

ENZYME KINETIC MODELLING AND ANALYTICAL SOLUTION OF NONLINEAR RATE EQUATION IN THE TRANSFORMATION OF D-METHIONINE INTO L-METHIONINE IN BATCH REACTOR USING THE NEW HOMOTOPY PERTURBATION METHOD

Pavithra Sivasamy, Jansi Rani Palaniyandi Ganapathy, Iswarya Thinakaran and Rajendran Lakshmanan*

Department of Mathematics, Sethu Institute of Technology, Pulloor, Kariapatti – 626115, Virudunagar District – Tamilnadu, India

Recebido em 18/04/2016; aceito em 27/06/2016; publicado na web em 26/08/2016

A mathematical model of biotransformation of D-methionine into L-methionine in the cascade of the enzymes such as, D-amino acid oxidase (D-AAO), L-phenylalanine dehydrogenase (L-PheDH) and formate dehydrogenase (FDH) is discussed. The model is based on a system of coupled nonlinear reaction equations under non steady-state conditions for biochemical reactions occurring in the batch reactor that describes the substrate and product concentration within the catalyst. 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). Enzyme reaction rate in terms of concentration and kinetic parameters are also reported. The analytical results are also compared with experimental and numerical ones and a good agreement is obtained. The graphical procedure for estimating the kinetic parameters is also reported.

Keywords: mathematical model; non-linear equations; enzyme membrane reactor; L-phenylalanine dehydrogenase; D-aminoacidoxidase; formate dehydrogenase.

INTRODUCTION

The development of cascade conversions (i.e. enzyme membrane catalytic reactions without intermediate recovery steps as taking place in living cells) is considered as one of the important future directions for carrying out sustainable organic syntheses with inherently safer designs.^{1,2} Hence, the enzyme is very important for the industrial application in the purification and determination of certain amino acids. Bae *et al.*, developed a multi-enzyme system composed of glutamate racemase, thermo stable D-amino acid amino transferase, glutamate dehydrogenase and formate dehydrogenase for the production of aromatic D-amino acids, D-phenylalanine and D-tyrosine, from the corresponding α -keto acids, phenylpyruvate and hydroxyl phenylpyruvate, respectively.³

Hummel *et al.* discussed the biocatalytic membrane reactors using laboratory scale in enzyme systems. Mathematical models for separate reactions steps, as well as for the complete system are developed and validated in the batch reactor experiments.⁴ 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.^{5,6}

The mathematical model of mono-enzymatic biosensor involving Michaelis-Menten kinetics is presented.⁷ The theoretical model for amperometric enzymes reactions for steady state condition is discussed and the various analytical methods for solving the non-linear reaction diffusion equation in enzyme biosensors has been reviewed recently.⁸ 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.⁹ The steady state concentration and current occurring at microdisk and the microcylinder enzyme electrodes for amperometric biosensor using

homotopy perturbation method is obtained.¹⁰ All the above models have two enzyme systems.

Mathematical modellings of two enzyme systems are quite common.^{11,12} But it is not easy in the case of three or more enzyme systems. Findrik *et al.* developed a kinetic model of amino acid oxidation catalyzed by a new D-amino acid oxidase from *Arthrobacter protophormiae*. In developing the enzyme-catalyzed reaction for large-scale production, mathematical modeling of the reaction of kinetics plays an important role. Therefore, the subject of this study is focused on the kinetics of the oxidative deamination, a very complex reaction system, which is catalyzed by D-AAO from *Arthrobacter protophormiae* using its natural substrate D-methionine and the aromatic amino acid 3,4-dihydroxyphenyl-D-alanine (*D-DOPA*).¹³⁻¹⁵

Findrik *et al.* have developed a mathematical model for biotransformation of D-methionine into L-methionine in the cascade of four enzymes systems.¹ This model is formulated as a set of non-linear differential equations describing the mass balance of the concentration of D-methionine, 2-oxo-4-methylthiobutyric acid, L-methionine, ammonium, nicotinamide adenine dinucleotide coenzyme and nicotinamide adenine dinucleotide hydrogen. It is observed that even though the results are compared with experimental results, they are only be obtained at discrete points depending on the step size provided. This creates a shortcoming since four cascade enzyme models are represented as a dynamical system using coupled PDE.

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 four enzyme systems based on new homotopy perturbation method. This is an effective tool for solve the nonlinear problems in chemical sciences.¹⁶ 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.

*e-mail: dr.rajendran.l@gmail.com

NOMENCLATURE

Symbol	Meaning
r_1	Enzyme reaction rate of <i>D</i> -AAO catalyzed D-methionine oxidation (mmol dm ⁻³ min ⁻¹ mg ⁻¹)
r_2	Enzyme reaction rate of <i>L</i> -PheDH catalyzed 2-oxo-4-methylthiobutyric acid reductive amination (mmol dm ⁻³ min ⁻¹ mg ⁻¹)
r_3	Enzyme reaction rate of <i>L</i> -PheDH catalyzed L-methionine oxidation (mmol dm ⁻³ min ⁻¹ mg ⁻¹)
r_4	Enzyme reaction rate of <i>FDH</i> catalyzed NAD ⁺ reduction (mmol dm ⁻³ min ⁻¹ mg ⁻¹)
V_{m1}	Maximum enzyme reaction rate (mmol dm ⁻³ min ⁻¹ mg ⁻¹)
V_{m2}	Maximum enzyme reaction rate (mmol dm ⁻³ min ⁻¹ mg ⁻¹)
V_{m3}	Maximum enzyme reaction rate (mmol dm ⁻³ min ⁻¹ mg ⁻¹)
V_{m4}	Maximum enzyme reaction rate (mmol dm ⁻³ min ⁻¹ mg ⁻¹)
c_{D-met}	Concentration of D-methionine (mmol dm ⁻³)
c_{2-oxo}	Concentration of 2-oxo-4-methylthiobutyric acid (mmol dm ⁻³)
c_{L-met}	Concentration of L-methionine (mmol dm ⁻³)
$c_{NH_4^+}$	Concentration of ammonium (mmol dm ⁻³)
c_{NAD^+}	Concentration of nicotinamide adenine dinucleotide coenzyme (mmol dm ⁻³)
c_{NADH}	Concentration of nicotinamide adenine dinucleotide hydrogen (mmol dm ⁻³)
c_F	Concentration of formate (mmol dm ⁻³)
K_m^{D-met}	Michaelis-Menten constant of D-methionine (mmol dm ⁻³)
K_i^{2-oxo}	Product inhibition constant of 2-oxo-4-methylthiobutyric acid (mmol dm ⁻³)
K_i^{NADH}	Product inhibition constant of Nicotinamide adenine dinucleotide hydrogen (mmol dm ⁻³)
K_m^{2-oxo}	Michaelis-Menten constant of 2-oxo-4-methylthiobutyric acid (mmol dm ⁻³)
K_m^{NADH}	Michaelis-Menten constant of Nicotinamide adenine dinucleotide hydrogen (mmol dm ⁻³)
$K_m^{NH_4^+}$	Michaelis-Menten constant of Ammonium (mmol dm ⁻³)
K_m^{L-met}	Michaelis-Menten constant of L-methionine (mmol dm ⁻³)
$K_m^{NAD^+}$	Michaelis-Menten constant of Nicotinamide adenine dinucleotide coenzyme (mmol dm ⁻³)
K_m^F	Michaelis-Menten constant of Formate (mmol dm ⁻³)
a_1 to a_5	Enzyme reaction rate (mmol dm ⁻³ min ⁻¹)
K_1 to K_{10}	Michaelis-Menten constant (min ⁻¹)

MATHEMATICAL FORMULATION OF THE PROBLEM

The reaction scheme of four enzyme systems *D*-AAO, *L*-PheDH, *FDH* and coupled system of D-methionine into L-methionine are represented in Figure 1. The mass balance equations with corresponding

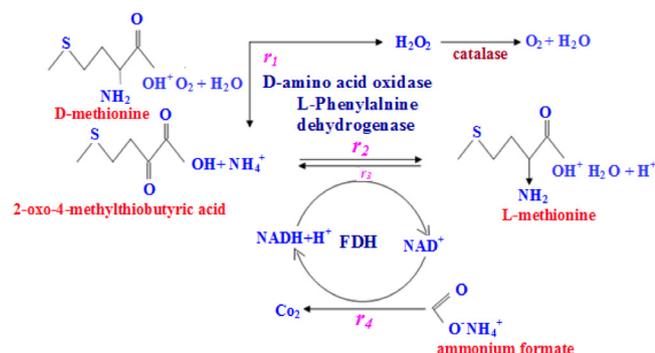


Figure 1. Kinetic scheme of *D*-AAO, *L*-PheDH, *FDH* and Coupled system of *D*-methionine bioconversion into *L*-methionine¹

boundary conditions and analytical expressions for the concentrations are shown in the following sections.

D-AAO Kinetics

Mass balances equations for D-methionine and 2-oxo-4-methylthiobutyric acid, in the batch reactor with the Michaelis-Menten kinetics are formulated as:¹

$$\frac{dc_{D-met}}{dt} = -r_1(t) \quad (1)$$

$$\frac{dc_{2-oxo}}{dt} = r_1(t) \quad (2)$$

The enzyme reaction rate of *D*-AAO catalyzed by D-methionine oxidation (r_1) is

$$r_1(t) = \frac{V_{m1} \cdot c_{D-met}(t) \gamma_{D-AAO}}{K_m^{D-met} \cdot \left(1 + \frac{c_{2-oxo}(t)}{K_i^{2-oxo}}\right) + c_{D-met}(t)} \quad (3)$$

The initial conditions for above equations (1) and (2) are given below:

$$c_{D-met} = (c_{D-met})_0, c_{2-oxo} = (c_{2-oxo})_0, at t = 0 \quad (4)$$

where c_{D-met} , c_{2-oxo} are the concentration of D-methionine and 2-oxo-4-methylthiobutyric acid (competitive product). V_{m1} is the maximal enzyme reaction rate, K_m^{D-met} is the Michaelis-Menten constant of D-methionine and K_i^{2-oxo} is the product inhibition constant of 2-oxo-4-methylthiobutyric acid. Solving the above nonlinear equations (1)-(2) using new homotopy perturbation method (Appendix A), we can obtain the analytical expressions of concentration of D-methionine and 2-oxo-4-methylthiobutyric acid as follows:

$$c_{D-met}(t) = (c_{D-met})_0 \exp[-(K_0 t)] \quad (5)$$

$$c_{2-oxo}(t) = (c_{D-met})_0 + (c_{2-oxo})_0 - (c_{D-met})_0 \exp[-(K_0 t)] \quad (6)$$

where,

$$K_0 = \frac{V_{m1} \gamma_{D-AAO}}{K_m^{D-met} \left[1 + \frac{(c_{2-oxo})_0}{K_{i2}^{2-oxo}}\right] + (c_{D-met})_0} \quad (\text{min}^{-1}) \quad (7)$$

Substituting c_{2-oxo} from the Equation 6 in the Equation 1, we can write the rate Equation 8a as

$$\frac{dc_{D-met}}{dt} = r_1(t) = -\frac{V_{m1} c_{D-met}(t) \gamma_{D-AAO}}{K_m^{D-met} + \frac{K_m^{D-met}}{K_{i2}^{2-oxo}} \left[(c_{D-met})_0 + (c_{2-oxo})_0 - (c_{D-met})_0 \exp[-(K_0 t)] \right] + c_{D-met}(t)} \quad (8a)$$

Similarly the rate equation r_1 in terms of concentration c_{2-oxo} can be obtained as follows:

$$\frac{dc_{2-oxo}}{dt} = r_1(t) = \frac{V_{m1} \cdot (c_{D-met})_0 \cdot (c_{2-oxo})_0 + (c_{D-met})_0 \exp[-(K_0 t)] \gamma_{D-AAO}}{K_m^{D-met} + \frac{K_m^{D-met}}{K_{i2}^{2-oxo}} c_{2-oxo}(t) + (c_{D-met})_0 (c_{2-oxo})_0 + (c_{D-met})_0 \exp[-(K_0 t)]} \quad (8b)$$

Equation 8a represents the new simple expression of the enzyme reaction rate of D-methionine in terms of c_{D-met} , whereas the Equation 8b represents enzyme reaction rate of 2-oxo-4-methylthiobutyric acid in terms of c_{2-oxo} .

L-PheDH Kinetics

In this case, the rate equations of concentration of L-methionine, 2-oxo-4-methylthiobutyric acid, ammonium, nicotinamide adenine dinucleotide and nicotinamide adenine dinucleotide hydrogen are given as follows:¹

$$\frac{dc_{L-met}}{dt} = r_2(t) - r_3(t) \quad (9)$$

$$\frac{dc_{2-oxo}}{dt} = -r_2(t) + r_3(t) \quad (10)$$

$$\frac{dc_{NH_4^+}}{dt} = -r_2(t) + r_3(t) \quad (11)$$

$$\frac{dc_{NAD^+}}{dt} = r_2(t) - r_3(t) \quad (12)$$

$$\frac{dc_{NADH}}{dt} = -r_2(t) + r_3(t) \quad (13)$$

The enzyme reaction rate of reductive amination of 2-oxo-4-methylthiobutyric acid (r_2 in Equation 14) is described by three substrate Michaelis-Menten kinetics. The enzyme reaction rate of the reverse reaction, L-methionine oxidation (r_3 in Equation 15) is described by double-substrate Michaelis-Menten equation with the inclusion of the competitive NADH inhibition.

$$r_2(t) = \frac{V_{m2} \cdot c_{2-oxo}(t) \cdot c_{NADH}(t) \cdot c_{NH_4^+}(t) \cdot \gamma_{L-pheDH}}{(K_m^{2-oxo} + c_{2-oxo}(t)) \cdot (K_m^{NADH} + c_{NADH}(t)) \cdot (K_m^{NH_4^+} + c_{NH_4^+}(t))} \quad (14)$$

$$r_3(t) = \frac{V_{m3} \cdot c_{L-met}(t) \cdot c_{NAD^+}(t) \cdot \gamma_{L-pheDH}}{(K_m^{NAD^+} \cdot (1 + c_{NADH}(t)/K_m^{NADH}) + c_{NAD^+}(t)) \cdot (K_m^{L-met} + c_{L-met}(t))} \quad (15)$$

The initial conditions for above equations are given below:

$$c_{NAD^+} = (c_{NAD^+})_0, c_{NADH} = (c_{NADH})_0, c_{2-oxo} = (c_{2-oxo})_0, \\ c_{L-met} = (c_{L-met})_0, c_{NH_4^+} = (c_{NH_4^+})_0, \text{ at } t = 0 \quad (16)$$

where V_{m2} and V_{m3} are maximum enzyme reaction rate, K_m^{2-oxo} , K_m^{NADH} , $K_m^{NH_4^+}$, $K_m^{NAD^+}$, K_m^{L-met} are the Michaelis-Menten constant of 2-oxo-4-methylthiobutyric acid, Nicotinamide adenine dinucleotide hydrogen, Ammonium, Nicotinamide adenine dinucleotide respectively. K_{i2}^{NADH} is the product inhibition constant of nicotinamide adenine dinucleotide hydrogen. Solving the above non-linear equations 9-13 using new HPM (Appendix A), the analytical expression of concentration of L-methionine, 2-oxo-4-methylthiobutyric acid, ammonium, nicotinamide adenine dinucleotide (NAD^+) and nicotinamide adenine dinucleotide hydrogen ($NADH$) are obtained as follows:

$$c_{L-met}(t) = \left(\frac{a_1}{K_1}\right) + \left[(c_{L-met})_0 - \left(\frac{a_1}{K_1}\right)\right] \exp(-K_1 t) \quad (17)$$

$$c_{2-oxo}(t) = \left(\frac{a_2}{K_2}\right) + \left[(c_{2-oxo})_0 - \left(\frac{a_2}{K_2}\right)\right] \exp(-K_2 t) \quad (18)$$

$$c_{NH_4^+}(t) = \left(\frac{a_2}{K_3}\right) + \left[(c_{NH_4^+})_0 - \left(\frac{a_2}{K_3}\right)\right] \exp(-K_3 t) \quad (19)$$

$$c_{NAD^+}(t) = \left(\frac{a_1}{K_4}\right) + \left[(c_{NAD^+})_0 - \left(\frac{a_1}{K_4}\right)\right] \exp(-K_4 t) \quad (20)$$

$$c_{NADH}(t) = \left(\frac{a_2}{K_5}\right) + \left[(c_{NADH})_0 - \left(\frac{a_2}{K_5}\right)\right] \exp(-K_5 t) \quad (21)$$

where the parameters a_1 , a_2 and K_1 to K_5 are given in the Appendix B.

FDH Kinetics

In this case, the mass balance equation for formate, nicotinamide adenine dinucleotide coenzyme and nicotinamide adenine dinucleotide hydrogen oxidase enzyme membrane reactor are represented as follows:¹

$$\frac{dc_F}{dt} = r_4(t) \quad (22)$$

$$\frac{dc_{NAD^+}}{dt} = r_2(t) - r_3(t) - r_4(t) \quad (23)$$

$$\frac{dc_{NADH}}{dt} = r_2(t) - r_3(t) - r_4(t) \quad (24)$$

The enzyme reaction rate of NAD^+ reduction by FDH kinetics (Eqn (25)) is

$$r_4(t) = \frac{V_{m4} \cdot c_F(t) \cdot c_{NAD^+}(t) \cdot \gamma_{FDH}}{(K_{m2}^{NAD^+} \cdot (1 + c_{NADH}(t)/K_{i3}^{NADH}) + c_{NAD^+}(t)) \cdot (K_m^F + c_F(t))} \quad (25)$$

The Eqns (22-24) are solved for the following initial conditions:

$$c_F = (c_F)_0, c_{NAD^+} = (c_{NAD^+})_0, c_{NADH} = (c_{NADH})_0, \text{ at } t = 0 \quad (26)$$

where V_{m4} is a maximum enzyme reaction rate and $K_{m2}^{NAD^+}$, K_m^F are the Michaelis-Menten constant of Nicotinamide adenine dinucleotide and Formate. K_{i3}^{NADH} is the product inhibition constant of Nicotinamide adenine dinucleotide hydrogen. Solving the above mentioned non-linear equations (22) - (24) using new Homotopy perturbation method (Appendix A), we get the analytical expression of concentrations of formate, nicotinamide adenine dinucleotide (NAD^+) and nicotinamide adenine dinucleotide hydrogen ($NADH$) oxidase in enzyme membrane reactor as follows:

$$c_F(t) = (c_F)_0 \exp(K_6 t) \quad (27)$$

$$c_{NAD^+}(t) = \left(\frac{a_3}{K_7}\right) + \left[(c_{NAD^+})_0 - \left(\frac{a_3}{K_7}\right)\right] \exp(-K_7 t) \quad (28)$$

$$c_{NADH}(t) = \left(\frac{a_4}{K_8}\right) + \left[(c_{NADH})_0 - \left(\frac{a_4}{K_8}\right)\right] \exp(-K_8 t) \quad (29)$$

where the parameters a_3 , a_4 and K_6 to K_8 are given in the Appendix B

Coupled kinetics

In this case, the rate equation of 2-oxo-4-methylthiobutyric acid and ammonium are given as follows:¹

$$\frac{dc_{2-oxo}}{dt} = r_1(t) - r_2(t) + r_3(t) \quad (30)$$

$$\frac{dc_{NH_4^+}}{dt} = r_1(t) - r_2(t) + r_3(t) \quad (31)$$

where the reaction rate is

$$r_1(t) = \frac{V_{m1} \cdot c_{D-met}(t) \cdot \gamma_{D-AAO}}{\left(K_m^{D-met} \cdot (1 + c_{2-oxo}(t)/K_{i2}^{2-oxo} + c_{NADH}(t)/K_i^{NADH}) + c_{D-met}(t)\right)} \quad (32)$$

The reaction rate r_2 and r_3 are given in the Equations 14 and (15). The initial conditions for the above equations are given below:

$$c_{2-oxo} = (c_{2-oxo})_0, c_{NH_4^+} = (c_{NH_4^+})_0, \text{ at } t=0 \quad (33)$$

Solving the non-linear Equations 30 and (31) using new Homotopy perturbation method, we can obtain the approximate analytical expression for the concentration of 2-oxo-4-methylthiobutyric acid and ammonium as follows:

$$c_{2-oxo}(t) = \left(\frac{a_5}{K_9}\right) + \left[(c_{2-oxo})_0 - \left(\frac{a_5}{K_9}\right) \right] \exp(-K_9 t) \quad (34)$$

$$c_{NH_4^+}(t) = \left(\frac{a_5}{K_{10}}\right) + \left[(c_{NH_4^+})_0 - \left(\frac{a_5}{K_{10}}\right) \right] \exp(-K_{10} t) \quad (35)$$

where the parameters a_5 and K_9 to K_{10} are given in the Appendix B.

NUMERICAL SIMULATION

The non-linear differential equations 1, 2, 9-13, 22-24, 30 and 31 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_{D-met} using Equation 5 and c_{2-oxo} using Equation 6 for the D-AAO kinetics are compared with simulation results (Scilab program 4.1) in Tables 1-2. The Scilab program is also given in Appendix C. We run scilab 4.1 on apple imac core i5. The maximum error between our analytical results and simulation results 0.5%. Similarly our analytical results for the concentration of ammonium, nicotinamide adenine dinucleotide coenzyme and nicotinamide adenine dinucleotide hydrogen are compared with

Table 1. Comparison of analytical result with simulation result for the concentration of c_{D-met} using Equation 5

Time t (min)	Concentration of c_{D-met} (mmol dm ⁻³)		% of deviation between simulation result and Equation 5
	Simulation result	Analytical result Equation 5	
0	9.992700	10	0.07
1	9.402076	9.401787	0.003077
10	5.396787	5.396398	0.007204
20	2.910123	2.912111	-0.06832
30	1.572126	1.571491	0.040373
40	0.849807	0.848039	0.208076
50	0.457956	0.457636	0.070032
60	0.245974	0.246958	-0.40035

Table 2. Comparison of analytical result with Simulation result for the concentration of c_{2-oxo} using Equation 6

Time t (min)	Concentration of c_{2-oxo} (mmol dm ⁻³)		% of deviation between simulation result and Equation 6
	Simulation result	Analytical result Equation 6	
0	0.100200	0.100000	0.20
1	0.697964	0.698213	-0.03572
10	4.703253	4.703602	-0.00742
20	7.189917	7.187889	0.02821
30	8.527914	8.528509	-0.00697
40	9.250233	9.251961	-0.01868
50	9.642084	9.642364	-0.00291
60	9.854066	9.853042	0.010399

numerical results and available experimental results¹ in Figures 2-7. Satisfactory agreement is found for all values of time t .

RESULTS AND DISCUSSION

D-AAO Kinetics

Equations 5 and 6 are the new and simple approximate analytical expression of the concentrations of D-methionine and 2-oxo-4-methylthiobutyric acid. The rate of reaction can be determined from

Table 3. Experimental Values of Parameter Use in This Work and Findrik *et al.*¹

Parameter	Value	Parameter	Value
D-AAO kinetics		FDH kinetics	
V_{m1}	37.93 (U mg ⁻¹) or 63.2167 (mmol dm ⁻³ mg ⁻¹ min ⁻¹)	V_{m4}	0.63 (U mg ⁻¹) or 1.05 (mmol dm ⁻³ mg ⁻¹ min ⁻¹)
K_m^{D-met}	0.23 (mmol dm ⁻³)	K_m^F	12.80 (mmol dm ⁻³)
K_i^{2-oxo}	1.26 (mmol dm ⁻³)	$K_{m2}^{NAD^+}$	0.039 (mmol dm ⁻³)
K_i^{NADH}	0.022 (mmol dm ⁻³)	K_{i3}^{NADH}	0.289 (mmol dm ⁻³)
γ_{D-AAO}	0.01 (mg)	γ_{FDH}	0.85 (mg)
L-pheDH kinetics			
V_{m2}	59.211 (U mg ⁻¹) or 98.65 (mmol dm ⁻³ mg ⁻¹ min ⁻¹)	V_{m3}	0.42 (U mg ⁻¹) or 0.7 (mmol dm ⁻³ mg ⁻¹ min ⁻¹)
K_m^{2-oxo}	1.06 (mmol dm ⁻³)	K_m^{L-met}	4.02 (mmol dm ⁻³)
K_m^{NADH}	0.05 (mmol dm ⁻³)	$K_m^{NAD^+}$	0.44 (mmol dm ⁻³)
$K_m^{NH_4^+}$	76.99 (mmol dm ⁻³)	K_{i2}^{NADH}	0.0027 (mmol dm ⁻³)
$\gamma_{L-pheDH}$	10 (mg)		

the graphs for a specified time, by measuring the gradient of the graphs using a tangent. Figure 2(a) signifies the comparison of the concentration of 2-oxo-4-methylthiobutyric acid and D-methionine versus time t . From Figure 2(b), it is observed that when concentration $D\text{-met}$ increases when the parameter $\gamma_{D\text{-AAO}}$ decreases. From Figure 2(c), it is inferred that when concentration 2-oxo increases when the parameter $\gamma_{D\text{-AAO}}$ increases. Our analytical results for the enzyme reaction rate (r_1) versus concentration of D-methionine (Equation 8a) and concentration of 2-oxo-4-methylthiobutyric acid (Equation 8b) are compared with experimental results for some fixed values of parameters in Figure 2(d) and 2(e) and satisfactory agreement is noted.

L-PheDH Kinetics

Equations 17-21 represent the analytical expressions of the concentrations of L-methionine, 2-oxo-4-methylthiobutyric acid, Ammonium, Nicotinamide adenine dinucleotide and Nicotinamide adenine dinucleotide hydrogen. Figures 3(a)-3(g) signifies the concentrations of L-methionine, 2-oxo-4-methylthiobutyric acid, Ammonium, Nicotinamide adenine dinucleotide and Nicotinamide adenine dinucleotide hydrogen for various values of parameter $\gamma_{L\text{-PheDH}}$ and for all time it remains domain. Figure 3(a), signifies that the comparison of L-methionine, 2-oxo-4-methylthiobutyric acid versus time t . Figure 3(b), represents the comparison of the concentration of ammonium, Nicotinamide adenine dinucleotide and Nicotinamide adenine dinucleotide hydrogen versus time t . From Figures 3(c) and 3(f), it is noted that the concentrations of L-methionine and Nicotinamide adenine dinucleotide decreases when the parameter $\gamma_{L\text{-PheDH}}$ increases. From Figures 3(d), (e) and 3(g), it is obvious that the concentration of 2-oxo-4-methylthiobutyric acid, Ammonium and Nicotinamide adenine dinucleotide hydrogen increases when the parameter $\gamma_{L\text{-PheDH}}$ increases. Our analytical results for enzyme reaction rate (r_2) versus the concentrations of 2-oxo-4-methylthiobutyric acid, Nicotinamide adenine dinucleotide hydrogen, Ammonium are compared with experimental results for some fixed values of parameters in Figures

4(a)-4(c). The enzyme reaction rate (r_3) versus the concentrations of L-methionine, Nicotinamide adenine dinucleotide and Nicotinamide adenine dinucleotide are compared with the experimental results for some fixed values of other parameters in Figures 4(d)-4(f).

FDH Kinetics

Equations 27-29 identify the analytical expressions of the concentrations of formate, Nicotinamide adenine dinucleotide and Nicotinamide adenine dinucleotide hydrogen. Figure 5(a) represents the comparison of the concentration of Nicotinamide adenine dinucleotide and Nicotinamide adenine dinucleotide hydrogen versus time t . From Figure 5(b) it is clear that when the parameter γ_{FDH} increases the concentration of Nicotinamide adenine dinucleotide decreases. From Figure 5(c) it is evident that when the concentration γ_{FDH} increases the concentration of Nicotinamide adenine dinucleotide hydrogen also increases. In our analytical results, enzyme reaction rate (r_4) versus the concentration of formate c_{F_3} , Nicotinamide adenine dinucleotide and Nicotinamide adenine dinucleotide coenzyme are compared with the experimental results for some fixed values of other parameters in Figures 5(d)-5(f).

Coupled kinetics

Equations 34 and 35 characterizes the analytical expressions of the concentration of 2-oxo-4-methylthiobutyric acid and Ammonium. From Figure 6(a) and 6(b) show the concentration of 2-oxo-4-methylthiobutyric acid and Ammonium versus time t for some fixed values of other parameters. Concentrations of 2-oxo-4-methylthiobutyric acid and Ammonium decreases when the parameter $\gamma_{D\text{-AAO}}$ increases. Influence of the parameter on the concentration is also reported.

Estimation of Kinetic Parameters

To check the validity of the model against the experimental

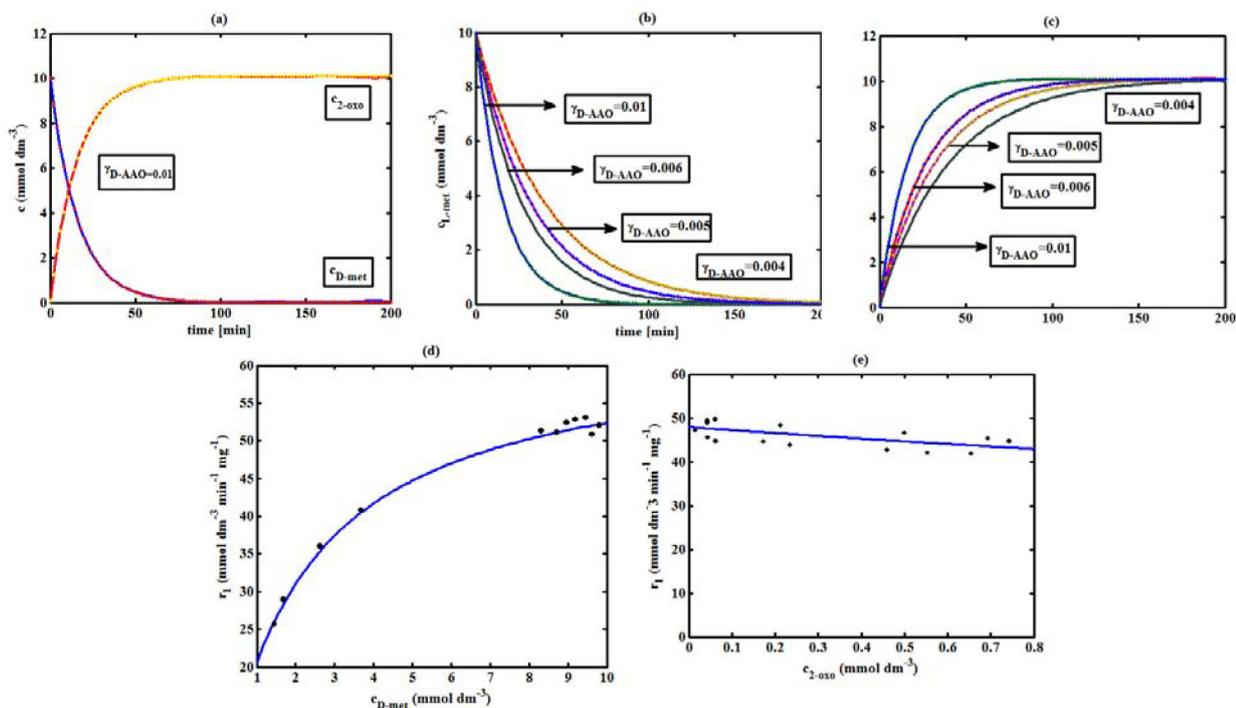


Figure 2. D-AAO kinetics (a) Comparison between the theoretical results (Equations 5 and (6)) and simulation results. (b) Comparison between theory (Equation 5) and simulation results. (c) Comparison between theory (Equation 6) and simulation results. (d)-(e) Comparison of theoretical results with experimental results¹ for the reaction rate r_1 . The numerical value of the kinetic parameters used for the above figure is given in Table 1

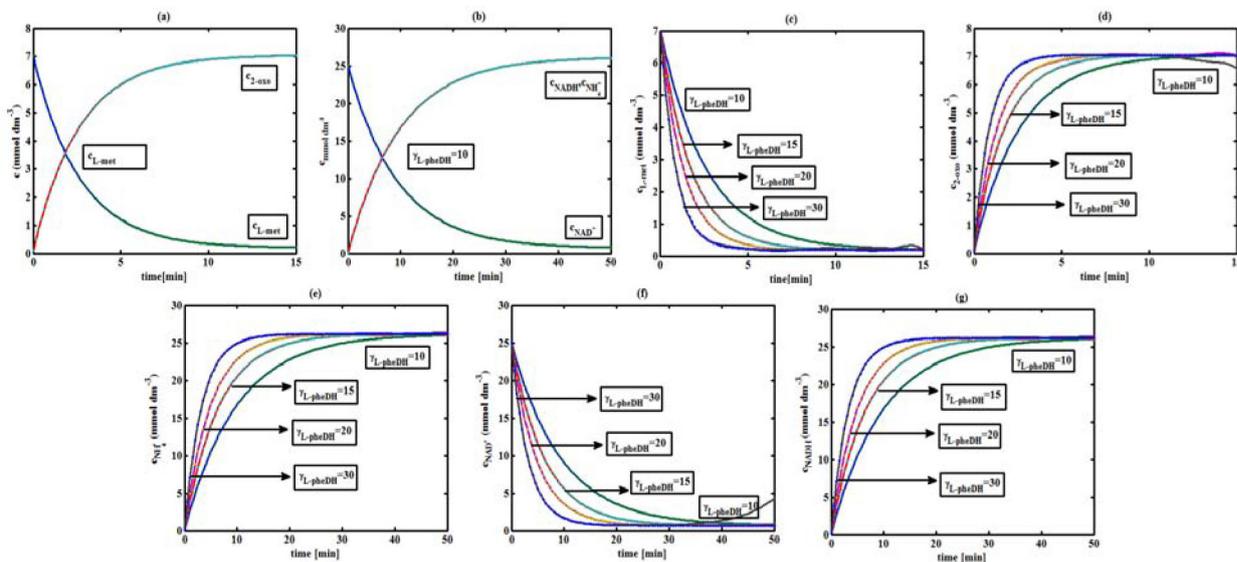


Figure 3. *L*-pHeDH kinetics (a) Comparison between the theoretical results (Equations 17 and (18)) and simulation results. (b) Comparison between the theoretical results (Equations 19, (20) and (21)) and simulation results. Concentration of (c) *L*-methionine (Equation 17), (d) 2-oxo-4-methylthiobutyric acid (Equation 18), (e) Ammonium (Equation 19), (f) Nicotinamide adenine dinucleotide (Equation 20), (g) Nicotinamide adenine dinucleotide hydrogen (Equation 21) versus time *t*. The numerical value of the kinetic parameters used for the above figure is given in Table 1. Key to the graph: (—) represents the (Equations 17-(21)) and (....) represents the numerical results

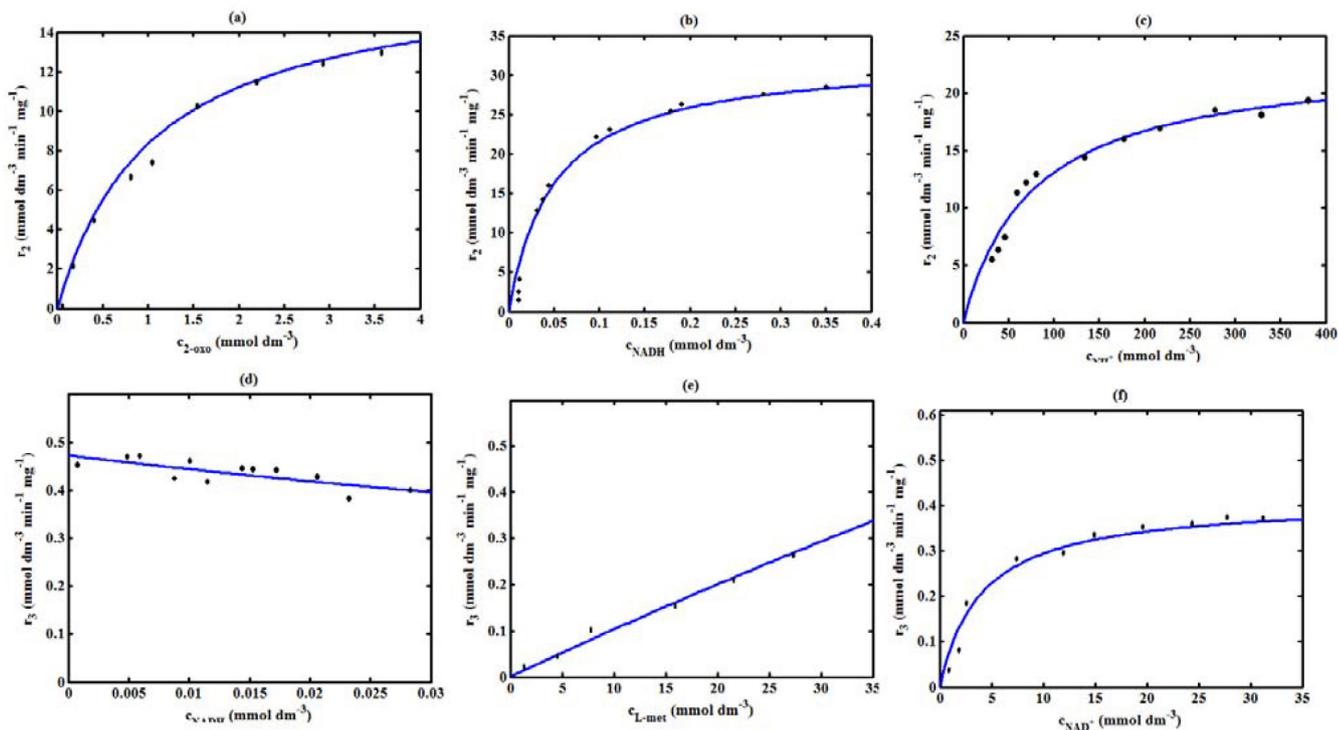


Figure 4. Comparison of theoretical results with the experimental results for the reaction rate r_2 and r_3 . The numerical value of the kinetic parameters used for the above figure is given in Table 1

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 three parameters in Equation 1 can be evaluated by means of non-linear least-squares fit. Equation 3 can be rewritten as

$$\frac{1}{r_1} = \left[\frac{K_m^{D-met} - K_i^{2-oxo}}{V_{m1} \gamma_{D-AAO} K_i^{2-oxo}} \right] - \left[\frac{K_m^{D-met} (K_i^{2-oxo} + c_i)}{V_{m1} \gamma_{D-AAO} K_i^{2-oxo}} \right] \frac{1}{c_{D-met}} \quad (36)$$

As shown in Figure 7, Plot of $1/r_1$ versus $1/(c_{D-met})$ gives the slope $\left[\frac{K_m^{D-met} (K_i^{2-oxo} + c_i)}{V_{m1} \gamma_{D-AAO} K_i^{2-oxo}} \right]$ and y-intercept $\left[\frac{K_m^{D-met} - K_i^{2-oxo}}{V_{m1} \gamma_{D-AAO} K_i^{2-oxo}} \right]$. If

we know the maximum enzyme reaction rate V_{m1} , we can obtain the other parameter Michaelis-Menten constant of D-methionine K_m^{D-met} and product inhibition constant of 2-oxo-4-methylthiobutyric acid K_i^{2-oxo} . As K_i tends to infinity the equation reduces to the form of Michaelis-Menten kinetics¹⁰ for which the Lineweaver–Burk plot¹⁷ is commonly used to determine the parameter values.

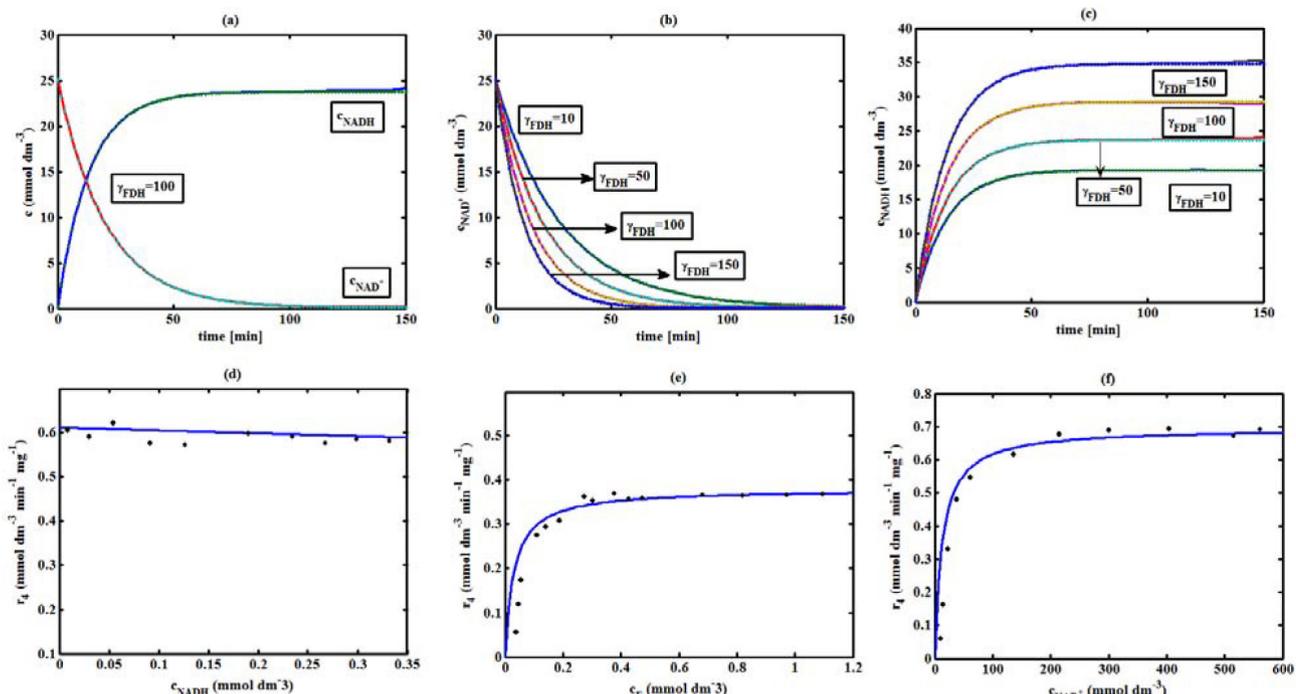


Figure 5. FDH kinetics (a) Comparison between the theoretical results (Equations(28) and (29)) and simulation results. (b) Concentration of Nicotinamide adenine dinucleotide (Equation 28), (c) Nicotinamide adenine dinucleotide hydrogen (Equation 29) versus time t . (d-f) Comparison of theoretical results with the experimental results ¹ for the reaction rate r_i . The numerical value of the kinetic parameters used for the above figure is given in Table 3

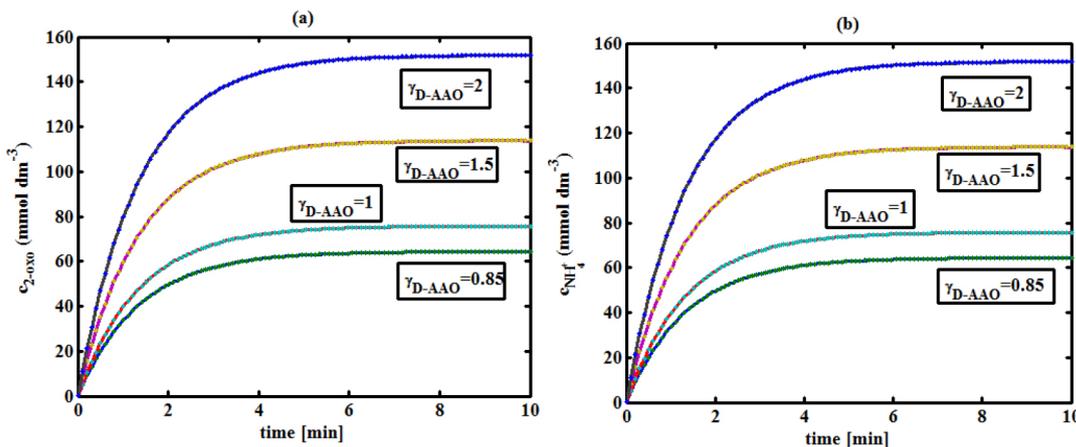


Figure 6. (a) Variation of the concentration of 2-oxo-4-methylthiobutyric acid (c_{2-oxo}) with time t at different γ_{D-AAO} using Equation 34. (b) Variation of the concentration of ammonium ($c_{NH_4^+}$) with time t at different γ_{D-AAO} using Equation 35

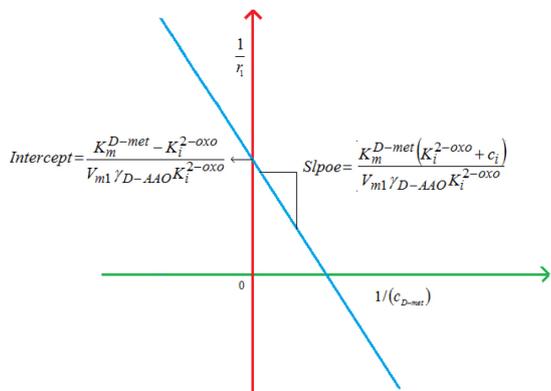


Figure 7. Estimation of kinetic parameters K_m^{D-met} and K_i^{2-oxo} using Equation 36. The numerical value of the kinetic parameters used for the above figure is given in Table 3

Also the rate equation (1) can be written as

$$\frac{dc_{D-met}}{dt} \approx b(1) + (b(2)/c_{D-met}) \tag{37}$$

where the constant

$$b(1) = \frac{V_{m1} \gamma_{D-AAO} K_{i2}^{2-oxo}}{\alpha_2}, \quad b(2) = \frac{V_{m1} \gamma_{D-AAO} K_{i2}^{2-oxo} \alpha_1}{\alpha_2^2},$$

$$\alpha_1 = K_m^{D-met} (K_{i2}^{2-oxo} + c_i), \quad \alpha_2 = K_{i2}^{2-oxo} - K_m^{D-met} \quad \text{and} \tag{38}$$

$$c_i = (c_{D-met})_0 + (c_{2-oxo})_0$$

If we know the experimental data i.e (t_i, c_{D-met}), we can obtain the numerical values of the constant $b(1)$ ($=0.7721$) and $b(2)$ ($=1.9645$) using the Matlab program (Appendix D). From the numerical values, the kinetic constant K_m^{D-met} , K_{i2}^{2-oxo} , V_{m1} and γ_{D-AAO} can be obtained.

Estimation of parameter from our analytical result Eqn (5)

The Equation 5 can be rewritten as,

$$\log(c_{D-met}) = n \log[(c_{D-met})_0] - K_0 t \quad (39)$$

Using the method of Least chi-square we get,

$$\sum \log(c_{D-met}) = n \log[(c_{D-met})_0] - K_0 \sum t \quad (40)$$

The parameter K_0 can be obtained from the above Equation 40. From this parameter we can obtain the V_{m1} , γ_{D-AAO} , K_m^{D-met} , K_i^{2-oxo} by changing the initial concentration of c_{D-met} . Similarly the parameter for other kinetics scheme can be obtained. The numerical value of the parameter $b(1)$ estimated from the Equation 39 is 0.773 where as the value of the parameter estimated from the Matlab/Scilab program is 0.07721. This validate our analytical model against the experimental data.

CONCLUSIONS

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 2-oxo-4-methylthiobutyric acid, L-methionine, Ammonium, Nicotinamide adenine dinucleotide, Nicotinamide adenine dinucleotide hydrogen and Formate. The analytical expressions are compared to the numerical simulation using Scilab software. Good agreement is noted. Theoretical evaluation of the kinetic parameter is also reported.

SUPPLEMENTARY MATERIAL

The supplementary data associated with this article can be found at <http://qumicanova.sbg.org.br> in pdf format with free access.

ACKNOWLEDGEMENTS

The author very much grateful to the referees for the valuable suggestions. The authors are thankful to Mr. S. Mohamed Jaleel, The Chairman, Dr. A. Senthilkumar, The Principal, Sethu Institute of Technology, Kariapatti-626115, Tamilnadu, India for their encouragement.

REFERENCES

- Findrik, Z.; Vasić-Rački, Đ.; *Biotechnol. Bioeng.* **2007**, *98*, 956.
- Bruggink, A.; Schoevaart, R.; Kieboom, T.; *Org. Process Res. Dev.* **2003**, *7*, 622.
- Bae, H. S.; Lee, S. G.; Hong, S. P.; Kwak, M. S.; Esaki, N.; Soda, K.; *J. Mol. Catal. B: Enzym.* **1999**, *6*, 241.
- Hummel, W.; Schmidt, E.; Wandrey, C.; Kula, M. R.; *Appl Microbiol Biotechnol.* **1986**, *25*, 175.
- Michaelis, L.; Menten, M. L.; *Biochem. Z.* **1913**, *49*, 333.
- Murray, J. D.; *Mathematical Biology*, Springer-Verlag: New York, **2002**.
- Kirthiga, O. M.; Rajendran, L.; *J. Electroanal. Chem.* **2015**, *751*, 119.
- Rajendran, L.; Anitha, S.; *Electrochim. Acta* **2013**, *102*, 474.
- Rajendran, L.; Rahamathunissa, G.; *J. Math. Chem.* **2008**, *44*, 849.
- Eswari, A.; Rajendran, L.; *J. Electroanal. Chem.* **2010**, *641*, 35.
- Cho, B. K.; Cho, H. J.; Park, S. H.; Yun, H.; Kim, B. G.; *Biotechnol. Bioeng.* **2003**, *81*, 783.
- Cho, B. K.; Seo, J. H.; Kang, T. J.; Kim, J.; Park, H. Y.; *Biotechnol. Bioeng.* **2006**, *94*, 842.
- Findrik, Z.; Vasić-Rački, Đ.; Lütz, S.; Daubmann, T.; Andrey, C.; *Biotechnol. Lett.* **2005**, *27*, 1087.
- Findrik, Z.; Vasić-Rački, Đ.; Geueke, B.; Kuzu, M.; Hummel, W.; *Eng. Life Sci.* **2005**, *5*, 550.
- Findrik, Z.; Geueke, B.; Hummel, W.; Vasić-Rački, Đ.; *Biochem. Eng. J.* **2006**, *27*, 275.
- He, H. J.; *Electrochim. Acta* **2013**, *109*, 617.
- Lineweaver, H.; Burk, D.; *J. Am. Chem. Soc.* **1934**, *56*, 658.