Control and Cybernetics

vol. **42** (2013) No. 2

Mathematical modeling to optimize the product in enzyme kinetics*

by

Sumit Nandi†, Mithun Kumar Ghosh††, Rupa Bhattacharya† and Priti Kumar Roy††0

† Narula Institute of Technology, Kolkata 700109, India
††Centre for Mathematical Biology and Ecology,
Department of Mathematics, Jadavpur University,
Kolkata 700032, India

Abstract: Optimization of product in enzyme kinetics is successful by the showers of mathematical analysis with control measures. Enzymes are an important functional aspects of all biochemical processes, as they catalyze numerous reaction taking place within living organisms. With this view, optimization and quantification of product is stressed upon and in such a context, optimal control approaches have been applied in our study. In this article, we have formulated a mathematical model of enzymatic system dynamics with control measures with a view to optimize the product as well as process conditions. Here, Pontryagin Minimum Principle is used for determination of optimal control with the help of Hamiltonian. We discuss the relevant numerical solutions for the concentration of substrate, enzyme, complex and product with respect to a specified time interval by varying control factors.

Keywords: enzyme kinetics, optimization, optimal control approach, reversible reaction

1. Introduction

Enzyme kinetics in the light of mathematical modeling has played a significant role for optimization of product in dynamical reaction systems. For smooth completion of reaction, all biochemical and most chemical reactions require catalyst or biocatalyst like enzymes. By acting as a catalyst, an enzyme dramatically increases the rate of reaction by lowering the activation energy of the reaction without forming any side products. In the system dynamics of enzymatic reaction, enzyme binds target molecules or substrates through the active sites, the most vibrant part of an enzyme. After binding with the substrate, it forms

*Submitted: September 2012; Accepted: April 2013
0Corresponding author: pritiju@gmail.com

enzyme-substrate complex, finally transformed into products through a series of steps by enzymatic mechanism.

The advantages of enzymatic reactions correlate with the fact that these reactions occur in mild conditions and desirable product is obtained due to specificity of enzymes. Extensive research has been made in the kinetics of enzymatic processes and it has a significant role in optimizing the rate of reactions, rate of formation of intermediate complex as well as products. Enzyme kinetics with a mathematical bent has been discussed in various books (Rubinow, 1975; Murray, 1989; Segel, 1980; Roberts, 1977). Later, enzyme kinetics based on mathematical foundation were studied in various disciplines led by the pioneer work of Sharpe and Lotka (Sharpe and Lotka, 1923) in Epidemiology and Ecology. Recently, different analytical techniques have been adopted for better understanding of enzyme kinetic models (Alicea, 2010; Varadharajan and Rajendran, 2011; Tzafriri and Edelman, 2004). So, mathematical analysis has an important role in enzymatic reaction environment and helps us to realize the evaluation of control parameters, optimum control of reaction conditions and product optimization in relation to kinetically controlled enzymatic systems. In other words, mathematical models, especially when coupled with modern computer technique, prove to be effective in searching to optimize and quantify productivity (Vasic-Racki et al., 2003).

One of the most important aspects of enzyme kinetics is the formation of enzyme-substrate complex of different nature. In 1902, Brown (Brown, 1902) proposed the existence of an enzyme-substrate complex in a purely kinetic context with a fixed lifetime to form the product. This was the first time that the existence of the complex was proposed in an enzymatic dynamics. Later, formation of complex by the interaction of substrate and enzyme was found a reversible process. A complex may either form products or revert back again to substrate. Control measure in this aspect contributes significantly. In this perspective, optimal control approach has been applied in the backward reaction in this study for product optimization. Another control input has been introduced in the stage of conversion of complex to product for the complete quantification of enzymatic reaction product.

In our consideration of the enzyme kinetics, a mathematical approach aims at product optimization by introducing control measures. For continuously operated reaction process, optimization of productivity by mathematical analysis is still an emerging field of research. As the nature, stability and conversion rate of complex are the most fundamental aspects for optimization and quantification of product, so double control approaches have been applied in case of backward reversible stage and final forward reaction. Here the model equation is analyzed in two different avenues, analytical and numerical. For the determination of optimal control, Pontryagin Minimum Principle has been adopted and it has been solved using Hamiltonian. Numerical analysis was done to find out the system

parameters for which the product can be optimized. Our model parameters reflect more specifically an enzyme's true kinetic properties and affinity for a substrate and thus provide a better characterization of the enzymatic dynamics. Numerical findings are in agreement with the results of theoretical analysis.

2. Control theoretic approach

One of the most basic enzymatic reactions, proposed by Leonor Michaelis and Maud Menten (1913), is schematically given by

$$S + E \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} [SE] \xrightarrow{k_2} P + E.$$

That is, one molecule of the enzyme (E) combines with one molecule of the substrate (S) to form one molecule of the complex (SE). The complex (SE) can dissociate into one molecule of each of the enzyme and substrate, or it can produce a product and a recycled enzyme. k_1 is the rate of formation of enzyme-substrate complex, k_{-1} is the rate of dissociation of the complex and k_2 is the rate of creation of the product.

Denoting the concentrations [S], [E], [SE] and [P] by s, e, c and p respectively, the law of mass action applied to this system leads to the following four non-linear differential equations:

$$\begin{array}{l} \frac{ds}{dt} = -k_1 es + k_{-1} c \\ \frac{de}{dt} = -k_1 es + k_{-1} c + k_2 c \\ \frac{dc}{dt} = k_1 es - k_{-1} c - k_2 c \\ \frac{dp}{dt} = k_2 c \end{array} \tag{2}$$

with initial condition $s(0) = s_0$, $e(0) = e_0$, $c(0) = 0$ and $p(0) = 0$.
Here k_1, k_2, k_{-1}, s_0 and e_0 are positive constants.
Now we are introducing two control inputs $u_1(t)$ and $u_2(t)$. $u_1(t)$ is introduced to reduce the rate of backward reaction and $u_2(t)$ is introduced to maximize product formation. That is, here, we are using both of the control variables to get the maximum amount of product as fast as possible. The schematic diagram is given by

$$S + E \underset{k_{-1}, u_1}{\overset{k_1}{\rightleftharpoons}} [SE] \overset{k_2, u_2}{\longrightarrow} P + E.$$

The cost function is thus formulated as

$$J(u_1, u_2) = \int_{t_i}^{t_f} [A u_1^2(t) + B u_2^2(t) - N p^2(t)] dt \tag{3}$$

subject to the state system

$$\begin{array}{l} \frac{ds}{dt} = -k_1 es + k_{-1}(1 - u_1(t)) c \\ \frac{de}{dt} = -k_1 es + k_{-1}(1 - u_1(t)) c + k_2 u_2(t) c \\ \frac{dc}{dt} = k_1 es - k_{-1}(1 - u_1(t)) c - k_2 u_2(t) c \\ \frac{dp}{dt} = k_2 u_2(t) c. \end{array} \tag{4}$$

The parameters A and B represent the weight constant related to the cost of production and N is the penalty multiplier. Our aim is to find the optimal control pair $u^*=(u_1^*, u_2^*)$ such that

$$J(u_1^*, u_2^*) = min\ (J(u_1, u_2) : (u_1, u_2) \in U),$$

where $U = U_1 \times U_2$,
$U_1 = (u_1(t) :\ u_1$ is measurable and $0 \leq u_1 \leq 1,\ t \in [t_i, t_f])$
and $U_2 = (u_2(t) :\ u_2$ is measurable and $0 \leq u_2 \leq 1,\ t \in [t_i, t_f])$.

Here we use Pontryagin Minimum Principle (Pontryagin et al., 1986; Bonnans and Hermant, 2009) to find $u_1^*(t)$ and $u_2^*(t)$. The Hamiltonian is given by

$$\begin{aligned} H = {} & Au_1^2(t) + Bu_2^2(t) - Np^2(t) \\ & + \xi_1(-k_1es + k_{-1}(1 - u_1(t))c) \\ & + \xi_2(-k_1es + k_{-1}(1 - u_1(t))c + k_2u_2(t)c) \\ & + \xi_3(k_1es - k_{-1}(1 - u_1(t))c - k_2u_2(t)c) \\ & + \xi_4k_2u_2(t)c. \end{aligned} \tag{5}$$

By using the Pontryagin Minimum Principle and the existence condition of the optimal control theory (Fleming and Rishel, 1975; Fister et al., 1998; Kirschner et al., 1997; Bonnard and Sugny, 2009), we obtain the theorem stated below:

Theorem 1. *The cost function $J(u_1, u_2)$ over U is minimum for the optimal control $u^*=(u_1^*, u_2^*)$ corresponding to the interior equilibrium (s^*, e^*, c^*, p^*). There exist also adjoint functions ξ_1, ξ_2, ξ_3 and ξ_4 which satisfy equation* (4).

Proof. Using Pontryagin Minimum Principle (Fleming and Rishel, 1975), the unconstrained optimal control variables u_1^* and u_2^* satisfy

$$\frac{\partial H}{\partial u_1^*} = \frac{\partial H}{\partial u_2^*} = 0. \tag{6}$$

Here,

$$\begin{aligned} H = {} & (Au_1^2 + \xi_1k_{-1}(1 - u_1(t))c + \xi_2k_{-1}(1 - u_1(t))c \\ & - \xi_3k_{-1}(1 - u_1(t))c) \\ & + (Bu_2^2 + \xi_2k_2u_2(t)c - \xi_3k_2u_2(t)c + \xi_4k_2u_2(t)c) \\ & + \text{terms without } u_1(t) \text{ and } u_2(t). \end{aligned} \tag{7}$$

Thus, from (6) and (7), we have

$$\begin{aligned} \frac{\partial H}{\partial u_1^*} &= 2Au_1^* - k_{-1}c(\xi_1 + \xi_2 - \xi_3) = 0 \\ \frac{\partial H}{\partial u_2^*} &= 2Bu_2^* + k_2c(\xi_2 - \xi_3 + \xi_4) = 0. \end{aligned}$$

By solving, we get

$$\begin{aligned} u_1^*(t) &= \frac{k_{-1}c(\xi_1+\xi_2-\xi_3)}{2A} \\ u_2^*(t) &= \frac{k_2c(\xi_3-\xi_2-\xi_4)}{2B}. \end{aligned} \tag{8}$$

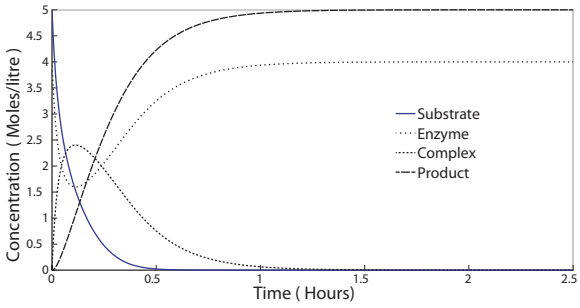

Figure 1. Normalized concentration profiles of substrate, enzyme, complex and product as a function of time for various values of reaction parameters

Due to the boundedness of the standard control,

$$u_1^*(t) = \begin{cases} 0, & \frac{k_{-1}c(\xi_1+\xi_2-\xi_3)}{2A} \leq 0; \\ \frac{k_{-1}c(\xi_1+\xi_2-\xi_3)}{2A}, & 0 < \frac{k_{-1}c(\xi_1+\xi_2-\xi_3)}{2A} < 1; \\ 1, & \frac{k_{-1}c(\xi_1+\xi_2-\xi_3)}{2A} \geq 1. \end{cases} \tag{9}$$

Hence the compact form of $u_1^*(t)$ is

$$u_1^*(t) = max(0,\ min(1,\ \tfrac{k_{-1}c(\xi_1+\xi_2-\xi_3)}{2A})). \tag{10}$$

In a similar way we can have the compact form of $u_2^*(t)$ as

$$u_2^*(t) = max(0,\ min(1,\ \tfrac{k_2c(\xi_3-\xi_2-\xi_4)}{2B})). \tag{11}$$

According to Pontryagin Minimum Principle (Pontryagin et al., 1986)

$$\tfrac{d\xi}{dt} = -\tfrac{\partial H}{\partial x}, \tag{12}$$

and

$$H(x(t), u^*(t), \xi(t), t) = \min_{u\in U}(H(x(t), u(t), \xi(t), t)). \tag{13}$$

The above equations are the necessary conditions for the optimal control input $u_1(t)$, $u_2(t)$ and the state system variables. The existence condition for the adjoint variable is given by

$$\begin{aligned} \tfrac{d\xi_1}{dt} &= -\tfrac{\partial H}{\partial s} = k_1e(\xi_1+\xi_2-\xi_3) \\ \tfrac{d\xi_2}{dt} &= -\tfrac{\partial H}{\partial e} = k_1s(\xi_1+\xi_2-\xi_3) \\ \tfrac{d\xi_3}{dt} &= -\tfrac{\partial H}{\partial c} = k_{-1}(1-u_1(t))(\xi_3-\xi_1-\xi_2) \\ &\quad + k_2u_2(t)(\xi_3-\xi_2-\xi_4) \\ \tfrac{d\xi_4}{dt} &= -\tfrac{\partial H}{\partial p} = 2Np. \end{aligned} \tag{14}$$

The optimality of the system involves the state system with the adjoint system together with the initial condition. The transversality condition implies $\xi_i(t_f)$=0 (i=1,2,3,4) and $s(0)$=s_0, $e(0)$=e_0, $c(0)$=0 and $p(0)$=0. ■

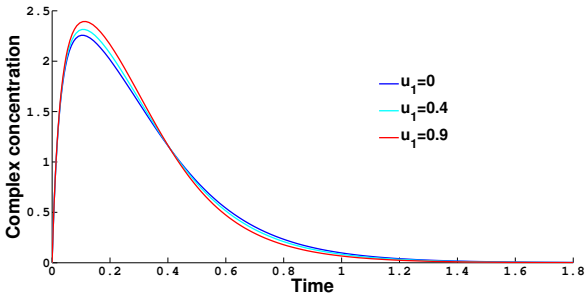

Figure 2. Normalized concentration profile of enzyme-substrate complex as a function of time for various values of control parameters $u_1 = 0$, $u_1 = 0.4$ and $u_1 = 0.9$

3. Numerical simulation of the model equations

The dynamics of reaction system kinetics are analyzed using numerical methods. The present study deals with the application of control measures in the enzyme kinetic theory with an objective to maximize the product yield by minimizing the backward reaction of the intermediate complex. In this section, we show the analytical expressions for the time dependent variation of the substrate s, enzyme e, substrate-enzyme complex c and product p as a function of time t for various values of reaction parameters to characterize the reaction process. Also, we investigate the effect of change of the dynamical reaction system with changes in the reaction parameters taking into consideration the optimal control approach.

Fig. 1 represents the kinetic profile diagram of the substrate s, enzyme e, complex c and product p considering the parameter values of $k_1 = 5$, $k_2 = 5$ and $k_{-1} = 0.1$ in the absence of any control measures. As expected, according to the enzyme kinetic behavior, the substrate concentration falls off with time and becomes zero (s=0) when $t \geq 1$ hour as it is consumed with the progress of the reaction. Consumption is rapid at the initial stages due to initial higher rate of collision between substrate and enzyme but gradually slows off with time possibly due to the backward reaction. The enzyme is the catalyst with concentration diminishing as the reaction proceeds but is recovered at the end of the reaction when the formation of the enzyme-substrate reaction is complete. The enzyme-substrate complex concentration c increases gradually from its initial point ($c(0) = 0$) and reaches maximum in the interval [0, 0.3]. This complex is then further transformed into the desired product but it also tends to revert back to the substrate and hence if a control measure is applied this backward process to arrest reversibility, yield of the product is expected to be at its maximum value. Furthermore, product yield is also expected to be optimum by implementing control parameters to the product forming process from the complex. From Fig. 1, it is inferred that the formation of product increases

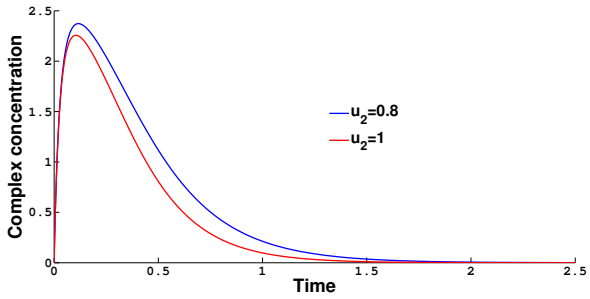

Figure 3. Normalized concentration profile of enzyme-substrate complex as a function of time for various values of control parameters $u_2 = 0.8$ and $u_2 = 1$

progressively from the initial stage of reaction ($p(0) = 0$) and finally attains a maximum value when $t = 1.5$ hours. The yield of the product rises with reaction time and finally becomes steady at its optimum value.

Table 1. Values of parameters used for models dynamics calculations.

Parameter	Definition	Recommended Value
k_1	Rate of forward reaction	5 $(mole/litre)^{-1}hour^{-1}$ (Alicea, 2010; Varadharajan, 2011)
k_2	Rate of product formation	5 $hour^{-1}$ (Alicea, 2010; Varadharajan, 2011)
k_{-1}	Rate of backward reaction	2 $hour^{-1}$ (Alicea, 2010)

Now, the effect of increasing the value of the control parameter u_1, the changes in concentration of the enzyme-substrate complex, are observed in Fig. 2. It is noticed here that the yield of the complex rises by a significant proportion as the control value is raised from $u_1 = 0$ to $u_1 = 0.4$ and finally 0.9. This is in accordance with the real system in that as the control for the backward reaction is raised, the tendency of the intermediate complex to revert back to the substrate and enzyme decreases and this contributes to accumulation of more of the complex, as noted. Due to this reason, the complex remains somewhat more time in the reaction dynamics and longer retention of complex means it directs the probability of maximum conversion of enzyme-substrate to enzyme-product intermediate.

Variation in the control parameter u_2 contributes to the conversion of the enzyme-substrate complex to the ultimate product. In Fig. 3, the variation in concentration of the complex with varying u_2 values is exhibited. It is found that for a lower value of u_2, i.e., at $u_2 = 0.8$, the complex concentration is

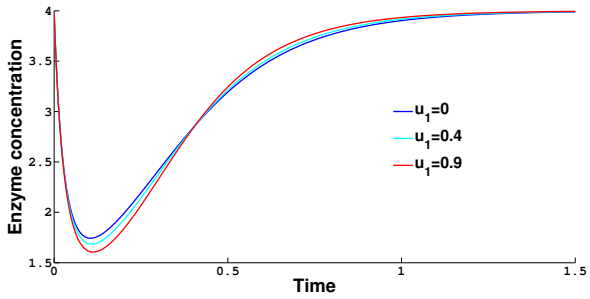

Figure 4. Normalized concentration profile of enzyme as a function of time for various values of control parameters $u_1 = 0$, $u_1 = 0.4$ and $u_1 = 0.9$

higher than that for $u_2 = 1$. This is in corroboration with the fact that for higher values of u_2, the accumulation of the complex is much lower as it is rapidly converted into the product. So, minimization of the reverse reaction by employing the control parameter u_1 and maximizing the complex breakdown process by using the control u_2, significantly focuses on product optimization and maximum yield of it. So, completion of reaction takes much less time by enhancing the rate of degradation of complex to product with specified control measures.

Fig. 4 displays the change in concentration of the free enzyme required for the enzymatic reaction with different values of u_1. When the value of u_1 changes from $u_1 = 0$ to $u_1 = 0.4$ and finally 0.9, the consumption of the enzyme increases. As a result, the concentration of the free enzyme decreases. This may be attributed to the fact that when the backward reaction is controlled by u_1, the formation of the enzyme-substrate complex is favored. So, the feasibility of the forward reaction increases due to which more substrate molecules collide with more of the enzyme to generate the intermediate complex molecules. Consequently, more enzyme molecules are consumed in the process so that a lower amount of enzyme is observed. However, the original amount of enzyme is recovered in all the cases irrespective of control application. The favorable formation of the complex is associated with the optimizing amount of the product and so it is apparent that the change in control parameter u_1 broadly influences the enzyme kinetic behavior regarding quantity of the product.

In Fig. 5, the concentration of the enzyme with control parameter u_2 is given. The curves show a rise in the concentration of the enzyme at higher values of $u_2 = 1$, which corresponds to a fast recovery of the enzyme when the complex breaks down to generate the product and releases the enzyme. The reaction is thus speeded up and it is possible to obtain greater amount of product within a specified time period ($t \cong 2$ hours).

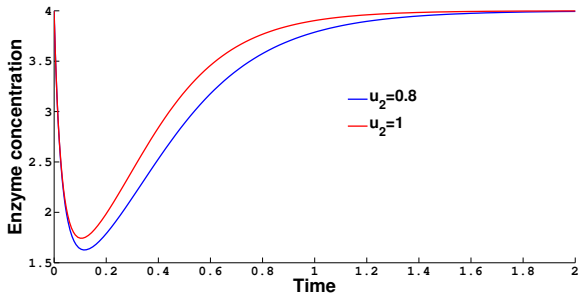

Figure 5. Normalized concentration profile of enzyme as a function of time for various values of control parameters $u_2 = 0.8$ and $u_2 = 1$

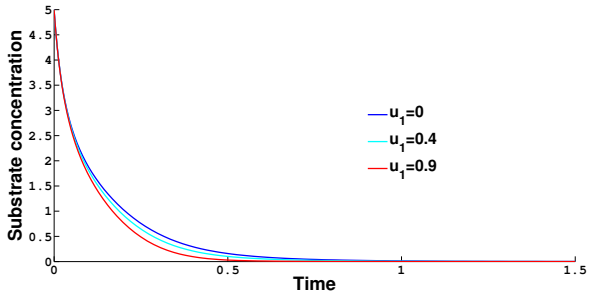

Figure 6. Normalized concentration profile of substrate as a function of time for various values of control parameters $u_1 = 0$, $u_1 = 0.4$ and $u_1 = 0.9$

Fig. 6 represents the change in substrate concentration with variation of control parameter u_1. It is indicated that with increasing control for the backward reaction, the substrate is readily consumed up and the equilibrium of the reaction tends to shift towards the formation of the enzyme-substrate complex. Here the control parameter u_2 is not considered as it is insignificant with respect to substrate.

At the end, we have come to the most important stage of the dynamics i.e., product formation. The former explanations regarding control are quite factual for the optimization and quantity of product too. As indicated in Fig. 7 and 8, both the rate of product formation and yield of the product are higher for. Higher value of u_1=0.9 induces the higher growth rate of enzyme-substrate complex from the initial stage of reaction ($t = 0$). So, one can get maximum concentration of enzyme-substrate complex within a very limited period of time by adopting this control approach. Again, accumulation of complex pushes the reaction towards forward direction which ultimately leads to the formation of the desired product. Now, if we apply control u_2=1, the synchronized effect re-

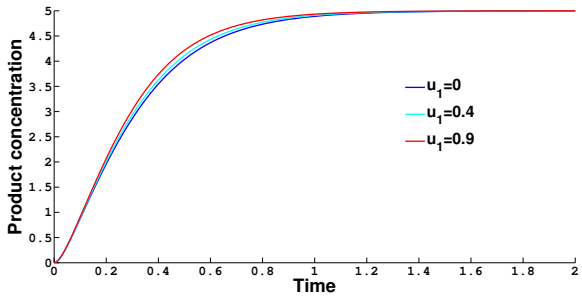

Figure 7. Normalized concentration profile of product as a function of time for various values of control parameters $u_1 = 0$, $u_1 = 0.4$ and $u_1 = 0.9$

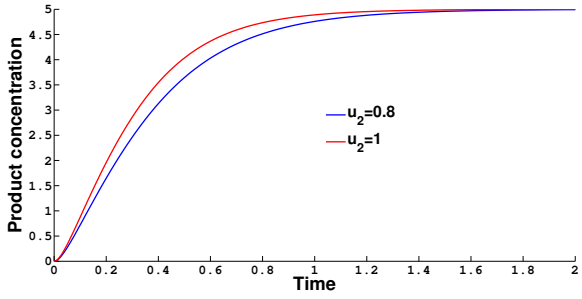

Figure 8. Normalized concentration profile of product as a function of time for various values of control parameters $u_2 = 0.8$ and $u_2 = 1$

sults in product optimization within a very limited period of time ($t \cong 1.5$ hours). An important facet is that here the kinetics undergoes irreversible shift from reversible dynamics which leads to the higher progression rate of reaction with time. So, optimal control strategy is successful for optimization and quantity increase of the product by influencing the enzyme reaction dynamics. Our approximate analytical expression of concentrations of substrate, complex, enzyme and product are compared with numerical results and satisfactory agreement is noted.

4. Discussion and conclusion

The dynamic profile of the basic enzyme kinetic reactions has been presented. A control theoretic approach has been adopted with a view to optimize product formation. Here, analytical solutions of non-linear reaction dynamics are presented using Pontryagin Minimum Principle to determine the optimal control and Hamiltonian method was used to solve it.

The new approach in this article is that the optimal control theory is applied to the backward process and to the final product formation from the complex. Optimization and quantitative increase of the product are observed with higher controls. Control involved in the backward process actually influences the reaction in the forward direction so that formation of the substrate-enzyme intermediate complex is favored. Moreover, control applied in the second stage of the reaction enables the rapid breakdown of the complex to yield the product and recover the enzyme. So, for higher controls, we get an earlier binding between the substrate and the enzyme, which ultimately gives the product within much less time. Higher control in the reaction dynamics signifies the conversion of a substantial amount of substrate to the product due to irreversibility when eventually the enzyme is recovered and the complex concentration is diminished.
In conclusion, the numerical analysis of enzyme kinetic reaction offers better predictability and understanding of control with respect to product optimization. Thus, the proposed control model of enzymatic reaction is more functional and provides an idea for faster product formation and its optimization. The parameters of the model correlate significantly with the physical factors affecting reaction dynamics. In this way, accurate prior prediction of system dynamics by analytical analysis and numerical simulation regarding product formation can be achieved for purposes of experimental studies.

References

ALICEA, R. M. (2010) A mathematical model for enzyme kinetics: multiple timescale analysis. *Asymptotics and Perturbations* **2A**, 1-9.

BONNANS, J. F. and HERMANT, A. (2009) Revisiting the analysis of optimal control problems with several state constraints. *Control and Cybernetics* **38**(4A).

BONNARD, B. and SUGNY, D. (2009) Geometric optimal control and two-level dissipative quantum systems. *Control and Cybernetics* **38**(4A).

BROWN, A. J. (1902) Enzyme action. *J. Chem. Soc. Trans.* **81**, 373–386.

FISTER, K. R., LENHART, S. and MCNALLY, J. S. (1998) Optimizing Chemotherapy in an HIV Model. *Electronic Journal of Differential Equations* **1998**(32), 1-12.

FLEMING, W. H. and RISHEL, R. W. (1975) *Deterministic and Stochastic Optimal Control*. Springer Verlag.

KIRSCHNER, D., LENHART, S. and SERBIN, S. (1997) Optimal control of the chemotherapy of HIV. *J. Math. Biol.* **35**, 775–792.

MURRAY, J. D. (1989) *Mathematical Biology*. Springer, Berlin, 109-113.

PONTRYAGIN, L. S., BOLTYANSKII, V. G., GAMKRELIDZE, R. V. and MISHCHENKO, E. F. (1986) *Mathematical Theory of Optimal Processes*. Gordon and Breach Science Publishers **4**.

ROBERTS, D. V. (1977) *Enzyme Kinetics*. Cambridge University Press.

RUBINOW, S. I. (1975) *Introduction to Mathematical Biology*. Dover Publi-

cations.

SEGEL, L. A. (1980) *Mathematical Models in Molecular and Cellular Biology.* Cambridge University Press.

SHARPE, F. R. and LOTKA, A. J. (1923) Contributions to the analysis of malaria epidemiology. IV. Incubation log. *Amer. J. Hyg.* 3 (Suppl.1), 96–112.

TZAFRIRI, A. R. and EDELMAN, E. R. (2004) The total quasi-steady-state approximation is valid for reversible enzyme kinetics. *Journal of Theoretical Biology* **226**, 303-313.

VARADHARAJAN, G. and RAJENDRAN, L. (2011) Analytical solution of coupled non-linear second order reaction differential equations in enzyme kinetics. *Natural Science* **3**(6), 459-465.

VASIC-RACKI, D., KRAGL, U. and LIESE, A. (2003) Benefits of enzyme kinetics modelling. *Chem. Biochem. Eng. Q.* **17**(1), 7-18.