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ESTIMATION OF EFFECTIVENESS FACTORS IN HETEROGENEOUS BIOCATALIST SYSTEMS WITH NON-UNIFORM ENZYME DISTRIBUTION

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ABSTRACT

An approximate method for effectiveness factor estimation using analytical expressions (perturbation and matching techniques) is applied to immobilized enzymes responding to intrinsic Michaelis-Menten kinetics disturbed by internal diffusion. The effect of different enzyme distribution profiles is considered. The method reproduces with good accuracy experimental results and numerical solutions.

INTRODUCTION

Immobilized enzymes, attached to solid supports or trapped into them, are of great scientific and technological importance (Buchholz, 1992). They can be easily removed from the reacting system and repeatedly used for the transformation of additional amounts of substrate. They can be also used in convenient reactors for performing continuous enzymatic processes. Porous materials have the advantage of a great available specific surface; however, the negative effect of internal diffusion and a non uniform enzyme distribution can be present in these cases.

Diffusion is a very common problem in heterogeneous catalysis, which is generally expressed through the concept of effectiveness factor (Aris, 1975). Calculation of effectiveness factors can be carried out analytically only in simple cases, such as first and zero order reactions. When the kinetic equation is more complex, complicate numerical solutions are required. Therefore, approximate expressions were used in order to solve this problem in the case of immobilized biocatalyst (Yamané, 1981). Gottifredi et al. (1981, 1986) have employed analytical expressions for approximations of effectiveness factor. These expressions make possible simple evaluations with an acceptable accuracy. Although the technique takes into account arbitrary kinetics and pellet geometry, the particular case where a non-uniform biocatalyst activity inside the particle arises and where a portion of the catalyst pellet near the center is not active, were not investigated. The effect of different type of enzyme distribution, including the case previously indicated and taken into account a Michaelis-Menten kinetics, is reported in this paper. Results are compared with those of other authors obtained experimentally or using numerical calculations .

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ANALYSIS

Let us consider an immobilized biocatalytic reaction that is carried out in an isothermal spherical porous pellet. The reaction rate per unit pellet volume will be given by the Michaelis-Menten intrinsic kinetic expression:

$$r = \frac{V_m\, C'}{K_m + C'} \,, \qquad (1)$$

where C' denotes the dimensional substrate concentration while its corresponding value at the pellet surface is C'_s, K_m holds for the Michaelis constant and V_m for the maximum velocity.

The dimensionless rate of reaction r^* can be obtained by dividing equation (1) by its surface value r_s

$$r^* = \frac{r}{r_s} = \frac{C\,(K+1)}{K+C} \,, \qquad (2)$$

where

$$K = K_m/C'_s, \qquad C = C'/C'_s \quad \text{and}$$

$$r_s = V_m C'_s/(K_m + C'_s) \,. \qquad (3)$$

Assuming steady state condition, the dimensionless mass balance inside the biocatalyst pellet can be written as :

$$\frac{1}{x^2}\frac{d}{dx}x^2\frac{dC}{dx} = h^2 f(x) r^* . \quad (4)$$

Here, x is the dimensionless normalized distance measured from the pellet center and

$$h^2 = \frac{R_p^2 r_s}{D_e C_s'} , \quad (5)$$

h being the Thiele modulus, D_e the substrate effective diffusivity and R_p the pellet radius.

To take into account the non uniform distribution of the enzyme into the catalyst pellet, a function f(x) is introduced. The restriction of constant enzyme load to compare results requires the normalization of f(x) in such a way that:

$$3\int_0^1 f(x)\, x^2\, dx = 1 . \quad (6)$$

Equation (4) is solved with the following boundary conditions:

$$C=1 \text{ at } x = 1; \quad \frac{dC}{dx} = 0 \text{ at } x=0 . \quad (7)$$

Under realistic conditions, Eqn. (4) does not have an analytical solution and the numerical solution requires a strong computational effort, even with novel numerical techniques. Nevertheless, a general solution can be obtained using the perturbation and matching technique developed by Gottifredi and Gonzo (1987), which is based on the knowledge of the asymptotic behavior of the solutions for small and large values of the Thiele modulus (h).

Following the procedure sketched for the technique just indicated, when (h -> 0) it is proposed that

$$C = 1 + A(x)\, h^2 + O(h^4) , \quad (8)$$

which after introduction into Eqn. (2) and taking into account only terms up to the order of h^2 leads to:

$$r^* = 1 + h^2\, A(x)(K/(K+1)) , \quad (9)$$

where A(x) is the solution of the differential equation obtained by replacing Eqns. (8)-(9) into Eqn. (4) and equating terms of the same power of h :

$$\frac{1}{x^2}\frac{d}{dx}x^2\frac{dA}{dx} = f(x) \quad (10)$$

with boundary conditions

$$A(x) = 0 \quad \text{at} \quad x = 1 \quad \text{and}$$
$$\frac{dA}{dx} = 0 \quad \text{at } x = 0 . \quad (11)$$

Since the effectiveness factor η is defined as the ratio of the pellet volume averaged reaction rate to the rate under outside surface conditions, then (Froment and Bischoff, 1990) we have

$$\eta = 3\int_0^1 f(x)\, r^*\, x^2\, dx =$$

$$\eta = (3/h^2)\,(dC/dx)_{x=1} . \quad (12)$$

By solving differential equation (10) and taken into account Eqn. (9) we obtain

$$\eta = 1 - \sigma\, h^2 + O(h^4) , \quad (13)$$

where

$$\sigma = -\frac{3K}{(K+1)}\int_0^1 f(x)\, A(x)\, x^2\, dx . \quad (14)$$

The asymptotic solution for (h -> ∞) can be found using the Clareaut substitution in Eqn. (4). As the reaction is irreversible and considering Eqn. (12):

$$\eta = \frac{\rho}{h} \quad (15)$$

with

$$\rho = 3\left[2\, f(1)\int_0^1 r^*\, dC\right]^{1/2} \quad (16)$$

for this specific case:

$$\rho = 3\left[2\, f(1)\,(1+K)\left(1+K.\ln\left(\frac{K}{K+1}\right)\right)\right]^{1/2} . \quad (17)$$

With the aim of obtaining an expression which permit the estimation of η for all range of h values, the formula proposed by Gottifredi et al. (1986) was used to match the asymptotic solutions equations (15) and (13)

$$\eta = [\hat{h}^2 + \exp(-a\hat{h}^2)]^{-1/2} \qquad (18)$$

in which

$$\hat{h} = h/\rho \quad . \qquad (19)$$

The unknown parameter " a" can be determined by comparing the expansion of Eqn. (18) when h is very small, with Eqn. (13). This yields

$$a = 1 - 2\,\sigma\,\rho^2 . \qquad (20)$$

On the other hand, when ($h \to \infty$) , Eqn. (18) reproduces exactly Eqn. (15).

One of the most important features of Eqn. (18) is that the unknowns "a" and " " are determined in a very simple and explicit form through Eqns. (17) and (20), respectively.

In the case of $a < 0$ it is assumed that $a = 0$ in Eqn. (18) to avoid inconsistency of the proposed matching expression. Then, a very simple expression results instead of Eqn. (18):

$$\eta = (1 + \hat{h}^2)^{-1/2} . \qquad (21)$$

These expressions can be easily modified in order to generalize the treatment for several pellet geometries, accordingly, if $f(x) = 1$:

$$\rho = (N+1)[2.f(1)(1+K)(1+K.\ln(K/K+1))]^{1/2} \qquad (22)$$

$$a = 1 - 2\,\rho^2 K/((N+1)(N+3)(K+1)), \qquad (23)$$

where N = 0; N = 1 and N = 2 stand for slab, cylindrical and spherical pellets respectively. The value of Rp in Eqn. (5) is the radius of spherical and cylindrical pellets, but it must be replaced by the half thickness in the case of slab particle.

RESULTS AND DISCUSSION

Equation (4) has been solved numerically by several authors for the case of uniform enzyme distribution ($f(x)=1$). A comparison between the results of Horvarth and Engasser (1974) for a slab geometry with those predicted through Eqn. (18), are presented in Fig. 1 in order to illustrate the performance of the approximate approach. It is clear that Eqn. (18) is able to predict values in very close agreement with the corresponding values obtained by numerical technique for the entire range of modified Thiele modulus Φ or catalyst pellet size, being Φ defined as $\Phi = R_p(V_m/D_e.K_m)^{1/2}$.

Experimental data on the activity of immobilized chymotrypsin on DEAE-cellulose was also presented by Regan et al. (1974). For this case (V_m=5.10^3 μmol/min cm^3 of support; K_m = 8 mM; mean particle size 40 μm and D_e = 5.4 10^{-5} cm^2/min), the authors found experimentally that as K_m/C_s decreased from 3.4 to 0.43 the effectiveness factor changed from 0.46 to 0.72. For these extreme values of K_m/C_s, the effectiveness factor estimated using Eqn. (18) are 0.463 and 0.734, respectively.

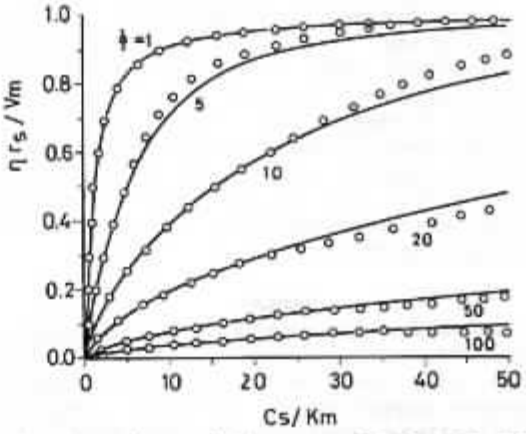

Fig.1 : Plots of the normalized rate of reaction $\eta r_s/V_m$ against the dimensionless substrate concentration C_s/K_m at different values of $\Phi=R_p(V_m/D_eK_m)^{1/2}$. Trace line draw from Eqn.(18). o o o Numerical calculation (Horvarth and Engasser, 1974). Abbreviations are as in Tables 1 and 2.

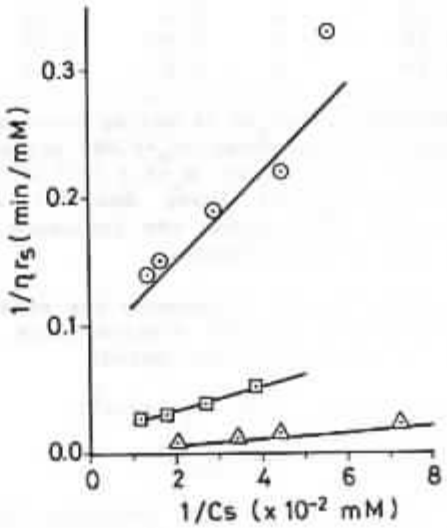

Fig.2 : Comparison of experimental results (open symbols) with values calculated according to Eqn.(18) (trace lines) for sucrose inversion by immobilized yeast cells (Toda and Shoda, 1975). Maximum rate V_m(μmol/min.cm^3):○12; . □ 57; △ 455. R_p=0.047 cm; K_m=38 mM; D_e=4.10^{-4} cm^2/min. Abreviations are as in Tables 1 and 2.

Toda and Shoda (1975) presented experimental data for the sucrose inversion by yeast cells immobilized in spherical agar pellets. Some of their results together with curves calculated with Eqn. (18) are shown in Fig. 2. A very good fit of the experimental data can be observed in this case.

Hooijmans et al. (1990) determined, using a microsensor, the oxygen concentration profiles in spherical agarose gels pellets containing entrapped L-lactate mono-oxygenase. After calculating the intrinsic kinetic parameters, they estimated values of effectiveness factors for several pellet diameter and enzyme concentrations. Some of their results are compared in Table 1 with those obtained by Eqn. (18), the values were corrected considering the external film diffusion present in this case.

TABLE 1

Calculation of experimentally determined effectiveness factors (η) using Eqn. (18). Data for L-lactate monooxygenase supported in spherical agarose gel pellets (Hooijmans et al., 1990)[a].

Protein con. $kg/m^3.10^2$	$2R_p$ $m.10^3$	exp.η	theor.η[b]
0.25	4	0.98	0.97
0.25	5	1	0.95
0.25	6	0.9	0.92
0.25	4	0.98	0.97
0.50	4	0.9	0.93
1.25	4	0.75	0.79
2.50	4	0.67	0.61

[a] Maximum rate: V_m=0.36 mol/kg protein.s Michaelis-Menten const.: K_m=0.049 mol/m^3 Effective diffusivity: D_e=2.3 10^{-9} m^2/s

[b] was η calculated using Eqn.(18) and corrected considering the influence of external film diffusion.

With the aim to examine the effect of different enzyme distributions on η, two functions were tested:

i) polynomial $f(x) = b_1(1+x+x^2)$,

ii) parabolic $f(x) = b_2 x^2$.

Taking into account condition (6) the values of parameters b_1 and b_2 are:

$b_1 = 20/47$ $\quad$ $b_2 = 5/3$

A graphical representation of these enzyme distributions are shown in Fig. 3. The effect of the nonuniform enzyme distribution on the bioparticle effectiveness factor evaluated according to Eqn. (18) is important as shown in Table 2, where results for catalyst with the non uniform distributions (b and c in Fig. 3) are compared with that correspondent to uniform distribution for the case of immobilized Chymotrypsin on DEAE-cellulose (kinetic data from Regan et al., 1974). As can be seen, the biocatalyst with nonuniform enzyme distribution exhibits higher effectiveness compared with that of uniform distribution. The improvement on the effectiveness factor can be as high as 30%, specially under conditions of considerable internal mass transfer limitation.

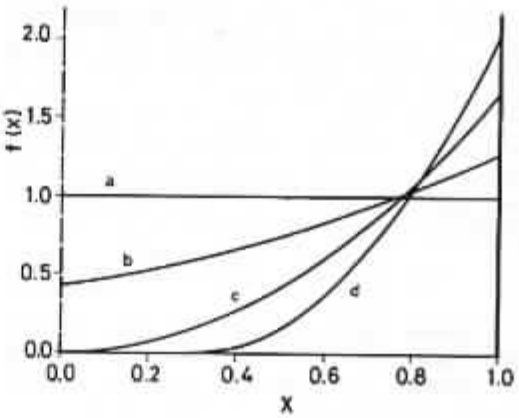

Fig.3 : Enzyme distribution used in the estimation of effectiveness factors.
a) Uniform: $f(x) = 1$
b) Polynomial $f(x) = 20/47(1+x+x^2)$
c) Parabolic $f(x) = 5/3\ x^2$
d) Parabolic in the range $0.3 \le x \le 1$

TABLE 2

Effect of enzyme distribution on effectiveness factor (η).
V_{max} = $5x10^{-3}$ $\mu mol/min.cm^3$; K_m = 8 mM ; D_e = $5.4.10^{-5}$ cm^2/min; C_s = 10 mM(Data for immobilized chymotrypsin in DEAE-cellulose, Regan et al., 1974).

R_p (μm)	η Unif.[a]	Para.[b]	Poly.[c]
1	0.9984	0.9992	0.9988
5	0.9636	0.9793	0.9715
10	0.8719	0.9227	0.8970
20	0.6504	0.7697	0.7001
30	0.4802	0.5932	0.5320
40	0.3693	0.4694	0.4145
50	0.2970	0.3817	0.3350

[a] $f(x) = 1$
[b] $f(x) = 5/3\ x^2$
[c] $f(x) = 20/47(1+x+x^2)$
C_s = substrate concentration at the pellet surface; R_p pellet's radius; other abbreviations are as in Table 1.

Borchert and Buchholz (1984) have

shown that the ratio of effectiveness factors for nonuniform and uniform enzyme distribution is around 1.43 for the hydrolysis of BAEE on pellets with immobilized Trypsin. They found that the enzyme profile is of the parabolic type but with the enzyme concentrated in the outer shell of the carrier. Using our procedure for the effectiveness factor estimation and an enzyme distribution of the form:

$$f(x) = 4.1709\ (x-0.3)^2$$
$$\text{for } 1 \geq x \geq 0.3,$$

and $f(x) = 0$ for $0.3 \geq x \geq 0$,

i.e. profile (d) in figure 3, and taking : $K_m = 1.205.10^{-8}$ mol/cm^3 ; $V_m = 3.8.10^{-3}$ mol/min.g and $R_p = 8.75.10^{-3}$ cm, the following value was obtained $\eta_{NH} = 0.3785$. Meanwhile for $f(x) = 1$ (uniform distribution) $\eta_H = 0.2648$. The ratio of both values is 1.43, which is the value found experimentally.

CONCLUSIONS

As was shown, expression (18) accurately predicts effectiveness factor values when non uniform enzyme distribution is considered even in the case where a inner shell of the catalyst particle is not active.
It can be concluded that our general procedure presented in this contribution can be safely used to produce effectiveness factors estimation when these kinds of kinetics and enzyme distribution are to be considered.

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