

8.1

The reaction between [E], [S], and [ES] is reversed with k_1 as forwarding rate and k_2 as backward rate. The reaction between [ES] and [P] is the one-way reaction with k_3 as the rate. Therefore, the equations of their rate of changes are written below (Formula.1).

$$\left\{ \begin{array}{l} \frac{d[E]}{dt} = -k_1[E][S] + (k_2 + k_3)[ES] \\ \frac{d[S]}{dt} = -k_1[E][S] + k_2[ES] \\ \frac{d[ES]}{dt} = k_1[E][S] - (k_2 + k_3)[ES] \\ \frac{d[P]}{dt} = k_3[ES] \end{array} \right.$$

Formula.1 The equations of each component in the reaction

[E], [S], [ES], and [P] mean the concentration of each component. k_1 , k_2 , and k_3 mean the reaction rate.

8.2

Based on the equations from 8.1, each differential equation contains the variables of [E], [S], and [ES] (Only $d[P]/dt$ has one variable of [ES]). Combining with the initial value conditions, we can rewrite the system of equations (Formula.2).

$$\left\{ \begin{array}{l} E' = f([E],[ES],[S]), E_0 = 1\mu m \\ S' = g([E],[ES],[S]), S_0 = 10\mu m \\ ES' = j([E],[ES],[S]), ES_0 = 0\mu m \\ P' = l([E],[ES],[S]), P_0 = 0\mu m \end{array} \right.$$

Formula.2 The system of equation with initial value conditions

Then, the fourth-order Runge-Kutta method is applied directly to this system of equations. Taking $d[E]/dt$ as an example, we can give the iteration function based on a certain step length h (Formula.2).

$$\left\{ \begin{array}{l} F_1 = f(E_n, ES_n, S_n) \\ F_2 = f\left(E_n + \frac{h \cdot F_1}{2}, ES_n + \frac{h \cdot J_1}{2}, S_n + \frac{h \cdot G_1}{2}\right) \\ F_3 = f\left(E_n + \frac{h \cdot F_2}{2}, ES_n + \frac{h \cdot J_2}{2}, S_n + \frac{h \cdot G_2}{2}\right) \\ F_4 = f\left(E_n + \frac{h \cdot F_3}{2}, ES_n + \frac{h \cdot J_3}{2}, S_n + \frac{h \cdot G_3}{2}\right) \\ E_{n+1} = E_n + \frac{h}{6} \cdot (F_1 + 2F_2 + 2F_3 + F_4) \end{array} \right.$$

Formula.3 The iteration functions of d[E]/dt based on fourth-order Runge-Kutta method

The iteration functions of other components such as [ES], [S], and [P] can be written below based on the structure of [E] (Formula.4-6).

$$\left\{ \begin{array}{l} G_1 = g(E_n, ES_n, S_n) \\ G_2 = g\left(E_n + \frac{h \cdot F_1}{2}, ES_n + \frac{h \cdot J_1}{2}, S_n + \frac{h \cdot G_1}{2}\right) \\ G_3 = g\left(E_n + \frac{h \cdot F_2}{2}, ES_n + \frac{h \cdot J_2}{2}, S_n + \frac{h \cdot G_2}{2}\right) \\ G_4 = g\left(E_n + \frac{h \cdot F_3}{2}, ES_n + \frac{h \cdot J_3}{2}, S_n + \frac{h \cdot G_3}{2}\right) \\ S_{n+1} = S_n + \frac{h}{6} \cdot (G_1 + 2G_2 + 2G_3 + G_4) \end{array} \right.$$

Formula.4 The iteration functions of d[S]/dt based on fourth-order Runge-Kutta method

$$\left\{ \begin{array}{l} J_1 = j(E_n, ES_n, S_n) \\ J_2 = j\left(E_n + \frac{h \cdot F_1}{2}, ES_n + \frac{h \cdot J_1}{2}, S_n + \frac{h \cdot G_1}{2}\right) \\ J_3 = j\left(E_n + \frac{h \cdot F_2}{2}, ES_n + \frac{h \cdot J_2}{2}, S_n + \frac{h \cdot G_2}{2}\right) \\ J_4 = j\left(E_n + \frac{h \cdot F_3}{2}, ES_n + \frac{h \cdot J_3}{2}, S_n + \frac{h \cdot G_3}{2}\right) \\ ES_{n+1} = ES_n + \frac{h}{6} \cdot (J_1 + 2J_2 + 2J_3 + J_4) \end{array} \right.$$

Formula.5 The iteration functions of d[ES]/dt based on fourth-order Runge-Kutta method

$$\left\{ \begin{array}{l} L_1 = l(E_n, ES_n, S_n) \\ L_2 = l\left(E_n + \frac{h \cdot F_1}{2}, ES_n + \frac{h \cdot J_1}{2}, S_n + \frac{h \cdot G_1}{2}\right) \\ L_3 = l\left(E_n + \frac{h \cdot F_2}{2}, ES_n + \frac{h \cdot J_2}{2}, S_n + \frac{h \cdot G_2}{2}\right) \\ L_4 = l\left(E_n + \frac{h \cdot F_3}{2}, ES_n + \frac{h \cdot J_3}{2}, S_n + \frac{h \cdot G_3}{2}\right) \\ P_{n+1} = P_n + \frac{h}{6} \cdot (L_1 + 2L_2 + 2L_3 + L_4) \end{array} \right.$$

Formula.6 The iteration functions of d[P]/dt based on fourth-order Runge-Kutta method

Based on the equations above, I used Rstudio to design the program. In the R file, it contains three functions: function_sets, Runge_Kutta, and Solution. At first, function_sets will return the rate of changes based on the concentration of each component. Then, the Runge_Kutta function can compute the iteration function and give the estimation of the next value. Finally, the Solution function makes the whole steps literately in defined step length and time steps.

In this experience, the step length **h** is defined as 0.001 min with time step equaling 100. Using ggplot2 in R to draw the concentration curves of each component. The result is stored in the figure file named 'Figure.1 The ggplot2 of concentration curves' (Figure.1).

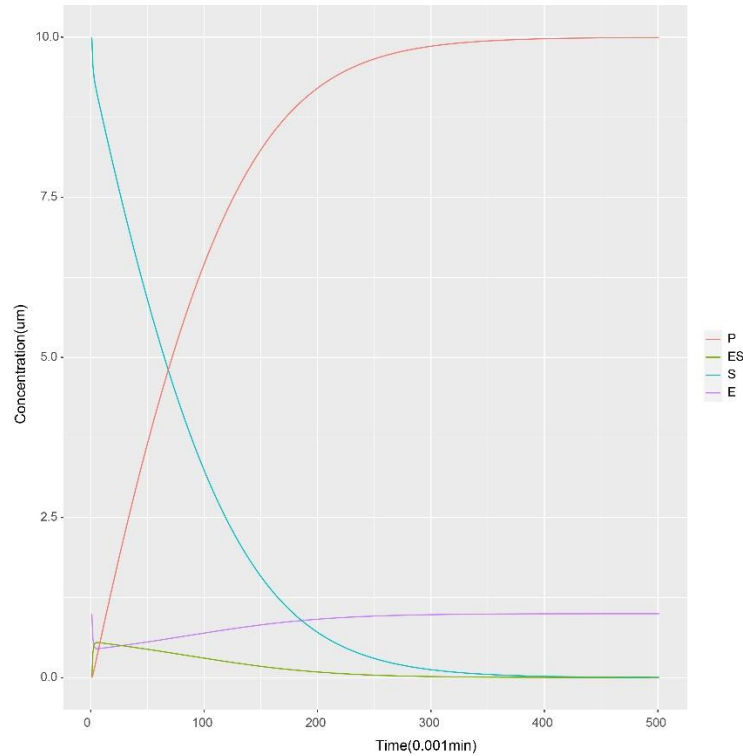


Figure.1 The concentrations of each component in iterations

8.3

1. Deduction of Michaelis-Menten equation

These equations can be described as ‘**Michaelis-Menten equation**’ in biodynamics. It gives a hint in how to judge the relationship between reaction rate and concentration of substrate. The deduction of the reaction formula can be written below.

The production of [P] is only associated with the concentration of [ES] and k_3 (Formula.7). However, [ES] is hard to estimate in the real environment. Formula.7 should be rewritten based on the theorem in quasi-steady state and enzyme conversion.

$$\frac{d[P]}{dt} = k_3 [ES]$$

Formula.7 The reaction rate of the Michaelis-Menten equation

While reaction is in a quasi-steady state, the reaction rate of $d[ES]/dt$ should equal 0 (Formula.8.1). Combining the enzyme conversion theorem, we can define the concentration function of [E] (Formula.8.2).

$$\begin{cases} \frac{d[ES]}{dt} = k_1 [E] [S] - (k_2 + k_3) [ES] = 0 & (1) \\ [E_t] = [ES] + [E] & (2) \end{cases}$$

Formula.8 The quasi-steady state and enzyme conversion theorem

$$\begin{cases} [ES] = \frac{[E_t] [S]}{K_m + [S]} \\ K_m = \frac{k_2 + k_3}{k_1} \end{cases}$$

Formula.9 The formula of [ES]. K_m is catalytic constant which can be applied to assess the ability of enzyme

Finally, combine the formula.7 and formula.9 together, we can obtain the final function of $d[P]/dt$ (Formula.10). With the fixing of k_1 , k_2 , k_3 , and $[E_t]$, the rate of enzyme reaction is only associated with [S].

$$\begin{cases} \frac{d[P]}{dt} = \frac{V_{\max}[S]}{K_m + [S]} \\ K_m = \frac{k_2 + k_3}{k_1} \\ V_{\max} = k_3 E_t \end{cases}$$

Formula.10 The formula of $d[P]/dt$. K_m is catalytic constant while V_{\max} is the maximum speed of the enzyme reaction

Formula.10 is not simple enough for us to judge the relationship between $d[P]/dt$ and $[S]$. Therefore, the **Lineweaver-Burk double inverse graphing method** can make the formula more obvious (Formula.11).

$$\frac{1}{V_p} = \frac{K_m}{V_{\max}} \cdot \frac{1}{[S]} + \frac{1}{V_{\max}}$$

Formula.11 The Lineweaver-Burk double inverse graphing method

2. Results

Using ggplot2 in Rstudio to plot the relationship between $d[P]/dt$ and $[S]$. Compared the estimation with the real function in formula.10. We can find that the fourth-order Runge-Kutta method has almost perfect performance on this dataset. Due to the start value of $[P]=0$, there might have some unexpected points in $[S]=10\mu\text{m}$.

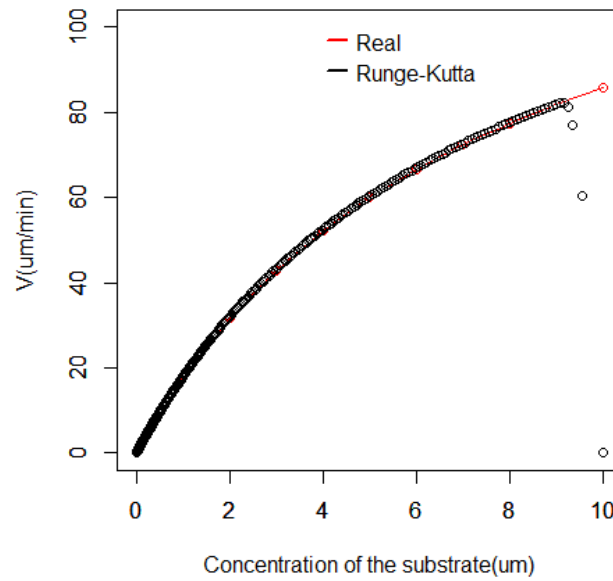


Figure.2 Comparison between real function and estimation

3. Estimation of Vmax

Due to the limitation of [S], we cannot further expand the estimated curve. However, both Lineweaver-Burk double inverse graphing method and the real function of $d[P]/dt$ can figure out the value of Vmax.

Using lm function in R to do the linear regression between $1/V_p$ and $1/[S]$. The result indicate that the slope (K_m/V_{max}) equals 0.048798 while Intercept ($1/V_{max}$) equals 0.006919 (Figure.3.1). Therefore, based on Lineweaver-Burk double inverse graphing method, the value of Vmax should be **144.5296 $\mu\text{m/min}$** . Also, based on the definition, the Vmax equals $k_3 \cdot E_t$ (Figure.3.2). Vmax should be **150 $\mu\text{m/min}$** . There is little difference between estimation and real function which should be normal.

