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KINETICS ANALYSIS OF THERMAL INACTIVATION OF ENZYME USED IN BIOTECHNOLOGY USING VARIATION ITERATION METHOD

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Abstract: In this work, variation iteration method is used to develop analytical solutions for the prediction of molar concentration of native and denatured jack bean urease (EC 3.5.1.5) through the three–reaction steps kinetic model of thermal inactivation of the urease. The obtained analytical solutions are used to study the kinetics of thermal inactivation of the enzyme as applied in biotechnology. The analytical solutions are verified with numerical solutions using Runge–Kutta with shooting method and good agreements are established between the solutions. The information given in this theoretical investigation will assist in the kinetic analysis of the experimental results over handling rate constants and molar concentrations. The analytical solutions as developed in this work can serve as a starting point for a better understanding of the relationship between the physical quantities of the problems.
Keywords: Kinetics; Thermal activation; Jack bean urease; Enzyme; Variation iteration method

1. INTRODUCTION

Urease (urea amino hydrolase E.C.3.5.1.5) is an enzyme usd in biotechnology. It is a part of the superfamily of amidohydrolases and phosphotriesterases. It catalyzes the hydrolysis of urea to produce ammonia and carbamate. The produced carbamate is subsequently degraded by spontaneous hydrolysis to produce another ammonia and carbonic acid [1]. Consequently, the pH of its environment increases as ammonia is produced. Ureases catalyzes at a rate approximately 10^{14} times faster than the rate of the non–catalyzed reaction [2]. As a nickel–containing metalloenzymes of high molecular weight [3], it can be found in numerous bacteria, fungi, algae, plants and some invertebrates, as well as in soils, as a soil enzyme.

Jack bean urease, which is the most widely used plant urease, is a nickel containing oligomeric enzyme exhibiting a high degree of specifity to urea [4]. The importance and applications of the urease as a good catalyst for hydrolysis of urea has attracted several research interests [1–19] especially in biotechnology and biomedical engineering studies. Also, the thermostability of jack bean urease has often been a subject of investigation. However, there are few studies where the temporal loss of enzyme activity and the kinetic analysis of heat induced decay of enzyme activity were presented. Moreover, none of these studies involved consistent evaluation of kinetics of the urease inactivation. Most of the past studies described the complex mechanisms of thermal deactivation of enzymes as a "one step – two states" process where the native (active) form is transformed in the denaturated (inactive) form by a first order unimolecular irreversible reaction [18]. This unifying simplification is of interest for people focusing attention to phenomenological rather than mechanistic description of the kinetics of heat induced enzyme deactivation. However, the multi–temperature evaluation revealed that an adequate kinetic model had to incorporate at least three reaction steps [18]. Although, three–step mechanism model of inactivation of the enzyme has been developed by Illeova et al. [18], there is no provision of analytical solutions (except by Ananthi et al. [19]) for the predictions of model concentrations of the native enzyme, denature enzyme and temperature for thermal inactivation of urease. Ananthi et al. [19] applied homotopy analysis method to develop approximate analytical solutions for the analysis of kinetic and thermal inactivation of the enzyme. Although, the homotopy analysis method is a reliable and efficient semi–analytical technique, but it suffers from a number of limiting assumptions such as the requirements that the solution ought to conform to the so–called rule of solution expression and the rule of coefficient ergodicity. Also, the use of HAM in the analysis of linear and nonlinear equations requires the determination of auxiliary parameter which will increase the computational cost and time. Also, the lack of rigorous theories or proper guidance for choosing initial approximation, auxiliary linear operators, auxiliary functions, and auxiliary parameters limits the applications of HAM. Moreover, such method requires high skill in mathematical analysis and the solution comes with large number of terms.

The wide range of applications due to its flexibility and high accuracy have made VIM a promising approximate analytical method in nonlinear analysis of physical and real life problems. It is established that with few number

of iteration, even, in some cases, a single iteration, VIM can converge to correct solutions or results [20–27]. Therefore, in this work, variation parameter method is applied to the kinetics analysis of thermal inactivation of enzyme. The developed analytical solutions are used to study the effects of the models parameters on the molar concentration of the native and denatured enzyme. The analytical solutions as developed in this work can serve as a starting point for a better understanding of the relationship between the physical quantities of the problems as it provides continuous physical insights into the problem than pure numerical or computation methods.

2. MODEL FORMULATION

The three – step mechanism of inactivation with a dissociation reaction of the native form of the enzyme, N, into a denatured form, D, and with two parallel association reactions of the native and denatured forms into irreversible denatured enzymes forms $\mathbf{I_1}$ and $\mathbf{I_2}$, respectively.

$$N \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} 2D \qquad 2D \xrightarrow{k_2} I_1 \qquad 2N \xrightarrow{k_3} I_2 \tag{1}$$

where $\mathbf{k_{+1}}$, $\mathbf{k}$, $\mathbf{k_2}$ and $\mathbf{k_3}$ represent the rate constants of individual reactions. The material balances equations for $\mathbf{N}$, $\mathbf{D}$ and temperature are given as follows [28, 29]:

$$\frac{dc_N}{dt} = -k_{+1}c_N + k'_{-1}c_D^2 - 2k'_3c_D^2 \tag{2a}$$

$$\frac{dc_D}{dt} = 2k_{+1}c_N - 2\left(k'_{-1} + k'_2\right)c_D^2 \tag{2b}$$

$$\frac{dT}{dt} = K\left(T - T_B\right) \tag{2c}$$

Initial conditions are

$$t = 0,\ \ c_N = 1,\ \ c_D = 0\ ,\ T = 30 + T_B, \tag{3}$$

The kinetic model was formed by the set of nonlinear ordinary differential equations (eqs. (2a)–(2c)). The core of the kinetic model was formed by the material balances of the forms N and D (eqs. (2a) and (2b)). The third equation of the model was the enthalpy balance (Eq. (2c)) describing the initial heating period

Let c_N, c_D, $\mathbf{k_{+1}}$, $\mathbf{k_{-1}}$, $\mathbf{k_2}$ and $\mathbf{k_3}$ by X, Y, $\mathbf{a, b, c\ and\ d}$, respectively, eq. 2a and 2b become

$$\frac{dX}{dt} = -aX + bY^2 - 2dX^2 \tag{4a}$$

$$\frac{dY}{dt} = 2aX - 2\left(b + c\right)Y^2 \tag{4b}$$

$$t = 0, \quad X = 1, \quad Y = 0 \tag{5}$$

while the exact solution of Eq. (2c) is given as

$$T\left(t\right) = T_B + 30e^{-Kt} \tag{6}$$

3. METHOD OF SOLUTION: VARIATIONAL ITERATION METHOD

In finding direct and practical solutions to the problem, variational iteration method is applied to the simultaneous nonlinear equations. As pointed previously, the variational iteration method is an approximate analytical method for solving differential equations. The basic definitions of the method are as follows.

The differential equation to be solved can be written in the form

$$Lu + Nu = g(t) \tag{7}$$

where *L* is a linear operator, N is a nonlinear operator and g(t) is an inhomogeneous term in the differential equation.

Following VIM procedure, we have a correction functional as

$$u_{n+1}(t) = u_n(t) + \int_0^t \lambda \left\{ Lu_n(\tau) + N\tilde{u}(\tau) - g(t) \right\} d\tau \tag{8}$$

λ is a general Lagrange multiplier, the subscript *n* is the nth approximation and $\tilde{u}$ is a restricted variation $\delta\tilde{u} = 0$

Applying the above VIM procedures to eqs. (4a) and (4b), the following iteration formulations are constructed,

$$X_{n+1}(t) = X\left(0\right) + \int_0^t \lambda\left(t,\xi\right)\left[-aX_n + bY_n^2 - 2dX_n^2\right]d\xi \tag{9a}$$

$$Y_{n+1}(t) = Y\left(0\right) + \int_0^t \lambda\left(t,\xi\right)\left[2aX_n - 2\left(b+c\right)Y_n^2\right]dt, \tag{9b}$$

where it was found that $\lambda(\eta,\xi)=1$
Therefore, eqs. (9a) and (9b)

$$X_{n+1}(t)=X(0)+\int_0^t\left[-aX_n+bY_n^2-2dX_n^2\right]d\xi \tag{10a}$$

$$Y_{n+1}(t)=Y(0)+\int_0^t\left[2aX_n-2(b+c)Y_n^2\right]dt, \tag{10b}$$

From the initial condition

$$X_0=1,\quad Y_0=0 \tag{11}$$

Using the iterative scheme in eqs. (10a) and (10b), we have

$$X_1=-(a+2d)t \tag{12a}$$

$$Y_1=2at \tag{12b}$$

$$X_2=1+\frac{1}{6}\left(4abt+3a(a+2d)-4d(a+2d)^2t\right)t^2 \tag{13a}$$

$$Y_2=-\frac{a}{3}\left[3(a+2d)+8a(b+c)t\right]t^2 \tag{13b}$$

$$\begin{aligned}X_3=1&+\frac{a^2b}{63}\left[8a(b+c)^2t^2+56a(b+c)(a+2d)t+63a^2(a+2d)\right]t^5\\&-\frac{at}{6}\left[\left(ab-4ad^2-a^2d-4d^3\right)t^3+a(a+2d)t^2+6\right]\\&-2dt\left\{\begin{array}{l}\frac{1}{63}\left(\begin{array}{l}4a^4d^2-8a^3bd+32a^3d^3+4a^2b^2-32a^2bd^2\\+96a^2d^4-32abd^3+128ad^5\end{array}\right)t^6\\+\frac{1}{9}\left(ba^3-6a^3d^2-a^4d-12a^2d^3+2a^2bd-8ad^4\right)t^5\\+\frac{1}{20}\left(a^4+4a^3d+4a^2d^2\right)t^4+\frac{1}{3}\left(ba-4ad^2-a^2d-4d^3\right)t^3\\+\frac{a}{20}(a+2d)t^2+1\end{array}\right\}\end{aligned} \tag{14a}$$

$$\begin{aligned}Y_3=&\frac{a^2t}{3}\left[\left(ab-4ad^2-a^2d-4d^3\right)t^3+a(a+2d)t^2+6\right]\\&-\frac{2a^2(b+c)t^5}{9}\left[8at(b+c)(a+2d)+\frac{64a^2t^2(b+c)}{7}+\frac{1}{5}\right]\end{aligned} \tag{14b}$$

Similarly X_4, Y_4, X_5, Y_5, X_6, Y_6, X_7, Y_7, X_8, Y_8, X_9, Y_9, X_{10}, Y_{10}... are determined using the iterative schemes in Eqs. (10). The solutions of X_n and Y_n form the approximate analytical solutions of concentrations of native and denatured enzyme. The analytical solutions are simulated and the results are shown below.

Table 1: Comparison of results

The results of VIM and Numerical methods for X(t) for $a=1, b=0.01, c=0.001, d=0.05$			
	X(t)		
X	VIM	NUM	NM–VIM
0.00	1.000000	1.000000	0.000000
0.10	0.896320	0.896320	0.000000
0.20	0.804239	0.804239	0.000000
0.30	0.722362	0.722362	0.000000
0.40	0.649479	0.649479	0.000000
0.50	0.584542	0.584542	0.000000
0.60	0.526637	0.526637	0.000000
0.70	0.474965	0.474965	0.000000
0.80	0.428834	0.428824	0.000000
0.90	0.387599	0.387599	0.000000
1.00	0.350878	0.350748	0.000000

Table 2: Comparison of results

The results of VIM and Numerical methods for X(t) for $a=1,\ b=0.01, c=0.001, d=0.05$			
	Y(t)		
X	VIM	NUM	NM–VIM
0.00	0.000000	0.000000	0.000000
0.10	0.189399	0.189399	0.000000
0.20	0.359101	0.359101	0.000000
0.30	0.511178	0.511178	0.000000
0.40	0.647477	0.647477	0.000000
0.50	0.769644	0.769644	0.000000
0.60	0.879150	0.879150	0.000000
0.70	0.977311	0.977311	0.000000
0.80	1.065300	1.065300	0.000000
0.90	1.144180	1.144170	0.000000
1.00	1.214880	1.214840	0.000000

4. RESULTS AND DISCUSSION

Tables 1 and 2 show the comparison between the results of VIM and NM. The obtained results of concentrations using VIM as compared with the numerical procedure using Runge–Kutta method coupled with shooting method are in good agreements. The high accuracy of VIM gives high confidence about validity of the method in providing solutions to the problem.

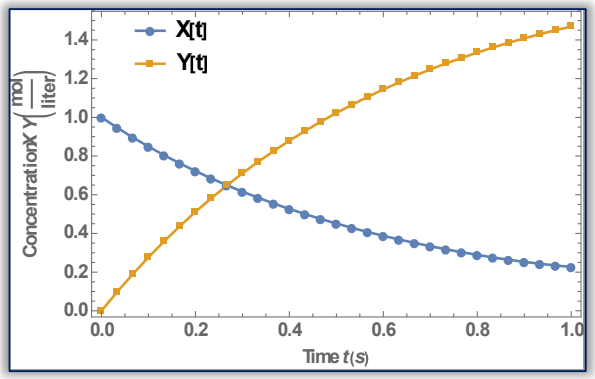

Figure 1: Molar concentrations of native and denatured enzyme when$k_{-1} = 1, k_{+1} = 0.01,\ k_2 = 0.001,\ k_3 = 0.05$

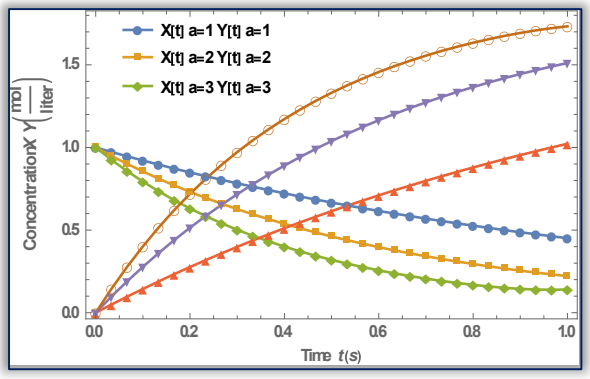

Figure 2: Effects of dissociation native rate constant (k_{-1}) on molar concentration of denatured enzyme

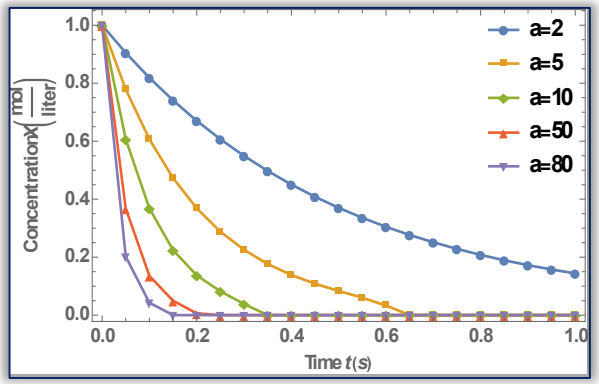

Figure 3: Effects of dissociation native rate constant (k_{-1}) on mmolar concentration of native enzyme when $k_{+1} = 0.01,\quad k_2 = 0.001,\quad k_3 = 0.001$

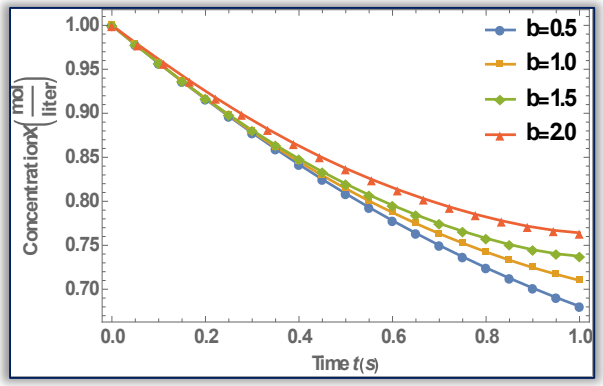

Figure 4: Effects of dissociation native rate constant (k_{+1}) on molar concentration of native enzyme When $k_{-1} = 0.88,\quad k_2 = 0.001,\quad k_3 = 0.00028$

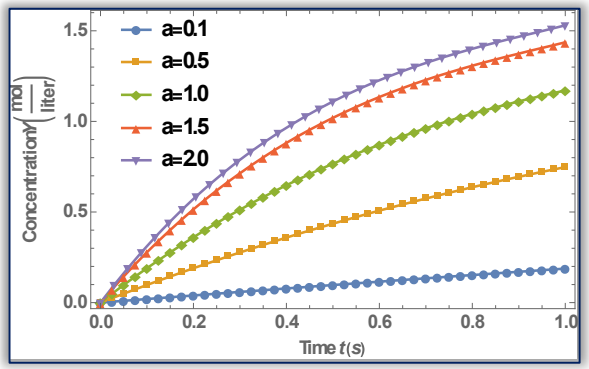

Figure 5: Effects of dissociation native rate constant (k_{-1}) on molar concentration of denaturedenzyme when $k_{+1} = 0.1,\quad k_2 = 0.00026,\quad k_3 = 0.001$

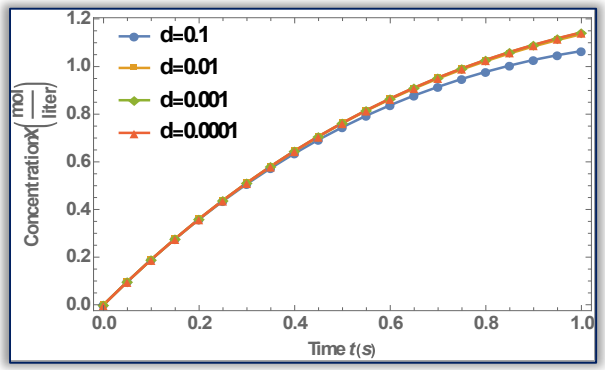

Figure 6: Effects of dissociation native rate constant (k_{-1}) on molar concentration of native enzyme when $k_{-1} = 1,\quad k_2 = 0.1,\quad k_3 = 0.001$

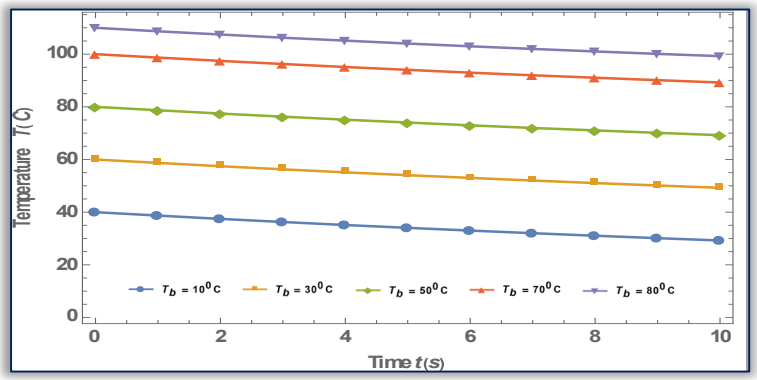

Figure 7: Temperature variation with time of the enzyme when $k_{-1} = 1,\quad k_2 = 0.1,\quad k_3 = 0.001$

Figure 1 shows variation of the molar concentration of native and denatured enzyme with time when $\mathbf{k_{-1}} = 1, \mathbf{k_{+1}} = 0.01, \mathbf{k_2} = 0.001, \mathbf{k_3} = 0.05$. As depicted in the figure, the molar concentration of native enzyme decreases as the time increases while the molar concentration of the denatured enzyme increases as the time increases. The time taken to reach the maximum value of the molar concentration of native enzyme is the same as the time taken to reach the minimum value of the molar concentration of the denature enzyme. The steady values of molar concentrations of native and denatured enzyme depend upon the rate constants.

Figure 2 show the effects of dissociation native rate constant ($\mathbf{k_{-1}}$) on molar concentration of denatured enzyme while Figure 3 depict the effects of dissociation native rate constant ($\mathbf{k_{-1}}$) on molar concentration of native enzyme when $\mathbf{k_{+1}} = 0.01, \mathbf{k_2} = 0.001, \mathbf{k_3} = 0.001$.

From these figures, it is found that, the value of molar concentration of the denatured enzyme initially increases and reaches the steady state value when $t \geq 5$. Also, the molar concentration of the denatured enzyme increases when k increases and the molar concentration becomes zero when $k_{+1} \leq 0.01 s^{-1}$. Figure 4 presents the effects of dissociation native rate constant ($\mathbf{k_{+1}}$) on molar concentration of native enzyme when $\mathbf{k_{-1}} = 0.88, \mathbf{k_2} = 0.001, \mathbf{k_3} = 0.00028$ while Figure 5 shows the effects of dissociation native rate constant ($\mathbf{k_{-1}}$) on molar concentration of denatured enzyme when $\mathbf{k_{+1}} = 0.1,\ \mathbf{k_2} = 0.00026, \mathbf{k_3} = 0.001$.Effects of dissociation native rate constant ($\mathbf{k_{-1}}$) on molar concentration of native enzyme when $\mathbf{k_{-1}} = 1, \mathbf{k_2} = 0.1, \mathbf{k_3} = 0.001$ are shown in Figure 6.

Figure 7 show the temperature history of the enzyme when $\mathbf{k_{-1}} = 1,\ \mathbf{k_2} = 0.1,\ \mathbf{k_3} = 0.001$. Also, effects of bath temperature on the temperature history are depicted in the figure. The temperature of the enzyme decreases linearly with time. It could be seen that as the bath temperature, T_B increases, the temperature of the enzyme increase.

4. CONCLUSION

In this work, approximate analytical solutions for the analysis of kinetic model of thermal inactivation of the jack bean urease (E.C.3.5.1.5) have been developed using variation iteration method. The analytical solutions are verified with numerical solution using Runge–Kutta with shooting method and good agreements are established. The information given in this theoretical investigation will assist in the kinetic analysis of the experimental results over handling rate constants and molar concentrations. The analytical solutions as developed in this work can serve as a starting point for a better understanding of the relationship between the physical quantities of the problems as it provides continuous physical insights into the problem than pure numerical or computation methods.

Nomenclature

c_N: Molar concentration of the native enzyme form (mole/cm)
c_D: Molar concentration of the denaturedenzyme form (mole/cm
k_{-1}, k_{+1}, k_2, k_3: Rate constants of individual reaction (s^{-1})
k'_{-1}, k'_2, k'_3: Modified rate constants (s^{-1})
K: Coefficient in the enthalpy balance (s)
T_B: Bath temperature (K)
T: Temperature (K)
t: Time

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