MASS TRANSFER IN HETEROGENEOUS SYSTEM ENZYME–SUBSTRATE; APPROXIMATE ANALYTICAL MODEL FOR THE CASE OF LIQUID SUBSTRATE–IMMOBILIZED ENZYME

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Calculation procedure is suggested for flow intensity of substrate toward reaction interface of immobilised enzyme at simultaneous effect of enzymatic reaction and internal diffusion. The approximate model is presented in an analytical form for the basic type of Michaelis–Menten kinetics and for the case of inhibition in excess of substrate.

Shortage of raw materials of fossil origin as sources of energy and increasing strategic significance of food and fodder are the reasons of orientation toward exploitation of untraditional sources of carbon. It becomes obvious at present that the only practical possibility is the use of vegetable products especially on basis of sugar, cellulose and all-lignin wastes (production of ethanol, single-cell proteins *etc.*). For their low-energy consumption seem as quite perspective technologies based on enzymatic reactions, which have a wide application both for the mentioned purposes and also in other branches, *e.g.* in environment protection (waste water treatment, denitrification of drinking water *etc.*). Application of accumulated knowledge of reactor technique for the needs of enzymatic reactors usually faced the problems of effective separation both of enzymes and reaction products. But at present the technique of immobilisation of enzymes on carriers has considerably improved and enables application of knowledge from the region of theory of heterogeneous catalytic reactors and hydrodynamic behaviour of multiphase mixtures while respecting specific behaviour of enzymes and reacting substrates.

From the point of view of industrial application it is possible to specify some basic enzyme reactor types:

I) Liquid (substrate in solution)-immobilized enzyme on solid, stationary carrier; fixed bed, moving bed, trickle bed reactors, with the freely packed or oriented packing (internals).

II) Liquid (substrate in solution)-immobilised enzyme on solid nonstationary carrier (moving particles); fluidised bed, spouted bed and slurry reactors.

III) Suspensions up to pastes (solid substrate in liquid media)-immobilised enzyme on solid stationary carrier: fixed and moving bed reactors with freely packed or oriented packing, tubular reactors.

IV) Suspensions (solid substrate in liquid media)-immobilised enzyme on solid nonstationary carrier (moving particles); fluidised bed reactor, spouted bed, slurry and tubular reactors.

V) Solid substrate-enzyme in solution; any type of reactor with a section of ultrafiltration.

Widest application, but mostly only on laboratory scale, have reactors of type *I*. Arrangement *II* has the advantage in suppressing unfavourable resistance to mass transfer due to sedimentation and blocking of active surface or micropores, by insoluble products of enzymatic reaction (*e.g.* by proteins, impurities *etc.*) and in limitations of unfavourable growth of bacteria. Perspective are reactor types *III* and *IV*, enabling *e.g.* solution of significant problems of enzymatic hydrolysis of large volumes of solid wastes of vegetable (plant) origin (*e.g.* prehydrolysed wastes after treatment of sugar cane or wood to fermentable sugars, or directly to single cell proteins). Application of type *V* in industrial dimensions requires economic handling of an effective ultrafiltration of low-molecular products from the enzyme and solid substrate.

For practical calculations of enzymatic reactors are necessary adequate models preferably in the analytical form, demonstrating the effects of hydrodynamic parameters on rate of heterogeneous enzymatic reaction.

In this contribution is discussed the effect of internal diffusion on the rate of enzymatic reaction for heterogeneous systems of *I* and *II* types for the basic type of the Michaelis–Menten enzymatic kinetics and for the frequent case of enzymatic kinetics, when the substrate in excess is the reaction inhibitor. The exact analytical solution of substrate flow into particles of immobilised enzyme and approximate expression of substrate concentration in the core of a porous particle is presented. This problem has been studied by Horvath and Engasser[1] but they only presented the graphical results of a numerical solution for the simplest case of the Michaelis–Menten kinetics.

The enzymes can be fixed on the carriers in several ways; over an intermediary layer on the external surface of inert particle or bound to the internal surface of the porous particle or homogeneously entrapped in the gel particles of different hardness.

Approximate Model

Substrate must diffuse into the microporous medium or gel where it reacts on the catalytically active surface or inside the particle. The enzymatic catalyst can be exposed on one side (immobilised enzyme on stationary carrier *e.g.* on a plate) or it can be exposed over the whole surface (immobilised enzyme on moving carrier – particle). For theoretical solution the active catalytic bed can be considered as the

planparallel layer or sphere; the solution is similar for both cases. In steady state the concentration changes inside the enzymatic catalyst are given by relations:
Michaelis–Menten simple kinetics

$$D_{\text{eff}}\, \mathrm{d}^2S/\mathrm{d}x^2 = r_s = \mu'_{\max} S/(K_s + S)\,. \tag{1}$$

Michaelis–Menten enzymatic kinetics for noncompetitive inhibitions with substrate in excess is given

$$D_{\text{eff}}\, \mathrm{d}^2s/\mathrm{d}x^2 = r_s = \frac{\mu'_{\max}}{1 + (K_s/S) + (S/K_1)}\,. \tag{2}$$

Boundary conditions for specific dimension (*e.g.* bed thickness) of the catalyst δ are

$$x = 0\,, \quad S = S_i \tag{3}$$

$$x = \delta\,, \quad \mathrm{d}S/\mathrm{d}X = 0\,.$$

In general relations (*1*) to (*3*) do not have an analytical solution for substrate concentration S as a function of distance x in the enzymatically active bed and their numerical integration is necessary. As concerns the mass transfer it is significant to express the flow rate of substrate into the active bed and for the component S there exists for relations (*1*) to (*3*) an exact analytical solution in the form

$$R_S = -D_{\text{eff}}\,(\mathrm{d}S/\mathrm{d}x)_{x=0} = \left(2D_{\text{eff}} \int_{S_L}^{S_i} r_s\, \mathrm{d}S\right)^{1/2}, \text{(mol/s m}^2\text{)}\,, \tag{4}$$

The value S is given as the result of hydrodynamic conditions in the liquid phase. When external diffusion is not the rate controlling step, S_i is equal to composition of substrate in the bulk. Concentration S_L in the point $x = \delta$ can be approximated by the relation resulting from solution of simplified forms of Eqs (*1*) and (*2*), *i.e*)

$$D_{\text{eff}}\, \mathrm{d}^2S/\mathrm{d}x^2 = \mu'_{\max} S/(K_s + S_L) \tag{5}$$

corresponding to relation (*1*) and

$$D_{\text{eff}}\, \mathrm{d}^2S/\mathrm{d}x^2 = \mu'_{\max} S/(S_L + K_s + S_L^2/K_1) \tag{6}$$

corresponding to relation (*2*).

Relations (*5*) and (*6*) with boundary conditions (*3*) lead to trivial solution

$$S_L = S_i/\cosh \phi\delta \tag{7}$$

i.e.

$$S_L = S_i/\cosh\left[\left(\frac{1}{D_{eff}} \cdot \frac{\mu'_{max}}{(K_s + S_L)}\right)^{1/2} \delta\right] \tag{7a}$$

or

$$S_L = S_i/\cosh\left[\left(\frac{1}{D_{eff}} \frac{\mu'_{max}}{(S_L + K_s + S_L^2(K_I))}\right)^{1/2} \delta\right], \tag{7b}$$

where values S_L can be found by iteration.

Relation (*4*) can be integrated, for the case of simple Michaelis–Menten kinetics, to the form for flow intensity of component S

$$R_s = \left\{2D_{eff}\mu'_{max}\left[(S_i - S_L) + K_s \ln\frac{K_s + S_L}{K_s + S_i}\right]\right\}^{1/2} \tag{8}$$

and for the case with inhibition, to the form

$$R_s = \left\{2D_{eff}\mu'_{max}\left((K_I/2)\left[\ln\left|\frac{S^2}{K_I} + S + K_s\right|_{S_L}^{S_i} - K_I I\right]\right)\right\}^{1/2}, \tag{9}$$

where

$$I = \frac{1}{(4K_s/K_I - 1)^{1/2}}\left[\operatorname{arctg}\left(\frac{(2S/K_I) + 1}{(4K_s/K_I - 1)^{1/2}}\right)\right]_{S_L}^{S_i}, \tag{10}$$

$$\text{for } 4K_s/K_I > 1$$

or

$$I = \frac{1}{2(1 - (4K_s/K_I))^{1/2}}\left[\ln\left|\frac{(2S/K_I) + 1 - (1 - 4K_s/K_I)^{1/2}}{(2S/K_I) + 1 + (1 - 4K_s/K_I)^{1/2}}\right|\right]_{S_L}^{S_i}, \quad \text{for} \quad 4K_s/K_I < 1 \tag{11}$$

In steady state the entire substrate S which enters into the active bed of enzymatic catalyst reacts inside (accumulation does not take place) and the relation for effectiveness factor η representing the effects of internal diffusion can be written in the form

$$\eta = -D_{eff}\left(\frac{dS}{dx}\right)_{x=0} \frac{1}{\delta}\frac{(K_s + S_i)}{(\mu'_{max}S_i)} = \frac{R_s(K_s + S_i)}{\delta(\mu'_{max} + S_i)} \tag{12}$$

or for the type of kinetics (*2*)

$$\eta = \frac{R_s}{\delta}\frac{(1 + K_s/S_i + S_i/K_I)}{\mu'_{max}}, \tag{13}$$

where η is the ratio of reaction rate measured at given hydrodynamic conditions and the intrinsic reaction rate corresponding to elimination of internal diffusion effect.

$$R_{s,kin} = \frac{\mu'_{max} S_i}{K_s + S_i} \text{ or } R_{s,kin} = \frac{\mu'_{max} \cdot S_i}{1 + (K_s/S_i) + (S_i/K_I)} \qquad (14)$$

Model Verification

Agreement of the given approximate solution with the exact one was tested for limiting cases of the Michaelis–Menten kinetics, which has an exact analytical solution

Case 1: $S_i \ll K_s$

Solution of relation (*1*) leads to the type of linear kinetics with respect to the component S

$$R_s = D_{eff} S_i (\mu'_{max}/K_s D_{eff})^{1/2} \tanh \left[(\mu'_{max}/K_s D_{eff})^{1/2} \delta\right], \qquad (15)$$

$$S_L = S_i / \cosh \left[(\mu'_{max}/K_s D_{eff})^{1/2} \delta\right] \qquad (16)$$

Case 2: $S_i \gg K_s$

Solution of relation (*1*) leads to the type of kinetics of the zero-th order with respect to component S

$$R_s = \mu'_{max} \delta \qquad (17)$$

$$S_L = S_i - \tfrac{1}{2}(\mu'_{max}/D_{eff})\, \delta^2 . \qquad (18)$$

For values

$$0{\cdot}3 \leqq \phi = (\mu'_{max}/K_s D_{eff})^{1/2} \delta \leqq 10 \qquad (19)$$

the difference between the approximate and exact solution is smaller than 5% rel, for both cases 1 and 2.

For values $\phi \ll 0{\cdot}3$ the approximation (*8*) is not suitable for the case 1 (it does not have a solution or it is unstable). But in this region it is not necessary to consider the effect of diffusion at all, as the values S_i and S_L always differ by less than 5% rel. The reaction rate can be then calculated directly from relations (*14*).

The limit $\phi \leqq 10$ is given by the possibility of agreement of approximate and exact solution for the case 2, when for $\phi > 10$ is the difference greater than 5% rel. But higher values of ϕ are practically not actual in this region, for large μ'_{max} Eq. (*18*) yields a negative value for S_L.

Relation (*19*) can be thus taken for the range of validity of this approximation for the general Michaelis–Menten model. This range of validity is sufficiently wide to cover the conditions encountered in real cases of application of immobilised enzymes.

LIST OF SYMBOLS

D_{eff} effective diffusivity of substrate in enzymatic media
k_L mass transfer coefficient
K_s Michaelis–Menten constant
K_I inhibition constant
r_s reaction rate
R_s intensity of substrate flow on particle surface
S substrate concentration
S_i concentration on external catalyst surface
S_L concentration inside the catalyst
S_b bulk concentration in liquid media
x distance in the bed of enzymatic catalyst
δ thickness of active bed of catalyst
μ'_{max} saturation rate per unit of catalyst volume
μ_{max} saturation rate per unit of external catalyst surface
ϕ modified Thiele factor
η efficiency
diff without effect of reaction rate constant
kin without diffusion effects

REFERENCES

1. Horvath C., Engassev J. M.: Biotechol. Bioeng. *16*, 909 (1974).

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