# Invariance of the Hybrid System in Microbial Fermentation

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**Abstract.** In this study, we propose a nonlinear hybrid dynamical system to describe the concentrations of extracellular and intracellular substances in the process of bio-dissimilation of glycerol to 1,3-propanediol. An invariance principle is established for the hybrid dynamical system. At the same time, we state and prove new stability criteria for the nonlinear hybrid system. These results provide less conservative stability conditions for hybrid system as compared to classical results in the literature and allow us to characterize the invariance of a class of nonlinear hybrid dynamical systems.

**Keywords:** invariance, hybrid system, microbial fermentation.

## 1 Introduction

1,3-propanediol(1,3-PD) possesses potential applications on a large commercial scale, especially as a monomer of polyesters or polyurethanes, its microbial production is recently paid attention to in the world for its low cost, high production and no pollution, etc. [1]. Among all kinds of microbial production of 1,3-PD, dissimilation of glycerol to 1,3-PD by *Klebsiella pneumoniae* has been widely investigated since 1980s due to its high productivity [2,3]. The experimental investigations showed that the fermentation of glycerol by *K. pneumoniae* is a complex bioprocess, since the microbial growth is subjected to multiple inhibitions of substrate and products. The researches about the fermentation include the quantitative description of the cell growth kinetics of multiple-inhibitions, the metabolic overflow kinetics of substrate consumption and product formation in continuous cultures, feeding strategy of glycerol in fed-batch culture, and so on [4]. In these researches on fed-batch culture, all numerical results are based on the continuous dynamical models and there exist big errors between computational and experimental results. In fact, there exist impulsive phenomena in fed-batch culture, so the process characterized by continuous models is not fit for the actual process any longer. In order to characterize the actual process, the impulsive differential equations are applied to the fed-batch fermentation [5].

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The parameters in continuous system are not fit for the impulsive system, so parameter identification is necessary. Usually, ranges of parameters change in the neighborhood of initial values during the identification process. But we can't ensure the system is stable under the given ranges of parameters. Thus, stability of the system becomes a fundamental issue in system analysis and design, that is necessary for system identification and optimal control.

This paper is organized as follows. In section 2, we formulate the problem of impulsive system. In Section 3, an invariance principle is established for the hybrid dynamical system. At the same time, we state and prove new stability criteria for the nonlinear hybrid system. These results provide less conservative stability conditions for hybrid system as compared to classical results in the literature.

## 2 Hybrid Nonlinear Dynamical System

In this paper, 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 [6], the hybrid nonlinear dynamical system $S(l, i)$ concerning enzymatic catalyses and transports of glycerol and 1,3-PD can be described as

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

where $x(t) \in R^k_+$ is the state variable with $k$ components of the $ith$ dynamical system, $f : R^k_+ \times L \times G \times Dc \times D_s(i) \rightarrow R^k$ is the rate of reactions, $L = \{1, 2, \cdots, l\}$ is the serial number set of experiments. $v(l) = (D(l), c_{s0}(l))^T \in D_c(l) \subset R^2_+$, $D_c(l)$ is the admissible set of dilution rate and initial glycerol concentration in $lth$ experiment. $G = \{1, 2, \cdots, g\}$ is the serial number set of possible metabolic pathways, and $g$ 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 $W_a \subset R^k_+$ such that solution $x(t; v(l), q(i))$ of $S(l, i)$ is in $W_a$. In addition, $D_c(l)$ and $D_s(i), (l, i) \in L \times G$, 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 $(g)$ of metabolic pathways is 72. For simplicity, we only discuss system $S(1, 1)$ corresponding to the first experiment and the first case. Other models have properties similar to those of $S(1, 1)$. $S(1, 1)$ is expressed as follows.

$$\begin{cases} \dot{x}_1 = (\mu - D)x_1 \\ \dot{x}_2 = D(c_{s0} - x_2) - p_2 x_1 \\ \dot{x}_3 = k_1(x_8 - x_3)x_1 - Dx_3 \\ \dot{x}_4 = p_4 x_1 - Dx_4 \\ \dot{x}_5 = p_5 x_1 - Dx_5 \\ \dot{x}_6 = \frac{1}{k_2}(k_3 \frac{x_2}{x_2+k_4} + k_5(x_2 - x_6) - p_2) - \mu x_6 \\ \dot{x}_7 = k_6 u_1 \frac{x_6}{k_{m1}^*(1+\frac{x_7}{k_7})+x_6} - k_8 u_2 \frac{x_7}{k_{m2}^*+x_7(1+\frac{x_7}{k_9})} - \mu x_7 \\ \dot{x}_8 = k_8 u_2 \frac{x_7}{k_{m2}^*+x_7(1+\frac{x_7}{k_9})} - k_{10}(x_8 - x_3) - \mu x_8 \end{cases} \quad (2)$$

In (2), $D$ and $c_{s0}$ 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 $\mu$, 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.

$$\mu = \mu_m \frac{x_2}{x_2 + k_s^*} \prod_{i=2}^{5} (1 - \frac{x_i}{x_i^*}), \quad (3)$$

$$p_2 = m_2 + \frac{\mu}{Y_2} + \Delta_2 \frac{x_2}{x_2 + k_2^*}, \quad (4)$$

$$p_4 = m_4 + \mu Y_4 + \Delta_4 \frac{x_2}{x_2 + k_4^*}, \quad (5)$$

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

$$u_1 = k_{11} + k_{12}\mu + k_{13} \frac{x_2}{x_2 + k_{14}}, \quad (7)$$

$$u_2 = k_{15} + k_{16}\mu + k_{17} \frac{x_2}{x_2 + k_{18}}. \quad (8)$$

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. $q = (k_1, k_2, \cdots, k_{18}) \in D_s(1) \subset R^{18}$ 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 1.* For any pair $(l, i) \in L \times G$, $v(l) \in D_c(l)$ and $q(i) \in D_s(i)$, function $f(x(t), l, i, v(l), q(i))$ satisfies that $f \in C([t_0, t_f]; R^k)$ and $f$ is locally Lipschitz continuous in $x$ on $R_+^k$.

*Property 2.* For any pair $(l, i) \in L \times G$, $v(l) \in D_c(l)$ 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 $x(t) \in R^k_+, v(1) \in D_c(1)$ 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 $L_1 = |\mu_m| + |D|$, we obtain that $|f_1(x(t), 1, 1, v(1), q(1))| \leq L_1\|x\|$.

Furthermore, let $L_2 = \max\{|D|, |m_2| + |\mu_m||Y_2| + |\Delta_2|\}$. Since

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

we must conclude that $|f_2(x(t), 1, 1, v(1), q(1))| \leq |D||C_{s0}| + L_2\|x\|$.

Let $L_3 = \max\{\ |k_1||M_2|, |D|\}$. Then

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

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

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

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 $L_6 = |\frac{k_5}{k_2}| + |\mu_m|$. Since

$$|f_6(x(t), 1, 1, v(1), q(1))| \leq |\frac{k_{k_5}}{k_2}||x_2| + (|\frac{k_5}{k_2}| + |\mu_m|)|x_6| + (|\frac{q_2}{k_2}| + |\frac{k_3}{k_2}|),$$

we obtain that $|f_6(x(t), 1, 1, v(1), q(1))| \leq L_6\|x\| + (|\frac{q_2}{k_2}| + |\frac{k_3}{k_2}|)$.

Let $L_7 = |\mu_m|$. So

$$|f_7(x(t), 1, 1, v(1), q(1))| \leq |k_6||u_1| + |k_8||u_2| + |\mu_m||x_7|,$$

and we see that $|f_7(x(t), 1, 1, v(1), q(1))| \leq L_7\|x\| + |k_6||u_1| + |k_8||u_2|$.

Let $L_8 = |k_9| + |\mu_m|$. Then

$$|f_8(x(t), 1, 1, v(1), q(1))| \leq L_8\|x\| + |k_8||u_2|.$$

Let $\beta = \frac{\sqrt{2}}{2}\max\{L_1, \cdots, L_8\}$ and $\alpha = \frac{\sqrt{2}}{2}\max\{|D(1)||c_{s0}(1)|, |k_8|(|k_{15}| + |k_{16}||\mu_m| + |k_{17}|) + |k_6|(|k_{11}| + |k_{12}||\mu_m| + |k_{13}|), \frac{|m_2|+|\mu_m||Y_2|+|\Delta_2|}{|k_2|} + |\frac{k_3}{k_2}|\}$. In view of the boundedness of $D_c(1)$ and $D_s(1)$, we must conclude that

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

In the same way, it can be proved that for any $(l, i) \in L \times G$ 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 3.* For any pair $(l, i) \in L \times G$, $v(l) \in D_c(l)$ 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 1 and Property 2 that system (2) 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.

## 3 Stability Criteria

In this section, an invariance principle is established for the hybrid dynamical system. At the same time, we state and prove new stability criteria for the nonlinear hybrid system. These results provide less conservative stability conditions for hybrid system as compared to classical results in the literature.

**Definition 1.** *A function $V(t, x)$ is said to be*

(i) *decrescent if there exists a function $a : R_+ \to R_+$ such that*

$$V(t, x) \le a(\|x\|), \quad (t, x) \in R_+ \times s(\rho).$$

(ii) *positive definite if there exists a continuous function $b : R_+ \to R_+$ such that*

$$\begin{aligned} b(\|x\|) &\le V(t, x), \quad (t, x) \in R_+ \times s(\rho) \\ V(t, 0) &\equiv 0, \qquad\qquad t \in R_+. \end{aligned}$$

**Lemma 1.** *[7] Assume that*

(i) *$V \in \sum$, there exist $\lambda_k \in R$ and a continuous function $c_k : R_+ \to R_+$ such that*

$$D^+ V(t, x) \le \frac{\lambda_k}{\Delta t_k} c_k(V(t, x)), \quad (t, x) \in (t_{k-1}, t_k) \times s(\rho);$$

(ii) *there exist $v_k \in R$ and a continuous function $d_k : R_+ \to R_+$ such that*

$$V(t_k^+, x + I_k(x)) \le V(t_k, x) + v_k d_k(V(t_k, x)), \quad x \in s(\rho);$$

(iii) *$\lambda_k + v_k \le 0$, for $s \in (0, \rho)$, $c_k(s) \le d_k(s)$ if $v_k < 0$ and $c_k(s) \ge d_k(s)$ if $u_k < 0$.*

*Then system (1) is stable. Suppose further that*

(iv) *$V(t, x)$ is decrescent and for any $\eta > 0$, there exists a $\sigma > 0$ such that*

$$s + |v_k| d_k(s) < \eta, \qquad \forall s \in (0, \sigma), \quad k = 1, 2, \cdots$$

*Then system (1) is uniformly stable.*

**Theorem 1.** *The dynamical system* (2) *is impulsive stable if parameters in system* (2) *satisfy that* $\frac{b_1}{c_1}+\frac{b_2}{c_2}<1$ *and* $1+\frac{1}{Y_2}(a+1)+Y_3+Y_4>0$.

*Proof.* Define Lyapunov function

$$V(x)=\frac{1}{2}(x_1-x_2)^2+x_1x_3+x_1x_4+x_1x_5, \tag{9}$$

Then $V(x)$ is positive definite, decrescent. Along solutions of (4), we have

$$D^+V(x)=\frac{\partial}{\partial x}V(x)\cdot f(x)=(x_1-x_2+x_3+x_4+x_5)\mu x_1$$
$$-q_2x_1(x_2-x_1)+q_3x_1^2+q_4x_1^2+q_5x_1^2$$

Let $a=\frac{b_1}{c_1}+\frac{b_2}{c_2}$ in expressing $q_5$. Since $a<1$, $1+\frac{1}{Y_2}(a+1)+Y_3+Y_4>0$, and $\frac{\Delta_i x_2}{x_2+k_i}$, $i=2,3,4$, is increasing about $x_2$, we have

$$D^+V(x)\le[(a+1)m_2+m_3+m_4+(1+\frac{1}{Y_2}(a+1)+Y_3+Y_4)\mu_m$$
$$+(a+1)\frac{\Delta_2x_2^*}{x_2^*+k_2}+\frac{\Delta_3x_2^*}{x_2^*+k_3}+\frac{\Delta_4x_2^*}{x_2^*+k_4}]x_1^2$$
$$+\mu_mx_1x_2+\mu_mx_1x_3+\mu_mx_1x_4+\mu_mx_1x_5$$

Let $\lambda_0=(a+1)m_2+m_3+m_4+(1+\frac{1}{Y_2}(a+1)+Y_3+Y_4)\mu_m+\frac{\Delta_3x_2^*}{x_2^*+k_3}+\frac{\Delta_4x_2^*}{x_2^*+k_4}$ and $\lambda^*=\max\{2\lambda_0,\mu_m\}$, thus by (6)

$$D^+V(x)\le\lambda^*V(x) \tag{10}$$

This implies, by the definition of $I_i(x(t_i))$, that

$$V(x+I_i(x))=V(x_1(1-u_i),cu_i+x_2(1-u_i),x_3(1-u_i),$$
$$x_4(1-u_i),x_5(1-u_i))$$
$$=(1-u_i)^2V(x)-cu_i(1-u_i)x_1+\frac{1}{2}c^2u_i+cu_ix_2(1-u_i) \tag{11}$$

Hence $V\in\sum$, let $\lambda_i=\Delta t_i\lambda^*$, $c_k(s)=s$, the Condition (i) of Lemma 1 are satisfied from (7).

There are two cases to consider for Condition (ii) of Lemma 1.

Case 1. $x_1<x_2$. Choose $u_i$ that satisfies $u_i\le\frac{2x_1-2x_2-c}{2x_1-2x_2}$, which implies, in view of (8), that

$$V(x+I_i(x))\le V(x)-u_i(2-u_i)V(x) \tag{12}$$

Case 2. For $x_1\ge x_2$, we can also choose the controllable variable $u_i$ such that (9) is satisfied.

Let $v_i = -u_i(2 - u_i)$, $d_k(s) = s$, the Condition (ii) of Lemma 1 is satisfied from (9).

We can also choose the controllable variable $u_i$ such that $u_i \geq 1-\sqrt{1 - \lambda^* \Delta t_i}$, when $\lambda_i + v_i = \Delta t_i \lambda^* - u_i(2 - u_i) \leq 0$, we get $c_i(s) = d_i(s)$ if $v_i < 0$. Thus Condition (iii) of Lemma 1 is satisfied. Hence system (5) is impulsive stable by Lemma 1.

**Theorem 2.** *Under the conditions of Theorem 1, system* (2) *is uniformly stable.*

*Proof.* From Definition 1, the function $V(x)$ of Theorem 1 is decrescent and $s + |v_i|d_i(s) = s + u_i(2 - u_i)s$. Let $\sigma = \eta/2$, we have

$$s + |v_i|d_i(s) \leq 2s < \eta.$$

This implies that the condition (iv) of Lemma 1 is satisfied, and the system (5) is uniformly stable.

## 4 Conclusion

The conditions of parameters are given by Theorem 1 in the paper. Under these conditions, the parameter identification is realized for the fermentation process. Numerical simulation shows that the impulsive dynamical system presented in this paper can characterize the process of 1,3-propanediol production by fermentation. We conclude that the impulsive system is more fit for formulating fed-batch fermentations.

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