J. appl. Chem. Biotechnol. 1975, **25**, 623–640

Analysis of Diffusional Effects in Immobilised Two-enzyme Systems

R. Krishna[a] and P. A. Ramachandran[b]

[a] *Department of Chemical Engineering, University of Manchester Institute of Science and Technology, Manchester M60 1QD, and* [b] *Department of Chemical Engineering, University of Salford, Salford M5 4WT*

(*Paper received 18 November 1974 and accepted 22 January 1975*)

The effect of diffusional resistances (both inter- and intramembrane) on the kinetic behaviour of an immobilised two-enzyme system carrying out a consecutive sequence of reactions $S \rightarrow P_1 \rightarrow P_2$ is analysed for the case in which the intrinsic kinetics can be considered to be first order. The method of solution consists in first representing the diffusion–reaction equations in matrix notation and applying the similarity transformation to reduce the equations to a set of uncoupled equations in pseudoconcentrations. The set of uncoupled equations are solved analytically for the appropriate boundary conditions. The solution to the original problem is then recovered by applying the reverse transformation. Two geometries have been treated, the spherical capsule and the flat plate membrane. The diffusional resistances may be described adequately by two dimensionless numbers, the Thiele modulus and modified Sherwood number. The influence of these parameters on the concentration profiles and "effectiveness factors" have been presented graphically. The analysis is also extended to the prediction of the performance of a packed bed enzyme reactor.

1. Introduction

Water-insoluble enzyme derivatives (immobilised enzymes) are finding increasing scientific and technological applications. The study of immobilised enzymes is also of importance in understanding the *in vivo* behaviour of enzymes because many enzymes act *in vivo* while embedded in membranes or attached to subcellular particles. The kinetic behaviour of immobilised enzymes is different from that of the enzyme in solution because of the rate limitations imposed by diffusion. In addition, the intrinsic kinetics of some enzymic reactions may be modified on immobilisation due to steric effects, chemical modification of the active groups of the enzyme due to covalent bonding with the solids, etc. The mathematical analysis of the diffusion with chemical reaction in enzymes and packed enzyme columns is necessary for understanding the quantitative behaviour of these systems. The main theoretical work in this area has been confined to reactions with single enzyme systems catalysing simple single step reactions.[1, 2]

Reactions in biological systems often follow a consecutive reaction sequence or a complex network of consecutive, parallel and/or cyclic reactions. Each of these reactions is catalysed by different enzymes each specific to a particular reaction and the product of an enzymic reaction acts as a substrate for another enzyme. A number of approaches can be used for the simulation of the behaviour and industrial application of multi-enzyme systems. The enzymes can be arranged in a sequence in a packed column or alternatively a battery of enzyme columns may be used. For example, Brown *et al.*[3] have studied four entrapped enzymes participating in the oxidation of glucose, namely hexokinase, phosphogluco-isomerase, phosphofructo-kinase and aldolase, each enzyme arranged in separate sections within a column. The kinetic investigation of a two-enzyme system, pyruvate kinase and lactate dehydrogenase with each enzyme bound to separate filter paper disks, has also been made.[4] The kinetic behaviour of these systems can be modelled on lines similar to that for single enzyme systems, since only one enzyme is acting at any position in the column or in any given battery.

A more efficient method of operation is to bind two or more enzymes to the same matrix so that the substrate for the second enzyme is generated *in situ* as the first step in the reaction occurs. Mosbach and Mattiasson[5] have developed techniques for the preparation of matrix bound multi-enzyme systems. Kinetic investigation of a two-enzyme system consisting of hexokinase and glucose-6-phosphate dehydrogenase covalently bound to acrylamide beads has been carried out by these authors. A three-enzyme system, β-galactosidase, hexokinase and glucose-6-phosphate-dehydrogenase, all covalently bound to the same matrix has also been investigated.[6] A useful new approach in the biochemical processing industry is the utilisation of entrapped bacterial cells. This has the advantage that both the enzymes and the coenzymes required for the reaction are present at the same site and expensive operating procedures involving separate addition of the coenzymes to the reacting media is avoided. This might open up new areas in industrial production of many organic chemicals. For example, industrial production of steroids often involves two or more steps, each step involving a specific enzyme. Mosbach and Larsson[7] have studied the behaviour of entrapped cells which catalyse a sequence of consecutive reactions involved in the production of steroids such as 11β-hydroxylation and Δ-1,2-dehydrogenation.

From the above discussion it is evident that the kinetics of systems where two or more enzymes are bound to the same matrix is of immense importance. However, a detailed theoretical study of these systems has not yet been made. Goldman and Katchalski[8] have analysed a simple set of consecutive reactions, $S \rightarrow P_1 \rightarrow P_2$, for a two-enzyme system attached to an impermeable membrane. The kinetic behaviour of this system is affected by diffusion through the unstirred layer at the membrane–solution interface. If the membrane were permeable to both the substrate and the products, then intramembrane diffusion would be a significant factor to consider in addition to the resistance offered by the unstirred layer. It is the purpose of this paper to attempt a detailed theoretical study of two-enzyme systems and enzyme columns. Such an analysis will be necessary in both interpretation of laboratory experimental data and in the design of industrial enzyme reactors.

2. Outline of model

Consider a consecutive reaction scheme

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

taking place inside an immobilised two-enzyme capsule or membrane. The two enzymes 1 and 2 are considered to be uniformly distributed inside the membrane, which is assumed to be permeable to all three species. S denotes the substrate for enzyme 1 and P_1 and P_2 denote the products formed by enzyme 1 and enzyme 2 respectively. In the first instance the analysis of one such membrane (capsule) is presented and the treatment for a packed bed enzyme reactor is given in section 7. Two specific geometries are considered (Figure 1), the spherical capsule and the

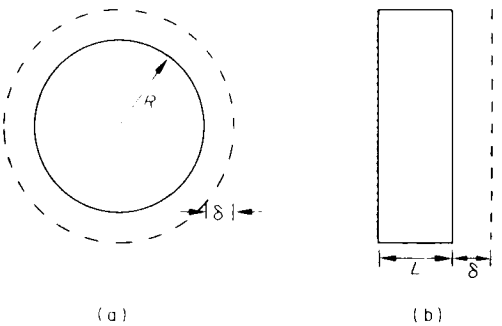

Figure 1. Geometry of immobilised two-enzyme system: (a) spherical capsule of radius R; (b) flat plate membrane of thickness L. δ is the thickness of the Nernst diffusion layer.

flat plate membrane. These two shapes are the ones most commonly encountered in practice. By defining appropriate shape factors the theoretical analysis of this paper can be extended to systems consisting of other geometrical shapes.

The membrane system is immersed in a solution in which the bulk concentrations are S_b, P_{1b} and P_{2b}. If there are no diffusional limitations of any kind for the transport of each of the three species, then the rates of formation of the individual species may be written down using the usual Michaelis–Menten kinetic model

$$r_S = \frac{dS}{dt} = -\frac{k_1 E_1 S}{K_{m1} + S} \tag{2}$$

$$r_{P_1} = \frac{dP_1}{dt} = \frac{k_1 E_1 S}{K_{m1} + S} - \frac{k_2 E_2 P_1}{K_{m2} + P_1} \tag{3}$$

$$r_{P_2} = \frac{dP_2}{dt} = \frac{k_2 E_2 P_1}{K_{m2} + P_1} \tag{4}$$

where r_S, r_{P_1}, r_{P_2} represent the rates of formation of each species (mol cm^{-3} s^{-1}); S, P_1, P_2 represent the concentration of the species at any reacting site (mol/cm^3);

E_1, E_2 are the enzyme concentrations (mol/cm^3); and K_{m1}, K_{m2} are the Michaelis–Menten constants associated with enzymes 1 and 2 respectively (mol/cm^3).

In practice one is usually interested in the concentration–time paths and the rates of formation of the products. Given the initial conditions, the above equations may be integrated by appropriate numerical techniques to obtain the required information. However, the assumption of no diffusional limitations is one which cannot usually be substantiated. There are two types of diffusional rate limitations in practice:

a. *Intermembrane mass transfer resistance:* i.e. resistance to transport of reactant species from the bulk solution to the solid–fluid interface and the resistance to the transport of product species from the interface to the bulk solution. If one adopts the usual "film" model for mass transfer, such resistances are assumed to be localised in a thin region near the solid surface (Nernst diffusion layer). The bulk solution surrounding this region is assumed to be well mixed. The interparticle resistance is described by a mass transfer coefficient, k_b, which can usually be estimated from the geometry, system properties and flow conditions. The mass transfer coefficient corresponds to the quantity D_b/δ, where D_b is the diffusion coefficient in the bulk fluid phase and δ is the thickness of the diffusion layer. It is assumed that each species experiences the same mass transfer limitation, which is equivalent to assuming equal diffusion coefficients for the three species.

b. *Intramembrane diffusional resistance:* the reacting species arriving at the solid–fluid interface has to diffuse inside the permeable membrane (or enzyme capsule) to the various reactive sites within it. The products have similarly to diffuse out of the reacting sites to arrive at the solid–fluid interface whence they are transferred back to the main fluid stream. The intramembrane diffusional resistance is usually described by a diffusion coefficient D. Again, it is assumed that the diffusion coefficient is identical for each species. This is a realistic assumption especially when the three species S, P_1 and P_2 have molecular sizes close to one another.

The inclusion of two diffusional transport resistances makes an analytical analysis of the general problem difficult. It was thought instructive first to tackle the problem for the case where the kinetics of the reaction may be considered linear. This situation would correspond to the case when the following conditions are satisfied: (a) the enzyme concentrations E_1 and E_2 are constant; (b) $K_{m1} \gg S$, and (c) $K_{m2} \gg P_1$. The first requirement is easily met with an insoluble immobilised enzyme. The other two conditions are usually satisfied near the end of the reaction or for the case of low initial substrate concentration.

In a pioneering paper, Goldman and Katchalski[8] have analysed the two-enzyme system based on the linear kinetic approximation mentioned above. Their analysis is restricted to the case where: (a) the enzymes are attached to a flat membrane, impermeable to both reactants and products; (b) the concentration of substrate S in the bulk of the solution is constant throughout the enzymic reaction, and (c) the initial concentrations of the products in the bulk solution (P_{1b} and P_{2b}) are zero. In practice, it is usual to have a spherical encapsulated enzyme in addition to the flat plate configuration. Further, it is difficult to keep the substrate concentration in the bulk liquid constant as required by the Goldman–Katchalski analysis. It is

necessary to relax these assumptions and develop a general analysis incorporating both inter- and intramembrane (capsule) diffusional resistances, while retaining a linear kinetic scheme. The linearised analysis should provide a limiting case of the general non-linear kinetics and may have usefulness in determining the experimental kinetics when the initial substrate concentration may be chosen to be sufficiently small. One major advantage of the linear analysis is that an *analytical* solution to the problem can be obtained.

3. Theoretical analysis

The differential equations describing the intramembrane transport and reaction can be formulated as follows

$$D\nabla^2(S)=k_1S \tag{5}$$

$$D\nabla^2(P_1)=-k_1S+k_2P_1 \tag{6}$$

$$D\nabla^2(P_2)=-k_2P_1 \tag{7}$$

which emphasise the fact that at any point within the membrane the rate of diffusional transport and chemical reaction must be balanced at steady state. The Laplacian (∇^2) of the concentrations assumes different forms in different coordinate systems. k_1 and k_2 are the first order rate constants.

The equations (5), (6) and (7) may be most conveniently represented in matrix notation as follows

$$D\nabla^2(C)=[K](C) \tag{8}$$

where (C) represents the column matrix of concentrations,

$$(C)\equiv\begin{pmatrix}S\\P_1\\P_2\end{pmatrix} \tag{9}$$

and $[K]$ is the matrix of reaction rate constants,

$$[K]\equiv\begin{bmatrix}k_1 & 0 & 0\\-k_1 & k_2 & 0\\0 & -k_2 & 0\end{bmatrix} \tag{10}$$

The boundary conditions for the differential equation (8) are specified for the spherical and flat plate membranes (Figure 1) as follows:

$$b.c.\ 1:\ \textit{sphere}\ \text{at}\ r=0,\ \frac{\partial(C)}{\partial r}=(0) \tag{11a}$$

$$\textit{flat plate}\ \text{at}\ x=0,\ \frac{\partial(C)}{\partial x}=(0) \tag{11b}$$

Equation (11a) expresses the fact that at the centre of the sphere there can be no flux of mass and similarly equation (11b) shows that there can be no mass flux penetrating the solid wall ($x=0$) on to which the membrane is attached.

$$b.c.\ 2:\quad \textit{sphere}\ \text{at}\ r=R,\ (C)=(C)_R \tag{12a}$$

$$\textit{flat plate}\ \text{at}\ x=L,\ (C)=(C)_L \tag{12b}$$

The solid-fluid interface compositions, $(C)_R$ and $(C)_L$, are usually unknown and therefore these must be expressed in terms of the bulk solution concentrations, $(C)_b$. This is done by invoking the fact that there must be continuity of fluxes at the solid surface, that is, the diffusive flux into the membrane must equal the external mass transport flux,

$$b.c.\ 2'\text{:}\quad \textit{sphere}\quad D\left.\frac{\partial(C)}{\partial r}\right|_{r=R}=k_b\{(C)_b-(C)_R\} \tag{13a}$$

$$\textit{flat plate}\quad D\left.\frac{\partial(C)}{\partial x}\right|_{x=L}=k_b\{(C)_b-(C)_L\} \tag{13b}$$

The major problem associated with the solution of the differential equation (8) with the boundary conditions (11)–(13) is that the equations are coupled to one another, for the matrix of reaction rate constants, $[K]$, has non-diagonal elements. Wei[9] has discussed a method by which linear reaction–diffusion equations may be uncoupled. The technique used is a standard transformation in linear algebra, the similarity transformation.[10] The basic procedure involved is described in the following.

a. The first step involves the determination of the eigenvalues of the matrix $[K]$. There will be three eigenvalues, $\hat{k}_0$, $\hat{k}_1$ and $\hat{k}_2$ which are the three roots of the determinantal equation

$$\det\begin{vmatrix} k_1-\hat{k} & 0 & 0 \\ -k_1 & k_2-\hat{k} & 0 \\ 0 & -k_2 & 0-\hat{k} \end{vmatrix}=0 \tag{14}$$

Solving equation (14) we obtain

$$\hat{k}_0=0;\ \hat{k}_1=k_1;\ \hat{k}_2=k_2 \tag{15}$$

b. Corresponding to each eigenvalue $\hat{k}_i$ is an eigenvector, $(\mathbf{X})_i$, which satisfies the matrix equation

$$[K](\mathbf{X})_i=\hat{k}_i(\mathbf{X})_i \qquad i=0,\ 1,\ 2 \tag{16}$$

From equations (10) and (15), the eigenvectors are found to be

$$(\mathbf{X})_0=\begin{pmatrix}0\\0\\1\end{pmatrix};\quad (\mathbf{X})_1=\begin{pmatrix}\frac{k_2}{k_1}-1\\1\\-\frac{k_2}{k_1}\end{pmatrix};\quad (\mathbf{X})_2=\begin{pmatrix}0\\1\\-1\end{pmatrix} \tag{17}$$

Placing each eigenvector side by side, in order, a square matrix $[X]$ is formed and is called the modal matrix of $[K]$,

$$[X]=\begin{bmatrix}0 & \frac{k_2}{k_1}-1 & 0\\ 0 & 1 & 1\\ 1 & -\frac{k_2}{k_1} & -1\end{bmatrix} \tag{18}$$

The inverse of $[X]$ is determined to be

$$[X]^{-1}=\begin{bmatrix} 1 & 1 & 1 \\ \left(\frac{k_2}{k_1}-1\right)^{-1} & 0 & 0 \\ -\left(\frac{k_2}{k_1}-1\right)^{-1} & 1 & 0 \end{bmatrix} \tag{19}$$

c. Pre-multiplying both sides of equation (8) by $[X]^{-1}$ we obtain

$$D[X]^{-1}\nabla^2(C)=[X]^{-1}[K][X][X]^{-1}(C) \tag{20}$$

where we have used the property that

$$[X][X]^{-1}=\lceil I\rfloor=\begin{matrix} \lceil 1 & & \\ & 1 & \\ & & \underline{1}\rfloor \end{matrix} \tag{21}$$

where $\lceil I\rfloor$ is the identity matrix (having unity everywhere on the main diagonal and zeroes elsewhere) and multiplication with $\lceil I\rfloor$ leaves a matrix product unchanged.

We now define pseudoconcentrations, $(\hat{C})$ by

$$(\hat{C})=\begin{pmatrix} \hat{S} \\ \hat{P}_1 \\ \hat{P}_2 \end{pmatrix}=[X]^{-1}(C) \tag{22}$$

The pseudoconcentrations, $\hat{S}$, $\hat{P}_1$ and $\hat{P}_2$, represent linear combinations of the original concentrations S, P_1 and P_2. These pseudovariables have no physical significance and are only mathematical conveniences.

The product $[X]^{-1}[K][X]$ yields a *diagonal* matrix $\lceil\hat{k}\rfloor$ consisting of the eigenvalues $\hat{k}_i$, on the main diagonal and zeros elsewhere,

$$[X]^{-1}[K][X]=\lceil\hat{k}\rfloor=\begin{matrix} \lceil\hat{k}_0 & & \\ & \hat{k}_1 & \\ & & \hat{k}_2\rfloor \end{matrix}=\begin{matrix} \lceil 0 & & \\ & k_1 & \\ & & k_2\rfloor \end{matrix} \tag{23}$$

The similarity transformation therefore reduces the original coupled problem (8) to an uncoupled one in terms of pseudoconcentrations,

$$D\nabla^2(\hat{C})=\lceil\hat{k}\rfloor(\hat{C})=\begin{matrix} \lceil 0 & & \\ & k_1 & \\ & & k_2\rfloor \end{matrix}\quad(\hat{C}) \tag{24}$$

which represents a set of three uncoupled equations

$$D\nabla^2\hat{C}_i=\hat{k}_i\hat{C}_i \qquad i=0,\ 1,\ 2 \tag{25}$$

The pseudospecies $\hat{S}$, $\hat{P}_1$ and $\hat{P}_2$ behave as though they were participating in independent irreversible reactions, each with a different first order rate constant $\hat{k}_i$. The first eigenvalue $\hat{k}_0$ is zero and therefore the pseudospecies $\hat{S}$ does not react.

The original set of consecutive irreversible reactions (1) is therefore analysed now in a pseudoconcentration space in which we have three *independent* reactions:

$\hat{S}$ does not react
$\hat{P}_1$ decays with a first order constant $\hat{k}_1$ $(=k_1)$
$\hat{P}_2$ decays with a first order constant $\hat{k}_2$ $(=k_2)$

The boundary conditions for the transformed problem (25) is obtained by multiplying equations (11)–(13) by $[X]^{-1}$. Thus we have

$$b.c.\ 1: \quad sphere \text{ at } r=0, \ \frac{\partial \hat{C}_i}{\partial r}=0 \qquad i=0, 1, 2 \tag{26a}$$

$$flat\ plate \text{ at } x=0, \ \frac{\partial \hat{C}_i}{\partial x}=0 \qquad i=0, 1, 2 \tag{26b}$$

$$b.c.\ 2: \quad sphere \text{ at } r=R, \ \hat{C}_{ir}=\hat{C}_{iR} \qquad i=0, 1, 2 \tag{27a}$$

$$flat\ plate \text{ at } x=L, \ \hat{C}_{ix}=\hat{C}_{iL} \qquad i=0, 1, 2 \tag{27b}$$

$$b.c.\ 2': \quad sphere \ D\left.\frac{\partial \hat{C}_i}{\partial r}\right|_{r=R}=k_b\{\hat{C}_{ib}-\hat{C}_{iR}\} \tag{28a}$$

$$flat\ plate \ D\left.\frac{\partial \hat{C}_i}{\partial x}\right|_{x=L}=k_b\{\hat{C}_{ib}-\hat{C}_{iL}\} \tag{28b}$$

The concentration profiles and rates of formation can be obtained by solving the set of independent equations (25) with the boundary conditions (26)–(28) for a specified geometry.

4. Concentration profiles and reaction rates for spherical capsules

Using a spherical coordinate system, the differential equations (25) reduce to

$$D\left\{\frac{1}{r^2}\frac{\partial}{\partial r}\left(r^2\frac{\partial \hat{C}_i}{\partial r}\right)\right\}=\hat{k}_i\hat{C}_i \qquad i=0, 1, 2 \tag{29}$$

for each pseudospecies. The above equations are solved with boundary conditions (26a)–(28a) to give the pseudoconcentration profiles within the spherical capsule as

$$\hat{C}_{ir}=\frac{\hat{C}_{ib}\sinh\left(\phi_i\dfrac{r}{R}\right)}{\left(\dfrac{r}{R}\right)\sinh\phi_i\left\{1+\dfrac{1}{Sh}(\phi_i\coth\phi_i-1)\right\}}, \qquad i=0, 1, 2 \tag{30}$$

where $\hat{C}_{ir}$ is the concentration at any radius r within the membrane; $\phi_i=\sqrt{(\hat{k}_i/D)}R$, is the Thiele modulus portraying the relative importance of reaction and intracapsule diffusion resistance, and $Sh=(k_b R/D)$, is a modified Sherwood number giving the relative importance of external mass transfer resistance and intraparticle diffusion resistance.

If there were no diffusional limitations within the capsule, realisable with very small sized capsules, large diffusion coefficient D and slow reaction rates, the Thiele modulus assumes a very low value ($\phi_i < 0.1$) and the concentrations will be uniform everywhere within the enzyme system,
for

$$0 < r < R, \quad \hat{C}_{ir} = \hat{C}_{iR} \qquad i = 0, 1, 2 \tag{31}$$

On the other hand, for large values of the modified Sherwood number ($Sh > 50$) the external mass transfer resistance is negligible and we must have the bulk concentrations equal to the solid–fluid interface concentrations,

$$\hat{C}_{ir} = \hat{C}_{ib} \qquad i = 0, 1, 2 \tag{32}$$

In practice both diffusional resistances would be important and the profiles in terms of pseudoconcentrations would have to be obtained from equation (30). The original concentration profiles in terms of S, P_1 and P_2 are obtained by employing the inverse transformation

$$(C)_r = [X](\hat{C})_r \tag{33}$$

where the elements of $[X]$ are given by equation (18). Concentration profiles for two typical cases are plotted in Figure 2(a) and (b) in terms of dimensionless concentrations and dimensionless distances. In Figure 2(a) the bulk concentrations are

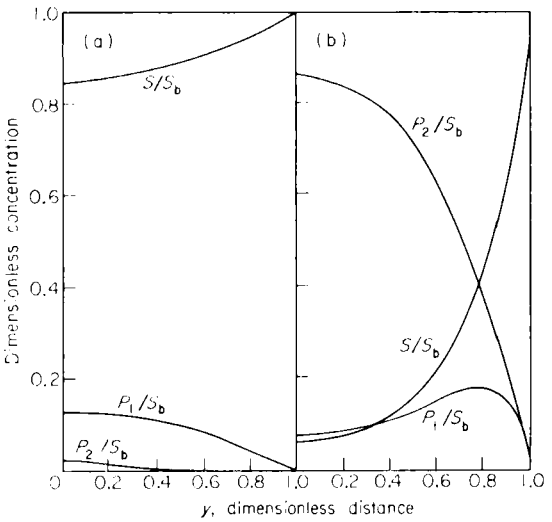

Figure 2. Concentration profiles within a spherical capsule for bulk concentrations $S_b/S_b = 1.0$; $P_{1b}/S_b = 0.0$; $P_{2b}/S_b = 0.0$; ratio of kinetic constants $k_1/k_2 = 0.64$ and Sherwood number, $Sh = 50$. (a) Profiles for Thiele modulus $\phi_1 = 1.0$; (b) profiles for Thiele modulus $\phi_1 = 5.0$.

chosen as $S_b/S_b = 1.0$, $P_{1b}/S_b = 0.0$ and $P_{2b}/S_b = 0.0$ with the external mass transfer resistance described by a Sherwood number of 50. The ratio of kinetic constants $k_1/k_2 = 0.64$ and the Thiele modulus ϕ_1 is taken as unity. For these parameters,

Figure 2(a) shows that the drop in the concentration of S inside the capsule is negligible and P_2 hardly makes an appearance. P_1 is produced to a greater extent than P_2. Figure 2(b) depicts the profiles obtained with a Thiele modulus $\phi_1 = 5$ and keeping all other parameters identical as in case (a). The situation is now changed dramatically. S drops steeply in concentration towards the centre of the capsule to a value less than 0.1. P_2 is now produced in appreciable quantities, especially towards the interior of the capsule and remains greater than the concentration of P_1 at any point. A large value of the Thiele modulus therefore favours the production of the end product P_2. The following conclusions may therefore be drawn: (a) if P_2 is the desired product then it is advisable to have a large value of ϕ_1. This can be achieved by using a faster reacting enzyme or by using a large sized capsule (large R); (b) small values of ϕ_1 favour the production of P_1 and therefore maximisation of P_1 can be achieved by use of slower reacting enzymes and smaller capsules.

In order to calculate the actual rates of production of P_1 and P_2 it becomes convenient to define an "effectiveness factor", η_i, for the ith reacting pseudospecies as

$$\eta_i = \frac{\text{Actual rate of reaction of pseudospecies } i}{\text{Rate of reaction if there were no resistance (external or internal) to mass transfer}}$$

$$= \frac{\int_0^R 4\pi r^2 \hat{k}_1 \hat{C}_{ir}\, dr}{\frac{4\pi}{3} R^3 \hat{k}_1 \hat{C}_{ib}} \qquad i = 0, 1, 2 \tag{34}$$

An expression for η_i can be obtained by integrating the concentration profiles (30) over the radius of the sphere

$$\eta_i = \frac{\frac{3}{\phi_1^2}\{\phi_1 \coth \phi_1 - 1\}}{1 + \frac{1}{\mathrm{Sh}}\{\phi_1 \coth \phi_1 - 1\}} \qquad i = 0, 1, 2 \tag{35}$$

The effectiveness factor η_i provides a convenient method for taking account of mass transfer resistances. The variation of the effectiveness factor η_i with ϕ_i and Sh is given in Figure 3(a). It may be observed that η_i is always less than unity and therefore the actual rate of reaction is always lowered due to mass transfer resistances. The greater the value of ϕ_i the lower is the value of η_i. This essentially means that the faster reaction is affected (slowed down) to a greater extent. This situation is analogous to progressive taxation—the more money you earn, the larger is the proportional amount of tax you pay. The external mass transfer resistance, characterised by Sh, also lowers the effectiveness factor. For values of Sherwood number greater than 50, the effect on η_i is negligible and the system becomes "controlled" by *intra*capsule diffusion resistance.

The advantage of defining η_i is that the system of reacting–diffusing pseudospecies (described by equation (25)) can be considered to react (independently) in a completely

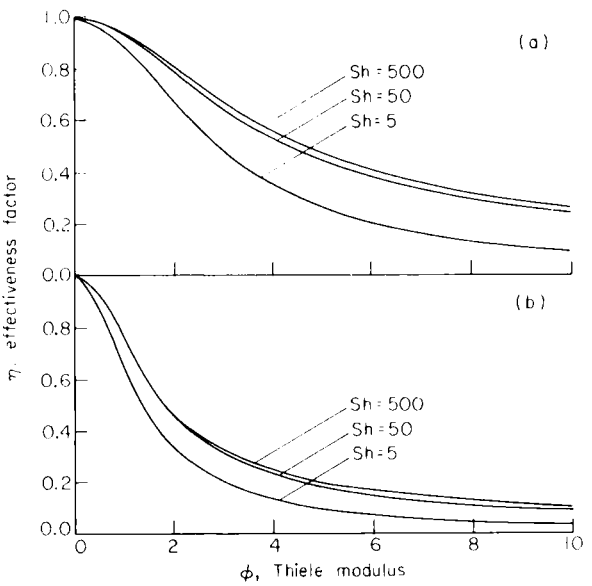

Figure 3. Plots of effectiveness factor versus Thiele modulus with the Sherwood number as parameter. (a) Spherical capsule, equation (35); (b) flat plate membrane, equation (48).

homogeneous system with first order rate constants $\eta_i \hat{k}_i$. The rates of formation of the pseudospecies may therefore be written as

$$\frac{d\hat{C}_i}{dt} = -\eta_i \hat{k}_i \hat{C}_i \qquad i = 0, 1, 2 \tag{36}$$

which can be written in matrix notation as

$$\frac{d(\hat{C})}{dt} = -\begin{vmatrix} \overline{0} & & \\ & \eta_1 k_1 & \\ & & \underline{\eta_2 k_2} \end{vmatrix} (\hat{C}) \tag{37}$$

The actual rates of reaction of species S, P_1 and P_2 can be obtained by multiplying equation (17) by $[X]$ and using the relation (33). Thus we have

$$\frac{d(C)}{dt} = -[K^*](C) \tag{38}$$

where $[K^*]$ is the observed overall matrix of reaction rate constants incorporating both diffusional resistances,

$$[K^*] = [X] \begin{vmatrix} \overline{0} & & \\ & \eta_1 k_1 & \\ & & \underline{\eta_2 k_2} \end{vmatrix} [X]^{-1} \tag{39}$$

Carrying out the matrix multiplication using the matrices $[X]$ and $[X]^{-1}$ given by equations (18) and (19) we obtain

$$[K^*]=\begin{bmatrix} \eta_1 k_1 & 0 & 0 \\ -\eta_1 k_1 \dfrac{\dfrac{\eta_2 k_2}{\eta_1 k_1}-1}{1-\dfrac{k_2}{k_1}} & \eta_2 k_2 & 0 \\ -\eta_1 k_1 \dfrac{\dfrac{k_2}{k_1}-\dfrac{\eta_2 k_2}{\eta_1 k_1}}{\dfrac{k_2}{k_1}-1} & -\eta_2 k_2 & 0 \end{bmatrix} \tag{40}$$

Comparison of equations (10) and (40) shows that the effect of diffusion is not only to reduce the reaction rates but also to change the observed reaction *scheme*. The matrix $[K^*]$ corresponds to the diffusion disguised reaction scheme shown below

$$\begin{array}{ccccc} S & \xrightarrow{k_1^*} & P_1 & \xrightarrow{k_2^*} & P_2 \\ & \underset{k_3^*}{\searrow} & & \nearrow & \end{array} \tag{41}$$

where the observed rate constants are given by

$$k_1^* = \eta_1 k_1 \frac{\dfrac{\eta_2 k_2}{\eta_1 k_1}-1}{1-\dfrac{k_2}{k_1}} \tag{42}$$

$$k_2^* = \eta_2 k_2 \tag{43}$$

$$k_3^* = \eta_1 k_1 \frac{\dfrac{k_2}{k_1}-\dfrac{\eta_2 k_2}{\eta_1 k_1}}{1-\dfrac{k_2}{k_1}} \tag{44}$$

Diffusional resistances introduce an apparent linkage between S and P_2 (with a first order rate constant k_3^*) where none originally existed (*cf* equation (1)). Physically this means that just by measuring changes in the bulk concentration and treating the system as a homogeneous one (described by equation (38)) will not only give low kinetic constants but even the true mechanistic picture will not be obtained. Once the disguised constants are known (from equations (42)–(44)) then the rates of reaction can be obtained from equation (38).

5. Concentration profiles and reaction rates for flat plate membranes

Equation (25) reduces for rectangular coordinate system to

$$D\frac{\partial^2 C_i}{\partial x^2} = k_i \hat{C}_i \qquad i=0,\ 1,\ 2 \tag{45}$$

The concentration profiles within the membrane may be obtained by solving (45) with the boundary conditions (26b)–(28b). Thus we get

$$\hat{C}_{ix} = \frac{\hat{C}_{ib} \cosh\left(\phi_i \frac{x}{L}\right)}{\cosh \phi_i \left\{1 + \frac{1}{Sh} \phi_i \tanh \phi_i\right\}}, \qquad i = 0, 1, 2 \tag{46}$$

where $\hat{C}_{ix}$ is the pseudoconcentration at any position x within the membrane; $\phi_i = \sqrt{(\hat{k}_i/D)}L$ is the Thiele modulus giving the relative importance of reaction and intramembrane diffusion; $Sh = (k_b L/D)$ is the modified Sherwood number showing the relative importance of external mass transfer resistance and intramembrane transport.

For low values of the Thiele modulus ($\phi_i < 0.1$) the membrane offers no resistance to diffusional transport and the concentrations are everywhere uniform inside the membrane (*cf* equation (31)). For large values of Sherwood number ($Sh > 50$) the external mass transfer resistance is neligible compared to the intramembrane transport limitation and the solid–fluid interface concentration equals the bulk concentration (*cf* equation (32)).

The effectiveness factor η_i for the flat plate membrane (of unit cross-sectional area) can be calculated from

$$\eta_i = \frac{\int_0^L \hat{k}_i \hat{C}_{ix} \, dx}{L \hat{k}_i \hat{C}_{ib}} \qquad i = 0, 1, 2 \tag{47}$$

Using the expression for C_{ix} given by equation (46) we obtain

$$\eta_i = \frac{\frac{\tanh \phi_i}{\phi_i}}{1 + \frac{1}{Sh} \phi_i \tanh \phi_i} \qquad i = 0, 1, 2 \tag{48}$$

The reaction rate is obtained exactly as before for the spherical capsule from equation (38) with the effectiveness factors η_1 and η_2 given by equation (48). Figure 3(b) shows the influence of the parameters ϕ_i and Sh on η_i for a flat membrane and the discussions following equation (35) would be applicable here as well. The concentration profiles analogous to Figure 2(a) and (b) may be drawn but these are not included here because the same qualitative conclusions are reached (*cf* discussion following equation (33)).

6. Prediction of the performance of a two-enzyme system

If the intrinsic first order reaction rate constants k_1 and k_2 are known and estimates of the diffusion coefficient D and mass transfer coefficient k_b are available, together with the information on system geometry, it is a straightforward procedure to predict the system performance. The steps involved here are: (a) calculation of η_1 and η_2 for the appropriate geometry; (b) calculation of the disguised rate constants k_1^*, k_2^*

and k_3* from equations (42)–(44) giving the disguised rate constants matrix $[K^*]$; (c) the concentration–time paths are described by the equation (38) which can be integrated to give the concentration at any time t after the start of the experiment as

$$(C)_t = \exp\{-[K^*]t\}(C)_0 \tag{49}$$

where $(C)_0$ and $(C)_t$ are the column matrices of bulk concentrations at the start of the experiment and at time t respectively. The matrix exponentiation can be performed either directly or by use of the following relationship

$$\exp\{-[K^*]t\} = [X]\begin{bmatrix}1 & & \\ & \exp(-\eta_1 k_1 t) & \\ & & \exp(-\eta_2 k_2 t)\end{bmatrix}[X]^{-1} \tag{50}$$

From equations (9), (18), (19), (49) and (50) the bulk concentrations of S, P_1 and P_2 may be obtained as

$$S_t = \exp(-\eta_1 k_1 t) S_0 \tag{51}$$

$$P_{1t} = \frac{\exp(-\eta_1 k_1 t) - \exp(-\eta_2 k_2 t)}{\frac{k_2}{k_1} - 1} S_0 + \exp(-\eta_2 k_2 t) P_{10} \tag{52}$$

$$P_{2t} = (S_0 + P_{10} + P_{20}) - (S_t + P_{1t}) \tag{53}$$

Equation (53) expresses the law of conservation of mass and shows that there are only two independent concentrations in the system.

The concentration profiles given by equations (51)–(53) have been plotted in Figure 4

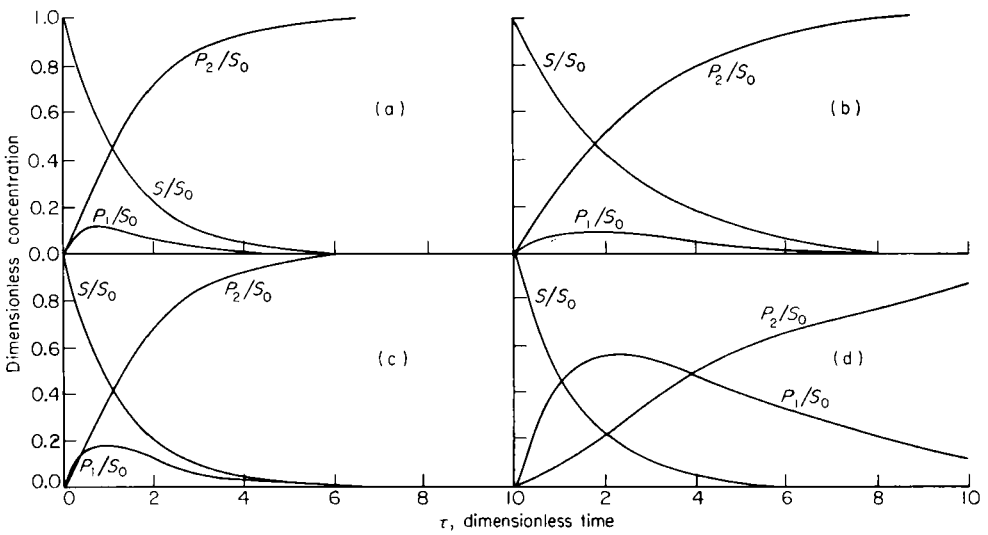

Figure 4. Transient (non-dimensional) concentration profiles for spherical capsule. Initial concentrations are $S_0/S_0 = 1.0$, $P_{10}/S_0 = 0.0$ and $P_{20}/S_0 = 0.0$. External mass transfer resistance is described by Sh = 50. (a) $k_2/k_1 = 4.0$ and $\phi_1 = 2.0$; (b) $k_2/k_1 = 4.0$ and $\phi_1 = 5.0$; (c) $k_2/k_1 = 2.25$ and $\phi_1 = 2.0$; (d) $k_2/k_1 = 0.25$ and $\phi_1 = 2.0$.

as non-dimensional variables S_t/S_0, P_{1t}/S_0 and P_{2t}/S_0 as functions of dimensionless time $\tau = k_1 t$. The effects of two parameters are considered in Figure 4(a)–(d), namely the Thiele modulus ϕ_1 and the ratio of the kinetic constants k_2/k_1. The initial concentrations are chosen in all four cases to be $S_0/S_0 = 1.0$, $P_{10}/S_0 = 0.0$ and $P_{20}/S_0 = 0.0$. The external mass transfer resistance is described by a Sherwood number of 50. All the computations were carried out for a single sphere. Figure 4(a) shows the concentration–time trajectories for the case where the ratio $k_2/k_1 = 4$ and the modulus $\phi_1 = 2.0$. In Figure 4(b) the ratio of kinetic parameters is kept the same but the Thiele modulus is increased to 5.0. Comparison of the two graphs shows that for short reaction times ($\tau < 1$) the larger Thiele modulus increases the selectivity of the reaction scheme in favour of P_2. In both cases the concentration of P_1 increases with time, reaches a maximum and then tends to decrease. This observation was not made by Goldman and Katchalski[8] because in their analysis the concentration of S was kept constant. Short reaction times and small Thiele modulus ϕ_1 therefore favours the production of P_1. Decreasing the ratio k_2/k_1 to a value 2.25 [Figure 4(c)] shows, predictably, that a larger amount of P_1 is produced. In fact during the initial period the concentration of P_1 is greater than that of P_2. The maxima in P_1 concentration is again observed. Finally, for $k_2/k_1 = 0.25$, Figure 4(d) shows a lag period in the formation of P_2 which is not observed for larger values of k_2/k_1.

The transient analysis presented in this section should be useful in the simulation of the performance of a batch reaction system. The optimum operating conditions under which the reaction should be carried out to maximise either P_1 or P_2 can be gleaned from the qualitative discussion above.

7. Performance of a packed bed two-enzyme reactor

For a packed bed two-enzyme reactor consisting of spherical immobilised two-enzyme capsules the concentration variation with height of the bed is given by

$$\frac{d(C)_z}{dz} = \frac{f}{v}[K^*](C)_z \tag{54}$$

where $(C)_z$ represents the concentration level at any height z in the bed; f is the fraction of the bed occupied by the enzyme particles; v is the superficial velocity of flow of reactants through the bed. Equation (54) assumes that there is no back-mixing of liquid in the column and can be integrated from the inlet concentration $(C)_{in}$ to the outlet concentration $(C)_{out}$ to give

$$(C)_{out} = \exp\left\{-[K^*]\frac{f}{v}Z\right\}(C)_{in} \tag{55}$$

where Z is the height of the bed. The expression (55) is exactly analogous to equation (49) with the time t replaced by the residence time in the packed bed, fZ/v. The matrix exponentiation can be done using (50) and the outlet compositions would be given by equations (51)–(53) replacing t by fZ/v. The profiles shown in Figure 4 also represent the concentration profiles along the height of the bed and the theoretical analysis is useful in the design of such packed bed enzyme reactors.

8. Analysis of laboratory experimental data

In many cases of interest the biologist or biochemist needs to obtain the kinetic rate constants free from diffusion disguises like the ones discussed in this paper. To achieve this the experiments have to be done under conditions in which the mass transfer resistances are negligible. The external mass transfer resistance can be reduced by agitating the bulk solution till the "stagnant" diffusion layer near the solid membrane surface is effectively reduced to zero. The intramembrane diffusion resistance can be diminished by having a membrane matrix with large pores or by using small sized membranes (or capsules). If such limiting experiments cannot be performed then there exists the problem of extracting the intrinsic kinetic data from the diffusion disguised case. This may be achieved as follows.

a. Estimate the values of D and k_b by performing experiments under non-reacting conditions.

b. From the observed rate data obtain the values of the disguised constants k_1^*, k_2^* and k_3^*.

c. The intrinsic constants k_1 and k_2 can be obtained by use of equations (42)–(44). This involves a trial and error procedure because η_1 and η_2 in these equations require a prior knowledge of k_1 and k_2.

9. Conclusions

The matrix method described in this paper provides a convenient method for analysing the effect of diffusional limitations on the kinetic behaviour of consecutive reaction systems taking place within an immobilised membrane or capsule. The strength of the method lies in the simplicity with which analytical solutions can be obtained. The same method may be used to analyse complex reaction networks typical of many enzymic reactions, provided the kinetics of each step in the reaction path can be considered to be first order. The procedure in this case would be first to construct the matrix of reaction rate constants, use the similarity transformation to reduce the diffusion–reaction equations to a series of *independent* reactions in terms of pseudoconcentrations, solve the differential equations to obtain the concentration profiles and reaction rates in terms of pseudovariables and then recover the solution to the original problem by using the inverse transformation. For the more general problem where the linear kinetic approximation does not apply one has to resort to numerical techniques to solve the non-linear equations. Such analyses should be useful in the design of enzyme reactors.

Appendix

Nomenclature

(C)	Column matrix of concentrations of species S, P_1 and P_2 (mol/cm^3)
(C)	Column matrix of pseudoconcentrations (mol/cm^3)
D	Diffusion coefficient of any species through the membrane (cm^2/s)
E_1, E_2	Enzyme concentrations (mol/cm^3)

f	Fraction of packed bed occupied by enzyme particles
$[I]$	Identity matrix
k_1	Intrinsic first order rate constant for the reaction $S \rightarrow P_1$ (s^{-1})
k_2	Intrinsic first order rate constant for the reaction $P_1 \rightarrow P_2$ (s^{-1})
k_i	ith eigenvalue of $[K]$ (s^{-1})
k_i^*	Disguised rate constants (s^{-1})
k_h	Mass transfer coefficient (cm/s)
$[K]$	Matrix of reaction rate constants (s^{-1})
$[K^*]$	Matrix of disguised rate constants (s^{-1})
L	Thickness of flat plate membrane (cm)
P_1	Concentration of P_1 (mol/cm^3)
P_2	Concentration of P_2 (mol/cm^3)
r	Radial distance (cm)
R	Radius of spherical capsule (cm)
S	Concentration of S (mol/cm^3)
Sh	Sherwood number
t	Time (s)
v	Superficial velocity of liquid through packed bed (cm/s)
x	Distance along flat plate (cm)
$[X]$	Modal matrix of $[K]$
y	Dimensionless distance
z	Height of packed bed (cm)

Greek letters

δ	Thickness of diffusion layer (cm)
η	Effectiveness factor
τ	Dimensionless time $= k_1 t$
ϕ	Thiele modulus

Subscripts

b	Bulk variable
i	Index
in	Inlet
L	Surface of flat plate
0	Initial
out	Outlet
r	At radius r
R	At surface of capsule
t	At time t
x	Position x inside plate

Superscripts

^	Transformed variable

References

1. Ramachandran, P. A. *J. appl. Chem. Biotechnol.* 1974, **24**, 265.
2. Ramachandran, P. A. *Biotech. Bioeng.* 1975, **17**, 211.
3. Brown, H. D.; Patel, A. B.; Chattopadhyay, S. K. *J. Chromatogr.* 1968, **35**, 103.
4. Wilson, R. J. H.; Kay, G.; Lilly, M. D. *Biochem. J.* 1968, **109**, 137.
5. Mosbach, K.; Mattiasson, B. *Acta. Chem. Scand.* 1970, **24**, 2093.
6. Mattiasson, B.; Mosbach, K. *Biochem. Biophys. Acta* 1971, **235**, 253.
7. Mosbach, K.; Larsson, P. O. *Biotech. Bioeng.* 1970, **12**, 19.
8. Goldman, R. ; Katchalski, E. *J. Theor. Biol.* 1971, **32**, 243.
9. Wei, J. *J. Catalysis* 1962, **1**, 526, 538.
10. Amundson, N. R. *Mathematical Methods in Chemical Engineering* Prentice Hall, Englewood Cliffs, NJ, 1966.