J. appl. Chem. Biotechnol. 1976, **26**, 214–224

Analysis of Immobilised Multi-enzyme Reaction Systems with Michaelis–Menten Kinetics

P. A. Ramachandran,[a] R. Krishna and C. B. Panchal

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

(*Paper received 30 April 1975, amended paper accepted 15 July 1975*)

The consecutive sequence of reactions $S \rightarrow P_1 \rightarrow P_2$ taking place inside a permeable spherical particle is analysed for the case in which the enzyme-catalysed steps in the reaction follow Michaelis–Menten type kinetics. The case in which each step in the reaction is inhibited by its product is also considered. The theoretical analysis takes into account both interparticle and intraparticle diffusional limitations and utilises an orthogonal collocation technique to obtain the effectiveness factor and selectivity of the reaction sequence. The orthogonal collocation method is found to be both convenient and simple and the analysis should prove useful in the design of immobilised multi-enzyme reactors.

1. Introduction

The advantages of immobilised enzymes relative to soluble enzymes in industrial biochemical processing applications are well known. Increasing attention has been given in recent years to immobilised enzymic systems in which two or more enzymes are bound to the same matrix or support. A number of experimental investigations of these systems have been published in the literature recently.[1–3] A new application in biochemical processing is the utilisation of entrapped bacterial cells and these often involve multi-enzyme systems. A theoretical analysis of the problem is of importance in a better understanding of the behaviour of these systems.

Kuchel *et al.*[4] and Easterby[5] have analysed the performance of consecutive reactions involving two or more enzymes in solution with special reference to coupled enzymic assay. The system analysed was a batch process. Immobilised enzyme systems carrying out a sequence of consecutive reactions have the additional complication of diffusional effects and the only published theoretical analysis of these systems appears to be confined to cases where the intrinsic reaction kinetics are first order. Thus, Goldman and Katchalski[6] have analysed a set of consecutive reactions, $S \rightarrow P_1 \rightarrow P_2$, for a two-enzyme system attached to an impermeable membrane. Krishna and Ramachandran[7] have analysed the problem in which the membrane is permeable and intramembrane diffusion is important.

The assumption of first order kinetics is generally valid when the Michaelis constants for the two reaction steps are greater than the bulk substrate concentrations. This requirement is usually satisfied for the case of low initial substrate concentration or towards the end of the reaction in a batch system. First order reactions have a characteristic that the "effectiveness factor" is independent of the substrate or product concentration in the bulk liquid and can be calculated analytically for simple geometries. In cases where each step in the consecutive reaction system follows Michaelis–Menten kinetics (with added complexities due to substrate or product inhibition), analytical expressions for the effectiveness factor cannot be obtained. The purpose of this paper is to analyse diffusional (inter- and intraparticle) effects in immobilised two-enzyme systems following complex kinetics and to show the relative importance of the various parameters on the rate of reaction. The problem is solved by orthogonal collocation methods.

2. Formulation of the problem

The analysis of the following section is based on a consecutive reaction scheme:

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

taking place inside an immobilised spherical pellet (or capsule) of radius R. The theoretical treatment is also valid for the case of enzymes attached to a flat plate membrane. The two enzymes 1 and 2 are considered to be distributed uniformly inside the pellet which is assumed to be permeable to all three species. The effects of both inter- and intrapellet diffusional resistance have been considered.

The differential mass balance for the transport and subsequent reaction of the substrate and intermediate is as follows:

$$D_s \frac{1}{r^2}\left\{\frac{d}{dr}\left(r^2 \frac{dS}{dr}\right)\right\} = f_s(S, P_1) \tag{1}$$

$$D_p \frac{1}{r^2}\left\{\frac{d}{dr}\left(r^2 \frac{dP_1}{dr}\right)\right\} = -f_s(S, P_1) + f_p(P_1, P_2) \tag{2}$$

where f_s and f_p are the rates of reaction of the substrate S and intermediate P_1 respectively (see Appendix for nomenclature).

The boundary conditions are obtained from the physical requirement that there can be no mass flux at the centre of the pellet ($r=0$) and that there must be continuity of mass flux at the surface of the capsule ($r=R$). These are expressed mathematically as follows:
At $r=0$,

$$\frac{dS}{dr} = \frac{dP_1}{dr} = 0 \tag{3}$$

At $r=R$,

$$D_s \left(\frac{dS}{dr}\right)_{r=R} = (k_b)_s (S_0 - S_{r=R}) \tag{4}$$

and

$$D_p \left(\frac{dP_1}{dr}\right)_{r=R} = (k_b)_p (P_{10} - P_{1r=R}) \tag{5}$$

Introducing the following dimensionless parameters,

$$s = \frac{S}{S_0}, \quad p = \frac{P_1}{S_0}, \quad y = \frac{r}{R}, \quad q = \frac{D_p}{D_s}$$

equations (1) and (2) reduce to

$$\frac{1}{y^2}\left[\frac{d}{dy}\left(y^2 \frac{ds}{dy}\right)\right] = \frac{R^2}{D_s S_0} f_s(s, p) \tag{6}$$

and

$$\frac{q}{y^2}\left[\frac{d}{dy}\left(y^2 \frac{dp}{dy}\right)\right] = \frac{R^2}{D_s S_0} \left[-f_s(s, p) + f_p(p, p_2)\right] \tag{7}$$

The boundary conditions (3) and (4) now become:

At $y=0$,

$$\frac{ds}{dy} = \frac{dp}{dy} = 0 \tag{8}$$

At $y=1$,

$$s=1-\frac{1}{\mathrm{Sh_s}}\frac{\mathrm{d}s}{\mathrm{d}y} \tag{9}$$

and

$$p=p_0-\frac{1}{\mathrm{Sh_p}}\frac{\mathrm{d}p}{\mathrm{d}y} \tag{10}$$

where

$$\mathrm{Sh_s}=\frac{(k_b)_s R}{D_s} \quad \text{and} \quad \mathrm{Sh_p}=\frac{(k_b)_p R}{D_p}$$

Two quantities are of interest in the present problem: (a) the rate of reaction of substrate S, and (b) the ratio of the rate of formation of the intermediate P_1 to the rate of formation of the product P_2.

The rate of reaction of S can be characterised by an "effectiveness factor", η, defined as the ratio of the actual rate of reaction of S to the rate of reaction in the absence of diffusional (external or internal) effects. Thus,

$$\eta=\frac{\int_0^R 4\pi r^2 f_s(S, P_1)\,\mathrm{d}r}{(4\pi/3)\,R^3 f_s(S_0, P_{10})}$$

$$=\frac{3}{f_s(1, p_0)}\int_0^1 y^2 f_s(s, p)\,\mathrm{d}y \tag{11}$$

The second quantity of interest is the selectivity defined by

$$\sigma=\frac{\text{net rate of formation of } P_1}{\text{net rate of formation of } P_2}$$

$$=\frac{\int_0^R 4\pi r^2(f_s-f_p)\,\mathrm{d}r}{\int_0^R 4\pi r^2 f_p\,\mathrm{d}r}$$

$$=\frac{\int_0^1 y^2(f_s-f_p)\,\mathrm{d}y}{\int_0^1 y^2 f_p\,\mathrm{d}y} \tag{12}$$

3. Method of solution

The differential equations (6) and (7) are non-linear and may be solved by a number of different techniques. The orthogonal collocation method proposed by Villadsen and Stewart[8] is particularly suitable for the solution of differential equations of this type and has been used for solution of single immobilised enzyme problems for various complex kinetics by Ramachandran.[9] Application to the modelling of a packed bed encapsulated enzyme reactor has also been considered.[10] The collocation method has been applied to the solution of the present problem as it has computational advantages and gives accurate and reliable answers.

Using the collocation expansion of the Laplacian operator, equations (6) and (7) become:

$$\sum_{j=1}^{N+1} B_{ij}s_j-\frac{R^2}{D_sS_0}f_s(s_i, p_i)=0, \qquad i=1, 2, \ldots N+1 \tag{13}$$

and

$$\sum_{j=1}^{N+1} qB_{ij}s_j-\frac{R^2}{D_sS_0}[f_p(p_i, p_{2i})-f_s(s_i, p_i)]=0, \qquad i=1, 2, \ldots N+1 \tag{14}$$

where B_{ij} values are elements of the collocation matrix for the Laplacian defined as:

$$\frac{1}{y^2}\left[\frac{d}{dy}\left(y^2\frac{ds}{dy}\right)\right]_{y=y_i} = \sum_{j=1}^{N+1} B_{ij}s_j \tag{15}$$

where y_i represents the collocation point i.

The point $y=1$ refers to the collocation $N+1$, the surface of the pellet. At this point the boundary conditions (9) and (10) may be expanded as:

$$\sum_{j=1}^{N+1} A_{1j}s_j = \mathrm{Sh_s}(1-s_{N+1}) \tag{16}$$

with a similar expression for p_{N+1}. In equation (16), the A_{ij} values are the elements of the collocation matrix for the derivative and are defined by

$$\left(\frac{ds}{dy}\right)_{y=y_i} = \sum_{j=1}^{N+1} A_{ij}s_j \tag{17}$$

Rearranging equation (16) we obtain

$$s_{N+1} = \frac{\mathrm{Sh_s} - \sum_{j=1}^{N} A_{N+1,j}s_j}{A_{N+1,N+1}+\mathrm{Sh_s}} \tag{18}$$

and a similar equation for p_{N+1}. Substituting these values of the surface concentrations s_{N+1}, p_{N+1} in equations (13) and (14) and rearranging we obtain:

$$\sum_{j=1}^{N}\left(B_{ij}-\frac{B_{i,N+1}A_{N+1,j}}{A_{N+1,N+1}+\mathrm{Sh_s}}\right)s_j+\frac{B_{i,N+1}\mathrm{Sh_s}}{A_{N+1,N+1}+\mathrm{Sh_s}}-\frac{R^2}{D_sS_0}f_s(s_i,p_i)=0, \qquad i=1,2,\ldots N \tag{19}$$

and

$$\sum_{j=1}^{N}\left(B_{ij}-\frac{B_{i,N+1}A_{N+1,j}}{A_{N+1,N+1}+\mathrm{Sh_p}}\right)qp_j+\frac{qB_{i,N+1}\mathrm{Sh_p}p_0}{A_{N+1,N+1}+\mathrm{Sh_p}}-\frac{R^2}{D_sS_0}[f_p,(p_i,p_{2i})-f_s(s_i,p_i)]=0,$$
$$i=1,2,\ldots N \tag{20}$$

The simultaneous solution of the $2N$ algebraic non-linear equations (19) and (20) gives the concentration distribution of S, P_1 and P_2 within the pellet. Once the concentration distribution is known, the effectiveness factor and selectivity can be calculated by the use of "weights", w_i, defined as follows:

$$\int_0^1 y^2 f(s)\,dy = \sum_{i=1}^{N+1} w_i f(s_i) \tag{21}$$

Thus, the effectiveness factor and the selectivity can be expressed in terms of the concentrations at the collocation points as:

$$\eta = \frac{3}{f_s(1,p_0)}\sum_{i=1}^{N+1} w_i f_s(s_i,p_i) \tag{22}$$

and

$$\sigma = \frac{\sum_{i=1}^{N+1} w_i f_s(s_i,p_i) - f_p(p_i,p_{2i})}{\sum_{i=1}^{N+1} w_i f_p(p_i,p_{2i})} \tag{23}$$

The collocation matrices B_{ij}, A_{ij}, w_i can be calculated from the procedure suggested by Villadsen and Stewart.[8] It is worth noting here that for many problems an approximation using a value of

$N=1$ is sufficiently accurate.[8] For such cases the problem of calculating η and σ reduces to the solution of two algebraic equations for $s(y_1)$ and $p(y_1)$. In the following section, we shall solve the problem for the case of Michaelis–Menten kinetics and also consider systems exhibiting product inhibition and discuss some of the features of the solutions.

4. Analysis of Michaelis–Menten kinetics

When each intrinsic step in the reaction sequence follows Michaelis–Menten kinetics the rate expressions are given by

$$f_s = \frac{k_1 E_1 S}{K_{m1} + S} \tag{24}$$

and

$$f_p = \frac{k_2 E_2 P_1}{K_{m2} + P_1} - \frac{k_1 E_1 S}{K_{m1} + S} \tag{25}$$

The dimensionless parameters characterising this system are as follows:

$$\phi_1^2 = \frac{k_1 E_1 R^2}{D_s K_{m1}}, \qquad \phi_2^2 = \frac{k_2 E_2 R^2}{D_s K_{m2}},$$

$$\gamma_1 = \frac{S_0}{K_{m1}}, \qquad \gamma_2 = \frac{S_0}{K_{m2}} \qquad \text{and} \qquad \beta = \frac{k_1 E_1}{k_2 E_2}.$$

Numerical computations were carried out for a wide range of values of the above dimensionless parameters using three collocation points ($N=3$). In order to check the accuracy of the numerical technique the parameters γ_1 and γ_2 were each assigned a value zero. For this particular case the problem reduces to a case of first order kinetics for which analytical solutions are available for the

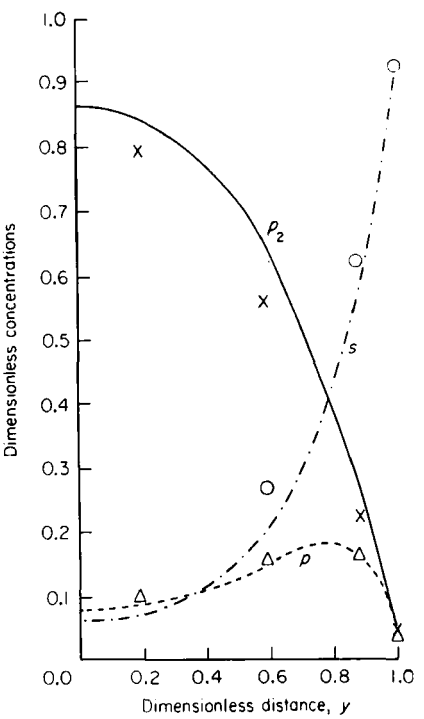

Figure 1. Concentration profiles within a spherical capsule for first order kinetics: comparison of numerical solution with analytical solution ($\phi_1=5.0$, $Sh_s=Sh_p=50.0$, $q=1.0$, $\beta=0.64$, $\gamma_1=0.0$, $\gamma_2=0.0$, $p_0=0.0$, $p_{20}=0.0$). ○, △, × = Numerical solutions.

concentration profiles within the pellet and the effectiveness factor.[7] It was found that numerical and analytical solutions were in close agreement over a wide range of values of the Thiele moduli ϕ_1 and ϕ_2. Figure 1 shows the concentration profiles for the three species for one particular set of values ($\phi_1 = 5.0$ and $\beta = 0.64$). It can be seen that there is good agreement in the values given by the two methods. The effectiveness factor, η, an important parameter in the design of enzyme reactors, when calculated by the numerical and analytical techniques is almost identical (Figure 2).

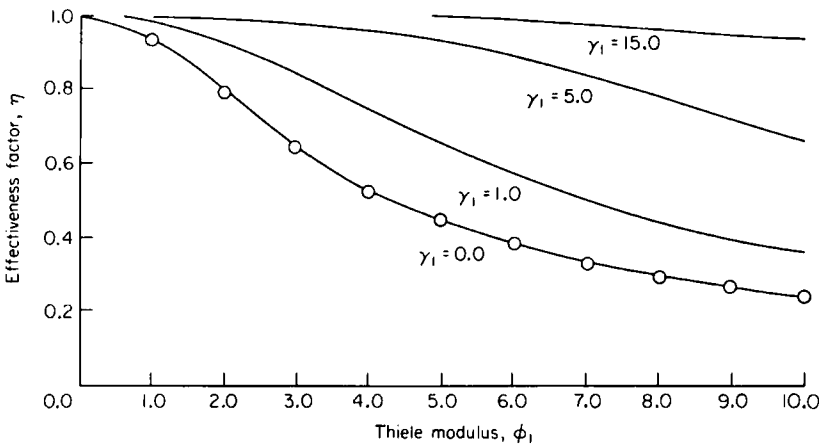

Figure 2. Variation of effectiveness factor η with ϕ_1 and γ_1 ($Sh_s = Sh_p = 50.0$, $q = 1.0$, $p_0 = 0.0$, $p_{20} = 0.0$). ○ = Numerical solution.

When the value of γ_1 is increased from zero, the effectiveness factor is also increased and for large values of γ_1 (say $\gamma_1 > 10$) the reaction kinetics can be approximated by a zero order mechanism with η approaching unity (Figure 2). The influence of γ_1 on the selectivity of the reaction scheme is depicted in Figure 3. For a constant value of γ_2 it is seen that the selectivity σ is significantly (adversely) affected by the increase in the parameter γ_1. The relative decrease in selectivity is considerable on increasing γ_1 for the case of low Thiele modulus while the decrease is not so significant for large value of ϕ_1.

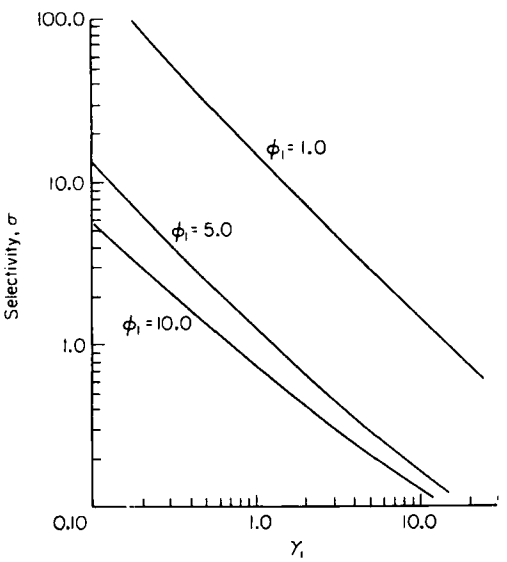

Figure 3. Effect of γ_1 on the selectivity σ ($Sh_s = Sh_p = 50.0$, $q = 1.0$, $\beta = 1.0$, $\gamma_2 = 1.0$, $p_0 = 0.0$, $p_{20} = 0.0$).

The influence of γ_2 can be similarly observed by changing γ_2 keeping other parameters constant. It can be seen (Table 1) that on increasing γ_2, the selectivity is increased considerably because of the suppression of the second reaction.

Table 1. Effect of γ_2 on the selectivity σ ($\phi_1=5.0$, $Sh_s=Sh_p=50.0$, $\beta=1.0$, $\gamma_1=1.0$, $q=1.0$, $p_0=0.0$, $p_{20}=0.0$)

γ_2	η	σ
0.0	0.6512	1.0984
1.0	0.6512	1.2337
10.0	0.6512	41.070
100.0	0.6512	3368.500

The influence of the Michaelis constants K_{m1} and K_{m2} can be observed by varying the values of γ_1 and γ_2 keeping the ratio γ_1/γ_2 constant. The effectiveness factor is increased but the selectivity is not affected greatly (Table 2).

Table 2. Relative influence of K_{m1} and K_{m2} on selectivity σ ($\phi_1=5.0$, $Sh_s=Sh_p=50.0$, $\beta=1.0$, $q=1.0$, $p_0=0.0$, $p_{20}=0.0$)

$\gamma_1=\gamma_2$	η	σ
0.0	0.483	1.379
0.5	0.562	1.284
1.0	0.651	1.233
5.0	0.931	1.206
10.0	0.981	1.250
100.0	0.999	1.307

5. Analysis of systems with product inhibition

We consider here the case in which each step in the reaction is competitively inhibited by its product. For simplicity we assume each species diffuses with equal facility. Analysis of the case with unequal diffusivities is straightforward. The reaction rate expressions are:

$$f_s=\frac{k_1E_1S}{K_{m1}(1+P_1/K_{I1})+S} \tag{26}$$

and

$$f_p=\frac{k_2E_2P_1}{K_{m2}(1+P_2/K_{I2})+P_1}-\frac{k_1E_1S}{K_{m1}(1+P_1/K_{I1})+S} \tag{27}$$

If we non-dimensionalise the reaction rate expressions as before we obtain two additional dimensionless groups. The first

$$\gamma_{I1}=\frac{S_0}{K_{I1}}$$

characterises the inhibition of the first reaction by its product P_1 and the second

$$\gamma_{I2}=\frac{S_0}{K_{I2}}$$

characterises the inhibition of the second reaction by the final product P_2.

The stoichiometric relation

$$P_2=S_0+P_{10}+P_{20}-S-P_1 \tag{28}$$

can be used to eliminate P_2 from the rate equation (27). The differential equations (6) and (7) can be solved as in the previous case using orthogonal collocation.

The relative importance of γ_{I1} and γ_{I2} was examined by varying these parameters. The influence of γ_{I1} is mainly on the effectiveness factor which decreases with increasing γ_{I1}. The selectivity is almost unaltered by changing the value of γ_{I1} (Table 3). This is a peculiar effect observed in the

Table 3. Influence of the product inhibition parameters γ_{I1} and γ_{I2} and the external (bulk) concentration of P_1 on the effectiveness factor and selectivity ($\phi_1=5.0$, $Sh_s=Sh_p=50.0$, $\beta=1.0$, $\gamma_1=1.0$, $\gamma_2=1.0$, $q=1.0$, $p_{20}=0.0$)

γ_{I1}	γ_{I2}	p_0	η	σ
0.0	0.0	0.0	0.651	1.2337
0.1	0.0	0.0	0.647	1.2335
0.5	0.0	0.0	0.633	1.2328
1.0	0.0	0.0	0.617	1.2324
4.0	0.0	0.0	0.549	1.2345
0.0	0.1	0.0	0.651	1.2556
0.0	1.0	0.0	0.651	1.4391
4.0	0.0	0.01	0.556	1.1572
4.0	0.0	0.1	0.614	0.0634
4.0	0.0	0.30	0.724	0.0375

case of sequential reactions each inhibited by its product. This may be further elucidated by comparing the concentration profiles of S and P_1 for two values of γ_{I1} of 0.0 and 4.0 (Figure 4). As the product inhibition parameter increases, the rate of the first reaction is lowered resulting in an increased concentration of S in the pellet and a decreased production of P_1. This decreased production of P_1 affects the rate of the second reaction ($P_1 \rightarrow P_2$) adversely. Thus there is a compensating effect due to lowering of both the first and second step of the reaction sequence resulting in only minor variations in the selectivity.

The influence of γ_{I2} is on the selectivity and not on the effectiveness factor η. As γ_{I2} increases the value of selectivity σ increases due to the suppression of the second reaction.

In the above discussions, the external (bulk) concentration of the intermediate P_1 was taken as zero. This is valid for a batch reactor at the start of the reaction or in a continuous packed bed reactor near the entrance to the reactor. It would be interesting to examine the influence of P_{10} for the case of product inhibition. This was done by varying $p_0=P_1/P_{10}$ (Table 3).

6. Analysis of systems with substrate inhibition

The rates of many biological systems are inhibited by the substrate. The inhibition is generally of a non-competitive nature. Systems with substrate inhibition have a characteristic that multiple steady states are possible within some range of values of ϕ_1. The analysis of this problem can be done by a graphical procedure suggested in the paper by Ramachandran.[9] Basically, this involves using a single collocation approximation and solving the resulting algebraic equations graphically.

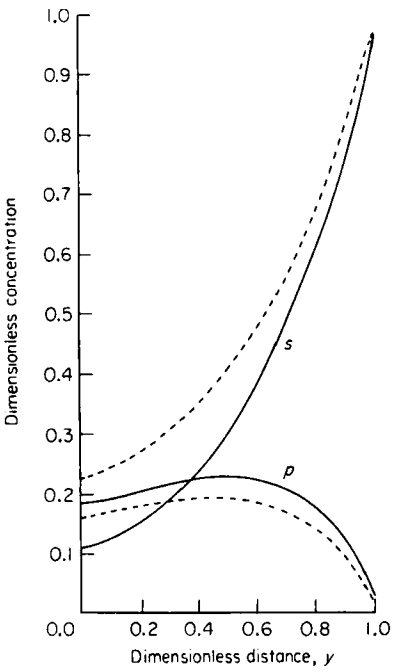

Figure 4. Effect of the product inhibition parameter γ_{I1} on the concentration distribution of S and P_1 inside a spherical capsule ($\phi_1 = 5.0$, $Sh_s = Sh_p = 50.0$, $q = 1.0$, $\beta = 1.0$, $\gamma_1 = 1.0$, $\gamma_2 = 1.0$, $\gamma_{I2} = 0.0$, $p_0 = 0.0$, $p_{20} = 0.0$). ———, $\gamma_{I1} = 0.0$; – – – – –, $\gamma_{I1} = 4.0$.

Accuracy is increased by using more collocation points. One may expect multiple steady states for both S and P_1 to occur under certain conditions.

7. Application to a backmix reactor

In this section we shall indicate the application of the above method in predicting the performance of a backmix reactor carrying out consecutive enzymic reactions. The material balance equations for a backmix reactor are:

For S

$$vS_{10} - vS_0 = V(1 - \epsilon_L)\,\eta f_s(S_0, P_0) \tag{29}$$

For P_1

$$vP_{110} - vP_{10} = V(1 - \epsilon_L)\,\eta\,\frac{\sigma}{1+\sigma} f_p(P_{10}, P_{20}) \tag{30}$$

where S_{10}, P_{110} are the inlet concentrations and S_0, P_{10} are the reactor exit concentrations.

For a given reactor size and inlet conditions, the outlet conditions can be obtained as follows:

a. Assume the outlet concentrations S_0 and P_{10} and calculate $\gamma_1 (= S_0/K_{m1})$ and $\gamma_2 (= S_0/K_{m2})$.

b. Now from a knowledge of the size of the catalyst pellets and intrinsic kinetics (i.e. ϕ_1 and ϕ_2), we can calculate η and σ using the collocation technique.

c. Equations (29) and (30) can now be used to obtain a new estimate of the outlet concentrations and the steps **a**, **b** and **c** repeated till convergence is obtained.

7. Conclusions

The effect of diffusional limitations on the rates of a consecutive reaction sequence catalysed by two enzymes immobilised inside a spherical pellet has been analysed. The problem is solved by use of

an orthogonal collocation technique which requires the solution of a set of non-linear *algebraic* equations. The analysis should prove useful in obtaining the optimum conditions for improving the rates and selectivity of consecutive reactions and also in the design of enzyme reactors. Systems exhibiting more complex kinetics can also be formulated in a similar manner and the same solution method would apply.

Appendix

Nomenclature

A_{ij}	Collocation matrix for the derivative
B_{ij}	Collocation matrix for the Laplacian
D_s, D_p	Diffusion coefficient of the substrate S and intermediate P_1 inside the pellet
E_1, E_2	Concentrations of enzymes 1 and 2 within the pellet
f_s	Rate of first reaction, $S \rightarrow P_1$
f_p	Rate of second reaction, $P_1 \rightarrow P_2$
k_1, k_2	Enzyme reaction rate constant for the first and second reaction respectively
$(k_b)_s$, $(k_b)_p$	External mass transfer coefficients for the transport of species S and P_1 respectively
K_{m1}, K_{m2}	Michaelis constant for first and second reaction respectively
K_{I1}, K_{I2}	Product inhibition parameters for first and second reaction respectively
N	Number of interior collocation points
P_1, P_2	Concentrations of the intermediate and product respectively
P_{10}	Concentration of P_1 in the bulk liquid
P_{1i0}	Concentration of P_1 in the inlet stream of the backmix reactor
p	Dimensionless concentration P_1/S_0
p_0	Dimensionless concentration P_{10}/S_0
p_2	Dimensionless concentration P_2/S_0
q	Ratio of diffusion coefficients D_p/D_s
r	Radial position in the pellet
R	Radius of pellet
S	Concentration of the substrate
S_0	Concentration of the substrate in the bulk solution
S_{i0}	Concentration of substrate in the inlet to backmix reactor
s	Dimensionless concentration S/S_0
Sh_s, Sh_p	Modified Sherwood numbers
v	Volumetric flow rate of reactant stream
V	Volume of reactor
w_i	Weights defined as in equation (21)
y	Dimensionless distance r/R

Greek letters

β	$= \dfrac{k_1 E_1}{k_2 E_2}$
ϕ_1	$= \left(\dfrac{k_1 E_1 R^2}{K_{m1} D_s}\right)^{1/2}$
ϕ_2	$= \left(\dfrac{k_2 E_2 R^2}{K_{m2} D_s}\right)^{1/2}$
γ_1	$= S_0/K_{m1}$
γ_2	$= S_0/K_{m2}$
γ_{I1}	$= S_0/K_{I1}$
γ_{I2}	$= S_0/K_{I2}$

η	Effectiveness factor defined by equation (11)
σ	Selectivity defined by equation (12)
ϵ_L	Volume fraction of liquid in the reactor

References

1. Mosbach, K.; Mattiasson, B. *Acta chem. scand.* 1970, **24**, 2093.
2. Mattiasson, B.; Mosbach, K. *Biochim. biophys. Acta* 1971, **235**, 253.
3. Mosbach, K.; Larsson, P. O. *Biotech. Bioeng.* 1970, **12**, 19.
4. Kuchel, P. W.; Nichol, L. W.; Jeffrey, P. D. *J. theor. Biol.* 1974, **48**, 39.
5. Easterby, J. S. *Biochim. biophys. Acta* 1973, **293**, 552.
6. Goldman, R.; Katchalski, E. *J. theor. Biol.* 1971, **32**, 243.
7. Krishna, R.; Ramachandran, P. A. *J. appl. Chem. Biotechnol.* 1975, **25**, 623
8. Villadsen, J. V.; Stewart, W. E. *Chem. Engng Sci.* 1967, **22**, 1483.
9. Ramachandran, P. A. *Biotech. Bioeng.* 1975, **17**, 211.
10. Ramachandran, P. A. *J. appl. Chem. Biotechnol.* 1974, **24**, 265.