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OPTIMAL METABOLIC REGULATION BY TEMPORAL VARIATION OF ENZYME ACTIVITIES: A CONTROL THEORETIC APPROACH

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Abstract: In this paper we use an optimal control approach to analyze time dependent enzyme concentrations that minimize the transition time of a metabolic pathway while respecting the natural constraints imposed by a limited biosynthetical capacity. Our main result states that, under appropriate assumptions, at each time instant all the available enzyme production capacity is *allocated to a single reaction*, a finding reminiscent of bang-bang control laws typical in classical time-optimal control.

Keywords: Metabolic pathways, Metabolic regulation, Optimal control.

1. INTRODUCTION

Metabolic pathways consist of networks of biochemical reactions which are regulated by a set of enzymes catalyzing each interaction (Heinrich and Schuster 1996). The overall behavior of such metabolic networks depends substantially on each of the enzymes involved in the process.

In this paper we address the mechanism responsible for the distribution of the enzyme concentrations in a metabolic network. This approach relies on the assumption that evolution has adapted this mechanism to satisfy certain optimality criteria. Previous studies have tackled this problem by considering a number of objective functions, e.g., flux optimization (Heinrich *et al.* 1991, Heinrich and Klipp 1996, Holzhütter 2004), minimization of total enzyme concentration (Klipp and Heinrich 1999) and maximization of growth rate (Bilu *et al.* 2006). All these works focus on the steady state properties of the pathways and consider time independent enzyme concentrations. However, recent experiments have revealed well defined hierarchical temporal patterns in enzyme expression levels in amino acid (Zaslaver *et al.* 2004) and flagella biosynthesis of *E. coli* (Kalir and Alon 2004), which suggest that the temporal distribution of the enzyme concentrations may have an important impact on the behavior of some metabolic networks.

Klipp and co-workers considered the problem of optimizing time varying enzyme concentrations in an insightful paper (Klipp *et al.* 2002). Interestingly, they concluded that the enzyme profiles that minimize the transition time of the pathway obey a clear hierarchical pattern: each enzyme is expressed at the maximum possible level in the same sequence as they appear in the pathway. A valuable complement to these results can be found in (Zaslaver *et al.* 2004), in which the authors de-

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rive enzyme profiles which optimize an objective function accounting for the total cost of enzyme production and the time taken by the pathway in reaching its presumed goal. A key feature of the latter work is that the results are supported by experimental data that validates this sequential or *just-in-time* behavior. These findings establish a one-to-one temporal relationship between the sequence of reaction steps in the pathway and the temporal sequence in which the enzymes are expressed.

An important limitation of the approach taken in (Klipp *et al.* 2002) is that the optimization is done by discretizing the time scale and assuming that the enzyme profiles are piecewise constant functions. This *ad-hoc* method leaves open the question of whether optimization over more general classes of temporal profiles might lead to different conclusions.

This paper complements the investigations of (Klipp *et al.* 2002) and (Zaslaver *et al.* 2004). We aim to gain insight into the time dependent enzyme concentrations which minimize the transition time in a metabolic pathway. The motivation for this work is the observation that the problem dealt with in (Klipp *et al.* 2002) can be naturally posed and solved in terms of classical optimal control theory (Pontryagin *et al.* 1962). It is in this sense that this work intends to be a rigorous extension of (Klipp *et al.* 2002). Furthermore, by posing the problem in a standard control theoretic framework, a direct link between the problem under consideration and traditional concepts and abstractions pertaining to control theory is made evident. In this context, the enzyme profiles correspond to the *control inputs* that drive the *state* of the system, i.e. metabolite concentrations, from a certain initial condition to a final state. The problem then consists of determining the optimal control inputs that steer the state to the desired final state while ensuring that the transition time is kept minimal and the input constraints are satisfied.

The main theoretical tool used throughout this paper is Pontryagin's Minimum Principle (PMP) (Pontryagin *et al.* 1962). This result will be used to derive generally applicable qualitative conclusions about the optimal enzyme profiles by using simple geometric arguments, as well as to derive optimal solutions to particular problem statements. We deal with the case of metabolic pathways whose objective is to convert a single initial substrate into a single final product. Our main result is that, if the optimal enzyme concentrations are uniquely determined, then they are switching sequences between zero and the maximum available enzyme concentration, which gives a strong control theoretic support for the *ad-hoc* procedure of (Klipp *et al.* 2002). This result is derived with no assumptions on the stoichiometry of the pathway, and only requires the kinetic laws to be linear in the enzyme concentrations. It also implies that for any time instant, all the available enzyme is allocated to a single reaction and so provides further evidence of the link between the activation sequence and the specific topology of the network.

We emphasize that our approach can encompass a wide range of situations and, since the application of the PMP is fairly general, it can ultimately lead to a systematic procedure for finding the exact form of the optimal enzyme concentrations.

2. PONTRYAGIN'S MINIMUM PRINCIPLE

In this section, we briefly state the main results of optimal control theory developed by Pontryagin and co-workers (Pontryagin *et al.* 1962). This will provide the basic concepts, notation and results to be used in the remainder of this paper.

We are interested in dynamical systems of the form

$$\dot{x}(t) = f\left(x(t), u(t)\right), \tag{1}$$

$$x(0) = x_0, \tag{2}$$

where the dot denotes the time derivative, $x(t) \in \mathbb{R}^n$ is the state vector, $u(t) \in \mathbb{R}^m$ is the control input vector, $f\left(x(t), u(t)\right)$ is a continuously differentiable function and $x_0 \in \mathbb{R}^n$ is the initial condition of the system. Suppose that the control objective is to drive the state $x(t)$ from x_0 to a final state $x(t_f)$ that must lie in a certain set $\mathcal{S}$. This allows us to tackle problems in which the final condition is not fully specified. Since in this framework the final time t_f is not specified *a priori*, this is referred to as a *free final time* problem.

The magnitude of the control input is usually bounded by some extremal values arising from the nature of the problem itself. If we denote the set of admissible values for $u(t)$ as $\mathcal{U} \subseteq \mathbb{R}^m$, then it is required that $u(t) \in \mathcal{U}$, $\forall t \in [0, t_f]$. The objective is then to determine an optimal control input, $u^*(t)$, such that

$$u^*(t) = \arg \min_{u(t) \in \mathcal{U}} \mathcal{J} \quad , \forall t \in [0,\, t_f] \tag{3}$$

where

$$\mathcal{J} = h\left(x(t_f)\right) + \int_0^{t_f} g\left(x(t), u(t)\right) dt, \tag{4}$$

is the target cost functional.

This problem is difficult and in general every case must be treated on an individual basis. However, a useful result due to Pontryagin and his co-workers (Pontryagin *et al.* 1962) provides a set

of necessary conditions for optimality, i.e. *if* an optimal solution exists, then it must satisfy the conditions given by the minimum principle. For that purpose we define a scalar valued function, called the *Hamiltonian*, as

$$\mathcal{H}\left(x(t), u(t), p(t)\right) = g\left(x(t), u(t)\right) + p(t)^T f\left(x(t), u(t)\right), \quad (5)$$

where the vector $p(t) \in \mathbb{R}^n$ is called the system's *co-state*. The PMP states that, if an optimal $u^*(t)$ exists for the problem of interest, then there exists a nontrivial co-state trajectory $p^*(t)$ such that:

(a) The set of differential equations

$$\dot{x}^*(t) = \frac{\partial \mathcal{H}\left(x^*(t), u^*(t), p^*(t)\right)}{\partial p}, \quad (6)$$

$$\dot{p}^*(t) = -\frac{\partial \mathcal{H}\left(x^*(t), u^*(t), p^*(t)\right)}{\partial x}, \quad (7)$$

is satisfied by the state and co-state trajectories subject to the boundary conditions $x^*(0) = x_0$ and $x^*(t_f) \in \mathcal{S}$. Because of the nature of the constraints on the solutions, equations (6) and (7) typically constitute a *two point boundary value problem* (BVP).

(b) The Hamiltonian is minimized by the optimal control input $u^*(t)$ for all $t \in [0, t_f]$, i.e.

$$u^*(t) = \arg \min_{u(t) \in \mathcal{U}} \mathcal{H}\left(x^*(t), u(t), p^*(t)\right). \quad (8)$$

(c) The Hamiltonian for the optimal control input is zero for all $t \in [0, t_f]$, that is,

$$\mathcal{H}\left(x^*(t), u^*(t), p^*(t)\right) = 0. \quad (9)$$

This is a consequence of the fact that the final time is not specified in the problem formulation, but rather is a parameter to be optimized over.

(d) The co-state vector is transversal to $\mathcal{S}$ in the final time, that is,

$$p^*(t_f)^T \left(q - x^*(t_f)\right) = 0, \, \forall q \in \mathcal{M} \quad (10)$$

where $\mathcal{M}$ is the tangent hyper-plane of $\mathcal{S}$ at $x^*(t_f)$.

From the definition of the Hamiltonian in (5) it should be noted that (6) is just a convenient way of rewriting the state equation (1). However, (7) through (10) give us additional information useful to obtain the optimal control input $u^*(t)$. The solution procedure for a particular optimal control problem using the PMP is far from being standard and usually requires some problem-specific analysis. Nevertheless, a general outline to accomplish this task would be as follows:

- Derive the form of the optimal control law from (8) and (9).
- Solve the two point BVP comprised in (6) and (7). The original optimal control problem has been transformed into a set of $2n$ differential equations.
- The $2n$ integration constants arising from the BVP in (6) and (7) and the final time t_f can be computed by solving the system of $2n + 1$ algebraic equations composed by $x^*(0) = x_0$, $x^*(t_f) \in \mathcal{S}$, (9) at $t = t_f$ and (10). This emphasizes the importance of the *transversality condition* in (10), which is key for the complete solution of the problem.

3. OPTIMAL METABOLIC REGULATION

Consider a metabolic pathway with a single substrate x_1 and a single final product P. The objective of the pathway is to convert x_1 into P through a set of p chemical reactions involving the metabolites $\{x_1, x_2, \ldots, x_n\}$. Unlike the theory of Metabolic Control Analysis (Heinrich and Schuster 1996), we allow the substrate and product concentrations to change in the same time scale as the intermediate metabolites. Hence, a fundamental relation arising from this formulation is that the total metabolite concentration remains fixed, i.e.

$$P(t) + \sum_{i=1}^{n} x_i(t) = C, \quad (11)$$

where $x_i(t)$ and $P(t)$ denote the metabolite and product concentrations as functions of time, respectively.

A dynamical description for the metabolic pathway is

$$\dot{x}(t) = \boldsymbol{N} v\left(x(t), u(t)\right), \quad (12)$$

$$P(t) = C - qx(t), \quad (13)$$

where $x(t) = [x_1(t)\ x_2(t)\ \cdots\ x_n(t)]^T \in \mathbb{R}^n$ is the vector of metabolite concentrations, $u(t) \in \mathbb{R}^m$ is the vector of enzyme concentrations, $v\left(x(t), u(t)\right) \in \mathbb{R}^p$ is the vector of reaction rates, $\boldsymbol{N} \in \mathbb{R}^{n \times p}$ is the stoichiometry matrix, $q = [1\ 1\ \cdots\ 1] \in \mathbb{R}^n$ and $m \geq n$.

We also assume that the rate laws comprising $v\left(x(t), u(t)\right)$ are linear in the enzyme concentrations, as in e.g. Mass Action and Michaelis-Menten kinetics. In addition, since the enzyme production capacity of the cell is finite, it is plausible to posit that the enzyme concentrations must satisfy

$$\sum_{i=1}^{m} u_i(t) \leq E_T, \quad (14)$$

where E_T stands for the total available enzyme abundance. Moreover, all enzymes must satisfy $u_i(t) \geq 0$, which together with (14) implies that the set of admissible enzyme concentrations $\mathcal{U}$ is given by a simplex in $\mathbb{R}^m$.

We are interested in finding time dependent enzyme concentrations $u^*(t)$ that drive the pathway from the initial state

$$x(0) = \begin{bmatrix} C & 0 & \cdots & 0 \end{bmatrix}^T, \tag{15}$$

to the terminal condition $P(t_f) = P_f < C$, $t_f < \infty$ over some time interval $[0,\, t_f]$, such that

$$u^*(t) = \arg \min_{u(t)\in\mathcal{U}} \mathcal{T} \quad , \forall t \in [0,\, t_f] \tag{16}$$

where

$$\mathcal{T} = \frac{1}{C}\int_0^{t_f} (C - P(t))\, dt, \tag{17}$$

is the transition time of the substrate-product conversion (Klipp *et al.* 2002).

It is important to note that, as in the framework described in Section 2, the terminal condition $P(t_f) = P_f$ does not completely specify the final state, but rather gives the surface where the final state must lie. Using (11), this surface is described by

$$\mathcal{S} = \left\{ x(t_f) \in \mathbb{R}^n : \sum_{i=1}^{n} x_i(t_f) = C - P_f \right\}. \tag{18}$$

This kind of terminal condition also implies that the value of the final state arises from the optimization itself, instead of being pre-specified.

The transition time defined in (17) is slightly different from the one used in (Klipp *et al.* 2002), since by forcing $P_f < C$ we are implicitly excluding the case when $t_f = \infty$. This is done in order to ensure that the transition time is finite for any control input whose values belong to the set $\mathcal{U}$. As shall be seen later, this issue has consequences for the behavior of the optimal enzyme concentrations that lead to a difference between our results and the ones in (Klipp *et al.* 2002).

Referring to (5), the Hamiltonian for this dynamical system is given by

$$\mathcal{H}\left(x(t), u(t), p(t)\right) = 1 - \frac{P(t)}{C} + p(t)^T \boldsymbol{N} v\left(x(t), u(t)\right), \tag{19}$$

where $p(t) \in \mathbb{R}^n$ is the co-state trajectory. An inspection of (19) shows that, since the rate is linear in the enzyme concentrations, the Hamiltonian is linear in every control input. This fact together with the geometry of the set $\mathcal{U}$ has interesting consequences in the form of the optimal enzyme profiles, as shown in next proposition. For this purpose and under the assumption than $m \geq n$, it is convenient to write the Hamiltonian as

$$\mathcal{H}\left(x^*(t), u(t), p^*(t)\right) = 1 - \frac{P(t)}{C} + \sum_{i=1}^{m} h_i(t) u_i(t), \tag{20}$$

where $h_i(t)$ is, in general, a nonlinear function of $x^*(t)$ and $p^*(t)$.

Proposition 1. The optimal enzyme profile $u^*(t)$ satisfies

$$\sum_{i=1}^{m} u_i^*(t) = E_T, \quad \forall t \in [0,\, t_f] \tag{21}$$

Proof. The proof follows using simple geometrical facts of linear functions defined over convex polyhedrons. For ease of notation, we will denote the set of vertexes of $\mathcal{U}$ as

$$\mathcal{V} = \{e_1, e_2, \ldots, e_m\} \cup \{\mathbf{0}\}, \tag{22}$$

where e_i has E_T in its i^{th} entry and 0 elsewhere. Similarly, the set of $(m-1)-$dimension faces of $\mathcal{U}$ is defined as

$$\mathcal{F} = \{F_1, F_2, \ldots, F_n\} \cup \{\mathcal{P}\}, \tag{23}$$

where F_i and $\mathcal{P}$ are the faces defined by

$$F_i = \{u(t) \in \mathcal{U} : u_i(t) = 0\}, \tag{24}$$

$$\mathcal{P} = \left\{ u(t) \in \mathcal{U} : \sum_{i=1}^{m} u_i(t) = E_T \right\}, \tag{25}$$

respectively.

From (9) it holds that $\mathcal{H}$ must vanish along the optimal trajectory, but

$$1 - \frac{P(t)}{C} > 0, \quad \forall t \in [0, t_f], \tag{26}$$

which from (20) implies that

$$\sum_{i=1}^{m} h_i(t) u_i^*(t) < 0, \quad \forall t \in [0, t_f], \tag{27}$$

$$\Rightarrow u^*(t) \neq \mathbf{0}, \forall t \in [0, t_f]. \tag{28}$$

Since $\mathcal{H}$ is linear in $u(t)$ and $\mathcal{U}$ has linear boundaries, from (8) it follows that $u^*(t) \in \mathcal{V}, \forall t \in [0, t_f]$, which is sometimes referred as the fundamental theorem of linear programming. Let $u^{*1}(t)$ be the optimal solution for $t \in [t_a, t_b]$ such that $u^{*1}(t)$ is located at vertex e_i. If there exists another optimal solution $u^{*2}(t) \neq u^{*1}(t)$ for $t \in [t_a, t_b]$ such that $u^{*2}(t) \in F_i \setminus \mathcal{V}$, then the linearity of $\mathcal{H}$ implies that any point in F_i is also optimal for $t \in [t_a, t_b]$. In particular, since $\mathbf{0} \in F_i, \forall i$, this implies that the origin would also be optimal, which contradicts (28). Hence it follows that $u^*(t) \notin F_i \setminus \mathcal{V}$, $\forall i$, so that $u^*(t) \in \mathcal{P}$ and (21) holds. □

Proposition 1 implies that, in order to keep a minimal transition time, the total available enzyme must be always fully used. This result resembles the nature of *bang bang* control laws in classical time-optimal control. However, it must be pointed out that the problem under consideration is not exactly a time-optimal control problem in its classical conception, because the transition time $\mathcal{T}$ is a measure of the average time taken by the pathway in achieving its goal.

Additional qualitative insight into the form of the optimal enzyme profiles may be gathered by exploiting the properties of the Hamiltonian and the set of admissible control inputs $\mathcal{U}$, as shown in next proposition.

Proposition 2. If the optimal enzyme profile $u^*(t)$ is unique, then it is a piecewise constant function. Moreover, if $\min\{h_1(t), h_2(t), \ldots, h_m(t)\}$ is unique, then each component of $u^*(t)$ follows a switching sequence defined by

$$u_i^*(t) = \begin{cases} E_T \ , \forall t: \ h_i(t) = \min\{h_1(t), h_2(t), \\ \qquad\qquad\qquad\qquad \ldots, h_m(t)\} \\ 0 \quad \text{, any other case} \end{cases} \tag{29}$$

Proof. Here we follow the notation in the proof of Proposition 1. If the optimal input $u^*(t)$ is unique, then $u^*(t) \notin \mathcal{P} \setminus \mathcal{V}, \forall t \in [0, t_f]$, since otherwise the linearity of $\mathcal{H}(x^*(t), u(t), p^*(t))$ would imply that there exists a time interval over which any input value in $\mathcal{P}$ is optimal. Furthermore, the only case in which $u^*(t)$ is not piecewise constant is when there exist $0 \leq t_a < t_b \leq t_f$ such that $u^*(t) \in \mathcal{P} \setminus \mathcal{V}, \forall t \in [t_a, t_b]$, which contradicts our first conclusion. Therefore, if $u^*(t)$ is unique then it is a piecewise constant function. The form (29) can be derived using the fact that, from (9), (20) and (26) it follows that $h_i(t) < 0$, for some $i \in \{1, 2, \ldots, m\}$, $\forall t \in [0, t_f]$. □

Equation (29) reveals that the optimal enzyme profiles are essentially a set of switching sequences between 0 and the maximum enzyme concentration E_T. In the context of the general metabolic pathway of our interest, this has major significance in its behavior, since it essentially means that at any time instant, only a single biochemical reaction of the whole network is *active*. This behavior is closely related with the just-in-time promoter activity described in (Zaslaver *et al.* 2004), and also gives a strong control theoretic support to the methodology developed in (Klipp *et al.* 2002), which precisely assumed that the optimal enzyme concentrations are piecewise constant functions. It should be remarked that we do not make any assumptions on the stoichiometry of the pathway and just require the kinetics to be linear in the enzyme concentrations.

Our results are strongly connected with the ones of (Klipp *et al.* 2002), but we must point out an essential distinction between the statement of Proposition 2 and the the analysis given in (Klipp *et al.* 2002). Under uniqueness of the solution, Proposition 2 does not allow active enzymes with concentrations lower than E_T, i.e. only full activity is permitted. However, the results in (Klipp *et al.* 2002) state that, for the case of an unbranched pathway, the optimal solution is such that there is a time instant t' such that $0 < u_i^*(t) < E_T$, $\forall t > t'$. The difference might arise from the fact that the problem dealt with in the current paper considers a finite time horizon in the cost functional, while in (Klipp *et al.* 2002) the authors address the infinite time horizon case.

The uniqueness of the optimal enzyme profiles in Proposition 2 is a strong assumption which is always guaranteed, provided that $\min\{h_1(t), h_2(t), \ldots, h_m(t)\}$ is non-unique only for isolated time instants. Indeed, at those isolated time instants, the optimal solution is undefined and they correspond to the switching times of $u^*(t)$. If this does not hold for some time interval T, i.e.

$$h_i(t) = \min\{h_1(t), h_2(t), \ldots, h_m(t)\}, \tag{30}$$

$\forall i \in \mathbb{I}$, $\forall t \in T$, where $\mathbb{I}$ is a certain index set with at least two elements and $T \subseteq [0, t_f]$, then the optimal solution is not unique and is described by the hyperplane

$$\begin{cases} \sum_{i \in \mathbb{I}} u_i^*(t) = E_T, \ \forall t \in T \\ u_i^*(t) = 0, \qquad \forall i \notin \mathbb{I}, \forall t \in T \end{cases} \tag{31}$$

In the case in which the solution is unique, Proposition 2 can be interpreted as that the optimal solution *jumps* from one vertex of $\mathcal{U}$ to another depending on how the functions $h_i(t)$ evolve in time. Thus, since each vertex of $\mathcal{U}$ (with exception of the origin) can be regarded as a full expression of a single enzyme, we conclude that these jumps correspond to a bang bang switching sequence that determines the order in which the reactions are activated. A further step in revealing the nature of this switching sequence is to determine the precise ordering in which the switchings take place. As it has been previously reported in (Zaslaver *et al.* 2004) for the case of unbranched pathways, the sequence of the activations can be closely related to the topology of the biochemical network, so in view of our results it should be possible to reveal such links for other pathway topologies.

An interesting extension of our results would be to add transcriptional dynamics of the enzyme biosynthesis to the pathway described in (12). This would allow the gathering of insightful information about the gene promoter activity behind the expression of each of the involved enzymes. Taken directly, such an approach would neglect the time scale difference between enzyme biosynthesis and metabolism, as was done in (Zaslaver *et al.* 2004).

So far we have focused only on the properties of the Hamiltonian, but in order to determine a precise switching sequence it turns out that the core information lies in the solution of the BVP comprised in (6) and (7), as shown in the next section for the case of an an unbranched pathway.

4. ILLUSTRATIVE EXAMPLE

As an illustrative example of our derivations, we apply our results to optimize time dependent enzyme concentrations in an unbranched pathway as depicted in Figure 1.

$$x_1 \xrightarrow{u_1} x_2 \longrightarrow \cdots \longrightarrow x_n \xrightarrow{u_n} P$$

Fig. 1. Unbranched metabolic pathway.

Assuming Mass Action enzyme kinetics, i.e. $v_i = k_i u_i(t) x_i(t)$, and using the result of Proposition 2 we derive analytical solutions of the BVP intervalwise for $n = 2$, $k_1 = 0.1$, $k_2 = 0.05$, $C = 1$, $P_f = 0.8$ and $E_T = 1$. The details of the derivation are omitted due to length constraints, but it can be noted that the optimal solution is such that there is only a single switching instant. The plot of both optimal metabolite and enzyme concentrations is shown in Figure 2. The results show that the product effectively reaches the desired level by means of a single switching at $t = t_1$. The switching time t_1, the final time t_f and the exact value of the final state arise from the optimization itself.

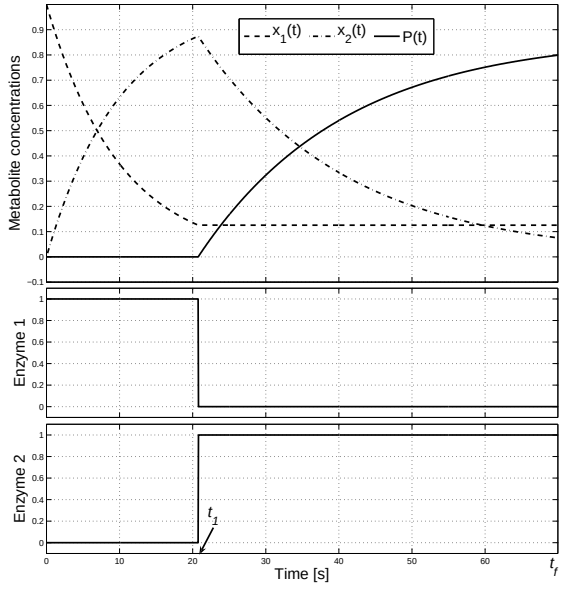

Fig. 2. Optimal solution for $n = 2$.

5. CONCLUSIONS

We have tackled the issue of determining time dependent enzyme concentrations that minimize the transition time of a metabolic pathway subject to a limited total enzyme abundance. It is shown that this problem can be naturally posed within an optimal control framework and solved by means of Pontryagin's Minimum Principle.

By studying properties of the Hamiltonian function and the set of feasible enzyme concentrations, qualitative insights regarding the optimal enzyme distributions can be derived. Our main result states that these are essentially switching sequences between zero and maximum activity. This result is valid for general stoichiometries, provided that there is a single substrate and a single product and that the kinetics are linear in the enzyme concentrations, as in e.g. Mass Action and Michaelis-Menten kinetics.

The problem may also be posed in terms of a two point boundary value problem. However, this might turn out to be a difficult task itself and will depend crucially on the enzyme kinetics. As an illustration, a simple example was presented for the case of an unbranched pathway.

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