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ORIGINAL PAPER

Sensitivity analysis of a linear and unbranched chemical process with n steps

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Abstract In this paper, we present a quasi-analytical method to calculate the optimal enzyme concentrations in a chemical process, considering the minimization of the operation time. The resulting constrained optimal control problem is solved using Pontryagin's Minimum Principle. First, our method allows us to obtain the generalized solution of an n-step system with an unbranched scheme and bilinear kinetic models and non-equal catalytic efficiencies of the enzymes. Second, we discuss the sensitivity analysis of these catalytic parameters in detail.

Keywords Optimal control · Chemical process · Sensitivity analysis

Mathematics Subject Classification 49J30 · 49M05 · 92E20 · 80A30 · 92C40

1 Introduction

A fast and reliable mathematical model derived from chemical principles is needed to optimize a chemical process. Kinetic investigations of multi-step enzymatic reactions are a crucial part of these studies. In this context, one of the most important problems is the study of enzyme concentrations, while minimizing the operation time during which the substrate is converted into the product. A very common kinetic model for an enzymatic reaction is the first-order kinetic model. Let us consider an unbranched metabolic pathway composed of n irreversible reaction steps converting substrate x_1 into product p. We denote as x_i $(i = 2, \ldots, n)$ the concentration of the intermediate compounds, and as u_i $(i = 1, \ldots, n)$, the concentration of the enzyme catalyzing the i-th reaction.

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An explicit solution for the simplest case, i.e. $n = 2$, can be found in [1], while for longer pathways, the authors solved the optimization problem numerically. The solution is obtained quasi-analytically in [2], though with the constraint of considering only the case of $n = 3$, with two intermediate compounds. In [3] several theoretical results on the qualitative properties of the solution for the general case of n steps are presented. These authors prove that the optimal enzyme concentration profile is of the " bang-bang" type (except in the last interval), though they do not present the analytical solution.

In a previous paper [4], we extended the theoretical analysis of [1], [2] and [3], presenting the quasi-analytical solution for the more general case of n steps while assuming equal catalytic efficiencies of the enzymes ($k_i = 1$). In [4], we addressed the minimization of the transition time. This transition time is defined by a improper integral running until infinite time. As this model may be considered somewhat unrealistic (even if this integral is approximated by a proper integral over a long, finite time), in the present paper we shall consider a more realistic situation in chemistry or biology. We shall minimize the operation time, defined by specifying the final product concentration. It clearly makes more sense to define an operation time according to the reasoning that a certain percentage, say 90 %, of the initial substrate should be converted into product. Moreover, we shall substantially extend the theoretical analysis of [4], considering nonequal catalytic efficiencies, k_i.

Identification of the most sensitive parameters influencing the system conditions is likewise of major importance for optimizing process control. Sensitivity analysis (SA) investigates the relations between model parameters and a property of the outcome. Classically (see, for example, [5] and [6]), SA is performed by the partial derivatives of the outcome with respect to its parameters. This SA method is called differential SA (also known as the direct method) and belongs to the so-called local methods, as the derivative is taken at a fixed point in the state space of the model parameters. When a closed-form equation describes the relationship between the independent variables and the dependent variable, this SA is easy to perform. This is precisely the major advantage of our method, which allows us to obtain the partial derivatives of the concentration of the compounds, x_i, with respect to the catalytic efficiencies of the enzymes, k_i. When the analytical solution is not available, a SA can be obtained by increasing each parameter while leaving all others constant and analyzing the change in model outcome. This method belongs to the class of one-at-a-time (OAT) methods and is the method we shall use in this paper for the SA of the operation time, t_f, with respect to k_i.

The paper is organized as follows. Section 2 presents the statement of the problem, analyzing the kinetic model and the objective function in detail. In Sect. 3, the resulting mathematical problem is presented as an optimal control problem. We prove a theorem based on Pontryagin's Minimum Principle that allows us to obtain the optimal solution. The two SA methods used for the optimal solution are presented in Sect. 4. The optimal solutions of several numerical examples are then presented in Sect. 5, as is the SA of the optimal solution. Finally, we present the conclusions drawn in Sect. 6.

2 Statement of the problem

2.1 Model formulation

Let us consider the following unbranched metabolic pathway composed of n irreversible reaction steps converting substrate x_1 into product p:

$$x_1 \overset{u_1}{\rightarrow} x_2 \overset{u_2}{\rightarrow} x_3 \overset{u_3}{\rightarrow} \cdots \rightarrow x_{n-1} \overset{u_{n-1}}{\rightarrow} x_n \overset{u_n}{\rightarrow} p \tag{1}$$

where $x_1(t)$ is the substrate concentration at time t, $p(t)$ the concentration of the final product at time t, $x_i(t)$ $(i = 2, \ldots, n)$ the concentration of the intermediate compounds at time t, and $u_i(t)$ $(i = 1, \ldots, n)$ the concentration at time t of the enzyme catalyzing the i-th reaction. In this kind of problem, the rate laws $v_i(x_i(t), u_i(t))$ (v_i being the rate of the i-th reaction) characterize the kinetic properties of the enzymes catalyzing the pathway. Most enzyme kinetic models satisfy the following assumptions:

(i) The rate laws are linear in the enzyme concentrations, u_i:

$$v_i(x_i(t), u_i(t)) = w_i(x_i(t)) \cdot u_i(t) \tag{2}$$

(ii) The functions $w_i(x_i(t)) \geq 0$ are continuous and:

$$\frac{dw_i}{dx_i} > 0 \text{ for } x_i > 0, \quad \text{with } w_i(0) = 0, (i = 1, \ldots, n) \tag{3}$$

These two conditions are satisfied by the following common kinetic models:

$$\begin{aligned} w_i(x_i) &= k_i x_i && \text{(Mass action)} \\ w_i(x_i) &= \frac{k_i x_i}{K_i + x_i} && \text{(Michaelis-Menten)} \\ w_i(x_i) &= \frac{k_i x_i^m}{K_i + x_i^m} && \text{(Hill)} \\ w_i(x_i) &= k_i x_i^c && \text{(Power law)} \end{aligned} \tag{4}$$

where $k_i > 0$, $K_i > 0$, $m \geq 0$ and $c > 0$. The dynamic model for the pathway shown in (1) is given by mass conservation as:

$$\dot{x}_i(t) = v_{i-1}(x_{i-1}(t), u_{i-1}(t)) - v_i(x_i(t), u_i(t)); \quad (i = 1, \ldots, n) \tag{5}$$

Many papers address these models. For example, in [1] and [2], the authors assumed both bilinear (linear in the metabolite concentrations, x_i, and linear in the enzyme concentrations, u_i) and irreversible rate laws. Both papers present the solution for very simple cases: in [1], for $n = 5$, and in [2], for $n = 3$. In [3], however, it is used the Michaelis-Menten model, a nonlinear model in x_i, though also for a particular case ($n = 4$).

In this paper, we assume the mass action equation:

$$w_i(x_i) = k_i x_i \tag{6}$$

and hence use a bilinear kinetic model to solve the problem analytically for the general case of n reactions. For the sake of simplicity, we employ normalized quantities, i.e. enzyme levels are divided by the maximum total enzyme concentration, and substrate, intermediate and product levels are divided by the initial substrate concentration. The model of the reactions in (1) can then be described using (2), (5), and (6) by the set of differential equations:

$$\begin{cases} \dot{x}_1 = -k_1 u_1 x_1 & x_1(0) = 1,\ x_1(t) \geq 0 \\ \dot{x}_2 = k_1 u_1 x_1 - k_2 u_2 x_2 & x_2(0) = 0,\ x_2(t) \geq 0 \\ \dot{x}_3 = k_2 u_2 x_2 - k_3 u_3 x_3 & x_3(0) = 0,\ x_3(t) \geq 0 \\ \cdots & \\ \dot{x}_n = k_{n-1} u_{n-1} x_{n-1} - k_n u_n x_n & x_n(0) = 0,\ x_n(t) \geq 0 \end{cases} \tag{7}$$

As an initial condition, for $t = 0$, we shall consider the concentrations of the intermediate compounds and of the product to be equal to zero. We assume that the enzymes can be switched on and off instantaneously. Finally, we shall consider the concentrations of the compounds, x_i, as well as those of the enzymes, u_i, to be positive limited quantities and, after normalization, that the upper bound on the enzymatic concentration is 1.

In a previous paper [4], we assumed equal catalytic efficiencies of the enzymes ($k_i = 1$). In this paper, we shall substantially generalize the study, considering nonequal catalytic efficiencies.

2.2 Objective function

The optimization of enzyme concentrations in metabolic pathways can be calculated using the optimality criterion of minimizing the time period during which an essential product is generated. Our goal is to convert substrate x_1 into product p as fast as possible, taking into consideration several cost functions.

In [1], [2] and [4], the authors use the *transition time*, τ (defined in [7] and [8]). This transition time is defined by a time integral running until infinite time:

$$\min_{u_1,\ldots u_n} \tau = \min_{u_1,\ldots u_n} \int_0^\infty \frac{1}{x_1(0)} (x_1(0) - p(t)) dt \tag{8}$$

Due to normalization, $x_1(0) = 1$, and the conservation relation:

$$x_1(t) + x_2(t) + \cdots + x_n(t) + p(t) = 1, \quad \forall t \geq 0 \tag{9}$$

the objective function may be written as:

$$\min_{u_1,\cdots u_n} \tau = \min_{u_1,\ldots u_n} \int_0^\infty (x_1(t) + x_2(t) + \cdots + x_n(t))dt \tag{10}$$

where concentrations $x_1, x_2, \ldots, x_n$ are the state variables (p is eliminated) and the enzyme concentrations $u_1, u_2, \ldots, u_n$ comprise the control variables. Evidently, this improper integral is unrealistic and hence other functionals must be taken into consideration. In [3] it is considered a combined optimization of the time taken to reach the new steady state and a measure of enzyme usage:

$$\min_{u_1,\ldots u_n} \int_0^{t_f} (1 + \alpha^T \mathbf{u}(t))dt \tag{11}$$

where α is the vector of weights and $\mathbf{u} = (u_1(t), ...,u_n(t))$, the vector of enzyme concentrations.

In this paper, we shall consider a more realistic situation in biology, in which the product $p(t)$ need not be fully synthesized, but rather synthesized to a pre-defined concentration. We therefore minimize the *operation time* (to distinguish it from the transition time), defined by specifying the final product concentration, e.g. $p(t_f) = 0.9$, with t_f as the final time. This definition does not require unrealistic improper integration until infinite time. In the case of an exhaustible initial substrate, x_1, from the conservation relation (9), we have that:

$$x_1(t_f) + x_2(t_f) + \cdots + x_n(t_f) + p(t_f) = 1 \tag{12}$$

and imposing $p(t_f) = 0.9$, we obtain:

$$x_1(t_f) + x_2(t_f) + \cdots + x_n(t_f) = 0.1 \tag{13}$$

The objective function of the optimization problem may thus be defined as:

$$\tau_{90} = \min_{u_1,\ldots u_n} t_f = \min_{u_1,\ldots u_n} \int_0^{t_f} dt \tag{14}$$

As we see, this objective coincides with (11) if we choose $\alpha = 0$.

3 Optimal solution

In this section, we present the solution to the problem defined in the previous section:

$$\tau_{90} = \min_{u_1,\ldots u_n} t_f = \min_{u_1,\ldots u_n} \int_0^{t_f} dt \tag{15}$$

subject to:

$$\begin{cases} \dot{x}_1 = -k_1 u_1 x_1 & x_1(0) = 1,\ x_1(t) \geq 0 \\ \dot{x}_2 = k_1 u_1 x_1 - k_2 u_2 x_2 & x_2(0) = 0,\ x_2(t) \geq 0 \\ \dot{x}_3 = k_2 u_2 x_2 - k_3 u_3 x_3 & x_3(0) = 0,\ x_3(t) \geq 0 \\ \dots & \\ \dot{x}_n = k_{n-1} u_{n-1} x_{n-1} - k_n u_n x_n & x_n(0) = 0,\ x_n(t) \geq 0 \end{cases} \tag{16}$$

and $(u_1(t), ..., u_n(t)) \in \Omega$, being:

$$\Omega = \{\mathbf{u} = (u_1(t), \dots, u_n(t)) \in \mathbb{R}^n | u_1 \geq 0, \dots u_n \geq 0; \quad u_1 + \cdots + u_n \leq 1\} \tag{17}$$

We have thus stated an optimal control problem (OCP). Our standard Lagrange-type OCP may be mathematically formulated as follows:

$$\min_{\mathbf{u}(t)} \int_0^{t_f} F\,(t, \mathbf{x}(t), \mathbf{u}(t))\, dt \tag{18}$$

subject to satisfying:

$$\dot{\mathbf{x}}(t) = f\,(t, \mathbf{x}(t), \mathbf{u}(t))\,; \quad \mathbf{x}(0) = \mathbf{x}_0 \tag{19}$$

$$\mathbf{u}(t) \in \Omega, \quad 0 \leq t \leq t_f \tag{20}$$

where $F \equiv 1$ is the objective function, $\mathbf{x} = (x_1(t), \dots, x_n(t)) \in \mathbb{R}^n$ is the *state vector*, with initial conditions $\mathbf{x}_0$, $\mathbf{u} \in \mathbb{R}^n$ is the *c*ontrol vector, Ω denotes the set of admissible control values, and t is the operating time, which starts from 0 and ends at t_f (the value to minimize). The state variables must satisfy the state equation (19), with given initial conditions, and we consider the final state to be free. Let H be the Hamiltonian function:

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

where $\lambda = (\lambda_1(t), \dots, \lambda_n(t)) \in \mathbb{R}^n$ is called the *costate vector*. Using Pontryagin's Minimum Principle [9], the necessary conditions lead to a two-point boundary value problem (TPBVP):

$$\dot{\mathbf{x}} = \frac{\partial H}{\partial \lambda};\ \mathbf{x}(0) = \mathbf{x}_0 \tag{22}$$

$$\dot{\lambda} = -\frac{\partial H}{\partial \mathbf{x}};\ \lambda(t_f) = \mathbf{0} \tag{23}$$

$$\min_{\mathbf{u} \in \Omega} H(t, \mathbf{x}, \mathbf{u}, \lambda) \tag{24}$$

When control $\mathbf{u}$ appears linearly, the last optimality condition (24) leads to the minimization of a linear function of n variables of the following type:

$$\min_{\mathbf{u}\in\Omega} H = \min_{\mathbf{u}\in\Omega} \{-\mu_1 u_1 - \mu_2 u_2 - \cdots - \mu_n u_n\} \tag{25}$$

where the functions μ_i are called the *switching functions*. It is known that control u_i will be activated when the switching function, μ_i, reaches its maximum value. If u_i switches between its upper and lower bounds only at isolated points in time, then the optimal control is said to be a *bang-bang type control* [10]. The times are called *switching times*.

We now present the solution to the OCP defined above using Pontryagin's Minimum Principle [9]. The fundamental result to obtain may be summarized as follows:

Theorem 1 *There exists a set of switching times* $\{t_1, t_2, \cdots, t_{n-1}\}$, *(with* $0 < t_i < t_j$, *for* $i < j$*) which partition the optimization interval as:*

$$[0, t_1) \cup [t_1, t_2) \cup \cdots \cup [t_{n-2}, t_{n-1}) \cup [t_{n-1}, t_f] \tag{26}$$

such that the optimal profile of the i*-th enzyme is of the bang-bang type and satisfies:*

$$u_i^*(t) = \begin{cases} 1 & \text{for } t \in [t_{i-1}, t_i) \\ 0 & \text{for } t \notin [t_{i-1}, t_i) \end{cases}; \quad i = 1, \ldots, n \tag{27}$$

with $t_0 = 0$ *and* $t_n = t_f$. *In each interval* $[t_{i-1}, t_i]$, $i = 1, \ldots, n$, *the optimal metabolite concentration is given by:*

$$x_1(t) = \begin{cases} e^{-k_1 t} & i = 1 \\ e^{-k_1 t_1} & i > 1 \end{cases} \tag{28}$$

$$x_j(t) = \begin{cases} \prod_{h=1}^{j-1} (1 - e^{-k_h(t_h - t_{h-1})}) \cdot e^{-k_j(t_j - t_{j-1})} & j = 2, \ldots, i-1 \\ \prod_{h=1}^{j-1} (1 - e^{-k_h(t_h - t_{h-1})}) \cdot e^{-k_j(t - t_{i-1})} & j = i \\ \prod_{h=1}^{i-1} (1 - e^{-k_h(t_h - t_{h-1})}) \cdot (1 - e^{-k_i(t - t_{i-1})}) & j = i+1 \\ 0 & j = i+2, \ldots, n \end{cases} \tag{29}$$

Proof As the proof is quite similar to that presented in [4], we shall omit some steps which the reader may consult in more detail in [4]. In our case, when the Hamiltonian, H:

$$H = 1 + \lambda_1(-k_1 u_1 x_1) + \lambda_2(k_1 u_1 x_1 - k_1 u_2 x_2) + \cdots + \lambda_n(k_{n-1} u_{n-1} x_{n-1} - k_n u_n x_n) \tag{30}$$

is minimized w.r.t. the control variables, we have that:

$$\min_{\mathbf{u}\in\Omega} H = \min_{\mathbf{u}\in\Omega} \{-\mu_1 u_1 - \mu_2 u_2 - \cdots - \mu_n u_n\} \tag{31}$$

$$= \min_{\mathbf{u}\in\Omega} \{k_1(\lambda_1 - \lambda_2) x_1 u_1 - k_2(\lambda_2 - \lambda_3) x_2 u_2 - \cdots - k_n \lambda_n x_n u_n\} \tag{32}$$

Hence, control u_i will be activated when the function μ_i reaches its maximum. Moreover, according to the optimality condition (23), we have:

$$\dot{\lambda}_1 = k_1(\lambda_1 - \lambda_2)u_1, \ldots, \dot{\lambda}_{n-1} = k_{n-1}(\lambda_{n-1} - \lambda_n)u_{n-1}, \dot{\lambda}_n = k_n\lambda_n u_n \tag{33}$$

When control u_i is activated, the coefficient μ_i has to be positive: $\mu_i \geq 0$, $(\forall i = 1, \ldots, n)$ (otherwise $u_i = 0$). The following condition can thus be easily seen to hold:

$$\lambda_1 \geq \lambda_2 \geq \lambda_3 \geq \cdots \geq \lambda_n \tag{34}$$

We shall obtain the optimal solution constructively by intervals, starting from $t = 0$ and concatenating the results.

– First interval $[0, t_1]$. For $t = 0 \Rightarrow u_1 = 1, u_2 = 0, u_3 = 0, \cdots, u_n = 0$, since, if $u_1 = 0$ from (16), $\dot{x}_1 = 0 \Rightarrow x_1(t) = 1, \forall t$, and the product will not be produced. Therefore, from (16), we have:

$$\begin{cases} \dot{x}_1 = -k_1 x_1 & x_1(0) = 1 \\ \dot{x}_2 = k_1 x_1 & x_2(0) = 0 \\ \dot{x}_3 = 0, \ldots, \dot{x}_n = 0 & x_3(0) = 0, \ldots, x_n(0) = 0 \end{cases} \Rightarrow \begin{cases} x_1(t) = e^{-k_1 t} \\ x_2(t) = 1 - e^{-k_1 t} \\ x_3(t) = 0, \ldots, x_n(t) = 0 \end{cases} \tag{35}$$

Moreover, from (16), (31) and (33), the following holds:

$$\dot{\lambda}_1 = k_1 (\lambda_1 - \lambda_2), \dot{\lambda}_2 = 0, \dot{\lambda}_3 = 0 \tag{36}$$

and

$$\dot{\mu}_1 = 0, \dot{\mu}_2 \geq 0, \mu_3 = 0, \ldots, \mu_n = 0 \tag{37}$$

Hence,

$$\mu_1 = cte, \mu_2 = \text{increasing}, \ \mu_3 = 0, \ldots, \mu_n = 0 \tag{38}$$

– Second interval $[t_1, t_2]$. For $t = t_1 \Rightarrow u_1 = 0, u_2 = 1, u_3 = 0, \ldots, u_n = 0$. Thus, from (16):

$$\begin{cases} \dot{x}_1 = 0 & x_1(t_1) = e^{-k_1 t_1} \\ \dot{x}_2 = -k_2 x_2 & x_2(t_1) = 1 - e^{-k_1 t_1} \\ \dot{x}_3 = k_2 x_2 & x_3(t_1) = 0 \\ \dot{x}_4 = 0, \ldots, \dot{x}_n = 0 & x_4(t_1) = 0, \ldots, x_n(t_1) = 0 \end{cases}$$
$$\Rightarrow \begin{cases} x_1(t) = e^{-k_1 t_1} \\ x_2(t) = \left(1 - e^{-k_1 t_1}\right) e^{-k_2 (t - t_1)} \\ x_3(t) = \left(1 - e^{-k_1 t_1}\right) \left(1 - e^{-k_2 (t - t_1)}\right) \\ x_4(t) = 0, \ldots, x_n(t) = 0 \end{cases} \tag{39}$$

Once again, using (16), (31) and (33), we have that:

$$\dot{\lambda}_1 = 0, \dot{\lambda}_2 = k_2\,(\lambda_2 - \lambda_3)\,, \dot{\lambda}_3 = 0, \dot{\lambda}_4 = 0 \tag{40}$$

and

$$\dot{\mu}_1 \leq 0, \dot{\mu}_2 = 0, \dot{\mu}_3 \geq 0, \mu_4 = 0, \ldots, \mu_n = 0 \tag{41}$$

Hence,

$$\mu_1 = \text{decreasing},\ \mu_2 = cte, \mu_3 = \text{increasing},\ \mu_4 = 0, \ldots, \mu_n = 0 \tag{42}$$

The successive intervals are similarly obtained. In the last step, which simply involves concatenating the solutions, we obtain the following result.

– Interval $[t_{n-1}, t_f]$. For $t = t_{n-1} \Rightarrow u_1 = 0, u_2 = 0, u_3 = 0, \cdots, u_{n-1} = 0, u_n = 1$. From the state equations:

$$\dot{x}_1 = 0, \ldots, \dot{x}_{n-1} = 0, \dot{x}_n = -k_n x_n \tag{43}$$

$$\begin{cases} x_1(t) = e^{-k_1 t_1} \\ x_2(t) = \left(1 - e^{-k_1 t_1}\right) e^{-k_2 (t_2 - t_1)} \\ x_3(t) = \left(1 - e^{-k_1 t_1}\right) \left(1 - e^{-k_2 (t_2 - t_1)}\right) e^{-k_3 (t_3 - t_2)} \\ \cdots \\ x_{n-2}(t) = \left(1 - e^{-k_1 t_1}\right) \cdots (1 - e^{-k_{n-3}(t_{n-3} - t_{n-4})}) e^{-k_{n-2}(t_{n-2} - t_{n-3})} \\ x_{n-1}(t) = \left(1 - e^{-k_1 t_1}\right) \cdots (1 - e^{-k_{n-2}(t_{n-2} - t_{n-3})}) e^{-k_{n-1}(t_{n-1} - t_{n-2})} \\ x_n(t) = \left(1 - e^{-k_1 t_1}\right) \cdots (1 - e^{-k_{n-1}(t_{n-1} - t_{n-2})}) e^{-k_n (t - t_{n-1})} \end{cases} \tag{44}$$

$$\mu_1 = cte,\ \mu_2 = cte, \ldots \mu_{n-3} = cte, \mu_{n-2} = cte, \mu_{n-1} = \text{decreasing}, \mu_n = cte \tag{45}$$

We now have to determine the switching times: $t_1, t_2, \ldots, t_{n-1}$, and the value of t_f. In order to do so, we apply the method of Lagrange multipliers to the augmented functional:

$$L(t_1, t_2, \ldots, t_{n-1}, t_f, \beta) = t_f + \beta(x_1(t_f) + x_2(t_f) + \cdots + x_n(t_f) - 0.1) \tag{46}$$

where the values of concentrations $x_1(t_f), x_2(t_f), \ldots, x_n(t_f)$ are given by (44). We now need to solve the non-linear system:

$$\frac{\partial L}{\partial t_1} = 0; \frac{\partial L}{\partial t_2} = 0; \ldots; \frac{\partial L}{\partial t_{n-1}} = 0; \frac{\partial L}{\partial t_f} = 0; \frac{\partial L}{\partial \beta} = 0 \tag{47}$$

which may be done by means of any widely used program. The problem is completely solved. □

The Table 1 presents the results developed from the formula (29) for ease of comprehension.

Table 1 Metabolite concentration

$t \in [0, t_1]$:

$$\begin{cases} x_1(t) = e^{-k_1 t} \\ x_2(t) = 1 - e^{-k_1 t} \\ x_3(t) = 0; \ldots; x_n(t) = 0 \end{cases}$$

$t \in [t_1, t_2]$:

$$\begin{cases} x_1(t) = e^{-k_1 t_1} \\ x_2(t) = \left(1 - e^{-k_1 t_1}\right) e^{-k_2 (t - t_1)} \\ x_3(t) = \left(1 - e^{-k_1 t_1}\right) \left(1 - e^{-k_2 (t - t_1)}\right) \\ x_4(t) = 0; \ldots; x_n(t) = 0 \end{cases}$$

$\ldots$

$t \in \left[t_{n-2}, t_{n-1}\right]$:

$$\begin{cases} x_1(t) = e^{-k_1 t_1} \\ x_2(t) = \left(1 - e^{-k_1 t_1}\right) e^{-k_2 (t_2 - t_1)} \\ x_3(t) = \left(1 - e^{-k_1 t_1}\right) \left(1 - e^{-k_2 (t_2 - t_1)}\right) e^{-k_3 (t_3 - t_2)} \\ \ldots \\ x_{n-2}(t) = \left(1 - e^{-k_1 t_1}\right) \cdots (1 - e^{-k_{n-3}(t_{n-3} - t_{n-4})}) e^{-k_{n-2}(t_{n-2} - t_{n-3})} \\ x_{n-1}(t) = \left(1 - e^{-k_1 t_1}\right) \cdots (1 - e^{-k_{n-2}(t_{n-2} - t_{n-3})}) e^{-k_{n-1}(t - t_{n-2})} \\ x_n(t) = \left(1 - e^{-k_1 t_1}\right) \cdots (1 - e^{-k_{n-2}(t_{n-2} - t_{n-3})})(1 - e^{-k_{n-1}(t - t_{n-2})}) \end{cases}$$

$t \in \left[t_{n-1}, t_f\right]$:

$$\begin{cases} x_1(t) = e^{-k_1 t_1} \\ x_2(t) = \left(1 - e^{-k_1 t_1}\right) e^{-k_2 (t_2 - t_1)} \\ x_3(t) = \left(1 - e^{-k_1 t_1}\right) \left(1 - e^{-k_2 (t_2 - t_1)}\right) e^{-k_3 (t_3 - t_2)} \\ \ldots \\ x_{n-2}(t) = \left(1 - e^{-k_1 t_1}\right) \cdots (1 - e^{-k_{n-3}(t_{n-3} - t_{n-4})}) e^{-k_{n-2}(t_{n-2} - t_{n-3})} \\ x_{n-1}(t) = \left(1 - e^{-k_1 t_1}\right) \cdots (1 - e^{-k_{n-2}(t_{n-2} - t_{n-3})}) e^{-k_{n-1}(t_{n-1} - t_{n-2})} \\ x_n(t) = \left(1 - e^{-k_1 t_1}\right) \cdots (1 - e^{-k_{n-1}(t_{n-1} - t_{n-2})}) e^{-k_n (t - t_{n-1})} \end{cases}$$

4 Sensitivity analysis

Sensitivity analysis investigates the effect of parameter change on the solution of mathematical models. The literature (see, for example, [5,6,11] and [12]) contains details on the types of SA employed in various modeling situations. More than a dozen SA techniques have been reported, two of which will be employed in this paper. A generalized model is considered throughout the following definitions that contains several independent variables, $X = (X_1, \ldots, X_n)$, and one dependent variable, Y, where $Y = f(X)$. The SA methods that we shall employ are:

(i) *Differential SA*. In this case, the sensitivity coefficient, ϕ_i, for a particular independent variable, X_i, can be calculated from the partial derivative of the dependent variable, Y, with respect to X_i:

$$\phi_i = \frac{dY}{X_i} \tag{48}$$

If a measure that is independent of the units used for Y and X_i is needed, SX_i can be used:

$$SX_i = \frac{dY}{X_i}\frac{X_i}{Y} \tag{49}$$

where the quotient X_i/Y is introduced to normalize the coefficient by removing the effects of units. When an explicit algebraic equation describes the relationship $Y = f(X)$, the differential SA is easy to perform. Employing the analytical formulas (28) and (29) obtained in the previous section, we are able to obtain the sensitivity coefficient, ϕ_i, of the concentration of the intermediate compounds and substrate x_i with respect to the catalytic efficiencies, k_i $(i = 1, \ldots, n)$.

(ii) *One-at-a-Time SA*. The simplest SA method is to repeatedly vary one parameter at a time while keeping the others fixed. A SA can be easily obtained by increasing each catalytic efficiency, k_i, by a given percentage, while leaving all others constant and quantifying the change in model output one factor at a time (OAT). We shall use this method to study the SA of the operation time, t_f, with respect to k_i, since, as stated in the previous section, we do not possess the analytical relationship between them.

These two types of analysis have been defined as *local* SA, as they address the point chosen and not the entire parameter distribution.

5 Example: discussion of the results

Using the results presented in the previous section, we developed a program using the Mathematica® package that allows us to obtain the optimal solution. We shall perform three examples. The first presents the optimum solution as the number n of intermediate compounds increases. The second example studies the sensitivity of the concentrations with respect to the catalytic efficiencies, while the third studies the influence of these efficiencies on the operation time.

5.1 Example 1: optimal solution

Let us consider the following values for the nonequal catalytic efficiencies, $k_i(s^{-1})$:

$$k_1 = 10;\ k_2 = 10;\ k_3 = 9;\ k_4 = 9;\ k_5 = 8;\ k_6 = 7;\ k_7 = 5;\ k_8 = 3;\ k_9 = 12 \tag{50}$$

In Table 2, we present the optimal solution for the cases $n = 3, \ldots, 9$. Let us now look at the switching times, t_i $(i = 1, \ldots, n)$, and the operation time, $\tau = t_n$. Recall that u_i is given by 1 in all the intervals (when it is active). Moreover, the substrate concentration, x_1, the concentrations of the intermediate compounds, $x_2, \ldots, x_n$, and

Table 2 Switching times and operation time of the optimal solution

n	3	4	5	6	7	8	9
t_1	0.3401	0.3702	0.3958	0.4188	0.4440	0.4755	0.4821
t_2	0.6803	0.7404	0.7917	0.8376	0.8880	0.9510	0.9642
t_3	1.0469	1.1404	1.2201	1.2915	1.3698	1.4677	1.4883
t_4	–	1.5404	1.6485	1.7453	1.8516	1.9845	2.0124
t_5	–	–	2.1160	2.241	2.3792	2.5512	2.5874
t_6	–	–	–	2.7898	2.9633	3.1801	3.2257
t_7	–	–	–	–	3.7150	3.9942	4.0529
t_8	–	–	–	–	–	5.1845	5.2648
t_9	–	–	–	–	–	–	5.6817

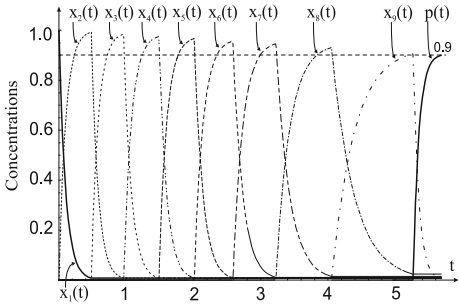

Fig. 1 Metabolite and product profile. Case n = 9

the concentration of the final product, p, are immediately obtained in any interval using the formulas presented in Theorem 1. Figure 1 shows the optimal solution for the case $n = 9$.

The results show that during each of the n intervals, only one enzyme is active and present at its maximum value ($u_i = 1$), corresponding to intermediate compound x_i, which we wish to convert into x_{i+1}, $(i = 1, \ldots, n-1)$. The solution is hence of the bang-bang type. In the first interval, starting from $x_1(0) = 1$ and using u_1, substrate x_1 is converted into x_2. At the first switching time, t_1, x_2 reaches its maximum value, $x_2(t_1)$, while x_1 takes on a constant value, $x_1(t_1)$, which it will maintain until the final instant, t_f. The process is repeated in the second interval, though now it is x_2 which, starting from this maximum value, $x_2(t_1)$, is converted into x_3. The process is likewise repeated for all x_i. From the qualitative point of view, we may present the following conclusions:

(i) The maximum values obtained by all the compounds, $x_i(t_{i-1})$, become progressively smaller as the process advances, regardless of the values of k_i (see Fig. 1).
(ii) The constant values, $x_i(t_i)$, which they maintain during a good part of the chain reaction depend on the values of k_i. If k_i decreases, $x_i(t_i)$ increases, and *vice versa*. Moreover, it is seen that if the k_i are equal (cases $k_1 = k_2$ and $k_3 = k_4$), the values decrease very slightly with i. In the example under consideration, the values obtained are present in Table 3.

Table 3 The constant values, $x_i(t_i)$ (10^{-3})

$x_1(t_1)$	$x_2(t_2)$	$x_3(t_3)$	$x_4(t_4)$	$x_5(t_5)$	$x_6(t_6)$	$x_7(t_7)$	$x_8(t_8)$	$x_9(t_9)$
8.06	7.99	8.80	8.72	9.71	10.97	15.12	24.53	6.09

(iii) In this paper, using different k_i, the intervals between switching times (ST) are directly related to the values of k_i. It can be easily seen in Table 2 that if the k_i are equal (cases $k_1 = k_2$ and $k_3 = k_4$), the intervals between ST are also equal ($t_1 - t_0 = t_2 - t_1$, and $t_3 - t_2 = t_4 - t_3$). It can also be seen that if k_i decreases (see, for example: $k_4 > k_5 > k_6 > k_7 > k_8$), the intervals between ST increase, and *vice versa* (case $k_8 < k_9$). This result is logical from the point of view of reaction kinetics. Recall that in [4], with $k_i = 1$, $\forall i$, and also with a different functional (the transition time), these intervals become progressively smaller as the chain reaction advances.

(iv) Product p is only generated in the last interval $[t_{n-1}, t_f]$ and the concentration of the substrates in this time period are ordered as stated in (ii).

(v) The minimal operation time logically increases as the number of intermediate compounds, n, increases.

(vi) Finally, it can also be seen that all the switching times are increasingly delayed with increasing n and the intervals between ST also increase with n, regardless of the values of k_i.

5.2 Example 2: differential SA

This section presents the sensitivity properties of the optimal solution. Let us now see how the Differential SA of our problem can be performed immediately, employing analytic formulas to do so (28) and (29). The sensitivity coefficient, ϕ_{ij}, defined from the partial derivative of the dependent variable x_i $(i = 1, \ldots, n)$ with respect to k_j $(j = 1, \ldots, i)$:

$$\phi_{ij} = \frac{dx_i}{k_j} \tag{51}$$

was calculated using the Mathematica® package. The result obtained for the sensitivity coefficient, ϕ_{ij}, $(i = 1, 2, 3)$, is shown in Fig. 2.

The main results are:

(i) The major influence on each x_i corresponds to the parameters k_i and k_{i-1}. Regarding the former has a negative derivative and the second, a positive derivative.

(ii) The main influence of k_{i-1} on x_i is produced during the reaction: $x_{i-1} \rightarrow x_i$, the sensitivity coefficient subsequently being virtually negligible.

(iii) The influence of k_i on x_i is noted most in the time interval during which the reaction: $x_i \rightarrow x_{i+1}$ takes place. It subsequently remains constant.

(iv) The rest of the parameters k_j ($j < i - 1$) exert a much smaller influence over x_i. In this case, the sensitivity coefficients are always positive, much smaller that the previous ones, and also follow the shape of the metabolites.

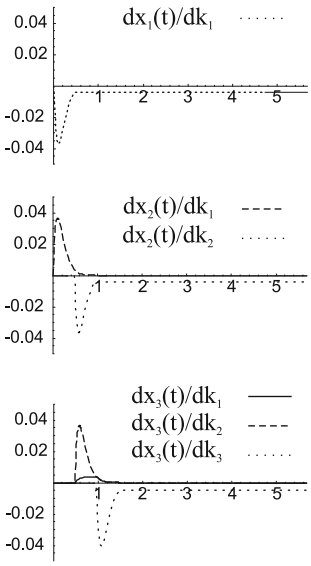

Fig. 2 Sensitivity coefficient ϕ_{ij} $(i = 1, 2, 3)$

Figure 3 shows the results obtained for the remaining compounds: x_i $(i = 4, \ldots, 9)$. The performance is similar to that already seen. The graph is not so clear, as the sensitivity coefficients $\frac{dx_i}{k_j}$ sometimes overlap for $j < i - 1$. The last result can, however, be clearly distinguished in Fig. 3.

(v) The sensitivity coefficients increase progressively, in absolute values, as the reaction advances.

5.3 Example 3: OAT SA

In this example, we analyze the influence of each k_i on the operation time, t_f. As t_f is obtained by solving the nonlinear system using approximate methods (47) (in our case, using the Newton method included in Mathematica), we shall use the well-known OAT SA. Varying one catalytic efficiency, k_i, at a time and setting the others to their nominal values, we obtain the optimal solutions of t_f for different values of k_i. For ease of comprehension, we now choose a example with $n = 6$, the value of the parameters $k_i(s^{-1})$ (all non-equal) being:

$$k_1 = 10;\ k_2 = 9;\ k_3 = 8;\ k_4 = 7;\ k_5 = 6;\ k_6 = 5 \tag{52}$$

The results are shown in Fig. 4. We first solved the problem starting from the base values (52), obtaining an optimal value of $t_f = 3.40187(s)$. Next, keeping the remaining k_j $(j \neq i)$ constant, we successively varied each k_i until duplicating its base value (52). We represent Δk_i , defined as the relative increment, in per unit, on the x-axis, while the operation time, $t_f(s)$, is represented on the y-axis. The main conclusions drawn are:

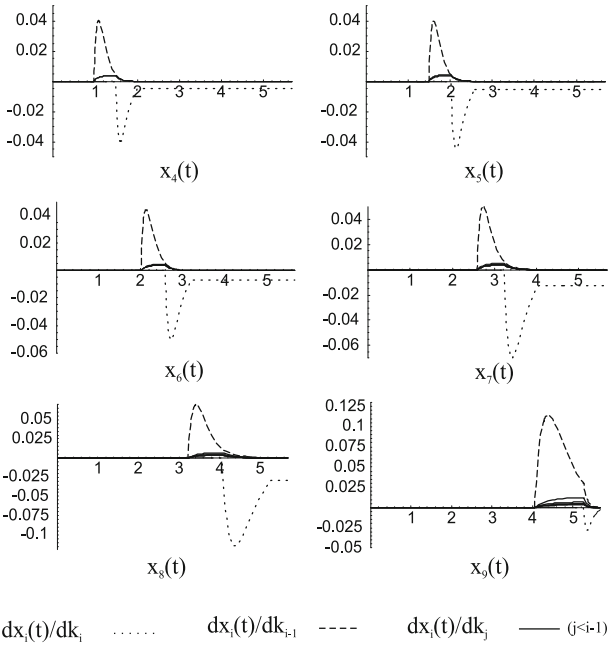

Fig. 3 Sensitivity coefficient ϕ_{ij} $(i = 4, \ldots, 9)$

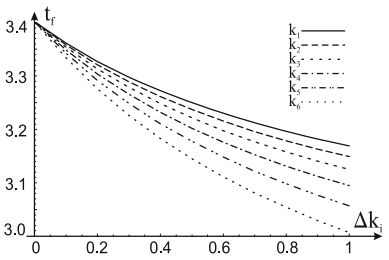

Fig. 4 Optimal cost, t_f, as a function of catalytic efficiencies, k_i

(i) As expected, the operation time, t_f, always decreases with increasing k_i.

(ii) The operation time, t_f, is less sensitive to higher values of k_i. For example, increasing $k_1 = 10$ to 100% of its nominal value ($k_1^* = 20$) yields a $t_f = 3.16877(s)$, whereas the same percentage increase in k_6 ($k_6 = 5 \rightarrow k_6^* = 10$) yields a $t_f = 3.00707(s)$.

6 Conclusions

Our paper supposes the generalization of the optimal control problem that arises when considering a linear unbranched chemical process with n steps. We provide a quasi-analytical solution to the case of n steps, considering the minimization of the operation time and non-equal catalytic efficiencies of the enzymes. The model used for the cost functional and the kinetic model used in the reaction are thus much more realistic.

Moreover, using our closed-form equation for the optimal solution, the SA is very easy to perform. This type of analysis is very important for researchers when quantifying the importance of the parameters employed. We believe that the results presented in this paper constitute a fundamental tool for comparing any other approximate method. Moreover, in this respect, it is essential no longer be subject to the constraint of equal k_i. Finally, as regards future lines of research, it would be very interesting to carry out similar analytical studies, though using nonlinear models such as the Michaelis-Menten model.

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