

Decreases in molecularity promote conversion when reactions are catalyzed by enzymes immobilized in slab-shaped beads

R.M. Barros, F.X. Malcata

Abstract Application of Fick's first law to substrate undergoing chemical reaction catalyzed by an enzyme immobilized in a porous slab-shaped bead leads to substrate concentration profiles that are flatter when the ratio of stoichiometric coefficients of product to reactant (ν) is lower. Since the actual effectiveness factor decreases when ν increases (at approximately the same rate irrespective of the value taken by the Michaelis-Menten parameter), then considerable overprediction of the conversion levels obtained within a given reaction timeframe will occur if the simplistic (and more easily modelled) situation of equimolar counterdiffusion is taken for modelling purposes when $\nu > 1$.

List of symbols

A	cross-sectional area of the catalyst slab [m^2]
C_{tot}	total molar density of the mixture [$\text{mol} \cdot \text{m}^{-3}$]
D_{SP}	binary diffusivity of S and P [$\text{m}^2 \cdot \text{s}^{-1}$]
E	enzyme
k_{cat}	first order kinetic constant associated with formation of P [s^{-1}]
K_{m}	Michaelis-Menten constant associated with dissociation of enzyme/substrate complex [$\text{mol} \cdot \text{m}^{-3}$]
K_{m}^*	normalized value of K_{m} [—]
L	half-thickness of the slab [m]
N	total number of iterations [—]
N_{P}	molar flux of P [$\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$]
N_{S}	molar flux of S [$\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$]
$N_{\text{S},z}$	uni-dimensional component of vector N_{S} [$\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$]
P	product
S	substrate
Th	Thiele modulus [—]
v_{max}	maximum rate of reaction [$\text{mol} \cdot \text{m}^{-3} \cdot \text{s}^{-1}$]
v_{P}	molar volume of P [$\text{m}^3 \cdot \text{mol}^{-1}$]
v_{S}	molar volume of S [$\text{m}^3 \cdot \text{mol}^{-1}$]
x_{S}	molar fraction of S [—]
y_{S}	auxiliary variable [—]
$y_{\text{S}}^{(i)}$	value of y_{S} at the i -th iteration [—]
z	unidimensional linear coordinate [m]
z^*	normalized value of z [—]

Greek letters

η	effectiveness factor of catalyst slab [—]
ν	stoichiometric coefficient of P [—]

1

Introduction

Although enzymes are synthesized and operate in vivo at concentration levels that are high when compared with the actual concentration levels of their substrates, industrial practice has it that the concentration of substrate is to be raised to the highest possible value, so as to approach the maximum possible rate ever, and the concentration of catalyst is to be maintained as low as possible, so as to approach the minimum operating costs ever. The reason for these apparently opposing behaviors hinges on the constraints posed on the aforementioned processes, which are metabolic in the former case and economic in the latter. Since enzymes are molecules possessing a paramount number of degrees of freedom in virtue of their size and tridimensional nature as peptide bonds, partial or total deactivation is easy and occurs at high rates unless the rigidity of their amino acid residue backbone is constrained via an externally engineered microenvironment; the easiest way to accomplish this deed is via immobilization of the enzyme onto a solid support.

Since the shape of the immobilization bead is not of crucial importance provided that the characteristic length scale for intraparticle diffusion and the specific volumetric activity of enzyme are similar (Smith, 1981), a slab-shaped bead seems appropriate for modelling purposes because of the mathematical simplicity derived from its unidimensional nature and constant cross section along the direction of flow, and has accordingly been considered by several authors (e.g. Malcata, 1991; Moreira and Malcata, 1996). The case of 1:1 stoichiometry has dominated essentially all theoretical analyses; in situations where a different stoichiometry exists, description of molecular transport has traditionally been effected via Fick's law of equimolar counterdiffusion, but the results obtained in terms of effectiveness factors may be excessively conservative when the molecularity of the chemical reaction increases considerably from reactants to products.

It is the purpose of this communication to mathematically assess the effect of stoichiometry upon the substrate concentration gradient within a slab-shaped bead where an enzyme-catalyzed reaction following Michaelis-Menten kinetics takes place, and use such reasoning to predict the variation of the effectiveness factor of the enzyme bead

R.M. Barros, F.X. Malcata
Escola Superior de Biotecnologia, Rua Dr. António Bernardino de Almeida, P-4200 Porto, Portugal

Correspondence to: F. Xavier Malcata

with stoichiometric coefficients and physicochemical and processing parameters.

2 Theory

Consider a chemical reaction that takes place in a binary solution of substrate S and product P and is brought about by an enzyme E according to the following mechanism:



where K_m is the equilibrium constant associated with dissociation of the enzyme-substrate complex (ES), k_{cat} is the intrinsic kinetic constant associated with formation of product, and ν is the stoichiometric coefficient of product P . Consider, in addition, that said enzyme is uniformly immobilized in a microporous bead; in this situation, transport of molecular species S and P occurs by diffusion only, according to Fick's first law:

$$N_S = x_S(N_S + N_P) - C_{tot} D_{SP} \nabla x_S, \quad (2)$$

where N_S and N_P denote the diffusion flux of S and P , respectively, relative to stationary coordinates, x_S the molar fraction of S , C_{tot} the total molar density of the mixture of S and P , and D_{SP} the diffusivity in the binary system formed by S and P .

The stoichiometry relationship between S and P as apparent in Eq. (1) allows one to write:

$$N_P = -\nu N_S, \quad (3)$$

which, upon combination with Eq. (2), restriction to a single (dominant) direction, and algebraic rearrangement, yields:

$$N_{S,z} = -\frac{C_{tot} D_{SP}}{1 - (1 - \nu)x_S} \frac{dx_S}{dz}, \quad (4)$$

where $N_{S,z}$ is the uni-dimensional counterpart of N_S and z is an uni-dimensional linear coordinate.

A shell mass balance to S within the slab-shaped bead (Bird et al., 1979), which considers molar transport by diffusion as given by Eq. (4) and transformation by chemical reaction as depicted in Eq. (1), gives:

$$D_{SP} \frac{d}{dz} \left\{ \frac{C_{tot}}{1 - (1 - \nu)x_S} \frac{dx_S}{dz} \right\} - \frac{\nu_{max} C_{tot} x_S}{K_m + C_{tot} x_S} = 0,$$

$$x_S\{z = L\} = 1,$$

$$\frac{dx_S}{dz}\{z = 0\} = 0, \quad (5)$$

where L is the half-thickness of the slab and ν_{max} is the maximum rate of reaction, calculated as the product of k_{cat} by the total concentration of available active enzyme.

Assume, for the sake of simplicity, that the total molar density of the mixture is approximately constant, which will be the case if S and P are not too dissimilar compounds; in this case, Eq. (5) becomes:

$$\frac{d}{dz^*} \left\{ \frac{1}{1 - (1 - \nu)x_S} \frac{dx_S}{dz^*} \right\} - \text{Th}^2 \frac{x_S}{K_m^* + x_S} = 0,$$

$$x_S\{z^* = 1\} = 1,$$

$$\frac{dx_S}{dz^*}\{z^* = 0\} = 0, \quad (6)$$

where the normalized Michaelis-Menten constant is defined as:

$$K_m^* \equiv \frac{K_m}{C_{tot}}, \quad (7)$$

the Thiele modulus as:

$$\text{Th} \equiv L \sqrt{\frac{\nu_{max}}{C_{tot} D_{SP}}}, \quad (8)$$

and the dimensionless coordinate as:

$$z^* \equiv \frac{z}{L}. \quad (9)$$

Equation (6) may be rewritten as:

$$\frac{d^2 y_S}{dz^{*2}} - \text{Th}^2 \frac{1 - \exp\{-(1 - \nu)y_S\}}{(1 - \nu)K_m^* + 1 - \exp\{-(1 - \nu)y_S\}} = 0,$$

$$y_S\{z^* = 1\} = -\frac{\ln\{\nu\}}{1 - \nu},$$

$$\frac{dy_S}{dz^*}\{z^* = 0\} = 0, \quad (10)$$

provided that the auxiliary variable y_S is defined as:

$$y_S \equiv -\frac{\ln\{1 - (1 - \nu)x_S\}}{1 - \nu}. \quad (11)$$

The effectiveness factor of the porous catalyst slab, η , defined as:

$$\eta \equiv \frac{2A \int_0^L \frac{\nu_{max} C_{tot} x_S\{z\}}{K_m + C_{tot} x_S\{z\}} dz}{2AL \frac{\nu_{max} C_{tot}}{K_m + C_{tot}}}, \quad (12)$$

where A denotes the cross-sectional area of the catalyst slab, may, in view of the dimensionless variables introduced above, be redefined as:

$$\eta \equiv (K_m^* + 1) \times \int_0^1 \frac{1 - \exp\{-(1 - \nu)y_S\{z\}\}}{(1 - \nu)K_m^* + 1 - \exp\{-(1 - \nu)y_S\{z\}\}} dz^*. \quad (13)$$

Since Eqs. (10) and (13) do not possess any analytical solution, a numerical method based on finite differences was employed to solve them:

$$y_S^{(0)} = y_0,$$

$$y_S^{(1)} = y_S^{(0)},$$

$$y_S^{(i+2)} = 2y_S^{(i+1)} - y_S^{(i)}$$

$$+ \text{Th}^2 \frac{1 - \exp\{-(1 - \nu)y_S^{(i+1)}\}}{(1 - \nu)K_m^* + 1 - \exp\{-(1 - \nu)y_S^{(i+1)}\}};$$

$$i = 0, 1, \dots, N - 2, \quad (14)$$

where superscript (i) denotes the i -th iteration, N the total number of iterations, and y_0 an arbitrary initial estimate, which is to be updated if $y_S^{(N)} \neq -(\ln\{v\}/(1-v))$, and:

$$\eta = \frac{1 + K_m^*}{N} \sum_{i=0}^{N-1} \frac{1 - \exp\left\{- (1-v) y_S^{(i+1)}\right\}}{(1-v) K_m^* + 1 - \exp\left\{- (1-v) y_S^{(i+1)}\right\}}, \quad (15)$$

respectively. The molar fraction profiles of S throughout the catalyst slab as obtained via Eq. (14) for the intermediate case of Th and K_m^* both equal to unity are plotted in Fig. 1, whereas the variation of the effectiveness factors with the Thiele modulus for various values of K_m^* and v as obtained via Eq. (15) are depicted in Fig. 2.

3 Discussion and conclusions

Assumption that the total molar density of the binary mixture of S and P remains constant may be seen as a result of the implicit requirement that $v_S = v v_P$, where v denotes molar volume; in the case of liquids, which are the normal substrates for enzymatic reactions, the molar volume is usually proportional to the volume of the molecules, which in turn results from an approximate summation of the volumes of all atoms in question; since the total number of atoms of each kind remains constant during occurrence of a chemical reaction, then the approximation set forth just prior to the derivation of Eq. (6) is reasonable.

Fig. 2a-d. Variation of the effectiveness factor, η , versus the Thiele modulus, Th , for various values of the product stoichiometric coefficient, v (from top to bottom: $v = 0.33$, $v = 0.5$, $v = 1$, $v = 2$, and $v = 3$) with (a) $K_m^* = 0.1$, (b) $K_m^* = 1$, (c) $K_m^* = 10$, and (d) $K_m^* = 100$

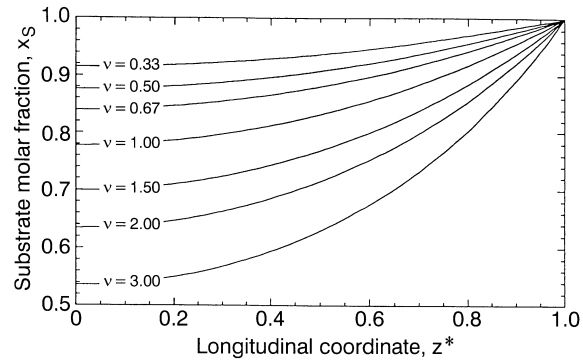
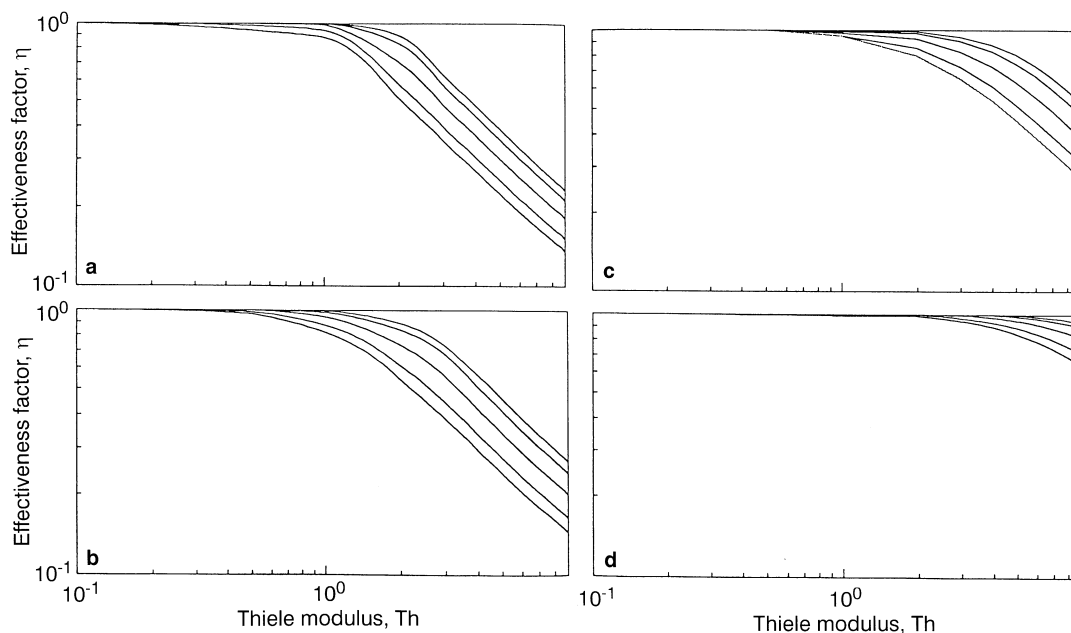


Fig. 1. Profile of molar fraction of substrate, x_S , versus the normalized longitudinal coordinate, z^* , for various values of the product stoichiometric coefficient, v , with $\text{Th} = 1$ and $K_m^* = 1$

In the case of equimolarity and consequent countermolar equidiffusion (i.e. when $v = 1$), then the differential equation denoted as Eq. (6) reduces to:

$$\frac{d^2 x_S}{dz^{*2}} - \text{Th}^2 \frac{x_S}{K_m^* + x_S} = 0, \quad (16)$$

which was used in Fig. 1 to obtain the corresponding profile since Eq. (6) would lead to a mathematically indeterminate behavior as v approached unity.

Inspection of Fig. 1 indicates that increases in the molecularity of the reaction, which, after normalization of the stoichiometric coefficient of S , is measured by v , lead to a distortion of the mole fraction profile of substrate within the porous bead in the direction of the molecular flux. This distortion is the result of the fact that the diffusion flux of substrate relative to stationary coordinates is the resultant of two vector quantities, as apparent in Eq. (2): the vector $x_S(N_S + N_P)$, which is the molar flux of S resulting from the bulk motion of the fluid, and the vector $-C_{\text{tot}} D_{SP} \nabla x_S$, which is the molar flux of S resulting from the diffusion superimposed on

the bulk flow. In fact, as the molar flow rate of product relative to that of substrate increases owing to a higher molecularity change during the reaction, a steeper molar fraction gradient of substrate S will be required in order to maintain a rate of diffusional transport compatible with the intrinsic rate of reaction. This observation is somewhat parallel to the existence of a convective flow through a (macro)porous bead, with the sole difference that the molecular bulk flow occurs in a balanced fashion in two opposite directions, which implies that the center of gravity is not displaced due to this flow, whereas the actual convective flow due to an externally applied pressure field occurs in a single direction, which leads to a finite displacement of the center of gravity; hence, our results are consistent with those reported by Moreira and Malcata (1996), who provided theoretical evidence to support the claim that convective flow through slab-shaped beads containing an immobilized enzyme catalyzing a Michaelis-Menten reaction actually decreases the rate of substrate inlet to the slab-shaped bead across the outer surface where bulk motion and diffusional motion take place in opposite directions.

The general shape for the η vs. Th bilogarithmic plot can be easily ascertained from inspection of Fig. 2. It is remarkable that (i) an increasing v leads to a decreasing η for every given combination of Th and K_m^* , (ii) the effect of v becomes of a lesser importance as its value lies on the very high side or alternatively on the very low side, and (iii) the η vs. Th curve undergoes vertical downward shifts with increasing v , following the same amplitude pattern irrespective of the value of K_m^* . As expected, increasing values of K_m^* make the apparent reaction order of the Michaelis-Menten rate expression change from pseudo-zero to pseudo-first order, and so a corresponding upward shift of the η vs. Th plots is observed in the diffusional regime, which leads to attainment of the kinetic regime at higher values of Th .

It should be noted that temperature gradients, pressure gradients and external field force gradients also contribute to the diffusion flux; however, in most situations of practical interest their effects are minor, so development of the mathematical reasoning was deliberately restricted to the case where the diffusion flux results solely from concentration gradients. On the other hand, only the case of a binary mixture was considered; although the classical situation of enzyme-catalyzed reactions encompasses dilute solutions of substrates, the current trend of industrial practice is towards use of substrates in the highest possible concentrations, a situation that is most easily attained via use of solvent-free systems. It should be noted that use of a solvent, which is an essentially stationary species, slows down the bulk motion of the fluid; hence the effect of v upon the concentration gradients would not have been so notorious and the mathematical rationale would not be so straightforward due to the need to inclusion of multi-component diffusivities.

References

1. Bird, R.B.; Stewart, W.E.; Lightfoot, E.N.: Transport Phenomena, McGraw-Hill, New York, USA (1979)
2. Moreira, N.E.; F.X. Malcata: "Use of convective flow to enhance the effectiveness factor of porous slab-shaped beads with immobilized enzyme," J. Chem. Eng. Japan 29 (1996) 392
3. Malcata, F.X.: "On the analytical solution of a problem of optimization in the field of immobilized enzymes," Int. J. Math. Educ. Sci. Technol. 22 (1991) 177
4. Smith, J.M.: Chemical Engineering Kinetics, McGraw-Hill, New York, USA (1981)