PARAMETER IDENTIFICATION OF NONLINEAR DELAYED DYNAMICAL SYSTEM IN MICROBIAL FERMENTATION BASED ON BIOLOGICAL ROBUSTNESS

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Abstract. In this paper, the nonlinear enzyme-catalytic kinetic system of batch and continuous fermentation in the process of glycerol bio-dissimilation is investigated. On the basis of both glycerol and 1,3-PD pass the cell membrane by active and passive diffusion under substrate-sufficient conditions, we consider the delay of concentration changes on both extracellular substances and intracellular substances. We establish a nonlinear delay dynamical system according to the batch and continuous fermentation of bio-dissimilation of glycerol to 1,3-propanediol(1,3-PD) and we propose an identification problem, in which the biological robustness is taken as a performance index, constrained with nonlinear delay dynamical system. An algorithm is constructed to solve the identification problem and the numerical result shows the values of time delays of glycerol, 3-HPA, 1,3-PD intracellular and extracellular substances. This work will be helpful for deeply understanding the metabolic mechanism of glycerol in batch and continuous fermentation.

1. Introduction. Over the past several years, 1,3-propanediol(1,3-PD) has attracted much attention in microbial production throughout the world because of its lower cost, higher production and no pollution. Zeng\cite{19, 20} carried out including the quantitative description of the cell growth kinetics of multiple inhibitions, the metabolic overflow kinetics of substrate consumption and product formation, Ye investigated the existence of equilibrium points and stability\cite{17}, Tian and Liu considered the dynamical behavior for the models of the continuous cultures and the feeding strategy of glycerol\cite{10, 5}.

However, due to less information about intracellular behavior, less attempt has been made on the glycerol metabolic system. In 2008, Sun et al. proposed a
mathematical model to describe the concentration changes of extracellular and intracellular substances [9]. Then, Wang proposed a complex metabolic network and the corresponding nonlinear hybrid dynamical system to determine the most possible metabolic system based on the biological robustness [13]. So, the quantitative descriptions of biological robustness become a feasible method to overcome the shortcoming. Biological robustness is a property that allows a system to maintain its functions despite external and internal perturbations. Kitano and Stelling qualitatively described the robustness of biological systems [2, 8].

Parameter identification and multistage modeling are widely discussed in fed-batch and continue culture, see [15, 16, 21, 18, 11, 12] and the references therein. However, the investigations above tend to ignore an essential aspect, time delay. Time delay is considered to play an important role in occurrence of oscillation, although the mechanism of oscillation is very complex. In many biological systems, time lag exists between a large change of environmental conditions and metabolic responses of cells. Although the dynamic behaviour of microorganisms in continuous culture has been the objective of a number of theoretical works [6, 7, 1], more thorough and comprehensive experimental studies have been reported only recently. These experimental studies revealed several phenomena, such as sustained oscillation and multiple stable steady states, which can hardly be explained by previous theoretical studies. Lian and Li, respectively, consider a finite time delay (discrete delay) and a continuous time delay (distributed delay) in continuous culture and the corresponding oscillatory behavior [4, 3]. In the paper, a finite time delay vector between the biomass formation and the operating conditions on both extracellular substances and intracellular substances is taken into account in the kinetic system. We establish a nonlinear delay dynamical system according to the batch and continuous fermentation of bio-dissimilation of glycerol to 1,3-propanediol (1,3-PD), then we propose an identification problem, in which the biological robustness is taken as a performance index, constrained with nonlinear delay dynamical system. An algorithm is constructed to solve the identification problem and the numerical result shows the value of time delays of glycerol, 3-HPA, 1,3-PD intracellular and extracellular substances. This work will be helpful for deeply understanding the metabolic mechanism of glycerol in batch and continuous fermentation.

This paper is organized as follows. In Section 2, the model of the delay differential system in continuous and batch culture is proposed. In section 3, investigates the biological robustness and parameter identification model are proposed. In Section 4, we develop a computational approach to solve the parameter identification model and illustrates the numerical results. Finally, conclusions are provided in Section 5.

2. Nonlinear dynamical system with delay. In this paper, based on assumptions that the transport of glycerol and 1,3-PD across cell membrane are both active transport coupled with passive diffusion, considering the process of substrate taking up and products secreting across the cell membrane, a finite time delay \( \tau = (\tau_1, \tau_2, \tau_3, \tau_4) \) between the biomass formation and the operating conditions are taken into account in the kinetic system as follows:

\[
\begin{align*}
\dot{x}_1(t) &= (\mu - D)x_1(t) \\
\dot{x}_2(t) &= D(C_{s0} - x_2(t)) - q_2x_1(t) \\
\dot{x}_3(t) &= q_3x_1(t) - Dx_3(t - \tau_1)
\end{align*}
\]
\[
x_4'(t) = q_4 x_1(t) - D x_4(t) \\
x_5'(t) = q_5 x_1(t) - D x_5(t) \\
x_6'(t) = \frac{1}{u^7} (u^8 x_2(t) + u^9 + u^{10} (x_2(t) - x_6(t - \tau_2))) N_+ (x_2(t) - x_6(t - \tau_2) - q_{20}) \\
x_7'(t) = \frac{u_{11} x_6(t - \tau_2)}{K_m^G + K_m^G \left( \frac{x_7(t - \tau_3)}{u^8} \right) + x_6(t - \tau_3)} - \frac{u_{13} x_7(t - \tau_3)}{K_m^P + x_7(t - \tau_3) + \frac{x_2(t - \tau_3)}{u^4}} - \mu x_7(t - \tau_3) \\
x_8'(t) = \frac{u_{15} x_8(t - \tau_4)}{x_8(t - \tau_4) + \mu x_8(t - \tau_4)} - \frac{u_{17} (x_8(t - \tau_4) - x_3(t - \tau_1)) N_+ (x_8(t - \tau_4) - x_3(t - \tau_1))}{x_8(t - \tau_4) + \mu x_8(t - \tau_4)} \]

Here \( x(t; \tau) = (x_1(t), x_2(t), x_3(t - \tau_1), x_4(t), x_5(t), x_6(t - \tau_2), x_7(t - \tau_3), x_8(t - \tau_4))^T \) are concentrations of biomass, glycerol, 1,3-PD, acetic acid, ethanol in reactor and intracellular concentrations of glycerol, 3-HPA, 1,3-PD, respectively. \( D \) and \( C_{s0} \) are, respectively, the dilution rate and substrate concentrate in feed. In the continuous culture, \( C_{s0} = 675 \text{mmol/L.D} = 0.15 \text{h}^{-1} \). Based on the mechanism of fermentation, \( C_{s0} = 0 \text{mmol/L.D} = 0 \text{h}^{-1} \) in the batch culture. According to the factual experiments, we consider the properties of the system on a subset \( W_a := \prod_{k=1}^4 [x_k*, x_k^*] \) which is admissible set of state vector \( x(t; \tau) \). \( x_k*, x_k^* \) are the lower and upper bounds of \( x_k(t) \). Let \( U = (u^1, \ldots, u^{17})^T \), the value of parameters can be found in [14]. \( \tau_1, \tau_2, \tau_3, \tau_4 \) are the delay value of 1,3-PD and in reactor intracellular concentrations of glycerol, 3-HPA, 1,3-PD, respectively and \( \tau_2 < \tau_3 < \tau_4 < \tau_1 \). Let \( \Gamma = \{ \tau | \tau = (\tau_1, \tau_2, \tau_3, \tau_4) \} \) is the parameter need to be identified, \( \Gamma_a := \prod_{i=1}^4 [\tau_i*, \tau_i^*] \) is admissible set of \( \tau, \tau_1^* = 0.001, \tau_4^* = 0.3 \) are the lower and upper bounds of the delay value.

The specific cellular growth rate appeared in Eq.(2.1) can be expressed as follows:

\[
\mu = \mu_m \frac{x_2(t)}{x_2(t) + K_s (1 - \frac{x_2(t)}{x_2^*}) (1 - \frac{x_3(t - \tau_1)}{x_3^* (t - \tau_1)}) (1 - \frac{x_4(t)}{x_4^* (t)}) (1 - \frac{x_5(t)}{x_5^* (t)})}
\]

Using the Monod equation for describing active transport and Fick diffusion law for passive diffusion[9], we can express the specific substrate consumption rate \( q_2 \) and specific product formation rate \( q_3 \) as follows:

\[
q_2 = u^1 \frac{x_2(t)}{x_2(t) + u^2} + u^3 (x_2(t) - x_6(t - \tau_2)) N_+ (x_2(t) - x_6(t - \tau_2)) \\
q_3 = u^4 \frac{x_8(t - \tau_4)}{x_8(t - \tau_4) + u^5} + u^6 (x_8(t - \tau_4) - x_3(t - \tau_1)) N_+ (x_8(t - \tau_4) - x_3(t - \tau_1))
\]

Here

\[
N_+(y) = \begin{cases} 1, & y > 0 \\ 0, & y \leq 0 \end{cases}
\]

While the uptake of extracellular glycerol is considered as a black box model, its specific consumption rate \( q_{20} \), the specific product formation rates of acetate \( q_4 \) and ethanol \( q_5 \) are shown as follows:
\[ q_{20} = m_2 + \frac{\mu}{Y_2} + \Delta q_2 \frac{x_2(t)}{x_2(t) + K_2^*} \]
\[ q_4 = m_4 + \mu Y_4 + \Delta q_4 \frac{x_2(t)}{x_2(t) + K_4^*} \]
\[ q_5 = m_5 + \mu Y_5 \]

Then, we can describe the process of fermentation by the following nonlinear delay dynamical system (denoted by NDS).

\[
\begin{aligned}
\dot{x}(t) &= f^b(x; \tau) = (f_1^b(x; \tau), \ldots, f_8^b(x; \tau))^T, \quad t \in [t_0, t_b] \\
x(t_0) &= x_0 \\
x_0(t; \tau) &= \phi_0(t), t \in [-\tau, 0], i = 3, 6, 7, 8 \\
\dot{x}(t) &= f^c(x; \tau) = (f_1^c(x; \tau), \ldots, f_8^c(x; \tau))^T, \quad t \in [t_b, t_f] \\
x(t_b) &= x_b \\
x_b(t; \tau) &= \phi_0(t), t \in [t_b - \tau, t_b], i = 3, 6, 7, 8
\end{aligned}
\]

Where \([t_0, t_b] \subset R_+\) and \([t_b, t_f] \subset R_+\) be the period of time of batch and continuous stage, respectively. Apparently, \(0 < t_b < t_f < \infty\). \(x_0\) is the initial state of the batch stage; \(x_b\) is the initial state of the continuous stage, which is also the terminal state of the batch stage.

Similarly to the result in [16], it is easy to verify the following property.

**Proposition 1.** The vector-valued functions defined above \(f^b : W_a \times \Gamma_a \to R^8_+\) and \(f^c : W_a \times \Gamma_a \to R^8_+\) are continuous on \(W_a \times \Gamma_a\).

3. **Properties of the nonlinear dynamical system.** The purpose of this section is to demonstrate the existence, uniqueness and continuous dependence of solution with respect to parameters. According to the factual process of continuous culture, we make the following assumptions:

H1: The set \(W_a \subset R^8_+, \Gamma_a \subset R^4_+\) are compact, respectively.

H2: The absolute difference between extracellular and intracellular glycerol concentration and that of 1,3-PD concentration are bounded, i.e., \(\exists M_1 > 0, M_2 > 0\), such that

\[
\begin{aligned}
|x_2(t) - x_6(t - \tau_2)| &\leq M_1, \forall t \in [t_0, t_f] \\
|x_8(t - \tau_4) - x_3(t - \tau_1)| &\leq M_2, \forall t \in [t_b, t_f]
\end{aligned}
\]

**Proposition 2.** Under the assumptions (H2), \(\forall \tau \in \Gamma_a, x(t; \tau) \in W_a\), the vector function \(f^b\) and \(f^c\) satisfy

1. \(f^b\) and \(f^c\) about \(x(t; \tau) \in W_a\) are Lipschitz continuous, respectively;
2. \(f^b\) and \(f^c\) satisfy the linear growth condition, i.e., \(\exists a_1, a_2, c_1, c_2 > 0\), such that

\[
\begin{aligned}
f^b(x; \tau) \leq a_1 + e_1 \|x\|, \quad f^c(x; \tau) \leq a_2 + e_2 \|x\|
\end{aligned}
\]

**Proof.** (1) Letting \(L_1 = \mu - D + 1, L_2 = D + C_2, L_3 = D + C_3, L_4 = D + C_4, L_5 = D + C_5\), then

\[
\begin{aligned}
|f_1^c(x; \tau) - f_1^c(y; \tau)| &\leq L_1 \|x_1(t) - y_1(t)\| \\
|f_2^c(x; \tau) - f_2^c(y; \tau)| &\leq L_2 \|x_2(t) - y_2(t)\|
\end{aligned}
\]
3.1 and 3.2, we can obtain the following theorem.

\[|f_3^r(x; \tau) - f_3^e(y; \tau)| \leq C_3 \|x_1(t) - y_1(t)\| + D \|x_3(t - \tau) - y_3(t - \tau)\| \]
\[\leq L_3(\|x(t) - y(t)\| + \|x_3(t - \tau) - y_3(t - \tau)\|)\]
\[|f_3^r(x; \tau) - f_3^e(y; \tau)| \leq C_4 \|x_1(t) - y_1(t)\| + D \|x_4(t) - y_4(t)\| \leq L_4(\|x(t) - y(t)\|)\]
\[|f_3^r(x; \tau) - f_3^e(y; \tau)| \leq C_5 \|x_1(t) - y_1(t)\| + D \|x_5(t) - y_5(t)\| \leq L_5(\|x(t) - y(t)\|)\]

Letting \( L_6 = K_1 + u^{10} + \mu \), when \( N_+(x_2(t) - x_6(t - \tau_2)) = 1 \), we know that
\[\exists K_1 > 0, \text{ such that } \|u^a(x_2(t) + u^b(y_2(t) + u^c))\| \leq K_1, \text{ which implies}\]
\[|f_6^e(x; \tau) - f_6^e(y; \tau)| \leq L_6(\|x_2(t) - y_2(t)\| + \|x_6(t - \tau) - y_6(t - \tau)\|)\]

When \( N_+(x_2(t) - x_6(t - \tau_2)) = 0 \), in the same way, we can prove that it is lipschitz continuous.

Letting \( L_7 = K_2 + K_3 + K_4 \), \( \exists K_2, K_3, K_4, \text{ s.t. } |u^{13} K_2^c| \leq K_2, |u^{13}| \leq K_3, |\mu| \leq K_4 \), then
\[|f_7^r(x; \tau) - f_7^e(y; \tau)| \leq L_7(\|x_7(t - \tau_3) - y_7(t - \tau_3)\| + \|x_7(t - \tau_2) - y_7(t - \tau_2)\|)\]

Letting \( L_8 = K_5 + K_6 + K_7 + K_8 \), \( \exists K_5, K_6, K_7, K_8, \text{ s.t. } |u^{13}| \leq K_5, |u^{15}| \leq K_6, |\mu| \leq K_8 \), when \( N_+(y_8(t - \tau_4) - y_8(t - \tau_1)) = 1 \) or \( 0 \), then
\[|f_8^e(x; \tau) - f_8^e(y; \tau)| \leq L_8(\|x(t; \tau) - y(t; \tau)\|)\]

Finally, we can see that \( \exists L = \sum_{i=1}^8 L_i \), \( \text{s.t. } \|f^c(x; \tau) - f^c(y; \tau)\| \leq L(\|x(t; \tau) - y(t; \tau)\|) \). Accordingly, we demonstrate that the vector function \( f^c \) is lipschitz continuous. In the same way, letting \( D = 0 \), the vector function \( f^b \) is lipschitz continuous. The proof is completed.

(2) Firstly, we must prove the linear growth condition of the vector function \( f^c \).

Letting \( C_{20} = |m_2| + |\frac{1}{\tau_7}| + |\triangle q_2|, C_2 = u^1 + u^3 M_1, C_3 = u^4 + u^6 M_2, C_4 = |m_4| + |Y_4| + |\triangle q_4|, C_5 = |m_5| + |Y_5| \), since \( \|\frac{x_2}{x_2 + K_1}\| < 1, i = 2, 4, |q_0| \leq C_{20}, |q_1| \leq C_i, i = 2, 3, 4, 5 \).

Letting \( L'_1 = \mu_{\text{max}} - D, L'_2 = \max\{D + C_2, D C_{s0}\}, L'_3 = C_3 + D, L'_4 = C_4 + D, L'_5 = C_5 + D, L'_6 = \max\{\frac{u}{u^8}(u^8 + u^{10} M_1 + C_{20}), \mu_{\text{max}}\}, L'_7 = \max\{u^{11} + u^{13}, \mu_{\text{max}}\}, L'_8 = \max\{u^{13} + u^{15} + u^{17} M_2, \mu_{\text{max}}\}, \text{then,}\)
\[\|f^c\| = \|(\mu - D)x_1(t)\| \leq |\mu - D||x_1(t)\| \leq L'_1(\|x(t; \tau)\| + 1)\]
\[\|f^c\| \leq D C_{s0} + D ||x_2(t)|| + C_2 ||x_1(t)|| \leq L'_2(\|x(t; \tau)\| + 1)\]
\[\|f^c\| \leq ||q_3 x_1(t)|| + D ||x_3(t - \tau_1)|| \leq C_5 ||x_1(t)|| + D ||x_3(t - \tau_1)|| \leq L'_3(\|x(t; \tau)\| + 1)\]
\[\|f^c\| \leq ||q_4 x_1(t)|| + D ||x_4(t)|| \leq C_4 ||x_1(t)|| + D ||x_4(t)|| \leq L'_4(\|x(t; \tau)\| + 1)\]
\[\|f^c\| \leq ||q_5 x_1(t)|| + D ||x_5(t)|| \leq C_5 ||x_1(t)|| + D ||x_5(t)|| \leq L'_5(\|x(t; \tau)\| + 1)\]
\[\|f^c\| \leq \frac{1}{u_7}(u^8 + u^{10} M_1 + C_{20}) + \mu_{\text{max}} \|x_6(t - \tau_2)\| \leq L'_6(\|x(t; \tau)\| + 1)\]
\[\|f^c\| \leq u^{11} + u^{13} + \mu_{\text{max}} \|x_7(t - \tau_3)\| \leq L'_7(\|x(t; \tau)\| + 1)\]
\[\|f^c\| \leq u^{13} + u^{15} + u^{17} M_2 + \mu_{\text{max}} \|x_8(t - \tau_4)\| \leq L'_8(\|x(t; \tau)\| + 1)\]

Letting \( L' = \sum_{i=1}^8 L'_i \), then we can obtain: \( \|f^c\| \leq L'(\|x(t; \tau)\| + 1) \). Letting \( a_2 = L', c_2 = L' \) and \( D = 0 \), then we can obtain that the linear growth condition of the vector function \( f^b \), which completes our proof.

Based on the classical theory of differential equations and the above properties 3.1 and 3.2, we can obtain the following theorem.
Theorem 3.1. Under the above assumptions (H1), (H2), for \( \forall \tau \in \Gamma_\alpha \), there exists a unique solution to the system \( NDS \), denoted by \( x(t; \tau) \), satisfy initial conditions \( x(t_0) = x_0, x_n(t; \tau) = \phi_0(t), t \in [-\tau_1, 0], i = 3, 6, 7, 8 \) and \( x(t; \tau) \) is continuously differentiable about \( \tau \in \Gamma_\alpha \).

4. Description of biological robustness and parameter identification. To determine the validity of the delay system, the computational values of the state vector should be consistent with experimental data. On the one hand, since only extracellular data can be measured in experiments, we define the relative error between computational concentrations and experimental data of extracellular substances. On the other hand, how to evaluate the validity of the computational concentrations of intracellular substances becomes the heart of the matter. Firstly, we recall some fundamental definitions, which are similar with the work in [14]. On the basis of definitions, the solution of the system should reach the steady state, which is referred to as the approximately steady state defined as follows.

Definition 4.1. \( \forall \varepsilon > 0, \tau \in \Gamma_\alpha \), if there is \( t_\delta := \inf \{ t_s : \| f_c(x(t; \tau)) \| < \varepsilon, \forall t \in [t_s, t_f] \} \) such that \( x(t; \tau) \) is the solution of \( NDS \) reaches approximately steady state at \( t_\delta \) with the accuracy \( \varepsilon \).

Definition 4.2. With uniform distribution, the set of sample points randomly generated in \( \Gamma_\alpha \), namely \( \Gamma_1 := \{ \tau^k | \tau^k = (\tau^k_1, \tau^k_2, \tau^k_3, \tau^k_4), k = 1, 2, \ldots, q \} \), here \( q \) is a sufficiently large positive integer.

Definition 4.3. Letting \( S(\tau^k) := \{ x(t; \tau^k) | x(t; \tau^k) \) is the solution of \( NDS \) with \( \tau^k \in \Gamma_0 \} \), \( \Gamma_0 \subseteq \Gamma_0 \) denotes the state set of approximate steady state can be reached; \( S_w(\tau^k) \) is set of steady state solutions at \( t_\delta \) with respect to \( \tau^k \in \Gamma_w \).

Next, we will prove the important property of the compactness of the sets \( \Gamma_0 \) and \( \Gamma_w \).

Theorem 4.4. Under assumptions (A1) and (A2), the subsets \( S(\tau^k) \) and \( S_w(\tau^k) \) are compact in \( C([t_0, t_f], R^4_+) \), respectively. If \( \Gamma_0 \) and \( \Gamma_w \) are both non-empty, the sets \( \Gamma_0 \) and \( \Gamma_w \) are compact in \( R^4_+ \), respectively.

Proof. On the basis of assumptions, \( \Gamma_\alpha \) is a non-empty bounded and closed subset in \( R^4_+ \). According to the Proposition 2.1, the map \( \tau^k \in \Gamma_\alpha \rightarrow x(\cdot; \tau^k) \in S(\tau^k) \) is continuous, so \( S(\tau^k) \) is compact in functional space \( C([t_0, t_f], R^4_+) \). Let \( \{ \tau^k_j \}(\subseteq \Gamma_0 \subseteq \Gamma_\alpha) \) be any sequence, \( W_a \) and \( \Gamma_a \) be the non-empty bounded subset of \( R^8_+ \) and \( R^4_+ \), respectively. It is easy to verify the sequence \( \{ \tau^k_j \} \) is also bounded, so it must has the convergent sequence, namely \( \{ \tau^k_j \} \rightarrow \tau^* \). From the definition of \( \Gamma_0, x(\cdot; \tau^k) \in S(\tau^k) \) and \( x(\cdot; \tau^k_j) \in W_a \), so \( x(\cdot; \tau^*) \in S(\tau^k) \) and \( x(\cdot; \tau^*) \in W_a, \tau^* \in \Gamma_0 \). Summing up the above, \( \Gamma_0 \) is compact in \( R^4_+ \). Similarly, we can prove that \( S_w(\tau^k) \) is compact in \( C([t_0, t_f], R^8_+) \) and \( \Gamma_w \) is compact in \( R^4_+ \). □

4.1. Quantitative definition of biological robustness.

Definition 4.5. Let the computational concentrations and experimental data of extracellular substances at steady stage be \( y = (y_1, y_2, y_3, y_4, y_5)^T \) and \( x(t_\delta; \tau^k), k = \)}
1, 2, \ldots, q$, respectively, then the relative error of the extracellular substance concentrations is defined as

$$SSD(\tau^k) := \frac{1}{5} \sum_{s=1}^{5} \frac{|x_s(t_\delta; \tau^k) - y_s|}{|y_s|}. \quad (5)$$

Let $\Gamma_{sw} := \{ \tau^k \in \Gamma_w : x(t_\delta; \tau^k) \in S_w(\tau^k) \}$ and the $SSD(\tau^k) \leq \alpha$ holds, $\alpha$ is the tolerance of relative error $SSD(\tau^k)$, $S_{sw} := \{ x(\cdot; \tau^k)|x(\cdot; \tau^k) \}$ is the approximate solution of the system $NDS$ with respect to $\tau^k \in \Gamma_{sw}$. We have the definition to describe the differences about the intracellular substances at steady stage.

**Definition 4.6.** $\forall \tau^m, \tau^n \in \Gamma_{sw}, (m \neq n), x(t_\delta m; \tau^m)$ and $x(t_\delta n; \tau^n)$ are approximate solutions of the system $NDS$ at $t_\delta m$ and $t_\delta n$ corresponding to $\tau^m, \tau^n$, respectively. The average relative difference of intracellular states is defined by:

$$MSD(\tau^m, \tau^n) := \frac{1}{3} \sum_{s=6}^{8} \frac{|x_s(t_\delta m; \tau^m) - x_s(t_\delta n; \tau^n)|}{\|\tau^m - \tau^n\|}^2. \quad (6)$$

$\forall \tau^{b'} \in \Gamma_{sw}$, the average relative difference of intracellular states with $\tau^{b'}$ is defined by:

$$MSD(\tau^{b'}) = \frac{1}{q} \sum_{p=1}^{q} MSD(\tau^{b'}, \tau^p). \quad (7)$$

**Definition 4.7.** Given $\tau^a \in \Gamma_{sw}, a \in I_q$, the maximum deviation of intracellular substances $x(\cdot; \tau^a)$ at steady stage $\tau^a$ in $B(\tau^a; \delta)$ is defined by:

$$MSD_{max}(\tau^a) = \max\{MSD(\tau^b) | \tau^b \in B(\tau^a; \delta)\}, \quad (8)$$

for all $k \in I_q$ and any $\tau^k \in \Gamma_{sw}$. The robust performance of the dynamical system is defined by:

$$J(\tau^*) := \min\{MSD_{max}(\tau^k) | \tau^k \in \Gamma_{sw}\}, \quad \tau^* = \text{argmin}\{J(\tau^k) | \tau^k \in \Gamma_{sw}\}. \quad (9)$$

**Remark 1.** If there exists $\tau^1, \tau^2 \in \Gamma_{sw}$, and $\tau^1 \neq \tau^2$, such that $J(\tau^1) < J(\tau^2)$, then will say $\tau^1$ is better than $\tau^2$.

4.2. **Parameter identification model.** Since only extracellular data can be measured in experiments, a quantitative definition of biological robustness was proposed and it is as the part of performance index of an identification model. That is, the performance index is composed of $SSD$ and $MSD$. So the parameter identification model of the nonlinear delay dynamical system is shown as follows:

$$(\text{IP}) : \inf J(\tau^k) := SSD(\tau^k) + \min\{MSD_{max}(\tau^k)\}
\text{s.t.} \quad x(t; \tau^k) \in S_{sw}, t \in [t_0, t_f]
\tau^k \in \Gamma_{sw},
\forall \varepsilon > 0, \exists t_\delta, s, t \parallel f(x(t; \tau^k); \tau^k) \parallel < \varepsilon, \forall t \in (t_\delta, t_f). \quad (10)$$

**Theorem 4.8.** Parameter identification model $IP$ is identifiable.

**Proof.** On the basis of the compactness of $S_{sw}$ and $\Gamma_{sw}$, we can obtain the desired result. □
5. Algorithm and numerical result. The algorithm of the model $IP$ is presented as follows:

**Algorithm 1**

\[\text{step 1} \quad \text{Given } N > 0, \text{ let } n = 0, \Gamma_{sw} = \emptyset;\]

\[\text{step 2} \quad \text{Let } n = n + 1, \text{ if } n > N, \text{ goto step 5; otherwise, generate } q \text{ (see Definition 4.2) stochastic samples } \tau^k, k = 1, 2, \ldots, q \text{ following the uniform distribution } \Gamma_q;\]

\[\text{step 3} \quad \text{For each } \tau^k, \text{ solve the nonlinear delay differential equation by Euler method.}\]

\[\text{If the solution } x(t) \in W_\alpha, \text{ and } \| f'(x(t); \tau^k) \| \leq \varepsilon \text{ (see Definition 4.1), } \forall t \in [t_e, t_f], \text{ goto step 4; else goto step 2;}\]

\[\text{step 4} \quad \text{Based on (3.1), compute } SSD(\tau^k), \text{ if } SSD(\tau^k) \leq \alpha (\alpha \text{ is the tolerance of relative error } SSD(\tau^k)), \text{ then } \Gamma_{sw} = \Gamma_{sw} \cup \{\tau^k\}, \text{ goto step 2; }\]

\[\text{step 5} \quad \text{Compute } MSD(\tau^m, \tau^n), \tau^m, \tau^n \in \Gamma_{sw}, \tau^m \neq \tau^n \text{ and } MSD_{\max}(\tau^k), \tau^k \in \Gamma_{sw} \text{ on the basis of (3.2) and (3.3);}\]

\[\text{step 6} \quad J(\tau^k) = MSD_{\max}(\tau^k), \tau^k \in \Gamma_{sw}, \text{ and } J(\tau^k) = \min\{J(\tau)|\tau^k \in \Gamma_{sw}\}, \tau^* = \arg\min\{J(\tau^k)|\tau^k \in \Gamma_{sw}\}.\]

Where $n, N \times q$ stands for the sequence and maximum of the stochastic samples, respectively. $\Gamma_{sw}$ is the set of the constraints are satisfied. According to the model and algorithm mentioned above, we have programmed the software and applied it to the optimal control problem of microbial fermentation in batch culture. Figs. 1 and 2 show the simulating results of biomass concentration, extracellular and intracellular glycerol, 1,3-PD concentration in batch and continuous fermentation of the system with respect to fermentation time.

The basic data is listed as follows:

- **boundary value of state vector:**
  
  \[x_{s1} = 0.001 \text{ mmol}/L, x_{s1}^* = 2039 \text{ mmol}/L, x_{s2} = 0.001 \text{ mmol}/L, x_{s2}^* = 939.5 \text{ mmol}/L, x_{s3} = 0.01 \text{ mmol}/L, x_{s3}^* = 10 \text{ mmol}/L, u_{s4} = 0.01 \text{ mmol}/L, u_{s4}^* = 1026 \text{ mmol}/L, u_{s5} = 200, u_{s5}^* = 360.9 \text{ mmol}/L.\]

We adopt $\alpha = 0.2$ in the procedure. Then, by **Algorithm 1**, the optimal value of delay vector $\tau$ and the approximately steady solution $x = (x_1, x_2, \cdots, x_8)^T$ are $(0.27956, 0.0150799, 0.0185566, 0.0249532)^T$ and $(4.00206, 4.56946, 277.769, 121.471, 130.227, 4.55788, 136.994, 0.420106)^T$, respectively.

| Number of the sample points | Value of the performance index | time(second) |
|-----------------------------|-------------------------------|--------------|
| 10000                       | 0.35249                       | 1093.4       |
| 30000                       | 0.43487                       | 3407.7       |
| 50000                       | 0.32474                       | 5508.6       |
| 80000                       | 0.37008                       | 8725.3       |
| 100000                      | 0.29489                       | 10298.5      |
| 300000                      | 0.29273                       | 31012.1      |
| 500000                      | 0.289185                      | 50986.4      |
| 800000                      | 0.291286                      | 81027.6      |
| 1000000                     | 0.288143                      | 100573.3     |

The Fig. 1 and Table 1 show the convergence curve of the algorithm for the performance index. From Fig. 1, the value of the performance index basically lives up to stabilization, so the number of the sample points with $N \times q = 100000$ are appropriate. The comparisons between the computational values and experimental
data under steady states for the first three substances are depicted in Fig. 2. The computational values of intracellular substances (glycerol, 3-HPA, 1,3-PD) are depicted in Fig. 3.

**Figure 1.** The convergence curve of the algorithm for the performance index.

**Figure 2.** Comparison of the steady state solutions for the concentration of biomass, glycerol, and 1,3-PD between experimental (red real line) and calculated (blue point line) results.
Figure 3. Simulating results of intracellular glycerol, 3-HPA and 1,3-PD concentrations in batch and continuous fermentation of the system with respect to fermentation time.

6. Conclusion. In this paper, the nonlinear enzyme-catalytic kinetic system of batch and continuous fermentation in the process of glycerol bio-dissimilation is investigated. The delay of concentration changes on both extracellular substances and intracellular substances is considered to establish a nonlinear delay dynamical systems. According to the batch and continuous fermentation of bio-dissimilation of glycerol to 1,3-propanediol (1,3-PD), we propose an identification problem, in which the biological robustness is taken as a performance index, constrained with nonlinear delay dynamical system. An algorithm is constructed to solve the identification problem and the numerical result shows the value of time delays of glycerol, 3-HPA, 1,3-PD intracellular and extracellular substances.

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