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L. Bayón, J. A. Otero, P. M. Suárez & C. Tasis

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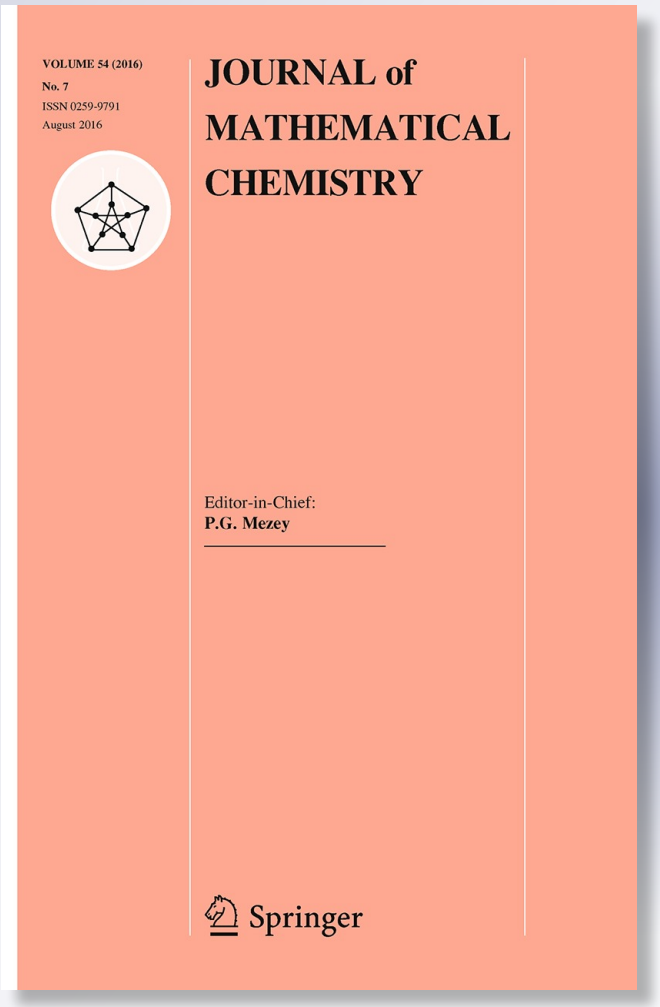

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ORIGINAL PAPER

Solving linear unbranched pathways with Michaelis–Menten kinetics using the Lambert W-function

L. Bayón[1] · J. A. Otero[1] · P. M. Suárez[1] · C. Tasis[1]

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Abstract In this paper, an n-step, linear and unbranched pathway with Michaelis–Menten kinetics is solved in a quasi-analytical way. The method, based on the optimal control theory, calculates the optimal enzyme concentrations while minimizing the operation time. In the computation of the solution, the Lambert W-function plays a fundamental role, due to the presence of a non-linear kinetic model. Our method allows us to obtain the generalized solution and to perform the sensitivity analysis of the catalytic parameters.

Keywords Optimal control · Michaelis–Menten kinetic · Lambert W-function

Mathematics Subject Classification 49J30 · 49M05 · 80A30

1 Introduction

This paper presents a method for obtaining the generalized solution of an n-step system with an unbranched scheme and non-linear kinetic model in an almost exclusively analytical way. Most of the previous papers use a bilinear (linear in the metabolite concentrations, x_i, and linear in the enzyme concentrations, u_i) kinetic model for the solution. For example, an explicit solution for $n = 2$, can be found in [1], while, for $n = 5$, the authors solved the optimization problem numerically. The solution for $n = 3$ is obtained quasi-analytically in [2]. In a previous paper [3], we addressed the minimization of the transition time, and generalized the works of [1] and [2], presenting the quasi-analytical solution for the general case of n steps, but under the assumption

✉ L. Bayón
bayon@uniovi.es

1 Department of Mathematics, University of Oviedo, EPI Gijón, Gijón, Spain

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of equal catalytic efficiencies of the enzymes ($k_i = 1$). Later, in [4], and addressing the minimization of the operation time, we extended the theoretical analysis of [3], considering unequal catalytic efficiencies k_i.

There are few works dealing with nonlinear models in the x_i. Among these, [5] used the Michaelis–Menten (MM) model [6], though for a particular case ($n = 4$). In [7], a metabolic control analysis is used to obtain the optimal behavior both in the setting of an unbranched linear pathway and one of MM type. A mathematical model of an unbranched reaction chain obeying MM kinetics is used in [8] for $n = 3$. Another numerical example with a three-step pathway and reversible MM kinetics is shown in [9]. Besides these numerically solved examples, [10] derives analytic equations but for a very simple example, modeling a single enzyme that follows MM kinetics and operates in the middle of an unbranched metabolic pathway.

Focusing on the kinetic models, MM has proven to be a powerful approach for describing enzyme processes. Due to difficulties in obtaining closed form solutions for this model, several papers based upon effective scaling and singular perturbation techniques have been written, giving fairly accurate solutions [11]. A closed form solution to the MM equation was found, for the first time, in [12], using the Lambert W-function. A generalization that is still valid when the initial substrate concentration is close to that of the enzyme was recently presented in [13]. In [14] the Lambert W-function is employed to estimate the catalytic parameters.

In this paper we present both the solution for the general case of n steps and a sensitivity analysis of the catalytic parameters (the K_m and k_{cat} constants). Using optimal control techniques, a functional that takes into account the operation time is minimized. We prove that the optimal enzyme concentration profile (in a quasi-closed form) is of "bang-bang" type. The paper is organized as follows: In Sect. 2 we abridge the fundamental theoretical results about three issues: the kinetic model, the Lambert W-function and Pontryagin's minimum principle (PMP); in Sect. 3, we state the problem and, applying PMP, we obtain the optimal solution; numerical simulations of the solution and a sensitivity analysis based on the catalytic parameters, are presented in Sect. 4; finally, Sect. 5 summarizes the main contributions of this paper.

2 Theoretical foundations

2.1 Kinetic model

The kinetics of the Michaelis–Menten (MM) model [6] describes the velocity (rate) of lots of enzymatic reactions. This model assumes a simple 2-step reaction: step 1 (Binding), in which the enzyme E interacts with the substrate S to form the enzyme-substrate complex ES; step 2 (Catalysis), decomposition of the ES to regenerate the free enzyme E and the new product P.

$$E + S \underset{k_{-1}\ bind.}{\overset{k_1}{\rightleftarrows}} [ES] \underset{cat.}{\overset{k_2 = k_{cat}}{\rightarrow}} E + P \tag{1}$$

The rate equation of the MM kinetic model is:

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$$V_0 = \frac{d\,[P]}{dt} = \frac{V_{\max}.\,[S]}{K_m + [S]} = \frac{k_2.\,[S]}{K_m + [S]}[E_T] \tag{2}$$

where $d[P]/dt$ or V_0 is the initial rate of product generation, $V_{\max}$ is the maximum rate and $[E_T]$ is the total enzyme concentration. The following ratio of rate constants is called the MM constant, K_m:

$$K_m = \frac{k_{-1} + k_2}{k_1} \tag{3}$$

The MM equation (2) describes how the initial reaction rate V_0 depends on the substrate concentration, $[S]$. From (2) follows that K_m can also be defined as the substrate concentration at which the rate $V_{\max}/2$ is reached. Several simplifying assumptions are required to derive the MM equation:

(1) The binding step is fast and the catalytic step is slower.
(2) At an early stage, when the initial velocity (V_0) is measured, $[P] \approx 0$. Hence, the inverse transformation of the product can be ignored.
(3) ES reaches steady state immediately, so that $[ES]$ is constant.
(4) The fraction of S that binds to E (to form ES) is negligible, and $[S]$ is constant at early times.
(5) The total enzyme concentration $[E_T]$ is the sum of the free and substrate-bound concentrations: $[E_T] = [E] + [ES]$.

The constant K_m is characteristic of each enzyme and specific for each substrate. It is directly related to the affinity of the enzyme for that substrate and does not vary with the concentration of the former. A small (resp. large) value of K_m indicates a high (resp. low) affinity of E for the specific S, because at a low (resp. high) concentration of the substrate, the enzyme has already (resp. only) developed half the maximum rate.

When $[S] \ll K_m$, the rate and the substrate concentration are directly proportional to each other and the reaction has first-order kinetics. When $[S] \gg K_m$, the rate is equal to the maximum velocity and is independent of the substrate concentration. The reaction has zero-order kinetics.

2.2 The Lambert W-function

The Lambert W-function, $W(z)$ is a set of functions which are the branches of the inverse of the function:

$$z = f(W) = We^W \tag{4}$$

where W is a complex variable. In this paper we focus on real-valued $W(x)$, which is defined only for $x \geq -1/e$ and is double-valued on $(-1/e, 0)$.

Adding the condition $W \geq -1$, we get a single-valued function $W_0(x)$ which is the principal branch of the W-function. In this case, $W_0(0) = 0$ and $W_0(-1/e) = -1$. For $W \leq -1$, one gets the lower branch, denoted $W_{-1}(x)$, which is decreasing from $W_{-1}(-1/e) = -1$ to $W_{-1}(0-) = -\infty$.

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We refer the reader to [15] for a survey on existing results on this function. For example, by implicit differentiation, one proves easily that all branches of W satisfy:

$$\frac{dW}{dx} = \frac{W(x)}{x(1+W(x))}; x \notin \{0, -1/e\} \tag{5}$$

In [12], a closed solution to equation (2) is given:

$$[S](t) = K_m W\left(\frac{[S_0]}{K_m}\exp\left(\frac{-V_{\max}t + [S_0]}{K_m}\right)\right) \tag{6}$$

which we are going to use extensively in this work for an n-step system with an unbranched scheme.

2.3 Pontryagin's minimum principle

We provide a summary of Optimal Control Theory in this section. More specifically, we state Pontryagin's Minimum Principle (PMP). An optimal control problem, in the multidimensional case, with free end-time t_f and free end state $\mathbf{x}(t_f)$ can be posed as the following equation:

$$\min_{t_f, \mathbf{u}(t)} J = \int_0^{t_f} F(\mathbf{x}(t), \mathbf{u}(t), t)dt + B[t_f, \mathbf{x}(t_f)] \tag{7}$$

subject to:

$$\dot{x}_i(t) = f_i(\mathbf{x}(t), \mathbf{u}(t), t); \quad x_i(0) = x_{i0}; \quad i = 1, \ldots, n \tag{8}$$

$$\mathbf{u}(t) \in U(t), \quad 0 \le t \le t_f \tag{9}$$

with $\mathbf{x}(t) = (x_1(t), \ldots, x_n(t)) \in \mathbb{R}^n$ the state vector, and $\mathbf{u}(t) = (u_1(t), \ldots, u_n(t)) \in \mathbb{R}^n$ the control vector. The optimum t_f^* is unknown and to be determined. The following hypotheses are assumed: (i) F and $\mathbf{f} = (f_1(t), \ldots, f_n(t))$ are continuous. (ii) F and $\mathbf{f}$ have partial first derivatives with respect to continuous t and $\mathbf{x}$. They may have discontinuous derivative in $\mathbf{u}$. (iii) The control variable, $\mathbf{u}(t)$, may be have discontinuities it only needs to be piecewise continuous. (iv) The state variable, $\mathbf{x}(t)$, is continuous, but its derivative only needs to be piecewise continuous ($\mathbf{x}(t)$ admits corner points). And (v) B has continuous partial first derivatives. The set of admissible controls, U, is often compact and convex. The Hamiltonian is defined as:

$$H(\mathbf{x}(t), \mathbf{u}(t), \lambda(t), t) = F(\mathbf{x}(t), \mathbf{u}(t), t) + \lambda(t)\mathbf{f}(\mathbf{x}(t), \mathbf{u}(t), t) \tag{10}$$

where $\lambda(t) = (\lambda_1(t), \ldots, \lambda_n(t))$ is the costate vector. The following theorem [16] establishes the necessary conditions for optimality for the problem being addressed here:

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Theorem 1 Pontryagin's minimum principle (PMP) *Let* $\mathbf{u}^*(t)$ *be the optimal piecewise control path, and* $\mathbf{x}^*(t)$*, the optimal associated state path, defined in the interval* $[0, t_f]$*. There is a continuous function,* $\lambda^*(t)$*, which has piecewise continuous first derivatives, such that for each* $t \in [0, t_f]$*, the following conditions are verified, for each* $i = 1, \ldots, n$*:*

$$\begin{aligned}
&\text{(i)} \quad \dot{\lambda}_i^*(t) = -\frac{\partial H(\mathbf{x}^*(t), \mathbf{u}^*(t), \lambda^*(t), t)}{\partial x_i}; \quad \lambda_i^*(t_f^*) = \frac{\partial B[t_f^*, \mathbf{x}^*(t_f^*)]}{\partial x_i} \\
&\text{(ii)} \quad H(\mathbf{x}^*(t), \mathbf{u}^*(t), \lambda^*(t), t) \leq H(\mathbf{x}^*(t), \mathbf{u}(t), \lambda^*(t), t); \quad \mathbf{u}(t) \in U(t) \\
&\text{(iii)} \quad \dot{x}_i^*(t) = f_i(\mathbf{x}^*(t), \mathbf{u}^*(t), t); \quad x_i^*(0) = x_{i0} \\
&\text{(iv)} \quad H(\mathbf{x}^*(t_f^*), \mathbf{u}^*(t_f^*), \lambda^*(t_f^*), t_f^*) + \frac{\partial B[t_f^*, \mathbf{x}^*(t_f^*)]}{\partial t_f} = 0
\end{aligned} \tag{11}$$

The solution may not be interior so that minimizing the Hamiltonian does not necessarily imply $\partial H/\partial \mathbf{u} = \mathbf{0}$. If the dynamic function $\mathbf{f}$, and the integrand F, have no explicit time-dependence, the problem is said to be *autonomous*. In this case, $H_t \equiv 0$, which implies that the Hamiltonian is constant throughout said solution:

$$H(\mathbf{x}^*(t), \mathbf{u}^*(t), \lambda^*(t)) = const. \tag{12}$$

When the control $\mathbf{u}$ appears linearly in $F(\mathbf{x}(t), \mathbf{u}(t), t)$ and in $\mathbf{f}(\mathbf{x}(t), \mathbf{u}(t), t)$, then:

$$H(\mathbf{x}(t), \mathbf{u}(t), \lambda(t), t) = \nu(\mathbf{x}(t), \lambda(t), t) - \mu(\mathbf{x}(t), \lambda(t), t)\mathbf{u} \tag{13}$$

As we shall eventually see, in our specific case the optimality condition (ii) leads to the minimization of a linear function of n variables of the following type:

$$\min_{\mathbf{u} \in U} H = \min_{\mathbf{u} \in U} \left\{ -\sum_{i=1}^{n} \mu_i u_i \right\} \tag{14}$$

where the functions $\mu_i = -\partial H/\partial u_i$ are called the *switching functions*. Minimizing H with respect to u_i leads to:

$$u_i^*(t) = \begin{cases} u_{i\,\max} & \text{if } \partial H/\partial u_i < 0 \\ u_{\text{sing}} & \text{if } \partial H/\partial u_i = 0 \\ u_{i\,\min} & \text{if } \partial H/\partial u_i > 0 \end{cases} \tag{15}$$

If u_i switches between its upper and lower limits only at isolated points in time, then the optimal control is said to be a *bang-bang type control*. Those times are called the *switching times*. If $\partial H/\partial u_i = 0$ for every t in some open subinterval, then the original problem is called a *singular control* problem and the corresponding trajectory, a *singular arc* u_{sing}.

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3 Statement of the problem and optimal solution

We are going to focus on unbranched metabolic pathways with MM kinetics as described below. Consider the following unbranched metabolic pathway composed of n irreversible reactions converting substrate x_1 into product p:

$$x_1 \xrightarrow{u_1} x_2 \xrightarrow{u_2} x_3 \xrightarrow{u_3} \cdots \rightarrow x_{n-1} \xrightarrow{u_{n-1}} x_n \xrightarrow{u_n} p \tag{16}$$

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 each intermediate compound at time t, and $u_i(t)$ $(i = 1, \ldots, n)$ the concentration at time t of the enzyme catalyzing the i-th reaction. For the sake of simplicity, we use normalized quantities: the u_i are divided by the maximum total enzyme concentration, and the x_i and p are divided by $x_1(0)$. Using (2) we get:

$$v_i(x_i(t), u_i(t)) = \frac{k_i x_i(t)}{K_{mi} + x_i(t)} u_i(t) \tag{17}$$

where v_i is the rate of the i-th reaction $(i = 1, \ldots, n)$, and the dynamical model for the pathway shown in (16) is given by conservation of mass:

$$\dot{x}_i(t) = v_{i-1}(x_{i-1}(t), u_{i-1}(t)) - v_i(x_i(t), u_i(t)); \quad (i = 1, \ldots, n) \tag{18}$$

So, the reactions in (16) can then be modeled by the set of differential equations:

$$\begin{cases} \dot{x}_1 = -\dfrac{k_1 x_1}{K_{m1} + x_1} u_1 & x_1(0) = 1 \\ \dot{x}_2 = \dfrac{k_1 x_1}{K_{m1} + x_1} u_1 - \dfrac{k_2 x_2}{K_{m2} + x_2} u_2 & x_2(0) = 0 \\ \cdots \\ \dot{x}_n = \dfrac{k_{n-1} x_{n-1}}{K_{mn-1} + x_{n-1}} u_{n-1} - \dfrac{k_n x_n}{K_{mn} + x_n} u_n & x_n(0) = 0 \end{cases} \tag{19}$$

with $x_i(t) \geq 0$. Due to normalization, we have $x_1(0) = 1$, and:

$$x_1(t) + x_2(t) + \cdots + x_n(t) + p(t) = 1, \quad \forall t \geq 0 \tag{20}$$

Our goal is to transform x_1 into product p as fast as possible. Thus, we shall minimize the operation time, which is defined in terms of the concentration of the final product, $p(t_f)$, with t_f as the final time. In the case of an exhaustible initial substrate, x_1, from (20), and imposing $p(t_f) = C_f$ $(0 < C_f < 1)$, we obtain:

$$x_1(t_f) + x_2(t_f) + \cdots + x_n(t_f) = 1 - C_f \tag{21}$$

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So that the optimization problem may thus be defined as the following control problem (Pr):

$$(Pr): \quad \tau_{C_f} = \min_{u_1,\ldots,u_n} \int_0^{t_f} dt = \min_{u_1,\ldots,u_n} t_f$$

subject to (19), (21) and:

$$U(t) = \{\mathbf{u} \in \mathbb{R}^n | u_1 \geq 0, \ldots, u_n \geq 0; \quad u_1 + \cdots + u_n \leq 1\} \tag{22}$$

Using PMP, we get the following solution to (Pr):

Theorem 2 Optimal solution *The optimal i-enzyme profile is of bang-bang type and satisfies:*

$$u_i^*(t) = \begin{cases} 1 \; for \; t \in [t_{i-1}, t_i) \\ 0 \; for \; t \notin [t_{i-1}, t_i) \end{cases}; \quad i = 1, \ldots, n \tag{23}$$

where $\{t_0, t_1, t_2, \ldots, t_n\}$ are the switching times, with $t_0 = 0$ and $t_n = t_f$. If we denote by $x_{ji}(t)$ the optimal j-th metabolite concentration in the i-th interval $[t_{i-1}, t_i]$, $i = 1, \ldots, n$, with $x_{10}(t_0) = 1$, then the optimal solution is:

$\mathbf{x_{j1}(t)}$	*for*	**j**
$K_{m1} W \left(\frac{x_{10}(t_0)}{K_{m1}} e^{\frac{x_{10}(t_0)}{K_{m1}}} e^{-\frac{k1}{K_{m1}}(t-t_0)} \right)$		1
$x_{10}(t_0) - x_{11}(t)$		2
0		$3, \ldots, n$

(24)

$\mathbf{x_{ji}(t)}$	*for*	**j**
$x_{jj}(t_j)$		$1, \ldots, i-1$
$K_{mi} W \left(\frac{x_{ii-1}(t_{i-1})}{K_{mi}} e^{\frac{x_{ii-1}(t_{i-1})}{K_{mi}}} e^{-\frac{k_i}{K_{mi}}(t-t_{i-1})} \right)$		i
$x_{ii-1}(t_{i-1}) - x_{ii}(t)$		$i+1$
0		$i+2, \ldots, n$

(25)

$\mathbf{x_{jn}(t)}$	*for*	**j**
$x_{jn}(t_j)$		$1, \ldots, n-1$
$K_{mn} W \left(\frac{x_{nn-1}(t_{n-1})}{K_{mn}} e^{\frac{x_{nn-1}(t_{n-1})}{K_{mn}}} e^{-\frac{k_n}{K_{mn}}(t-t_{n-1})} \right)$		n

(26)

Proof See Appendix 1. □

4 Numerical simulations

We have developed a program using Mathematica® which allows us to easily obtain the optimal solution for problems of any dimension n. As Mathematica® includes the function ProductLog[z], which is a symbolic version of the Lambert W-function, it permits us to perform the main operations with it: derivation, integration, plotting, etc.

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4.1 Example of optimal solution

As an illustrative example, first we consider $n = 4$ and the following values for the catalytic efficiencies $k_i\,(s^{-1})$ and the MM constants K_{mi}(mM):

i	1	2	3	4
k_i	1	2	4	3
K_{mi}	1	0.9	0.8	0.7

(27)

Assume $p(t_f) = C_f = 0.9$. We shall minimize the operation time according to the specific ratio (90 % in this case) of the initial substrate to be converted into the product.

In Table 1, the optimal solutions for $n = 2, 3, 4$ are given. The switching times t_i for $i = 1, \ldots, n$ are given as are the total operation times $\tau_{C_f} = t_n = t_f$, in boldface. These values are computed solving the nonlinear system (54) (see Appendix 1). To this end, we make use of the Mathematica® command FindRoot[·]. The fact that we obtain the solution by successively increasing the value of n is not accidental: the FindRoot[·] command is based on Newton-Raphson's method and requires an initial seed for finding local solutions. We have verified that working this way, the values of t_i computed for case $n - 1$ can be used as seeds for case n. This way, the convergence of the method is guaranteed without requiring any initial estimation of the solution.
Figure 1 shows the optimal solution obtained for the enzyme concentration for the case $n = 4$. As Theorem 2 states, due to the linearity of the control, the solution of the optimal control problem is of bang-bang type and all the u_i are 1 in all the intervals where they are active.
Figure 2 shows the optimal solution for the substrate concentration, x_1, the concentrations of the intermediate compounds, x_2, x_3, x_4, and the concentration of the final product, p, for the case $n = 4$. It is relevant to point out that once the optimal values shown in Table 1 are obtained numerically, the remaining values of the solution are immediately obtained analytically using the closed-form formulas of Theorem 2. The time required by the program to complete this example was 0.094 s on a budget computer (Intel Core 2/2.66 GHz).

Table 1 Switching times and operation time of the optimal solution

n	t_1	t_2	t_3	t_4
2	3.57301	**5.58414**	–	–
3	3.70160	5.77137	**6.87851**	–
4	3.83301	5.96261	7.09668	**8.42125**

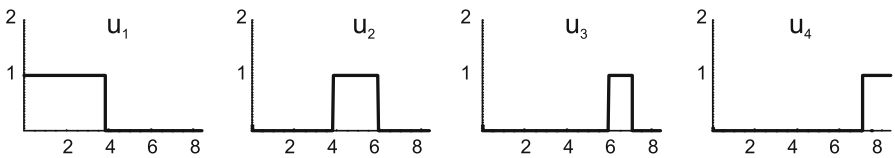

Fig. 1 Optimal enzyme profile

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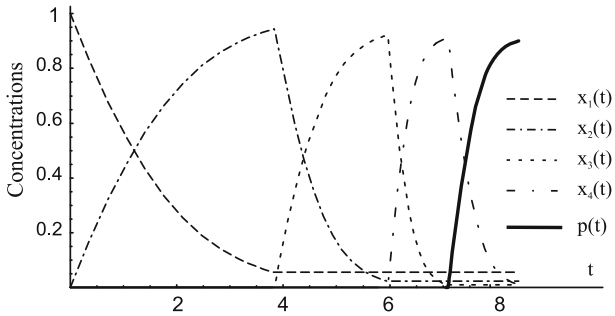

Fig. 2 Profiles of Metabolite and product

Table 2 Switching times and operation time of the optimal solution

n	t_1	t_2	t_3	t_4	t_5	t_6	t_7	t_8
2	0.3569	**1.0091**	–	–	–	–	–	–
3	0.4118	1.1919	**1.9909**	–	–	–	–	–
4	0.4446	1.3019	2.1810	**3.0039**	–	–	–	–
5	0.4810	1.4242	2.3928	3.2975	**4.5606**	–	–	–
6	0.4960	1.4749	2.4806	3.4193	4.7354	**5.5486**	–	–
7	0.5108	1.5248	2.5669	3.5390	4.9072	5.7482	**6.6839**	–
8	0.5189	1.5522	2.6145	3.6050	5.0019	5.8582	6.8113	**7.4628**

Bold values indicate the total operation time

To verify the behaviour of our method when increasing n, we present a second example, considering $n = 8$ and the following values for the catalytic efficiencies $k_i\,(s^{-1})$ and the MM constants K_{mi}(mM):

i	1	2	3	4	5	6	7	8
k_i	100	50	60	70	50	100	60	90
K_{mi}	10	12	15	16	18	19	13	12

(28)

We assume once again $p(t_f) = C_f = 0.9$. In Table 2, the optimal solutions for all the values of n are given.

The convergence of the method without using our method is very difficult, due the complexity of the Lambert Function. However, using for n, the initial estimation of the solution given by $n - 1$, the convergence is always achieved. The time required to complete this example was 20.905 s on a budget computer (Intel Core 2/2.66 GHz).

After extensive testing on the above example we have analyzed in detail the influence of the values of the constants in both the catalytic and the Michaelis–Menten. As regards the interpretation of the results, we may present the following conclusions from the qualitative point of view:

(1) If k_i decreases, ceteris paribus, then the intervals between switching times, $t_i - t_{i-1}$, increase and vice versa. This result is logical from the point of view of

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reaction kinetics, because if k_i is small, then so is the reaction rate and hence the active interval of each u_i must be larger.

(2) if K_{mi} increases, ceteris paribus, then the intervals between switching times, $t_i - t_{i-1}$, increase and vice versa. This result is also logical, because if K_{mi} is big, then the reaction rate is small and hence the active interval of u_i must be larger.
   – These two results are independent of the position of the constants(k_i and K_{mi}) in the reaction.

(3) In the first interval, starting from $x_1(0) = 1$, substrate x_1 is converted into x_2, which reaches its maximum value $x_2(t_1)$ at the first switching time, t_1. At this moment, x_1 stays constant with value $x_1(t_1)$, which it will keep until the end, at time t_f. The process is likewise repeated for all x_i, as follows from the solution being of bang-bang type.

(4) The maximum value obtained by each compound $x_i(t_{i-1})$, becomes progressively smaller as the process advances, regardless of the values of k_i and K_{mi}.
   – The values $x_i(t_{i-1})$ increase for increasing k_i, ceteris paribus, and *vice versa*. The rest of values $x_j(t_{j-1})$, $(j \neq i)$ also increase w.r.t. the value obtained in (4).
   – The values $x_i(t_{i-1})$ decrease for increasing K_{mi}, ceteris paribus, and *vice versa*. The rest of values $x_j(t_{j-1})$, $(j \neq i)$ also decrease w.r.t. the value obtained in (4).

(5) If $k_i = k_j$ and $K_{mi} = K_{mj}$ (for all i, j), the constant value reached by each compound $x_i(t_i)$, becomes progressively smaller as the process advances.
   – The values $x_i(t_i)$ decrease for increasing k_i, ceteris paribus, and *vice versa*. The rest of values $x_j(t_j)$, $(j \neq i)$ increase w.r.t. the value obtained in (5).
   – The values $x_i(t_i)$ increase for increasing K_{mi}, ceteris paribus, and *vice versa*. The rest of values $x_j(t_j)$, $(j \neq i)$ decrease w.r.t. the value obtained in (5).

(6) Product p is only generated in the last interval $[t_{n-1}, t_f]$ and the concentrations of the substrates in this time period are constant.

(7) The minimal operation time τ_{C_f} increases—as is natural—with the number of intermediate compounds, n.

(8) The switching times are increasingly delayed for increasing n, regardless of the values of k_i and K_{mi}.

Table 3 summarizes the qualitative influence of k_i and K_{mi} on several characteristics of the optimal solution.

In Table 4 we present the execution times obtained for increasing values of n. We have taken, in this case, $k_i = 50\,(s^{-1})$ and $K_{mi} = 10\,(\text{mM})$.

The execution times suggest the exponential character, $O(4^n)$, of the algorithm's computational complexity.

Table 3 Qualitative influence of k_i and K_{mi}

		$x_i(t_{i-1})$	$x_i(t_i)$	$t_i - t_{i-1}$	τ_{C_f}
k_i	↗	↗	↘	↘	↘
K_{mi}	↗	↘	↗	↗	↗

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Table 4 Influence of n in the execution time

n	2	3	4	5	6	7	8	9	10
$t(s)$	0.015	0.032	0.109	0.343	1.279	5.007	20.748	84.848	342.438

4.2 Sensitivity analysis

In this section, we present the sensitivity analysis of the optimal solution. Sensitivity analysis (SA) (see, for example, [17,18] and [19]) explores the effect of the change of a parameter on the solution of a mathematical model. Consider a general model with one dependent variable Y and several independent variables, $X = (X_1, \ldots, X_n)$, where $Y = f(X)$. One the most usual methods of SA is Differential SA. In it, the sensitivity coefficient, ϕ_i, for the independent variable, X_i, is defined as:

$$\phi_i = \frac{dY}{dX_i} \tag{29}$$

When a closed form expression for $Y = f(X)$ is known, this coefficient is very easy to compute. In our case, employing the analytical formulas (24), (25) and (26) we can calculate the sensitivity coefficient, ϕ_{ij}, of the concentration of the intermediate compounds and substrate x_i with respect to the catalytic efficiencies, k_j:

$$\phi_{ij} = \frac{dx_i}{dk_j}; \quad (i = 1, \ldots, n), \quad (j = 1, \ldots, i) \tag{30}$$

As we have already pointed out, the derivative of the Lambert W-function is easily computed using (5), so that the above ϕ_{ij} can be explicitly obtained. Their values for $i = 1, 2, 3, 4$ are plotted in Fig. 3.

The main results are:

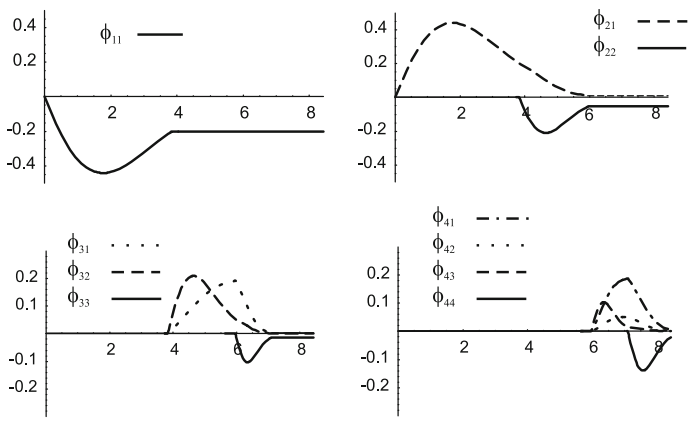

Fig. 3 Sensitivity coefficient ϕ_{ij}

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(a) If all the k_i are equal, then the greatest influence on each x_i corresponds to the parameters k_i and k_{i-1}, the other k_j for $j < i-1$ exerting a lesser influence than the last two. Moreover, this latter influence is almost the same for all of them and follows the shape of the metabolites.
(b) The influence of k_i on x_i is most relevant in the time interval during which the reaction $x_i \rightarrow x_{i+1}$ takes place. It thence remains constant with a value which may be considered as significant (in the example, $\simeq$30 % of the maximum value for ϕ_{11}).
(c) The influence of k_{i-1} on x_i is relevant almost exclusively during the time when the reactions $x_{i-1} \rightarrow x_i \rightarrow x_{i+1}$ take place. The sensitivity coefficient in the remaining times is constant and practically negligible.
(d) The influence of k_j on x_i for $j < i-1$ is also only relevant during the time when the reactions $x_{i-1} \rightarrow x_i \rightarrow x_{i+1}$ take place.
(e) If all the k_i are equal, then the values of the sensitivity coefficients stay within a range during all the reaction and their time interval of influence is shifted.
(f) If k_j decreases, then ϕ_{ij} increases. This is why, in the example (with $k_i = 1$ the smallest one), we see that, despite (a), ϕ_{41} is greater than all the other coefficients.

One can also perform the Differential SA with respect to the MM constants K_{mj}:

$$\Phi_{ij} = \frac{dx_i}{dK_{mj}}; \quad (i = 1, \ldots, n), \quad (j = 1, \ldots, i) \tag{31}$$

Figure 4 shows the corresponding results. Conclusions are very similar to those obtained for for ϕ_{ij}:

(a) If the K_{mi} are equal for all i, then the greatest influence on each x_i corresponds to the parameters K_{mi} (now with positive derivative) and K_{mi-1} (with negative derivative). The remaining parameters for K_{mj} (for $j < i-1$) exert again the least influence and their graphs have a similar profile to the metabolites.
(b) The influence of K_{mi} on x_i is most relevant in the time interval when the reaction $x_i \rightarrow x_{i+1}$ takes place and remains constant afterwards, with a value which

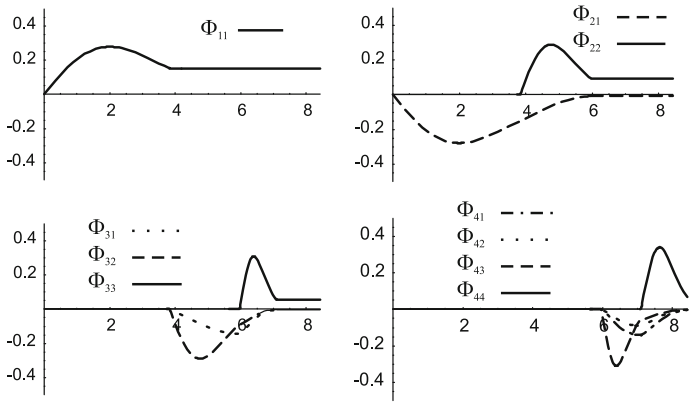

Fig. 4 Sensitivity coefficient Φ_{ij}

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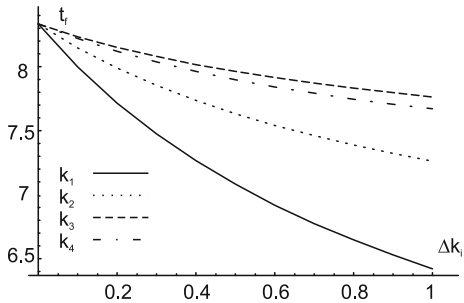

Fig. 5 Optimal cost, t_f, as a function of k_i

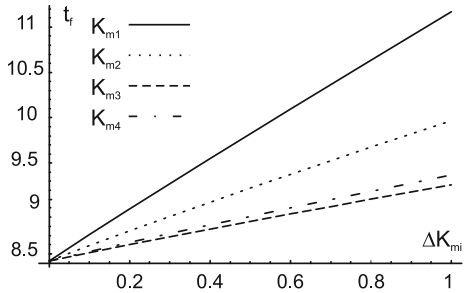

Fig. 6 Optimal cost, t_f, as a function of K_{mi}

is even more significant than those of the k_i (in our example, $\simeq$52 % of the maximum value for Φ_{11}).

(c) The greatest influence of K_{mi-1} and K_{mj} (for $j < i - 1$) on x_i happens during the reactions $x_{i-1} \rightarrow x_i \rightarrow x_{i+1}$ and afterwards the sensitivity coefficient is virtually negligible.

(d) If K_{mj} decreases, then the coefficient Φ_{ij} increases.

We finish this section with the simplest SA method: the one-at-a-time (OAT) method. The idea is to iteratively vary one parameter at a time while keeping the others fixed. We must to use this method to perform the SA of the operation time, t_f, with respect to k_i and K_{mi}, since the analytic relation among them is unknown.
Figure 5 represents the OAT SA for the catalytic efficiencies, k_i. Keeping the remaining k_j (for $j \neq i$) constant, we successively vary each k_i until doubling the initial value given in (27). For a better understanding and comparison, we represent on the x-axis, Δk_i (the relative increment) in per unit and on the y-axis, the operation time $t_f(s)$. The main conclusions are:

(i) The operation time t_f always decreases for increasing k_i. This is a totally natural result, given the kinetic interpretation of these constants.

(ii) The operation time t_f is less sensitive to higher values of k_i. For example, doubling k_1 to $k_1 = 1$ yields a decrease in t_f of 23.75 %, whereas the same percentage increase in k_3 yields a decrease in t_f of just 7.12 %.

(iii) The least values of t_f are always obtained for the largest k_i (in this case k_3).

Analogue results are shown in Fig. 6, for the OAT SA for the K_{mi}.

In this case, the operation time t_f always increases for increasing K_{mi}. The reason was explained in Sect. 2.1, when we gave the kinetic interpretation of these constants.

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As regards the sensitivity of t_f, unlike the k_i, it is greater the greater the value of each K_{mi} is: doubling K_{m1} to $K_{m1} = 1$ yields an increase of 32.6 % in the value of t_f, whereas doubling K_{m4} to $K_{m4} = 0.75$, only increases t_f by 11.2 %. Moreover, we notice a remarkable fact related to the influence of k_i on K_{mi}: as $k_3 > k_4$, the total time t_f remains minimum for the greatest k_i (in this case, k_3) and this despite K_{m3} being greater than K_{m4}.

5 Conclusions

We have presented in this paper for the first time the quasi-analytical solution of an n-step linear unbranched pathway with Michaelis–Menten kinetics. As objective function we minimize the operation time, defined by specifying the final concentration of the product. Traditionally, kinetics with non-linear equations, like Michaelis–Menten, have only been solved approximately. The closed-form formulae of Theorem 2 allow finding the solution for problems of arbitrary dimension, with the only limitation of solving the nonlinear system. With the proposed iterative method of progressively finding the solution for increasing values of n, we have verified that the solution of the system poses no special difficulty from the numerical point of view. The reason is that at each step n, the starting seeds for unknowns $1, \ldots, n-1$ can be estimated to high precision by using the switching times computed at the previous step and the value of the new unknown can also be easily estimated. All the issues related to convergence of numerical processes, frequent in other methods, are thus prevented.

Appendix 1: Proof of Theorem 2.

We now prove that the solution obtained using Pontryagin's Minimum Principle is effectively a solution of our problem. In (22), we have $F = 1$, $B = 1$ and the Hamiltonian H is:

$$H = 1 + \lambda_1 \left[-\frac{k_1 x_1}{K_{m1} + x_1} u_1 \right] + \lambda_2 \left[\frac{k_1 x_1}{K_{m1} + x_1} u_1 - \frac{k_2 x_2}{K_{m2} + x_2} u_2 \right] + \cdots + \lambda_n \left[\frac{k_{n-1} x_{n-1}}{K_{mn-1} + x_{n-1}} u_{n-1} - \frac{k_n x_n}{K_{mn} + x_n} u_n \right] \tag{32}$$

which is autonomous, so that $H_t \equiv 0 \Rightarrow H(t) = ct$. This condition together with (iv) implies that $H(t) = 0$. Now the optimality condition (ii) leads to:

$$\min_{\mathbf{u} \in U} H = \min_{\mathbf{u} \in U} \left\{ -\sum_{i=1}^{n} \frac{k_i (\lambda_i - \lambda_{i+1}) x_i}{K_{mi} + x_i} u_i \right\} = \min_{\mathbf{u} \in U} \left\{ -\sum_{i=1}^{n} \mu_i u_i \right\} \tag{33}$$

with $\lambda_{n+1} = 0$. According to the optimality condition (i), we have:

$$\dot{\lambda}_i = k_i K_{mi} (\lambda_i - \lambda_{i+1}) \frac{u_i}{(K_{mi} + x_i)^2}; \quad (i = 1, \ldots, n) \tag{34}$$

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It is known from (33) that the control u_i is activated when the switching function μ_i reaches its maximum. Moreover, when this happens, the coefficient μ_i must be positive, because otherwise $u_i = 0$. Hence, it follows that λ_i is decreasing. From (33):

$$\mu_i = \frac{k_i(\lambda_i - \lambda_{i+1})x_i}{K_{mi} + x_i} \geq 0 \Rightarrow \lambda_i \geq \lambda_{i+1} \tag{35}$$

We obtain the optimal solution constructively by intervals, starting at $t = 0$ and concatenating the results. This procedure will prove essential in order to obtain a simple solution to the problem. We shall also see that using the following condition in (22):

$$U(t) = \{\mathbf{u} \in \mathbb{R}^n | u_1 \geq 0, \ldots, u_n \geq 0; \quad u_1 + \cdots + u_n \leq 1\} \tag{36}$$

we are not going to require either the final condition (i) $\lambda_i^*(t_f^*) = 0$ for the costate variables, or the transversality condition (iv) $H(t) = 0$. As a matter of fact, we shall see that it will not be necessary to compute λ_i^*, so that we shall not compute $H(t)$ either.

(**1**) **Interval:** $[0, t_1]$.

We reason by contradiction. Assume that $u_1 = 0$. From (19):

$$\left.\begin{array}{l} \dot{x}_1 = -\dfrac{k_1 x_1}{K_{m1} + x_1} u_1 = 0 \\ x_1(0) = 1 \end{array}\right\} \Rightarrow x_1(t) = 1, \quad \forall t \tag{37}$$

and the product would not be produced. Hence, we have $u_1 = 1$ and from condition (36) we get:

$$u_i = 0, \quad i = 2, \ldots, n \tag{38}$$

Once the optimal values for the enzymes are computed, we can solve now (19):

$$\begin{array}{ll} \left.\begin{array}{l} \dot{x}_1 = -\dfrac{k_1 x_1}{K_{m1} + x_1} \\ x_1(0) = 1 \end{array}\right\} & \Rightarrow x_1(t) = K_{m1} W\left(\frac{1}{K_{m1}} e^{\frac{1-k_1 t}{K_{m1}}}\right) \\ \left.\begin{array}{l} \dot{x}_2 = \dfrac{k_1 x_1}{K_{m1} + x_1} \\ x_2(0) = 0 \end{array}\right\} & \Rightarrow x_2(t) = 1 - x_1(t) \\ \left.\begin{array}{l} \dot{x}_i = 0 \\ x_i(0) = 0 \end{array}\right\} & \Rightarrow x_i(t) = 0; \quad i = 3, \ldots, n \end{array} \tag{39}$$

In Appendix 2 we give the details of the solution. In order to generalize the formula, it is interesting to use the following notation: we denote by $x_{ji}(t)$ the concentration of j-metabolite in the i-interval $[t_{i-1}, t_i]$, $i = 1, \ldots, n$, with $x_{10}(t_0) = 1$. So, we have:

$$\begin{array}{l} x_{11}(t) = K_{m1} W\left(\frac{x_{10}(t_0)}{K_{m1}} e^{\frac{x_{10}(t_0)}{K_{m1}}} e^{-\frac{k1}{K_{m1}}(t-t_0)}\right) \\ x_{21}(t) = 1 - x_{11}(t) \\ x_{i1}(t) = 0; \quad i = 3, \ldots, n \end{array} \tag{40}$$

From (34), the following holds:

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$$\dot{\lambda}_1 = \frac{k_1 K_{m1}(\lambda_1 - \lambda_2)}{(K_{m1} + x_1)^2}; \quad \dot{\lambda}_i = 0, \quad i = 2, \ldots, n \tag{41}$$

From (33) and (19), after some elementary computations and substituting $\dot{\lambda}_1$ and $\dot{x}_1$ by their values, we get

$$\mu_1 = \frac{k_1(\lambda_1 - \lambda_2)x_1}{K_{m1} + x_1} \Rightarrow \dot{\mu}_1 = \frac{k_1(\dot{\lambda}_1 - \dot{\lambda}_2)x_1}{K_{m1} + x_1} + \frac{k_1(\lambda_1 - \lambda_2)\dot{x}_1}{(K_{m1} + x_1)^2} = 0 \tag{42}$$

On the other hand:

$$\mu_2 = \frac{k_2(\lambda_2 - \lambda_3)x_2}{K_{m2} + x_2} \Rightarrow \dot{\mu}_2 = \frac{k_2(\lambda_2 - \lambda_3)K_{m2}}{(K_{m2} + x_2)^2} \frac{k_1}{K_{m1} + x_1} x_1 \geq 0 \tag{43}$$

So that

$$\begin{aligned} &\dot{\mu}_1 = 0 \Rightarrow \mu_1 = ct \\ &\dot{\mu}_2 \geq 0 \Rightarrow \mu_2 = \text{increasing} \\ &\mu_i = 0 \Rightarrow \mu_i = 0; \quad i = 3, \ldots, n \end{aligned} \tag{44}$$

(**2**) **Interval:** $[t_1, t_2]$.
With a reasoning analogue to the one used for the first interval:

$$u_1 = 0, \quad u_2 = 1; \quad u_i = 0, \quad (i = 3, \ldots, n) \tag{45}$$

and:

$$\begin{aligned} &\left.\begin{aligned} &\dot{x}_1 = 0 \\ &x_1(t_1) = x_{11}(t_1) \end{aligned}\right\} \Rightarrow x_{12}(t) = x_{11}(t_1) \\ &\left.\begin{aligned} &\dot{x}_2 = -\frac{k_2 x_2}{K_{m2} + x_2} \\ &x_2(t_1) = x_{21}(t_1) \end{aligned}\right\} \Rightarrow x_{22}(t) = K_{m2} W\left(\frac{x_{21}(t_1)}{K_{m2}} e^{\frac{x_{21}(t_1)}{K_{m2}}} e^{-\frac{k_2}{K_{m2}}(t - t_1)}\right) \\ &\left.\begin{aligned} &\dot{x}_3 = \frac{k_2 x_2}{K_{m2} + x_2} \\ &x_3(t_1) = 0 \end{aligned}\right\} \Rightarrow x_{32}(t) = x_{21}(t_1) - x_2(t) \\ &\left.\begin{aligned} &\dot{x}_i = 0 \\ &x_i(t_1) = 0 \end{aligned}\right\} \Rightarrow x_{i2}(t) = 0; \quad i = 4, \ldots, n \end{aligned} \tag{46}$$

which gives:

$$\dot{\lambda}_1 = 0; \quad \dot{\lambda}_2 = \frac{k_2 K_{m2}(\lambda_2 - \lambda_3)}{(K_{m2} + x_2)^2}; \quad \dot{\lambda}_i = 0, \quad i = 3, \ldots, n \tag{47}$$

and performing the adequate substitutions, one proves that:

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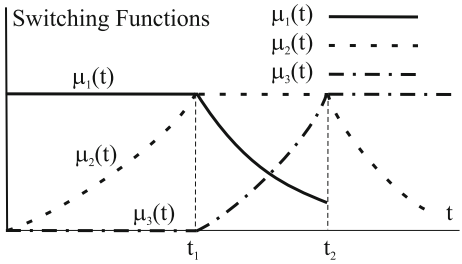

Fig. 7 Illustration of the behavior of the switching functions

$$\begin{aligned}
&\dot{\mu}_1 \le 0 \Rightarrow \mu_1 = \text{decreasing}\\
&\dot{\mu}_2 = 0 \Rightarrow \mu_2 = ct\\
&\dot{\mu}_3 \ge 0 \Rightarrow \mu_3 = \text{increasing}\\
&\mu_i = 0 \Rightarrow \mu_i = 0; \quad i = 4, \ldots, n
\end{aligned} \tag{48}$$

In Fig. 7, the behavior of the switching functions is shown.
The values for each successive interval are similarly obtained, by concatenating the solutions. For the sake of simplicity, we present only the solution for the last one.

(n) Interval: $[t_{n-1}, t_f]$.

In this case:

$$u_i = 0, \quad (i = 1, \ldots, n-1); \quad u_n = 1 \tag{49}$$

$$\left.\begin{aligned}
&\dot{x}_i = 0\\
&x_i(t_{n-1}) = x_{ii}(t_i)
\end{aligned}\right\} \Rightarrow x_{in}(t) = x_{ii}(t_i); \quad i = 1, \ldots, n-1 \tag{50}$$

and

$$\left.\begin{aligned}
&\dot{x}_n = -\frac{k_n x_n}{K_{mn} + x_n}\\
&x_n(t_{n-1}) = x_{nn-1}(t_{n-1})
\end{aligned}\right\} \Rightarrow$$

$$\Rightarrow x_{nn}(t) = K_{mn} W\left(\frac{x_{nn-1}(t_{n-1})}{K_{mn}} e^{\frac{x_{nn-1}(t_{n-1})}{K_{mn}}} e^{-\frac{k_n}{K_{mn}}(t-t_{n-1})}\right) \tag{51}$$

$$\begin{aligned}
&\dot{\mu}_i = 0 \quad \Rightarrow \mu_2 = ct; \quad i = 1, \ldots, n-2\\
&\dot{\mu}_{n-1} \le 0 \Rightarrow \mu_{n-1} = \text{decreasing}\\
&\mu_n = 0 \quad \Rightarrow \mu_n = ct
\end{aligned} \tag{52}$$

Once the optimum values for x_i^* and u_i^* have been obtained, it is still required to compute the values of the following unknowns: the switching times $t_1, t_2, \ldots, t_{n-1}$ and the operation time t_f. In order to do so, we use the restriction (21) which we have not used yet. The simplest way is to apply the Lagrange multipliers to the augmented functional:

$$L(t_1, t_2, \ldots, t_{n-1}, t_f, \beta) = t_f + \beta(x_{1n}(t_f) + x_{2n}(t_f) + \cdots + x_{nn}(t_f) - C_f) \tag{53}$$

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where the values of the concentrations $x_{1n}(t_f), x_{2n}(t_f), \ldots, x_{nn}(t_f)$ are given by (24), (25) and (26), and in which one sees that the unknowns $t_1, t_2, \ldots, t_{n-1}$ appear. We need to solve the non-linear system:

$$\frac{\partial L}{\partial t_1} = 0; \frac{\partial L}{\partial t_2} = 0; \ldots; \frac{\partial L}{\partial t_{n-1}} = 0; \frac{\partial L}{\partial t_f} = 0; \frac{\partial L}{\partial \beta} = 0 \tag{54}$$

which can be done with any computer algebra software. This is the only part of the solution which is not carried out analytically, whence our calling it "quasi-analytical." Now the problem is completely solved.

Appendix 2: Solution of the state equations

In order to shed some light on the solution of the state equations, we carry it out completely for the case of the interval $[0, t_1]$. First, we solve the differential equation for $x_1(t)$:

$$\frac{dx_1}{dt} = -\frac{k_1 x_1}{K_{m1} + x_1} \Rightarrow \left(\frac{K_{m1}}{x_1} + 1\right) dx_1 = -k_1 dt \tag{55}$$

Integrating:

$$K_{m1} \ln x_1 + x_1 = -k_1 t + C \tag{56}$$

Imposing the initial condition $x_1(0) = 1$, we get $C = 1$, so that

$$K_{m1} \ln x_1 + x_1 = -k_1 t + 1 \Rightarrow \ln x_1 + \frac{x_1}{K_{m1}} = -\frac{k_1}{K_{m1}} t + \frac{1}{K_{m1}} \tag{57}$$

By exponentiation:

$$e^{\ln x_1 + \frac{x_1}{K_{m1}}} = e^{\frac{1}{K_{m1}} - \frac{k_1}{K_{m1}} t} \Rightarrow x_1 e^{\frac{x_1}{K_{m1}}} = e^{\frac{1}{K_{m1}} - \frac{k_1}{K_{m1}} t} \tag{58}$$

Dividing by K_{m1}:

$$\frac{x_1}{K_{m1}} e^{\frac{x_1}{K_{m1}}} = \frac{1}{K_{m1}} e^{\frac{1}{K_{m1}} - \frac{k_1}{K_{m1}} t} \tag{59}$$

And from the definition of the Lambert W-function

$$x = W(x) e^{W(x)} \tag{60}$$

we get:

$$x_1(t) = K_{m1} W\left(\frac{1}{K_{m1}} e^{\frac{1}{K_{m1}}} e^{-\frac{k1}{K_{m1}} t}\right) \tag{61}$$

In order to obtain the closed form expression for $x_2(t)$, instead of integrating

$$\dot{x}_2 = \frac{k_1 x_1}{K_{m1} + x_1}; \quad x_2(0) = 0 \tag{62}$$

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it is much easier to realize that

$$\dot{x}_1 + \dot{x}_2 = 0 \tag{63}$$

from which follows, immediately, that

$$x_1(t) + x_2(t) = c \Rightarrow x_1(0) + x_2(0) = c \Rightarrow c = 1 \tag{64}$$

so that we get the recurrence relation:

$$x_1(t) + x_2(t) = 1 \Rightarrow x_2(t) = 1 - x_1(t) \tag{65}$$

And one proceeds similarly for the remaining intervals.

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