Modelling of Immobilized Glucoamylase Kinetics by Flow Calorimetry

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Received: 19 July 2012 / Accepted: 3 September 2012 / Published: 1 October 2012

A mathematical modeling of immobilized glucoamylase kinetics by flow calorimetry is discussed. The model is based on non-stationary diffusion equation containing a non-linear term related to kinetics of the enzymatic reaction. This paper presents the complex numerical methods (The modified Adomian decomposition method) to solve the non-linear differential equations that describe the diffusion coupled with a non-linear reaction terms. Approximate analytical expressions for substrate concentration have been derived for all values of enzyme reactions parameters.

Keywords: Flow calorimetry; Immobilized glucoamylase; Kinetic measurement; intrinsic kinetics; The modified Adomian decomposition method, Boundary value problems.

1. INTRODUCTION

Since the 1990s [1-7] there has been an important transition in the development of immobilized enzymes. Approaches used for the design of immobilized enzymes have become increasingly more rational; this is reflected in the use of more integrated and sophisticated immobilization techniques to solve problems that cannot be easily solved by previously developed single immobilization approaches. In this phase, the major focus of enzyme immobilization was on the development of robust enzymes that are not only active but also stable and selective in organic solvents. Although in the period from the 1970s to the 1980s it was recognized that many enzymes are active and stable in organic solvents under appropriate conditions, the enzymes used are usually less active or stable in organic solvents than in conventional aqueous media [8]. For this reason development of more robust
immobilized enzymes which can work under hostile conditions, especially in non-aqueous media came to the forefront of many research interests in this period [9-11]. Immobilization of enzymes on suitable support materials has resulted in their extended use in batch and continuous bioreactors. For immobilised enzymes, however, there are several factors which affect the observed kinetics that could be significantly different from the intrinsic kinetics of the free enzyme.

Immobilized biocatalysts (IMB) – enzymes are still in the interest of people working in different branches. They constitute principal parts of devices of very variable scale and application starting from microgram amounts of IMB in special analytical devices up to industrial reactors with IMB loading of hundreds of kilograms. This is valid in the stage of IMB screening and design, as for the specification of operational conditions in which they should be used. Therefore, there is always the need coming with a new IMB to find sufficiently accurate, simple and fast experimental technique of investigation of their kinetic properties.

Vladimir Stefuca et.al [12] described the principles and applications of flow calorimetry (FC) in the investigation of the IMB properties. The FC can be used practically for every enzyme-substrate system, under the condition that a sufficient reaction heat is produced and the substrate is in soluble form [13]. Wide applications of glucoamylase in starch industry research focused in the improvement of the enzyme properties by methods of enzyme screening, molecular biology and enzyme engineering. Research in this area can be facilitated by developing suitable methods for the investigation of kinetic properties of immobilized glucoamylase.

Vladimir Stefuca et.al [12] simplified this task by reducing the experiment to the initial rate measurement in combination with the FC avoiding the requirement of a more complicated chemical analysis. For the purpose of the methodology development, the enzyme was immobilized in controlled-pore glass (CPG) particles and a well defined substrate – maltodextrin (MDX) - was used. However, to the best of author’s knowledge, the steady state analytical expression of immobilized glucoamylase and effectiveness factor have not been derived. In this paper, we have obtained the analytical expression of immobilized glucoamylase and effectiveness factor for all values of parameters for steady state condition using the modified Adomian decomposition method.

2. MATHEMATICAL FORMULATION OF THE NON-LINEAR DIFFUSION PROBLEM

We assume that the glucoamylase was immobilized in porous particles. The experimental set-up used for the measurements is depicted in Fig. 1. The main part of the system is a thermostatic cell with the immobolished enzyme column. The column is operated as a small packed bed reactor. Since biocatalyst particle is spherical shape, the material balance reaction diffusion equation is given by [12]

\[
D_e \left( \frac{d^2 c_s}{dr^2} + \frac{2}{r} \frac{dc_s}{dr} \right) - \nu_D = 0
\]

(1)

with boundary conditions:
\[
\frac{dc_s}{dr} = 0 \text{ at } r = 0, \quad \text{(2a)}
\]
\[
c_s = c_{sb} \text{ at } r = R, \quad \text{(2b)}
\]

where \( r \) is the particle radial coordinate, \( R \) the particle radius and \( D_e \) the substrate (MDX) effective diffusion coefficient, \( c_s \) is the substrate concentration and \( c_{sb} \) is the bulk substrate concentration.

**Figure 1.** Experimental calorimetric recirculation system.

For substrate inhibition model the reaction rate, \( v_r \) is given by

\[
v_r = \frac{V_m c_s}{K_m + c_s + \frac{c_{sb}^2}{K_i}} \quad \text{(3)}
\]

where \( V_m, K_m \) and \( K_i \) are kinetic parameters. The steady state effectiveness factor is [12]

\[
v = \frac{3(1 - \varepsilon)D_e}{R\varepsilon} \left[ \frac{dc_s}{dr} \right]_{r=R} \quad \text{(4)}
\]

where \( \varepsilon \) is void fraction of IMB bed. The system governs the substrate concentration \( c_s \) when there is no competitive inhibition in the reaction. The non-linear equation is made dimensionless by defining the following parameters

\[
u = \frac{c_s}{c_{sb}}, \quad X = \frac{r}{R}, \quad k = \frac{V_m R^2}{K_m D_e}, \quad \alpha = \frac{c_{sb}}{K_m}, \quad \beta = \frac{c_{sb}^2}{K_i K_m} \quad \text{(5)}
\]
where \( u(X) \) represents dimensionless concentration, \( X \) is dimensionless distance. \( k, \alpha \) and \( \beta \) are dimensionless parameters. The equation (1) reduces to the following dimensionless form

\[
\frac{d^2 u}{dX^2} + \frac{2}{X} \frac{du}{dX} - \frac{ku}{1 + \alpha u + \beta u^2} = 0
\]  

(6)

The boundary conditions reduce to

\[
\frac{du}{dX} = 0 \quad \text{at} \quad X = 0
\]  

(7a)

\[
u = 1 \quad \text{at} \quad X = 1
\]  

(7b)

The dimensionless effective factor (\( \eta \)) is given by [1]

\[
\eta = \frac{R^2 \sigma v}{3(1 - \varepsilon)C_{sb}} = \left( \frac{du}{dX} \right)_{X=1}
\]  

(8)

3. ANALYTICAL SOLUTION OF BOUNDARY VALUE PROBLEM USING THE MODIFIED ADOMIAN DECOMPOSITION METHOD (MADM)

The modified adomian decomposition method is an extremely simple method to solve the nonlinear differential equations. In the recent years, much attention is devoted to the application of the adomian decomposition method to the solution of various scientific models [14]. The MADM yields, without linearization, perturbation, transformation or discretisation, an analytical solution in terms of a rapidly convergent infinite power series with easily computable terms.

The decomposition method is simple and easy to use and produces reliable results with some iteration used. The results show that the rate of convergence of modified Adomian decomposition method is higher than standard Adomian decomposition method [15-19]. Furthermore, the obtained result is of high accuracy. Using this modified Adomain decomposition method (see Appendix A), the solution of the boundary value problem (Eqs. 6 - 7) is

\[
u(X) = 1 - M + \frac{7NM}{60} + \left( \frac{6M - NM}{6} \right) X^2 + \frac{NM}{20} X^4
\]  

(9)

where

\[
M = \frac{k}{6(1 + \alpha + \beta)} \quad \text{and} \quad N = \frac{k(1 - \beta)}{(1 + \alpha + \beta)^2} \quad \text{for} \quad k \leq 1
\]  

(10)

The above expression is valid when \( k \) is small (\( k \leq 1 \)) and all possible values of parameters \( \alpha \) and \( \beta \). The experimental range of the numerical values of the dimensionless parameters are \( k = 0.001 \)
to 1000, \( \alpha = 0.001 \) to 0.1 and \( \beta = 0.001 \) to 0.1. When \( k \) is large (\( k > 1 \)), the substrate concentration becomes

\[
u(X) = \frac{\sinh(\sqrt{k}X)}{X \sinh(\sqrt{k})} \quad \text{for} \quad k > 1
\] (11)

The effective factor using eqns. (9) and (11) becomes

\[
\eta = \frac{2M(15 - N)}{15} \quad \text{for} \quad k \leq 1
\] (12)

\[
= \sqrt{k} (\coth \sqrt{k}) - 1 \quad \text{for} \quad k > 1
\] (13)

4. NUMERICAL SIMULATION

Figure 2. Dimensionless substrate concentration \( u(X) \). The concentrations were computed using Equation (11) for various values of \( \alpha \) and \( \beta \) and for a fixed value of \( k = 1 \).

An analytical solution for non-linear reaction diffusion equation in immobilized glucoamylase kinetics solved using the modified Adomian decomposition method. To show the efficiency of the present method, our problem is compared with the numerical solution (MATLAB program). We have used the function pdeX1 in MATLAB software, to solve the initial-boundary value problems numerically.

The default parameters employed in Vladimir Stefuca et.al [1] and in this study are given in Table 1. The numerical solution is compared with our analytical results in Figs 3-6 and Tables 2-4. The relative difference between the analytical dimensionless substrate concentration \( u \) and numerical reference results does not exceed 0.22 % for all values of the parameters. Upon comparison, it gives a
satisfactory agreement for all values of the dimensionless parameters $k$, $\alpha$ and $\beta$. The MATLAB program is also given in Appendix (C).

Table 1. Numerical values of the parameters used in this work. The fixed values of the dimensionless parameters are $c_{sb} = 100 \text{ g/l}$, $K_m = 0.15 \text{ gdm}^{-3}$ to $0.96 \text{ gdm}^{-3}$, $K_i = 470 \text{ gdm}^{-3} \text{s}^{-1}$ to $1910 \text{ gdm}^{-3} \text{s}^{-1}$, $V_m = 3.8 \text{ gdm}^{-3} \text{s}^{-1}$ to $4.2 \text{ gdm}^{-3} \text{s}^{-1}$ and $D_e = 9.4 \times 10^{-9} \text{ dm}^2 \text{s}^{-1}$. These are dimensionless parameters used in Vladimir Stefuca et al.[1]

<table>
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<tr>
<th>Parameter</th>
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<tr>
<td>$\beta = \frac{c_{sb}^2}{K_i K_m}$</td>
<td>$10^{-8}$ to $10^{-6}$</td>
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<td>$k = \frac{V_m R^2}{K_m D_e}$</td>
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Parameter | Numerical values of parameters used in this work
---|---
| Fig. 2 | Fig. 3 | Fig. 4 | Fig. 5 | Fig. 6 |
| (a) | (b) | (a) | (b) | (c) | (a) | (b) | (c) |

**Figure 3.** Dimensionless substrate concentration $u$. The concentrations were computed using Eq. (11) for various values of the reaction/diffusion parameter $k$ and for a fixed small value of dimensionless diffusion co-efficient $\alpha = 0.001$ and $\beta = 0.001$. The key to the graph: ‘- -’ represent Eq. 11 for $k \leq 1$ and Eq. 13 for $k > 1$ and ‘+++’ represents the simulation result.
Figure 4. Dimensionless substrate concentration $u$. The concentrations were computed using Eq. (11) for various values of the reaction/diffusion parameter $k$ and for a fixed small value of dimensionless diffusion co-efficients $\alpha = 0.01$ and $\beta = 0.01$. The key to the graph: ‘__’ represent Eq.11 for $k \leq 1$ and Eq.13 for $k > 1$ and ‘++’ represents the simulation result.

Figure 5. Dimensionless substrate concentration $u$. The concentrations were computed using Eq. (11) for various values of the reaction/diffusion parameter $k$ and for a fixed small value of dimensionless diffusion co-efficients $\alpha = 0.05$ and $\beta = 0.05$. The key to the graph: ‘__’ represent Eq.11 for $k \leq 1$ and Eq.13 for $k > 1$ and ‘++’ represents the simulation result.
Figure 6. Dimensionless substrate concentration $u$. The concentrations were computed using Eq. (11) for various values of the reaction/diffusion parameter $k$ and for a fixed small value of dimensionless diffusion coefficients $\alpha = 0.1$ and $\beta = 0.1$. The key to the graph: ‘__’ represent Eq.11 for $k \leq 1$ and Eq.13 for $k > 1$ and ‘+++’ represents the simulation result.

Table 2. Comparison of dimensionless concentration of analytical and numerical of $u$ for various small values of $k$ when $\alpha = 0.001$, $\beta = 0.001$

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Upon comparison, it gives a satisfactory agreement for all values of the dimensionless parameters $k, \alpha$ and $\beta$. The MATLAB program is also given in Appendix (C).
Table 3. Comparison of dimensionless concentration of analytical and numerical of $u$ for various small values of $k$ when $\alpha = 0.1, \beta = 0.1$

<table>
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Table 4. Comparison of dimensionless concentration of analytical and numerical of $u$ for various large values of $k$ when $\alpha = 0, \beta = 0$

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5. DISCUSSION

5.1 Concentration profile

The kinetics response of calorimetric recirculation systems depends on the concentrations of glucoamylase. The concentrations of glucoamylase depends on the following three factors $k, \alpha$ and $\beta$. Thiele modulus, $k$ represents the ratio of the characteristic time of the enzymatic reaction to that of substrate diffusion. The variation in the Thiele modulus $k$ can be achieved by varying either the radius of the particle or kinetic parameters. The Thiele modulus is indicative of the competition between the diffusion and reaction in the calorimetry. When $k$ is small, the kinetics dominates and the uptake of glucoamylase in the enzyme matrix is kinetically controlled. Under these conditions, the glucoamylase concentration profile across the membrane is essentially uniform. When $k$ is large, diffusion limitations are the principal resistance.

Eq.9 and Eq.11 represent the analytical expressions for the dimensionless concentration of glucoamylase $u(X)$. Eq. 9 is valid for $k \leq 1$ and Eq. 11 is valid for $k > 1$. Fig. 2 presents the dimensionless substrate concentration $u(X)$. The concentrations were computed using Eq. 9 for various
values of $\alpha$ and $\beta$ and for a fixed value of $k = 1$. From Fig. 2, it is inferred that the concentration increases when the values of $\alpha$ and $\beta$ increases. In Figs. 3-6, simulation and analytical results are compared. From these figures, it is inferred that the value of the concentration $u$ increases when $k$ decreases but there is no significant difference in the concentration. When $k \leq 1$, the concentration is uniform.

5.2 Effectiveness factor

![Dimensionless effectiveness factor](image)

**Figure 7(a).** Dimensionless effectiveness factor $\eta$. The dimensionless effectiveness factor were computed using Eq. (14) for various values of dimensionless diffusion co-efficients $\alpha$ and $\beta$.

![Dimensionless effectiveness factor](image)

**Figure 7(b).** Dimensionless effectiveness factor $\eta$. The dimensionless effectiveness factor were computed using Eq. (15) for large values of dimensionless parameter $k$. 
Eqs. 12 and 13 represent the analytical expressions for the dimensionless effectiveness factor $\eta$. The normalized effectiveness factor $\eta$ versus $k$ is plotted in Fig. 7(a). This figure illustrates the effectiveness factor $\eta$ for $1 \leq k$ and all practical values of $\alpha$ and $\beta$. In this figure, the effectiveness factor increases when the parameters $\alpha$ and $\beta$ decreases. But there is no significant difference in the effectiveness factor for all practical values of the parameters $\alpha$ and $\beta$. Fig 7(b) illustrates the effectiveness factor $\eta$ versus $k$ for various values of $\alpha$ for $k > 1$. In this figure the effectiveness factor increases when the value of $k$ increases. From these figures (7(a) and 7(b)), we conclude that the value of $\eta$ increases when the reaction diffusion parameter $k$ increases.

6. CONCLUSIONS

We have presented a theoretical model of immobilized glucoamylase kinetics by flow calorimetry. An approximate analytical expression of substrate concentration and effectiveness factor for all possible values of the kinetic parameters are derived using the Adomian decomposition method [15-17]. The accuracy of the approximate analytical solutions of non-linear differential equations has been verified by comparison with numerical solutions. The theoretical results is very much useful to determine the reaction rate and intrinsic kinetic parameters of immobilized glucomylase.

ACKNOWLEDGEMENTS

This work was supported by the University Grants Commission (F. No. 39-58/2010(SR)), New Delhi, India. The authors are thankful to Dr. R. Murali, The Principal, The Madura College, Madurai and Mr. M. S. Meenakshisundaram, The Secretary, Madura College Board, Madurai for their encouragement. The author S. Muthukaruppan is very thankful to the Manonmaniam Sundaranar University, Tirunelveli for allowing to do the research work.

References

Appendix A: Basic concepts of the modified Adomian decomposition method

Consider the non-linear differential equation in the form

\[ y^{(n)} + \frac{2n}{x} y' + \frac{n(n-1)}{x^2} y = g(x) - F(x, y) \quad n \geq 0 \]  \hspace{1cm} (A.1)

with initial condition

\[ y(0) = A, \quad y'(0) = B \]

where \( F(x, y) \) is a real function, \( g(x) \) is the given function and \( A \) and \( B \) are constants. We propose the new differential operator, as below

\[ L = x^{-n} \frac{d^2}{dx^2} (x^n y) \]  \hspace{1cm} (A.2)

So, the problem (A.1) can be written as,

\[ L(y) = g(x) - F(x, y) \]  \hspace{1cm} (A.3)

The inverse operator \( L^{-1} \) is therefore considered a two-fold integral operator, as below.

\[ L^{-1}(.) = x^{-n} \int_{0}^{x} \int_{0}^{x} x^n (. \hspace{.1cm}) \, dx \, dx \]  \hspace{1cm} (A.4)

Applying \( L^{-1} \) on both sides of Eq. (A.1), we find

\[
A + Bx + L^{-1} g(x) - L^{-1} F(x, y) = L^{-1}\left[ y^{(n)} + \frac{2n}{x} y' + \frac{n(n-1)}{x^2} y \right]
\]

\[ = x^{-n} \int_{0}^{x} \int_{0}^{x} x^n \left( y^{(n)} + \frac{2n}{x} y' + \frac{n(n-1)}{x^2} y \right) \, dx \, dx \]  \hspace{1cm} (A.5)

\[ = x^{-n} \int_{0}^{x} \left( x^n y' + nx^{n-1} y \right) \, dx \]

\[ = y(x) - y(0) \]

The Adomian decomposition method introduce the solution \( y(x) \) and the nonlinear function \( F(x, y) \) by infinity series
\[ y(x) = \sum_{n=0}^{\infty} y_n(x) \quad \text{(A.6)} \]

and

\[ F(x, y) = \sum_{n=0}^{\infty} A_n \quad \text{(A.7)} \]

where the components \( y_n(x) \) of the solution \( y(x) \) will be determined recurrently and the Adomian polynomials coefficients \( A_n \) of \( F(x, y) \) are evaluated using the formula

\[ A_n(x) = \frac{1}{n!} \frac{d^n}{dn^2} \left( \sum_{n=0}^{\infty} (\lambda^n y_n) \right) \bigg|_{\lambda=0} \quad \text{(A.8)} \]

By substituting Eq. (A.7) and Eq. (A.8) into Eq. (A.6),

\[ \sum_{n=0}^{\infty} y_n(x) = A + Bx + L^{-1}g(x) - L^{-1}\sum_{n=0}^{\infty} A_n \quad \text{(A.9)} \]

Using modified Adomian decomposition method, the components \( y_n(x) \) can be determined as

\[ y_0(x) = A + Bx + L^{-1}g(x) \]
\[ y_{n+1}(x) = -L^{-1}(A_n), \quad n \geq 0 \quad \text{(A.10)} \]

which gives

\[ y_0(x) = A + Bx + L^{-1}g(x) \]
\[ y_1(x) = -L^{-1}(A_0) \]
\[ y_2(x) = -L^{-1}(A_1) \]
\[ y_3(x) = -L^{-1}(A_2) \quad \text{(A.11)} \]
\[ \ldots \]

From Eq. (A.9) and Eq. (A.11), we can determine the components \( y_n(x) \), and hence the series solution of \( y(x) \) in Eq. (A.6) can be immediately obtained.

\textbf{Appendix B: Solution of non-linear Eq. (6) by using modified Adomian decomposition method}

In this appendix, we derive the general solution of nonlinear Eq. (6) by using Adomian decomposition method. We write the Eq. (6) in the operator form,

\[ L(u(X)) = kN[u(X)] \quad \text{(B.1)} \]

where
\[ L = X^{-1} \frac{d^2}{dX^2} X \quad \text{and} \quad N[u(X)] = \frac{u}{1 + \alpha u + \beta u^2} \]  
(B.2)

Applying the inverse operator \( L^{-1} \) on both sides of Eq. (B.1) yields

\[ u(X) = A X + B + k L^{-1} N[u(X)] \]  
(B.3)

where \( A \) and \( B \) are the constants of integration. We let,

\[ u(X) = \sum_{n=0}^{\infty} u_n(X) \]  
(B.4)

\[ N[u(X)] = \left( \frac{u}{1 + \alpha u + \beta u^2} \right) = \sum_{n=0}^{\infty} A_n \]  
(B.5)

In view of Eqs. (B.4) and (B.5), Eq. (B.3) gives

\[ \sum_{n=0}^{\infty} u_n(X) = A X + B + k \sum_{n=0}^{\infty} A_n L \]  
(B.6)

We identify the zeroth component as

\[ u_0(X) = A X + B \]  
(B.7)

and the remaining components as the recurrence relation

\[ u_{n+1}(X) = k L^{-1} A_n \quad n \geq 0 \]  
(B.8)

where \( A_n \) are the Adomian polynomials of \( u_0, u_1, u_2, \ldots, u_n \). We can find the first few \( A_n \) as follows:

\[ A_0 = N(u_0) = \frac{k}{1 + \alpha + \beta} \]  
(B.9)

\[ A_1 = \frac{d}{d\lambda} \left[ N(u_0 + \lambda u_1) \right] = \frac{k(1 - \beta)u_1}{(1 + \alpha + \beta)^2} \]  
(B.10)

The remaining polynomials can be generated easily, and so,

\[ u_0(X) = 1 \]  
(B.11)

\[ u_1(X) = \frac{k}{6(1 + \alpha + \beta)} (X^2 - 1) \]  
(B.12)
\[ u_2(X) = 1 + \frac{7k^2(1-\beta)}{360(1+\alpha+\beta)^3} - \frac{k^2(1-\beta)}{36(1+\alpha+\beta)^3} X^2 + \frac{k^2(1-\beta)}{120(1+\alpha+\beta)^3} X^4 \] (B.13)

Adding (B.11) to (B.13) we get Eq. (9) in the text.

**Appendix C: The Matlab program to find the numerical solution of Equation 6**

```matlab
function pdex4
m = 2;
x = linspace(0,1);
t = linspace(0,1000);
sol = pdepe(m,@pdex4pde,@pdex4ic,@pdex4bc,x,t);
u = sol(:,:,1);
figure
plot(x,u(end,:))
title('u(x,t)')

% function [c,f,s] = pdex4pde(x,t,u,DuDx)
c = 1;
f = 1.*DuDx;
r=1;
a=0.01;
b=0.1;
F = -(r*u)/((1+(u*a)+(b*u^2)));
s=F;

% function u0 = pdex4ic(x);
u0 = [1];

% function [pl,ql,pr,qr] = pdex4bc(xl,ul,xr,ur,t)
pl = 0;
ql = 1;
pr = ur-1;
qr = 0;
```

**Appendix D: Nomenclature**

- \( r \) particle radial coordinate
- \( R \) particle radius
- \( D_{se} \) substrate (MDX) effective diffusion coefficient \( t (=9.4 \times 10^{-9} \text{ dm}^2\text{s}^{-1}) \)
- \( v_r \) reaction rate
- \( V_m, K_m, \) and \( K_i \) are kinetic parameters
- \( u(X) \) dimensionless concentration
- \( X \) dimensionless distance
- \( c_s \) substrate concentration
ν effective factor
$c_{ib}$ bulk substrate concentration
η dimensionless effective factor
ε void fraction of IMB bed
$N, M$ dimensionless constants.