Modeling and Robustness Analysis of Biochemical Networks of Glycerol Metabolism by *Klebsiella Pneumoniae*

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Abstract. Glycerol bioconversion to 1,3-propanediol (1,3-PD) by *Klebsiella pneumoniae* (*K. pneumoniae*) can be characterized by an intricate network of interactions among biochemical fluxes, metabolic compounds, key enzymes and genetic regulatory. To date, there still exist some uncertain factors in this complex network because of the limitation in biotechniques, especially in measuring techniques for intracellular substances. In this paper, among these uncertain factors, we aim to infer the transport mechanisms of glycerol and 1,3-PD across the cell membrane, which have received intensive interest in recent years. On the basis of different inferences of the transport mechanisms, we reconstruct various metabolic networks correspondingly and subsequently develop their dynamical systems (S-systems). To determine the most reasonable metabolic network from all possible ones, we establish a quantitative definition of biological robustness and undertake parameter identification and robustness analysis for each system. Numerical results show that it is most possible that both glycerol and 1,3-PD pass the cell membrane by active transport and passive diffusion.

Keywords: Metabolic network inference, Biological robustness, Transport across cell membrane, Parameter identification.

1 Introduction

The bioconversion of glycerol to 1,3-PD is particularly attractive to industry because of the increasing glycerol surplus on the market and the potential uses of the product 1,3-PD. The latter is discussed as a bifunctional chemical reagent on a large commercial scale, especially as a monomer for polyesters, polyethers and polyurethanes. Since the 1980s, a number of computational models have been developed to describe the fermentation process of glycerol by *K. pneumoniae*. The latest ones proposed and modified by Zeng et al. [1,2,3] have been widely

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used in computer simulation. Nevertheless, some important intracellular intermediate substances (such as 3-hydroxypropionaldehyde) and enzymes (such as glycerol dehydratase and 1,3-PD oxydoreductase), which play important roles in glycerol metabolism, were not taken into consideration in the above models. Therefore, these models are limited in providing a qualitative understanding of the underlying metabolic network, even though they have successful predictions about the fermentation process.

Recently, metabolic network of glycerol by *K. pneumoniae* has received intensive interest [4,5,6,7], since it is helpful for strain selection and genetic modification for increasing 1,3-PD production. The metabolic pathways of glycerol in the intracellular environment consist of oxidative and reductive components. Sun et al. [8] proposed a mathematical model to describe the reductive pathway, in which the interrelationships among substrate, key enzymes, intermediates and target product were considered. By comparing *K. pneumoniae* with *E. coli*, Sun et al. assumed that glycerol passes the cell membrane by both passive diffusion and active transport and 1,3-PD is transported by passive diffusion. The model can successfully simulate experimental results and forecast intracellular metabolites concentration for continuous cultures. But fundamental properties of this metabolic network (such as robustness) were not considered in [8].

Robustness is a property that allows a system to maintain its functions despite external and internal perturbations. It is one of the fundamental characteristics of biological systems. Numerous reports have been published on this topic, especially in the past decades [9,10,11,12,13,14,15]. N. Barkai et al. [11] argued that the key properties of biochemical networks are robust, that is, they are relatively insensitive to the precise values of biochemical parameters. This point of view, which has been observed for a wide variety of experiments [16,17,18], is being gradually accepted by experts in the field of systems biology.

In this paper, we study the fermentations of glycerol covering both extracellular and intracellular environments. The two environments are linked by the transports of substrate (glycerol) and product (1,3-PD) across the cell membrane, which haven't been completely observed in experiments yet. All possible transport mechanisms are under consideration. Different metabolic networks are reconstructed based on distinct inferences of transport mechanisms and correspondingly different dynamic systems are developed. Then parameters in each system are identified to minimize the relative error between experimental data and computational results. Since only extracellular data can be measured in experiments, it is still hard to know which inference of the transport mechanisms is the most reasonable. To cope with this problem, robustness analysis is carried out for each system. We first propose a quantitative definition of biological robustness. After that, robust performance is calculated for each system and the reasonability of these systems are measured by their robust performances.

This paper is organized as follows. In Section 2, all possible dynamical systems of glycerol dissimilation are developed and parameters in each system are identified. In Section 3, a quantitative definition of biological robustness is established and robust performance of each system is calculated to determine the

most reasonable metabolic network. Some conclusions are presented at the end of this paper.

2 Modeling and Parameter Identification

In this section, taking the transports of glycerol and 1,3-PD across cell membrane into consideration, we develop three kinetic models to describe the reductive pathway of glycerol dissimilation. Moreover, parameters are identified for each system based on 30 groups of experimental data reported in [1,2,3].

2.1 Kinetic Models

During glycerol metabolism by *K. pneumoniae* under anaerobic condition, glycerol is first transported across the cell membrane from the extracellular environment to the intracellular environment, and then is further catabolized, reactions catalyzed by enzymes, to generate intermediates and final products, e.g., 3-hydroxypropionaldehyde (3-HPA), 1,3-PD, acetic acid, ethanol, etc. Finally, the products are transported across the cell membrane from the intracellular environment to the extracellular environment. As shown in Fig. 1, the transport mechanisms of glycerol and 1,3-PD across the membrane haven't been observed in experiments yet. Glycerol may pass the membrane by both passive diffusion and active transport or by passive diffusion only. So is the transport of 1,3-PD. In addition, since the molecular weight of glycerol is larger than that of 1,3-PD, it is thought that 1,3-PD will pass the cell membrane only by passive diffusion if glycerol is transported in this pattern [19]. Therefore, three possible cases are discussed, and kinetic systems are developed according to different inferences of the transport mechanisms.

All dynamical models developed in this paper are power-law systems (S-systems), which were first proposed by Savageau et al. [20]. As is known to us, Michaelis-Menten kinetics have been widely used in the study of biological dynamical systems. However, if several substrates or reactions are involved, and if several modulators affect the pathway, the system quickly becomes quite complicated. Another disadvantage of Michaelis-Menten kinetics is that one should clearly know the mechanisms of the constituent enzymes in the pathway before formulating its mathematical model. It is hard to image that complex pathways could be successful described and numerically identified in terms of their detailed enzyme mechanisms by Michaelis-Menten kinetics. Different from Michaelis-Menten kinetics, S-systems are equivalent to linearization in logarithmic coordinates, which are especially convenient for steady-state analysis in large-scale systems.

According to the factual experiments, we make the following assumptions.

(**H1**) The extracellular and intracellular concentrations of substances are uniform in reactor and in cells, respectively.
(**H2**) The effect of the microbial growth on the concentrations of intracellular substances is ignored.

(**H3**) The oxidative pathway can afford the reductive pathway enough reducing power.

Under the above assumptions and according to Fig. 1, three S-systems are developed as follows.

Case 1. Glycerol and 1,3-PD pass cell membrane by both passive diffusion and active transport. Then the dynamical system, denoted by S(1), is formulated as:

$$\begin{cases} \dot{x_1}(t) = a_{1,1}x_1^{g_{1,1}}x_2^{g_{1,2}}x_3^{g_{1,3}}x_4^{g_{1,4}}x_5^{g_{1,5}} - b_{1,1}x_1^{h_{1,1}}D^{h_{1,2}} \\ \dot{x_2}(t) = a_{1,2}(\frac{c_{s0}}{x_2})^{g_{1,6}}D^{g_{1,7}} - b_{1,2}x_1^{h_{1,3}}x_2^{h_{1,4}}(\frac{x_2}{x_6})^{h_{1,5}} \\ \dot{x_3}(t) = a_{1,3}x_1^{g_{1,8}}x_8^{g_{1,9}}(\frac{x_8}{x_3})^{g_{1,10}} - b_{1,3}D^{h_{1,6}}x_3^{h_{1,7}} \\ \dot{x_4}(t) = a_{1,4}x_1^{g_{1,11}}x_2^{g_{1,12}} - b_{1,4}D^{h_{1,8}}x_4^{h_{1,9}} \\ \dot{x_5}(t) = a_{1,5}x_1^{g_{1,13}}x_2^{g_{1,14}} - b_{1,5}D^{h_{1,10}}x_5^{h_{1,11}} \\ \dot{x_6}(t) = a_{1,6}x_2^{g_{1,15}}(\frac{x_2}{x_6})^{g_{1,16}} - b_{1,6}x_6^{h_{1,12}}x_7^{h_{1,13}} \\ \dot{x_7}(t) = a_{1,7}x_6^{g_{1,17}}x_7^{g_{1,18}} - b_{1,7}x_7^{h_{1,14}} \\ \dot{x_8}(t) = a_{1,8}x_7^{g_{1,19}} - b_{1,8}x_8^{h_{1,15}}(\frac{x_8}{x_3})^{h_{1,16}} \\ x(t_0) = x^0 \end{cases} \quad t \in [t_0, t_f^1], \quad (1)$$

where x_1, x_2, x_3, x_4, x_5 are concentrations of biomass, glycerol, 1,3-PD, acetic acid, ethnol in reactor, respectively. x_6, x_7, x_8 are intracellular concentrations of glycerol, 3-HPA, 1,3-PD, respectively. x^0 is the initial state, which is restricted in

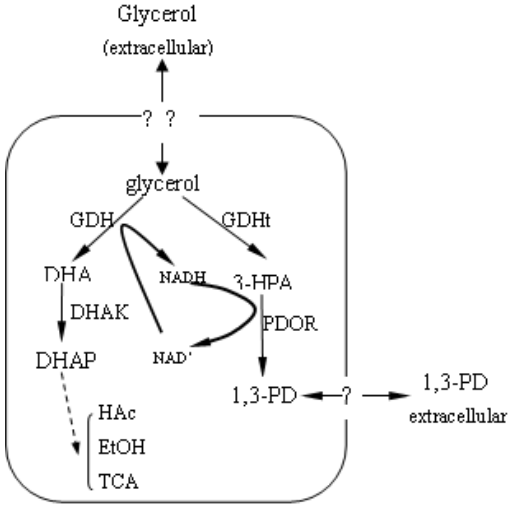

Fig. 1. Anaerobic metabolic pathways of glycerol fermentation. *Abbreviations*: GDHt, glycerol dehydratase; 3-HPA, 3-hydroxypropionaldehyde; 1,3-PD, 1,3-propanediol; PDOR, 1,3-PD oxydoreductase; GDH, glycerol dehydrogenase; DHA, dihydroxyacetone; DHAK, dihydroxyacetone kinase; DHAP, dihydroxyacetonephosphate; HAc, acetic acid; ETOH, ethnol; TCA, TCA cycle.

$S_0 := [0.001, x_1^*] \times [100, x_2^*] \times [0, x_3^*] \times [0, x_4^*] \times [0, x_5^*] \times [0, x_6^*] \times [0, x_7^*] \times [0, x_8^*]$. x_i^*, $i = 1, 2, \cdots, 8$, are the critical values of x_i, respectively. Biomass, glycerol, intermediate substances and products can not exceed their critical concentrations according to the practical production. t_f^1 is the terminal moment of the fermentation, i.e., the moment when the system S(1) reaches its steady state. D is dilution rate and c_{s_0} is substrate concentrate in feed, which are two of the main operating conditions in continuous fermentations. $a_{1,i}$ and $b_{1,i}$ ($i \in I_8 := \{1, 2, \cdots, 8\}$) are rate constants. $g_{1,j}$ ($j \in I_{19}$) and $h_{1,k}$ ($k \in I_{16}$) are kinetic orders.

For convenience, let $x := (x_1, x_2, \cdots, x_8)^T$ and $u^1 := (a_{1,1}, \cdots, a_{1,8}, b_{1,1}, \cdots, b_{1,8}, g_{1,1}, \cdots, g_{1,19}, h_{1,1}, \cdots, h_{1,16})^T$. Generally speaking, kinetic orders representing biochemical reactions or transport steps are very often in the range between 0 and 1, and inhibitory effects typically call for kinetic orders between 0 and -0.5. Rate constants are set in the range between 0 and 1000 in this paper. From the above statements, we set the range of the parameter vector u^1 as $\mathcal{U}^1$. In addition, according to experimental limitation, the range of the operating conditions vector $v = (D, c_{s_0})^T$ and the admissible set of state vector x are denoted by W_{ad} and Λ_{ad}, respectively. Let $f^1 := (f_1^1, f_2^1, \cdots, f_8^1)^T$, $f_i^1 : \Lambda_{ad} \times W_{ad} \times \mathcal{U}^1 \to R$, $i = 1, \cdots, 8$. Then we rewrite S(1) as:

$$\begin{cases} \dot{x}(t) &= f^1(x(t), v, u^1), \\ x(t_0) &= x^0 \ . \end{cases} \qquad t \in [t_0, t_f^1], \tag{2}$$

Case 2. Glycerol passes the cell membrane by both passive diffusion and active transport but 1,3-PD passes the cell membrane by passive diffusion only. Then we just need amend S(1) at:

$$\dot{x_3}(t) = a_{2,3} x_1^{g_{2,8}} (\frac{x_8}{x_3})^{g_{2,10}} - b_{2,3} D^{h_{2,6}} x_3^{h_{2,7}}, \tag{3}$$

$$\dot{x_8}(t) = a_{2,8} x_7^{g_{2,19}} - b_{2,8} (\frac{x_8}{x_3})^{h_{2,16}}, \tag{4}$$

where $a_{2,i}$ and $b_{2,i}$ ($i \in I_8$) are rate constants, $g_{2,j}$ ($j \in I_{19} \setminus \{9\}$) and $h_{2,k}$ ($k \in I_{16} \setminus \{15\}$) are kinetic orders. Denote the terminal moment by t_f^2, the parameter vector by u^2 and its range by $\mathcal{U}^2$. The amended system is denoted by S(2) with its right hand term $f^2(x(t), v, u^2)$.

Case 3. Both glycerol and 1,3-PD pass the cell membrane by passive diffusion only. Then we just need amend S(1) at:

$$\dot{x_2}(t) = a_{3,2} (\frac{c_{s_0}}{x_2})^{g_{3,6}} D^{g_{3,7}} - b_{3,2} x_1^{h_{3,3}} (\frac{x_2}{x_6})^{h_{3,5}}, \tag{5}$$

$$\dot{x_3}(t) = a_{3,3} x_1^{g_{3,8}} (\frac{x_8}{x_3})^{g_{2,10}} - b_{2,3} D^{h_{2,6}} x_3^{h_{2,7}}, \tag{6}$$

$$\dot{x_6}(t) = a_{3,6} (\frac{x_2}{x_6})^{g_{3,16}} - b_{3,6} x_6^{h_{3,12}} x_7^{h_{3,13}}, \tag{7}$$

$$\dot{x_8}(t) = a_{3,8} x_7^{g_{3,19}} - b_{3,8} (\frac{x_8}{x_3})^{h_{3,16}}, \tag{8}$$

where $a_{3,i}$ and $b_{3,i}$ ($i \in I_8$) are rate constants, $g_{3,j}$ ($j \in I_{19} \setminus \{9, 15\}$) and $h_{3,k}$ ($k \in I_{16} \setminus \{4, 15\}$) are kinetic orders. Denote the terminal moment by t_f^3, the parameter vector by u^3 and its range by $\mathcal{U}^3$. The amended system is denoted by S(3) with its right hand term $f^3(x(t), v, u^3)$.

2.2 Parameter Identification

Let $I_l := \{1, \cdots, l\}$ be the serial number set of experiments, where l is the total experiment times. For given $v^s, s \in I_l$, denote the experimental values of extracellular concentrations of reactants at steady stage as $y_1^s, y_2^s, y_3^s, y_4^s, y_5^s$ correspondingly. Let $y^s := (y_1^s, y_2^s, y_3^s, y_4^s, y_5^s)^T \in R^5, s \in I_l$. While reaching the steady state t_f^m, the solution of the system S(m) for given v^s satisfies that

$$f^m(x(t_f^m), v^s, u^m) = 0, \qquad m \in I_3 \ . \tag{9}$$

Although the concentrations of biomass, glycerol, 1,3-PD, acetic acid and ethanol in reactor are measured in experiments, this paper is only concerned with the relative error between experimental data and computational values of the first three substances for the reason that alkali is intermittently fed into the reactor to maintain its pH value at 7 or so, which has great effect on the extracellular concentrations of acetic acid and ethanol. Therefore, the parameter identification problem can be formulated as follows:

$$\begin{aligned} P(m): \ \min \ & \frac{1}{3} \sum_{n=1}^{3} \frac{\sum_{s \in I_l} |x_n^s - y_n^s|}{\sum_{s \in I_l} y_n^s} \\ \text{s.t.} \ & f^m(x^s, v^s, u^m) = 0, \quad s \in I_l, \\ & (x^s, u^m) \in \Lambda_{ad} \times \mathcal{U}^m \ . \end{aligned} \tag{10}$$

After taking logarithms and rearranging, we can rewrite $P(m)$ in the following form:

$$\begin{aligned} P'(m): \ \min \ & \frac{1}{3} \sum_{n=1}^{3} \frac{\sum_{s \in I_l} |z_n^s - \ln(y_n^s)|}{\sum_{s \in I_l} \ln(y_n^s)} \\ \text{s.t.} \ & A(u^m) \cdot z^s = b(u^m, v^s), \quad s \in I_l, \\ & (z^s, u^m) \in \Lambda'_{ad} \times \mathcal{U}^m \ . \end{aligned} \tag{11}$$

where $z^s := (z_1^s, \cdots, z_8^s)^T = (\ln(x_1^s), \cdots, \ln(x_8^s))^T$. $A(u^m)$ is an 8×8 matrix determined by u^m and $b(u^m, v^s)$ is a vector in R^8 with its value determined by u^m and v^s. Λ'_{ad} is a set transformed from Λ_{ad} in logarithmic coordinates. The detailed technique can be seen in [20].

The parameter identification problem $P(m)$ is equivalent to $P'(m)$, which is solved by an improved real-coded genetic algorithm introduced in [21]. To

Table 1. Identified parameters for S(1)

$a_{1,1}$	$a_{1,2}$	$a_{1,3}$	$a_{1,4}$	$a_{1,5}$	$a_{1,6}$	$a_{1,7}$	$a_{1,8}$
466.765	364.617	468.720	492.180	138.326	98.737	105.090	435.973
$b_{1,1}$	$b_{1,2}$	$b_{1,3}$	$b_{1,4}$	$b_{1,5}$	$b_{1,6}$	$b_{1,7}$	$b_{1,8}$
345.555	394.919	83.097	414.958	351.909	124.152	171.561	398.340
$g_{1,1}$	$g_{1,2}$	$g_{1,3}$	$g_{1,4}$	$g_{1,5}$	$g_{1,6}$	$g_{1,7}$	$g_{1,8}$
0.0791	-0.0651	-0.0176	-0.0393	-0.0276	0.8128	0.9679	0.02346
$g_{1,9}$	$g_{1,10}$	$g_{1,11}$	$g_{1,12}$	$g_{1,13}$	$g_{1,14}$	$g_{1,15}$	$g_{1,16}$
0.2204	0.6301	0.0596	-0.0630	0.4374	-0.0510	0.4619	0.8843
$g_{1,17}$	$g_{1,18}$	$g_{1,19}$					
0.2815	-0.0702	0.3402					
$h_{1,1}$	$h_{1,2}$	$h_{1,3}$	$h_{1,4}$	$h_{1,5}$	$h_{1,6}$	$h_{1,7}$	$h_{1,8}$
0.4326	0.2242	0.5	0.0005	0.9984	0.7153	0.4697	0.7623
$h_{1,9}$	$h_{1,10}$	$h_{1,11}$	$h_{1,12}$	$h_{1,13}$	$h_{1,14}$	$h_{1,15}$	$h_{1,16}$
0.4936	0.3657	0.4189	0.3807	-0.1195	0.3016	0.4697	0.4985

Table 2. Identified parameters for S(2)

$a_{2,1}$	$a_{2,2}$	$a_{2,3}$	$a_{2,4}$	$a_{2,5}$	$a_{2,6}$	$a_{2,7}$	$a_{2,8}$
50.3511	407.6265	349.4654	356.7966	163.7408	188.1783	177.4258	175.9596
$b_{2,1}$	$b_{2,2}$	$b_{2,3}$	$b_{2,4}$	$b_{2,5}$	$b_{2,6}$	$b_{2,7}$	$b_{2,8}$
64.5248	105.5797	49.3736	407.1377	483.8713	475.0738	104.1135	392.4753
$g_{2,1}$	$g_{2,2}$	$g_{2,3}$	$g_{2,4}$	$g_{2,5}$	$g_{2,6}$	$g_{2,7}$	$g_{2,8}$
0.0010	-0.0875	-0.0278	-0.0451	-0.0948	0.6613	0.8835	0.02102
$g_{2,9}$	$g_{2,10}$	$g_{2,11}$	$g_{2,12}$	$g_{2,13}$	$g_{2,14}$	$g_{2,15}$	$g_{2,16}$
—	0.5042	0.2116	-0.0639	0.4907	-0.0501	0.4971	0.2
$g_{2,17}$	$g_{2,18}$	$g_{2,19}$					
0.2121	-0.0450	0.2146					
$h_{2,1}$	$h_{2,2}$	$h_{2,3}$	$h_{2,4}$	$h_{2,5}$	$h_{2,6}$	$h_{2,7}$	$h_{2,8}$
0.4844	0.6004	0.4585	0.1271	0.5980	0.4166	0.4751	0.5918
$h_{2,9}$	$h_{2,10}$	$h_{2,11}$	$h_{2,12}$	$h_{2,13}$	$h_{2,14}$	$h_{2,15}$	$h_{2,16}$
0.3959	0.3228	0.2512	0.3847	-0.0623	0.4599	—	0.4824

deal with the equality constraint, each parameter vector as an individual is substituted into the linear equations in (11) to compute the corresponding state vector and its performance index. Convex crossover operator is used to ensure the offspring still lie in $\mathcal{U}_{ad}$. In addition, ranking selection and multi-Gaussian mutation operators are adopted in the algorithm.

The relative errors between experimental data and computational values and the identified parameters for each system and are shown in Tables 1-4. Numerical results show that there exists no great difference among the relative errors of the three systems. So it is hardly to determine the best one only by parameter identification.

Table 3. Identified parameters for S(3)

$a_{3,1}$	$a_{3,2}$	$a_{3,3}$	$a_{3,4}$	$a_{3,5}$	$a_{3,6}$	$a_{3,7}$	$a_{3,8}$
123.6634	304.0117	401.7615	39.1099	143.7021	291.7930	31.2899	452.1026
$b_{3,1}$	$b_{3,2}$	$b_{3,3}$	$b_{3,4}$	$b_{3,5}$	$b_{3,6}$	$b_{3,7}$	$b_{3,8}$
358.2629	114.8660	72.8336	417.4015	369.0153	180.3583	244.8731	334.8029
$g_{3,1}$	$g_{3,2}$	$g_{3,3}$	$g_{3,4}$	$g_{3,5}$	$g_{3,6}$	$g_{3,7}$	$g_{3,8}$
0.08264213	-0.0845	-0.022	-0.0378	-0.089	0.7546	0.9945	0.00293
$g_{3,9}$	$g_{3,10}$	$g_{3,11}$	$g_{3,12}$	$g_{3,13}$	$g_{3,14}$	$g_{3,15}$	$g_{3,16}$
—	0.4925	0.4995	-0.0993	0.3783	-0.09589	—	0.8209
$g_{3,17}$	$g_{3,18}$	$g_{3,19}$					
0.3113	-0.0141	0.1232					
$h_{3,1}$	$h_{3,2}$	$h_{3,3}$	$h_{3,4}$	$h_{3,5}$	$h_{3,6}$	$h_{3,7}$	$h_{3,8}$
0.4985	0.6927	0.4985	—	0.2156	0.3345	0.4614	0.3095
$h_{3,9}$	$h_{3,10}$	$h_{3,11}$	$h_{3,12}$	$h_{3,13}$	$h_{3,14}$	$h_{3,15}$	$h_{3,16}$
0.45797	0.22502	0.1334	0.38563	-0.010	0.4917	—	0.3294

Table 4. Relative errors between experimental data and computational results

S(m)	$m = 1$	$m = 2$	$m = 3$
Error	36.9985%	35.032%	37.1262%

3 Robustness Analysis

On the study of biological systems, one always faces a situation that the interrelationships of the pools (substrates, enzymes, products, etc.) can't be clearly known even though all pools have been detected. In terms of biological networks, we just know the nodes of the networks but have incomplete information of their edges. The true network need to be identified from all possible ones. In this context, some basic features of biological systems (such as robustness) should be taken into consideration.

In this section, the most reasonable dynamical system of those listed in previous section is obtained by means of robustness analysis. We first present a quantitative definition of biological robustness (call it robust performance). Then an algorithm is constructed to calculate the robust performances of the systems discussed in previous section.

3.1 Mathematical Definition of Robustness

Assume that M possible networks with N nodes are taken into consideration, which can be formulated as M dynamical systems with N state variables. $N_{ob} := \{n_1, n_2, \cdots, n_d\} \subset I_N$ is an index set of state variables that can be measured in experiment. For the mth system (denoted by S(m), $m \in I_M$), u^m is a vector of kinetic parameters. S_0 and $\mathcal{U}^m$ still denote the ranges of initial state x^0 and parameter vector u^m, respectively. Λ_{ad} and W_{ad} denote the admissible sets of state vector x and control vector v, respectively. $y^{s,k} := (y^{s,k}_{n_1}, y^{s,k}_{n_2}, \cdots, y^{s,k}_{n_d})$ is

experimental result measured at the time point $t_{s,k}$ with control vector v taking value v^s, $s \in I_l$, $k \in I_{l_s}$, where l_s is the total measurement times of the sth experiment. For convenience, we also assume that the mth dynamical system is in the following form:

$$\begin{cases} \dot{x}(t) &= f^m(x(t), v, u^m), \\ x(t_0) &= x^0, \end{cases} \qquad t \in [t_0, t_f^m], \tag{12}$$

where $f \in C^1(\Lambda_{ad}, W_{ad}, \mathcal{U}^m; R^N)$, t_f^m is the moment when the system S(m) reaches its steady state, i.e.,

$$f^m(x(t_f^m), v, u^m) = 0 \ . \tag{13}$$

For given $x^0 \in S_0$, $v \in W_{ad}$ and $u^m \in \mathcal{U}^m$, let $x(t; x^0, v, u^m)$ denote the solution of (12) starting from x^0. Then, define

$$p(u^m) = \frac{1}{|N_{ob}|} \sum_{i \in N_{ob}} \frac{\sum\limits_{s \in I_l} \sum\limits_{k \in I_{l_s}} |x_i^s(t_{s,k}; x^0, v^s, u^m) - y_i^{s,k}|}{\sum\limits_{s \in I_l} \sum\limits_{k \in I_{l_s}} y_i^{s,k}}, \tag{14}$$

where $|N_{ob}|$ is the cardinal number of N_{ob}. Let u^{m*} denote an optimal parameter vector for the system S(m) with regard to $\{y^{s,k}\}_{s \in I_l, k \in I_{l_s}}$, i.e., u^{m*} satisfies that

$$u^{m*} \in \mathrm{Argmin}\{p(u^m) | u^m \in \mathcal{U}^m\} \ . \tag{15}$$

Now, we shall discuss the robustness of S(m) with regard to its optimal parameter vector u^{m*} against a set of perturbations in $\mathcal{U}^m$. In most cases, the robustness can be measured by the variation between the state vector with perturbed parameter vector and that with optimal parameter vector. More generally, assume that only some state variables are under consideration, the index set of which are denoted by N_{rb}. For specified $u^m \in \mathcal{U}^m$, define

$$dp(u^m) = \frac{1}{|N_{rb}|} \sum_{i \in N_{rb}} \frac{\sum\limits_{s \in I_l} \sum\limits_{k \in I_{l_s}} |x_i^s(t_{s,k}; x^0, v^s, u^m) - x_i^s(t_{s,k}; x^0, v^s, u^{m*})|}{\sum\limits_{s \in I_l} \sum\limits_{k \in I_{l_s}} x_i^s(t_{s,k}; x^0, v^s, u^{m*})} \tag{16}$$

and let $\mathcal{U}_{ad}^m := \{u^m \in \mathcal{U}^m | \ \exists \ x^0 \in S_0 \ \text{ such that } \ x(t; x^0, v, u^m) \in \Lambda_{ad}, \ \forall \ v \in W_{ad}, \ t \in [t_0, t_f^m]\}$ be the feasible set of u^m. Randomly generate q sample points from $\mathcal{U}^m$ by uniform distribution, denoted by $u^{m,1}, \cdots, u^{m,q}$, where q is a sufficiently large positive integer number. Let $\mathcal{U}_{ss}(m) := \{u^{m,i} \in \mathcal{U}_{ad}^m \mid i \in I_q\}$. $|\mathcal{U}_{ss}(m)|$ denotes the cardinal number of $\mathcal{U}_{ss}(m)$. Then the robust performance of the biological dynamical system S(m) can be defined as follows.

Definition 1. *The robust performance (R) of the system S(m) with regard to its optimal parameter vector u^{m*} against a set of perturbations in $\mathcal{U}^m$ is described as:*

$$R_{u^m, \mathcal{U}^m}^m = \frac{1}{|\mathcal{U}_{ss}(m)|} \sum_{u^{m,i} \in \mathcal{U}_{ss}(m)} dp(u^{m,i}) \ . \tag{17}$$

We can conclude that the larger the value of (17) is, the more robust the system is. This conclusion can be explained in the following two aspects of analysis. On the one hand, observing (16), we know that the value of $dp(u^m)$ is small if $x(t; x^0, v, u^m)$ is insensitive to u^m and is large otherwise; on the other hand, the larger the value of $|\mathcal{U}_{ss}(m)|$ is, the higher the probability of the randomly generated parameter vector being in its feasible set is. Naturally, the comparison of the robustness between two systems can be defined as follows.

Definition 2. *A system S(i) is said to be more robust than a system S(j) when*

$$R^i_{u^i,\mathcal{U}^i} < R^j_{u^j,\mathcal{U}^j} \ . \tag{18}$$

3.2 Algorithm and Numerical Results

Since only steady states are considered in the study of glycerol continuous fermentations, i.e., $l_s = 1$ for all $s \in I_l$, we have

$$dp(u^m) = \frac{1}{|N_{rb}|} \sum_{i \in N_{rb}} \frac{\sum_{s \in I_l} |x^s_i(t^m_f; x^0, v^s, u^m) - x^s_i(t^m_f; x^0, v^s, u^{m*})|}{\sum_{s \in I_l} x^s_i(t^m_f; x^0, v^s, u^{m*})}, \tag{19}$$

where $N_{rb} = \{1, 2, 3, 6, 7, 8\}$. u^{m*}, $m = 1, 2, 3$, take values from Tables 1-3, respectively. Recall that $x(t^m_f)$ satisfies

$$f^m(x(t^m_f), v^s, u^m) = 0, \qquad s \in I_l \ . \tag{20}$$

As is mentioned in last section, (20) can be converted into a group of linear equations in logarithmic coordinates.

According to the above analysis, we construct the following algorithm to calculate the robust performances of the system S(m), $m = 1, 2, 3$.

Algorithm 1.

Step 1. Set $w = 0$, $q = 5000$ and $q_1 = 0$. Compute $x^s(t^m_f; x^0, v^s, u^{m*})$ from Eqs.(20) with $u^m = u^{m*}$, $s = 1, 2, \cdots, l$, then goto Step 2.
Step 2. If $w > q$, goto Step 5, else generate a sample point $u^{m,w}$ from $\mathcal{U}^m$ and let $w := w + 1$, then goto Step 3.
Step 3. Compute $x^s(t^m_f; x^0, v^s, u^{m,w})$ from Eqs.(20) with $u^m = u^{m,w}$, $s = 1, 2, \cdots, l$. If $x^s(t^m_f; x^0, v^s, u^{m,w}) \in \Lambda_{ad}$ for all $s \in I_l$, then let $q_1 := q_1 + 1$, goto Step 4, else goto Step 2.
Step 4. Compute $dp(u^{m,w})$ from (19), then goto Step 2.
Step 5. Let $|\mathcal{U}_{ss}(m)| := q_1$ and compute $R^m_{u^m,\mathcal{U}^m}$ from (17), stop.

For the system S(m), according to the above algorithm, we calculate its robust performance 50 times, denoted by $R^m_{u^m,\mathcal{U}^m}(k)$, $k = 1, \cdots, 50$. Then we compute the expectation and variance of $\{R^m_{u^m,\mathcal{U}^m}(k)\}^{50}_{k=1}$, denoted by $\overline{R}^m_{u^m,\mathcal{U}^m}$

Table 5. Expectations and variances of robust performances for the three systems

S(m)	$m=1$	$m=2$	$m=3$
$\overline{R}^m_{u^m,\mathcal{U}^m}$	5.9023	22.766	21.625
$VR^m_{u^m,\mathcal{U}^m}$	0.04	0.25	0.574

and $VR^m_{u^m,\mathcal{U}^m}$. As shown in Table 5, on the one hand, $VR^m_{u^m,\mathcal{U}^m}$, $m=1,2,3$, are small enough, which implies that $R^m_{u^m,\mathcal{U}^m}$ is independent of the sample points when q is sufficiently large and therefore the rationality of our definition of robust performance in (17); on the other hand, since the robust performance of the system S(1) is much smaller than that of the other systems, it demonstrates that S(1) is the most reasonable, i.e., it is most possible that both glycerol and 1,3-PD pass the cell membrane by active transport and passive diffusion.

4 Conclusions and Discussions

Metabolic network of glycerol enzyme-catalytic dissimilation by *K. pneumoniae* includes some uncertain factors since the transport mechanisms of glycerol and 1,3-PD across the cell membrane haven't been completely observed, which leads to various inferences of the metabolic network. In this study, different dynamical systems were developed based on distinct inferences of the metabolic network and parameters were identified for each system based on 30 groups of experimental data. To infer the most reasonable metabolic network in the context of lack of intracellular information, we carried out robustness analysis for these systems. A quantitative definition of biological robustness was established and the robust performances of our proposed systems were calculated. Numerical results show that it is most possible that both glycerol and 1,3-PD pass the cell membrane by active transport and passive diffusion.

In the future, we will attempt to search for grounded theoretical basis for the robustness definition proposed in this article and put more numerical examples to verify its rationality and feasibility. It is worthwhile to emphasis that the robustness definition developed in this work can only be applied to the biological systems whose robustness can be reflected in their state vectors, which, of course, is not the only case. So we will be engaged in exploring new feasible theoretical and computational avenues to provide a broad and unified account of robustness of biological systems. In addition, on the basis of our theoretical study of biological systems, we will take further study on the whole metabolic network of glycerol bioconversion to 1,3-PD, including reductive and oxidative pathways and genetic regulation on the metabolic process.

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