J Biochem Tech (2011) 3(3): 285-288
ISSN: 0974-2328

On the dynamics of immobilized enzyme kinetics in a microreactor: A study of AP-catalyzed reactions

Pratap R Patnaik*

Received: 01 November 2011 / Received in revised form: 08 November 2011, Accepted: 08 November 2011, Published online: 25 January 2012,

Abstract

The kinetics of immobilized enzyme-catalyzed reactions in microreactors differ from those in macro-scale reactors. Recognizing this, a recent study (Patnaik 2011) based on a new interpretation of the kinetics of AP-catalyzed reactions showed that dynamic behavior is feasible only certain loci relating key kinetic parameters. That work has been extended here, and the kinetic parameters have now been related to bulk phase concentrations, thereby providing a link with the reaction system *per se*. It has also been shown that under certain conditions the reaction may become self-quenching but either monotonically or as damped oscillations. These two studies thus establish the importance of understanding kinetic dynamics in microreactors and in selecting feasible operating conditions.

Key words: Microreactor, Enzyme catalysis, Immobilized alkaline phosphatse, Dynamic behavior.

Introduction

An article published recently in this journal (Patnaik 2011) analyzed constraints on some key parameters so as to enable the dynamic behavior of enzymatic reactors catalysed by immobilized alkaline phosphate (AP) to be feasible in a microreactor. Even within these constraints, it is important to understand the nature of the dynamics, as explained later; this is therefore the subject of the present communication.

Enzymatic reactions in microreactors are growing rapidly in both analyses and applications. The basic microcreactor system itself has many benefits over conventional large-scale (or macro-scale) reactors. These benefits include lower production costs, higher efficiency, greater reproducibility, and more effective temperature control. The last advantage may be attributed to the large aspect ratio of the capillary tubes, thereby providing large surface areas and efficient heat exchange with the environment (Haeberle and Zengerle 2007; Hessel and Lowe 2010). Thus, microreactors have been employed favourably for highly exothermic rectories such as Fischer-Tropsch synthesis (Guettel et al. 2008) and steam reforming of methane or methanol (Arzamendi et al. 2009).

Exothermic reactions are, however, not the only significant class of applications for microreactors. Biological applications are currently a rapidly growing area of microreactor applications. The choice of microreactors here is driven by other features such as the absence of turbulent mixing, the implementation of sophisticated controls and the lower volumes of production than is chemical and petrochemical processes (Beebe et al. 2002). Many of these reactions are catalyzed by cells or enzymes, with major applications for protein and peptide mapping (Palm and Novotny 2004), combinatorial synthesis (Watts and Haswell 2004), DNA analyses (Paegel et al. 2003) and immunoassays (Kim and Park 2005).

Both enzyme-catalyzed and chemically catalyzed processes in microreactors have been accelerated by recent advances in microelectronic manufacturing systems and in the integration of microreactors with other micro-devices and or with macro-scale analytic units such as immunnoanalysers and mass spectrometers (Erickson and Li 2004; Ziaie et al. 2004). While expanding considerably the scope of microreactor applications, they also make it difficult to analyse and optimize the complex interactions among the integrated components. Therefore it is important to understand how individual micro-components perform before multi-unit architectures can be analyzed and designed.

Microreactors for Immobilized Enzymes

In general, all the advantages of immobilized enzymes in conventional reactors also apply to capillaries and networks of microchannels (Miyazaki et al. 2008). In addition, owing to the large surface to volume ratios, interfacial interactions play a significant role (Matosevic et al. 2011). Because of this difference, the predominantly laminar flow and much stronger diffusion control, the observed kinetics in micr fluidic reactors is different from that in large reactors. The differences are more critical in the

Pratap R. Patnaik

Department of Chemical Engineering, C. V. Raman College of Engineering, Bidyanagar, Mahura, Janla, Bhubaneswar-752054, Odisha

Tel: 09040930337; Fax: 0674-2113593
E-mail: pratap.r.patnaik@gmail.com

unsteady state than in the steady state. Nevertheless, most analyses of immobilized enzyme microreactors has focused on the steady state behavior (Matosevic et al. 2011; Miyazaki et al. 2008). The importance of analyzing the dynamics is also underlined by the fact that the large surface to reduce ratios, the small residence times and the significant interactions make microreactors more sensitive to disturbances than the more robust macroreactors.

These inferences are supported by a number of studies of enzymatic reactions catalysed by immobilized AP. Mao et al. (2002) studied the kinetics of AP immobilized in a microchannel under no flow conditions. While the Michaelis-Menten constant, K_m, was close to its solution-phase value, the turn-over rate, $k_{cat,}$ was six times smaller. Seong et al. (2003) reported similar observations for horseradish peroxidase and β-galactopyranoside, both under continuous flow conditions. To obtain "true" K_m values, they extrapolated their data to the asymptotic situation of zero flow. The agreement of Seong et al.'s parameters with those of Mao et al. (2002) is puzzling because fluid flow should reduce mass transfer resistance and thus increase the observed value of K_m. Seong et al.'s (2003) observations also contradict those of Lilly et al. (1996); for the hydrolysis of benzoylarginine ethyl ester, Lilly and coworkers observed a decrease in K_m with increasing flow rate of the substrate. However, one difference between the methods employed by these two groups is that while Seong et al. (2003) extrapolated their data to zero flow, Lilly et al. (1996) went the other way and extrapolated to high flow rates, arguing that theis minimized mass transfer resistance.

The argument in favor of high flow rates was also exploited by Gleason and Carbeck (2004); surprisingly, their observations matched the no-flow results of Mao et al. (2002), i.e. K_m values were close to those in solution phase and k_{cat} values were much smaller. Koh and Pishko (2005) also studied immobilized AP-catalyzed kinetics in a microreactor and reported K_m values much lower than in the solution phase; this may be attributed to the presence of diffusion resistance, which Koh and Pischko did not eliminate.

These results indicate that there is still considerable disagreement on the kinetics of AP catalysed reactions in immobilized microreactors. Conventional arguments suggest that the differences between solution phase kinetics and that for the immobilized enzyme may be due to the presence of mass transfer resistance in the latter case. However, a recent study by Kerby et al. (2006) has argued against this explanation. Unlike previous studies, these authors analysed the problem at the fundamental level of the occupation of active sites on the biocatalyst by the substrate and the products. They hypothesized that the dynamics of site occupation, and not the presence of diffusion or matter transfer effects, is the main reason for the differences between immobilized-phase kinetics and solution phase kinetics. Kerby et al.'s (2006) model is presented below, and it was used recently by this author (Patnaik 2011) to show that the dynamic behavior of the immobilized enzyme-driven reactor is feasible only in certain ranges of some key kinetic parameters. Steady state analyses do not reveal this, thereby illustrating the relevance of analyzing the dynamic behavior.

Kinetics of AP-catalyzed Reactions

This work, and it proceeding study (Patnaik 2011) are based on the kietics proposed by Kerby et al. (2006) for reactions catalyzed by AP. Like other research workers (Gleason and Carbeck 2004; Koh and Pishko 2005; Mao et al. 2002; Seong et al. 2003), Kerby et al. began with the assumption that the kinetics follow the Michaelis-Menten mechanism.

$$A + E \underset{\alpha_1}{\overset{\beta_1}{\leftrightarrow}} AE \xrightarrow{\beta_2} E + B + P \quad (1)$$

Here A is the substrate, E is the enzyme AP, and B and P are products.

A mole balance across a differential section of a microreactor packed with immobilized AP yields the following steady state equation.

$$u\frac{dc_A^b}{dz} = -\frac{v_{max}c_A^b}{K_m + c_A^b} \quad (2)$$

Upon integration over the length L of the reactor, we obtain from Eqn.(2):

$$K_m \ln\frac{c_{AL}^b}{c_{A0}^b} + c_{AL}^b - c_{A0}^b = -\frac{v_{max}V_R}{Q} \quad (3)$$

To test the validity of Eqn. (3), Kerby et al. applied it to the dephosphrylation of 6,8–difluoro–4-methylumbelliferyl phosphate (DiFMUp) by AP to 6,8-difuloro–4- methylumbelliferone (DiFMU). Therefore, in the present application A is DiFMUp, P is phosphate (PO_4^{-2}) and E is the enzyme AP. Kerby et al.'s (2006) study revealed that while Eqn. (3) was adequate at low conversions, it could not portray the reactor behavior at high conversions. The latter weakness is significant because an economically viable process requires high conversions of the substrate. This weakness of a classical Michaelis-Menten model arises from its ignorance of the occupancy of a significant proportion of active sites by phosphate molecules. To overcome this limitation, Kerby et al. (2006) proposed an alternate model derived from a mechanism proposed earlier by Labow et al. (Labow et al. 1993). This mechanism expands Eqn. (1) by introducing enzyme-bound PO_4^{-2} as another intermediate.

$$A + E \underset{\alpha_1}{\overset{\beta_1}{\leftrightarrow}} AE \xrightarrow{\beta_2} EP + B \quad (4a)$$

$$EP \underset{\alpha_3}{\overset{\beta_3}{\leftrightarrow}} E + P \quad (4b)$$

By analogy with the le Chatelier principle for reactions in solution phase, the rates of adsorption and desorption of substrate, A, and phosphate, P, were considered to be proportional to the number of unoccupied and occupied enzyme sites respectively. On this basis Kerby et al. derived the equations presented below for the time-evolution of the concentrations of active sites.

$$\frac{dG_A}{dt} = \beta_1(G_\infty - G_A - G_P)c_A^b - (\alpha_1 + \beta_2)G_A \quad (5)$$

$$\frac{dG_P}{dt} = \beta_2 G_A + \alpha_3(G_\infty - G_A - G_P)c_P^b - \beta_3 G_P) \quad (6)$$

To compare active site dynamics for A and P, it is useful to nondimensnonilize Eqns. (5) and (6) by dividing through by G_∞. The nondimensional model thus becomes:

$$\frac{dg_A}{dt} = \beta_1^*(1 - g_A - g_P) - (\alpha_1 + \beta_2)g_A \quad (7)$$

$$\frac{dg_P}{dt} = \beta_2 g_A + \alpha_3^*(1 - g_A - g_P) - \beta_3 g_P \quad (8)$$

where $\beta_1^* = \beta_1 c_A^b$ and $\alpha_3^* = \alpha_3 c_P^b$.

Results and Discussion

In a previous study (Patnaik 2011), Eqns (7) and (8) were simplified by using the observation that an economically profitable process requires high conventions; whereas Kerby et al.'s (2006) model is applicable under these conditions, other models (Gleason and Carbeck 2004; Koh and Pishko 2005; Mao et al. 2002; Seong et al. 2003), have limitations. At a high conversion, a significant proportion of phosphate ions are bound to active sites on the immobilized enzyme, thereby reducing its concentration in the bulk liquid phase to low values (relative to that of the substrate). This inference led to the assumption that $c_P^b \ll 1$. It was also assumed that a favorable equilibrium required $\alpha_3 << \beta_3$. Here we relax both assumptions and analyze the full model expressed by Eqns. (7) and (8). In matrix form this may be written as:

$$\begin{bmatrix} \frac{dg_A}{dt} \\ \frac{dg_P}{dt} \end{bmatrix} = \begin{bmatrix} -(\alpha_1 + \beta_2 + \beta_1^*) & -\beta_1^* \\ (\beta_2 - \alpha_3^*) & -(\alpha_3^* + \beta_3) \end{bmatrix} \begin{bmatrix} g_A \\ g_P \end{bmatrix} + \begin{bmatrix} \beta_1^* \\ \alpha_3^* \end{bmatrix} \quad (9)$$

For ease of analysis, Eqn. (9) may be written compactly as:

$$\frac{dx}{dt} = Ax + b \quad (10)$$

where $\mathbf{x} = [g_A \quad g_P]^T$, $\mathbf{b} = [\beta_1^* \quad \alpha_3^*]^T$, and

$$\mathbf{A} = \begin{bmatrix} a_{11} & a_{12} \\ a_{21} & a_{22} \end{bmatrix} = \begin{bmatrix} -(\alpha_1 + \beta_2 + \beta_1^*) & -\beta_1^* \\ (\beta_2 - \alpha_3^*) & -(\alpha_3^* + \beta_3) \end{bmatrix}$$

Equation (10) has the solution

$$x_j = p_j \exp(\lambda_j t) + q_j; j = 1,2 \quad (11)$$

where p_j and q_j are integration constants and the λ_j are the eigenvalues of **A**.

Mathematically, Eqn. (11) implies that x_1 and x_2 may increase monotonically or decrease monotonically or oscillate, depending on whether the corresponding x_j is real and positive or real and negative or imaginary. Our interest here is on the physical interpretation of some important cases of Eqn. (10). We begin with the general solution

$$\lambda_{1,2} = \frac{(a_{11}+a_{22}) \pm \sqrt{(a_{11}-a_{22})^2 + 4a_1a_{21}}}{2} \quad (12)$$

Case (1). $\beta_2 = \alpha_3^* = \alpha_3 c_P^b$
In this case $a_{21} = 0$, so Eqn. (12) simplifies to

$$\lambda_1, \lambda_2 = -(\alpha_1 + \beta_2 + \beta_1^*), -(\alpha_3^* + \beta_3)$$

Since both eigenvalues are negative, x_1 (=g_A) and x_2 ((=g_P) decrease as time progresses and eventually the reaction becomes too slow to the sustainable. Since β_2 and α_3 are kinetic parameters, the equality specified in case (1) effectively implies that once the bulk phase concentration of P, i.e. the phosphate ions PO_4^{-2}, equals β_2 / α_3, the dephosphorylation process is no longer feasible. It may be recalled here that an earlier analysis (Patnaik 2011) had shown that dynamic feasibility is possible only on specific loci relating β_1^*, β_2 and β_3. The present condition prescribes the important caveat that even on these loci the choice of β_2 is restricted by $\beta_2 \neq \alpha_3 c_P^b$ or, more practically, $c_P^b \neq \beta_2/\alpha_3$. This restriction is plausible because the bulk phase concentrations of A and P cannot be independent of the kinetic parameters.

Case (2). $\beta_2 < \alpha_3 \Rightarrow a_{12}a_{21} > 0$

This conditions guarantees that $x_1 > 0$; however, x_2 may be positive or negative, depending on the values of a_{11}, a_{22}, a_{12} and a_{21}. In other words, while the concentrations of bound A, i.e. DiFMUp, increase monotonically, that of bound phosphate may either increase or reduce with time. The latter situation is obviously not favorable for a high conversion, and hence the operating conditions should be such that a substantial fraction of PO_4^{-2} ions remain immobilized at all times. This requirement thus limits the choices for a_{11}, a_{22}, a_{12} and a_{21}, i.e. $\alpha_1, \beta_1^*, \beta_2, \beta_3$ and α_3^*, to regions that generate positive values of λ_2.

Case (3). $\beta_2 > \alpha_3^* \Rightarrow a_{12}a_{21} < 0$

This case presents a number of interesting possibilities, depending on the sign of the discriminate $\Delta = (a_{11} - a_{22})^2 + 4a_{12}a_{21}$. By considering both positive and negative signs for Δ and accounting for the facts that $a_{11} + a_{22} = -(\alpha_1 + \beta_2 + \beta_1^*) - (\alpha_3^* + \beta_3) < 0$ and $(a_{11} - a_{22})^2 > 0$, the range of possibilities is depicted in the table below.

Sign of Δ	Nature of eigenvalues λ_1 and λ_2					
	$\Delta > \lvert a_{11}+a_{22}\rvert^2$		$\Delta < \lvert a_{11}+a_{22}\rvert^2$		$\Delta = \lvert a_{11}+a_{22}\rvert^2$	
	λ_1	λ_2	λ_1	λ_2	λ_1	λ_2
+	+	-	-	-	0	0
-	-p+iq	-p-iq	-p+iq	-p-iq	-p+iq	-p-iq

In this Table, p and q are positive constants and $i = \sqrt{-1}$. We note that if $\Delta > 0$ then $\lambda_2 \leq 0$; this implies, as in case (2) above, that the concentration of immobilized PO_4^{-2} diminishes as the reaction progresses, and eventually all active site are occupied by the substrate DiFMUp and the reaction stops. Thus $\Delta > 0$ is not a feasible condition. A negative Δ, on the contrary, leads to a pair of complex conjugate roots with negative real parts. Therefore here too the enzyme-bound substrate and phosphate decrease with time but in an oscillating manner, i.e. the immobilized concentrations of both exhibit damped oscillations. The overall inference from this discussion is that case (3) does not promote a long term reaction and is therefore not practically useful.

A previous analysis of the AP–catalysed dephosphorylation of DiFMUp (Patnaik 2011) derived loci that demarcate regions where dynamic kinetic behavior in feasible. The present work has gone further and derived additional conditions on dynamic feasibility and shown how, even under feasible conditions, the variation of the concentration of active sites occupied by the primary substrate and the product may differ according to the relationship between kinetic parameters.

Conclusions

The differences between the kinetics of free and immobilized enzymatic reactions in microreactors is exemplified here by the AP–catalysed system. Recent work has shown that the differences are due to the lack of recognition by kinetic models of finite occupancy of active sites by phosphate molecules and not due to mass transfer effects. This weakness was overcome by an improved kinetic model proposed by Kerby et al. (2006). With their model, a previous analysis had derived feasible loci for kinetic dynamics in a 3-parameter space. The present study has extended that work and derived additional conditions on feasibility, which now provide a correspondence between the kinetic parameters and the reaction system by relating the former to bulk phase concentrations. Under certain conditions the rate of the dephosphorylation reaction studied

here may gravitate slowly to unsustainable levels; interestingly, this slowing down may be either monotonic or oscillatory, a feature not revealed by previous studies.

Nomenclature

c_A^b	concentration of A in the bulk phase
c_{A0}^b	initial value of c_A^b
G_∞	total concentration of active sites on the enzyme
G_A	concentration of active sites occupied by A
G_P	concentration of active sites occupied by P
g_A	dimensionless G_A ($=G_A/G_\infty$)
g_P	dimensionless G_P ($=G_P/G_\infty$)
K_m	Michaelis-Menten equilibrium constant
L	total length of the microreactor
Q	flow rate through the microreactor
t	time
u	fluid velocity through the microreactor
v_{max}	maximum rate of reaction
V_R	volume of the microreactor
z	distance along the microreactor
$\alpha_1, \alpha_3, \beta_1, \beta_2, \beta_3$	kinetic parameters

References

Arzamendi G, Dieguez M, Montes M, Centeno A, Odriozola JA, Gandia LM (2009) Integration of methanol steam reforming and combustion in a microchannel reactor for H_2 production: A CFD simulation study. Catal Today 143: 25-31.

Beebe DJ, Mensing GA, Walker GM (2002) Physics and applications of microfluidics in biology. Ann Rev Biomed Eng 4: 261

Erickson D, Li D (2004) Integrated microfluidic devices. Analytica Chim Acta 507:11

Gleason NJ, Carbeck JD (2004) Measurement of enzyme kinetics using microscale steady state kinetic analysis. Langmuir 20: 6374-6381.

Haeberle S, Zengerle R (2007) Microfluidic platforms for lab-on-a chip applications. Lab Chip 7: 1094-1110.

Hessel V, Knobloch C, Lowe H (2008) Review on patents in microreactor and micropocess engineering. Recent Patents Chem Eng 1: 1-16.

Hessel V, Lowe H (2010) Microreactor technology: Applications in pharma/chemical processing. Innov Pharma Technol 2: 88-92.

Guettel R, Kunz U, Turek T (2008) Fisher-Tropsch synthesis in monolithic honeycomb reactors. Chem Eng Technol 31: 746-754.

Kerby MB, Legge RS, Tripathi A (2006) Measurements of kinetic parameters in a microfluidic reactor. Analyt Chem 78: 8273-8280.

Kim KS, Park J-K (2005) Magnetic force-based multiplexed immunoassay using superparamagnetic nanoparticles in microfluidic channel. Lab Chip 5: 657-664.

Koh WG, Pishko M (2005) Immobilization of multi-enzyme microreactors inside microfluidic devices. Sensors Actuators B 106 : 335-342.

Labow BI, Herschlag D, Jencks WP (1993) Catalysis of the hydrolysis of phosphorylated pyridines by alkaline phosphatase. Biochemistry 32: 8737-8741.

Lilly MD, Hornby WE, Crook EM (1968) Some changes in the reactivity of enzymes resulting from their chemical attachment to water-insoluble derivatives of cellulose. Biochem J 102: 669-674.

Lin W-Y, Wang Y, Wang S, Tseng H-R (2009) Integrated microfluidic reactors. Nano Today 4: 470-481.

Mao H, Yang T, Cremer PS (2002) Design and characterization of immobilized enzymes in microfluidic systems. Analyt Chem 74: 379-385.

Matosevic S, Szita N, Baganz F (2010) Fundamentals and applications of immobilized microfluidic enzymatic reactors. J Chem Technol Biotechnol 86: 325-334.

McMullen JP, Jensen KF (2010) Integrated microreactors for reaction automation: new approaches to reaction development. Ann Revs Analyt Chem 3: 19-42.

Microfluidics Corporation (2010) http://www.microfluidicscorp.com.

Mindbranch, Microfluidic Technologies (2010) http://www.mindbranch.com/Microfluidics-Technologies-R2-768.

Miyazaki M, Honda T, Yamaguchi H, Briones MPP, Maeda H (2008) Enzymatic processing in microfluidic reactors. Biotechnol Genet Eng Revs 25: 405-428.

Ohno K, Tachikawa K, Manz A (2008) Microfluidics: Applications for analytical purposes in chemistry and biochemistry. Electrophoresis 29: 4443-4453.

Paegel BM, Blazej RG, Mathies RA (2003) Microfluidic devices for DNA sequencing: Sample preparation and electrophoretic analysis. Curr Opin Biotechnol 14: 42-50.

Palm AK, Novotny MV (2004) Analytical characterization of a facile porous polymer moinolithic trypsin microreactor enabling peptide mass mapping using mass spectrometry. Rapid Commun Mass Spectrom 18: 1374-1382.

Patnaik PR (2011) Parametric constraints on the dynamic behavior of immobilized enzyme kinetics in a microreactor. J Biochem Technol (In press).

Seong GH, Heo J, Crooks RM (2003) Measurement of enzyme kinetics using a continuous-flow microfluidic system. Analyt Chem 75: 3161-3167.

Zatts P, Haswell SJ (2004) Combinatorial synthesis in microreactors. Comb Chem High Throughput Screening 7: 397

Ziaie B, Baldi A, Lei M, Guy Y, Siegel RA (2004) Hard and soft micromachining for BioMEMS: review of techniques and examples of applications in microfluidics and drug delivery. Adv Drug Dely Rev 56:145