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Modelling of the Non-linear Biotransformation Process

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Abstract: Mathematical modelling of production of l-malic acid from fumaric acid which is performed by the action of the fumarase isolated from porcine heart and the fumarase in permeabilized baker's yeast cells are discussed. This model is based on nonlinear system of equations containing a non-linear term related to rate of the reactions. The effects of various parameters on the concentrations are also discussed. Our analytical results are also compared with numerical results (Sci lab program) and satisfactory agreement is noted. Simple analytical expressions for the concentration of substrate and product have been derived for all values of reaction parameters using the new homotopy perturbation method (NHPM). Kinetic parameters are also obtained from the enzyme reaction rate.

Keywords: l-Malic acid; Fumarase; Baker's yeast; Permeabilization; Mathematical Modelling; non-linear differential equation.

1. Introduction

Biotransformation, using either the whole cells or isolated enzyme as biocatalysts, have been extensively applied [1–3]. Enzymes are large complex protein molecules, which proceed as a catalyst to speed up chemical reactions in living organisms. In biochemistry, Michaelis-Menten kinetics is one of the simplest and important models to enzyme kinetics. In this model the rate of enzymatic reactions is a nonlinear function of concentration of a substrate. Also these reactions are essential in biochemistry because most of cell processes need enzymes to find a significant rate [4, 5].

Figueiredo and Carvalho [6] produce the L-malic acid using immobilized Saccharomyces cerevisiae. Peleg et al. [7] amplified the inducible over expression of the FUM1gene in Saccharomyces cerevisiae: localization of fumarase and efficient fumaric acid bioconversion to l-malic acid. Zambianchi et. al mainly used in the pharmaceutical and agrochemical industries, because of the great need for optical pure molecules [8, 9]. The use of whole cell biocatalysts over purified enzymes is an advantage in terms of cost, isolation, and stability [10]. Pavithra Sivasamy et. al. [11] discussed a mathematical model of biotransformation of D-methionine into L-methionine in the cascade of four enzymes such as, D-amino acid oxidase (D-AAO), L-phenylalanine dehydrogenase (L-PheDH) and formate dehydrogenase (FDH).

The mathematical model of mono-enzymatic biosensor involving Michaelis-Menten kinetics is presented [12]. The theoretical model for amperometric enzymes reactions for steady state condition is discussed and the various analytical methods (HPM, HAM, Vim and Adomin decomposition method) for solving the non-linear reaction diffusion equation in enzyme biosensors has been reviewed recently [13]. The approximate expression of steady state current for amperometric polymer molecular electrodes for the first-order and zero-order kinetics using Danckwert's expression is derived [14]. Eswari et. al

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obtain. The steady state concentration and current occurring at microdisk and the microcylinder enzyme electrodes for amperometric biosensor using Homogony perturbation method [15]. Findrik et. al., have developed a mathematical model for biotransformation of D-methionine into L-methionine in the cascade of four enzymes systems [16].

However, to the best of our knowledge, till date there is no general analytical result corresponding to the non-steady state concentration for four enzyme system has been reported. The purpose of this communication is to derive the analytical expression of concentration of fumaric acid and malic acid in batch reactor and fed-batch reactor enzyme systems based on new Homotopy perturbation method. This is an effective tool for solve the nonlinear problems in chemical sciences. These analytical results are helpful to understand the mechanism and physical effects of parameters through the model problem. It is also useful to validate the numerical results and the experimental data.

2. Mathematical Formulation of The Problem

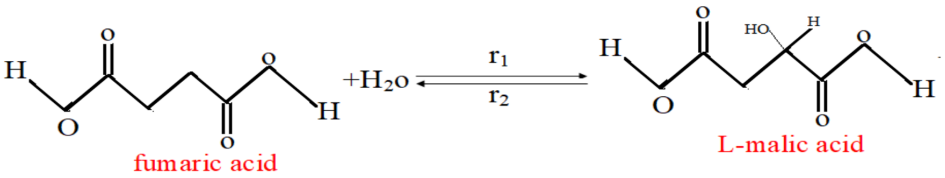

Figure 1: Kinetic and mass balance equations of the batch and fed-batch reactor for the l-malic production [1].

The kinetics of purified fumarase from porcine heart and fumarase in permeabilized baker's yeast cell for fumaric acid hydration, as for the reverse reaction of l-malic acid dehydration was described by Michaelis-Menten equation with competitive product inhibition (Equations (1) and (2)). This model is formulated as a set of non-linear differential equations describing the mass balance of the concentration of fumaric acid and malic acid. The mass balance equations for the l-malic acid production in batch (Equations (3) and (4)) and fed-batch reactor (Equations (5)-(7)) are given as follows:

Mass balance equations-batch reactor

$$\frac{dc_{fumaric\,acid}}{dt} = r_2 - r_1 \tag{1}$$

$$\frac{dc_{malic\,acid}}{dt} = r_1 - r_2 \tag{2}$$

Mass balance equations-fed-batch reactor

$$\frac{dc_{fumaric\,acid}}{dt} = \frac{-c_{fumaric\,acid} + c_{0,fumaric\,acid}}{V} q_{c_0} + r_2 - r_1 \tag{3}$$

$$\frac{dc_{malic\,acid}}{dt} = \frac{-c_{malic\,acid}}{V} q_{c_0} + r_1 - r_2 \tag{4}$$

$$\frac{dV}{dt} = q_{c_0} \tag{5}$$

where,

$$r_1 = \frac{V_m^{fumaric\,acid}\,\gamma_{biocatalyst}\,c_{fumaric\,acid}}{\left[K_m^{fumaric\,acid}\left(1 + \left(c_{malic\,acid}/K_i^{malic\,acid}\right)\right)\right] + c_{fumaric\,acid}} \tag{6}$$

$$r_2 = \frac{V_m^{malic\,acid}\,\gamma_{biocatalyst}\,c_{malic\,acid}}{\left[K_m^{malic\,acid}\left(1 + \left(c_{fumaric\,acid}/K_i^{fumaric\,acid}\right)\right)\right] + c_{malic\,acid}} \tag{7}$$

The initial conditions for above equations are given below:

$$c_{fumaric\,acid} = (c_{fumaric\,acid})_0\,,\ c_{malic\,acid} = (c_{malic\,acid})_0\ at\quad t = 0 \tag{8}$$

3. Analytical Solutions of the Concentrations by Using New Homotopy Perturbation Method

Many problems in the fields of physics, engineering and biology are modeled by linear and nonlinear PDEs. In recent years, the new homotopy perturbation method (NHPM) has been employed to solve these PDEs [18–23]. HPM has been used extensively to solve nonlinear boundary and initial value problems. Therefore HPM is of great interest to many researchers and scientists. HPM, first presented by Ji Huan He [24–26], is a powerful mathematical tool to investigate a wide variety of problems arising in different fields. It is obtained by successfully coupling homotopy theory in topology with perturbation theory. In HPM, a complicated problem under study is continuously deformed into a simple problem which is easy to solve to obtain an analytic or approximate solution [27]. Using the NHPM we can obtain the concentration of fumaric acid and malic acid (Appendix B) as follows:

Mass balance equation-batch reactor

$$c_{fumaric\,acid}(t) = \frac{a}{b} + \left[(c_{fumaric\,acid})_0 - \frac{a}{b}\right] e^{-bt} \tag{9}$$

$$c_{maic\,acid}(t) = \frac{D}{f} + \left[(c_{malic\,acid})_0 - \frac{D}{f}\right] e^{-ft} \tag{10}$$

Mass balance equation-fed-batch reactor

$$c_{fumaric\,acid}(t) = \frac{AV}{bV + q_{c_0}} + \left[(c_{fumaric\,acid})_0 - \frac{AV}{bV + q_{c_0}}\right] e^{-\left(bt - \frac{q_{c_0}}{V}\right)} \tag{11}$$

$$c_{malic\,acid}(t) = \frac{DV}{eV + q_{c_0}} + \left[(c_{malic\,acid})_0 - \frac{DV}{eV + q_{c_0}}\right] e^{-\left(et - \frac{q_{c_0}}{V}\right)} \tag{12}$$

$$V(t) = q_{c_0\,t} \tag{13}$$

Where,

$$a = \frac{V_m^{malic\,acid}\,\gamma_{biocatalyst}\ (c_{malic\,acid})_0}{\left[K_m^{malic\,acid}\left(1 + \left((c_{fumaric\,acid})_0 \Big/ K_i^{fumaric\,acid}\right)\right)\right] + (c_{malic\,acid})_0} \tag{14}$$

$$b = \frac{V_m^{fumaric\,acid}\,\gamma_{biocatalyst}}{\left[K_m^{fumaric\,acid}\left(1 + \left((c_{malic\,acid})_0 \,/ K_i^{malic\,acid}\right)\right)\right] + (c_{fumaric\,acid})_0} \tag{15}$$

$$D = b\ (c_{fumaric\,acid})_0 \tag{16}$$

$$f = a/\ (c_{malic\,acid})_0 \tag{17}$$

4. Estimation of Kinetic Parameters

To check the validity of the model against the experimental data, the model equation, which contains three kinetic parameters, is transformed so that a linear plot of the data can be made. The plot has yielded reasonable linearity, and the parameter values can be estimated from the plot. The two parameters in Equation (1) can be evaluated by means of non-linear least-squares fit. Equation (5) can be rewritten as

$$\frac{1}{r_1} = \left[\frac{K_m^{fumaric\,acid} + \frac{K_m^{fumaric\,acid}}{K_i^{malic\,acid}}\left[(c_{fumaric\,acid})_0 + (c_{malic\,acid})_0\right]}{\left(V_m^{fumaric\,acid}\,\gamma_{biocatalyst}\right)}\right] \frac{1}{(c_{fumaric\,acid})} + \frac{\left(\frac{K_m^{fumaric\,acid}}{K_i^{malic\,acid}} - 1\right)}{\left(V_m^{fumaric\,acid}\gamma_{biocatalyst}\right)} \tag{18}$$

Plot of $1/r_1$ versus $1/\left(c_{fumaric\,acid}\right)$ gives the slope

$$\left[K_m^{fumaric\,acid}+\frac{K_m^{fumaric\,acid}}{K_i^{malic\,acid}}\left[\left(c_{fumaric\,acid}\right)_0+\left(c_{malic\,acid}\right)_0\right]/\left(V_m^{fumaric\,acid}\,\gamma_{\text{biocatalyst}}\right)\right] \tag{19}$$

y-intercept $\left(\frac{K_m^{fumaric\,acid}}{K_i^{malic\,acid}}-1\right)/\left(V_m^{fumaric\,acid}\,\gamma_{biocatalyst}\right)$. If we know the maximum enzyme reaction rate$V_m^{fumaric\,acid}$, we can obtain the other parameter Michaelis-Menten constant of fumaric acid $K_m^{fumaric\,acid}$ and product inhibition constant of malic acid $K_i^{malic\,acid}$. As K_i tends to infinity the equation reduces to the form of Michaelis-Menten kinetics (Eswari et al.,2010) [15] for which the Lineweaver–Burk plot (Lineweaver et al., 1934) [28] is commonly used to determine the parameter values.

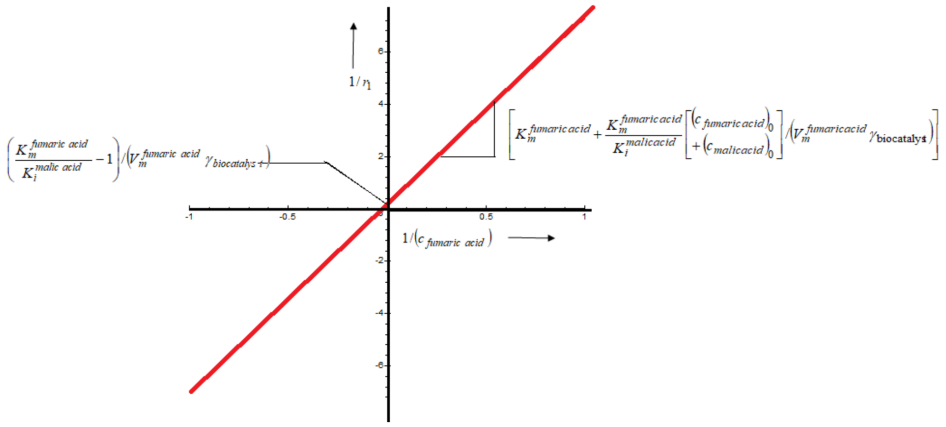

Figure 2: Estimation of kinetic parameters $K_m^{fumaric\,acid}$ and $K_i^{malic\,acid}$ using equation (19). The numerical value of the kinetic parameters used for the above figure is given in Appendix D.

5. Numerical Simulation

The non-linear differential equations (1)-(5) are also solved using numerical methods. The function pdex4 in Scilab software which is the function of solving the initial value problems for ordinary differential is used to solve this equation. Our theoretical results for the concentration of $c_{fumaric\,acid}$ using Equation (9) and $c_{malic\,acid}$ using Equation (10) for the mass balance equations of batch reactor are compared with simulation results (Scilab program 4.1). The Scilab program is also given in Appendix C. We run scilab 4.1 on apple imac core i5. Similarly our analytical results for the concentration of fumaric acid, malic acid in mass balance equation in batch reactor and fed bacth reactor are compared with numerical results and available experimental results [1] in Figures 2-5 satisfactory agreement is found for all values of time t.

6. Result and Discussion

Mass balance equation-batch reactor: Equations (9) and (10) are the new and simple approximate analytical expression of the concentrations of fumaric acid and malic acid in batch reactor. From Figure 3(a), it is observed that when concentration fumaric acid increases when the parameter $\gamma_{biocatalysit}$ decreases. From Figure 3(b), it is inferred that when concentration fumaric acid increases when the parameter $V_m^{fumaric\,acid}$ increases. From Figure 3(c), signifies that when concentration fumaric acid increases when the parameter $K_m^{fumaric\,acid}$ decreases. From Figure 3(d), it is clear that when concentration fumaric acid has no changes when the parameter $K_i^{malic\,acid}$ decreases. From Figure 4(a), it is observed

that when concentration malic acid increases when the parameter $\gamma_{biocatalysit}$ decreases. From Figure 4(b), it is inferred that when concentration malic acid decreases when the parameter $V_m^{malic\,acid}$ increases. From Figure 4(c), signifies that when concentration mal ic acid increases when the parameter $K_m^{malic\,acid}$ decreases. From Figure 4(d), it is clear that when concentration fumaric acid decreases when the parameter $K_i^{fumaric\,acid}$ increases.

Mass balance equation-fed-batch reactor: Equations (11) and (12) are the new and simple approximate analytical expression of the concentrations of fumaric acid and malic acid in batch reactor. From Figure 5(a), it is observed that when concentration fumaric acid decreases when the parameter $\gamma_{biocatalysit}$ increases. From Figure 5(b), it is inferred that when concentration fumaric acid increases when the parameter $V_m^{fumaric\,acid}$ increases. From Figure 5(c), signifies that when concentration fumaric acid increases when the parameter $K_m^{fumaric\,acid}$ decreases. From Figure 5(d), observed that when concentration fumaric acid decreases when the parameter q_{c_0} decreases. From Figure 5(e), it is clear that when concentration fumaric acid has no changes when the parameter $K_i^{malic\,acid}$ decreases. From Figure 5(f), it is clear that when concentration malic acid has no changes when the parameter V increases.

From Figure 6(a), it is observed that when concentration malic acid increases when the parameter $\gamma_{biocatalysit}$ decreases. From Figure 6(b), it is inferred that when concentration malic acid decreases when the parameter $V_m^{malic\,acid}$ decreases. From Figure 6(c), signifies that when concentration mal ic acid increases when the parameter $K_m^{malic\,acid}$ decreases. From Figure 6(d), it is clear that when concentration fumaric acid decreases when the parameter $K_i^{fumaric\,acid}$ increases. From Figure 6(e), it is observes that when concentration fumaric acid increases when the parameter q_{c_0} increases. From Figure 6(f), it is clear that when concentration fumaric acid has no changes when the parameter V increases.

7. Conclusion

A non-linear time dependent reaction equations in enzyme kinetics have been solved analytically using new Homotopy perturbation method. In this paper we have presented approximate analytical expression of the concentration of fumaric acid and malic acid. The analytical expressions are compared to the numerical simulation using Scilab software good agreement is noted. Theoretical evaluation of the kinetic parameters is also reported.

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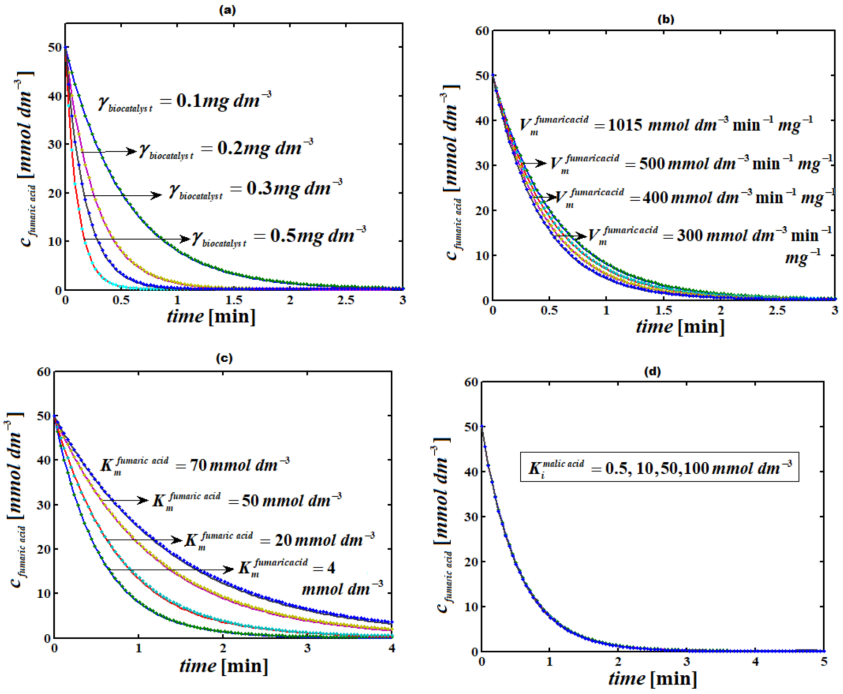

Figure 3: Concentration of fumaric acid in batch reactor versus time t for various values of parameters. Comparison between analytical result (Equation (9)) and simulation results. The numerical value of the kinetic parameters used for the above figure is given in Appendix D.

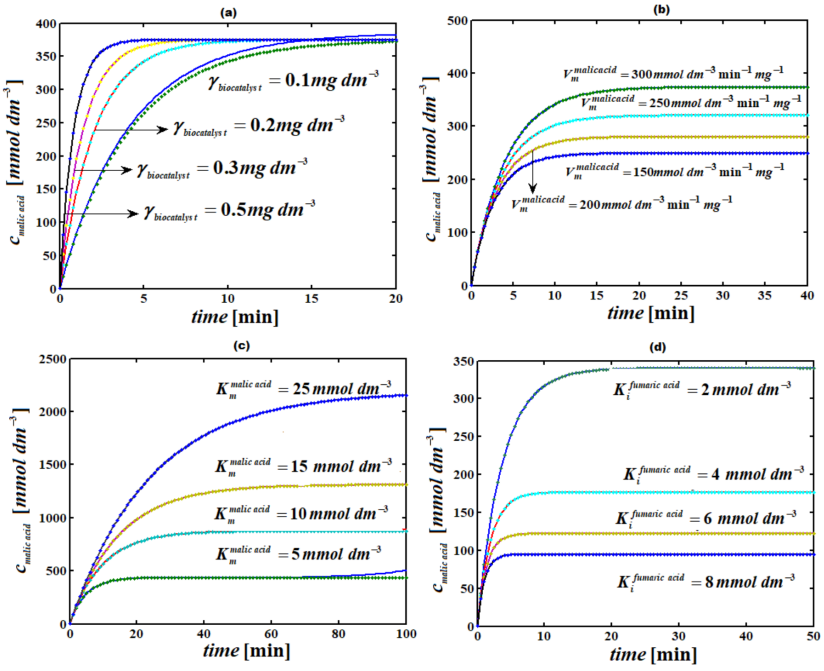

Figure 4: Concentration of malic acid in batch reactor versus time t for various values of parameters. Comparison between analytical result (Equation (10)) and simulation results. The numerical value of the kinetic parameters used for the above figure is given in Appendix D.

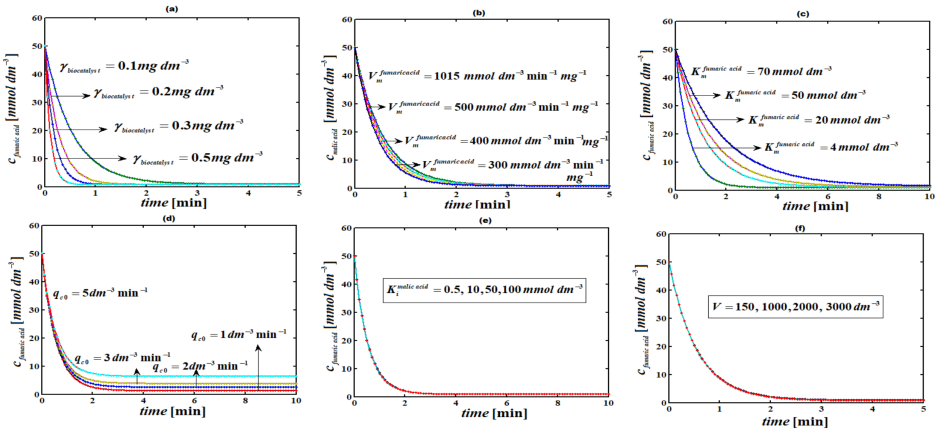

Figure 5: Concentration of fumaric acid in fed-batch reactor versus time t for various values of parameters. Comparison between analytical result (Equation (11)) and simulation results. The numerical value of the kinetic parameters used for the above figure is given in Appendix D.

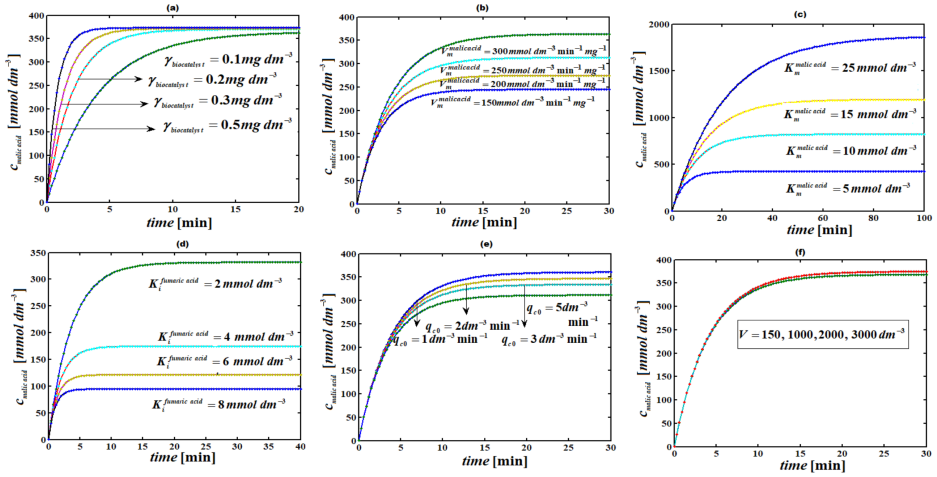

Figure 6: Concentration of malic acid in batch reactor versus time t for various values of parameter. Comparison between analytical result (Equation (12)) and simulation results. The numerical value of the kinetic parameters used for the above figure is given in Appendix D.

Appendix A: Basic Concept of the New Homotopy Perturbation Method (NHPM)

To explain this method, let us consider the following function:

$$D_o(u) - f(r) = 0, \qquad \mathrm{r} \in \Omega \tag{A1}$$

with the boundary conditions of

$$B_o\left(u, \frac{\partial u}{\partial n}\right) = 0, \quad \mathrm{r} \in \Gamma \tag{A2}$$

where D_o is a general differential operator, B_o is a boundary operator, $f(r)$ is a known analytical function and Γ is the boundary of the domain Ω. In general, the operator D_o can be divided into a linear part L and a non-linear part$N$. The equation (A1) can therefore be written as

$$L(u) + N(u) - f(r) = 0 \tag{A3}$$

By the Homotopy technique, we construct a Homotopy $v(r,p):\Omega\times[0,1]\to\Re$ that satisfies

$$H(v,p)=(1-p)[L(v)-L(u_0)]+p[D_o(v)-f(r)]=0. \tag{A4}$$

$$H(v,p)=L(v)-L(u_0)+pL(u_0)+p[N(v)-f(r)]=0. \tag{A5}$$

Where $p\in[0,1]$ is an embedding parameter, and u_0 is an initial approximation of equation (A1) that satisfies the boundary conditions. From equations (A4) and (A5), we have

$$H(v,0)=L(v)-L(u_0)=0 \tag{A6}$$

$$H(v,1)=D_o(v)-f(r)=0 \tag{A7}$$

When $p=0$, the equation (A4) and (A5) become linear equations. When $p=1$, they become non-linear equations. The process of changing p from zero to unity is that of $L(v)-L(u_0)=0$ to $D_o(v)-f(r)=0$. We first use the embedding parameter p as a "small parameter" and assume that the solutions of equations (A4) and (A5) can be written as a power series in p:

$$v=v_0+pv_1+p^2v_2+\ldots \tag{A8}$$

Setting $p=1$ results in the approximate solution of the equation (A1):

$$u=\lim_{p\to1}v=v_0+v_1+v_2+\ldots \tag{A9}$$

This is the basic idea of the HPM.

Appendix B: Approximate Analytical Solutions of the Equation (1) Using New Homotopy Perturbation Method

$$\frac{dc_{fumaric\,acid}}{dt}=r_2-r_1=\frac{V_m^{malic\,acid}\,\gamma_{biocatalyst}\,c_{malic\,acid}}{\left[K_m^{malic\,acid}\left(1+\left(c_{fumaric\,acid}\Big/K_i^{fumaric\,acid}\right)\right)\right]+c_{malic\,acid}}-\frac{V_m^{fumaric\,acid}\,\gamma_{biocatalyst}\,c_{fumaric\,acid}}{\left[K_m^{fumaric\,acid}\left(1+\left(c_{malic\,acid}/K_i^{malic\,acid}\right)\right)\right]+c_{fumaric\,acid}} \tag{B1}$$

The new Homotopy of the above Equation (B1) can be written as follows:

$$(1-p)\left\{\begin{array}{c}\frac{dc_{fumaric\,acid}}{dt}-\frac{V_m^{malic\,acid}\,\gamma_{biocatalyst}\,c_{malic\,acid}}{\left[K_m^{malic\,acid}\left(1+\left(\left(c_{fumaric\,acid}(t=0)\right)\Big/K_i^{fumaric\,acid}\right)\right)\right]+\left(c_{malic\,acid}(t=0)\right)}\\+\frac{V_m^{fumaric\,acid}\,\gamma_{biocatalyst}\,c_{fumaric\,acid}}{\left[K_m^{fumaric\,acid}\left(1+\left(\left(c_{malic\,acid}(t=0)\right)/K_i^{malic\,acid}\right)\right)\right]+\left(c_{fumaric\,acid}(t=0)\right)}\end{array}\right\}$$
$$+p\left\{\begin{array}{c}\frac{dc_{fumaric\,acid}}{dt}-\frac{V_m^{malic\,acid}\,\gamma_{biocatalyst}\,c_{malic\,acid}}{\left[K_m^{malic\,acid}\left(1+\left(\left(c_{fumaric\,acid}(t=0)\right)\Big/K_i^{fumaric\,acid}\right)\right)\right]+\left(c_{malic\,acid}(t=0)\right)}\\+\frac{V_m^{fumaric\,acid}\,\gamma_{biocatalyst}\,c_{fumaric\,acid}}{\left[K_m^{fumaric\,acid}\left(1+\left(\left(c_{malic\,acid}(t=0)\right)/K_i^{malic\,acid}\right)\right)\right]+\left(c_{fumaric\,acid}(t=0)\right)}\end{array}\right\}=0 \tag{B2}$$

The zeroth iteration of the above equation can be written as

$$p^0:\ \frac{dc_{fumaric\,acid}}{dt}=\frac{V_m^{malic\,acid}\,\gamma_{biocatalyst}\,c_{malic\,acid}}{\left[K_m^{malic\,acid}\left(1+\left(\left(c_{fumaric\,acid}\right)_0\Big/K_i^{fumaric\,acid}\right)\right)\right]+\left(c_{malic\,acid}\right)_0}-\frac{V_m^{fumaric\,acid}\,\gamma_{biocatalyst}\,c_{fumaric\,acid}}{\left[K_m^{fumaric\,acid}\left(1+\left(\left(c_{malic\,acid}\right)_0/K_i^{malic\,acid}\right)\right)\right]+\left(c_{fumaric\,acid}\right)_0} \tag{B3}$$

Using the zeroth iteration of homotopy we can obtain the concentration of fumaric acid and malic acid. Solving the above equation using the initial condition, we get

$$c_{fumaric\,acid}(t) = \frac{a}{b} + \left[(c_{fumaric\,acid})_0 - \frac{a}{b}\right] e^{-bt} \tag{B4}$$

where,

$$a = \frac{V_m^{malic\,acid}\,\gamma_{biocatalyst}\,(c_{malic\,acid})_0}{\left[K_m^{malic\,acid}\left(1 + \left((c_{fumaric\,acid})_0 \Big/ K_i^{fumaric\,acid}\right)\right)\right] + (c_{malic\,acid})_0} \tag{B5}$$

$$b = \frac{V_m^{fumaric\,acid}\,\gamma_{biocatalyst}}{\left[K_m^{fumaric\,acid}\left(1 + \left((c_{malic\,acid})_0 / K_i^{malic\,acid}\right)\right)\right] + (c_{fumaric\,acid})_0} \tag{B6}$$

The accuracy can be obtained by considering the higher iterations are simply proved.

Appendix C: Scilab program to find the numerical solution of Equations (1)-(5).

```
function main1
options= odeset('RelTol',1e-6,'Stats','on');
Xo = [50;0.1;50;0.1];
tspan = [0,10];
tic
[t,X] = ode45(@TestFunction,tspan,Xo,options);
toc
figure
hold on
%plot(t, X(:,1))
%plot(t, X(:,2))
plot(t, X(:,3))
%plot(t, X(:,4))
return
function [dx_dt]= TestFunction(t,x)
v1=1012.023,v2=299.09,k1=3.999,k2=0.279,k3=4.286,k4=1.807;
g=0.1,c1i=50;c2i=0.1,q=5,v=100;
a=(v2*g*c2i)/(k3*(1+(c1i/k4))+c2i);
b=(v1*g)/(k1*(1+(c2i/k2))+c1i);
d=(v1*g*c1i)/(k1*(1+(c2i/k2))+c1i);
e=(v2*g)/(k3*(1+(c1i/k4))+c2i);
dx_dt(1)=a-(b*x(1));
dx_dt(2)=d-(e*x(2));
dx_dt(3)=-((x(3)*q)/v)+((c1i*q)/v)+a-(b*x(3));
dx_dt(4)=-((x(4)*q)/v)+d-(e*x(4));
dx_dt = dx_dt';
return
```

Appendix D: Experimental values of parameter use in this work and Findrik and Vasic-Racki [1].

Parameter	Value
Fumaric acid hydration	
$V_m^{fumaric\,acid}$	607.214 (U mg^{-1}) or 1012.023$(mmol\,dm^{-3}mg^{-1}min^{-1})$
$K_m^{fumaric\,acid}$	3.999$(mmol\,dm^{-3})$
$K_i^{malic\,acid}$	0.279$(mmol\,dm^{-3})$
L-Malic acid dehydration	
$V_m^{malic\,acid}$	179.456 (U mg^{-1}) or 299.09$(mmol\,dm^{-3}mg^{-1}min^{-1})$
$K_m^{malic\,acid}$	4.286$(mmol\,dm^{-3})$
K_m^{NADH}	0.05$(mmol\,dm^{-3})$
$K_i^{fumaric\,acid}$	0.0027$(mmol\,dm^{-3})$

Nomenclature

c	molar concentration (mmol dm^{-3})
K_i	inhibition constant (mmol dm^{-3})
K_m	Michaelis–Menten constant (mmol dm^{-3})
q_{c_0}	flow rate (cm^3min^{-1})
r	reaction rate (U mg^{-1}, mmol $dm^{-3}min^{-1}mg^{-1}$)
t	time (min)
V_m	maximal reaction rate (U mg^{-1}, mmol $dm^{-3}min^{-1}mg^{-1}$)
γ	mass concentration (mgdm^{-3})