Effectiveness factor for immobilized biocatalysts: two substrates-two products reactions

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Abstract

Immobilized enzymes are being increasingly used as biocatalysts in numerous processes to obtain high-value products for the pharmaceutical, flavour and fragrance industries (Gandhi et al., 2000). The major advantages of immobilization include the increase in enzyme stability, the possibility of enzyme reutilization and the easy separation of the biocatalysts from the reaction mixture. However, it is necessary to account for mass transfer limitations that, under some conditions, may arise in these systems (Gómez et al., 2003; Jeison et al., 2003). These resistances comprise the effects of intraparticle diffusion and external mass-transfer. Given the complexity of the kinetics of multi-substrate enzyme reactions, reactor modelling studies that account for mass-transfer phenomena are so far limited to single-substrate ones (Gómez et al., 2003).

To compare the observed reaction rate with the reaction rate in the absence of mass-transfer limitations, an overall effectiveness factor is usually calculated (Gómez et al., 2003; Jeison et al., 2003). In this work, a model is developed to calculate the overall effectiveness factor for immobilized enzymes that carry out irreversible two substrates-two products reactions following kinetic mechanisms such as the Ternary Complex or the Ping-Pong Bi-Bi with inhibition by the second substrate.

The model has two dimensionless parameters for each substrate – Thiele modulus (reaction/intraparticle diffusion), Biot number (film diffusion/intraparticle diffusion) – and one related to the reaction kinetics (K_i/K_M ratio). Their influence on the effectiveness factor is analysed. The results obtained can be applied in the design and simulation of enzymatic reactors.

1 Introduction

Since the beginning of the last decade, ester synthesis by enzyme catalysis (esterification and transesterification reactions) became a major area of research (Yahya et al., 1998; Gandhi et al., 2000).

The advantages of the enzymatic processes over the traditional ones are well established (Gandhi et al., 2000) and comprise mostly of the enzymes high selectivity and their ability to conduce esterifications in mild and more environmentally friendly conditions. It is, however, of general knowledge that enzymes are less stable than the typically used catalysts. One way to overcome this drawback is through immobilization. Immobilization turns enzymes more stable, both mechanically and conformationally. It also enables the reuse of the catalyst and reduces the cost of downstream processing, making immobilized enzymes the preferred form for application in enzymatic reactors.

One of the downsides of enzyme immobilization generally lies in the introduction of internal (intraparticle diffusion) and external (film diffusion) mass transfer limitations.

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One valuable tool for rationalizing the reaction-mass transfer competition is the effectiveness factor. By comparing the actual reaction rate with the reaction rate that would be attained if the enzyme inside the particles was fully exposed to the bulk concentration one can have an actual measure of the extent of the mass transfer interference in the reaction process.

Reactor modeling studies that account for both internal and external mass-transfer phenomena are until now limited to single-substrate reactions (Bódalo et al., 1986; Bódalo et al., 1995; Abu-Reesh, 1997; Gómez et al., 2003; Jeison et al., 2003) mainly due to the complexity of the kinetics of the multi-substrate reactions. This work presents a model for the calculation of effectiveness factors for immobilized enzymes that catalyse irreversible esterification/transesterifications, or any other two substrates-two products reactions that follow the Ping-pong bi-bi with competitive inhibition by the second substrate or the Ternary complex kinetic mechanisms. These two mechanisms are amongst the most extensively used.

For irreversible reactions or for initial velocities of reversible reactions, the first mechanism results in the following rate equation (Segel, 1993):

$$v = \frac{v_{max}}{\frac{K_{M1}}{C_1}\left(1+\frac{C_2}{K_i}\right)+\frac{K_{M2}}{C_2}+1} \tag{1}$$

For the same conditions the Ordered ternary complex mechanism yields (Segel, 1993):

$$v = \frac{v_{max}}{\frac{K_{M2}}{C_2}\left(1+\frac{K_i}{C_1}\right)+\frac{K_{M1}}{C_1}+1} \tag{2}$$

where C_1 is the concentration of component 1 (acid or ester) and C_2 is the concentration of component 2 (alcohol).

2 Model

The first step is to calculate the steady state concentration profile of the substrates inside the catalyst particle. The model was developed taking into account the following assumptions:

i) The enzyme is immobilized, and homogeneously distributed, inside isothermal spherical porous particles of radius R;

ii) The substrate's diffusion inside the particles and through the stagnant film around them can be described by Fick's first law and the diffusivities are constant inside the support;

iii) The bulk phase is perfectly mixed and the batch reactor is isothermal;

iv) The enzyme's activity is constant throughout the process.

Given these assumptions, the steady state material balance equation for substrate i inside a spherical catalyst particle can be written as:

$$\varepsilon_p D_{pi} \frac{1}{r^2}\frac{d}{dr}\left(r^2 \frac{dc_i}{dr}\right) - v\rho_{app} = 0, \mathrm{i} = 1, 2 \tag{3}$$

with the following boundary conditions:

$$\begin{aligned} r = 0 \quad & \left.\frac{dc_i}{dr}\right|_{r=0} = 0, \mathrm{i} = 1, 2 \\ r = R \quad & \varepsilon_p D_{pi} \left.\frac{d c_i}{d r}\right|_{r=R} = k_{fi}\left[C_i - (c_i)_{r=R}\right], \mathrm{i} = 1, 2 \end{aligned} \tag{4}$$

The previous equations can be expressed in terms of the following dimensionless variables and parameters:

$$f_i = \frac{c_i}{K_{Mi}} \qquad F_i = \frac{C_b}{K_{Mi}} \qquad x = \frac{r}{R} \tag{5}$$

$$\phi_i = R\sqrt{\frac{v_{\max}\rho_{app}}{\varepsilon_p K_{Mi} D_{pi}}} \qquad Bim_i = \frac{k_f R}{\varepsilon_p D_p}\frac{V_L}{V_S} \tag{6}$$

Finally, the model equations become:

$$\frac{d^2 f_i}{dx^2} + \frac{2}{x}\frac{df_i}{dx} - \phi_i^2 \frac{v}{v_{\max}} = 0 \ , \mathrm{i} = 1, 2 \tag{7}$$

with boundary conditions:

$$\left.\frac{df_i}{dx}\right|_{x=0} = 0 \ , \mathrm{i} = 1, 2$$
$$\left.\frac{df_i}{dx}\right|_{x=1} = Bim_{\mathrm{i}} \frac{V_s}{V_L}\left[F_i - (f_i)_{x=1}\right], \mathrm{i} = 1, 2 \tag{8}$$

The overall effectiveness factor η compares the observed reaction rate to the reaction rate evaluated at bulk conditions, i.e., in the absence of both internal and external mass transfer resistances. In terms of dimensionless variables, the expression can be written as:

$$\eta = \frac{3}{\phi^2}\frac{v_{\max}}{v}\left.\frac{df}{dx}\right|_{x=1} \tag{9}$$

The term v/v_{max} depends on the kinetic mechanism considered and can also be expressed as a function of dimensionless variables. Therefore, equations (1) and (2) are equivalent to the equations (10) and (11), respectively:

$$\frac{v}{v_{\max}} = \frac{1}{\frac{1}{[f_1]}\left(1 + \frac{K_{M2}}{K_i} f_2\right) + \frac{1}{f_2} + 1} \tag{10}$$

$$\frac{v}{v_{\max}} = \frac{1}{\frac{1}{[f_2]}\left(1 + \frac{K_i}{K_{M1}}\frac{1}{f_1}\right) + \frac{1}{f_1} + 1} \tag{11}$$

Results and discussion

The steady state intraparticle concentration profile was obtained by solving Equations 7 subject to the boundary conditions 8, for both kinetic mechanisms, and for several sets of parameters. Then, the effectiveness factor was calculated for several levels of substrate concentration C_b and K_i/K_M ratio. For each C_b - K_i/K_M pair, the effectiveness factor can be represented as a function of the Thiele modulus ϕ and the Biot number *Bim*.

The initial concentrations and the parameters *Bim* and ϕ were considered equal for both substrates which is a reasonable assumption. The simulations were carried out using the physical properties of the commercially available Immobilized Lipase PS-C "Amano" I, from Amano Enzyme, kindly provided by the manufacturer.

Ternary complex mechanism

Figure 1 shows the effectiveness factor as a function of both Bim and ϕ for three K_i/K_{M1} ratios and for two bulk concentrations.

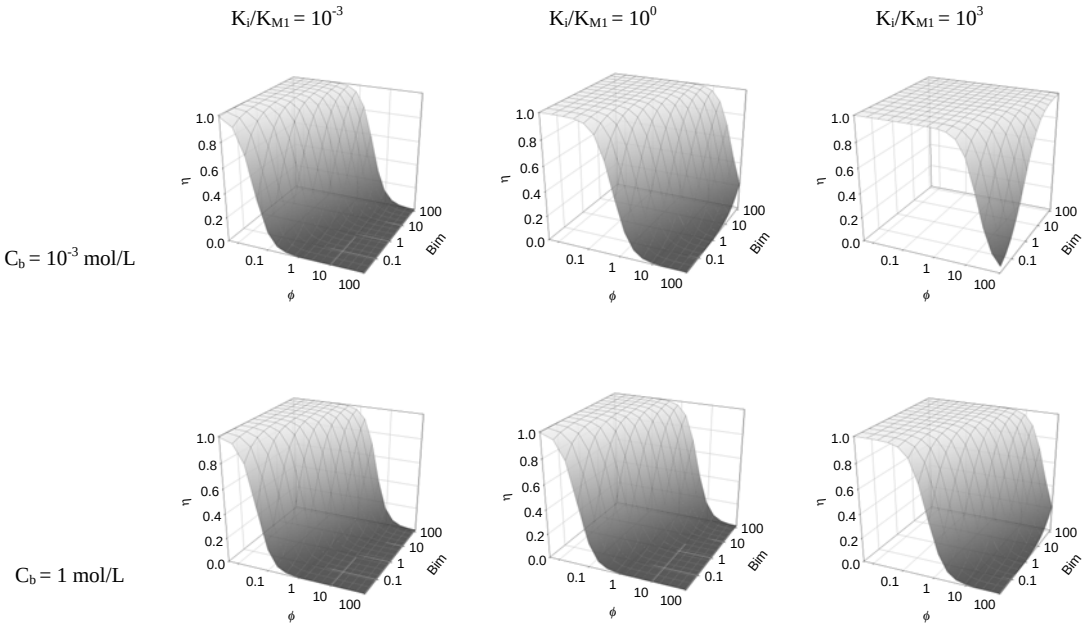

Figure 1. Effectiveness factor as a function of ϕ and Bim calculated for the ternary complex mechanism, for three inhibition ratios and two different bulk concentrations.

As expected, there is a lower bound for the influence of ϕ in the system that represents no significant intraparticle mass-transport, below which the effectiveness factor becomes unity, and an upper bound that stands for very fast reaction rate relative to the intraparticle diffusion phenomenon, leading to a very low effectiveness factor, since the substrates are consumed before reaching the enzyme that is immobilized in the more internal parts of the support. The value of these bounds depends on the K_i/K_{M1} ratio. In this mechanism K_i represents the equilibrium constant for the unbinding of the first substrate from the enzyme. A large K_i means that the substrate's affinity to bind to the enzyme's active sites is rather low. Since ϕ does not account for the influence of K_i in the reaction rate, when this parameter becomes significant, it's as if the reaction rate is slower than it was considered in the Thiele modulus definition. Hence, the ϕ bounds are moved to greater values as the K_i/K_{M1} ratio becomes larger. As can be seen, η increases with the K_i/K_{M1} ratio, for each ϕ-Bim pair. As the intrinsic reaction rate decreases, the reaction rate tends to be chemically controlled and less dependent on diffusional resistances.

In the region where the Bim number is low and, comparatively, much lower than ϕ, η tends to zero, indicating that severe film limitations control the reaction rate. This region is larger for lower K_i/K_{M1} ratios.

Ping-pong bi-bi mechanism with competitive inhibition by the second substrate

Similar results are obtained for this mechanism. Again, the effectiveness factor is presented as a function of both Bim and ϕ for three K_i/K_{M2} ratios and for two representative C_b, on Figure 2.

In this mechanism K_i represents the equilibrium constant for the unbinding of the second substrate from the enzyme, returning the catalyst to its original state. This is the competitive inhibition step. Since K_i is defined as the equilibrium constant for the unbinding reaction, it becomes smaller as the affinity of the enzyme for the second substrate becomes higher, leading to a larger effect of the competitive inhibition step in the process. Therefore, η increases with inhibition, i. e., for lower K_i/K_{M2} the intrinsic reaction rate decreases and the reaction rate is chemically controlled. In the case where the dimensionless concentration levels are significantly lower than K_i/K_{M2}, the effectiveness

factor curves become almost independent of the K_i/K_{M2} ratio as can be seen on the first row of figure 2.

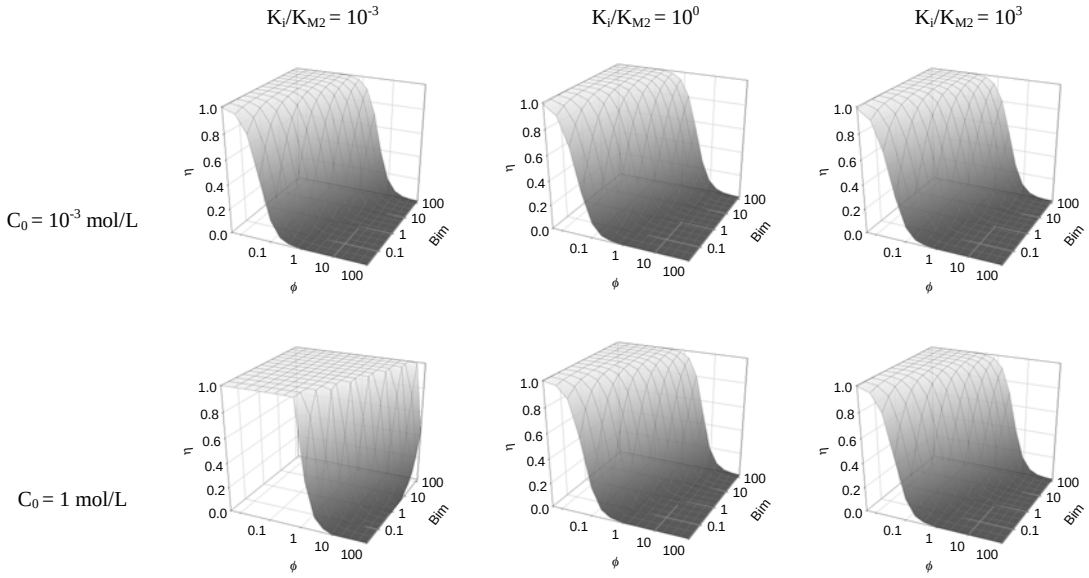

Figure 2. Effectiveness factor as a function of ϕ and Bim calculated for the Ping-pong bi-bi mechanism with competitive inhibition by the second substrate, for three inhibition ratios and two different bulk concentrations.

4 Conclusions

A model was developed for the calculation of the effectiveness factor for immobilized biocatalysts that perform irreversible esterification/transesterifications, or any other two substrates-two products reactions that follow either the Ternary complex or the Ping-pong bi-bi with competitive inhibition by the second substrate kinetic mechanisms; two dimensionless parameters for each substrate and another one related to the reaction kinetics were identified. The effect of both internal and external mass transfer limitations was discussed as well as the regions over which these phenomena affect the catalyst performance. This model constitutes a valuable tool in biocatalysis reaction engineering and can be applied in reactor design and optimization.

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