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Abstract

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Analysis of an Immoblised Enzyme Reactor with Catalysts Activation

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Abstract

We investigate the behavior of a reaction described by Michaelis-Menten kinetics in an immobilized enzyme reactor (IER). The IER is treated as a well stirred flow reactor, in which the immobilized bounded and unbounded enzyme species are constrained to remain within the reaction vessel. The product species leaves the IER in the reactor outflow. Before the substrate can react with the enzyme, the enzyme must first be activated by absorption of an activator. We use steady state analysis to identify the best operating conditions or the reactor. To this end, we show that the concentration of product is maximized at low residence time whereas the productivity of the reactor is maximized at high residence times.

KEYWORDS: bioreactors, nonlinear dynamics, reaction engineering

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1 Introduction

Immobilised enzyme technology (IET) is attractive to process industries in which either the enzyme (biocatalysts or microorganisms) involved are expensive or a large throughput of substrate is required. In a bioreactor using IET the enzymes are constrained to remain within the reactor. The advantages of IER include: the easy re-use of the enzymes, easier product recovery and purification, the use of high enzyme loads, prolonged enzyme activity, and the production of large amounts of product with relatively small amounts of catalyst (Katchalski-Katzir, 1993). When operated as a continuous process additional advantages include reductions in costs and energy, the ability to recycle products and to operate at high flow rates. This last point is particularly important in the food industry, especially in the treatment of perishable commodities (D'Souza, 1999). The high yield of pure material resulting from the use of IET is associated with a reduction in the production of waste products. Finally, immobilization often protects the enzyme from inactivation. This allows reactions to occur under harsher environmental conditions, such as pH or temperature or even allowing the use of organic solvents, than when the non-immobilised enzyme is used.

Enzymes can be constrained to remain in the reactor by attaching them to solid constraints. Techniques for immobilization can be broadly classed into four categories: adsorption, covalent bonding, cross-linking and entrapment in a polymer lattice or in microcapsules. Alternatively, the use of a continuous ultrafiltration-membrane reactor allows the enzyme to remain in solution if the membrane is permeable to the substrate and product and impermeable to the enzyme. The use of IET has increased steadily in the chemical, food and pharmaceutical industries. Furthermore, IET can be used for waste-water treatment when the contaminants have been well-characterised. Examples of immobilized reactor applications include (Balaceo *et al*, 1996, Durante *et al*, 2004, and Nighojkar *et al*, 1995). The use of immobilized enzymes is reviewed by (Katchalski-Katzir, 1993, and D'Souza, 1999)

We investigate a model for an enzyme-catalysed reaction occurring in an immobilised enzyme reactor (IER) in which the catalyst must be activated before the reaction can proceed. The reaction is assumed to proceed via Michaelis-Menten kinetics. The Michaelis-Menten mechanism is widely used in modeling studies because it has been shown to provide a useful interpretation of kinetic data from many enzyme-catalysed reactors (Campbell, 1988).

The reaction scheme is

$$E + W \underset{k_d}{\overset{k_a}{=}} [E-W] + [E-A], \tag{1}$$

$$[E-A] + S \underset{k_{-1}}{\overset{k_1}{=}} [E-S] \overset{k_2}{\rightarrow} [E-A] + P. \tag{2}$$

The process represented by equation (1) is the reversible activation of the enzyme (E) through the absorption of an activating species (W). The activation process produce an enzyme-activator complex ($[E-W]$) and an active site ($[E-A]$). The active site may be part of the enzyme-activator complex. Alternatively, it could could be a byproduct of the formation of the complex without being part of it. The process represented by equation (2) is the conversion of a substrate species (S) to a product species (P).

The first step of this conversion is the reversible formation of a bounded enzyme species, the enzyme-substrate complex $[E-S]$. The final step is the irreversible decay of the bounded enzyme species, $[E-S]$, to give an activated site, $[E-A]$, and the product. We interpret this process as representing the synthesis of a desired chemical species.

In an earlier paper we consider a related reaction scheme in which catalyst activation is not required (Nelson *et al*, 2005). Thus, in the earlier paper the kinetic scheme consisted of the process represented by equation (2).

2 Model equations

We model the IER as a perfectly mixed bioreactor. The substrate (S) flows through the IER whilst the reaction product (P), and unused substrate, are discharged from it. A key feature of IET is the retention of an immobilised enzyme within a zone of a flow reactor. This is reflected in the model as it is assumed that the unbounded and bounded enzymes are constrained to remain within the reactor. Thus there are no flow terms in equations (7)–(8): $-q[E-A]$ and $-q[E-S]$. We further assume that there are no enzyme complexes initially present in reactor, equations (11).

The dimensional and dimensionless forms of our model are stated in sections 2.1 & 2.2 respectively.

2.1 Dimensional Model

The governing equations of our system are given by

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Enzyme associated species

$$V\frac{\mathrm{d}}{\mathrm{d}t}[E] = V(k_d[E-W] - k_a[E]W) \tag{3}$$

$$V\frac{\mathrm{d}}{\mathrm{d}t}[E-A] = V(k_a[E]W - k_d[E-W] - k_1[E-A]S + k_{-1}[E-S]) + Vk_2[E-S] \tag{4}$$

$$V\frac{\mathrm{d}}{\mathrm{d}t}[E-S] = V(k_1[E-A]S - k_{-1}[E-S] - k_2[E-S]) \tag{5}$$

$$V\frac{\mathrm{d}}{\mathrm{d}t}[E-W] = V(k_a[E]W - k_d[E-W]) \tag{6}$$

Non-enzyme associated species

$$V\frac{\mathrm{d}S}{\mathrm{d}t} = q(S_0 - S) + V(k_{-1}[E-S] - k_1[E-A]S) \tag{7}$$

$$V\frac{\mathrm{d}W}{\mathrm{d}t} = q(W_0 - W) + V(k_d[E-W] - k_a[E]W) \tag{8}$$

$$V\frac{\mathrm{d}P}{\mathrm{d}t} = q(P_0 - P) + Vk_2[E-S] \tag{9}$$

initial conditions

$$[E](t=0) = E_0, \tag{10}$$

$$[E-A](t=0) = [E-S](t=0) = [E-W](t=0) = S(t=0) = W(t=0) = P(t=0) = 0. \tag{11}$$

The terms that appear in equations (3)–(11) are defined in the nomenclature.

Adding equations (3) & (6), integrating and applying the initial conditions we obtain that

$$[E](t) + [E-W] = E_0. \tag{12}$$

This relationship therefore enables the elimination of one of the enzyme species ($[E], [E-W]$) from the model We choose to eliminate the unbounded enzyme species ($[E]$).

Adding equations (3), (4) & (6), integrating and applying the initial conditions we obtain that

$$[E](t) + [E-A](t) + [E-W](t) = E_0.$$

Applying relationship (12) we obtain

$$[E-A](t) = [E-W](t) - [E-S](t) \tag{13}$$

We use this relationship to eliminate the concentration of active enzyme sites ($[E-A]$) from the model.

2.2 Dimensionless equations

We non-dimensionalise the concentration of enzyme and non-enzyme species relative to the initial concentration of free enzyme in the reactor (E_0). Time is non-dimensionalised using the reaction-rate constant k_{-1}. A feature of our scaled model is that there is a one-to-one correspondence between scaled and non-scaled variables. Therefore, in what follows, we will, for example, write about substrate concentration in the feed rather than 'scaled substrate concentration in the feed'. Equations (12) & (13) become

$$\theta(t^*) + \theta_W(t^*) = 1,$$
$$\theta_A(t^*) = \theta_W(t^*) - \theta_S(t^*).$$

The dimensionless model equations are then

$$\frac{d\theta_S}{dt^*} = k_1^*\theta_W S^* - k_1^*\theta_S S^* - (1+k_2^*)\theta_S \tag{14}$$

$$\frac{d\theta_W}{dt^*} = k_a^* W^* - k_a^* W^*\theta_W - k_d^*\theta_W \tag{15}$$

Non-enzyme associated species

$$\frac{dS^*}{dt} = \frac{1}{\tau^*}(S_0^* - S^*) + \theta_S + k_1^*\theta_S S^* - k_1^*\theta_W S^* \tag{16}$$

$$\frac{dW^*}{dt^*} = \frac{1}{\tau^*}(W_0^* - W^*) + k_d^*\theta_W + k_a^* W^*\theta_W - k_a^* W^* \tag{17}$$

$$\frac{dP^*}{dt} = \frac{1}{\tau^*}(P_0^* - P^*) + k_2^*\theta_S \tag{18}$$

initial conditions

$$\theta_S(t^*=0) = \theta_W(t^*=0) = S^*(t^*=0) = W^*(t^*=0) = P^*(t^*=0) = 0. \tag{19}$$

In these equations τ^*, a dimensionless residence time, is the main experimentally controllable parameter.

The product concentration (P^*) does not appear in equations (14)–(17). Thus we are free to study the subsystem (14)–(17) that contains four coupled ordinary differential equations.

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3 Results

In our analysis we assume that the concentration of product entering the reactor is zero, i.e. $P_0 = P_0^* = 0$.

In section 3.1 we show that the system has a unique, physically meaningful, steady-state solution. For a physically meaningful solution we require that the fractional coverage of the enzyme is between zero and one ($0 \leq \theta_S^*, \theta_W^* \leq 1$) and that all other concentrations are non-negative ($S^* > 0$, $W^* > 0$). In section 3.2 we show that this solution is always locally stable.

3.1 Steady-state analysis

The steady-state solutions of equations (14)–(15) are given by

$$(\theta_s, \theta_W, S^*, W^*) = \left(\hat{\theta}_S, \frac{k_a^* W_0^*}{k_a^* W_0^* + k_d^*}, \frac{(1+k_2^*)\,\hat{\theta}_S}{k_1^*\,(\theta_W - \hat{\theta}_S)}, W_0^* \right) \tag{20}$$

where the steady-state value $\hat{\theta}_S$ solves the quadratic equation

$$\mathscr{G}\left(\hat{\theta}_S\right) = a\hat{\theta}_S^{\,2} + b\hat{\theta}_S + c, \tag{21}$$

$$a = k_1^* k_2^* \tau^*, \tag{22}$$

$$b = -\left[k_1^*\left(k_2^* \theta_W \tau^* + S_0^*\right) + (1+k_2^*)\right], \tag{23}$$

$$c = k_1^* \theta_W S_0^*. \tag{24}$$

Calculation shows that

$$b^2 - 4ac = k_1^{*2}\left(k_2^* \theta_W \tau^* - S_0^*\right)^2 + (1+k_2^*)^2 + 2k_1^*\,(1+k_2^*)\left(k_2^* \theta_W \tau^* + S_0^*\right) > 0.$$

Consequently as $a > 0$, $b < 0$ and $c > 0$ we deduce that equation (21) has two positive solutions. We have

$$\mathscr{G}\hat{\theta}_S = 0 = k_1^* \theta_W S_0^* > 0, \tag{25}$$

$$\mathscr{G}\hat{\theta}_S = \theta_W = -\left(1+k_s^*\right)\theta_W < 0. \tag{26}$$

Thus we deduce that we have one solution with $0 < \hat{\theta}_S < \theta_W$ and one solution with $\theta_W < \hat{\theta}_S$. From the component of the steady-state solution in equation (20) that represents dimensionless substrate S^*) we deduce that only the former solution is

physically meaningful. For when $\theta_W < \hat{\theta}_S$ we have $S^* < 0$. Thus the steady-state solution for the dimensionless substrate-enzyme complex is given by

$$\theta_S = \frac{1}{2k_1^* k_2^* \tau^*}\left\{\left[k_1^*\left(k_2^*\theta_W\tau^* + S_0^*\right) + \left(1+k_2^*\right)\right] - \sqrt{k_1^{*2}\left(k_2^*\theta_W\tau^* - S_0^*\right)^2 + \left(1+k_2^*\right)^2 + 2k_1^*\left(1+k_2^*\right)\left(k_2^*\theta_W\tau^* + S_0^*\right)}\right\}. \tag{27}$$

Note that steady-state value for θ_W is given by a sigmoidal function that saturates for large values of the activator concentration in the feed (W_0^*).

3.2 Stability

The Jacobian matrix for equations 14–17 is

$$J = \begin{pmatrix} -k_1^* S^* - \left(1+k_2^*\right) & k_1^* S^* & k_1^*\left(\theta_W - \theta_S\right) & 0 \\ 0 & -k_a^* W^* - k_d^* & 0 & k_A^*\left(1-\theta_W\right) \\ 1+k_1^* S^* & -k_1^* S^* & -\frac{1}{\tau^*} + k_1^*\left(\theta_S - \theta_W\right) & 0 \\ 0 & k_d^* + k_a^* W^* & 0 & -\frac{1}{\tau^*} + k_A^*\left(\theta_W - 1\right) \end{pmatrix}$$

The characteristic polynomial of this matrix is given by

$$P(\Lambda) = P_1(\Lambda)\cdot P_2(\Lambda),$$

$$P_1(\Lambda) = \Lambda^2 + \left[\frac{1}{\tau^*} + \left(1-\theta_W\right)k_a^* + k_a^* W^* + k_d^*\right]\Lambda + \frac{1}{\tau^*}\left(k_a^* W^* + k_d^*\right),$$

$$P_2(\Lambda) = \Lambda^2 + \left[k_2^* + \left(\theta_W - \theta_S\right)k_1^* + 1 + k_1^* S^* + \frac{1}{\tau^*}\right]\Lambda + \frac{1+k_1^* S^* + k_2^*}{\tau^*} + k_1^* k_2^*\left(\theta_W - \theta_S\right).$$

Recall that the roots of a quadratic polynomial

$$\Lambda^2 + b\Lambda + c = 0$$

have strictly negative real part provided that

$$b > 0,$$
$$c > 0.$$

Then as

$$\theta_W < 1$$

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and

$$\theta_S < \theta_W$$

it follows that the steady-state solution (20) is always locally stable.

4 Discussion

Here we use the physically meaningful steady-state solution found in the previous section to investigate how the product concentration leaving the reactor system and the reactor productivity vary as a function of the residence time.

4.1 Product concentration leaving the reactor

The steady-state product concentration is obtained from equation (18) and equation (27) and is given by

$$P^* = +\frac{1}{2k_1^*}\left\{\left[k_1^*\left(k_2^*\theta_W\tau^* + S_0^*\right) + \left(1+k_2^*\right)\right] - \sqrt{k_1^{*2}\left(k_2^*\theta_W\tau^* - S_0^*\right)^2 + \left(1+k_2^*\right)^2 + 2k_1^*\left(1+k_2^*\right)\left(k_2^*\theta_W\tau^* + S_0^*\right)}\right\}. \tag{28}$$

From equation (28) it follows that

1. In the limit of small residence times ($\tau^* \ll 1$) we have
$$P^* = \frac{k_1^*k_2^*\theta_W S_0^*}{1+k_1^*S_0^*+k_2^*}\tau^* + O\left(\tau^{*2}\right).$$
2. In the limit of high residence times ($\tau^* \gg 1$) we have
$$P^* = S_0^* - \frac{\left(1+k_2^*\right)S_0^*}{k_1^*k_2^*\theta_W}\frac{1}{\tau^*} + O\left(\frac{1}{\tau^2}\right).$$
3. For $\tau^* \in (0,\infty)$
$$\frac{\mathrm{d}P^*}{\mathrm{d}\tau^*} > 0.$$
4. For $W_0^* \geq 0$
$$\frac{\mathrm{d}P_0^*}{\mathrm{d}W_0^*} > 0.$$

We therefore deduce that the product concentration leaving the reactor is an increasing function of the residence time and of the concentration of activator in the feed. Thus the maximum concentration is obtained at an infinite residence time and is independent of the concentration of activator in the feed.

4.2 Reactor productivity

An alternative way to define the performance of a reactor is to consider its dimensionless productivity (Pr^*) which is given by

$$\begin{aligned} Pr^* &= \frac{P^*}{\tau^*} \\ &= \frac{1}{2k_1^*\tau^*}\left\{\left[k_1^*\left(k_2^*\theta_W\tau^* + S_0^*\right) + \left(1+k_2^*\right)\right]\right. \\ &\quad \left. -\sqrt{k_1^{*2}\left(k_2^*\theta_W\tau^* - S_0^*\right)^2 + \left(1+k_2^*\right)^2 + 2k_1^*\left(1+k_2^*\right)\left(k_2^*\theta_W\tau^* + S_0^*\right)}\right\}. \end{aligned} \tag{29}$$

From equation (29) it is possible to deduce that

1. In the limit of small residence times ($\tau^* \ll 1$) we have

$$Pr^* \approx \frac{k_1^*k_2^*\theta_W S_0^*}{1+k_2^*+k_1S_0^*}\left\{1 - \frac{k_1^*k_2^*\theta_W\left(1+k_2^*\right)S_0^*}{\left(1+k_2^*\right)^2 + 2\left(1+k_2^*\right)k_1^*S_0^* + k_1^{*2}S_0^{*2}}\cdot\tau^*\right\} + O\left(\tau^{*2}\right)$$

2. In the limit of high residence times ($\tau^* \gg 1$) we have

$$Pr^* \approx \frac{S_0^*}{\tau^*} + O\left(\frac{1}{\tau^{*2}}\right).$$

3. For $\tau^* \in (0,\infty)$

$$\frac{dP^*}{d\tau^*} < 0.$$

To establish this we first obtain

$$\frac{dPr^*}{dt^*} = \frac{A(\tau^*)}{2k_1^*\tau^{*2}\sqrt{b^2-4ac}},$$

$$A(\tau^*) = (k_1S_0^* + 1 + k_2^*)^2 + (1 + k_2^* - k_1^*S_0^*)k_1^*k_2^*\theta_W\tau^* - (k_1S_0^* + 1 + k_2^*)\sqrt{b^2-4ac}.$$

It is clear that on the interval $\tau^* \in (0,\infty)$ that the derivative is continuous. Therefore in order for the derivative to change sign there must be a value of the residence time with $\tau^* \in (0,\infty)$ for which $A(\tau^*) = 0$. Assuming that there is such a value leads to the conclusion that

$$4k_a^{*3}k_2^{*2}\theta_W^2S_0^*\left(1+k_2^*\right)\tau^{*2} = 0,$$

but this is impossible for $\tau^* \in (0,\infty)$. Thus we conclude that the derivative is only ever of one sign in the required interval.

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By comparison of the asymptotic limits for the dimensionless productivity in the limits of low ($\tau^* \ll 1$) and high ($\tau^* \gg 1$) residence times we deduce that the derivative is always negative.

4. For $W_0^* \geq 0$

$$\frac{\mathrm{dPr}^*}{\mathrm{d}W_0^*} > 0.$$

We therefore deduce that the reactor productivity is a decreasing function of the residence time and an increasing function of the concentration of activator in the feed (W_0^*). The maximum productivity, obtained in the limit of a zero residence time, is a linear function of the proportion of the enzyme present in the form of activator-enzyme complex (θ_W). As the value for θ_W, equation (20), is a sigmoidal function of W_0^* there comes a point where increases in the value for W_0^* lead to negligible increases in the value for the productivity.

In this paper we have considered applications of immobilised enzyme technology in process industries. A recent application of IET is to design micro-scale immune-biosensor's, which have potential applications in clinical analyses, environmental control and food quality control. The kind of analysis reported here may have applications in the development of the novel immunosensing strategy proposed Liu *et al* (2006).

5 Conclusions

In this paper we have investigated the performance of an immobilised enzyme reactor. The kinetic scheme used was the Michaelis-Menten mechanism, in which the enzyme must first be activated by absorption of an activating species. This extends earlier work in which activation of the enzyme was not required (Nelson *et al*, 2005).

We have shown that the both the product concentration and the reactor productivity are increasing functions of the concentration of activator in the feed. However, whilst the product concentration is an increasing function of the residence time the reactor productivity is a decreasing function of the residence time.

The next step in this research program is to validate our model using experimental data. In particular, it will be instructive to compare the predictions of the current model with that of the standard model (Nelson *et al*, 2005), which does not require activation of the enzyme.

A Nomenclature

$[E]$	Concentration of immobilised enzyme	$(\mathrm{mol\,m^{-3}})$
$[E]_0$	Concentration of immobilised enzyme at time $t = 0$.	$(\mathrm{mol\,m^{-3}})$
$[E-S]$	Concentration of immobilised species $E-S$.	$(\mathrm{mol\,m^{-3}})$
P	Concentration of the product species.	$(\mathrm{mol\,m^{-3}})$
P^*	Dimensionless product concentration. $P^* = \frac{P}{E_0}$	(—)
Pr^*	Dimensionless productivity. $\mathrm{Pr}^* = \frac{P^*}{\tau^*}$	(—)
S	Concentration of substrate.	$(\mathrm{mol\,m^{-3}}))$
S^*	Dimensionless substrate concentration. $S^* = \frac{S}{E_0}$	(—)
V	Volume of the reactor.	$(\mathrm{m^3})$
W	Concentration of activating species.	$(\mathrm{mol\,m^{-3}}))$
W^*	Dimensionless concentration of activating species. $W^* = \frac{W}{E_0}$	(—)
k_1	Forward reaction-rate constant	$(\mathrm{m^3\,mol^{-1}\,s^{-1}})$
k_1^*	Dimensionless forward reaction-rate constant $k_1^* = \frac{k_1 E_0}{k_{-1}}$	(—)
k_{-1}	Backwards reaction-rate constant	$(\mathrm{s^{-1}})$
k_2	Reaction-rate constant.	$(\mathrm{s^{-1}})$
k_2^*	Dimensionless reaction-rate constant. $k_2^* = \frac{k_2}{k_{-1}}$	(—)
k_a	Reaction-rate constant for activation of enzyme active sites.	$(\mathrm{m^3\,mol^{-1}\,s^{-1}})$
k_a^*	Dimensionless reaction-rate constant for activation of enzyme active sites. $k_a^* = \frac{k_a E_0}{k_{-1}}$	(—)
k_d	Reaction-rate constant for deactivation of enzyme active sites.	$(\mathrm{s^{-1}})$
k_d^*	Dimensionless reaction-rate constant for deactivation of enzyme active sites. $k_d^* = \frac{k_d}{k_{-1}}$	(—)
q	Flow rate.	$(\mathrm{m^3\,s^{-1}})$
t	Time.	(s)
t^*	Dimensionless time.	(—)

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	$t^* = k_{-1}t$.	
θ	Proportion of the enzyme present in the form of unbounded enzyme. $\theta = \frac{[E]}{[E]_0}$	(—)
θ_S	Fraction of enzyme presented in the form of substrate-enzyme complex. $\theta_S = \frac{[E-S]}{[E]_0}$	(—)
θ_W	Proportion of the enzyme present in the form of activator-enzyme complex. $\theta_W = \frac{[E-W]}{[E]_0}$	(—)
τ^*	Dimensionless residence time. $\tau^* = \frac{V}{q} \cdot k_{-1}$	(—)

References

Balceo, V.M., Paiva, A.L., and Malcata, F.X. Bioreactors with immobilized lipases: State of the art *Enzyme and Microbial Technology*, 18(6), 392–416, 1996.

Campbell, I.M. *Catalysis at Surfaces*. Chapman and Hall, first edition, 1988.

Durante, D., Casadio, R., Martelli, L., Tasco, G., Portaccio, M., De Luca, P., Bencivenga, U., Rossi, S., Di Martino, S., Grano, V., Diano, N., and Mita, D.G. Isothermal and non-isothermal bioreactors in the detoxification of waste waters polluted by aromatic compounds by means of immobilised laccase from Rhus vernicifera. *Journal of Molecular Catalysis B: Enzymatic*, 27(4–6), 191–206, 2004.

Katchalski-Katzir, E. Immobilized enzymes — learning from past successes and failures. *Trends in Biotechnology*, 11(11):471–478, 1993.

Liu, Y., Qin, Z., Wu, X., and Jiang, H. Immune-biosensor for aflatoxin B_1 based bio-electrocatalytic reaction on micro-comb electrode. *Biochemical Engineering Journal*, 32:211–217, 2006.

Nelson, M.I., Chen, X.D., and Sexton, M.J. Analysis of the Michaelis-Menten mechanism in an immobilised enzyme reactor. *The ANZIAM Journal*, 47(2):173–184, 2005.

Nighojkar, A., Srivastava, S., and Kumar, A. Production of low methoxyl pectin using immobilized pectinesterase bioreactors. *Journal of Fermentation and Bioengineering*, 80(4), 346–349, 1995.

Souza, S.F. Immobilized enzymes in bioreactors. *Current Science*, 77:69–79, 1999.