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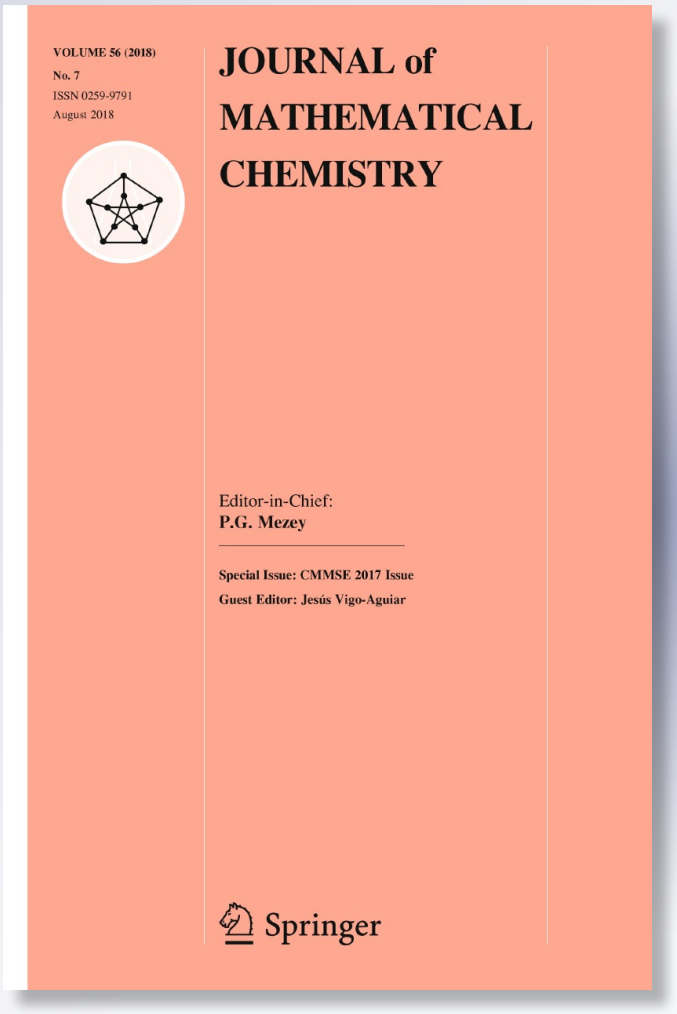

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ORIGINAL PAPER

CMMSE-17: general analytical laws for metabolic pathways

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Abstract In this paper a general formulation for the kinetics of multi-step enzymatic reactions is presented. The optimal enzyme and metabolite concentrations are studied for the problem of minimizing the operation time in which the substrate is converted into the product. We give an analytic solution for three different kinetic models for both the unbranched and branched cases. Sufficient conditions for the optimality of the solution are studied. Several examples are presented.

Keywords Optimal control · Kinetic models · Analytical laws

Mathematics Subject Classification 49J30 · 49M05 · 80A30

1 Introduction

The kinetics of multi-step enzymatic reactions is an ongoing research topic and, in it, the minimization of the operation time in which the substrate is transformed into the product is one of the classical problems which is being currently studied. In it, one measures the optimal profiles of both the enzyme and the metabolite. Our aim in this work is to obtain a general analytic solution for this problem. This way, we avoid the unwieldy numerical solutions, which are always tainted by the specific traits of each particular problem.

This is one of several papers published together in Journal of Mathematical Chemistry on the "Special Issue: CMMSE 2017".

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In the last years, several results have been presented on the topic. In many of them, an unbranched reaction chain of n irreversible reaction steps is studied (e.g. [1], where an explicit solution for the simplest case, $n = 2$ with linear kinetic model, is given). A mathematical model of an unbranched reaction chain with $n = 3$ and obeying the Michaelis–Menten (MM) kinetic model is used in [2]. In [3], a quasi-analytic solution is found for $n = 3$ and linear kinetics. In [4], the general case of n steps with MM kinetic model is analyzed and quantitative properties are presented, although the authors do not give an explicit analytical solution. In [5], we use a linear kinetic model for the solution of the general case of n steps. Later, in [6], we improve our results with a quasi-analytical solution for the n-steps MM model using the Lambert W-function.

Branched pathways have also been studied, certainly, but only specific cases. In [7], a network inspired in the glycolysis, with the MM model, is considered. The same example is revisited in [8] and a new pathway with two outputs is presented. Other objective functionals may also be considered, like maximizing the productivity of the metabolite [9] or the flux of a particular metabolite [10]. However, in this work we shall minimize the operation time to obtain a specified concentration of the final product.

The numerical methods for the solution of our dynamic optimization problem are usually classified into two groups: direct and indirect methods. Direct methods include complete parametrization [11], multiple shooting [12] or control vector parametrization [13]. In all of them, the basic idea is to transform the original problem into a non-linear programming problem by discretizing and approximating the control and the state variables.

On the other hand, the indirect methods solve the optimization problem using Pontryagin's Minimum Principle (PMP) taking into account the necessary optimality conditions. In this paper, the problem is stated as an Optimal Control Problem (OCP) and using PMP [14] we obtain the solution. Even more (and this is unusual in the literature), we shall also study the sufficient conditions to obtain an optimum. We also remark that we allow the possibility of using three different kinetic models in the same example. Finally, we consider not only an unbranched metabolic pathway but also a branched scheme. We obtain general, model-independent laws, for the first time.

The paper is organized as follows. In Sect. 2 we present the statement of the problem. The general laws of the optimal solution are obtained in Sect. 3, and a new kinetic model, the power law, is also presented. Section 4 contains a study on the verification of the sufficient conditions. Then we generalize the problem to the branched case with a statement valid for any graph satisfying some specific conditions. In Sect. 6 we present numerical examples for a well-known test-case. Finally, we end with a summary of the main conclusions.

2 Statement of the problem

For the sake of simplicity we start with the simplest case of an unbranched metabolic pathway composed of n irreversible reaction steps converting substrate x_1 into product p. The value $x_1(t)$ is the substrate concentration at time t, $p(t)$ the concentration of the final product, $x_i(t)$, $(i = 2, \ldots, n)$ the concentration of the intermediate compounds, and $u_i(t)$ $(i = 1, \ldots, n)$ the concentration of the enzyme catalyzing the ith reaction (see Fig. 1).

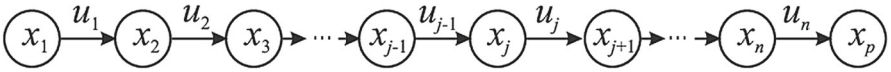

Fig. 1 Unbranched scheme

Once we have fully studied this case, we shall perform the generalization to the branched one. The rate of the ith reaction, $v_i(x_i(t), u_i(t))$ is linear in the enzyme concentrations, u_i:

$$v_i(x_i(t), u_i(t)) = w_i(x_i(t)) \cdot u_i(t) \tag{1}$$

The following are frequently used kinetic models:

$$\begin{aligned} w_i(x_i) &= k_i x_i && \text{(Mass action)} \\ w_i(x_i) &= \frac{k_i x_i}{K_i + x_i} && \text{(Michaelis-Menten)} \\ w_i(x_i) &= k_i x_i^c && \text{(Power law)} \end{aligned} \tag{2}$$

The dynamical model for the pathway is given by the law of conservation of mass:

$$\dot{x}_i(t) = v_{i-1}(x_{i-1}(t), u_{i-1}(t)) - v_i(x_i(t), u_i(t)); \ (i = 1, \ldots, n). \tag{3}$$

The objective is to transform x_1 into p as fast as possible; we denote t_f the final time. We assume an exhaustible initial substrate, x_1, and imposing $p(t_f) = C_f$ $(0 < C_f < 1)$, we obtain:

$$x_1(t_f) + x_2(t_f) + \cdots + x_n(t_f) = 1 - C_f \tag{4}$$

so that the optimization problem may thus be stated as the control problem (Pr):

$$\begin{aligned} &\text{(Pr)}: && \tau_{C_f} = \min_{u_1, \ldots u_n} \int_0^{t_f} dt = \min_{u_1, \ldots u_n} t_f \\ &\text{subject to:} && (3), (4) \\ & && u_1 \geq 0, \ldots u_n \geq 0; \ u_1 + \cdots + u_n \leq 1 \end{aligned} \tag{5}$$

3 Optimal solution

In two previous papers, we used PMP to obtain the solution to (Pr) for the mass action model [5] and for the MM model [6]. When the control appears linearly, as is the case for the problem under consideration, the control switches between its upper and lower bounds at discrete instants: the optimal control is said to be a *bang-bang type control* and those instants are called the *switching times*. The general form of the solution can be described as follows: there exist n switching times, as many as enzymes, so that the optimal i-enzyme profile is proved to be of bang-band type and satisfies:

$$u_i(t) = \begin{cases} 1 \text{ for } t \in [t_{i-1}, t_i) \\ 0 \text{ for } t \notin [t_{i-1}, t_i) \end{cases}; \ i = 1, \ldots, n \tag{6}$$

where $\{t_0, t_1, t_2, ..., t_n\}$ are the switching times, with $t_0 = 0$ and $t_n = t_f$.

We shall denote by $x_{ji}(t)$ (for $i, j = 1, \ldots, n$) the optimal jth metabolite concentration, in the ith interval $[t_{i-1}, t_i]$. The optimal solution of the complete system can be described on each interval, knowing that on the ith interval, $[t_{i-1}, t_i]$ (for $i = 2, \ldots, n-1$), there are 4 laws governing the metabolite concentrations:

(a) Metabolites before the ith remain at a constant value given by:

$$x_{ji}(t) = x_{jj}(t_j) \text{ for } j = 1, \ldots, i-1 \tag{7}$$

(b) The ith metabolite follows a law given by a function depending on: the parameters of the model, the previous switching time, and the value of the ith metabolite on the previous interval:

$$x_{ii}(t) = f(x_{ii-1}(t_{i-1}), t_{i-1}, t) \tag{8}$$

(c) The $i+1$-th metabolite follows a law obtained from the one of the previous metabolite as follows:

$$x_{i+1i}(t) = x_{ii-1}(t_{i-1}) - x_{ii}(t) \tag{9}$$

(d) Metabolites from the $i+2$-th on have not been activated yet, so that their value is zero:

$$x_{ji}(t) = 0 \text{ for } j = i+2, \ldots, n \tag{10}$$

A schematic idea is shown in Fig. 2.

On the first interval ($i = 1$), letting $x_{10}(t_0) = 1$, only Laws (b), (c) and (d) apply; on the last-but-one ($i = n-1$), only Laws (a), (b) and (c) apply; whereas on the last one ($i = n$), only Laws (a) and (b).

Notice that the formulas above are general and they only depend on the kinetic model [Law (b)]. In [5] we obtained the law for the mass action model (i.e. the linear one), getting:

$$x_{ii}(t) = f(x_{ii-1}(t_{i-1}), t_{i-1}, t) = x_{ii-1}(t_{i-1}) \exp(-k_i(t - t_{i-1})) \tag{11}$$

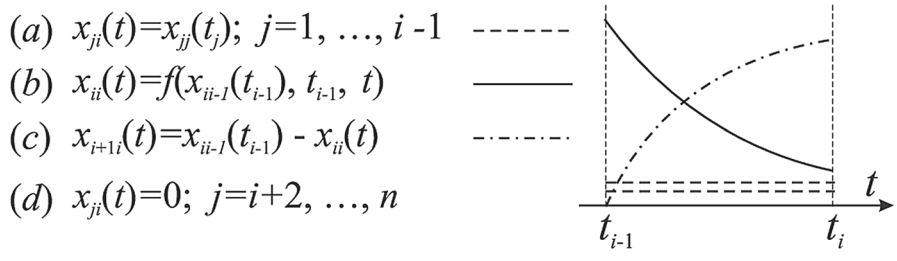

Fig. 2 Optimal concentration laws for the metabolites

Elsewhere, in [6], we obtained the law for the Michaelis–Menten model, which gives:

$$\begin{aligned} x_{ii}(t) &= f(x_{ii-1}(t_{i-1}), t_{i-1}, t) \\ &= K_{mi} W\left(\frac{x_{ii-1}(t_{i-1})}{K_{mi}} \exp\left(\frac{x_{ii-1}(t_{i-1})}{K_{mi}}\right) \exp\left(-\frac{k_i}{K_{mi}}(t - t_{i-1})\right)\right) \end{aligned} \tag{12}$$

where W is the Lambert W-function.

We present, for the first time, in this paper, the expression of Law (b) for the kinetic model given by the power law. The expression is now:

$$\begin{aligned} x_{ii}(t) &= f(x_{ii-1}(t_{i-1}), t_{i-1}, t) \\ &= \left[(x_{ii-1}(t_{i-1}))^{1-c} - (1-c)k_i(t - t_{i-1})\right]^{(1-c)^{-1}} \end{aligned} \tag{13}$$

The method for computing this optimal solution is analogous to the one we shown in our previous papers, and we refer the reader to them (mainly "Appendix 1" in [6], where it is given in detail).

The idea is to define the Hamiltonian $H(x_1, \ldots, x_n, u_1, \ldots, u_n, \lambda_1 \ldots, \lambda_n, t)$ associated to the problem (Pr):

$$\begin{aligned} H &= 1 + \sum_{i=1}^{n} \lambda_i \dot{x}_i(t) \\ &= 1 + \sum_{i=1}^{n} \lambda_i \left[w_{i-1}(x_{i-1}(t)) \cdot u_{i-1}(t) - w_i(x_i(t)) \cdot u_i(t)\right] \end{aligned} \tag{14}$$

and compute the optimum values for x_i and u_i applying the necessary conditions in PMP on that Hamiltonian. In this case:

$$\begin{aligned} &(i) \quad \dot{\lambda}_i(t) = -\frac{\partial H}{\partial x_i}; \lambda_i(t_f) = 0 \\ &(ii) \quad \min_{u_1, \ldots u_n} H \\ &(iii) \quad \dot{x}_i(t) = w_{i-1}(x_{i-1}(t)) \cdot u_{i-1}(t) - w_i(x_i(t)) \cdot u_i(t); x_i(0) = x_{i0}, \\ &(iv) \quad H(x_1, \ldots, x_n, u_1, \ldots, u_n, , \lambda_1 \ldots, \lambda_n, t_f) = 0 \end{aligned} \tag{15}$$

where $i = 1, \ldots, n$. Our system is autonomous, so that $H_t \equiv 0 \Rightarrow H(t) = cte$. This condition together with (iv) implies that $H(t) = 0$.

We obtain the optimal solution constructively by intervals, starting at $t = 0$ and concatenating the results. Once these values are computed, one still needs to calculate the switching times $t_1, t_2, \ldots, t_{n-1}$ and the operation time t_f. To this end, we use the restriction (4) and define the augmented functional:

$$L(t_1, t_2, \ldots, t_{n-1}, t_f, \beta) = t_f + \beta(x_{1n}(t_f) + x_{2n}(t_f) + \cdots + x_{nn}(t_f) - C_f) \tag{16}$$

where the values of the concentrations $x_{1n}(t_f) = x_{1n}(t_1)$, $x_{2n}(t_f) = x_{2n}(t_2), \ldots,$ $x_{nn}(t_f)$ are given, and where the unknowns $t_1, t_2, \ldots, t_{n-1}$ and t_f appear. Then we

solve the non-linear system:

$$\frac{\partial L}{\partial t_1} = 0; \frac{\partial L}{\partial t_2} = 0; \ldots; \frac{\partial L}{\partial t_{n-1}} = 0; \frac{\partial L}{\partial t_f} = 0; \frac{\partial L}{\partial \beta} = 0 \tag{17}$$

Once the optimal values of the switching times $t_1, t_2, \ldots, t_{n-1}$ and t_f are obtained numerically, the remaining values of the solution are immediately obtained analytically using the closed-form formulas (a)–(d), and the problem is completely solved.

Notice that, remarkably, the laws given above allow the simultaneous consideration of several models when studying the pathway: one just needs to use the appropriate function $f(x_{ii-1}(t_{i-1}), t_{i-1}, t)$ in Law (b).

4 Sufficient conditions

Considering the optimal control problem, with $x(t)$ and $u(t)$ denoting n -dimensional vectors:

$$\min_{u(t)} J = \int_0^T F(x(t), u(t), t)dt + B[T, x(T)] \tag{18}$$

$$\dot{x}(t) = f(x(t), u(t), t); \; x(0) = x_0 \tag{19}$$

$$u(t) \in U(t), \; 0 \leq t \leq T \tag{20}$$

we can guarantee the sufficient conditions for the existence of an optimal solution using Arrow's Theorem [14] The optimal solution found in the previous section is based on the use of the initial conditions imposed by PMP. When certain convexity conditions are satisfied, then the conditions stipulated by the PMP are also sufficient for minimization. Traditionally, the most frequently used sufficiency results are Mangasarian's and Arrow's Theorems [14]. We are going to study the sufficiency of the conditions using Arrow's result (this sufficiency is not considered in the previous works [4–6]).

Arrow's Theorem 1 *Let $u^*(t), x^*(t), \lambda^*(t)$ the solutions obtained upon applying PMP $\forall t \in [0, T]$. Let:*

$$H^0(x, \lambda, t) = \min_{u \in U(t)} H(x, u, \lambda, t) \tag{21}$$

be the Derived Hamiltonian. If the function $H^0(x, \lambda^*, t)$ is convex on x for each $t \in [0, T]$ and B is convex in x, then u^* is the optimal control of the problem and x^* is the optimal state trajectory. Moreover, λ^* the optimal trajectory of the adjoint variables.

In our case:

$$\begin{aligned}&\min_{u(t)} J = \min_{u_1,\ldots u_n} \int_0^{t_f} dt\\ &\dot{x}_i(t) = w_{i-1}(x_{i-1}(t)) \cdot u_{i-1}(t) - w_i(x_i(t)) \cdot u_i(t),\ i = 1, \ldots, n\\ &u_1 \geq 0, \ldots, u_n \geq 0;\ u_1 + \cdots + u_n \leq 1\end{aligned} \tag{22}$$

Upon minimizing $H(x, u, \lambda, t)$ in $u \in U(t)$ one obtains a function $u = u^0(x, \lambda, t)$ from which $H^0(x, u^0(x, \lambda, t), \lambda, t)$ can be computed. In each case, the equations of the model (Mass action, Michaelis–Menten and Power law) provide the sufficient conditions in one way or another. From the bang-bang nature of the solution given by PMP, we know that

$$u_1^0(t) = c_1, \ldots, u_n^0(t) = c_n \Rightarrow u^0(x, \lambda, t) = const \tag{23}$$

The results for each of the considered models follow. Let

$$H^0(x, \lambda, t) = H^0\left(x_1, \ldots, x_n, u_1^0, \ldots, u_n^0, \lambda_1 \ldots, \lambda_n, t\right) \tag{24}$$

Mass action The Derived Hamiltonian is:

$$\begin{aligned}&H^0(x, \lambda, t)\\ &= 1 + \lambda_1\left(-u_1^0 x_1\right) + \lambda_2\left(u_1^0 x_1 - u_2^0 x_2\right) + \cdots + \lambda_n\left(u_{n-1}^0 x_{n-1} - u_n^0 x_n\right)\\ &= 1 + \lambda_1(-c_1 x_1) + \lambda_2(c_1 x_1 - c_2 x_2) + \cdots + \lambda_n(c_{n-1} x_{n-1} - c_n x_n)\\ &= 1 + (-c_1\lambda_1 + c_1\lambda_2)x_1 + (-c_2\lambda_2 + c_2\lambda_3)x_2 + \cdots + (-c_n\lambda_n)x_n\end{aligned} \tag{25}$$

which is linear in x and so, convex.

Michaelis–Menten In this case:

$$\begin{aligned}H^0(x, \lambda, t) = 1 + \lambda_1\left(-c_1\frac{k_1 x_1}{K_{m1} + x_1}\right) + \lambda_2\left(c_1\frac{k_1 x_1}{K_{m1} + x_1} - c_2\frac{k_2 x_2}{K_{m2} + x_2}\right)\\ + \cdots + \lambda_n\left(\frac{k_{n-1} x_{n-1}}{K_{mn-1} + x_{n-1}} c_{n-1} - \frac{k_n x_n}{K_{mn} + x_n} c_n\right)\end{aligned} \tag{26}$$

Whose Hessian matrix

$$\mathbb{H} = \begin{pmatrix} \frac{\partial^2 H^0}{\partial x_1^2} & \cdots & \frac{\partial^2 H^0}{\partial x_1 \partial x_n} \\ \vdots & & \vdots \\ \frac{\partial^2 H^0}{\partial x_n \partial x_1} & \cdots & \frac{\partial^2 H^0}{\partial x_n^2} \end{pmatrix} = \begin{pmatrix} \frac{2(\lambda_1 - \lambda_2)c_1 k_1 k_{m1}}{(k_{m1} + x_1)^3} & 0 & \cdots & 0 \\ 0 & \ddots & & \vdots \\ \vdots & & \ddots & \\ 0 & \cdots & & \frac{2\lambda_n c_n k_n k_{mn}}{(k_{mn} + x_n)^3} \end{pmatrix} \tag{27}$$

happens to be diagonal, whence

$$x^T \mathbb{H} x = \frac{2(\lambda_1 - \lambda_2)c_1 k_1 k_{m1}}{(k_{m1} + x_1)^3} x_1^2 + \frac{2(\lambda_2 - \lambda_3)c_2 k_2 k_{m2}}{(k_{m2} + x_2)^3} x_2^2 + \cdots + \frac{2\lambda_n c_n k_n k_{mn}}{(k_{mn} + x_n)^3} x_n^2 \geq 0 \tag{28}$$

In [6] one can see that:

$$\lambda_1 \geq \lambda_2 \geq \lambda_3 \geq \lambda_n \geq 0 \tag{29}$$

which implies that $\mathbb{H}$ is positive semidefinite. Hence, $H^0(x, \lambda, t)$ is convex in x for all $t \in [t_0, t_f]$.

Power law For this model:

$$\begin{aligned} H^0(x, \lambda, t) = 1 &+ \lambda_1 \left(-u_1^0 x_1^c k_1\right) + \lambda_2 \left(u_1^0 x_1^c k_1 - u_2^0 x_2^c k_2\right) \\ &+ \cdots + \lambda_n \left(u_{n-1}^0 x_{n-1}^c k_{n-1} - u_n^0 x_n^c k_n\right) \end{aligned} \tag{30}$$

$$\mathbb{H} = \begin{pmatrix} (\lambda_1 - \lambda_2)c_1 k_1 c(1-c)x_1^{c-2} & 0 & \cdots & 0 \\ 0 & \ddots & & \vdots \\ \vdots & & \ddots & \\ 0 & \cdots & & \lambda_n c_n k_n c(1-c)x_n^{c-2} \end{pmatrix} \tag{31}$$

One has:

$$x^T \mathbb{H} x = (\lambda_1 - \lambda_2)c_1 k_1 c(1-c)x_1^c + (\lambda_2 - \lambda_3)c_2 k_2 c(1-c)x_2^c + \cdots + \lambda_n c_n k_n c(1-c)x_n^c. \tag{32}$$

In order to guarantee that $\mathbb{H}$ is positive semidefinite and $H^0(x, \lambda, t)$ is convex in x, we must impose $c \in [0, 1]$.

5 Generalization

After having solved the general case of the unbranched pathway, we present, in this section, the case of branched pathways. Non-linear pathways give rise to the following two sets for each node:

1. For the ith metabolite, the set Ω_i represents those produced from it (Fig. 3a).
2. The set Θ_i represents those metabolites which produce the ith one (Fig. 3b).

The chemical reactions we cover can be specified using the graph topology (see [15] for a general reference on Graph Theory). Let G be the directed graph describing the reaction, with vertices $\{x_1, \ldots, x_n\}$ and edges $\{e_1, \ldots, e_r\}$. The required property is:

Given two vertices x_i, x_j there is a directed path from i to j if and only if $i < j$.

Notice that in our graphs, an edge $e = (x_k, x_l)$ exists when reaction l happens after reaction l without intermediate products (in a temporal, *not chemical*, sense). Thus, the topology of our graphs is given by the *temporal dependence* of the reactions, not necessarily by the *chemical dependence*.

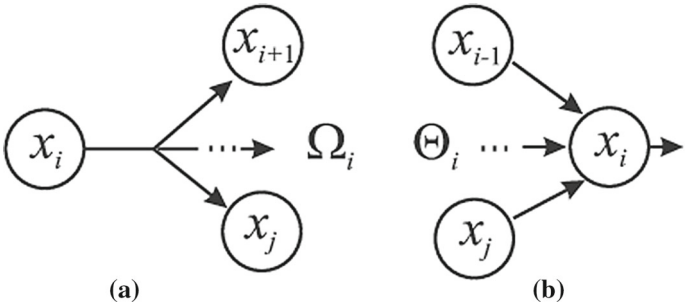

Fig. 3 Branched pathways

These graphs represent the fact that the synthesis of one enzyme requires the degradation of *all the previous ones*; this is the case, for example, of the glycolysis, see [8]. Following that paper, we assume that the optimal profile follows a pattern matching the topology of the pathway, reflecting the fact that the enzymes are activated sequentially. Notice that the type of graphs we deal with have a strictly upper-triangular adjacency matrix (and more conditions, but this one is easy to verify). The enzyme profiles show that for the synthesis of one enzyme the degradation of the previous is needed. So, the optimal profiles follow a pattern that matches with the pathway topology, In this way the sequential activation of the enzymes is reflected.
With the same notation as above, the optimal solution of these branched systems can be described on the ith interval $[t_{i-1}, t_i]$ ($i = 2, \ldots, n-1$) with these 4 laws:

– (a′) For the metabolites before the $i-$th one:

$$x_{ji}(t) = x_{jj}(t_j) \quad \text{for } j = 1, \ldots, i-1 \tag{33}$$

– (b′) For the ith metabolite:

$$x_{ii}(t) = f(x_{ii-1}(t_{i-1}), t_{i-1}, t) \tag{34}$$

– (c′) All the metabolites $j \in \Omega_i$ follow the same law:

$$x_{ji}(t) = x_{ji-1}(t_{i-1}) + x_{ii-1}(t_{i-1}) - x_{ii}(t) \tag{35}$$

– (d′) The metabolites jth such that $i < \min \Theta_j$, have not been activated yet:

$$x_{ji}(t) = 0 \tag{36}$$

The only differences with the unbranched system appear in (c′) and (d′).

6 Numerical examples

We provide several simulations which illustrate the general formulations above. We shall use a test example already studied by several authors and inspired by the upper

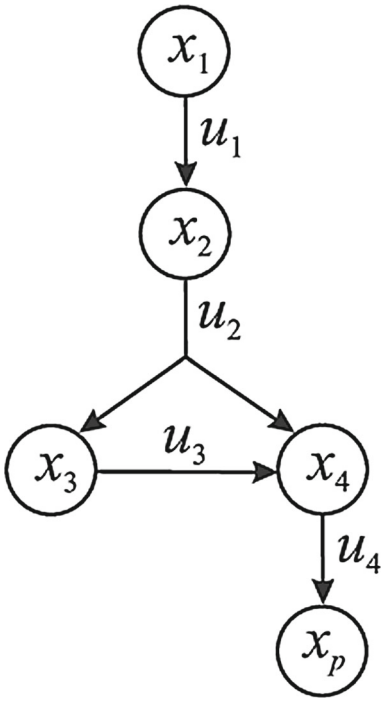

Fig. 4 Glycolysis inspired network

part of glycolysis. The original problem was stated by Bartl et al. [7] and was also considered in [8] with a new formulation incorporating the enzyme dynamics.
The pathway (Fig. 4) consists of four enzymatic reactions with one branch. Recall that x_1 corresponds to the substrate, x_2, x_3 and x_4 are the intermediate metabolites and x_p represents the product. The alternative route of the glycolysis is represented by u_2 (corresponding to the enzyme aldolase) metabolizing the intermediate x_2 to x_3 and x_4.

The single aim is to minimize the time needed to transform the substrate x_1 into a fixed amount (90%) of product x_p, (i.e. $C_f = 0.9$). We assume unbuffered or exhaustible substrate x_1 (i.e. the substrate is consumed during the process) and enzymes are assumed to become activated instantaneously (just-in-time activation). The following initial conditions at $t = 0$: $x_0 = [1, 0, 0, 0, 0]^{\mathrm{T}}$; $u_0 = [0, 0, 0, 0]^{\mathrm{T}}$ are imposed. Metabolites and enzymes are expressed in concentration units and time in seconds.

Additionally, restrictions on enzyme concentrations and their total amount are introduced in a normalized form as:

$$u_1 \geq 0, \ldots u_n \geq 0; \; u_1 + \cdots + u_n \leq 1 \tag{37}$$

This is in agreement with the assumption that the cell can only allocate a certain amount of protein to a pathway, and with experimental observations [16] in *Escherichia coli*.

The classical theoretical studies ([7,8]) are based on the Michaelis–Menten kinetics:

$$w_i(x_i) = \frac{k_i x_i}{K_i + x_i} \tag{38}$$

with unity rate constants for k_i and K_i: $k_i = 1(s^{-1})$, $K_i = 1(mM)$. However, we also give results for the other two laws we have studied: mass action and the power law. The case studies are based on the values obtained by a least-squares fit, taking the Michaelis–Menten model as the exact one. The parameters we obtain for the other models are:

$$w_i(x_i) = k_i x_i = 0.578258x \tag{39}$$

$$w_i(x_i) = k_i x_i^c = 0.516434x^{0.674676} \tag{40}$$

The fit (see Fig. 5) is, naturally, much better for the power law (which has two parameters) than for the linear mass-action model (which has only one). The optimal solution for the switching times t_i is given in Table 1, for $i = 1, \ldots, 4$. The total operation times $\tau_{C_f} = t_4 = t_f$ are written in boldface. These values are upon solving the nonlinear system (17) using Mathematica®. The solutions obtained using MM and the Power law are quite similar, due to the good fit of the latter to the former. This contrasts with the mass action model. The CPU running time on a budget computer (Intel Core 2/2.66 GHz) is 0.016 for the Mass action model, 0.156 for MM and 0.031 for the power law.

Recall that our method finds the solution in analytic form and in the case of the MM model, this implies the use of the Lambert W-function. Its symbolic use is what consumes most of the CPU time (up to 5 and 10 times the time of the other models).

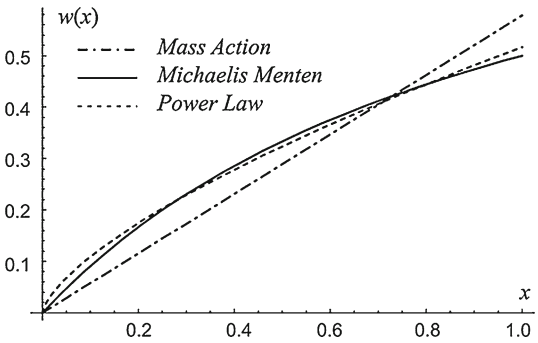

Fig. 5 Three different laws

Table 1 Switching times and operation time of the optimal solution

Model	t_1	t_2	t_3	t_f
Mass action	6.31172	12.6234	17.7365	24.0482
Michaelis–Menten	4.59187	9.18407	12.5971	17.1774
Power law	4.05568	8.13458	11.041	15.1464

The Mass action model is not a good choice due to its poor approximation but the power law model combines both a good fit and good running time. Moreover, this model can be considered an approximation of the Hill model:

$$w_i(x_i) = \frac{k_i x_i^n}{K_i + x_i^n} \tag{41}$$

Hill kinetics exhibit sigmoidal behaviour, leading to a switch-like behaviour for sufficiently high n. It is the typical model of some enzymes exhibiting a phenomenon known as cooperativity. Recall that the example we have considered contains just 4 enzymes. The effect would be much more remarkable for large n.

Figure 6 shows the optimal solution obtained for the enzyme concentration in the MM model. The solution of the optimal control problem is of bang-bang type and all the u_i are 1 on all the intervals where they are active. In our case, in which we minimize the operation time, the activation intervals of the enzymes are consecutive. This is not so when other functionals are considered (as, in [8], where they use the cost of the enzymes). Figure 7 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 MM model. We remark the consequence of the ramification of the pathway. During interval $[]t_1, t_2]$ enzyme u_2 is activated, producing simultaneously both x_3 and x_4. In Fig. 7 we have slightly moved their plots to highlight them (they are equal). Immediately later, enzyme u_3 is activated, which also produces x_4, whose increase is visible in the plot.

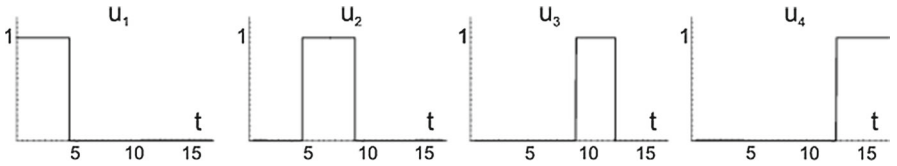

Fig. 6 Optimal enzyme profile

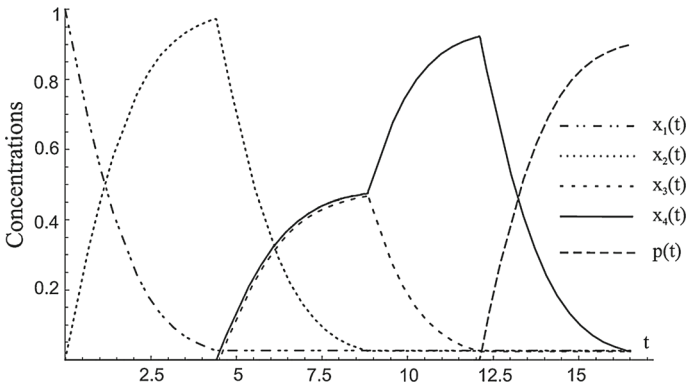

Fig. 7 Profiles of metabolite and product

7 Conclusions

We have developed in this paper the general laws governing the optimization of metabolic pathways. Our method finds an analytic solution, without recourse to numerical approximations. We study three different models: Mass action, Michaelis–Menten and, one specific contribution of this paper, the Power Law. The results we obtain highlight the importance of the analytic formulas that we provide for the Power Law, as it is an easy to implement model and which efficiently approximates complex models like Michaelis–Menten or Hill's. Despite the simplicity of the test case, we can already notice how our general laws permit the solution of network topologies allowing sequential orderings, including ramifications.

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