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REVIEW

Optimal inputs and sensitivities for parameter estimation in bioreactors

Lili Lu

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Abstract The paper presents optimal parametric sensitivity control design for estimation of parameters in bioreactors. We investigate the law of optimal parametric sensitivity control for the model of a single species for microbial growth on a single substrate. The nonlinear model is proposed and is shown to be structurally identifiable given its input. On the purpose of the estimation of the structural parameters the maximum specific growth rate $\mu_{\max}$ and the half-saturation coefficient K, we find out that an increase in the concentration of the system state results in the improvement of the exactness and the reliability of the parametric estimation through the theoretical analysis and numerical simulations.

Keywords Parametric sensitivity · Optimal control · Growth rate · Singular control · Bang–bang control

1 Introduction

Sensitivity analysis is used to determine how 'sensitive' a model is to the changes in the values of the parameters of the model and to the variability in the structure of the model. By showing how the model behavior responds to the changes in parameter values, sensitivity analysis is a useful tool in model building as well as in model evaluation. Sensitivity analysis helps to build confidence in the model by studying the uncertainties that are often associated with parameters in models. Many parameters in system dynamics models represent quantities that are very difficult, or even impossible to measure to a great deal of accuracy in the real world. Also, some parameter values change in the real world. Therefore, when building a system dynamics model, the

L. Lu (✉)
School of Mathematical Sciences, Fudan University, No.220, HandanRoad, 200433 Shanghai, China
e-mail: 071018031@fudan.edu.cn

modeler is usually at least somewhat uncertain about the parameter values he chooses and must use estimates. Sensitivity analysis allows him to determine what level of accuracy is necessary for a parameter to make the model sufficiently useful and valid.

Sensitivity tests help the modeler to understand dynamics of a system. Experimenting with a wide range of values can offer insights into the behavior of a system in extreme situations. Discovering that the system behavior greatly changes in correspondence of a change in a parameter value can identify a leverage point in the model—a parameter whose specific value can significantly influence the behavior mode of the system. Thus sensitivity analysis has been applied in many areas, such as complex engineering systems, economics, physics, social sciences, medical decision-making and so on. Although parametric sensitivity has been employed extensively in many fields, its role in biological reactions is of recent origin. Cobelli and Thomaseth [5] applied it to glucose utilization in the human body, and Geevan et al. [6] examined glucose-insulin feedback and β-cell kinetics in patients suffering from diabetes mellitus.

In many control models, we can select certain control laws so that the parameters in the models are optimally estimated. Observed data for parameter estimation is often both difficult and expensive to obtain. Thus, when an experiment is conducted, the input to the system should be such that the sensitivity to the parameter being estimated is maximized. A natural question in the context of parameter estimation arises: Given an experimental setup, how can the input sequence be chosen in such a way that the parameters are optimally estimated? This is the well known problem of optimal input design which is a classical problem in the identification literature [7]. Studies on optimal input signal synthesis for parameter estimation in linear systems started in the early 1970s [8]. Kalaba and Spingarn [9] presented an optimal solution for the case of a first order, linear model with a quadratic criterion in the input $u(t)$ and the parametric sensitivities. Birk and Medvedev dealt with a sensitivity analysis of a linear quadratic optimal multivariable controller for a fine coal injection vessel used in the blast furnace process [11]. When the model is linear in parameters, the larger the magnitude of the input, the greater will be the sensitivity. Then the input must be constrained, as in [8,9,11]; otherwise the optimal input is infinite. Stigter and Keesman [12] showed how the problem for a fed-batch reactor can be solved by means of singular control techniques. Ljung and Walter focused on the theory on the system parameter estimation and presented practical use of available techniques [21,22].

The description of microbial growth is a basic component of any mathematical model of a bioreactor. An accurate description would require many differential equations to express the rates of change of all intracellular and environmental variables. Indeed, the corresponding model is too complex and most models attempt a judicious simplification to retain key features of interest without irrelevant details. In this paper the model is simplified and we assume that the growth on a single substrate follows Monod kinetics [10] and the interaction between the growth rates is mutually inhibitory. Although models of microbial growth have been studied for many years especially in the field of stability [4,14], the application of the control theory in microbial growth models is still a new research field.

In early studies on the estimation of bio-kinetic parameters practical identifications have been done according to the experiments [15,16]. However, there are few topics

about the theoretical research on optimal input designs for parametric estimations considered to be of importance to microbial growth. The emphasis in the paper is on finding a control law that maximizes the parameter sensitivity for a specific parameter. The major goal of our work is to apply the optimal control theory to the parameter estimation of the nonlinear continuous microbial growth system. We will show that in the case of constant microbial population, the optimal control law is obtained analytically and in the case of microbial population that is changing with time, the optimal control law is not easy to obtain and we have to rely on numerical simulations.

The outline of the paper reads as follows. After the introduction, the problem statement is presented in Sect. 2. Section 3 is devoted to a brief introduction on the maximum principle with state constraints, which leads to a control strategy for the bioreactor in Sect. 4. Final conclusions on applications in Sect. 5 and a list of references close the work.

2 Problem statement

2.1 Concepts of parametric sensitivity

Consider a system of differential equations depending on a parameter α:

$$\begin{cases} \dot{x}(t) = f(t, x; \alpha), \\ x(0) = x_0. \end{cases} \tag{1}$$

If the system is multi-parameter, the discussion is similar. For convenience, we consider a single parameter system for discussion. The function $f(t, x; \alpha)$ is assumed to be continuous and continuously differentiable in all its arguments, the state x is continuously differentiable with respect to t and is differentiable with respect to the parameter α. The sensitivity $s(x; \alpha)$ of x with respect to the parameter α is defined as:

$$s(x; \alpha) = \frac{\partial x(t; \alpha)}{\partial \alpha}. \tag{2}$$

The sign of sensitivity $s(x; \alpha)$ can be positive or negative. Moreover, we assume time invariance of α so that interchange of differentiation with respect to α and time is allowed, leading to:

$$\frac{d(\partial x/\partial \alpha)}{dt} = \frac{\partial f}{\partial x}\frac{\partial x}{\partial \alpha} + \frac{\partial f}{\partial \alpha}, \tag{3}$$

that is,

$$\frac{ds(x; \alpha)}{dt} = \frac{\partial f}{\partial x} s(x; \alpha) + \frac{\partial f}{\partial \alpha}. \tag{4}$$

In a general way, it is assumed that the initial state x_0 is independent of the parameter α, then $s(x; \alpha)|_{t=0} = 0$.

2.2 Model

The cultivation with which we are dealing is a continuously stirred bioreactor. The tank contains a liquid mixture: water, nutrients and biological cells. Feed substrates or other nutrients are continuously introduced into the bioreactor.

We assume that the substrate is continuously fed into the bioreactor dynamically with a substrate feed rate $u(t)$ and the substrate concentration can be directly observable. In this study the growth dynamics include Monod kinetics, so that the dynamical model for the substrate dynamics reads:

$$\begin{cases} \dot{x}(t) &= -\mu_{\max}\frac{x(t)}{K+x(t)}X'(t) + u(t), \\ \dot{X}'(t) &= Y\mu_{\max}\frac{x(t)}{K+x(t)}X'(t) - DX'(t)\ . \end{cases} \tag{5}$$

where $\mu_{\max}$ is the maximum specific growth rate, K the half-saturation coefficient, $x(t)$ denotes the substrate concentration which can be directly observable, $X'(t)$ denotes the biomass concentration and Y is the yield rate constant, that is, the amount of the cells by the transformation of unit amount of the substrate. D is the output rate of the biomass.

Let $X(t) = X'(t)e^{Dt}$, then the system becomes:

$$\begin{cases} \dot{x}(t) &= -\mu_{\max}\frac{x(t)}{K+x(t)}X(t)e^{-Dt} + u(t), \\ \dot{X}(t) &= Y\mu_{\max}\frac{x(t)}{K+x(t)}X(t). \end{cases} \tag{6}$$

Generally, the output rate D of the biomass and the feed rate are of the same order of magnitude so that there is constant liquid volume in the tank. We assume that the order of magnitude of D is small enough so that the reaction between the biomass and the substrate can proceed completely by decreasing the output rate of the biomass. Thus, e^{-Dt} decays very slowly with time so it can be approximately regarded as a constant.

Let $e^{-Dt} \approx 1,\ t \in [0, T]$, then the model leads to:

$$\begin{cases} \dot{x}(t) &= -\mu_{\max}\frac{x(t)}{K+x(t)}X(t) + u(t), \\ \dot{X}(t) &= Y\mu_{\max}\frac{x(t)}{K+x(t)}X(t). \end{cases} \tag{7}$$

In this paper, we will consider the Eq. 7 for the continuously stirred tank reactor (CSTR) model.

2.3 Structural identifiability

Before developing a good input for parameter estimation, it should be examined whether the model is theoretically identifiable with the given input and output with respect to the unknown parameters or not.

A mathematical model is identifiable if and only if there is a unique relationship between each parameter value and the input–output behavior of the model, obtained by

means of numerical data, measured during an experiment. Structurally identifiability concept was first introduced by Bellman and Aström [17]. For linear systems, many approaches were developed and necessary and sufficient conditions were determined [18,19]. For nonlinear systems, there exist important results about the identifiability of the systems [20].

Consider the following parametric nonlinear system described by:

$$\begin{cases} \dot{x}(t) = f(x(t), u(t); \theta), \\ y(t) = h(x(t), u(t); \theta), \end{cases} \tag{8}$$

added by the initial conditions: $x(t_0) = x_0$, where $x \in R^n$, $u \in R^m$ and $y \in R^q$ are, respectively, the state vector, the input vector and output vector. θ is a p-dimensional parameter vector. Assume that the initial condition vector x_0 is well specified.

Identifiability is a concept that is central in identification problems. The problem is whether the identification procedure will yield a unique value of the parameter, and/or whether the resulting model is equal to the true system. We thus have the following definition:

Definition 1 A model structure $\mathscr{M}$ is a differentiable mapping from a connected, open subset $\mathscr{D}_{\mathscr{M}}$ of $\mathbb{R}^n$ to a model set, such that the gradients of the predictor functions are stable.

Definition 2 The structure $\mathscr{M}$ of system 8 is said to be globally identifiable at θ_* with respect to $\mathscr{D}_{\mathscr{M}}$ if there exists an input u at least such that $y(\theta_*, u) \equiv y(\theta, u)$, $\theta \in \mathscr{D}_{\mathscr{M}} \Rightarrow \theta_* = \theta$.

The question addressed in this section can be expressed as follows: Assuming that Y is known, that the input u can be manipulated experimentally, can the parameters $\mu_{\max}, K$ have unique values, given the parametric model 7 available experimental data of substrate concentrations and initial conditions on concentrations? This can be proved using the approach: Transformation of the nonlinear model on a linear model in the parameters.

This can be proved transforming the nonlinear model in a linear model in the parameters. In fact, it is matter of simple algebra to rewrite system 7 in the form:

$$\begin{cases} K(\dot{x} - u) + \mu_{\max} x X = (u - \dot{x})x, \\ K\dot{X} - \mu_{\max} Y x X = -x\dot{X}. \end{cases} \tag{9}$$

which is linear in $\mu_{\max}$ and K, i.e., the parameters to identify. It is proved that the model 7 is structurally identifiable.

2.4 Optimal parametric sensitivity input

In this study the growth dynamics include Monod kinetics and the dynamical model for the substrate dynamics reads:

$$\dot{x}(t) = -\mu_{\max} \frac{x(t)}{K + x(t)} X(t) + u(t), \tag{10}$$

We assume that $x(0), X(0) > 0$ are independent of $\mu_{\max}$ and K,and parameters $\mu_{\max}$ and K are independent of each other. Let $y(t) = \frac{\partial x(t)}{\partial K}$, $z(t) = \frac{\partial x(t)}{\partial \mu_{\max}}$, thus $y(0) = 0, z(0) = 0$.

In the following sections, we will consider two cases:

• Biomass concentration $X(t)$ is regarded as constant X;

Then the parameters K and $\mu_{\max}$ satisfy the sensitivity equations:

$$\dot{y}(t) = \mu_{\max} \frac{(x(t) - Ky(t))}{(K + x(t))^2} X, \tag{11}$$

$$\dot{z}(t) = -\mu_{\max} \frac{Kz(t)}{(K + x(t))^2} X - \frac{x(t)}{K + x(t)} X. \tag{12}$$

• Biomass concentration depends on time t.

The corresponding parameters K and $\mu_{\max}$ satisfy the sensitivity equations:

$$\dot{y}(t) = \mu_{\max} \frac{(x(t) - Ky(t))}{(K + x(t))^2} X(t) + Y\mu_{\max}^2 \frac{x(t)}{K + x(t)} \int_0^t \frac{(x(s) - Ky(s))}{(K + x(s))^2} ds X(t) \tag{13}$$

$$\dot{z}(t) = -\frac{x(t)(K + x(t)) + K\mu_{\max} z(t)}{(K + x(t))^2} X(t) - Y\mu_{\max} \frac{x(t)}{K + x(t)} \int_0^t \frac{x(s)(K + x(s)) + K\mu_{\max} z(s)}{(K + x(s))^2} ds X(t) \tag{14}$$

In order to estimate system parameters, such that the system can optimally excite the parametric sensitivities, we define the cost functional:

$$J = \int_0^T [py^2(t) + qz^2(t)] dt, \tag{15}$$

where $p, q \in (0, 1]$ represent the contribution of the parameters to the systematic sensitivities, respectively; T is the fixed terminal time which stands for the end of the chemical experiment. The terminal time is not free in the paper, allowing us to be able to find the optimal feed rate at any expected terminal time. If the terminal time is free, the optimal control problem will become different: we will not only find the optimal feed rate but also find the optimal terminal time.

Some constraints must be considered. Cell concentration must be nonnegative and the input u is bounded. Let $a<0<b$ and maximize the functional $J(\cdot)$ in the input region

$$\mathscr{U} \equiv \{u(\cdot): [0, T] \to [a, b] \mid x(t) \geq 0,\ u(\cdot) \text{ is measurable}\}.$$

That is, find a $u^*(\cdot) \in \mathscr{U}$, such that

$$J(u^*(\cdot)) = \max_{u(\cdot) \in \mathscr{U}} J(u(\cdot)). \tag{16}$$

We need to solve the optimal control problem with the state constraints in practice.

3 Maximum principle with state constraints

Here we consider a more general problem than 1 [1]:

$$\begin{cases} \dot{x}(t) = f(t, x(t), u(t)), \\ x(0) = x_0. \end{cases} \tag{17}$$

The cost functional is

$$J(u(\cdot)) = \int_0^T F(t, x(t), u(t))dt + S(T, x(T)), \tag{18}$$

subjected to the following state constraints

$$g(t, x(t), u(t)) \geq 0, \quad h(t, x(t)) \geq 0. \tag{19}$$

$$a(T, x(T)) \geq 0, \quad b(T, x(T)) = 0. \tag{20}$$

where F is the cost function, depending on the state and the control and S is the cost assigned to the terminal state. Assume that $F: \mathbb{R} \times \mathbb{R}^n \times \mathbb{R}^m \to \mathbb{R}$, $S: \mathbb{R} \times \mathbb{R}^n \to \mathbb{R}$, $f: \mathbb{R} \times \mathbb{R}^n \times \mathbb{R}^m \to \mathbb{R}^n$, $g: \mathbb{R} \times \mathbb{R}^n \times \mathbb{R}^m \to \mathbb{R}^s$, $h: \mathbb{R} \times \mathbb{R}^n \to \mathbb{R}^q$, $a: \mathbb{R} \times \mathbb{R}^n \to \mathbb{R}^l$, $b: \mathbb{R} \times \mathbb{R}^n \to \mathbb{R}^{l'}$ are continuously differentiable with respect to all their arguments. In addition, h is higher order differentiable as required.We are looking for a measurable control $u: [0, T] \to \mathbb{R}^m$ and a corresponding state trajectory $x(\cdot): [0, T] \to \mathbb{R}^n$ that is an absolutely continuous function, such that the constraints 17,19–20 are satisfied and the objective functional 18 takes its maximum value. For simplicity in exposition, we call $\{x(\cdot), u(\cdot)\}$ a *feasible pair* if $x(\cdot)$ is the state trajectory corresponding to $u(\cdot)$ and conditions 19–20 are satisfied. A feasible pair that globally maximizes 18 is called an *optimal pair*.

The Hamiltonian H and Lagrangian L are defined as follows :

$$H(t, x, u, \lambda) = F(t, x, u) + \langle \lambda, f(t, x, u) \rangle, \tag{21}$$

$$L(t, x, u, \lambda, \mu, \nu) = H(t, x, u, \lambda) + \langle \mu, g(t, x, u) \rangle + \langle \nu, h(t, x) \rangle. \tag{22}$$

where $\lambda \in \mathbb{R}^n$, $\mu \in \mathbb{R}^s$, $\nu \in \mathbb{R}^q$ and $\langle \cdot, \cdot \rangle$ are scalar products between vectors. Define the control region:

$$\Omega(t, x) = \{u \in \mathbb{R}^m : g(t, x, u) \geq 0\} \tag{23}$$

and the full rank condition:

$$\text{rank}[\partial g / \partial u, \ \text{diag}(g)] = s \tag{24}$$

Remark 1 In order to distinguish between the mixed constraints $g \geq 0$ and the pure state constraints $h \geq 0$, we assume that each component of the function g depends

explicitly on the control u. More precisely, we impose the full rank condition. The constraint qualification 24 means that the gradients with respect to u of all the active constraints $g \geq 0$ must be linearly independent.

Before stating a formulation of the maximum principle, for convenience in writing, we introduce the following abbreviation. For a certain solution $\{x^*(t), u^*(t)\}$, let $F^*[t] = F(t, x^*(t), u^*(t))$, and likewise for f, g and any of the other functions.

Theorem 1 [1] *Let $\{x^*(\cdot), u^*(\cdot)\}$ be an optimal pair of the above-mentioned problem in the fixed period of time $[0, T]$, such that $u^*(\cdot)$ is right-continuous with left-hand limits and the full rank condition holds for every triple $\{t, x^*(t), u\}$ $t \in [0, T]$ with $u \in \Omega(t, x^*(t))$. Assume that $x^*(\cdot)$ has finite discontinuities. Then there exist a piecewise absolutely continuous costate trajectory $\lambda(\cdot)$ and piecewise continuous multiplier functions $\mu(\cdot), \nu(\cdot)$, a vector $\eta(\tau_i) \in \mathbb{R}^q$ for each point τ_i of discontinuity of $\lambda(\cdot)$ and $\alpha \in \mathbb{R}^l$, $\beta \in \mathbb{R}^{l'}$, $\gamma \in \mathbb{R}^q$, such that for every t, we have $(\lambda(t), \mu(t), \nu(t), \alpha, \beta, \gamma, \eta(\tau_i)) \neq 0$ and the following conditions hold almost everywhere:*

$$u^*(t) = \arg \max_{u \in \Omega(t, x^*(t))} H(t, x^*(t), u, \lambda(t)), \tag{25}$$

$$L_u^*[t] = H_u^*[t] + \mu g_u^*[t] = 0, \tag{26}$$

$$\dot{\lambda}(t) = -L_x^*[t], \tag{27}$$

$$\mu(t) \geq 0, \quad \mu(t) g^*[t] = 0, \tag{28}$$

$$\nu(t) \geq 0, \quad \nu(t) h^*[t] = 0, \tag{29}$$

$$\frac{dH^*[t]}{dt} = \frac{dL^*[t]}{dt} = \frac{\partial L^*[t]}{\partial t}. \tag{30}$$

At the terminal time, the following transversality conditions hold:

$$\lambda(T^-) = S_x^*[T] + \alpha a_x^*[T] + \beta b_x^*[T] + \gamma h_x^*[T], \tag{31}$$

$$\alpha \geq 0, \ \gamma \geq 0, \quad \alpha a^*[T] = \gamma h^*[T] = 0. \tag{32}$$

At each point of discontinuity τ, it holds:

$$\lambda(\tau^-) = \lambda(\tau^+) + \eta(\tau) h_x^*[\tau], \tag{33}$$

$$H^*[\tau^-] = H^*[\tau^+] - \eta(\tau) h_t^*[\tau], \tag{34}$$

$$\eta(\tau) \geq 0, \quad \eta(\tau) h^*[\tau] = 0. \tag{35}$$

The maximum principle with state constraints has been introduced. Two questions of the problem arise:

1. *Existence*. Do the optimal inputs of the problem in the two cases exist?
2. *Analytical solution*. If the answer to the first question is positive, then can we find the analytical solutions of the problem in the two cases?

In the next section, Problems 1 and 2 will be solved for both cases.

4 Problem solution

As far as existence is concerned, due to the compactness of the control region, the set of the state trajectories is compact. Thus we can prove the existence of the optimal control of this problem in terms of the Filippov–Cesari theorem [13].

The control of the reactor is solved by the following steps, already mentioned in Sect. 3.

4.1 Case of constant cell concentration

In this situation, the system consists of one equation describing the model and two sensitivity equations, then it reads:

$$\begin{cases} \dot{x}(t) = -\mu_{\max}\frac{x(t)}{K+x(t)}X + u(t), \\ \dot{y}(t) = \mu_{\max}\frac{(x(t)-Ky(t))}{(K+x(t))^2}X, \\ \dot{z}(t) = -\mu_{\max}\frac{Kz(t)}{(K+x(t))^2}X - \frac{x(t)}{K+x(t)}X. \end{cases} \tag{36}$$

Here $g = \begin{pmatrix} u-a \\ b-u \end{pmatrix}$, $h = x$, so the corresponding Hamiltonian and Lagrangian functions are:

$$H(t,x,y,z,u,\lambda_1,\lambda_2,\lambda_3) = \lambda_1\left(-\mu_{\max}\frac{x}{K+x}X + u\right) + \lambda_2\mu_{\max}\frac{(x-Ky)}{(K+x)^2}X + \lambda_3\left(-\mu_{\max}\frac{Kz}{(K+x)^2} - \frac{x}{K+x}\right)X + py^2 + qz^2, \tag{37}$$

$$L(t,x,u,\lambda,\mu,\nu) = H(t,x,y,z,u,\lambda_1,\lambda_2,\lambda_3) + \mu_1(u-a) + \mu_2(b-u) + \nu x, \tag{38}$$

where

$$\nu = \begin{cases} \geq 0, & x^* = 0 \\ 0, & x^* > 0 \end{cases}, \quad \mu_1,\mu_2 \geq 0, \quad \mu_1(u^*-a) = \mu_2(b-u^*) = 0.$$

$$\dot{\lambda_1}(t) = -L_x^*[t] = \lambda_1\mu_{\max}\frac{K}{(K+x^*)^2}X - \lambda_2\mu_{\max}\frac{(K-x^*+2Ky^*)}{(K+x^*)^3}X - \lambda_3\left(\mu_{\max}\frac{2Kz^*}{(K+x^*)^3} - \frac{K}{(K+x^*)^2}\right)X - \nu\,, \tag{39}$$

$$\dot{\lambda_2}(t) = -L_y^*[t] = -2py^* + \lambda_2\mu_{\max}\frac{K}{(K+x^*)^2}X, \tag{40}$$

$$\dot{\lambda_3}(t) = -L_z^*[t] = -2qz^* + \lambda_3\mu_{\max}\frac{K}{(K+x^*)^2}X, \tag{41}$$

$$L_u^*[t] = H_u^*[t] + \mu g_u^*[t] = \lambda_1 + \mu_1 - \mu_2 = 0. \tag{42}$$

At each point of discontinuity τ, it holds:

$$\lambda_1(\tau^-) = \lambda_1(\tau^+) + \eta(\tau), \tag{43}$$
$$H^*[\tau^-] = H^*[\tau^+] - \eta(\tau)x_t^*(\tau), \tag{44}$$
$$\lambda_2(\tau^-) = \lambda_2(\tau^+),\ \lambda_3(\tau^-) = \lambda_3(\tau^+). \tag{45}$$

Since the system, the cost functional, g and h do not explicitly depend on t, then H must be constant for an optimal solution, namely

$$H^* = \text{const},\ t \in [0, T]. \tag{46}$$

We have

$$u^*(t) = \begin{cases} b, & \text{if } \lambda_1 > 0 \\ \text{undetermined}, & \text{if } \lambda_1 = 0 \\ a, & \text{if } \lambda_1 < 0 \end{cases} \tag{47}$$

By Eq. 47, the solution of $u^*(t)$ lies on the determination of the λ_1 sign. In optimal control, problems of singular control are problems that are difficult to solve because a straightforward application of Pontryagin's maximum principle fails to yield a complete solution. The most common difficulty in applying Pontryagin's principle arises when the Hamiltonian depends linearly on the control u (See Eq. 37) and the control is restricted to being between an upper and a lower bound: $a \le u(t) \le b$. To maximize $H(u)$, we need to make u as big or as small as possible, depending on the sign of λ_1, specifically:

If λ_1 is positive at some times, negative at others and is only zero instantaneously, then the solution is straightforward and is a bang–bang control that switches from a to b at times when λ_1 switches from negative to positive.

The case when λ_1 remains at zero for a finite length of time $[t_1, t_2]$ is called the singular control case. Between t_1 and t_2 the maximization of the Hamiltonian with respect to u gives us no useful information and the solution in that time interval is going to have to be found from other considerations. One approach would be to repeatedly differentiate $\partial H/\partial u$ with respect to time until the control u again explicitly appears, which is guaranteed to happen eventually. One can then set that expression to zero and solve for u. This amounts to saying that between t_1 and t_2 the control u is determined by the requirement that the singularity condition continues to hold. The resulting so-called singular arc will be optimal if it satisfies the condition 83.

We assume that $x(0) > 0$ is independent of the parameters $\mu_{\max}$ and K. We have $y(0) = z(0) = 0$ by the definitions of y, z. $\forall t \in [0, T]$, the expressions of $y(t)$ and $z(t)$ can be written as:

$$y(t) = \mu_{\max} X \int_0^t \exp\left(- \int_s^t \frac{K\mu_{\max} X}{(K + x(l))^2} dl \right) \frac{x(s)}{(K + x(s))^2} ds \tag{48}$$

$$z(t) = -X \int_0^t \exp\left(-\int_s^t \frac{K\mu_{\max} X}{(K + x(l))^2} dl \right) \frac{x(s)}{K + x(s)} ds \tag{49}$$

Theorem 2 *If the cell population X is constant and $qK^2 - p\mu_{\max}^2 \geq 0$, then the optimal pair $\{x^*(\cdot), u^*(\cdot)\}$ of the problem defined by Eq. 36 is such that $x^*(t) > 0$, $u^*(t) = b$ a.e. $t \in [0, T]$.*

Proof Case 1 $x^*(t) = 0$, a.e. $t \in [0, T]$

It is obtained that $y^*(t) = z^*(t) = 0$, a.e. $t \in [0, T]$ by the definitions of y and z, $u^*(t) = 0$, a.e. $t \in [0, T]$ by Eq. 36 and $\mu_1(t) = \mu_2(t) = 0$ under the Maximum Principle condition 38. Using 42, we have $\lambda_1(t) = 0$, a.e. $t \in [0, T]$ and the corresponding Hamiltonian H^* is 0.

However $x^*(0) > 0$, and then the control u^* is an impulse control at the initial time point which is a generalized solution, not a classical solution that we desire. So it does not meet the requirement in this case.

Case 2 $x^*(t) > 0$, a.e. $t \in [0, T]$

Now, $v(t) = 0$, $y^*(t) > 0$, $z^*(t) < 0$ a.e. $t \in [0, T]$. Under the terminal condition, it holds $\lambda_1(T^-) = \gamma$, $\lambda_2(T^-) = \lambda_3(T^-) = 0$, where $\gamma \geq 0$, $\gamma x^*(T^-) = 0$, and $x^*(T^-) > 0$, then $\gamma = 0$. Using Eqs. 39–41, we can get:

$$\lambda_1(t) = X \int_t^T \exp\left(\int_s^t \frac{K\mu_{\max} X}{(K + x^*(l))^2} dl \right) \frac{\Phi(s)}{(K + x^*(s))^3} ds, \tag{50}$$

where $\Phi(s) = [-\mu_{\max}\lambda_2(s) - K\lambda_3(s)]x^*(s) + \lambda_2(s)\mu_{\max}(K + 2Ky^*(s)) + \lambda_3(s)(2K\mu_{\max} z^*(s) - K^2)$.

$$\lambda_2(t) = 2p \int_t^T \exp\left(\int_s^t \frac{K\mu_{\max} X}{(K + x^*(l))^2} dl \right) y^*(s) ds, \tag{51}$$

$$\lambda_3(t) = 2q \int_t^T \exp\left(\int_s^t \frac{K\mu_{\max} X}{(K + x^*(l))^2} dl \right) z^*(s) ds. \tag{52}$$

Then Eqs. 51 and 52 lead to $\lambda_2(t) > 0$, $\lambda_3(t) < 0$ a.e. $t \in [0, T]$.

We assume that $qK^2 - p\mu_{\max}^2 \geq 0$, which is a natural assumption. In practice, $K \gg \mu_{\max}$ (For example $K = 1$, $\mu_{\max} = 4.09 \times 10^{-4}$ [2]) and p, q are over the same order of magnitude, so the assumption is reasonable.

Substitute Eqs. 48 and 49 into Eqs. 51 and 52, respectively, and yield:

$$\lambda_2(t) = 2p \int_t^T \int_0^s \exp\left(\left(\int_s^t - \int_l^s \right) \frac{K\mu_{\max} X}{(K + x^*(r))^2} dr \right) \frac{\mu_{\max} X x^*(l)}{(K + x^*(l))^2} dl ds, \tag{53}$$

$$\lambda_3(t) = -2q \int_t^T \int_0^s \exp\left(\left(\int_s^t - \int_l^s\right) \frac{K\mu_{\max} X}{(K + x^*(r))^2} dr\right) \frac{X x^*(l)}{K + x^*(l)} dl ds, \quad (54)$$

Using Eqs. 53 and 54 and the assumption condition $qK^2 - p\mu_{\max}^2 \geq 0$, thus, $\Phi(s) > 0$, a.e. $s \in [0, t]$, then $\lambda_1(t) > 0$, a.e. $t \in [0, T]$, hence, $u^*(t) = b$, a.e. $t \in [0, T]$.

Case 3 $x^*(t) \geq 0,\ t \in [0, T]$

Let $t_1 > 0, t_1 < t_2 \leq T$, and suppose that there exists at least one interval $[t_1, t_2] \subset [0, T]$, such that $x^*(t) = 0$, a.e. $t \in [t_1, t_2]$. By case 1, Hamiltonian is 0, then we have

$$\begin{aligned} & py^{*2}(T^-) + qz^{*2}(T^-) + \lambda_1(T^-)(-\mu_{\max} \frac{x^*}{K + x^*} X + u^*) \\ & \quad + \lambda_2(T^-)\mu_{\max} \frac{(x^* - Ky^*)}{(K + x^*)^2} X + \lambda_3(T^-) \left(-\mu_{\max} \frac{Kz^*}{(K + x^*)^2} - \frac{x^*}{K + x^*}\right) X \\ & = py^{*2}(T^-) + qz^{*2}(T^-) = 0. \end{aligned} \quad (55)$$

We infer that $y^*(T^-) = z^*(T^-) = 0$ which is in contradiction with $y^*(T^-) > 0, z^*(T^-) < 0$. Hence, case 3 is impossible. □

Remark 2 $x^*(T^-) \geq 0$, it holds $\lambda_1(T^-)\left(-\mu_{\max} \frac{x}{K+x} X + u\right) = 0$. If $x^*(T^-) > 0$, in terms of the terminal condition then $\lambda_1(T^-) = 0$; If $x^*(T^-) = 0$, then $u^*(T^-) = 0$.

In addition, we consider the factor of the substrate output into the model, the substrate model reads:

$$\dot{x}(t) = -\mu_{\max} \frac{x(t)}{K + x(t)} X - Dx(t) + u(t) \quad (56)$$

where the constant $D > 0$ is the output rate of the substrate.

Corollary 1 *The cost functional and the control region are the same as the above-mentioned discussion. On the hypothesis of* $qK^2 \geq p\mu_{\max}^2$, *there exists an optimal pair* $\{x^*(\cdot), u^*(\cdot)\}$ *satisfying* $x^*(t) > 0,\ u^*(t) = b$ *a.e.* $t \in [0, T]$.

4.1.1 Numerical results and discussion

In order to demonstrate the advantages of utilizing the optimal input, the parameter estimation accuracy is compared with and without the optimal input in the presence of observation noise. The analytical solution for the optimal input is first evaluated for

$$K = 1, \quad \mu_{\max} = 0.005, \quad x(0) = 2, \quad X = 1000, \quad T = 200, \quad b = 4$$

For the non-optimal input, a sine wave with angular frequency $\omega = 0.01$ rad/min is utilized. Then for the same power input as for the optimal input we have $u'(t) = b \sin \omega t$.

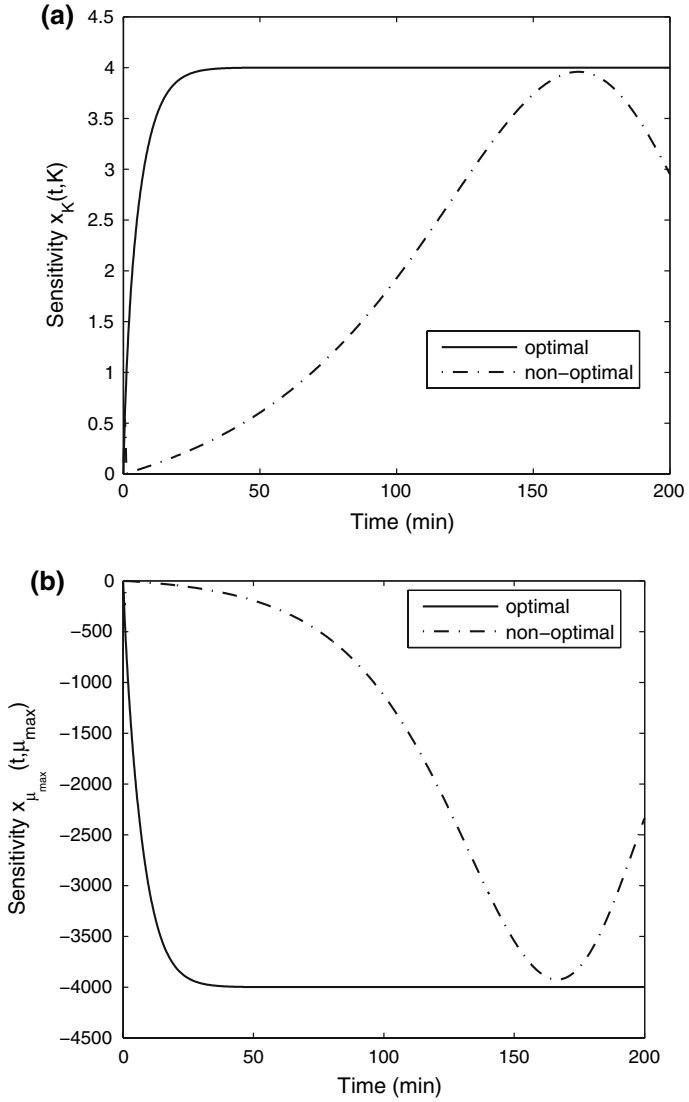

Fig. 1 Comparison of optimal and non-optimal inputs for $K = 1$mg/l, $\mu_{\max} = 0.005$, $x(0) = 2$mg/L, $X = 1000$mg/L, $T = 200$ min, $b = 4$mg/(L min). The optimal input: $u(t) = b$ and the non-optimal input: $u(t) = b \sin \omega t$ ($\omega = 0.01$ rad/ min). **a** Sensitivity $x_K(t, K)$ versus time. **b** Sensitivity $x_{\mu_{\max}}(t, \mu_{\max})$ versus time

Figure 1 a,b show the optimal and non-optimal sensitivities about parameters $K, \mu_{\max}$ as a function of time, corresponding to the inputs given in the above paragraph. It is seen that the absolute value of the sensitivity for the optimal input is higher than that for the non-optimal input.

The parameter estimation problem is solved via the method of quasi-linearization. First it is assumed that the true value of the parameters $K, \mu_{\max}$ are equal to 1 and

Table 1 Estimates of the Parameter $\mu_{\max}$ for a typical run

Cycle number	Estimate of $\mu_{\max}$	
	Optimal input	Non-optimal input
0	0.001	0.001
⋮	⋮	⋮
8	0.0050003	0.0049985
9	0.0050003	0.0049985
Percent error (cycle 9)	0.006%	0.03%
Average estimate (10 runs)	0.0044	0.0042
Standard deviation (10 runs)	0.0012	0.0013

$K = 1\text{mg/L}, x(0) = 2\text{mg/L}, X = 1000\text{mg/L}, T = 200\,\text{min}, b = 4\text{mg/(L min)}$. The optimal input: $u(t) = b$ and the non-optimal input: $u(t) = b \sin \omega t$ ($\omega = 0.01\,\text{rad/min}$). The first approximation of the parameter $\mu_{\max}$ is assumed to be $\mu_{\max} = 0.001$. 1000 additional observations are made at 0.2 min intervals. (True value of $\mu_{\max} = 0.005$)

0.005, respectively. The system has two unknown parameters. For simplicity, we fix one of the two parameters and estimate the other. Then for the conditions given, the optimal input is the maximal allowed input. The observations are formed by adding noise to the optimal input response, $x(t)$

$$m_i = x(t_i) + v(t_i), \tag{57}$$

where m_i is the observation at time t_i, $x(t_i)$ is the state variable, and $v(t_i)$ is the white Gaussian noise with zero mean and variance equal to 0.1. Starting with the initial condition, which is assumed to be known exactly at time $t = 0$, we made one thousand additional observations. The first approximations of the parameters K, $\mu_{\max}$ are assumed to be 0.5 and 0.001, respectively.

The procedure is repeated for the non-optimal input given in the above paragraph. The observations are formed according to Eq. 57 by adding noise to the non-optimal input response. As previously, one thousand observations are made, and the first approximations of the parameters K, $\mu_{\max}$ are assumed to be 0.5 and 0.001, respectively.

Tables 1 and 2 show parameter estimates for a typical run in presence of both the optimal and non-optimal inputs. Note that the additive noise is identical for both the optimal and non-optimal inputs. The percent error in the final estimate of $\mu_{\max}$ is 0.006% for the optimal input and 0.03% for the non-optimal input in Table 1. The percent error in the final estimate of K is 0.101% for the optimal input and 0.118% for the non-optimal input in Table 2. And the two tables show the average estimates and standard deviations for both the optimal and non-optimal inputs. While the average estimate of the optimal input is greater than that of the non-optimal input, the standard deviation of the optimal input is smaller than that of the non-optimal input in Table 1. The average estimates and the standard deviations are the same in Table 2. The results clearly show the advantages of utilizing the optimal input.

Table 2 Estimates of the Parameter K for a typical run

Cycle number	Estimate of K	
	Optimal input	Non-optimal input
0	0.5	0.5
1	0.99571	0.99617
2	0.99899	0.99882
3	0.99899	0.99882
Percent error (cycle 3)	0.101%	0.118%
Average estimate (5 runs)	0.8985	0.8985
Standard deviation (5 runs)	0.2228	0.2228

$\mu_{\max} = 0.005$, $x(0) = 2$mg/L, $X = 1000$mg/L, $T = 200$ min, $b = 4$mg/(L min). The optimal input: $u(t) = b$ and the non-optimal input: $u(t) = b \sin \omega t$ ($\omega = 0.01$ rad/min). The first approximation of the parameter K is assumed to be $K = 0.5$. 1000 additional observations are made at 0.2 min intervals. (True value of $K = 1$)

4.2 Case of time-varying cell concentration

In the previous section we supposed that the biomass is constant. In this part, we will consider the biomass X changing with the variation of the substrate concentration. The substrate x and the biomass X satisfy the following equation set:

$$\begin{cases} \dot{x}(t) = -\mu_{\max} \frac{x(t)}{K+x(t)} X(t) + u(t), \\ \dot{X}(t) = Y \mu_{\max} \frac{x(t)}{K+x(t)} X(t). \end{cases} \tag{58}$$

By the expression of X, we have $X(t) > 0, t \in [0, T]$. The corresponding parameters K and $\mu_{\max}$ satisfy the sensitivity Eqs. 13 and 14.

Let

$$\alpha(t) = \int_0^t \frac{(x(s) - Ky(s))}{(K + x(s))^2} ds, \tag{59}$$

$$\beta(t) = \int_0^t \frac{x(s)(K + x(s)) + K\mu_{\max} z(s)}{(K + x(s))^2} ds. \tag{60}$$

then y, z satisfy the following differential equations:

$$\dot{y}(t) = \mu_{\max} \dot{\alpha}(t) X(t) + \mu_{\max} \alpha(t) \dot{X}(t), \tag{61}$$

$$\dot{z}(t) = -\dot{\beta}(t) X(t) - \beta(t) \dot{X}(t). \tag{62}$$

Then

$$y(t) = \mu_{\max} \alpha(t) X(t), \tag{63}$$

$$z(t) = -\beta(t)X(t). \tag{64}$$

and substitute them into the expressions of α, β, we can obtain:

$$\alpha(t) = \int_0^t \frac{x(s)}{(K + x(s))^2} \exp\left(-\int_s^t \frac{\mu_{\max} K X(p)}{(K + x(p))^2} dp\right) ds \tag{65}$$

$$\beta(t) = \int_0^t \frac{x(s)}{K + x(s)} \exp\left(-\int_s^t \frac{\mu_{\max} K X(p)}{(K + x(p))^2} dp\right) ds \tag{66}$$

The system becomes:

$$\begin{cases} \dot{x}(t) &= -\mu_{\max} \frac{x(t)}{K+x(t)} X(t) + u(t), \\ \dot{X}(t) &= Y \mu_{\max} \frac{x(t)}{K+x(t)} X(t), \\ \dot{\alpha}(t) &= \frac{x(t) - K\mu_{\max}\alpha(t)X(t)}{(K+x(t))^2}, \\ \dot{\beta}(t) &= \frac{x(t)(K+x(t)) - K\mu_{\max}\beta(t)X(t)}{(K+x(t))^2}. \end{cases} \tag{67}$$

The corresponding cost functional is :

$$J = \int_0^T [p\mu_{\max}^2 \alpha^2(t) + q\beta^2(t)] X^2(t) dt. \tag{68}$$

The corresponding Hamiltonian and Lagrangian are:

$$\begin{aligned} H(t, x, X, \alpha, \beta, u, \lambda_1, \lambda_2, \lambda_3, \lambda_4) = \lambda_1 &\left(-\mu_{\max} \frac{x}{K + x} X + u\right) \\ &+ \lambda_2 Y \mu_{\max} \frac{x}{K + x} X + \lambda_3 \frac{(x - K\mu_{\max}\alpha X)}{(K + x)^2} \\ &+ \lambda_4 \frac{[x(K + x) - K\mu_{\max}\beta X]}{(K + x)^2} \\ &+ (p\mu_{\max}^2 \alpha^2 + q\beta^2) X^2, \end{aligned} \tag{69}$$

$$\begin{aligned} L(t, \mathcal{X}, u, \lambda, \mu, \nu) = H(t, x, X, \alpha, \beta, u, \lambda_1, \lambda_2, \lambda_3, \lambda_4) \\ + \mu_1(u - a) + \mu_2(b - u) + \nu x. \end{aligned} \tag{70}$$

where

$$\nu = \begin{cases} \geq 0, & x^* = 0 \\ 0, & x^* > 0 \end{cases}, \quad \mu_1,\ \mu_2 \geq 0, \quad \mu_1(u^* - a) = \mu_2(b - u^*) = 0.$$

$$\dot{\lambda_1}(t) = -L_x^*[t] = \lambda_1\mu_{\max}\frac{KX^*}{(K+x^*)^2} - \lambda_2 Y\mu_{\max}\frac{KX^*}{(K+x^*)^2} - \lambda_3\frac{(K-x^*+2K\mu_{\max}\alpha^*X^*)}{(K+x^*)^3} - \lambda_4\frac{[K(K+x^*)+2K\mu_{\max}\beta^*X^*]}{(K+x^*)^3} - \nu, \tag{71}$$

$$\dot{\lambda_2}(t) = -L_X^*[t] = -2(p\mu_{\max}^2\alpha^{*2}+q\beta^{*2})X^* + \lambda_1\mu_{\max}\frac{x^*}{K+x^*} - \lambda_2 Y\mu_{\max}\frac{x^*}{K+x^*} + \lambda_3\frac{K\mu_{\max}\alpha^*}{(K+x^*)^2} + \lambda_4\frac{K\mu_{\max}\beta^*}{(K+x^*)^2}, \tag{72}$$

$$\dot{\lambda_3}(t) = -L_\alpha^*[t] = -2p\mu_{\max}^2\alpha^*X^{*2} + \lambda_3\frac{K\mu_{\max}X^*}{(K+x^*)^2}, \tag{73}$$

$$\dot{\lambda_4}(t) = -L_\beta^*[t] = -2q\beta^*X^{*2} + \lambda_4\frac{K\mu_{\max}X^*}{(K+x^*)^2}, \tag{74}$$

$$L_u^*[t] = H_u^*[t] + \mu g_u^*[t] = \lambda_1 + \mu_1 - \mu_2 = 0. \tag{75}$$

Since the system, the cost functional, g and h do not explicitly depend on t, then H^* is constant, that is,

$$H^* = \text{const},\ t \in [0,T]. \tag{76}$$

We can obtain

$$u^*(t) = \begin{cases} b, & \text{if } \lambda_1 > 0 \\ \text{undetermined}, & \text{if } \lambda_1 = 0 \\ a, & \text{if } \lambda_1 < 0 \end{cases} \tag{77}$$

By 77, the solution of $u^*(t)$ lies on the determination of the λ_1 sign.

Theorem 3 *If the cell population X is changing with time, then the optimal pair $\{x^*(\cdot), u^*(\cdot)\}$ of the problem defined by Eq. 67 is such that $x^*(t) > 0$, a.e. $t \in [0,T]$.*

Proof Case 1 $x^*(t) = 0$, a.e. $t \in [0,T]$

We have $\alpha^*(t) = \beta^*(t) = 0$, $u^*(t) = 0$, a.e. $t \in [0,T]$. Under the Maximum Principle condition, it holds $\mu_1(t) = \mu_2(t) = 0$, by 75, we get $\lambda_1(t) = 0$, a.e. $t \in [0,T]$ and the corresponding Hamiltonian H^* is 0.

However $x^*(0) > 0$, and then the control u^* is an impulse control at the initial time point which is a generalized solution, not a classical solution that we desire. Thus the case does not meet the requirement.

Case 2 $x^*(t) > 0$, a.e. $t \in [0,T]$

Then, $\nu(t) = 0$, $\alpha^*(t) > 0$, $\beta^*(t) > 0$ a.e. $t \in [0,T]$. Under the terminal conditions, it is concluded that $\lambda_1(T^-) = \lambda_2(T^-) = \lambda_3(T^-) = \lambda_4(T^-) = 0$.

Using Eqs. 73 and 74, we can conclude

$$\lambda_3(t) = 2p\mu_{\max}^2 \int_t^T \alpha^*(s) X^{*2}(s) \exp\left(\int_s^t \frac{K\mu_{\max} X^*(p)}{(K + x^*(p))^2} dp \right) ds, \tag{78}$$

$$\lambda_4(t) = 2q \int_t^T \beta^*(s) X^{*2}(s) \exp\left(\int_s^t \frac{K\mu_{\max} X^*(p)}{(K + x^*(p))^2} dp \right) ds, \tag{79}$$

Further, $\lambda_3(t) > 0$, $\lambda_4(t) > 0$ a.e. $t \in [0, T]$.
Under the terminal conditions, using Eqs. 71,72, we can conclude

$$\begin{aligned} \lambda_1(t) = \int_t^T \exp\left(\int_s^t \frac{K\mu_{\max} X^*(p)}{(K + x^*(p))^2} dp \right) \Bigg\{ & \lambda_2(s) \frac{Y\mu_{\max} K X^*(s)}{(K + x^*(s))^2} \\ & + \lambda_3(s) \frac{[K - x^*(s) + 2K\mu_{\max}\alpha^*(s) X^*(s)]}{(K + x^*(s))^3} \\ & + \lambda_4(s) \frac{[K(K + x^*(s)) + 2K\mu_{\max}\beta^*(s) X^*(s)]}{(K + x^*(s))^3} \Bigg\} ds, \end{aligned} \tag{80}$$

$$\begin{aligned} \lambda_2(t) = \int_t^T \exp\left(- \int_s^t \frac{Y\mu_{\max} x^*(p)}{K + x^*(p)} dp \right) \Bigg\{ & 2[p\mu_{\max}^2 \alpha^{*2}(s) + q\beta^{*2}(s)] X^*(s) \\ & - \lambda_1(s)\mu_{\max} \frac{x^*(s)}{K + x^*(s)} - \lambda_3(s) \frac{K\mu_{\max}\alpha^*(s)}{(K + x^*(s))^2} - \lambda_4(s) \frac{K\mu_{\max}\beta^*(s)}{(K + x^*(s))^2} \Bigg\} ds. \end{aligned} \tag{81}$$

Case 3 $x^*(t) \geq 0,\ t \in [0, T]$

Let $t_1 > 0, t_1 < t_2 \leq T$, suppose that there exists at least one interval $[t_1, t_2] \subset [0, T]$, such that $x^*(t) = 0,$ a.e. $t \in [t_1, t_2]$. By case 1, Hamiltonian is 0. Thus, we get

$$\begin{aligned} & [p\mu_{\max}^2 \alpha^{*2}(T^-) + q\beta^{*2}(T^-)] X^{*2} + \lambda_1(T^-) \left(-\mu_{\max} \frac{x^*}{K + x^*} X^* + u^* \right) \\ & + \lambda_2(T^-) Y\mu_{\max} \frac{x^*}{K + x^*} X^* + \lambda_3(T^-) \frac{(x^* - K\mu_{\max}\alpha^* X^*)}{(K + x^*)^2} \\ & + \lambda_4(T^-) \frac{[x^*(K + x^*) - K\mu_{\max}\beta^* X^*]}{(K + x^*)^2} = [p\mu_{\max}^2 \alpha^{*2}(T^-) + q\beta^{*2}(T^-)] X^{*2} = 0, \end{aligned}$$

It is inferred that $\alpha^*(T^-) = \beta^*(T^-) = 0$ which is in contradiction with $\alpha^*(T^-) > 0$, $\beta^*(T^-) > 0$. Then case 3 is impossible. □

4.2.1 Law of the singular control

If there exists a singular control, then there exists a closed interval $I \subset [0, T]$, such that $\lambda_1(t) = 0$, a.e. $t \in I$, that is, $H_u = 0$, in that case $\frac{d\lambda_1(t)}{dt} = 0$, namely $\frac{dH_u}{dt} = 0$ and using Eq. 71 it can be derived as

$$0 = \lambda_2 Y \mu_{\max} K X^*(K + x^*) + \lambda_3(K - x^* + 2K\mu_{\max}\alpha^* X^*) + \lambda_4[K(K + x^*) + 2K\mu_{\max}\beta^* X^*]. \tag{82}$$

Furthermore, the existence of the singular control has to satisfy a necessary condition:

$$\frac{\partial}{\partial u}\left[\frac{d^2 H_u}{dt^2}\right] \geq 0, \quad \text{namely, } \frac{\partial}{\partial u}\left[\frac{d^2\lambda_1(t)}{dt^2}\right] \geq 0 \tag{83}$$

This condition of the optimal control problem is usually called generalized Legendre-Clebsch condition.

Let

$$\begin{aligned}
\Phi(t) &= 2X^{*2}(t)(K + x^*(t))\left\{Y\mu_{\max}K\left[p\mu_{\max}^2\alpha^{*2}(t) + q\beta^{*2}(t)\right](K + x^*(t))\right. \\
&\quad + p\mu_{\max}^2\alpha^*(t)\left[K - x^*(t) + 2K\mu_{\max}\alpha^*(t)X^*(t)\right] + q\beta^*(t)[K^2 + Kx^*(t) \\
&\quad \left. + 2K\mu_{\max}\beta^*(t)X^*(t)]\right\}, \\
\Psi(t) &= \mu_{\max}x^*(t)X^*(t)\left[\lambda_3(t)(1 + 2K\mu_{\max}Y\alpha^*(t)) + K\lambda_4(t)(4 + 2\mu_{\max}Y\beta^*(t))\right] \\
&\quad + Y\mu_{\max}^2 K^2 X^*(t)[\lambda_3(t)\alpha^*(t) + \lambda_4(t)\beta^*(t)] + \frac{\mu_{\max}X^*(t)}{K + x^*(t)}\left[\lambda_3(t)\left(2Kx^*(t)\right.\right. \\
&\quad \left. + K^2 - x^{*2}(t) + 2K\mu_{\max}\alpha^*(t)X^*(t)x^*(t)\right) - K\lambda_4(t)\left(Kx^*(t) + 2x^{*2}(t)\right. \\
&\quad \left.\left. - K^2 - 2\mu_{\max}\beta^*(t)X^*(t)x^*(t)\right)\right],
\end{aligned}$$

then the singular control becomes:

$$u^*(t) = \frac{\Psi(t) - \Phi(t)}{2K[\lambda_3(t)(1 + \mu_{\max}\alpha^*(t)X^*(t)) + \lambda_4(t)\mu_{\max}\beta^*(t)X^*(t)]}. \tag{84}$$

4.2.2 Numerical simulations

Because the differential equations of $\lambda_1(t)$ and $\lambda_2(t)$ are coupled, we are unable to determine the sign of the switch function $\lambda_1(t)$ analytically. So we fix all the system parameters except respectively $\mu_{\max}$ (Fig. 2) and K (Fig. 3) to perform the numerical simulations. The experiments with different values of $\mu_{\max}$ and K are done setting $u(t) = b$. As a result, $\lambda_1(t) > 0$, and then all of the optimal controls are $u^*(t) = b$.

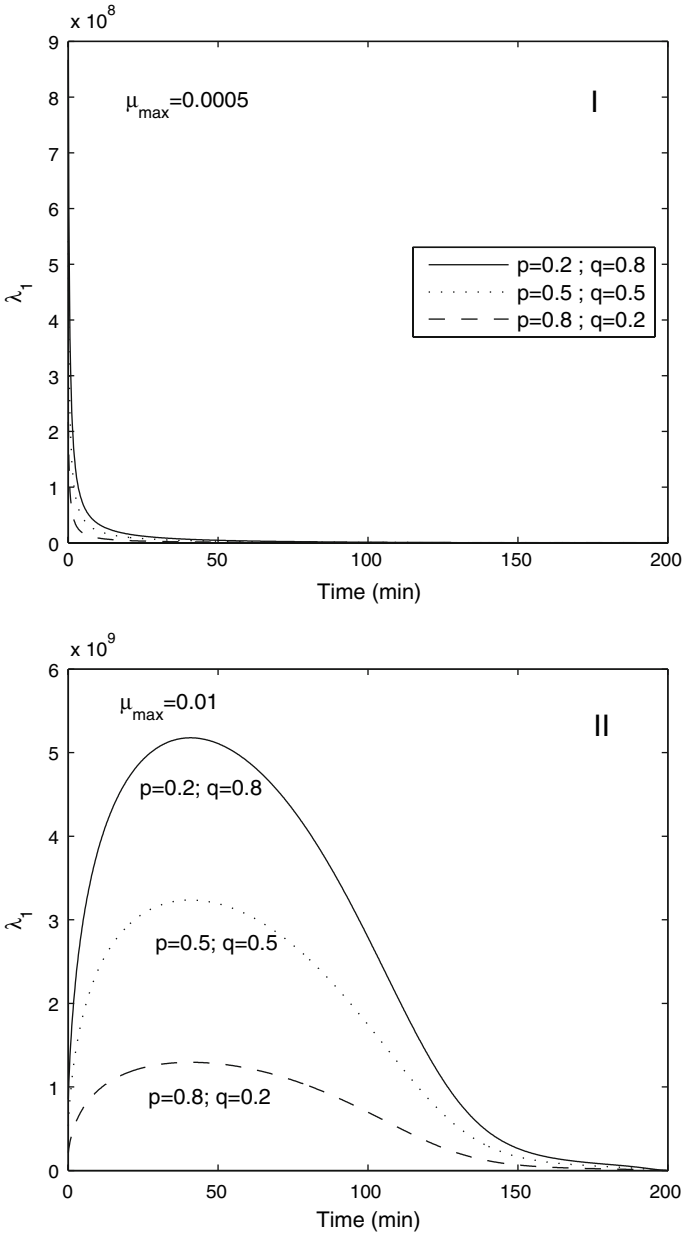

Fig. 2 The switch function λ_1 varying with p and q for various $\mu_{\max}$. K = 1mg/L, Y = 0.08, x(0) = 2mg/L, X(0)=1000mg/L. **a** = −4mg/(L min), **b** = 10mg/(L min). T=200 min, Δt = 0.2 min. (I) $\mu_{\max} = 0.0005\text{min}^{-1}$; (II) $\mu_{\max} = 0.01\text{min}^{-1}$

In Figs. 2 and 3 we show several plots, obtained varying the values of p and q. We can observe how the values of p and q affect the function $\lambda_1(t)$. The figures show that the smaller the value of p is and the larger the value of q is, the larger the value

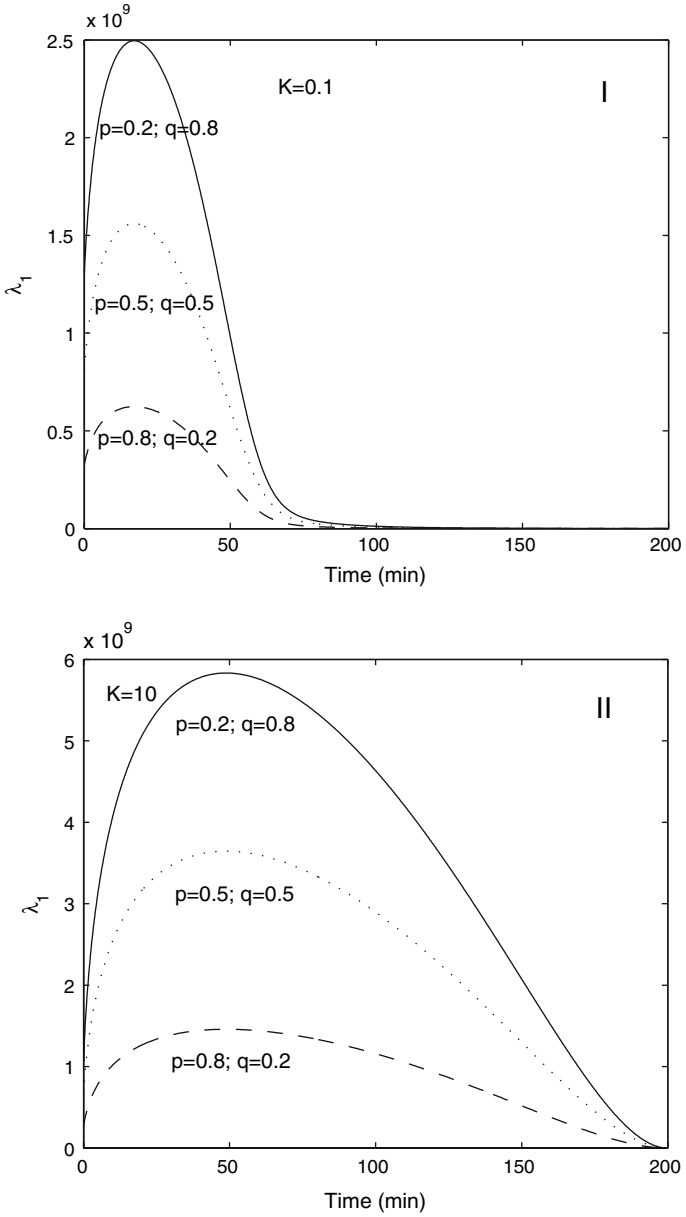

Fig. 3 The switch function λ_1 varying with p and q for various K. $\mu_{\max} = 0.01\text{min}^{-1}$, Y = 0.08, x(0) = 2mg/L, X(0) = 1000mg/L. **a** = −4mg/(L min), **b** = 10mg/(L min). T = 200 min, Δt = 0.2 min. (I) K = 0.1mg/L; (II) K = 10mg/L

of $\lambda_1(t)$ at each time point is. In addition, given all the parameters, the curves of the functions $\lambda_1(t)$ maintain similar shapes for different values of p and q.

Moreover, we can rewrite the equations of system 58 as follows:

$$X(t) = X(0)\exp\left(\int_0^t Y\mu_{\max}\frac{x(s)}{K + x(s)}ds\right)$$

and

$$x(t) = x(0) - \int_0^t \mu_{\max}X(s)\frac{x(s)}{K + x(s)}ds + \int_0^t u(s)ds$$

It is inferred that $X(0) \leq X(t) \leq X(0)e^{Y\mu_{\max}t}$. If the value of $\mu_{\max}$ is sufficiently small, the concentration of biomass varies small with time. Thus we can consider $X(t)$ as a constant $X(0)$ and we can classify such a case into Sect. 4.1. In addition, the second term on the right hand side in the substrate equation can not be neglected compared to the effect of the substrate. Because the order of magnitude of $X(t)$ is so large enough that $\mu_{\max}X(t)$ and $x(t)$ are of the same order of magnitude, thereby the second term is not close to zero.

If the value of $\mu_{\max}$ is large, $X(t)$ can not be considered a constant and we have to classify such a case into Sect. 4.2.

5 Conclusions

In this paper we show that the model of the growth of a single microbial species on a single substrate, here presented, is structurally identifiable, before developing a good input for parameter estimation. Then we investigate the design of optimal sensitivity input for the model. Moreover, we discuss two cases, where respectively the biomass X is a constant and is a variable. Whether the biomass in the model is constant or not depends on the value of the maximum specific growth rate $\mu_{\max}$. If the value of $\mu_{\max}$ is sufficiently small, the biomass can be regarded as a constant. Otherwise, X must be considered a variable; this fact increases the complexity of the problem, set in terms of an optimal control problem with state constraints.

In the paper the control problem is essentially the solution of the optimal control with state constraints. The corresponding optimal control consists of a bang–bang control and a singular control which depends on the sign of the costate λ_1. If the biomass in the model is a variable, then the costates of the corresponding optimal control problem are coupled and it is impossible to analytically determine the sign of λ_1. In addition, we perform some numerical simulation experiments. In Sect. 4.1.1, in order to demonstrate the advantages of utilizing the optimal input, the parameter estimation accuracy is compared with and without the optimal input in presence of observation noise. In Sect. 4.2.2, given the value of control, we analyze the sign of λ_1 and consequently establish whether the control is optimal or not. Our main goal is to find the optimal control by means of numerical simulations. However, up to now an

efficient algorithm for the search of the optimal control with state constraints has not yet been proposed and we should focus on the study of this algorithm in the future.

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