ANZIAM J. 47(2005), 173–184

ANALYSIS OF THE MICHAELIS-MENTEN MECHANISM IN AN IMMOBILISED ENZYME REACTOR

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(Received 30 January, 2004; revised 10 July, 2005)

Abstract

We investigate the behaviour of a reaction described by Michaelis-Menten kinetics in an immobilised enzyme reactor (IER). The IER is treated as a well-stirred flow reactor, with the restriction that bounded and unbounded enzyme species are constrained to remain within the reaction vessel. Our aim is to identify the best operating conditions for the reactor. The cases in which an immobilised enzyme reactor is used to either reduce pollutant emissions or to synthesise a product are considered. For the former we deduce that the reactor should be operated using low flow rates whereas for the latter high flow rates are optimal. It is also shown that periodic behaviour is impossible.

1. Introduction

Immobilised enzyme technology (IET) is attractive to process industries in which either the enzymes (biocatalysts) involved are expensive or a large throughput of substrate is required. We investigate a model for an enzyme-catalysed reaction, obeying Michaelis-Menten kinetics, occurring in an immobilised enzyme reactor (IER).

The Michaelis-Menten scheme is

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} [E - S] \overset{k_2}{\to} E + P. \tag{1.1}$$

The process represented by (1.1) 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, an enzyme-substrate complex, ($[E - S]$). The final step is the irreversible decay of the the bounded enzyme species to give unbounded

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enzyme ($[E]$) and the product. We interpret this process as representing either the removal of a pollutant from a feed stream or the synthesis of a desired chemical species.

The Michaelis-Menten mechanism is widely used in modelling studies because it has been shown to provide a useful interpretation of kinetic data from many enzyme-catalysed reactions [2]. This study is motivated by its use for modelling the production of hydrolysed lactose, a product in great demand by consumers who are lactose-intolerant, in a batch reactor [4].

1.1. Interpretation of the model A bioreactor is a vessel in which biological reactions are carried out by microorganisms or enzymes contained within the reactor itself. A membrane bioreactor is a vessel integrating membrane separations with biological transformation. In this paper we have in mind a membrane bioreactor in which the membrane walls constrain the freely-floating immobilised enzyme to remain within the reactor. However, our model applies equally well to bioreactors in which active enzyme groups are attached to the vessel walls through techniques such as adsorption, chemical bonding, polymer lattice entrapment *etc*. Although the models for these two types of IER are equivalent, there are some differences in the interpretation of the rate constants and the species concentrations between the two reactors. For instance, in the flow reactor the concentration of the species $[E]$ is measured in units of $\mathrm{mol\,m^{-2}}$, because the enzyme is dispersed over the surface of the reactor, and a new constant, A_s, the surface area over which the enzyme may be immobilised, is introduced.

2. Equations

We model the IER as a continuously stirred tank reactor. The substrate (S) flows through the IER whilst the reaction products are discharged from it — the retention of an immobilised enzyme within a zone of a flow reactor is one of the main advantages of IET. However, the unbounded and bounded enzyme are constrained to remain within the reactor. Thus there are no flow terms in Equations (2.1) and (2.3), such as $-q[E]$ and $-q[E-S]$. We further assume that there are no enzyme complexes initially present in the reactor, Equations (2.5) and (2.6).

2.1. Dimensional equations The model equations for the kinetic scheme (1.1) occurring in an IER are

unbounded enzyme concentration:

$$V\frac{\mathrm{d}[E]}{\mathrm{d}t} = V\left(k_{-1}[E-S] - k_1[E]S + k_2[E-S]\right), \tag{2.1}$$

substrate concentration:

$$V\frac{\mathrm{d}S}{\mathrm{d}t} = q\left(S_0 - S\right) + V\left(k_{-1}[E-S] - k_1[E]S\right), \tag{2.2}$$

concentration of bounded enzyme:

$$V\frac{\mathrm{d}[E-S]}{\mathrm{d}t} = V\left(k_1[E]S - k_{-1}[E-S] - k_2[E-S]\right), \tag{2.3}$$

product concentration:

$$V\frac{\mathrm{d}[P]}{\mathrm{d}t} = -qP + Vk_2[E-S] \quad \text{and} \tag{2.4}$$

initial conditions:

$$[E](t=0) = E_0, \tag{2.5}$$

$$S(t=0) = [E-S](t=0) = P(t=0) = 0. \tag{2.6}$$

We have assumed that enzyme species neither flow into nor out of the membrane bioreactor, a key feature of such a vessel. Furthermore, enzyme concentration is conserved by kinetic scheme (1.1) and therefore on physical grounds the total concentration of enzyme species ($[E]+[E-S]$) must equal its initial value (E_0). Mathematically, this follows from adding (2.1) and (2.3) and integrating to obtain

$$[E](t) + [E-S](t) = [E]_0. \tag{2.7}$$

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

The classical formulation of Michaelis-Menten kinetics applies a stationary state approximation to Equation (2.3), that is, it assumes that $\mathrm{d}[E-S]/\mathrm{d}t \approx 0$ for all t, and uses (2.7) to obtain the approximation

$$[E-S](t) = \frac{k_1 E_0 S}{k_1 S + k_{-1} + k_2}.$$

This expression, containing a saturating effect in the substrate concentration (S), is then substituted into (2.1), (2.2) and (2.4). We do not need to make the stationary state approximation for our analysis.

2.2. Dimensionless equations We non-dimensionalise the enzyme species using the conserved quantity $[E]_0$ by defining

$$\theta = \frac{[E]}{[E]_0} \quad \text{and} \quad \theta_S = \frac{[E-S]}{[E]_0},$$

where θ and θ_S are the proportion of unbounded and bounded enzyme respectively. Equation (2.7) becomes

$$\theta(t) + \theta_S(t) = 1.$$

The concentrations of liquid-phase species (S and P) are non-dimensionalised using the inflow substrate concentration (S_0) and time is non-dimensionalised using the reaction-rate constant k_{-1}.

The dimensionless model equations are then

$$\frac{\mathrm{d}S^*}{\mathrm{d}t^*} = q^*(1 - S^*) + \frac{E_0^*}{S_0^*}\theta_S - E_0^*(1 - \theta_S)S^*, \tag{2.8}$$

$$\frac{\mathrm{d}\theta_S}{\mathrm{d}t^*} = S_0^*(1 - \theta_S)S^* - (1 + k_2^*)\theta_S, \tag{2.9}$$

$$\frac{\mathrm{d}P^*}{\mathrm{d}t^*} = -q^*P^* + \frac{k_2^*E_0^*}{S_0^*}\theta_S. \tag{2.10}$$

The initial conditions are $S^*(t^* = 0) = \theta_S(t^* = 0) = P^*(t^* = 0) = 0$. In these equations q^*, a dimensionless flow rate, is the experimentally controllable parameter.

Observe that the product concentration (P^*) does not appear in (2.8) and (2.9). Thus the variables S^* and θ_S can be solved directly from the two-dimensional system comprised of Equations (2.8) and (2.9).

3. Results

The generation of periodic solutions by biochemical systems has been a matter of considerable experimental and theoretical interest. In Section 3.1 we establish that our model does not exhibit periodic solutions. In Section 3.2 we show that the system has a unique steady-state solution.

3.1. Non-existence of periodic solutions In this section we show that the system defined by (2.8) and (2.9) does not exhibit periodic solutions. In general it is very difficult to prove that a given system of ordinary differential equations *does not* exhibit periodic behaviour. However, the geometric constraints imposed upon solution trajectories in two-dimensional systems allow a powerful technique to be applied to obtain the desired result.

The non-existence proof proceeds in two parts.

(1) We first show that the region (Σ) in (S^*, θ_S) space defined by

$$0 \le \theta \le 1 \quad \text{and} \quad 0 \le S^* \le 1 + \frac{E_0^*}{S_0^*q^*}$$

is invariant. This means that for any initial condition in Σ the associated solution of the model remains in Σ. This result is established in Appendix A.

(2) We use the bounds $S^* \ge 0$ and $\theta_E \le 1$, established above in conjunction with the choice $B = 1$ in Dulac's theorem, to prove that Equations (2.8) and (2.9) cannot exhibit periodic solutions for any initial condition in the set Σ. This is proven in Appendix B.

3.2. Steady-state analysis From (2.9) the steady-state substrate concentration is given by

$$S^* = \frac{(1+k_2^*)}{S_0^*}\frac{\theta_S}{1-\theta_S}. \tag{3.1}$$

The steady-state bounded enzyme concentration is given by the solution of the equation

$$\theta_S^2 - \left[1 + \frac{q^*(1+k_2^*+S_0^*)}{k_2^*E_0^*}\right]\theta_S + \frac{q^*S_0^*}{k_2^*E_0^*} = 0. \tag{3.2}$$

Equation (3.2) has a positive root inside the invariant region Σ with $0 < \theta_S < 1$ and a physically meaningless root outside the invariant region with $1 < \theta_S < \infty$. Thus the steady-state solution for the fractional coverage is given by

$$\theta_S = \frac{1}{2}\left\{\left[1 + \frac{q^*(1+k_2^*+S_0^*)}{k_2^*E_0^*}\right] - \sqrt{\left[1 + \frac{q^*(1+k_2^*+S_0^*)}{k_2^*E_0^*}\right]^2 - \frac{4q^*S_0^*}{k_2^*E_0^*}}\right\}. \tag{3.3}$$

As we established in Section 3.1 that periodic solutions are impossible in Σ we conclude that the unique steady-state solution in Σ is attracting for all initial conditions within the invariant region.

Note that the steady-state corresponding to $\theta_S > 1$ has, from (3.1), $S^* < 0$. It is therefore non-physical.

4. Discussion

The steady-state product concentration is obtained from (2.10) and (3.3) and is given by

$$P^* = \frac{k_2^*E_0^*}{2S_0^*}\left\{\frac{(1+k_2^*+S_0^*)}{k_2^*E_0^*} + \frac{1}{q^*} - \frac{1}{q^*}\sqrt{1 + \frac{2q^*(1+k_2^*-S_0^*)}{k_2^*E_0^*} + \frac{q^{*2}(1+k_2^*+S_0^*)^2}{(k_2^*E_0^*)^2}}\right\}. \tag{4.1}$$

From (4.1) it follows that

(1) In the limit of small flow rates ($q^* \ll 1$) we have

$$P^* \approx 1 - \frac{(1+k_2^*)}{k_2^*E_0^*}q^* + O\left(q^{*^2}\right).$$

(2) In the limit of high flow rates ($q^* \gg 1$) we have

$$P^* \approx \frac{k_2^*E_0^*}{1+k_2^*+S_0^*}\left(\frac{1}{q^*} - \frac{2(1+k_2^*)k_2^*E_0^*}{(1+k_2^*+S_0^*)^2}\frac{1}{q^{*^2}}\right) + O\left(\frac{1}{q^{*^3}}\right).$$

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

$$\frac{\mathrm{d}P^*}{\mathrm{d}q^*} < 0. \tag{4.2}$$

Thus the product concentration decreases from 1 to 0 as the flow rate increases. Figure 1 shows the product concentration as a function of the flow rate.

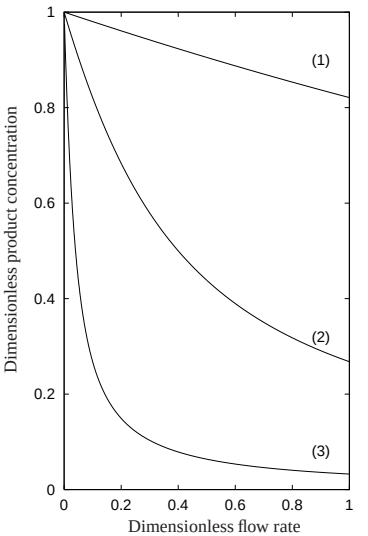

FIGURE 1. The dependence of the dimensionless product concentration (P^*) on the dimensionless flow rate (q^*), Equation (4.1). Enzyme concentration: (1) $E_0^* = 10$, (2) $E_0^* = 1$ and (3) $E_0^* = 0.1$. Parameter values: substrate concentration, $S_0^* = 1$; reaction rate constant, $k_2^* = 1$.

Equation (4.2) shows that product yield decreases with increasing flow rate. Suppose that the substrate species is a pollutant and that the immobilised enzyme reactor is a cleansing technology. If a maximum allowable substrate concentration leaving the reactor is specified, perhaps by legislation, then there exists a maximum flow rate at which the reactor can be operated: if the reactor is operated above this flow rate the emission of pollutant exceeds the specified value. This maximum flow rate can be obtained from (4.1). Thus if the aim is to minimise the emission of a pollutant the reactor must be operated at sufficiently 'low' flow rates. For a simple chemical mechanism such as Michaelis-Menten this is unsurprising: the lower the flow rate the longer that the substrate remains in the reactor, on average molecules have much more time to react.

Assume now that the membrane reactor is being used to synthesise a commercially viable product from the substrate. As the flow rate increases, the product-yield decreases. Do we again conclude that the reactor should be operated at low flow rates?

Consider a situation in which the parameter values are those of line (1) in Figure 1. When the flow rate is $q^* = 1$ the product concentration is $P^* = 0.8211$ whereas for a flow rate $q^* = 2$ the product concentration is $P^* = 0.6834$. Thus, although the product concentration has *decreased*, the steady-state reactor productivity per unit time, which is $R^* = q^*P^*$, has *increased*, from 0.8211 to 1.2668.

Hence if the reactor is being used to synthesise a product the appropriate quantity to consider is not the product concentration but rather the reactor productivity, which is given by

$$R^* = q^*P^* = \frac{k_2^*E_0^*}{2S_0^*}\left\{\frac{(1+k_2^*+S_0^*)q^*}{k_2^*E_0^*} + 1 - \sqrt{1 + \frac{2q^*(1+k_2^*-S_0^*)}{k_2^*E_0^*} + \frac{q^{*2}(1+k_2^*+S_0^*)^2}{(k_2^*E_0^*)^2}}\right\}. \tag{4.3}$$

From (4.3) it is possible to deduce that

(1) In the limit of small flow rates ($q^* \ll 1$) we have

$$R^* \approx q^* - \frac{2(1+k_2^*)}{k_2^*E_0^*}q^{*2} + O(q^{*3}).$$

(2) In the limit of high flow rates ($q^* \gg 1$) we have

$$R^* \approx \frac{k_2^*E_0^*}{1+k_2^*+S_0^*}\left(1 - \frac{2(1+k_2^*)k_2^*E_0^*}{(1+k_2^*+S_0^*)^2}\frac{1}{q^*}\right) + O\left(\frac{1}{q^{*2}}\right).$$

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

$$\frac{dR^*}{dq^*} > 0.$$

Thus the reactor productivity increases monotonically from zero as the flow rate increases, with limiting maximum value

$$(q^*P^*)_{\max} = \frac{k_2^*E_0^*}{1+k_2^*+S_0^*}. \tag{4.4}$$

Thus immobilised enzyme reactors should be operated at the highest possible flow rate (theoretically at an infinite flow rate) to maximise reactor productivity. Figure 2 shows the reactor productivity as a function of the flow rate for the same parameter values used in Figure 1. Note that saturation of the reactor productivity function, Equation (4.3), may occur at relatively low values of the flow rate, such as line (3) in Figure 2. In practice it may be desirable to operate at lower flow rates and accept a reduced reactor productivity. For instance, if there are significant costs associated

with the separation of the substrate and product species it may be more economical to maximise product yield, minimising substrate concentration in the outflow, rather than to maximise the reactor productivity. Operational considerations may impose restrictions on the maximum allowable flow rate, for example the stability of the immobilised enzyme is usually adversely effected at high flow rates.

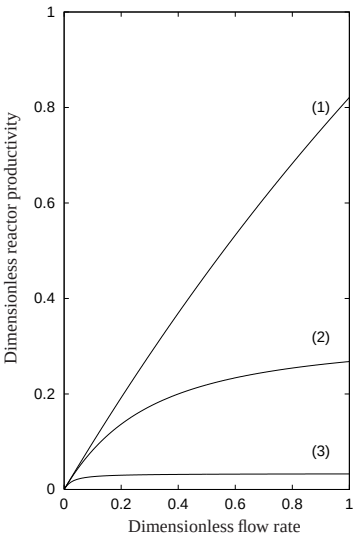

FIGURE 2. The dependence of the dimensionless reactor productivity (q^*P^*) on the dimensionless flow rate (q^*), Equation (4.3). Parameter values are the same as for Figure 1.

Equation (4.4) shows that the maximum reactor productivity is a linear function of the initial enzyme concentration. Very efficient enzymes have a high turnaround of substrate into product, corresponding to a large value for k_2^*. Equation (4.4) shows that the productivity of such enzymes is limited by their initial concentration.

5. Conclusion

We have examined the behaviour of the Michaelis-Menten mechanism in an immobilised enzyme reactor. We have established that periodic behaviour is impossible and that the system has a unique steady-state solution. We have shown that the product concentration inside the reactor is a monotonic decreasing function of the flow rate whilst the reactor productivity is a monotonic increasing function of the flow rate; in the limit that the flow rate becomes infinite the product concentration approaches zero and the reactor productivity obtains a non-zero maximum given by (4.4). Thus if the aim is to reduce the emission of a pollutant, low flow rates are required. Conversely,

if the aim is to maximise reactor productivity high flow rates should be used; this is not an obvious conclusion.

In future work we aim to extend the Michaelis-Menten mechanism to include product competitive inhibition and decomposition of the product via a side reaction. These mechanisms have been used to model the production of hydrolysed lactose in a batch reactor [4]. The ultimate intention of this analysis is to aid future experimental investigations in predicting efficient production techniques for hydrolysed lactose and other enzyme-based products.

Appendix A. Proof of invariant region

DEFINITION 1 (from [1]). Let Σ be a domain enclosed by a simple curve $\partial\Sigma$. Then Σ is an *invariant set* for the two-component system $\mathrm{d}\mathbf{u}/\mathrm{d}t = \mathbf{f}(\mathbf{u})$ if any solution of the system with initial conditions in Σ remains in Σ for all $t > 0$.

LEMMA 1 (from [1]). *If* $\mathbf{f}(\mathbf{u}) \cdot \mathbf{n}(\mathbf{u}) < 0$ *for all* $\mathbf{n}(\mathbf{u}) \in \partial\Sigma$, *where* $\mathbf{n}(\mathbf{u})$ *is the unit outward normal at* $\mathbf{u} \in \partial\Sigma$, *then* Σ *is an invariant set.*

Lemma 1 can be applied to domains containing points where $\mathbf{f} \cdot \mathbf{n} = 0$ or where the normal vector $\mathbf{n}$ is undefined provided that at these points the direction of the derivative vector does not point out of the invariant set.

CLAIM 1. *Using the coordinate system* (S^*, θ_S), *the rectangle defined by*

$$\left(0 \le S^* \le 1 + \frac{E_0^*}{q^* S_0^*},\ 0 \le \theta_S \le 1\right)$$

is invariant for the system defined by (2.8) *and* (2.9).

It is a straightforward calculation to determine whether the given region is invariant. Note that a unit outward normal is not defined at the corners of the region. At these points it is sufficient to show that the direction of the derivative vector does not point out of the invariant set.

We provide a sample calculation for one side of the rectangle to demonstrate the technique. Consider the edge $0 \le S^* \le 1 + (E_0^*/q^* S_0^*)$ with $\theta_S = 0$. The unit outward normal is the vector $(0, -1)$ and we have

$$\begin{aligned}\mathbf{f}(\mathbf{u}) \cdot \mathbf{n}(\mathbf{u}) &= -S_0^*(1-\theta_S)S^* + (1+k_2^*)\theta_S \\ &= -S_0 S^* \quad \text{as } \theta_S = 0 \text{ by assumption} \\ &< 0 \quad \text{by the assumption that } S^* \ge 0 \text{ except at the point } S^* = 0.\end{aligned}$$

We must now consider the direction of the derivative vector at the point $(S^*, \theta_S) = (0, 0)$. At this point we have $(\dot{S^*}, \dot{\theta_S}) = (q^*, 0)$ which points along the boundary of the invariant set. Thus it does not point out of the invariant set. The remaining sides and edge points can be similarly checked.

Appendix B. Dulac's Theorem

DEFINITION 2. Consider two coupled ordinary differential equations

$$\frac{\mathrm{d}x}{\mathrm{d}t} = f(x, y) \quad \text{and} \tag{B.1}$$

$$\frac{\mathrm{d}y}{\mathrm{d}t} = g(x, y). \tag{B.2}$$

The Dulac function $\mathscr{D}$ is defined as

$$\mathscr{D} = \frac{\partial(Bf)}{\partial x} + \frac{\partial(Bg)}{\partial y},$$

where $B(x, y)$ is a function having continuous first partial derivatives.

THEOREM 1 (from [3]). *If the Dulac function $\mathscr{D}$ never changes sign* (*and is not identically zero*) *in a simply connected region Σ then the system defined by* (B.1) *and* (B.2) *does not have a closed orbit* (*for example, periodic solutions*) *entirely within the region Σ.*

CLAIM 2. *The system defined by* (2.8) *and* (2.9) *does not exhibit periodic solutions.*

To prove this result we take the function B above to be the constant function $B = 1$ and use the bounds $\theta_S \leq 1$ and $S^* \geq 0$ that were established in Appendix A.

We have

$$\begin{aligned}
\mathscr{D} &= \frac{\partial}{\partial S^*}\frac{\mathrm{d}S^*}{\mathrm{d}t^*} + \frac{\partial}{\partial \theta_S}\frac{\mathrm{d}\theta_S}{\mathrm{d}t^*} \\
&= -q^* - E_0^*(1 - \theta_S) - S_0^* S^* - (1 + k_2^*) \\
&< -q^* - S_0^* S^* - (1 + k_2^*) \quad \text{as } \theta_S \leq 1 \\
&< -q^* - (1 + k_2^*) \quad \text{as } S^* \geq 0 \\
&< -1 \quad \text{as } q^* \geq 0 \text{ and } k_2^* \geq 0.
\end{aligned}$$

Thus the Dulac function is always of one sign in the invariant region Σ and periodic solutions are therefore impossible in this region.

Appendix C. Nomenclature

$[E]$	Concentration of immobilised enzyme	(mol m^{-3})
$[E]_0$	Concentration of immobilised enzyme at time $t = 0$ $[E]_0^* = (k_1/k_{-1})E_0$	(mol m^{-3})
$[E]_0^*$	Dimensionless concentration of immobilised enzyme at time $t^* = 0$	(—)
$[E - S]$	Concentration of immobilised species $E - S$	(mol m^{-3})
P	Concentration of the product species	(mol m^{-3})
P^*	Dimensionless product concentration $P^* = P/S_0$	(—)
$P(0)$	Concentration of the product species at time $t = 0$: $P(0) = 0$	(mol m^{-3})
R	The rate of production of the product $R = (k_2 E_0/S_0)\theta_S$	(—)
R^*	The dimensionless production rate of the product $R^* = (k_2^* E_0^*/S_0^*)\theta_S$	(—)
S	Concentration of substrate	(mol m^{-3})
S^*	Dimensionless substrate concentration $S^* = S/S_0$	(—)
$S(0)$	Concentration of substrate at time $t = 0$: $S(0) = 0$	(mol m^{-3})
$S^*(0)$	Dimensionless substrate concentration at time $t = 0$: $S^*(0) = S(0)/S_0$	(—)
S_0	Concentration of the substrate S in the inflow	(mol m^{-3})
S_0^*	Dimensionless concentration of the substrate in the inflow $S_0^* = (k_1/k_{-1})S_0$	(—)
V	Volume of the membrane reactor	(m^3)
k_1	Forward reaction-rate constant	$(\text{m}^3\text{mol}^{-1}\text{s}^{-1})$
k_{-1}	Backwards reaction-rate constant	(s^{-1})
k_2	Reaction-rate constant	(s^{-1})
k_2^*	Dimensionless reaction-rate constant $k_2^* = (k_2/k_{-1})$	(—)
q	Flow rate	$(\text{m}^3\text{s}^{-1})$
q^*	Dimensionless flow rate $q^* = q/k_{-1}V$	(—)
t	Time	(s)
t^*	Dimensionless time $t^* = k_{-1}t$	(—)
θ	Proportion of the enzyme present in the form of unbounded enzyme $\theta = [E - S]/[E]_0$	(—)
$\theta(0)$	Proportion of the enzyme present in the form of unbounded enzyme at time $t = 0$: $\theta(0) = [E - S](0)/[E]_0$	(—)
θ_S	Proportion of bounded enzyme present $\theta_S = [E - S]/[E]_0$	(—)

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