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Identification of intracellular kinetic parameters in continuous bioconversion of glycerol by *Klebsiella pneumoniae*

En-min Feng[1,*] Chong-yang Liu[1,†] Zhao-hua Gong[1]
Ya-qin Sun[2]

[1]Department of Applied Mathematics
Dalian University of Technology, Dalian 116024, Liaoning, China
[2]Department of Biotechnology
Dalian University of Technology, Dalian 116012, Liaoning, China

Abstract In this paper, we propose a hybrid nonlinear dynamical system to describe the concentration changes of extracellular and intracellular substances of glycerol bioconversion to 1,3-propanedol (1,3-PD) in microbial continuous cultures. It is proved that the solution to the system exists and is continuous with respect to kinetic parameters. Subsequently, a novel quantitative definition of biological robustness is investigated. We present a performance index based on experiment data of extracellular concentrations and biological robustness. Taking the proposed hybrid nonlinear dynamical system as a constraint, we establish an identification model to determine the most reasonable metabolic pathway and optimal kinetic parameters. The identifiability of the proposed model is also ascertained. This work provides theoretical basis for future numerical simulation and optimization computation.

Keywords Hybrid dynamical system; Continuous culture; Parameters identification; Robustness analysis

1 Introduction

1,3-PD possesses potential applications on a large commercial scale, especially as a monomer of polyesters or polyurethanes. Chemical synthesis, which uses valuable metal as catalyst, is the main approach to produce 1,3-PD. However, this approach has many shortcomings, such as environmental pollution, high cost and so on. Microbial production of 1,3-PD is particularly attractive to industry recently due to its low cost, high production, no pollution, etc. It is considered to be one of the bulk chemicals, which is likely to be produced by bio-processes on large scales. Recently, it is urgent to produce bio-diesel fuel due to energy scarce. Glycerol-containing water is expected to be produced in enormous quantities, indicating the significance of 1,3-PD production derived from this type of process [1].

*Email: emfeng@dlut.edu.cn
†Email: chongyangliu@yahoo.cn

The core of computational biology consists of mathematical modelling the dynamic behavior of the important intracellular components, numerical simulation, robust analysis, etc. At present, many researches mainly concern robustness simulation on specific dynamics. See [2, 3, 4] and references cited therein. However, all these researches fail to present quantitative descriptions of biological robustness. Recently, many studies about dynamic modelling for the bioconversion of glycerol to 1,3-PD have been carried out. An excess kinetic model of microbial production of 1,3-PD in 1995 was proposed in [5]. Later the model was improved by Xiu et al. in [6]. The optimality conditions, hybrid nonlinear system, parameters identification, and optimal control of 1,3-PD production in fed-batch culture were discussed in [7, 8, 9]. However, some important intermediate substances, intracellular substances, and enzymes, which play significant roles in glycerol metabolism, are not considered in the previous researches. In addition, the interrelationships among metabolic flows, substrates, enzymes, intermediates and products within cells are also not involved.

In this paper, based on the dynamical model concerning enzymatic catalyses and transports of glycerol and 1,3-PD across cell membrane [10], we propose a hybrid nonlinear dynamical system to describe the concentration changes of extracellular and intracellular substances in microbial continuous cultures. Then some properties of the system are proved. Subsequently, a novel quantitative formulation of biological robustness is investigated. We give a performance index on the basis of experimental data of extracellular concentrations and the biological robustness. Taking the proposed hybrid nonlinear dynamical system as constraints, an identification model to determine the most reasonable metabolic pathway and optimal kinetic parameters is established. The identifiability of the model is also proved. This work provides theoretical basis for future numerical simulation and optimization computation.

2 Hybrid Nonlinear Dynamical System

In this paper, omitting parts of enzymatic catalyses, we consider the effects of some enzymatic catalyses on substrates and products within cells. In this way, the computational load can be reduced greatly.

Based on [1] and [10], the hybrid nonlinear dynamical system $HNLD(l,i)$ concerning enzymatic catalyses and transports of glycerol and 1,3-PD can be described as

$$\begin{cases} \dot{x}(t) = f(x(t), l, i, v(l), q(i)), & t \in [t_0, t_f], \\ x(t_0) = x_0, & (l,i) \in L_{tri} \times G_{cn}, \end{cases} \tag{1}$$

where $x(t) \in R_+^{ks}$ is the state variable with ks components of the ith dynamical system, $f : R_+^{ks} \times L_{tri} \times G_{cn} \times Dc \times D_s(i) \to R^{ks}$ is the rate of reactions, $L_{tri} = \{1, 2, \cdots, tri\}$ is the serial number set of experiments, and $l \in L_{tri}$. $v(l) = (D(l), c_{s0}(l))^T \in D_c(l) \subset R_+^2$, $D_c(l)$ is the admissible set of dilution rate and initial glycerol concentration in lth experiment. $G_{cn} = \{1, 2, \cdots, gcn\}$ is the serial number set of possible metabolic pathways, and gcn is the total number of possible metabolic pathways. $q(i) = (k_{i,1}, k_{i,2}, \cdots, k_{i,ds(i)})^T \in D_s(i)$ is the kinetic parameter vector in the ith dynamical system, $ds(i)$ is the total number of kinetic parameters. Since each component of the state variable $x(t)$ represents a certain substance concentration, there exists a nonempty bounded closed region $W_a \subset R_+^{ks}$ such

that solution $x(t;v(l),q(i))$ of $HNLD(l,i)$ is in W_a. In addition, $D_c(l)$ and $D_s(i),(l,i) \in L_{tri} \times G_{cn}$, are nonempty bounded closed sets.

Because the relationships among substrates, intracellular substances and enzymes haven't been fully determined in experiments, the metabolic pathways have 72 possible cases according to mechanism analysis. That is, in the above model the total number (gcn) of metabolic pathways is 72. For simplicity, we only discuss system $HNLD(1,1)$ corresponding to the first experiment and the first case. Other models have properties similar to those of $HNLD(1,1)$. $HNLD(1,1)$ is expressed as follows.

$$\begin{cases} \dot{x}_1 = (\mu - D(1))x_1 \\ \dot{x}_2 = D(1)(c_{s0}(1) - x_2) - p_2 x_1 \\ \dot{x}_3 = k_{1,1}(x_8 - x_3)x_1 - D(1)x_3 \\ \dot{x}_4 = p_4 x_1 - D(1)x_4 \\ \dot{x}_5 = p_5 x_1 - D(1)x_5 \\ \dot{x}_6 = \frac{1}{k_{1,2}}(k_{1,3}\frac{x_2}{x_2 + k_{1,4}} + k_{1,5}(x_2 - x_6) - p_2) - \mu x_6 \\ \dot{x}_7 = k_{1,6}u_1\frac{x_6}{k^*_{m1}(1 + \frac{x_7}{k_{1,7}}) + x_6} - k_{1,8}u_2\frac{x_7}{k^*_{m2} + x_7(1 + \frac{x_7}{k_{1,9}})} - \mu x_7 \\ \dot{x}_8 = k_{1,8}u_2\frac{x_7}{k^*_{m2} + x_7(1 + \frac{x_7}{k_{1,9}})} - k_{1,10}(x_8 - x_3) - \mu x_8 \end{cases} \tag{2}$$

In (2), $D(1)$ and $c_{s0}(1)$ are the dilution rate and the initial glycerol concentration of the first experiment. k^*_{m1}, k^*_{m2} are given constants. The specific growth rate of cells μ, specific consumption rate of substrate p_2, specific formation rates of products p_i, $i = 4,5$, and u_i, $i = 1,2$, are expressed as follows.

$$\mu = \mu_m \frac{x_2}{x_2 + k^*_s}\prod_{i=2}^{5}(1 - \frac{x_i}{x^*_i}), \tag{3}$$

$$p_2 = m_2 + \frac{\mu}{Y_2} + \Delta_2\frac{x_2}{x_2 + k^*_2}, \tag{4}$$

$$p_4 = m_4 + \mu Y_4 + \Delta_4\frac{x_2}{x_2 + k^*_4}, \tag{5}$$

$$p_5 = p_2(\frac{c_1}{b_1 + Dx_2} + \frac{c_2}{b_2 + Dx_2}), \tag{6}$$

$$u_1 = k_{1,11} + k_{1,12}\mu + k_{1,13}\frac{x_2}{x_2 + k_{1,14}}, \tag{7}$$

$$u_2 = k_{1,15} + k_{1,16}\mu + k_{1,17}\frac{x_2}{x_2 + k_{1,18}}. \tag{8}$$

In (3)-(8), $\mu_m, k^*_s, k^*_2, k^*_4, \Delta_2, \Delta_4, m_2, m_4, c_i, b_i (i = 1,2)$ are given constants, respectively. Moreover, $x^*_i (i = 1,2,\cdots,5)$ are given critical concentrations. $q(1) = (k_{1,1}, k_{1,2}, \cdots, k_{1,18}) \in D_s(1) \subset R^{18}$ is the parameter vector in the first case.

According to the transport mechanisms of glycerol and 1,3-PD across cell membrane, we assume that

$(A1)$ The absolute values of differences between intracellular and extracellular concentrations of glycerol and 1,3-PD have upper bounds M_1 and M_2, respectively.

Under assumption $(A1)$, we can easily obtain the following properties of system (1).

Property 2.1 For any pair $(l,i) \in L_{tri} \times G_{cn}$, $v(l) \in D_c(l)$ and $q(i) \in D_s(i)$, function $f(x(t),l,i,v(l),q(i))$ satisfies that $f \in C([t_0,t_f];R^{ks})$ and f is locally Lipschitz continuous in x on R^{ks}_+.

Property 2.2 For any pair $(l,i) \in L_{tri} \times G_{cn}$, $v(l) \in D_c(l)$ and $q(i) \in D_s(i)$, function $f(x(t),l,i,v(l),q(i))$ satisfies linear growth condition, i.e., there exist positive constants $\alpha,\beta > 0$ such that

$$\|f(x(t),l,i,v(l),q(i))\| \leq \alpha + \beta\|x(t)\|, \ \forall\, t \in [t_0,t_f],$$

where $\|\cdot\|$ is Eulcidean norm.

Proof: For $f(x(t),1,1,v(1),q(1))$ the linear growth condition is satisfied as the following proof. For any $x(t) \in R^{ks}_+, v(1) \in D_c(1)$ and $q(1) \in D_s(1)$, we know that

$$|f_1(x(t),1,1,v(1),q(1))| \leq (|\mu_m| + |D(1)|)|x_1|.$$

Letting $L_1 = |\mu_m| + |D(1)|$, we obtain that $|f_1(x(t),1,1,v(1),q(1))| \leq L_1\|x\|$.
Furthermore, let $L_2 = \max\{|D(1)|, |m_2| + |\mu_m||Y_2| + |\Delta_2|\}$. Since

$$|f_2(x(t),1,1,v(1),q(1))| \leq |D(1)||c_{s0}| + |D(1)||x_2| + (|m_2| + |\mu_m||Y_2| + |\Delta_2|)|x_1|,$$

we must conclude that $|f_2(x(t),1,1,v(1),q(1))| \leq |D(1)||C_{s0}(1)| + L_2\|x\|$.
Let $L_3 = \max\{\ |k_{1,1}||M_2|, |D(1)|\}$. Then

$$|f_3(x(t),1,1,v(1),q(1))| \leq |k_{1,1}||M_2||x_1| + |D(1)||x_3|,$$

and we have that $|f_3(x(t),1,1,v(1),q(1))| \leq L_3\|x\|$.
Set $L_4 = \max\{|m_4| + |\mu_m||Y_3| + |\Delta_3|, |D(1)|\}$. Since

$$|f_4(x(t),1,1,v(1),q(1))| \leq (|m_4| + |\mu_m||Y_3| + |\Delta_3|)|x_1| + |D(1)||x_4|,$$

we obtain that $|f_4(x(t),1,1,v(1),q(1))| \leq L_4\|x\|$.
Let $L_5 = \max\{(|m_2| + |\mu_m||Y_2| + |\Delta_2|)(|\frac{c_1}{b_1}| + |\frac{c_2}{b_2}|), |D(1)|\}$. Since

$$|f_5(x(t),1,1,v(1),q(1))| \leq (|q_2|(|\frac{c_1}{b_1}| + |\frac{c_2}{b_2}|))|x_1| + |D(1)||x_5|,$$

we obtain that $|f_5(x(t),1,1,v(1),q(1))| \leq L_5\|x\|$.
Let $L_6 = |\frac{k_{1,5}}{k_{1,2}}| + |\mu_m|$. Since

$$|f_6(x(t),1,1,v(1),q(1))| \leq |\frac{k_{k_{1,5}}}{k_{1,2}}||x_2| + (|\frac{k_{1,5}}{k_{1,2}}| + |\mu_m|)|x_6| + (|\frac{q_2}{k_{1,2}}| + |\frac{k_{1,3}}{k_{1,2}}|),$$

we obtain that $|f_6(x(t),1,1,v(1),q(1))| \leq L_6\|x\| + (|\frac{q_2}{k_{1,2}}| + |\frac{k_{1,3}}{k_{1,2}}|)$.
Let $L_7 = |\mu_m|$. So

$$|f_7(x(t),1,1,v(1),q(1))| \leq |k_{1,6}||u_1| + |k_{1,8}||u_2| + |\mu_m||x_7|,$$

and we see that $|f_7(x(t),1,1,v(1),q(1))| \le L_7\|x\| + |k_{1,6}||u_1| + |k_{1,8}||u_2|$.
Let $L_8 = |k_{1,9}| + |\mu_m|$. Then

$$|f_8(x(t),1,1,v(1),q(1))| \le L_8\|x\| + |k_{1,8}||u_2|.$$

Let $\beta = \frac{\sqrt{2}}{2}\max\{L_1,\cdots,L_8\}$ and $\alpha = \frac{\sqrt{2}}{2}\max\{|D(1)||c_{s0}(1)|, |k_{1,8}|(|k_{1,15}| + |k_{1,16}||\mu_m| + |k_{1,17}|) + |k_{1,6}|(|k_{1,11}| + |k_{1,12}||\mu_m| + |k_{1,13}|), \frac{|m_2|+|\mu_m||Y_2|+|\Delta_2|}{|k_{1,2}|} + |\frac{k_{1,3}}{k_{1,2}}|\}$. In view of the boundedness of $D_c(1)$ and $D_s(1)$, we must conclude that

$$\|f(x(t),1,1,v(1),q(1))\| \le \alpha + \beta\|x(t)\|,\ \forall\, t \in [t_0,t_f].$$

In the same way, it can be proved that for any $(l,i) \in L_{tri} \times G_{cn}$ and $q \in D_s(i)$ the function $f(x(t),l,i,v(l),q(i))$ defined in (1) satisfies linear growth condition. □

Property 2.3 For any pair $(l,i) \in L_{tri} \times G_{cn}$, $v(l) \in D_c(l)$ and $q(i) \in D_s(i)$, system (1) has a unique solution, denoted by $x(t;v(l),q(i))$. Furthermore, $x(t;v(l),q(i))$ is continuous with respect to $q(i)$.

Proof: Since f is continuous with respect to $q(i) \in D_s(i)$, it follows from Property 2.1 and Property 2.2 that system (1) has a unique solution $x(t;v(l),q(i))$. In addition, $x(t;v(l),q(i))$ is continuous in q on $D_s(i)$ in term of the theory of continuous dependence of solution to differential equations on parameters. □

Now, let us denote the solution set of $HNLD(l,i)$ corresponding to $v(l) \in D_c(l)$ and $q(i) \in D_s(i)$ by

$$\begin{aligned} S(l,i) \triangleq \quad \{x(t;v(l),q(i)) \quad & |x(t;v(l),q(i)) \text{ is the solution of } HNLD(l,i) \\ & \text{corresponding to } v(l) \in D_c(l) \text{ and } q(i) \in D_s(i)\}. \end{aligned} \tag{9}$$

Furthermore, let $D_{ws}(i)$ be the set of the kinetic parameters $q(i)$ such that the solution of $HNLD(l,i)$ in W_a, i.e.,

$$D_{ws}(i) \triangleq \{q(i) \in D_s(i) | x(t;v(l),q(i)) \in S(l,i) \text{ and } x(t;v(l),q(i)) \in W_a\}. \tag{10}$$

3 Robust Performance

In this section, the robust performance is proposed in two steps. Firstly, the sum of square deviations between computational values and experimental data is computed. Secondly, a mathematical definition of biological robustness is established.

3.1 Square Deviation

Since only the concentrations of extracellular components are measured, we denote the index set of extracellular components of the state variable $x(t;v(l),q(i))$ by C_{out} ($C_{out} = \{1,2,\cdots,5\}$). Hence, the sum of square deviations between computational concentrations of extracellular components $x_k(t;v(l),q(i))(k \in C_{out})$ in the ith dynamical system and experimental concentrations $y(t_j,l,k)$ can be defined as

$$Dev(i,q) \triangleq \sum_{l \in L_{tri}} \sum_{k \in C_{out}} \sum_{j=1}^{nt(l)} (x_k(t_j;v(l),q(i))) - y(t_j,l,k))^2, \tag{11}$$

where $nt(l)$ is the number of measurements in the lth experiment. Moreover, we define

$$Devm(i) \triangleq \min\{Dev(i,q)|q \in D_{ws}(i)\}, \tag{12}$$

$$Devsa \triangleq \min_{i \in Gcn} Devm(i) + \gamma, \tag{13}$$

where γ is a given positive constant. Now, if $Devm(i) \geq Devsa$, then the ith dynamical system is invalid and is switched to the $i+1th$ one. Otherwise, we will calculate the robustness of the ith dynamical system.

3.2 Mathematical Definition of Biological Robustness

In order to investigate the robustness of dynamical systems, we only discuss the robustness of kinetic parameters in main state components. In this subsection, we propose an quantitative method to compute the robustness of some components in the ith dynamical system.

Randomly generate finite number of parameters $q(i)$ of the ith dynamical system by uniform distribution, and define the sample set of parameters as

$$D_{ss}(i) \triangleq \{q|Dev(i,q) \leq Devsa, q \in D_{ws}(i)\}. \tag{14}$$

Let $n_{spe}(i) \triangleq |D_{ss}(i)| < \infty$. For each $q \in D_{ss}(i)$, we compute state variable $x(t;v(l),q(i))$ of $HNLD(l,i)$. Therefore, the sample set $ss(i)$ of trajectory for component $k \in C_{rob}$ can be defined as

$$ss(i) \triangleq \{x_k(t;v(l),q(i)), t \in [t_0,t_f]|x(t;v(l),q(i)) \in S(l,i), \forall l \in Ltri, q(i) \in D_{ss}(i), k \in C_{rob}\}.$$

Obviously, $ss(i)$ is a subset of continuous function space $C([t_0,t_f];R)$.

Now, compute the expectation and variance of the sample curves set by the following definitions.

Definition 3.1 The deviation of sample curves corresponding to $q_1, q_2 \in D_{ss}(i)$ is

$$Var(i,q_1,q_2) \triangleq \sum_{l=1}^{L_{tri}} \sum_{k \in C_{rob}} \int_{t_0}^{t_f} (x_k(t;v(l),q_1(i)) - x_k(t;v(l),q_2(i)))^2 dt. \tag{15}$$

Definition 3.2 For given $q_0 \in D_{ss}(i)$, the maximal deviation between $x(t;v(l),q_0(i))$ and $x(t;v(l),q(i))$ is

$$Rob(i,q_0) \triangleq \max\{Var(i,q_0,q)|q \in D_{ss}(i)\}. \tag{16}$$

Let

$$Rob(i) \triangleq \min\{Rob(i,q)|q \in D_{ss}(i)\}, \tag{17}$$

$$q_e(i) \triangleq arg\min\{Rob(i,q)|q \in D_{ss}(i)\}. \tag{18}$$

For the ith $HNLD(l,i)$, we call $x_k(t;v(l),q_e(i)), k \in C_{rob}$, the expected sample curve of sample set $ss(i)$ and $q_e(i)$ the expected parameters.

If we denote the deviation set with regard to $q_e \in D_{ss}(i)$ by

$$Svar(i) \triangleq \{Var(i,q_e,q)|q \in D_{ss}(i)\}, \tag{19}$$

then

$$Svar(i) \subset [0, Rob(i,q_e)] \subset R^+. \tag{20}$$

Let n_d as the number of equal partition for $Rob(i,q_e)$. We obtain

$$drob \triangleq \frac{Rob(i,q_e)}{n_d}, \quad \upsilon_k \triangleq k \cdot drob, \text{ and } \upsilon_{n_d} = Rob(i,q_e).$$

For $k = 0, 1, \cdots, n_d - 1$, the set

$$Subv(i,k) \triangleq \{q \in D_{ss}(i) | \upsilon_k \leq Var(i,q_e,q) < \upsilon_{k+1}\} \tag{21}$$

can be computed. In addition, $nSubv(i,k) \triangleq |Subv(i,k)|$.

Basing on the above analysis, we give the measurement for the robustness of dynamical system as follows.

Definition 3.3 The robustness of dynamical system $HNLD(l,i)$ defined by (1) can be measured by the value

$$\frac{Rob(i,q_e)}{nSubv(i,0)}, \tag{22}$$

where $nSubv(i,0) \triangleq |Subv(i,0)|$. Furthermore, the smaller the value (22) is, the more robust the dynamical system is.

4 Identification Model Concerning Enzymatic Catalysis

For the *ith* dynamical system concerning enzymatic catalyses, which satisfies the requirement of deviation between computational value and experimental data of extracellular components, we can take (22) as a performance index. In view of (15), (16) and (17) in the previous section, the performance index can also be written as

$$J(i,q_e(i)) = \frac{\max\{\sum_{l=1}^{tri} \sum_{k \in C_{rob}} \int_{t_0}^{t_f} (x_k(t;v(l),q_e(i)) - x_k(t;v(l),q(i)))^2 dt | q(i) \in D_{ss}(i)\}}{nSubv(i,0)}. \tag{23}$$

Taking the hybrid nonlinear dynamical system as constraints, we can establish the identification model concerning enzymatic catalyses as follows.

$$\begin{array}{lll} \text{(IP)} & \min & J(i,q_e(i)) \\ & \text{s.t.} & x(t;v(l),q(i)), x(t;v(l),q_e(i)) \in S(l,i), \\ & & v(l) \in D_c(l), q(i),\ q_e(i) \in D_{ss}(i), \\ & & (l,i) \in L_{tri} \times G_{cn}. \end{array} \tag{24}$$

Theorem 4.1 Identification model (IP) is identifiable.

Proof: In view of the Property 2.1 and Property 2.2, the set $S(l,i)$ is nonempty. Furthermore, since $D_{ss}(i)$ and $D_c(l)$ are compact sets and L_{tri} and G_{cn} are finite sets, the identifiability of identification model must be obtained. □

5 Conclusion and Discussion

In this paper, a hybrid nonlinear dynamical system of bioconversion of glycerol to 1,3-propanediol by *Klebsiella pneumoniae* based on enzymatic catalyses is investigated. Some properties of the hybrid system are proved. Then a novel mathematical definition of biological robustness is proposed. To determine the most reasonable metabolic pathway and optimal kinetic parameters, we establish an identification model and prove its identifiablity.

In the future, we will consider the numerical simulation by discretizing the integer in performance index and construct optimization algorithms to obtain the optimal kinetic parameters. Optimal control problems in continuous cultures will be also discussed in later research works.

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