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Simulation of the effect of intrinsic reaction kinetics and particle size on the behaviour of immobilised enzymes under internal diffusional restrictions and steady state operation

D. Jeison [a,*], G. Ruiz [b], F. Acevedo [b], A. Illanes [b]

[a] Departamento de Ingeniería Química, Universidad de La Frontera, Casilla 54-D, Temuco, Chile
[b] Escuela de Ingeniería Bioquímica, Universidad Católica de Valparaíso, General Cruz 34, Valparaíso, Chile

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Abstract

The advantages of enzyme process are numerous and well established. However, enzymes are mostly used as soluble catalysts, being poorly stable and hard to recover. These problems may be solved by the use of immobilised enzymes, but new problems may arise, especially mass transfer limitations. Internal mass transfer limitations that may be especially significant in microporous and gel matrices, which, on the other hand, are superior in terms of protein loading capacity. This paper deals with the study, using mathematical simulation, of the effect of reaction kinetics and particle size on the effectiveness factor of immobilised enzymes, subjected to internal diffusional restrictions. The main conclusion of this work is that, depending on the enzyme reaction mechanism, a particle size may exist at which the effectiveness factor is a maximum and, therefore, the immobilised enzyme behaviour is optimum.

Keywords: Immobilised enzyme; Diffusional restrictions; Modeling; Kinetic models; Effectiveness factor

1. Introduction

During the last few years the use of enzymes as process catalysts has grown considerably, displacing in many cases classical chemical processes [1,2]. Nevertheless one of the disadvantages of the conventional industrial utilisation of enzymes dissolved in the reaction medium is related to their low stability under process conditions [3] and the difficulty of their separation from the product stream [4]. Those limitations can be circumvented by the use of immobilised enzymes. Immobilisation increases the enzyme stability and allows easy separation and reuse of the catalyst, favouring continuous reactor operation. This has an obvious economic impact and allows the utilisation of reactors with high enzyme loads [5,6].

The main limitation of the utilisation of immobilised enzymes lies in the existence of both external and internal mass transfer limitations. External mass transfer can be reduced by the manipulation of the reactors hydraulic conditions, for example by increasing the level of agitation [7]. However, intra-particle diffusional restrictions are generally more severe and much more difficult to overcome [8].

The utilisation of small catalytic particles complicates reactor operation, by increasing pressure drop (in the case of packed-bed reactors) or favouring catalyst washout (in the case of fluidised or well-mixed reactors). Nevertheless it is generally considered beneficial for reducing internal mass transfer limitations [9,10]. However, this criterion cannot be generalised for every enzyme system, and will depend on the kinetic mechan-

* Corresponding author.

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ism of the reaction. For example, in the case of enzymes that present substrate inhibition, diffusional restrictions can improve immobilised enzyme reactor performance by the reduction of the local substrate concentration, and therefore, its inhibitory effect. Furthermore, effectiveness factors greater than one can be obtained in such systems [11,12].

Consequently, the study and analysis of the behaviour of immobilised enzymes under different kinetic mechanisms by the use of simulation tools, has a great practical significance. This will help to establish the relationship between intrinsic enzyme reaction kinetics and mass transfer limitations, which is fundamental for the proper design and operation of immobilised enzyme reactors.

This study was undertaken under the hypothesis that steady state enzyme performance will be affected in a different way by the immobilisation process according to the mechanism of reaction. The different effects that immobilisation by entrapment presents over several reaction kinetic models are analysed, by the use of simulation tools.

2. Model development

The model considers the following assumptions:

- The temperature is constant.
- The enzyme is evenly distributed within the support.
- The reaction involves only one substrate, or two substrates in which one is in large excess.
- Diffusion coefficients are constant.
- Similar diffusion coefficients are considered for substrate and product.
- Catalytic particles are spherical.
- The diffusion of substrate and product within the support can be represented by Fick's first law.
- The system is in steady state (no enzyme inactivation is considered).
- External mass transfer resistance is neglected.

The mass differential balances for substrate and product under steady state, for the spherical catalytic particle are:

$$D_S\left(\frac{d^2S}{dr^2}+\frac{2}{r}\frac{dS}{dr}\right)-V=0 \quad (1)$$

$$D_P\left(\frac{d^2P}{dr^2}+\frac{2}{r}\frac{dP}{dr}\right)+V=0 \quad (2)$$

V will depend on the type of kinetic mechanism under study. Five kinetic mechanisms are considered:

- Simple irreversible Michaelis–Menten kinetics.
- Uncompetitive substrate inhibition.
- Total competitive product inhibition.
- Total non-competitive product inhibition.
- Reversible Michaelis–Menten reaction.

Table 1
Kinetic models considered in the simulations

Kinetic model	Equation
Simple Michaelis–Menten	$V=\frac{V_m \cdot S}{K_s+S}$
Uncompetitive substrate inhibition	$V=\frac{V_m \cdot S}{S(1+S/K_I)+K_S}$
Total competitive product inhibition	$V=\frac{V_m \cdot S}{K_s(1+P/K_I)+S}$
Total non-competitive product inhibition	$V=\frac{V_m \cdot S}{(1+P/K_I)(K_s+S)}$
Reversible Michaelis–Menten reaction	$V=\frac{V'_m(S-S_e)}{K_S+(S-S_e)}$

The model equations that describe these kinetic mechanisms are summarised in Table 1.

Table 2 presents the mass balance equations, in dimensionless form, for each of the five kinetic models presented in Table 1. Two mass balance equations are presented for product inhibition kinetic mechanisms (one for substrate and one for product). Therefore, two Thièle moduli are generated in those cases, one for substrate and one for product.

The boundary conditions for equations in Table 2 are:

$$\frac{d\beta}{d\varsigma}=0 \quad \text{when } \varsigma=0;\ \beta=\beta_0 \quad \text{when } \varsigma=1$$

For each kinetic model, the effectiveness factor was calculated at different values of the Thièle modulus (in the range from 0 to 10), and for several values of β_0 (in the range from 0 to 10). To study the effect of inhibition, the simulations were done at three values of inhibition degree (0.2, 1 and 5) and three values of substrate conversion (0.5, 0.7 y 0.9). For reversible Michaelis–Menten kinetics, the liquid phase steady state substrate conversion and the equilibrium conversion selected were 0.4 and 0.5, respectively.

The local effectiveness factor is determined as the local reaction rate in the support divided by the intrinsic reaction rate (this is, the reaction rate in the absence of mass transfer limitations, which corresponds to the rate evaluated at the concentration of substrate outside the support):

$$\eta=\frac{V}{V_0} \quad (3)$$

Table 2
Dimensionless mass balance equations for each kinetic model

Kinetic model	Mass balance equations
Simple Michaelis–Menten	$\frac{d^2\beta}{d\varsigma}+\frac{2}{\varsigma}\frac{d\beta}{d\varsigma}-9\Phi^2\frac{\beta}{1+\beta}=0$
Uncompetitive substrate inhibition	$\frac{d^2\beta}{d\varsigma}+\frac{2}{\varsigma}\frac{d\beta}{d\varsigma}-9\Phi_S^2\frac{\beta}{\beta(1+\beta\alpha)+1}=0$
Total competitive product inhibition	$\frac{d^2\beta}{d\varsigma}+\frac{2}{\varsigma}\frac{d\beta}{d\varsigma}-9\Phi_S^2\frac{\beta}{\beta+1+\gamma}=0,\ \frac{d^2\gamma}{d\varsigma}+\frac{2}{\varsigma}\frac{d\gamma}{d\varsigma}+9\Phi_P^2\frac{\beta}{\beta+1+\gamma}=0$
Total non competitive product inhibition	$\frac{d^2\beta}{d\varsigma}+\frac{2}{\varsigma}\frac{d\beta}{d\varsigma}-9\Phi_S^2\frac{\beta}{(1+\beta)(1+\beta\alpha)}=0,\ \frac{d^2\gamma}{d\varsigma}+\frac{2}{\varsigma}\frac{d\gamma}{d\varsigma}+9\Phi_P^2\frac{\beta}{(1+\beta)(1+\beta\alpha)}=0$
Reversible Michaelis–Menten reaction	$\frac{d^2\beta}{d\varsigma}+\frac{2}{\varsigma}\frac{d\beta}{d\varsigma}-9\Phi_S^2\frac{\beta-\beta_e}{1+\beta-\beta_e}=0$

The mean integrated effectiveness factor represents the average value of the effectiveness factor, considering the whole support. For a spherical catalyst particle:

$$\eta'=\frac{\int_0^1 \varsigma^2\cdot\eta\cdot d\varsigma}{\int_0^1 \varsigma^2\cdot d\varsigma} \tag{4}$$

For product inhibition mechanisms, the steady state product concentration outside the catalytic particle is:

$$P_0=S_0\frac{X}{1-X} \tag{5}$$

Mass balance equations were solved employing MATHCAD 2000 software, which uses fourth order Runge Kutta integration method.

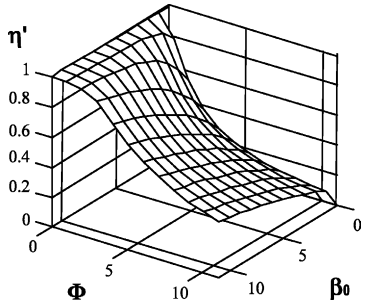

Fig. 1. Effect of the Thièle modulus and the dimensionless liquid-phase substrate concentration on the mean integrated effectiveness factor for simple Michaelis–Menten kinetics.

3. Results and discussion

3.1. Simple irreversible Michaelis–Menten kinetics

Fig. 1 shows the effect of the Thièle modulus and the dimensionless substrate concentration outside the support (β_0) on the mean integrated effectiveness factor for simple irreversible Michaelis–Menten kinetics.As expected, the effectiveness factor tends to one as the Thièle modulus decreases or the substrate concentration increases, as shown in Fig. 1.

Obviously, to utilise the maximum catalytic capacity of the enzyme, the reactor should operate at the highest value of the effectiveness factor, implying a lower value of the Thièle modulus. Substrate concentration is not considered as an operational parameter in this analysis, since it is determined by the particular requirements of each process. The value of the Thièle modulus can be manipulated by varying either V_m or R (D_S and K_S are intrinsic properties of the enzyme, substrate and support). V_m will be determined by the amount of enzyme immobilised per unit mass of support. Higher V_m values lead to smaller reactors, but poorer utilisation of the enzyme catalytic capacity (lower values of the effectiveness factor). On the other hand, lower values of V_m will generate reactors of higher volume, but a better use of the enzyme. The optimum V_m will be the one that minimises the cost of operation and should be determined on an economic basis. For a determined value of V_m, it will be of interest to operate with a particle size as small as possible, in order to reduce the internal mass transfer limitations. Lower limits of particle size will be dictated to a large extent by hydrodynamic considerations within the reactor.

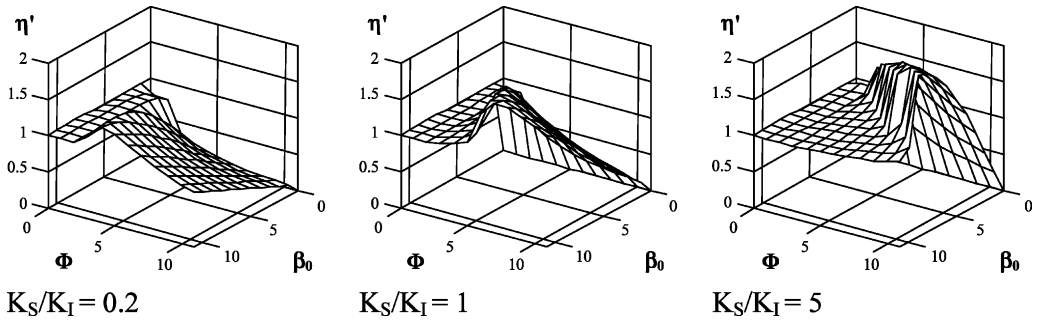

Fig. 2. Effect of the Thièle modulus and the dimensionless liquid-phase substrate concentration on the mean integrated effectiveness factor for substrate uncompetitive inhibition, at three levels of inhibition degree.

3.2. Uncompetitive substrate inhibition

Fig. 2 presents the mean integrated effectiveness factor as a function of Thièle modulus and the dimensionless substrate concentration, for substrate uncompetitive inhibition kinetics, at three levels of inhibition (K_S/K_I equals 0.2, 1 and 5).Fig. 2 shows that under certain conditions the mean integrated effectiveness factor is greater than 1. Even more, for a fixed substrate concentration there is a maximum mean integrated effectiveness factor for a determined value of Thièle modulus. As the inhibition degree (expressed as K_S/K_I) increases, this maximum moves to higher values of Thièle modulus. The maximum value of the mean integrated effectiveness factor increases as inhibition increases (higher K_S/K_I), rising up to close to 2 for $K_S/K_I = 5$.

In an enzymic system that presents substrate uncompetitive inhibition, because of diffusional restrictions, the enzyme is in contact with a lower substrate concentration than that in the fluid phase. Under certain conditions this can reduce enzyme inhibition, generating higher reaction rates. In this way, the substrate diffusional restriction protects the enzyme from the inhibitory effect caused by the high substrate concentration in the outside of the support, by lowering its concentration inside.

Furthermore, Fig. 2 shows that a larger portion of the surface presents values of mean integrated effectiveness factor equal or greater than 1, which means that the immobilisation of enzymes that present strong substrate inhibition allows a much efficient use of its catalytic capacity.

During the operation of an enzyme system with this type of kinetic behaviour, contrary to simple irreversible Michaelis–Menten kinetics, the highest mean integrated effectiveness factor will not be obtained at the lowest Thièle modulus. Then, there will be an optimum Thièle modulus, and therefore, an optimum particle size, with respect to catalytic effectiveness.

3.3. Product competitive inhibition

Fig. 3 presents the results for product competitive inhibition kinetics, at three different values of inhibition degree and substrate conversion.Fig. 3 shows that the mean integrated effectiveness factor increases with the inhibition degree. This is due to the reduction of the intrinsic reaction rate produced by the increase of inhibition that makes the system less sensitive to diffusional restrictions and controlled by the enzyme reaction kinetics. This is shown by the profiles of the Thièle modulus, which become less pronounced as the inhibition degree increases.

As shown in Fig. 3, the effectiveness factor increases with substrate conversion. This behaviour has the same explanation as above: a higher conversion generates a higher product concentration, which reduces the reaction rate by inhibition, reducing the influence of mass transfer on the apparent reaction rate.

In an enzymic system that presents product inhibition, mass transfer limitations affect the apparent reaction rate by affecting not only substrate diffusion to the reaction site but also product diffusion from it. Inside the support, product concentration will be higher than in the liquid phase, so the enzyme will be more inhibited than the inhibitor concentration in the fluid phase suggests. However, the negative effect of mass transfer limitations on the apparent reaction rate seems to be masked by the low intrinsic reaction rate that product inhibition causes, making the system less sensitive to diffusional restrictions both from substrate and product.

In the case of immobilised enzymes that present this type of inhibition, it will be convenient to work with small diameter particles, as in the case of simple irreversible Michaelis–Menten kinetics. However, the

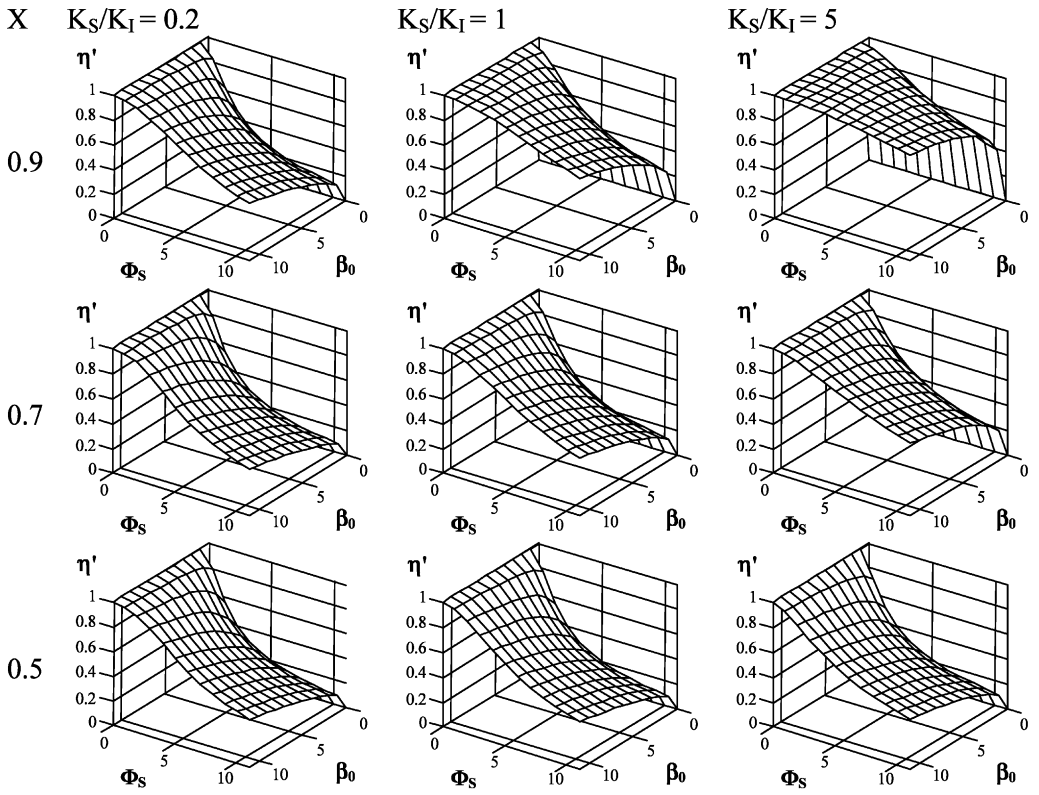

Fig. 3. Effect of the Thiële modulus and the dimensionless liquid-phase substrate concentration on the mean integrated effectiveness factor for product competitive inhibition, at three levels of inhibition degree and substrate conversion.

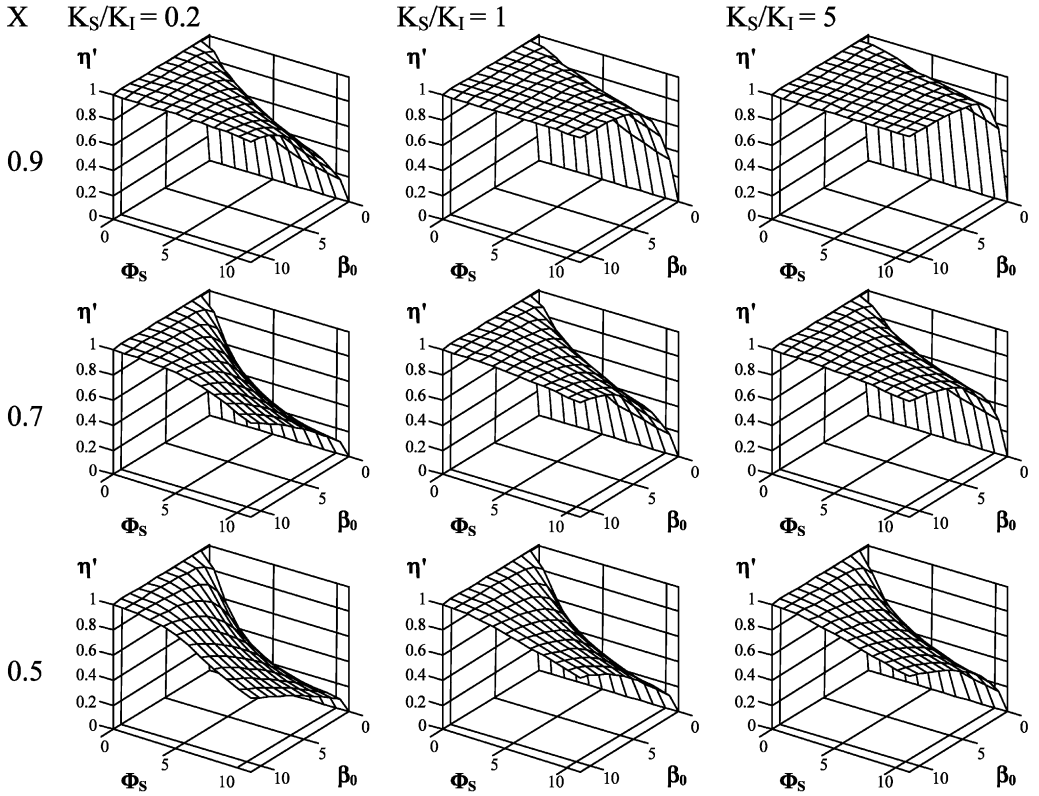

Fig. 4. Effect of the Thiële modulus and the dimensionless liquid-phase substrate concentration on the mean integrated effectiveness factor for product noncompetitive inhibition, at three levels of inhibition degree and substrate conversion.

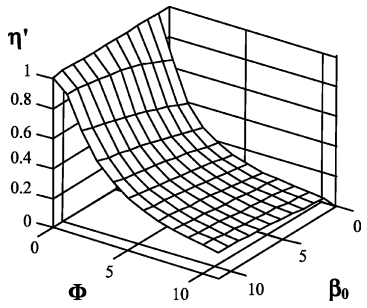

Fig. 5. Effect of the Thièle modulus and the dimensionless liquid-phase substrate concentration on the mean integrated effectiveness factor for a reversible Michaelis–Menten enzymatic reaction at a substrate conversion of 0.4 and an equilibrium substrate conversion of 0.5.

increase in the inhibition degree reduces the effect of the Thièle modulus, and therefore, the particle size, on the mean integrated effectiveness factor due to the reduction of the intrinsic reaction rate. When the system is severely inhibited, particle size has a marginal effect on the mean integrated effectiveness factor.

3.4. Product noncompetitive inhibition

Fig. 4 presents results for product noncompetitive inhibition kinetics, at three different values of inhibition degree and substrate conversion.The behaviour of an enzyme system with noncompetitive inhibition is similar to the case of competitive inhibition. The value of the mean integrated effectiveness factor increases with inhibition degree and decreases with substrate conversion, for a given pair of values of Thièle modulus and fluid-phase substrate concentration. However, as shown in Fig. 4, the effect of the parameters analysed on the mean integrated effectiveness factor is less pronounced than in the case of competitive inhibition kinetics. There is an ample region in which the mean integrated effectiveness factor is close to 1, and, in fact, the effect of particle size does not affect it so markedly as in other kinetic mechanisms.

3.5. Reversible Michaelis–Menten kinetics

Fig. 5 shows the effect of the Thièle modulus and fluid-phase substrate concentration on the mean integrated effectiveness factor for reversible Michaelis–Menten kinetics, considering a substrate conversion of 0.4 and an equilibrium substrate conversion of 0.5.As shown in Fig. 5 the effect of the Thièle modulus on the mean integrated effectiveness factor is quite strong, while the effect of fluid-phase substrate concentration is mild. Therefore, the immobilisation of an enzyme with reversible Michaelis–Menten kinetics can reduce significantly the apparent reaction rate. For the immobilisation of enzymes that catalyse reversible Michaelis–Menten reactions, the catalytic particle should be as small as possible in order to reduce mass transfer limitations. In this case, the profiles of substrate and product concentrations within the support will be less pronounced than in the other kinetic models studied. Then, mass transfer limitations will have a greater impact in reversible Michaelis–Menten reactions.

4. Conclusions

Results of simulations clearly show that the effect of internal diffusional restrictions has different effects on immobilised enzyme behaviour depending on the intrinsic kinetics of the reaction. For substrate uncompetitive inhibition kinetics, effectiveness factor greater than 1 can be obtained, and, for product inhibition, the effect of mass transfer limitations is reduced as the inhibition degree increases. For reversible Michaelis–Menten kinetics, immobilisation produces a higher impact on enzyme performance when compared with simple irreversible Michaelis–Menten kinetics.

Catalytic particle size plays a key role in the design of immobilised enzyme processes. Furthermore, the optimal particle size will depend on the intrinsic kinetics of the reaction: not always a smaller catalytic particle will produce a better performance of the immobilised enzyme.

The information obtained through simulation is a valuable tool for immobilised enzyme reactor design by providing a quantitative relation of enzyme performance with operational variables like substrate conversion and particle size.

5. List of symbols

D_S	diffusion coefficient for substrate within the support
D_P	diffusion coefficient for product within the support
K_I	intrinsic inhibition constant
K_S	intrinsic half saturation constant
P	product concentration
P_0	product concentration outside the support
r	radial distance within catalytic particle
R	radius of a catalytic particle
S	substrate concentration
S_0	substrate concentration outside the support
S_e	equilibrium substrate concentration (reversible reaction)
V	enzymatic reaction rate

V_0 intrinsic enzyme reaction rate
V_m maximum enzyme reaction rate
V'_m maximum enzyme reaction rate for reversible reactions
X substrate conversion
X_{eq} equilibrium substrate conversion (for reversible reactions)

Greek symbols
$\beta = S/K_S$ dimensionless substrate concentration
$\beta_0 = S_0/K_S$ dimensionless substrate concentration outside the support
$\gamma = P/K_I$ dimensionless product concentration
$\varsigma = r/R$ dimensionless radial distance
$\alpha = K_S/K_I$ dimensionless inhibition degree

$\Phi_S = \frac{R}{3}\left(\frac{V_m}{D_s K_s}\right)^{1/2}$ Thièle modulus for substrate

$\Phi_P = \frac{R}{3}\left(\frac{V_m}{D_P K_I}\right)^{1/2}$ Thièle modulus for product

$\eta = V/V_0$ local effectiveness factor
η' mean integrated effectiveness factor

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