Diyala Journal of Engineering Sciences

ISSN 1999-8716
Printed in Iraq

Vol. 06, No. 03, pp. 40-63, September 2013

SIMULATION OF ENZYME CATALYSIS IN CALCIUM ALGINATE BEADS

Ameel M. Al-Mayah
University of Baghdad/ Al-Khwarizmi College of Engineering/
Biochemical Engineering Department
(Received: 9/1/2012; Accepted: 8/10/2012)

ABSTRACT:- In the present study, a general mathematical model for a fixed bed–immobilized enzymereactor was developedto simulate the process of diffusion and reaction inside the biocatalyst particle. The modeling and simulation of starch hydrolysis using immobilized α–amylase was used as a model for this study. Corn starch hydrolysis was carried out at constant pH of 5.5 and temperature of 50°C. The substrate flowrate was ranging from 0.2 – 5.0 ml/min, substrate initial concentrations 1 to 100 g/L. α–amylase was immobilized on to calcium alginate hydro-gel beads of 2mm average diameter.In this work Michaelis–Menten kinetics has been considered. The effect of substrate flow rate (i.e. residence time) and initial concentration on intra-particle diffusion has been taking into consideration. The performance of the system is found to be affected by the substrate flow rate and initial concentrations. The reaction is controlled by the reaction rate. The model equation was a non-linear second order differential equation simulated based on the experimental data for steady state condition. The simulation was achieved numerically using FINITE ELEMENTS in MATLABSoftware package. The simulated results give satisfactory results for substrate and product concentration profile within the biocatalyst bead.

Keywords: **-**Enzyme, immobilization, biocatalysis, internal mass transfer, simulation, bioreactor

1. INTRODUCTION

Enzyme immobilization onto supports (or carriers) and their applications as catalysts has been grown considerably during the last three decades, and during the last few years becomes the most exciting aspects of biotechnology (Pedroet al., 2010; Djaffar, et al., 2009;

Dhanya, et al., 2009). Several methods of enzyme immobilization are exists and can be classified into three main categories, carrier binding, cross linking, and entrapment (Pedroet al., 2010). A number of advantages to enzyme immobilization on to support and several major reasons as, the ability to stop the reaction rapidly by removing the enzyme from the reaction solution (or vice versa), products are free of enzyme (especially useful in the food and pharmaceutical industries), reduces effluent disposal problems, suitable for continuous reactor operation, and multiple or respective use of a single batch of enzymes, especially if the enzymes are scarce or expensive, their applicability to continuous processes and the minimization of pH and substrate-inhibition effects.This has anobvious economic impact and allows the utilization of reactors with high enzyme loads (Illanes, 2011).

Enzyme entrapment within a gel matrix is one of the Enzyme immobilizationways. In this way, the enzyme is surrounded by a semi-permeable membrane. Enzyme support of a specific structure permit for the contact between the substrate and the biocatalyst in an appropriate way (Pauline, 1995).

The catalytic activity of immobilized enzyme is affected mainly by the limitations of internal and external mass transfer. External mass transfer limitations can be reduced by changing the reactor hydraulic conditions (for example, the level of agitation), while the internal mass transfer limitations are severe and much more difficult to solve (Illanes, 2011). Enzyme biochemical properties and reaction type and kinetics as well as support chemical and mechanical properties are all effect the internal mass transfer (Illanes, 2011).

To overcome these limitations, small carrier particle size proposed to be used. Uses small particle size complicate the reactor operation due to the increasing the pressure drop (i.e. in case of packed bed reactor) or increasing catalyst washout (i.e. fluidized bed reactors). However the idea cannot be generalized for every enzyme. For example, in the case of enzymes that inhibited by substrate concentration, the operation can be improved by the reduction of the local substrate concentration.

Many mathematical models have been developed by a large number of researchers supported by experimental data, for different types of reactors and mode of operation containing immobilized enzymes (Arturo et al., 2007; Jeisonet al., 2003; Mitra et al., 2002; Mitra et al., 2001). Simulation of these models can be contributed to improve the understanding of the immobilized systems as well as in the prediction of substrate consumption and product formation rates.

Some of these models consider the mass transfer of substrate from the bulk to the active sites where the enzyme immobilized inside the carrier where the reaction takes place.

Fick's law is used to model the mass transfer inside the biocatalyst particle (Loghambal et al., 2011; Jeisonet al., 2003). Enzyme catalysis is non—linear which makes mathematical models more complex, even for the simple Michaelis- Menten kinetics model (Loghambal et al., 2011; Pedro et al., 2010; Al-Muftahet al., 2005; Subhash et al., 2004).

The present study aimed to simulate the process of diffusion and reaction inside the biocatalyst particle. A set of differential equations will obtained for spherical immobilized biocatalyst particle allowing the determination of the concentration profile of substrate and product within the biocatalyst in terms of particle geometry (radius) and the concentration of substrate in the bulk liquid phase.

The hydrolysis reaction of corn starch catalyzed by immobilized α-amylase within hydrogel matrix was chosen and a mathematical model including the effects of diffusion rate and reaction rate parameters in a steady state conditions with scaling analysis were used in this study.

2. KINETICS PARAMETERS

Starch is a major component in many corps as, wheat, maize, tapioca, corn and potato (Djaffar, et al., 2009). Starch consists of a mixture of amylose (15-30%) and amylopectin (70-85%). Starch hydrolysed to low molecular weight of hydrocarbons by the action of either acids or enzymes (Huanxin et al., 2011).

α-amylase enzyme hydrolysis the internal α-1,4 glycosidic links that exists in amylase and amylopectin to produce low molecular weight products (Huanxin et al., 2011; Varatharajan et al., 2011; Abhishek et al., 2010). Starch hydrolysis is influenced by several factors as, crystal structure, particle size, amylase and amylopectin content, and the presence of enzyme inhibitors (Varatharajan et al., 2011; Djaffar, et al., 2009). The kinetics of starch hydrolysis follows the Michaelis-Menten model as confirmed by results of many authors (Varatharajan et al., 2011; Djaffar, et al., 2009) and as explained by Eqn. (1).

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P \quad \ldots (1)$$

where

E; is the enzyme concentration,

S; is the substrate concentration,

k_1 ; is the rate constant for the forward reaction between enzyme and substrate,

k_{-1} ; is the rate constant, for the backward reaction, and

k_2; denote the rate constant for the ES complex dissociation.

The rate of reactant consumption or product formation can be expressed as (Kumar ET AL., 2006; Konsoula ET AL., 2006; Alejandro, 2003):

$$v = V_{\max}\frac{[S]}{K_m + [S]} = \mathrm{k}_2[\mathrm{E}]_o\frac{[S]}{K_m + [S]} \quad ...(2)$$

where

$$K_m = \frac{k_{-1} + k_2}{k_1} \quad ...(3)$$

$$V_{\max} = \mathrm{k}_2[\mathrm{E}]_o \quad ...(4)$$

$[\mathrm{E}]_o$; denoted the initial enzyme concentration.

For plug flow reactor, Eqn. (2) can be rewritten as follows (Alejandro, 2003, Lee, 2001; Arica et al., 1995):

$$-\frac{d[S]}{dt} = V_{\max}\frac{[S]}{K_m + [S]} \quad ...(5)$$

Where $\left(-\frac{d[S]}{dt}\right)$ represent the rate of disappearance of substrate.

Rearranging Eqn. (5) and integration for the boundary conditions [S]=[So] at t=0 and [S]=[St] at time t,

$$-K_m\int_{S_o}^{S}\frac{d[S]}{[S]} - \int_{S_o}^{S} d[S] = V_{\max}\int_0^t dt \quad ...(6)$$

yields the integrated form of the Michaelis-Menten model:

$$K_m \ln\frac{[S_o]}{[S_t]} + [S_o] - [S_t] = V_{\max}t \quad ...(7)$$

For fixed bed reactor, the reaction time or the residence time of reactant spends in the reactor, which is equal to V_t / Q.

Where V_R, is the reactor volume and Q is the substrate volumetric flow rate. Reactor voidage can be expressed as $\varepsilon = V_b / V_R$ (where V_b denoted the volume of the immobilized enzyme beads), then the residence time can be expressed as $t = \left(\frac{V_b}{Q.\varepsilon}\right)$.

Rearranging Eqn. (7) yields:

$$V_{\max} = \frac{K_m}{t}\ln\frac{[S_o]}{[S_t]} + \frac{[S_o] - [S_t]}{t} \quad ...(8)$$

Eqn. (8) can be further be arranged in term of conversion as:

$$\frac{[S_o]}{K_m}\frac{X}{t}=\frac{\ln(1-X)}{t}+\frac{V_{\max}}{K_m} \quad ...(9)$$

or

$$\frac{\ln(1-X)}{t}=\frac{V_{\max}}{K_m}-\left(\frac{[S_o]}{K_m}\right)\frac{X}{\mathrm{t}} \quad ...(10)$$

Where $X=(([S_o]-[S])/[S_o])$.

A plot of $\frac{X}{t}$ versus $\frac{\ln(1-X)}{t}$ gives a straight line with slop equal to $\left(-\frac{[S_o]}{K_m}\right)$ and intercept of $\frac{V_{\max}}{K_m}$.

3. MATHEMATICAL MODEL

In the present study, the enzyme was immobilized on alginate beads; the intra-particle mass transfer resistance can be affecting the rate of enzyme reaction. In order to drive an equation that shows how the mass-transfer affects the effectiveness of an immobilized enzyme. The following assumptions were made in developing and solving the mathematical model:

1. *The reaction takes place at constant temperature.*
2. *Enzyme concentration is constant and uniformly distributed within the beads.*
3. *Enzyme activity is uniform within the beads.*
4. *The pressure drop across the reactor, and other mechanical effects are negligible.*
5. *All physical and transport properties are constant except rate constant.*
6. *Steady state conditions.*
7. *The substrate concentration is constant within the bulk liquid.*
8. *Enzyme kinetics is well described by Michaelis-Menten model.*
9. *External mass transfer limitations are negligible.*
10. *The immobilized enzyme bead is spherical axisymmetric geometry.*
11. *Constant diffusivities of substrate and products within the beads.*
12. *Mass transfer through the immobilized enzyme occurs via molecular diffusion.*

In this model cylindrical coordinates were used to locate the domain where diffusion and enzyme activity take place. Based on the above assumptions, the following unsteady state diffusion-reaction partial differential equations of mass balances for the product and substrate concentrations within the immobilized enzyme bead (and as shown in Fig. 1) can be written as (the details derivation of these equations are illustrated by Lee, 2001):

$$Accumulation = Input - Output + Generation \quad ...(10)$$

$$\frac{\partial S}{\partial t} = D_s\left(\frac{1}{r}\frac{\partial S}{\partial r}(r\frac{\partial S}{\partial r}) + \frac{\partial^2 S}{\partial z^2}\right) - \frac{V_{\max} S}{K_m + S} \quad ...(11)$$

$$\frac{\partial P}{\partial t} = D_P\left(\frac{1}{r}\frac{\partial P}{\partial r}(r\frac{\partial P}{\partial r}) + \frac{\partial^2 P}{\partial z^2}\right) + \frac{V_{\max} S}{K_m + S} \quad ...(12)$$

where

S and P denoted to the substrate and product concentration,

z and r; are the cylindrical coordinates, and

$D_s$andD_p; denoted the effective diffusivity of substrate and product.

The z-axis is the axis of symmetry of the enzyme bead that measures the distance from the planar bead. The variable r measure the radial distance from the z-axis.

The initial and boundary conditions to be assumed for the present problem are as follows:

$P = S = 0$ at $t = 0$ for $z < Y(r)$

$P = 0$ at $z = Y(r)$

$S = S_o$ at $z = Y(r)$

$(\partial P / \partial z) = (\partial S / \partial z) = 0$ at $z = 0$

$(\partial P / \partial r) = (\partial S / \partial r) = 0$ at $r = 0$

In order to simplify the solution of Eqns. (11) and (12), the following dimensionless parameters were introduced here as (Lee, 2001):

$$C_s = \frac{S}{S_o}, \qquad C_p = \frac{P}{S_o}, \qquad \tau = \frac{t}{R^2/D_s}$$

$$\dot{r} = \frac{r}{R}, \qquad \dot{z} = \frac{z}{R}, \qquad \phi = \frac{R}{3}\sqrt{\frac{V_{\max}}{D_s K_m}}$$

$$\beta = \frac{S_o}{K_m}, \qquad \lambda = \frac{D_p}{D_s} \qquad \overline{Y}(\dot{r}) = \frac{Y(r)}{R}$$

Equations (11) and (12) can be rewritten in the normalized form with the dimensionless parameters as:

$$\frac{\partial C_s}{\partial \tau} = \frac{1}{\dot{r}}\frac{\partial C_s}{\partial \dot{r}}(\dot{r}\frac{\partial C_s}{\partial \dot{r}}) + \frac{\partial^2 C_s}{\partial \dot{z}^2} - \frac{9\phi^2 C_s}{1+\beta C_s} \quad ...(13)$$

$$\frac{\partial C_p}{\partial \tau} = \lambda\left[\frac{1}{\dot{r}}\frac{\partial C_p}{\partial \dot{r}}(\dot{r}\frac{\partial C_p}{\partial \dot{r}}) + \frac{\partial^2 C_p}{\partial \dot{z}^2}\right] + \frac{9\phi^2 C_s}{1+\beta C_s} \quad ...(14)$$

The dimensionless parameter ϕ known as Thiele modulus is very important which relates the reaction rate with diffusion rate. In order to simplify the calculations by relating

the product concentration to substrate concentration Eqn. (13) and (14) can be reduced to one linear partial differential as follows:

Let $W = C_s + C_p$, then;

For a steady state conditions, the change in substrate concentration, $\frac{\partial C_s}{\partial \tau}$ and $\frac{\partial C_p}{\partial \tau}$ are equal to zero, then Eqn. (13) will becomes:

$$\frac{1}{\dot{r}}\frac{\partial C_s}{\partial \dot{r}}(\dot{r}\frac{\partial C_s}{\partial \dot{r}}) + \frac{\partial^2 C_s}{\partial \dot{z}^2} - \frac{9\phi^2 C_s}{1+\beta C_s} = 0 \quad ...(15)$$

To simulate the reaction inside the biocatalyst Eqn. (15) and $W = C_s + C_p$, are used in the present study with the following initial boundary conditions:

$W = C_s = 0$ at $\tau = 0$ and $\dot{z} < \bar{Y}(\dot{r})$

$W = C_s = 1$ at $\dot{z} = \bar{Y}(\dot{r})$

$\frac{\partial C_s}{\partial \dot{z}} = 0$ at $\dot{z} = 0$

$\frac{\partial C_s}{\partial \dot{r}} = 0$ at $\dot{r} = 0$

4. EXPERIMENTAL WORK

4.1 MATERIALS

Sodium alginate salt, (type Spectrum Chemical Mfg Corp), Calcium chloride, ($CaCl_2 \cdot 2H_2O$, of BDH type), Fungal amylase powder (MP Biomedicals) of 1 MU/g, Corn Starch (BDH), and HCl (BDH) were used in this study. All other chemical were of analytical grade reagents.

4.2 BUFFER SOLUTION

8 g of KH_2PO_4 (monobasic phosphate) and 0.2 g of K_2HPO_4 (dibasic phosphate) were dissolved in 0.5 liter of water to make a pH buffer solution in order to keep the pH value near to 5.5 during the enzymatic hydrolysis of the starch.

4.3 IODINE SOLUTION

100 ml of iodine solution (0.5% KI and 0.15% I_2) was diluted with distilled water till the final volume reached 300 ml. It was used to make a complex compound by reaction with residual starch in the collected samples. The final complex compound has a deep blue color in order to measure the absorbency of this compound.

4.4 PREPARATION OF SUBSTRATE SOLUTION

40 g of corn starch powder was mixed with 50 ml of water in a beaker. The slurry was added to 900 ml of warm water in a large beaker. During this period, the slurry was mixed well using a magnetic stirrer and then cooled to room temperature to get the final gelatinized starch solution. Additional volume of water was added in order to bring the total volume to 1 liter.

A few drops of iodine were added to the solution, the solution color changed to blue which indicates the presence of starch in the solution.

An equal volume of the above buffer and starch solutions were mixed. The resulting solution contains 20g/l of starch in a buffered environment.

4.5 ENZYME IMMOBILIZATION

3 % (w/v.) solution of sodium alginate was prepared by dissolving 15g of it in 500 ml of water. During the preparation, sodium alginate powder was added slowly (to prevent clomping) to the beaker of water while stirring on a magnetic stirrer. After that, 0.01 g of α-amylase enzyme powder was mixed with 150 ml of sodium alginate solution. The final mixture was dropped using a syringe into 500ml solution of 0.2M CaCl2. Finally, the beads were left for 2 hours in the calcium solution to get the final hardened form of 2mm average beads diameter. The final beads were removed from the calcium solution and washed five times with a distilled water to remove the excess calcium chloride.

4.6 IMMOBILIZED ENZYME ASSAY

20 ml of 3% starch solution and 2 g of calcium alginate beads was assayed by using Bernfeld method (1955) also this method is recommended by sigma-Aldrich Company. Details of this test are stated in this reference. One unit was defined as the amount of amylase that produced 1 mmole of reducing sugar under assay condition per gram of bead.

4.7 FIXED BED REACTOR

A 2cm×20cm glass column was used in the present study as a fixed bed reactor. The reactor surrounded with a jacket of water in order to keep the reaction in an isothermal conditions. Two layers cotton of 2 mm thick were placed at the two ends of the column in order to supports the beads and distribute the substrate solution uniformly. The substrate flow rate was adjusted using a dosing pump (B. Braun Melsungen AG, Model: 870602) and in a range of 0.2 – 5.0 ml/min. Samples of product effluents were collected at a specific time

intervals when the conditions was in steady state. In this study the hydrolysis of corn starch was carried out at constant temperature of 50°C, and atmospheric pressure while pH was kept constant at 5.5. Fig. 2 shows the experimental fixed bed reactor setup.

4.8 PACKED BED VOID FRACTION

The void fraction (ε) was determined experimentally using liquid impregnation method which can be illustrated as follows:

A 60 cm^3 reactor volume was used in this work. First, the reactor was filled with the immobilized enzyme beads, and then distilled water was poured in it till it covers all the beads. After that the reactor was drained to measure the volume of water which is equal to the volume of the void. The void fraction can be calculated using the following equation:

$$\varepsilon = \frac{Volume \text{ of voide}}{Reactor \text{ volume}}$$

4.9 ENZYME BEADS

Enzyme beads were place in the reactor between two layers of cotton. The beads have an average diameter of 2 mm and density of 1.2 g/cm^3. The packed bed has an average void fraction of 0.42.

4.10 STOPPING SOLUTION

0.1N HCl solution was used as a stopping solution to stop the hydrolysis reaction of starch for the collected samples in order to analyzed the starch content.

4.11 STARCH AND PRODUCTS ANALYSIS

The collected samples at timed intervals were analysed using Cintra 5 Double Beam UV-Spectrophotometer for residual starch content. Samples absorbency was measured at 620 nm.

5. RESULTS and DISCUSSION

5.1 STARCH HYDROLYSIS

The effect of substrate (starch) flow rate (i.e. residence time) on substrate concentration is explained in Fig. 3. It can be observed that as the substrate flow rate increases (i.e. low residence time) substrate conversion decreases and at different values of substrate initial concentration, becauseof the residence time is inversely proportional to substrate flow rate. In Fig. 3, it can also be observed that the relation is linear at the first

quarter of the time domain, and then the rate of change decreases rapidly. This is due to the fact that at the beginning of the reaction there is not a lot of substrate present near to enzymes, and the rate increases as the substrate increases because this will give the enzymes more substrate to work on, the rate of change continue till at a certain point, the rate of change decreases, because most of the active sites of enzyme within the bead will be saturated with the substrate to act on. After this point, the substrate concentration becomes too much for the enzyme to work on and the rate of change does not increase further so the rate of reaction becomes nearly constant.

5.2 KINETIC PARAMETERS

Kinetic parameters, Michaelis-Menten constant maximum activity V_{max},k_2 and K_m were determined at different substrate flow rates (i.e. residence times) and initial concentrations using Eqns. (4) and (10). These values are estimated from the slop and intercept of the straight lines shown in Fig. 4. The values of V_{max}, k_2 and K_m at different residence times were listed in Table 1. These values were drawn against residence time and the relation between them can be shown in Figs. 5 to 7. As the residence time increases (i.e. substrate flow rate decreases), V_{max} value decreases and it is also increases with increasing initial substrate concentration. The k_2, (which is named molecular activity, or turnover number of an enzyme is the number of substrate molecules converted to product by an enzyme molecule per unit time when the enzyme is fully saturated with substrate) value also decreases with increasing contact time and increases the initial substrate concentration because it is direct proportional to V_{max} value according to Eqn. (4). These observations can be attributed to the reason that with increasing residence time an increasing in the amount of substrate supplied to the enzyme and this phenomenon is not expected to continue for long where saturation has to be reached at some sites in the bead.

It can also be observed that K_m value increases with increasing residence time and initial substrate concentration. Thus, the lower the value of K_m, the higher the affinity of enzyme for substrate. The velocity of the enzyme-catalyzed reaction is limited by the rate of breakdown of the ES complex. Then with increasing $K_m$valuemore products are formed.

5.3 SIMULATION OF HYDROLYSIS REACTION WITHIN THE HYDROGEL BEAD

The solution for Eqn. (15) was achieved using FINITE ELEMENTS in MATLAB V. 2008A software package.

Figure 8 represent the algorithm for the computer simulation which is used to simulate substrate and product concentrations.

The effective diffusivity of substrate was calculated at the proposed conditions of the present work and according to the following references (Grunwald et al., 1997, Coulson et al., 1999). The details of method and equations are illustrated in these references. This value is equal to 7.8×10^{-8} cm^2/min.

The simulated dimensionless substrate and product concentration profiles are shown in Figs. (9-15). The color-scale map was used to study the substrate and product concentration profiles within the bead. It was assumed that two phases are exists solid bead phase and bulk liquid phase. At low substrate residence time (i.e. high substrate flow rate), the substrate drop rapidly only near the interface between the bead phase and the bulk liquid phase, and in this case the substrate reacts in a fast manner and it will never diffuse into the internal part of the bead and this is can be shown very well in Fig. (9-a) on the other hand the product is formed near the interface and diffuse at a very slow rate (as shown in Fig. (9-b). As the residence time increases (as shown in Fig. 9-a Fig. 15-a) the reaction region increases and the substrate diffuse into the internal part of the bead. On the other hand the product formed in a very slow manner and diffuse in a slow rate so its concentration remains nearly low (as shown in Fig. 9-b to Fig. 15-b). According to assumption IV listed above, the system is at steady state. Thus the composition and mass must be unchanged; substrate cannot accumulate in the shell.

The relation between Thiele modulus (ϕ) and the residence time can be well implemented by Fig. (16). the ϕ is a measure of whether the process is reaction rate controlled at low ϕ or diffusion rate controlled at high ϕ. It can be observed that ϕ increases with decreasing residence time. As ϕ increases, this means that mass transfer is much slower than the reaction; it is possible that all substrate entering the particle will be consumed before reaching the center of the bead. In this case, the concentration drops rapidly within the solid as illustrated by Fig. (17). The active sites occupied by the immobilized enzyme near the center are starved of substrate and the core of the bead becomes inactive. At low ϕ value the concentration of substrate within the bead is naturally higher or lower than in the liquid phase. It can be stated from Fig. (16), that starch hydrolysis at the proposed conditions is reaction rate controlled. At its reaction rate controllability decreases as the value of ϕ decreases.

6. CONCLUSIONS

In the present study, the performance of starch hydrolysis using α–amylase immobilized enzyme in a fixed bed reactor at steady state conditions was presented. The numerical solution of mass balances differential equations expressed in terms of a set dimensionless parameters with the assumed initial and boundary conditions, gave the following conclusions:

- The system performance is strongly affected by the substrate flow rate and initial concentration.
- The determined Thiele modulus (ϕ) values indicated that the reaction rate was controlled by reaction rate within the calcium alginate hydro-gel beads.
- A decreased in ϕ values determines an improvement of substrate conversion.
- The simulation, which has been performed with experimental data, gave satisfactory results for the substrate and product concentration profiles.
- The simulation results is a valuable tool for immobilized enzyme reactor design by providing a quantitative relation of enzyme performance with operational variables like substrate flow rate, initial concentration, conversion and particle size.

7. NOMENCLATURE

C	Dimensionless concentration	-
D	Effective diffusivity	cm^2/min
E	Enzyme concentration	g/l
$[E]_o$	Initial enzyme concentration	g/l
ES	Enzyme-substrate complex	-
k	Rate constant	s^{-1}
K_m	Michaelis constant	g/l
P	Product concentration	g/l
Q	Substrate flowrate	cm^3/min
r	Radius of immobilized enzyme bead	cm
R	Radius of immobilized enzyme bead	cm
$\dot{r}$	Dimensionless radius	-
S	Substrate concentration	g/l
t	time,	min
v	Rate of reactant decomposition	$gl^{-1}s^{-1}$
V	Rate of reaction in Eq. (2)	$gl^{-1}s^{-1}$

V	Volume	cm^3
W	Dimensionless concentration	-
X	Conversion	%
$\dot{z}$	Dimensionless height,	-

Subscript

1	Forward reaction between enzyme and substrate
-1	Backward reaction
2	ES complex dissociation
b	Bead in Eqn. $\varepsilon = V_b / V_R$
b	Bulk solution
max	Maximum value
o	Initial value
p	Product
R	Reactor
s	Substrate
t	Concentration at time t

Symbols

ϕ	Thiele's modulus, $\phi = \frac{R}{3}\sqrt{V_{max} / D_s K_m}$
τ	Dimensionless time
β	Dimensionlessratio
ε	Voidage

8. REFERENCES

1- Abhishek Mukherjee, Anil K. Ghosh, SubhabrataSengupta,(2010), *"Purification and characterization of a thiol amylase over produced by a non-cereal non-leguminous plant, Tinosporacordifolia"*, Carbohydrate Research, 345, 2731–2735.

2- Alejandro G. Marangoni, (2003), "Enzyme kinetics a modern approach", John Wiley & Sons, Inc.

3- Al-Muftah Ali E., Ibrahim M. Abu-Reesh, (2005), *"Effects of internal mass transfer and product inhibition on a simulated immobilized enzyme-catalyzed reactor for lactose hydrolysis"*, Biochemical Engineering Journal, 23,139–153.

4- Arica M.Y., V. Hasircl and N.G. Alaeddinoglu, (1995), *"Covalent immobilization of a-amylase onto pHEMA microspheres: preparation and application to fixed bed reactor"*, Biomaterials, 16, 761–768.

5- Arturo Horta, Jose´ R. A´ lvarez , Susana Luque, (2007), *"Analysis of the transient response of a CSTR containing immobilized enzyme particles Part I. Model development and analysis of the influence of operating conditions and process parameters"*, Biochemical Engineering Journal, 33, 72–87.

6- Bernfeld, P. (1955), Amylases, alpha and beta. In: Colowick, S. P. and Kaplan, N. O (eds.). *Methods in enzymology*. New Yorks: Academic Press. v. 1. pp.149 –158,

7- http://www.sigmaaldrich.com/sigma-aldrich/technical-documents/protocols/biology/enzymatic-assay-of-a-amylase.html.

8- Coulson J, M., Richardson J. F., J. R. Backhurst and J. H. Marker, (1999), *"Chemical Engineering"*, Volume 1, 6thed, Butter worth–Heinemann.

9- DhanyaGangadharan, K. MadhavanNampoothiri, SwethaSivaramakrishnan, Ashok Pandey, (2009), *"Immobilized bacterial a-amylase for effective hydrolysis of raw and soluble starch"*, Food Research International 42, 436–442.

10- DjaffarDjabali, NaimaBelhaneche, BoubekeurNadjemi, VirginieDulong, Luc Picton, (2009), *"Relationship between potato starch isolation methods and kinetic parameters of hydrolysis by free and immobilised a-amylase on alginate (from Laminariadigitata algae)"*, Journal of Food Composition and Analysis, 563–570.

11- Grunwald Peter, Kristin Hansen, Walter Gunber, (1997), *"The determination of effective diffusion coefficients in a polysaccharide matrix used for the immobilization of biocatalysts"*, Solid State Ionics, 101 – 103, 863 – 867.

12- Huanxin Zhang, Zhengyu Jin, (2011), *"Preparation of products rich in resistant starch from maize starch by an enzymatic method"*, Carbohydrate Polymers, 86, 1610– 1614.

13- Illanes A., (2011), *"Immobilized Biocatalysts"*Comprehensive Biotechnology (Second Edition), Volume 1, 25–39.

14- Jeison D., G. Ruiz, F. Acevedo, A. Illanes, (2003), *"Simulation of the effect of intrinsic reaction kinetics and particle size on the behaviour of immobilised enzymes under internal diffusional restrictions and steady state operation"*, Process Biochemistry, 39, 393–399.

15- Lee James M., (2011), *"Biochemical Engineering"*, Prentice-Hall Inc.

16- Loghambal S. and Rajendran L., (2011), *"Mathematical modeling in amperometric oxidase enzyme–membrane electrodes"*, Journal of Membrane Science, 373, 20–28.

17- Konsoula Zoe, Maria Liakopoulou-Kyriakides, (2006), *"Starch hydrolysis by the action of an entrapped in alginate capsules a-amylase from Bacillus subtilis"*, Process Biochemistry, 41, 343–349.

18- Kumar R. Siva Sai, K.S. Vishwanath, Sridevi Annapurna Singh, A.G. AppuRao, (2006), *"Entrapment of a-amylase in alginate beads: Single step protocol for purification and thermal stabilization"*, Process Biochemistry, 41, 2282–2288.

19- MitraDadvar, MortezaSohrabi, Muhammad Sahimi, (2001), *"Pore network model of deactivation of immobilized glucose isomerase in packed-bed reactors I: Two-dimensional simulations at the particle level"*, Chemical Engineering Science, 56, 2803–2819.

20- MitraDadvara, MuhammadSahimi, (2002), *"Pore network model of deactivation of immobilized glucose isomerase in packed-bed reactors II: three-dimensional simulation at the particle level"*, Chemical Engineering Science, 57, 939 – 952.

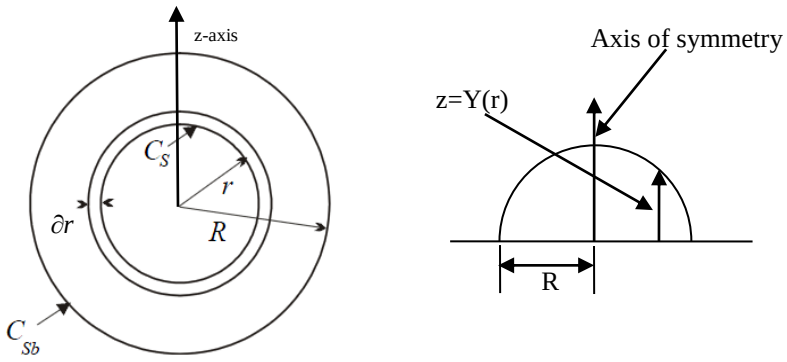
21- Pauline M. Doran, (1995), *"HeterogeneousReactions", Bioprocess Engineering Principles, Academic Press, 297–332*.Pedro Valencia, Lorena Wilson, Carolina Aguirre, A. Illanes, (2010), *"Evaluation of the incidence of diffusional restrictions on the enzymatic reactionsof hydrolysis of penicillin G and synthesis of cephalexin"*, Enzyme and Microbial Technology 47, 268–276.

22- Subhash Bhatia, Wei Sing Long, AzlinaHarunKamaruddin, (2004), *"Enzymatic membrane reactor for the kinetic resolution of racemic ibuprofen ester: modeling and experimental studies"*, Chemical Engineering Science 59 5061 – 5068.

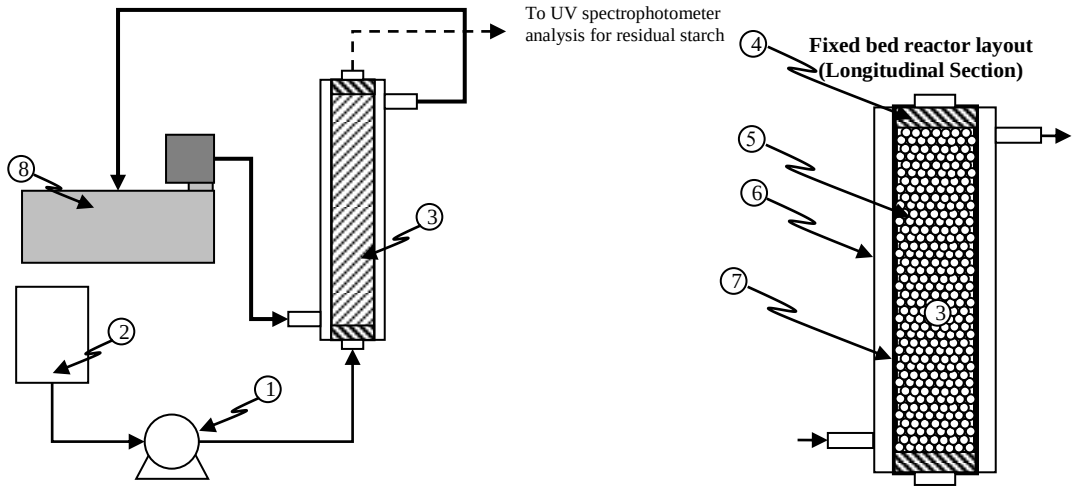
23- Varatharajan V., R. Hoover, Jihong Li, T. Vasanthan, K.K.M. Nantanga, K. Seetharaman, Q. Liu, E. Donner, S. Jaiswal, R.N. Chibbar, (2011), *"Impact of structural changes due to heat-moisture treatment at different temperatures on the susceptibility of normal and waxy potato starches towardshydrolysis by porcine pancreatic alpha amylase"*, Food Research International, paper in press.

Table (1): K_m, V_{max} and k_2 values of starch hydrolysis using immobilized amylase enzyme in fixed bed reactor at different values of initial substrate concentration and flow rate (pH=5.5, T=50°C).

[S] g/l	**K_m**						
	Residence time, min						
	5	**10**	**20**	**40**	**60**	**90**	**120**
1	0.340	0.428	0.510	0.658	0.790	0.926	1.010
5	1.690	2.137	2.550	3.290	3.940	4.630	5.060
10	3.380	4.270	5.100	6.580	7.870	9.260	10.13
50	16.89	21.37	25.51	32.90	39.37	46.30	50.66
100	33.78	42.74	51.00	65.79	78.74	92.60	101.3
V_{max}							
1	0.013	0.009	0.008	0.007	0.006	0.005	0.002
5	0.067	0.047	0.038	0.036	0.181	0.023	0.009
10	0.134	0.094	0.077	0.072	0.362	0.046	0.017
50	0.672	0.470	0.383	0.362	1.811	0.232	0.086
100	1.344	0.940	0.765	0.724	3.622	0.463	0.172
k_2							
1	0.747	0.522	0.425	0.402	0.350	0.257	0.096
5	3.734	2.611	2.126	2.010	10.06	1.286	0.478
10	7.467	5.223	4.252	4.020	20.12	2.572	0.957
50	37.34	26.12	21.26	20.10	100.6	12.86	4.784
100	74.68	52.23	42.52	40.20	201.2	25.72	9.569



Figure(1): Shell balance for a substrate in an immobilized enzyme (James Lee, 2001).



1	Peristaltic Pump	5	Calcium alginate beads
2	Substrate feed tank	6	Heating & cooling reactor jacket
3	Glass reactor	7	Reactor wall
4	Distributor (Cotton layer)	8	Water bath

Figure (2): Experimental fixed bed reactor setup.

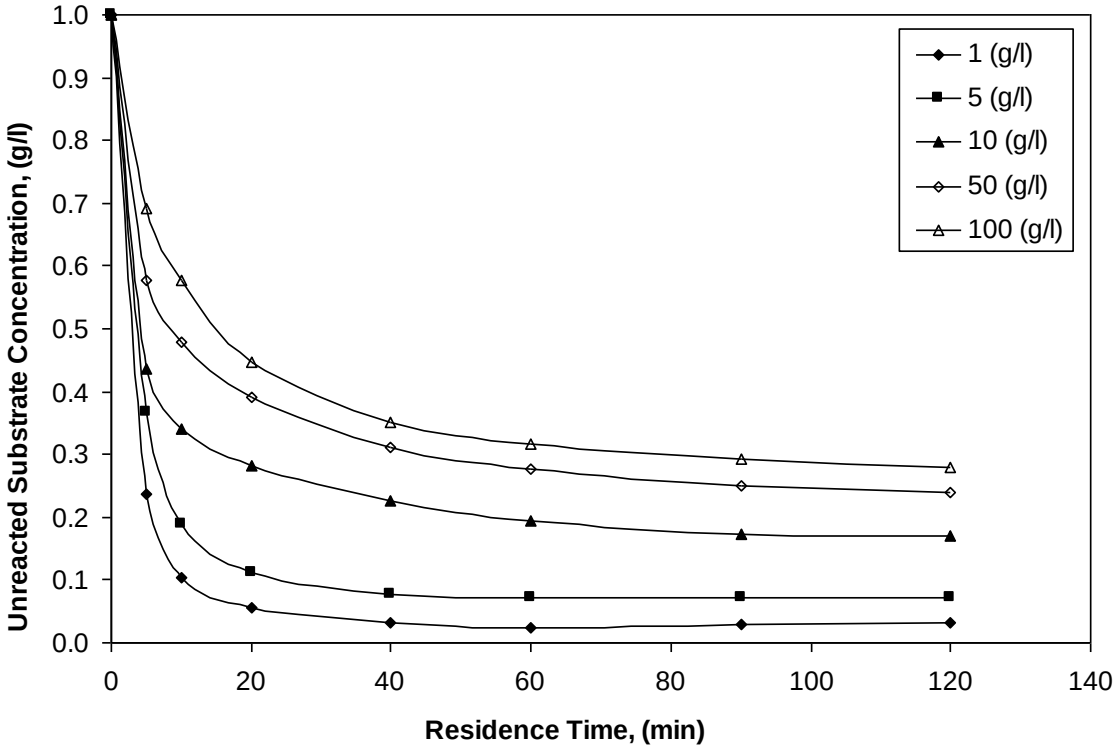


Figure (3): Effect of residence time on substrate concentration at different substrate initial concentration (pH=5.5, T=50°C).

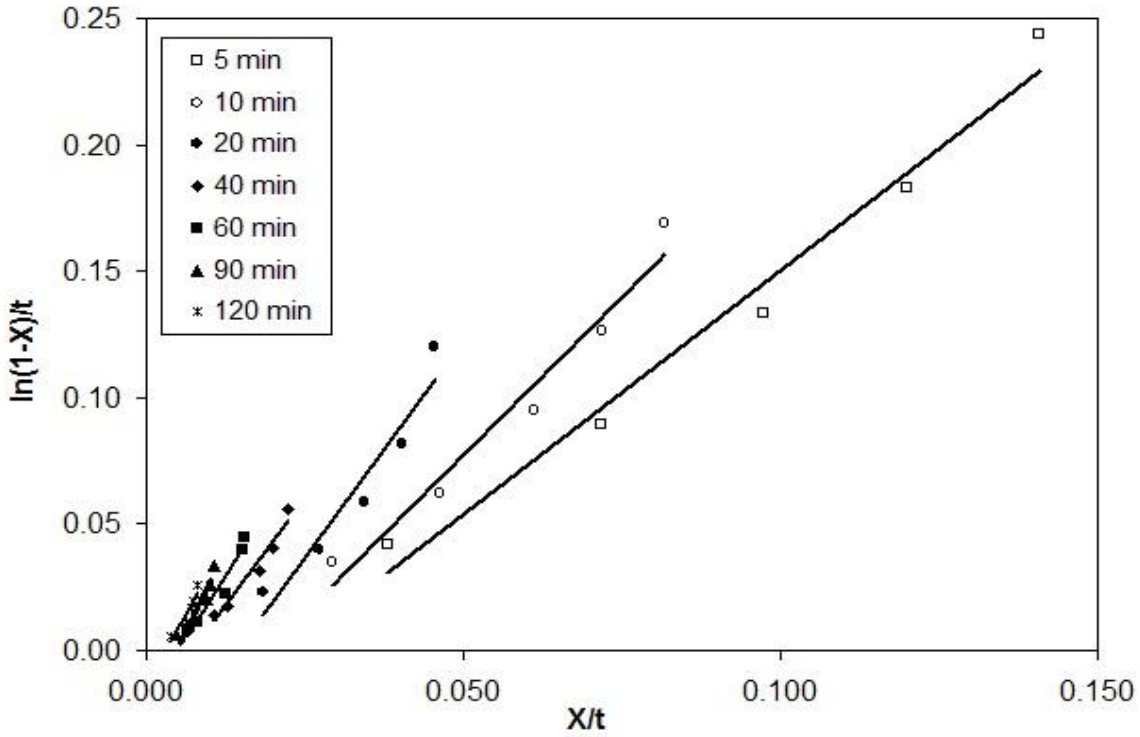


Figure (4): Linear plots of X/t vs. ln(1-X)/t for immobilized α-amylase enzyme in fixed bed reactor (pH=5.5, T=50°C).

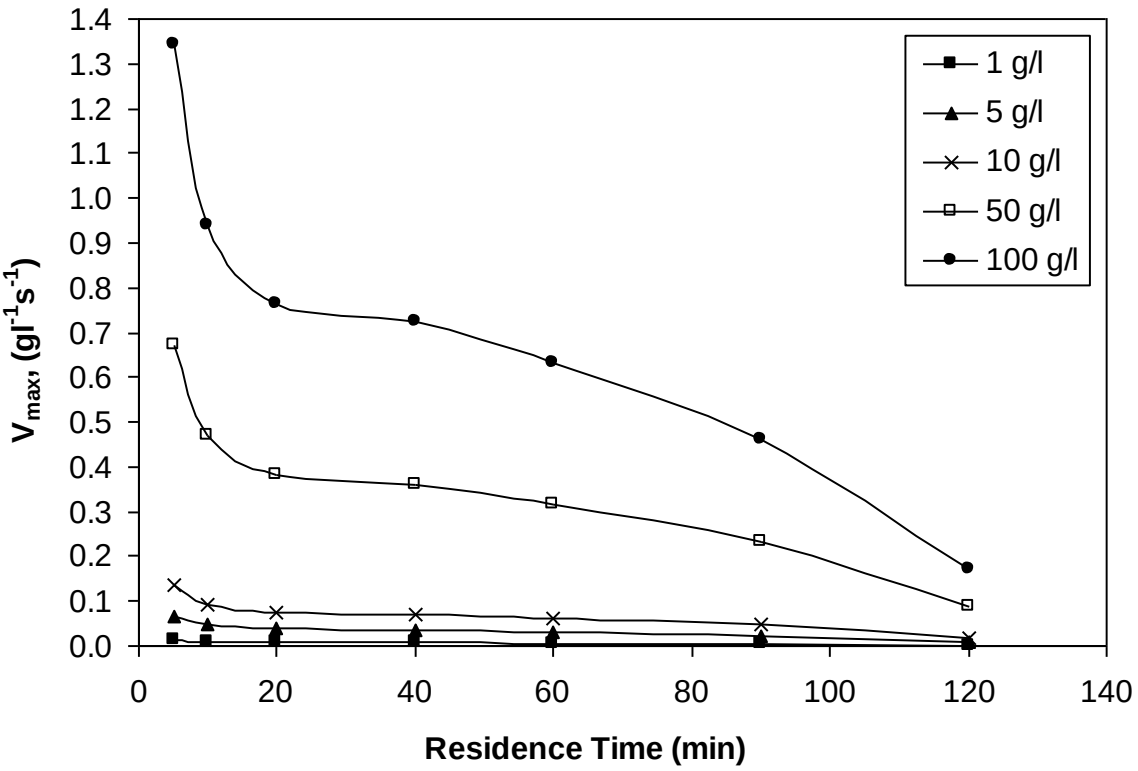


Figure (5): Effect of residence time on V_{max} value at different initial substrate concentration (pH= 5.5, T=50°C).

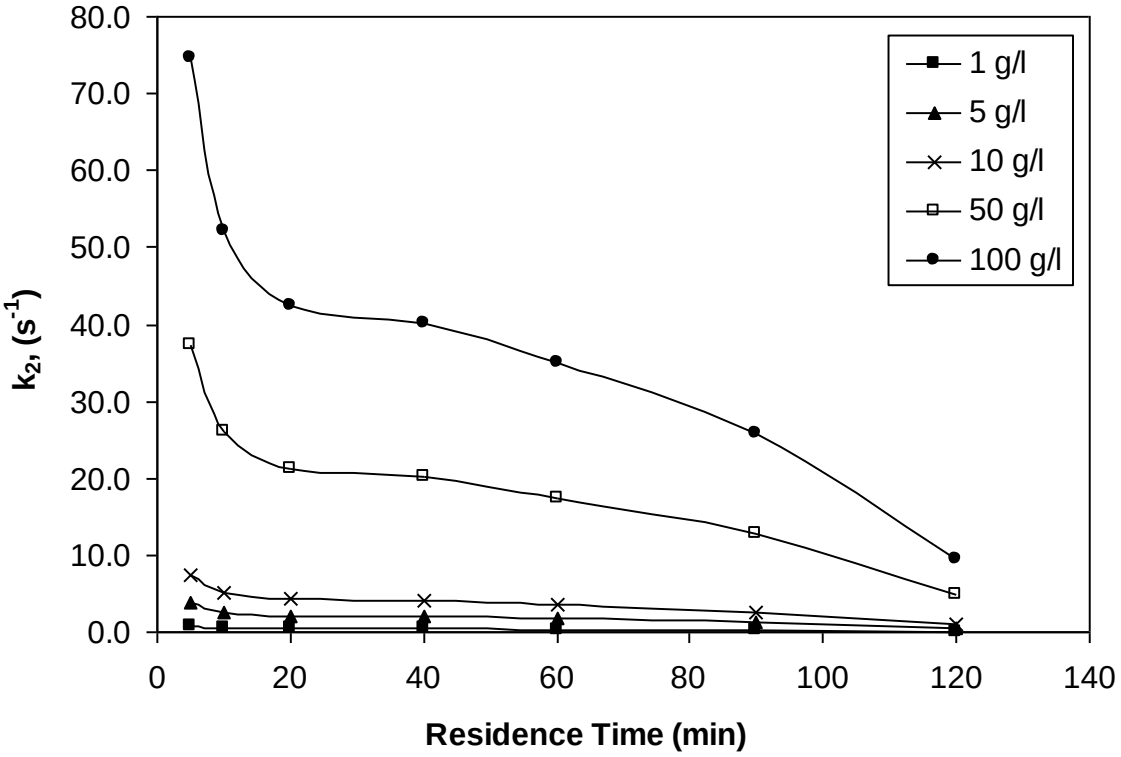


Figure (6): Effect of residence time on k_2 value at different initial substrate concentration (pH= 5.5, T=50°C).

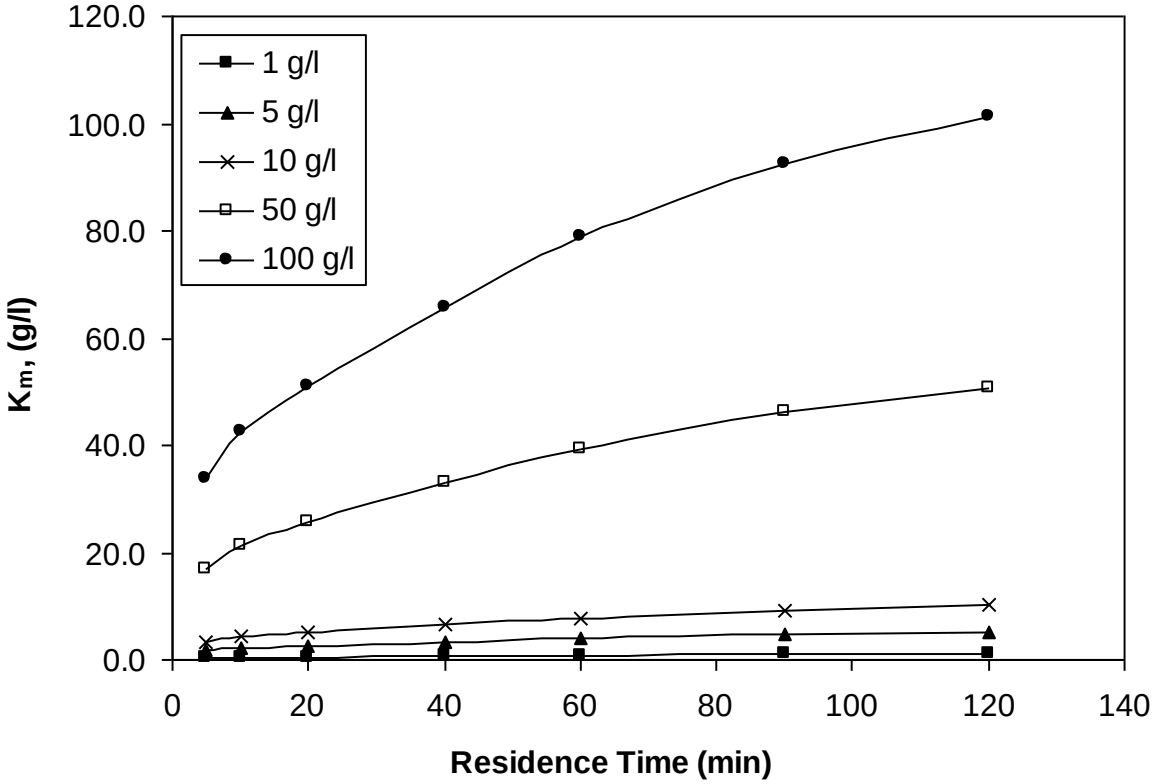


Figure (7): Effect of residence time on K_m value at different initial substrate concentration (pH= 5.5, T=50°C).

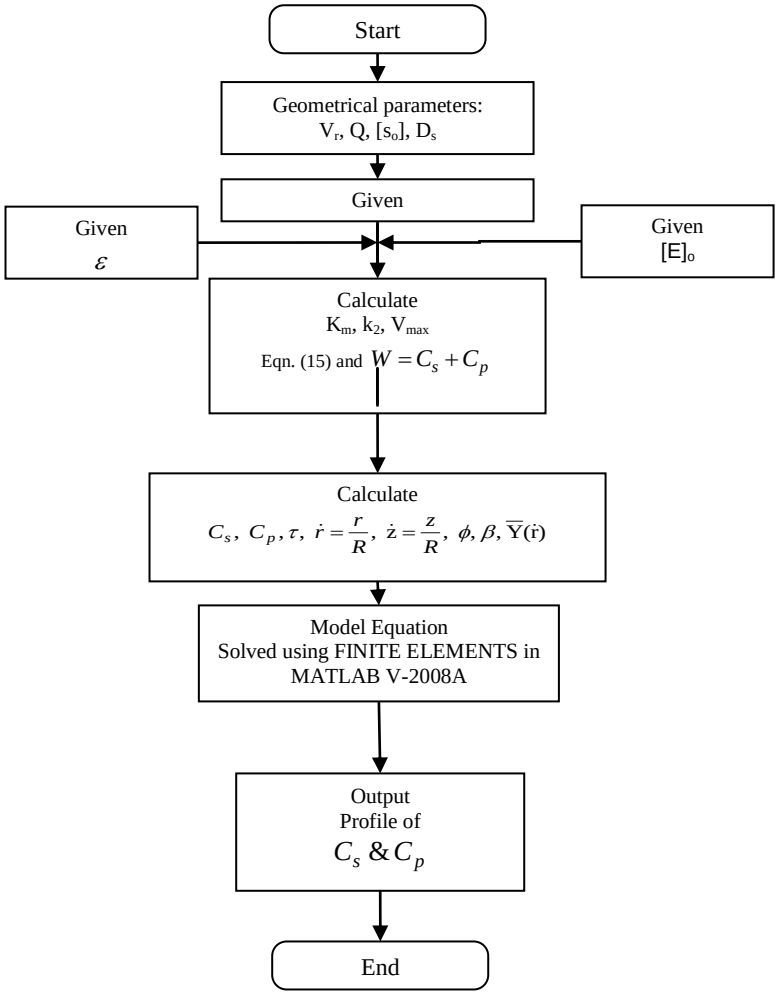
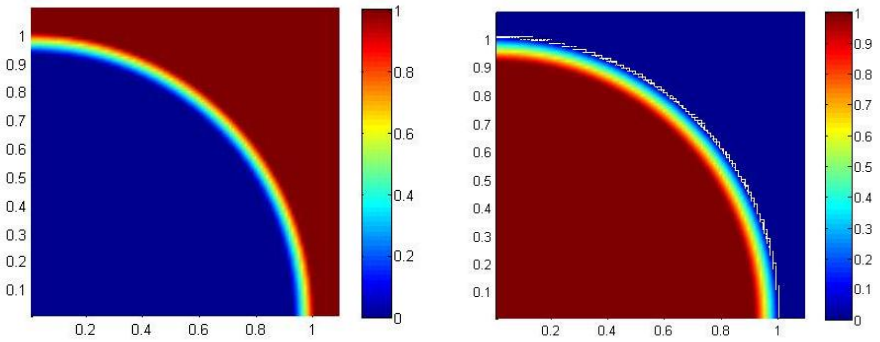
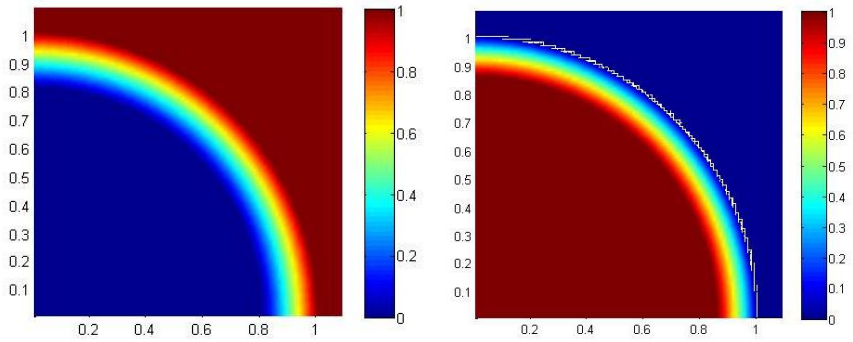


Figure (8): Algorithm to simulate substrate and product concentration profile in a fixed bed reactor.



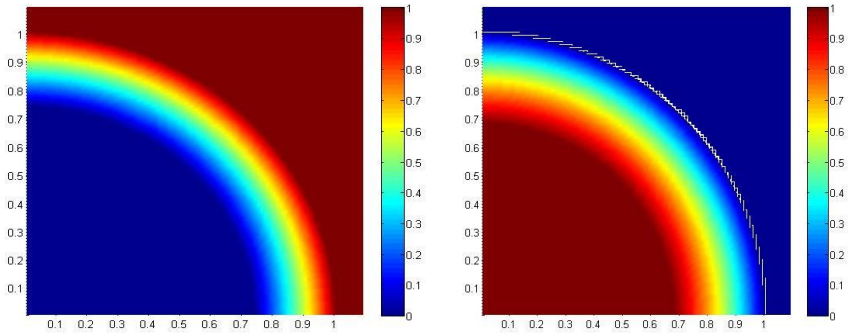
(a) Dimensionless substrate concentration (b) Dimensionless product concentration

Figure (9): Dimensionless substrate and product concentration profiles in 3D (at ϕ=47.6and t=5 min). The completely dark red area represents the region in which substrate or product at its maximum value (i.e. equilibrium value). x-axis represent dimensionless radius and y-axis represent z-axis.



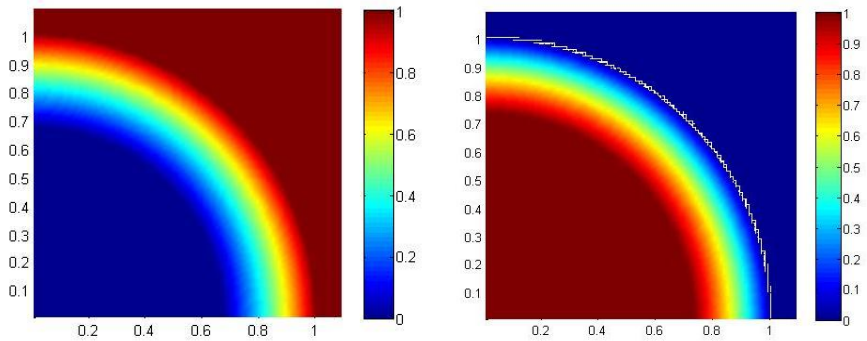
(a) Dimensionless substrate concentration (b) Dimensionless product concentration

Figure (10): Dimensionless substrate and product concentration profiles in 3D (at ϕ=35.4and t=10 min). The completely dark red area represents the region in which substrate or product at its maximum value (i.e. equilibrium value). x-axis represent dimensionless radius and y-axis represent z-axis.



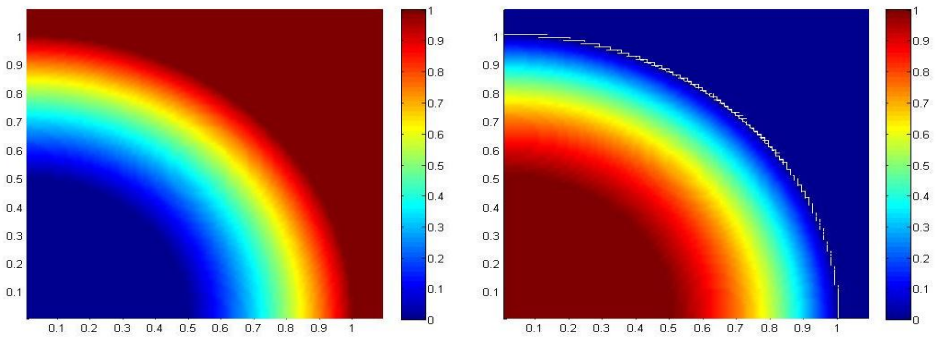
(a) Dimensionless substrate concentration (b) Dimensionless product concentration

Figure (11): Dimensionless substrate and product concentration profiles in 3D (at ϕ=29.2 and t=20 min). The completely dark red area represents the region in which substrate or product at its maximum value (i.e. equilibrium value). x-axis represent dimensionless radius and y-axis represent z-axis.



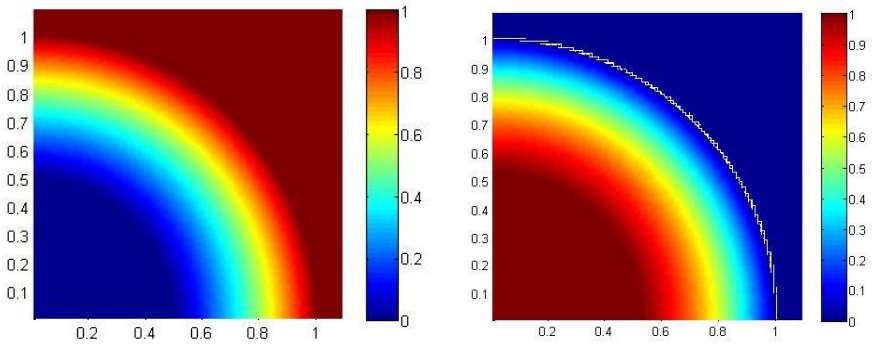
(a) Dimensionless substrate concentration (b) Dimensionless product concentration

Figure (12): Dimensionless substrate and product concentration profiles in 3D (at ϕ=25and t=40 min). The completely dark red area represents the region in which substrate or product at its maximum value (i.e. equilibrium value). x-axis represent dimensionless radius and y-axis represent z-axis.



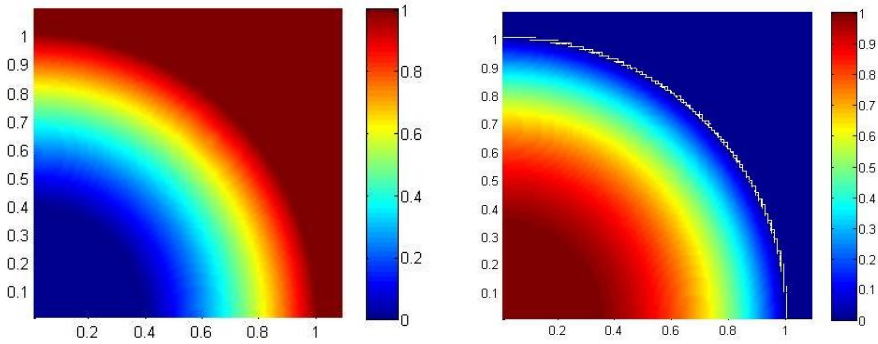
(a) Dimensionless substrate concentration (b) Dimensionless product concentration

Figure (13): Dimensionless substrate and product concentration profiles in 3D (at ϕ=21and t=60 min). The completely dark red area represents the region in which substrate or product at its maximum value (i.e. equilibrium value). x-axis represent dimensionless radius and y-axis represent z-axis.



(a) Dimensionless substrate concentration (b) Dimensionless product concentration

Figure (14): Dimensionless substrate and product concentration profiles in 3D (at ϕ=16.8and t=90 min). The completely dark red area represents the region in which substrate or product at its maximum value (i.e. equilibrium value). x-axis represent dimensionless radius and y-axis represent z-axis.



(a) Dimensionless substrate concentration (b) Dimensionless product concentration

Figure (15): Dimensionless substrate and product concentration profiles in 3D (at ϕ=9.8and t=120 min). The completely dark red area represents the region in which substrate or product at its maximum value (i.e. equilibrium value). x-axis represent dimensionless radius and y-axis represent z-axis.

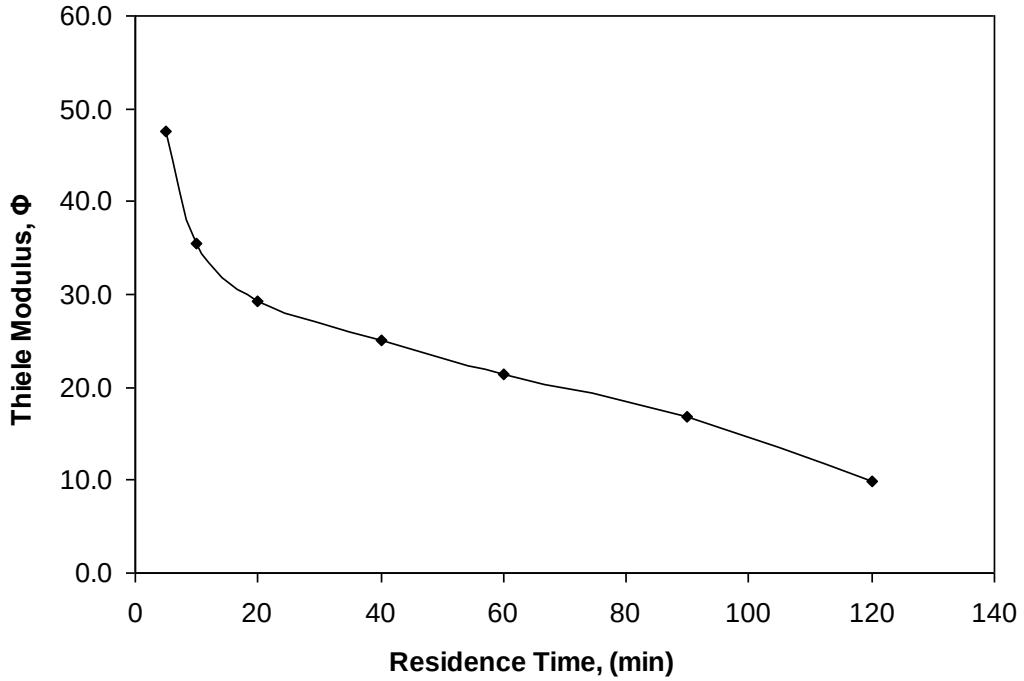


Figure (16): Effect of residence time on Thiele modulus (ϕ) value (pH= 5.5, T=50°C).

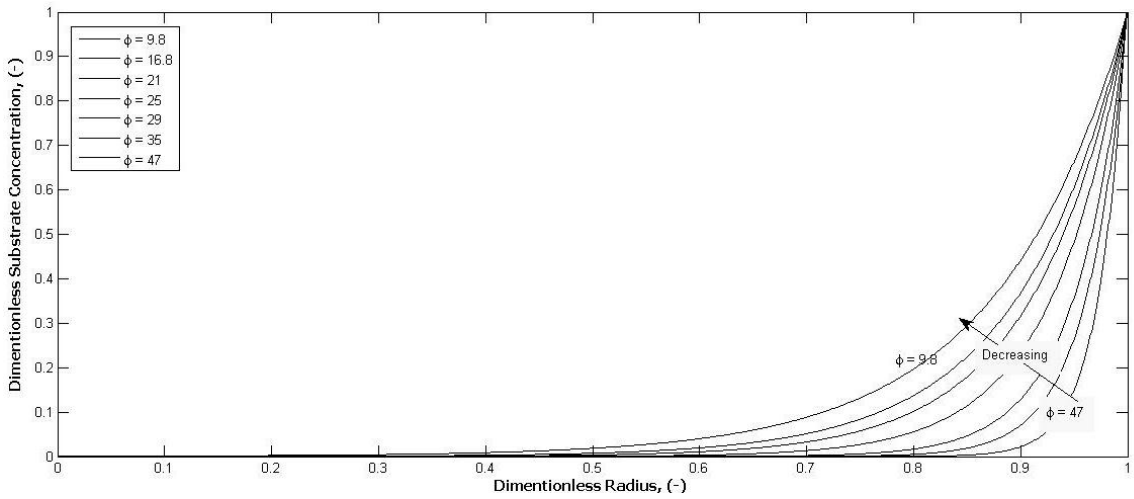


Figure (17): Dimensionless substrate concentration profile within the bead at study sate at different values of ϕ in 2D.

محاكاة تحفيز الانزيم في كريات من الجينات الكالسيوم

اميل محمد رحمن

مدرس

قسم الهندسة الكيميائية الاحيائية/ كلية الهندسة الخوارزمي/ جامعة بغداد

الخلاصة

طور في هذه الدراسة موديل رياضي لمحاكاة عملية التفاعل الكيمياوي والانتشار داخل العامل المساعد الحيوي المحمل في مفاعل ذو الحشوة الثابتة. تم استعمال تفاعل التحلل المائي للنشأ باستعمال انزيم الفا- اميليز لايجاد الموديل الرياضي والمحاكاة لهذه الدراسة. اجري تفاعل التحلل المائي للنشاء في دالة الرقم الهيدروجيني 5.5 وبدرجة حرارة 50°م. تراوح معدل جريان المادة المتفاعلة من 0.2 الى 5 مل/دقيقة، وتركيز المادة المتفاعلة الابتدائي من 1 الى 100 غم/لتر. تم تحميل انزيم الفا-اميليز على كريات من الجينات الكالسيوم الجيلاتينية بمعدل قطر 2 ملم. في هذه الدراسة، تم اعتماد حركية Michaelis–Menten. ان تاثير معدل جريان المادة المتفاعلة (زمن استبقاء المادة المتفاعلة) والتركيز الابتدائي على انتشار المواد داخل جسيمة العامل المساعد، تم اخذهما بنظر الاعتبار، وقد وجد بان النظام يتأثر بهذه المتغيرات. ان الخطوة الحاكمة لهذا التفاعل هي سرعة التفاعل. ان معادلة الموديل الرياضي كانت معادلة غير خطية من المرتبة الثانية واجريت المحاكات لها بالاعتماد على بيانات التجارب المختبرية وبظروف الحالة المستقرة. تم اجراء المحاكاة باستعمال طريقة FINITE ELEMENTS وباستعمال برنامج MATLAB. اعطت نتاج المحاكاة نتائج مرضية لتراكيز المادة المتفاعلة والناتجة خلال جسيمة العامل المساعد الحيوي.