{Sh_{4i-2}}{1 + \beta Sh_{4i-2}}(C_{4i-7} - C_{4i-2}) \qquad (21)$$

$$\frac{dC_{4i}}{dR} = \frac{Sh_{4i}}{1 + \beta Sh_{4i}}(C_{4i-5} - C_{4i}) \qquad (22)$$

$$i = 2, 3, 4, \ldots, N$$

and the total substrate conversion is given by

$$\text{Substrate conversion} = \beta \sum_{i=2}^{N} [(dC_{4i-2}/dR|_{R=1}) + (dC_{4i}/dR|_{R=1})] \qquad (23)$$

In the above equations, C_{4i-2} represents the concentration of the first substrate in the particle and C_{4i} that of the second substrate. It is tacitly assumed in the above formulation that all the reactors are identical in size and shape and under the same operating conditions. This set of equations can also be solved by the iterative Runge–Kutta method [10] for any number of CSTR in series.

Fig. 5 demonstrates the substrate conversion at different values of ϕ for five CSTRs in series. The sub-

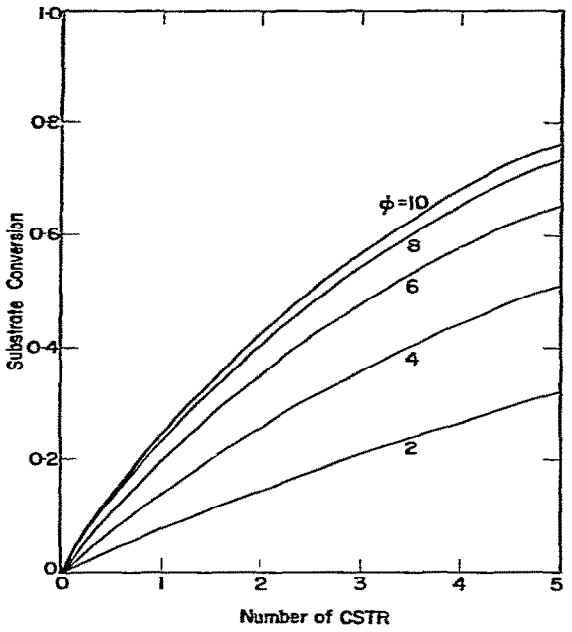


Fig. 6. Effect of the Thiele modulus on the substrate conversion in a five reactor system with $\beta = 0.5$, Sh = 100 and $K_m = 0.5$.

strate conversion increases with increasing number of CSTR, as anticipated. It appears that the substrate conversion increases linearly number of CSTR at a Thiele modulus less than two. However, nonlinear increase in the substrate conversion begins to appear at $\phi > 2$. For a fixed number of reactor, the substrate conversion seems do not improve much for a Thiele modulus greater than 8. This is in agreement with the previous observation that at high Thiele modulus, the reaction process becomes diffusion controlled and the substrate conversion can be improved only by reducing the external substrate diffusion resistance.

Similar information is displayed in fig. 6 for a higher modified Sherwood number of 100. The substrate conversion in this figure is much higher than the corresponding one in fig. 5. This is due to the reduced external substrate diffusion resistance at high modified Sherwood number. This figure shows similar substrate conversion characteristics to the previous one with the presence of diffusion controlling at high Thiele modulus.

4. Concluding remarks

An analytical procedure is presented in the present work for predicting the substrate conversion in a continuous stirred tank reactor. The present model is rigorous because of inclusion of the internal and external substrate diffusion resistances. The single CSTR model is also extended to describe the multiple CSTR system which enables the estimation of the number of CSTR in series needed to achieve a certain desired substrate conversion.

Numerical simulation of the model reveals that the reaction system is diffusion controlled for a Thiele modulus greater than ten. However, at a Thiele modulus less than three, the system becomes reaction controlled. Under most circumstances, neither reaction controlling nor diffusion controlling is expected to predominate. It is also observed that at low Thiele modulus and/or low value of β, multiple CSTR systems are needed if a certain prescribed substrate conversion is to be realized.

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