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Analytical Expression of Effectiveness Factor for Immobilized Enzymes System with Reversible Michaelis Menten Kinetics

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Abstract: **The mathematical model of immobilized enzyme system in porous spherical particle is presented. This model is based on a non-stationary diffusion equation containing a nonlinear term related to Michaelis-Menten kinetics of enzymatic reaction. A general and closed form of an analytical expression pertaining to the substrate concentration profile and effectiveness factor are reported for all possible values of Thiele modules ϕ and α. However, we have employed New Homotopy Perturbation Method (NHPM) to solve the non-linear reaction/diffusion equation in immobilized enzymes system. Therefore, analytical results were found to be in an appropriate agreement with simulation result.**
Keywords: **Diffusion-Reaction, Immobilized Enzymes, Biosensors/bio-fuel cells, New Homotopy perturbation method, Michaelis-Menten kinetics, Effectiveness factor.**

I.INTRODUCTION

The pros of using immobilized enzymes on a porous support is that the enzyme can be segregated easily from the reaction of bulk and recycled. Therefore, the reaction takes place inside the particles; the reaction rate can be influnced by the external diffusion processes and by diffusion within the particles. Most previously published enzymatic kinetic models involve non reversible Michaelis Menten kinetics are solved by numerical method. Among these models, some of the most relevant are those proposed by Engasser and Horvath [2], for a simple Michaelis Menten kinetics, modified by Tuncel [3]; the solution developed by Xiu et al. [4] for product competitive inhibition kinetics; or the two – substrate model formulated by Engasser and Hisland [5].

Hence, a little attention has been given to more complex kinetics such as reversible reactions [6]. The three-parameter model was developed by Bodalo et al. [7] and the two- parameter model reformulated by the same authors [8] could be considered the most general mathematical model published to date. The models were solved for reversible Michaelis – Menten kinetics, which also allows the evaluation of simple Michaelis – Menten and product competitive inhibition kinetics. The first model has been successfully applied in the design of heterogeneous enzymatic reactors: fixed bed reactors [9], continuous tank reactors[10] and fluidized bed reactors [11]. The same methodology applied to the simulation of a packed bed immobilized enzyme reactor performing lactose hydrolysis[12, 13]. All the above mentioned – cited kinetic models were solved by numerical method because reaction rate is a non- linear function of the substrate and product concentrations. Analytical solutions are obtained only for the limiting cases. The approximate analytical solutions, valid only in a limited range of the parameters, have also been published and elaborated. [14 -16].

Several numerical methods have been used to solve the boundary problems outlined in Eqs. (1) and (2). The most frequently used are finite differences [17] and orthogonal collocation [18], which transformed the problem into a system of algebraic equations. When the mass balance equations are non- linear, as in enzymatic kinetics, the result is also a non –linear equations system. The solution obtained by the predetermined differences method may not be exceptional; moreover, convergence problems could appear. On the other hand, since the orthogonal collocation method uses polynomial expressions to approach the concentration profiles, the method is not very reliable when high diffusional inadequacy occurs. Highlighting of the above, many authors have owned initial value procedure such as the Runge- Kutta method. Such techniques need to know the substrate concentration value at r =0. Since this value is unknown , the concentration profiles must be calculated based on an assumed value which would be adjusted by successive calculations (shooting method)[19].

Up to the excellence of our knowledge, no general analytical results for the steady-state substrate concentration and current for all values of parameters ϕ and α have been stated. The purpose of this communication is to derive expression for the steady-state substrate concentration and effectiveness factor in closed form for small values of parameters using the new Homotopy perturbation method.

II. MATHEMATICAL FORMULATION OF THE PROBLEM AND ANALYSIS

The mathematical models for estimating the effectiveness factor in heterogeneous enzymatic systems are based on the following assumptions: (i) The catalytic particle is spherical and its radius is R . (ii) The enzyme is uniformly

distributed throughout the whole catalytic particle. (iii) The system is stable and isothermal. Under these above assumptions, the differential mass balance equation for substrate and product in spherical co-ordinates are as follows [20]:

$$D_S\frac{d^2C_S}{dr^2}+\frac{2D_S}{r}\left(\frac{dC_S}{dr}\right)=V_S \qquad (1)$$

$$D_P\frac{d^2C_P}{dr^2}+\frac{2D_P}{r}\left(\frac{dC_P}{dr}\right)=-V_S \qquad (2)$$

The boundary conditions are

$$\frac{dC_S}{dr}=0;\ \frac{dC_P}{dr}=0 \text{ when } r=0 \qquad (3)$$

$$C_S=C_{SR};C_P=C_{PR} \text{ when } r=R \qquad (4)$$

where $V_S=\dfrac{V_m\left(C_S-\left(C_P/K_{eq}\right)\right)}{K_M+C_S+\left(K_M/K_P\right)C_P}$ (5)

and C_S and C_P denote the substrate and product concentration, *r* is the radial co-ordinate. The form of V_S determines the mathematical method to solve the above equations and its complexity. Most of them are already published articles on enzymatic solution were dealt with non-reversible Michaelis-Menten kinetics. The present model is an improvement based on the previously formulated three parameter model [21], since only two parameters are necessary to reach the solution. Adding equations (1) and (2) and using the boundary conditions the following relationship can be established:

$$C_P=C_{PR}+\frac{D_S}{D_P}\left(C_{SR}-C_S\right) \qquad (6)$$

Substituting the value of C_P , we can obtain

$$V_S=\frac{V_m\left(1+\dfrac{1}{K_{eq}}\dfrac{D_S}{D_P}\right)\left(C_S-C_{SE}\right)}{K_M+\dfrac{K_M}{K_P}C_{PE}+C_{SE}+\left(1-\dfrac{K_M}{K_P}\dfrac{D_S}{D_P}\right)\left(C_S-C_{SE}\right)} \qquad (7)$$

where

$$K_{eq}=\frac{C_{PE}}{C_{SE}} \qquad C_{SE}=\frac{C_{PR}+\left(D_S/D_P\right)C_{PR}}{K_{eq}+\left(D_S/D_P\right)} \qquad \text{and}$$

$$C_{PE}=K_{eq}C_{SE}=\frac{C_{PR}+\left(D_S/D_P\right)C_{PR}}{1+\left(1/K_{eq}\right)\left(D_S/D_P\right)}. \qquad (8)$$

Where C_{SE} and C_{PE} are the equilibrium substrate and product concentration. We make the non-linear differential equations outlined in equation (1) and (2) in dimensionless form by introducing the following dimensionless parameters:

$$S=\frac{C_S-C_{SE}}{C_{SR}-C_{SE}}\ ,\ \rho=\frac{r}{R},\ \ \varphi=\frac{R^2V_m}{\left(C_{SR}-C_{SE}\right)D_S}\frac{\left(1+\dfrac{1}{K_{eq}}\dfrac{D_S}{D_P}\right)}{\left(1-\dfrac{K_M}{K_P}\dfrac{D_S}{D_P}\right)} \text{ and}$$

$$\alpha=\frac{K_M+\dfrac{K_M}{K_P}C_{PE}+C_{SE}}{\left(C_{SR}-C_{SE}\right)\left(1-\dfrac{K_M}{K_P}\dfrac{D_S}{D_P}\right)} \qquad (9)$$

The mass balance differential equation for substrate in spherical co-ordinates for two parameter model is [21]:

$$\frac{d^2S}{d\rho^2}+\frac{2}{\rho}\left(\frac{dS}{d\rho}\right)-\phi\frac{S}{\alpha+S}=0 \qquad (10)$$

Where S is the substrate concentration and ρ is the dimensionless particle radial coordinate and ϕ and α are the dimensionless modules. The boundary conditions are represented as follows:

$$\frac{dS}{d\rho}=0 \text{ when } \rho=0 \qquad (11)$$

$$S=1 \text{ when } \rho=1 \qquad (12)$$

The effectiveness factor can be evaluated as [20]:

$$\eta=3(\alpha+1)\int_0^1\frac{S}{\alpha+S}\rho^2d\rho \qquad (13)$$

III. ANALYTICAL SOLUTION OF THE CONCENTRATION USING NEW HOMOTOPY PERTURBATION METHOD

Present Era, many authors have applied the new Homotopy perturbation method (NHPM) to solve the non-linear problem in physics and engineering sciences [22-26]. Recently this method is also used to solve some of the non-linear problem in physical sciences [27-31]. This method is a combination of homotopy in topology and classic perturbation techniques. Ji-Huan He introduced new Homotopy perturbation method to solve the Lighthill equation [32], the diffusion equation [33] and the blasius equation [34]. The NHPM is unique in its applicability, accuracy and efficiency. The NHPM uses the imbedding parameter *p* as a small parameter, and only a few iterations are needed to search for an asymptotic solution, NHPM yields a very swift convergence and usually, one iteration leads to high accuracy of solution. Solving equations (10) to (12) using Homotopy-perturbation method (Appendix A), we get the solution as

$$S(\rho)=\frac{\sinh\left(\sqrt{\dfrac{\phi}{\alpha+1}}\rho\right)}{\rho\sinh\left(\sqrt{\dfrac{\phi}{\alpha+1}}\right)} \qquad (14)$$

Using Eq. (13), we can obtain the effectiveness factor

$$\eta = \frac{3(\alpha+1)}{\phi}\left(\sqrt{\frac{\phi}{\alpha+1}}\coth\left(\sqrt{\frac{\phi}{\alpha+1}}\right)-1\right) \qquad (15)$$

The equations (13) and (14) represent the new and simple analytical expression of concentration of substrate and effectiveness factor of packed bed reactor.

IV. NUMERICAL SIMULATION

The non-linear differential equation (10) is solved by numerical methods. The function pdex4 in SCILAB software which is a function of solving the boundary value problems for ordinary differential equation is used to solve this equation. Its numerical solution is compared with new Homotopy perturbation method in figures 1 to 4 and it gives a satisfactory agreement with analytically result.

V.DISCUSSION

The Thiele modulus ϕ can be varied by changing either the particle radius or the amount of local concentration of substrate. This parameter estimates the relative importance of diffusion and reaction in the particle radius. When ϕ is small, the kinetics are the determining factor; the overall uptake of substrate in the enzyme matrix is kinetically controlled. Under these conditions, the substrate concentration profile across the membrane is essentially uniform. In contrast, when the Thiele modulus is large, diffusion limitations are the principal determining factor.

Figures. 1(a) and 1(b) show the dimensionless steady-state substrate concentration $S(\rho)$ for the different values of ϕ calculated using Eq. (14). From these figures, it is imply that the value of the concentration increases when ϕ decreases. Figures.2(a) and 2.(b) show the dimensionless steady-state substrate concentration $S(\rho)$ for the different values of ϕ calculated using Eq. (14). From these figures, it is inferred that the value of the concentration increases when α decreases. It is known that the value of the concentration of substrate increases gradually and attains the maximum at the boundary (ρ =1).

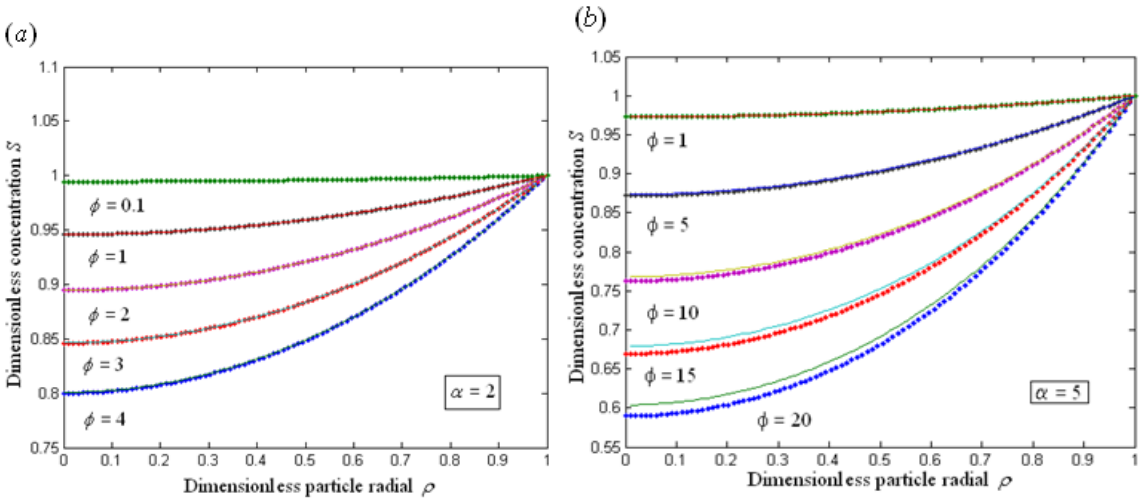

Fig.1 Influence of dimensionless module ϕ on the concentration profile of substrate S obtained from our approximate solution presented in this work (equation (14) solid line) and from the simulation result (dot line). The plot was constructed when (a) $\alpha = 2$, (b) $\alpha = 5$

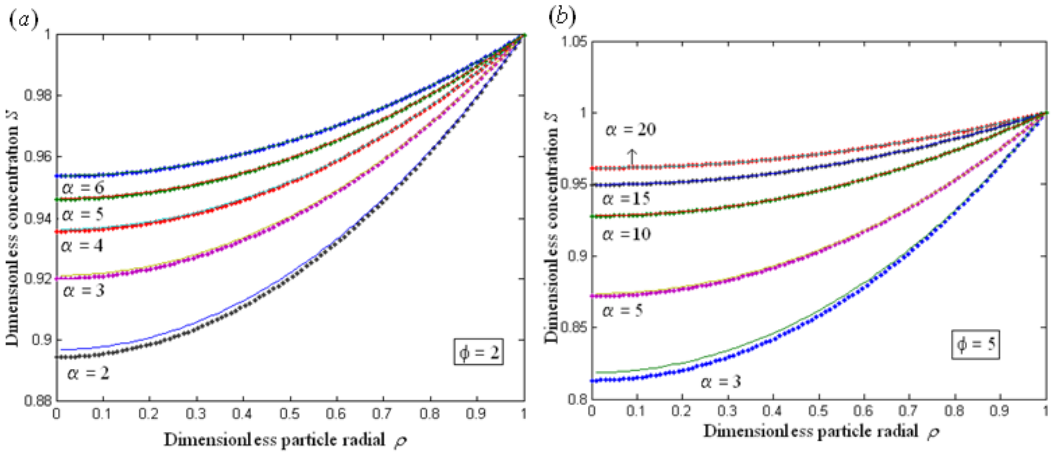

Fig.2 Influence of dimensionless module α on the concentration profile of substrate S obtained from our approximate solution presented in this work (equation (14), solid line) and from the simulation result (dot line). The plot was constructed for (a) $\phi = 2$, (b) $\phi = 5$

The normalized numerical simulation of three dimensional steady-state substrate concentration $S(\rho)$ is shown in Figures. 3.(a) and 3(b). The time independent concentration is designated in Figures.3 (a) for fixed value of $\alpha = 2$ and in Figures.3 (b) for some value of $\alpha = 5$. Concentration $S(\rho)$ is gradually diminishing when ϕ is enlarging. Then the concentration of $S(\rho) = 1$ when $\rho = 1$ and also for all values of α. In these figure, it should be noted that the value of the concentration of substrate decreases when ϕ increases. From this figures, it is apparent that the value of the concentration of substrate increases when α increases.

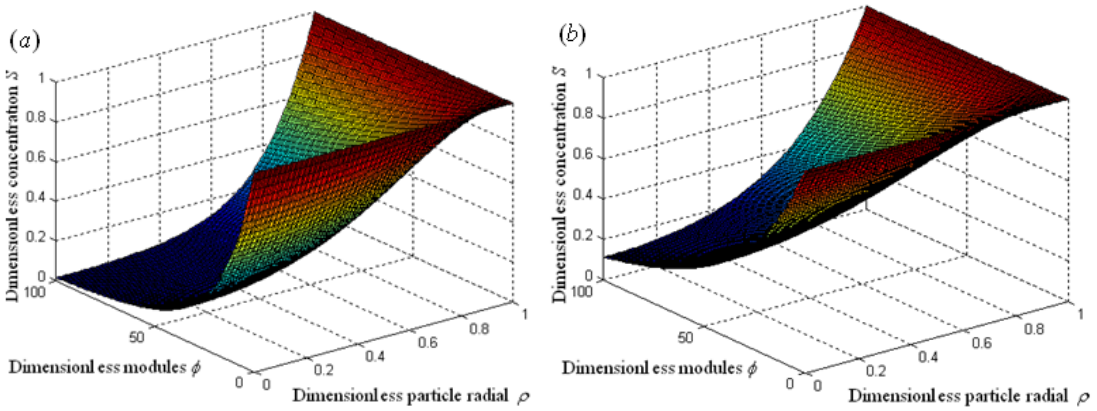

Fig.3 The normalized three dimensionless steady-state concentration profiles S calculated using equation (14) .The plot was constructed for the values of (a) $\alpha = 2$, (b) $\alpha = 5$

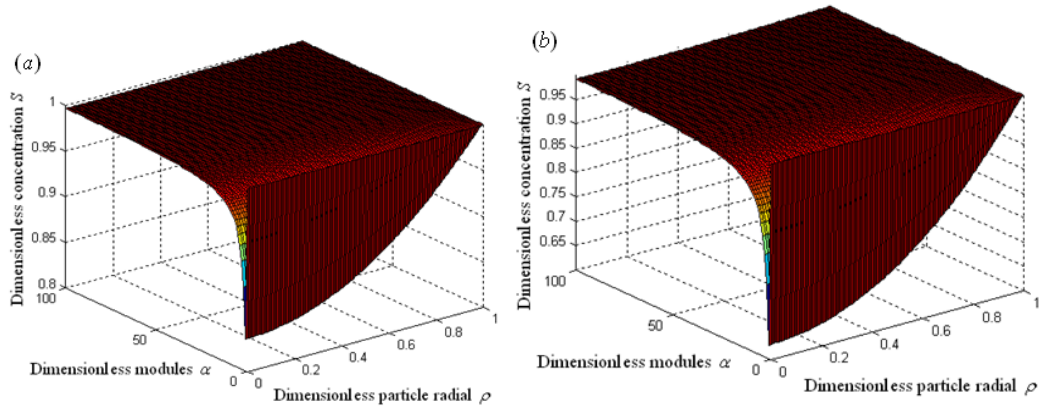

Fig.4 The normalized three dimensionless steady-state concentration profiles S calculated using equation (14) .The plot was constructed for the values of (a) $\phi = 2$, (b) $\phi = 5$

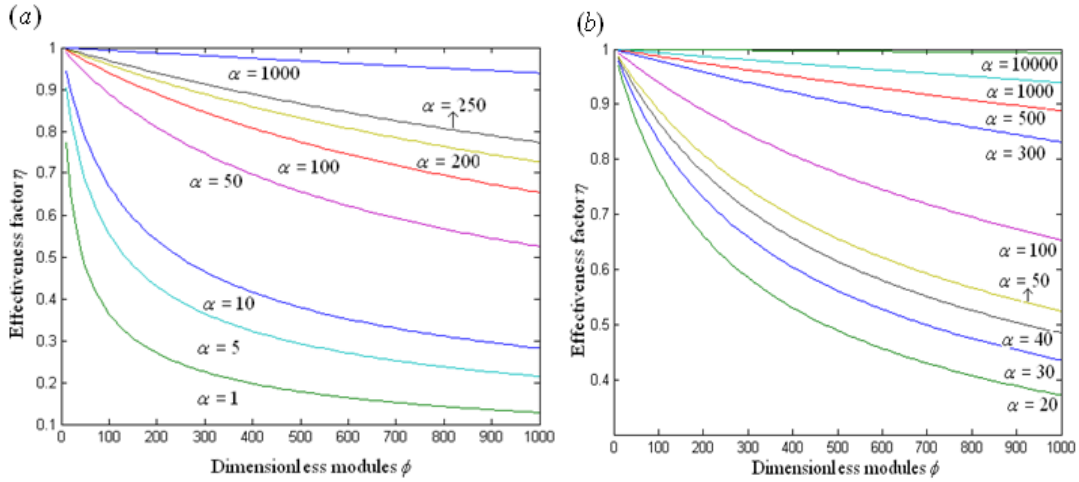

Fig.5 Plot of the effectiveness factor η versus dimensionless parameter ϕ . The effectiveness factor η were computed using equation (15)

The normalized numerical simulation of three dimensional steady-state substrate concentration $S(\rho)$ is shown in Figures.4(a) and 4(b). The time independent concentration ρ is represented in Figure.4(a) for fixed value of $\phi = 2$ and Figure.4(b) for fixed value of $\phi = 5$ Concentration $S(\rho)$ is slowly decreasing when α is deccreasing. Then the concentration of substrate is uniform when ρ =1 and all values of ϕ. In these figure, it should be provided that the value of the concentration of substrate decreases for all values of ϕ. The indifference in effectiveness factor η for various values of ϕ and α using Equation (15) is shown in Figures 5(a) and 5(b). From Figures, it is it is inferred that effectiveness factor η decreases when ϕ increases.

VI.CONCLUSION

The time independent non-linear reaction/diffusion equation in immobilized enzyme system has been formulated and solved analytically. An approximate analytical expression for the concentration and effectiveness factor under steady state conditions are obtained by using the new homotopy perturbation method (NHPM). The primary results of our work were simple approximate calculation of concentration and effectiveness factor for all values of parameters ϕ and α . This procedure can be applied to find the solution of all other non-linear reaction diffusion equations in immobilized enzymes for various complex boundary conditions.

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APPENDIX - I

Solution of the equation (8) using new Homotopy perturbation method

In this appendix, we indicate how Eq. (12) in this paper is derived. To find the solution of Eq. (8), we first construct a Homotopy as follows:

$$(1-p)\left[\frac{d^2S}{d\rho^2}+\frac{2}{\rho}\left(\frac{dS}{d\rho}\right)-\phi\frac{S}{\alpha+1}=0\right]+p\left[(\alpha+1)\left(\frac{d^2S}{d\rho^2}\right)+(\alpha+1)\left(\frac{2}{\rho}\frac{dS}{d\rho}\right)-\phi S\right]=0 \qquad \text{(A1)}$$

the initial approximations are as follows:

$$S = S_0 + pS_1 + p^2S_2 + p^3S_3 + ... \qquad \text{(A2)}$$

Substituting Eq. (A2) into Eq. (A1) and arranging the like coefficients of powers p, we can obtain the following differential equations

$$p^0: \quad \frac{d^2S}{d\rho^2}+\frac{2}{\rho}\left(\frac{dS}{d\rho}\right)-\phi\frac{S}{\alpha+1}=0 \qquad \text{(A3)}$$

The boundary condion are

$$\rho = 0;\ dS_0/d\rho = 0 \qquad \text{(A4)}, \qquad \rho = 1;\ S_0 = 1 \qquad \text{(A5)}$$

Solving equations (A1) using reduction of order the Eq. (A3), we can find the following results

$$S_0(\rho) = \frac{sinh(\sqrt{\frac{\phi}{\alpha+1}}\rho)}{\rho\, sinh(\sqrt{\frac{\phi}{\alpha+1}})} \qquad \text{(A6), According to the HPM, we can conclude that} \quad S(\rho) = \lim_{p\to 1} S(\rho) \cong S_0 \qquad \text{(A7)}$$

Nomenclature

Symbol	Meaning	Usual dimension
C_P	Product concentration inside the spherical particle	Mole/cm^3
C_{PE}	Equilibrium product concentration	Mole/cm^3
C_{PR}	Local product concentration at particle surface	Mole/cm^3
C_S	Substrate concentration inside the spherical particle	Mole/cm^3
C_{SE}	Equilibrium substrate concentration	Mole/cm^3
C_{SR}	Local substrate concentration at particle surface	Mole/cm^3
D_P	Effective product diffusivity inside the particle	Cm^2 sec^{-1}
D_S	Effective substrate diffusivity inside the particle	Cm^2 sec^{-1}
K_{eq}	Equilibrium constant	none
K_M	Michaelis constant	Mole/cm^3
K_P	Competitive product inhibition constant	none
r	Radial coordinate of the particle	Cm
R	Radius of the particle	Cm
S	Dimensionless substrate concentration, defined as C_S/C_{SR} for the two-parameters model	Mole/cm^3
V_m	Maximum reaction rate per unit of catalytic particle volume	Mole/cm^3 sec
V_S	Local reaction rate per unit of catalytic particle volume	Mole/cm^3 sec
Greek symbols		
α	Dimensionless module for two parameter model	none
ϕ	Dimensionless module for two parameter model	none
η	Effectiveness factor	none
ρ	Dimensionless particle radial coordinate	none