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JUST-IN-TIME ACTIVATION OF A GLYCOLYSIS INSPIRED METABOLIC NETWORK - SOLUTION WITH A DYNAMIC OPTIMIZATION APPROACH

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ABSTRACT

Optimization approaches can be used to study principles of metabolic regulation. Earlier studies considered mostly steady-state systems [1, 2]. The dynamic regulation, or just-in-time activation, of metabolic pathways has attracted increasing attention [3, 4] and was experimentally observed in the amino acid biosynthesis of Escherichia coli [5].

Minimizing the time for the activation of a pathway allows the organism to reduce the lag phase before optimal growth can be achieved [4]. Here we study a glycolysis inspired pathway with different physiological constraints on enzyme synthesis. This leads to a nonlinear dynamic optimization problem that we solve with the quasi-sequential approach [6].

The obtained just in time activation shows sequential synthesis of enzymes (similar to the behavior experimentally observed in [5]). It can be found that intermediates accumulate and are consumed later on, which could be harmful due to an increasing osmotic pressure. The results demonstrate the utility of optimization approaches in order to understand the regulation of metabolic pathways.

Index Terms – Nonlinear dynamic optimization, just in time expression of metabolic genes, enzyme synthesis constraints, metabolic pathways analysis

1. INTRODUCTION

The application of optimization in biological systems can provide important insights into the regulation of metabolic pathways in microorganisms. Optimization approaches have been applied to metabolic networks at steady state and have helped to explain the properties of these systems [1, 2]. Interestingly, a recent study using nonlinear optimization explains the zonation in liver tissue [7].

Dynamic optimization can be employed to study the time profiles or kinetic behaviors of enzymes within a metabolic network [3-5]. Since most metabolic reactions within an organism are catalyzed by enzymes, the time performance of metabolism strongly depends on the availability of these enzymes. Thus, activating a specific metabolic pathway requires a coordinated regulation of the synthesis of its enzyme components in order to reduce the time for the generation of the desired products through the pathway [4].

In previous studies the identification of optimal enzyme profiles to activate an unbranched metabolic pathway was derived using analytical methods like the variational calculus or Pontryagin's Maximum Principle (see e.g. [3] or [4]). Here we extend these results by studying a glycolysis inspired pathway occurring in many micro-organisms. The details of the model are presented in section 2.

In this work the just-in-time activation of a metabolic pathway is predicted by a dynamic optimization approach (see section 3). The objective is to minimize the time period in which an essential product is generated. In particular, we consider the transcriptional regulation of the metabolic network constrained by physiological boundaries during the enzyme synthesis. The optimization method used for the prediction is the quasi-sequential approach [6] with a moving finite element strategy to adapt the time element lengths.

Several optimization scenarios with different enzyme synthesis constraints are studied in section 4. The results of the optimization indicate that a sequential synthesis of enzymes within the pathway minimizes the time toward the product formation (even under boundaries during enzyme synthesis). These results are reminiscent of experimental results with the sequential activation of gene expressions which was observed in several amino acid biosynthetic pathways [5]. The introduction of a branching pathway leads to a new behavior: products of the branching reaction accumulates and is consumed later on. This may increases the osmotic pressure and could be harmful to the cell due to osmotic effects. Finally, in section 5, key results are summarized and areas of further works are outlined.

2. GLYCOLYSIS INSPIRED NETWORK

The metabolism of a cell can be considered as a chemical factory that produces desired energy and substances (building blocks) from the available substrates (growth media). In most cases each metabolic reaction is catalyzed by a specific enzyme

(summarized in **e**). Such reactions can be modeled by reaction stoichiometry to describe the relations between different metabolites (summarized in **x**), represented by the stoichiometric matrix **N** (positive or negative stoichiometric coefficients denotes formation of products or breakdown of substrates). With the dynamics of the reaction rates **v** follows:

$$\dot{\mathbf{x}}(t) = \mathbf{N} \cdot \mathbf{v}(\mathbf{x}(t), \mathbf{e}(t)),\ \mathbf{x}(t_0) = \mathbf{x}_0 . \qquad (1)$$

Where $\mathbf{v}(\mathbf{x}(t),\mathbf{e}(t))$ represents a function vector depending on the concentration profiles $\mathbf{x}(t)$ and $\mathbf{e}(t)$. Here we consider a glycolysis inspired network (Fig. 1) with substrate S, intermediates X_1 - X_3 and product P of the pathway (corresponding vector of metabolites: $\mathbf{x} = [S, X_1, X_2, X_3, P]^T$). Due to the task of just-in-time activation we assume the following initial condition at t_0: $\mathbf{x}_0 = [s_0, 0, 0, 0, 0]^T$ which means the substrate is newly introduced into the pathway (e.g. after a starvation period).

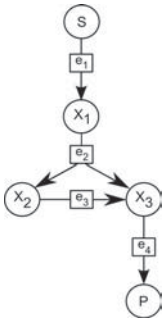

Figure 1: Metabolic pathway inspired by the upper part of glycolysis. The metabolites are denoted by circles and enzyme catalyzed reactions are indicated by rectangles. The typical alternative route of the glycolysis can be seen by e_2 (corresponding to the enzyme aldolase) metabolizing the intermediate X_1 to X_2 and X_3.

The stoichiometric matrix for the unbuffered or exhaustible substrate is defined by $\mathbf{N}_1$ and in the case of buffered substrate by $\mathbf{N}_2$:

$$\mathbf{N}_1 = \begin{bmatrix} -1 & 0 & 0 & 0 \\ 1 & -1 & 0 & 0 \\ 0 & 1 & -1 & 0 \\ 0 & 1 & 1 & -1 \\ 0 & 0 & 0 & 1 \end{bmatrix},\ \mathbf{N}_2 = \begin{bmatrix} 0 & 0 & 0 & 0 \\ 1 & -1 & 0 & 0 \\ 0 & 1 & -1 & 0 \\ 0 & 1 & 1 & -1 \\ 0 & 0 & 0 & 1 \end{bmatrix}. \qquad (2)$$

The theoretical studies in this paper are based on the Michaelis–Menten kinetics with unity rate constants for k_{cat} and K_M :

$$v_k(\mathbf{x}(t), \mathbf{e}(t)) = e_k(t) \frac{k_{cat} x_k(t)}{K_M + x_k(t)},\ k = 1,...,4 . \qquad (3)$$

3. IDENTIFICATION OF JUST-IN-TIME ACTIVATION

Optimization approaches can be used to explain the properties of biological systems [1-5, 7] and has become an integral part of Systems Biology. In [3] the just-in- time activation of metabolic pathways was firstly predicted by solving a dynamic optimization problem to identify these optimal enzyme profiles for pathway activation. Interestingly, the obtained sequential activation was confirmed later on by experiments of another group [5]. Here we extend the work of [3, 4] where infinite steepness in the enzyme profiles were allowed, by introducing different enzyme synthesis constraints (see section 3.1). The resulting nonlinear dynamic optimization problem is solved with the quasi-sequential approach from [6] and is outlined in section 3.2.

3.1. Identification formulated as optimization problem

In this work we consider the activation of metabolic pathways with a substrate that is newly introduced into the system. Klipp et al. [3] minimized the transition time from substrate to product. This transition time is defined by a time integral running to infinity. From a biological point of view it makes more sense to minimize the formation time (or final time t_f) of a product amount from the substrate [4]:

$$\min_{\mathbf{e}(t), t_f} t_f \quad x_5(t_f) = p_{t_f} . \qquad (4)$$

Additionally, boundaries on enzyme concentrations and their total amount are introduced in [3, 4]. These restrictions can be formulated in a normalized form as

$$e_k(t) \geq 0,\quad k = 1, 2, 3, 4 \qquad (5)$$

$$\sum_{k=1}^{4} e_k(t) \leq 1 . \qquad (6)$$

This is consistent with the reasonable assumption that the cell can only allocate a certain amount of protein to a pathway ([2, 3, 8]). In addition, this is in agreement with experimental observations [9] and other studies [10] in *Escherichia coli* where a protein degradation to synthesize another protein is shown. This is in contrast to the result of Zaslaver et al. [5] where these effects were not observed. However, due to the constraint of Eq. (6) it is necessary to redistribute the protein amount in time by the optimization approach, if the rate of enzyme synthesis is not limited. More meaningfully, we introduce individual enzyme synthesis rate constraints:

$$\dot{e}_{min,k} \leq \dot{e}_k(t) \leq \dot{e}_{max,k},\quad k = 1, 2, 3, 4 \qquad (7)$$

and constraints on the overall enzyme synthesis:

$$\sum_{k=1}^{4} \left(\dot{e}_k(t)\right)^2 \leq c_{max} . \qquad (8)$$

Since in Eq. (8) protein degradation to synthesize another enzyme can be rising or falling in enzyme profiles, we relax the constraint to:

$$\sum_{k=1}^{4} \dot{e}_k(t) \leq r_{max} . \qquad (9)$$

Therefore we need to consider simultaneously the capability of this recycling by

$$r_{min,k} \le \dot{e}_k(t) \quad k = 1, 2, 3, 4 \tag{10}$$

to avoid infinitesimal steepness in enzyme profiles during this reallocation of protein. As a result, the identification of the just-in-time activation of metabolic pathways leads to an optimization problem formulated by Eqs. (1)-(4) together with different combinations of enzyme synthesis constraints from Eqs. (5)-(10). In the next section the solution of the optimization problem is described.

3.2. Solution with the quasi-sequential approach

The dynamic optimization problem to be solved for the identification of just-in-time activation can be described by

$$\min_{\mathbf{e}(t), t_f} \; t_f \tag{11}$$

$$\text{s.t. } \dot{\mathbf{x}}(t) = \mathbf{F}\left(\mathbf{x}(t), \mathbf{e}(t)\right) \; ; \; \mathbf{x}_0 = \mathbf{x}(t_0) \tag{12}$$

$$\mathbf{x}(t_f) - p_{t_f} = 0 \tag{13}$$

$$\mathbf{G}\left(\mathbf{e}(t), \dot{\mathbf{e}}(t)\right) \le \mathbf{0} \tag{14}$$

where the production time as objective function is to be minimized (Eq. (11)) subject to the model Eqs. (12), to the final state condition in Eq. (13) and to the inequality constraints of enzyme synthesis (see Eq. (14)).

The solution approaches to such dynamic optimization problems can be separated into indirect and direct methods. The indirect methods (e.g. variational approaches, Maximum Principle) derive the solution based on necessary optimality conditions and the solution of a two-point-boundary-value problem. In this paper we use the quasi-sequential approach (direct method) from [6]. The infinite optimization problem is discretized into a finite dimensional problem and can then be solved with a nonlinear programming (NLP) algorithm. The NLP needs in addition the sensitivities of Eqs. (11)-(14).

Discretization

Both enzyme profiles and metabolites are discretizied using collocation on finite elements [11, 12]. Where the time period from t_0 to t_f is divided into NL finite elements

$$\Delta t_n = t_{n+1} - t_n \; ; \; n = 1, \ldots, NL \tag{15}$$

with t_n , t_{n+1} as start and end time of the n'th element. The metabolites (corresponding to ND differential states) are approximated by the linear combination of the Lagrange polynomials, i.e.

$$x_n^m(t) = \sum_{j=0}^{NC} \ell_j(t) \cdot x_{n,j}^m \; ; \; m = 1, \ldots, ND \; ; \; n = 1, \ldots, NL$$
$$\ell_j(t) = \prod_{\substack{i=0 \\ i \ne j}}^{NC} \frac{t - t_{n,i}}{t_{n,j} - t_{n,i}} \tag{16}$$

where NC denotes the collocation order and

$$x_{n,i}^m = x_n^m(t_{n,i}) \, . \tag{17}$$

From Eq. (16) the time derivatives of the metabolites are represented at the collocation points in an element by

$$\dot{x}_n^m(t_{n,i}) = \sum_{j=0}^{NC} \dot{\ell}_j(t_{n,i}) \cdot x_{n,j}^m \; ; \; i = 1, \ldots, NC \, . \tag{18}$$

We use Radau collocation to ensure continuity of the differential state profiles between two intervals:

$$x_{n-1,NC}^m = x_{n,0}^m \, , \tag{19}$$

i.e. the value on the last collocation point of an element is defined as initial condition of the next element. Here we denote the discrete values of the enzyme profiles in the vector $\mathbf{E}_n$ of the n'th interval and of the metabolites in the vector $\mathbf{X}_{n,i}$ of the i'th collocation points in the n'th interval. With the differential equations of the metabolites and the polynomial approximation (see Eqs. (12), (18)) we can present the residual equation by:

$$R_{n,i}^m\left(t_{n,i}\right) = \sum_{j=0}^{NC} \dot{\ell}_j(\tau_i) \cdot x_{n,j}^m - \Delta t_n \cdot F_m\left(\mathbf{X}_{n,i}, \mathbf{E}_n\right) = 0 \; , \quad i = 1, \ldots, NC \; ; \; m = 1, \ldots, ND \; ; \; n = 1, \ldots, NL \tag{20}$$

where τ represents a normalized time between one and zero within each element. The above discretization is applied to Eqs. (11)-(14) and leads to the corresponding discretized optimization problem:

$$\min_{\mathbf{E}, \mathbf{X}, \Delta\mathbf{T}} \; \sum_{n=1}^{NL} \Delta t_n \tag{21}$$

$$\text{s.t. } R_{n,i}^m\left(t_{n,i}\right) = 0 \; ; \; x_{n-1,NC}^m = x_{n,0}^m \; ; \; x_{1,0}^m = x^m(t_o) \tag{22}$$

$$i = 1, \ldots, NC \; ; \; m = 1, \ldots, ND \; ; \; n = 1, \ldots, NL \tag{23}$$

$$x_{NL,NC}^{ND} - p_{t_f} = 0 \tag{24}$$

$$\mathbf{G}\left(\mathbf{E}, \Delta\mathbf{T}\right) \le \mathbf{0} \, . \tag{25}$$

where in $\mathbf{X}$ and $\mathbf{E}$ all discrete values of metabolites and enzyme profiles are included. The handling of free final time problems (see Eqs. (11) and (21)) needs the extension of the optimization problem with the adaption of finite element lengths (so called moving finite elements - MFE) which are summarized in $\mathbf{\Delta T}$. The time derivatives of the enzyme profiles for Eq. (25) can be derived with a piecewise constant discretization by

$$\dot{e}_{n,k} \approx \frac{e_n - e_{n-1}}{\Delta t_n} ; \; e_{0,k} = 0 \quad k = 1, \ldots, 4 \; ; \; n = 1, \ldots, NL \, . \tag{26}$$

The solution of the NLP (21)-(25) represents the so called simultaneous approach with a MFE-strategy [13]. In the quasi-sequential approach the discretized model equations (22) are solved with a Newton method that reduces the dimension of the optimization problem. This leads to a two-layer

structure: the solution of the equation system is done in the simulation layer and the optimization problem in the optimization layer. As a result, the following reduced problem will be solved in the optimization layer

$$\min_{\mathbf{E},\Delta\mathbf{T}} \sum_{n=1}^{NL} \Delta t_n \tag{27}$$

$$x(\mathbf{E},\Delta\mathbf{T})_{NL,NC}^{ND} - p_{t_f} = 0 \tag{28}$$

$$\mathbf{G}(\mathbf{E},\Delta\mathbf{T}) \le \mathbf{0}\,. \tag{29}$$

where $x(\mathbf{E},\Delta\mathbf{T})_{NL,NC}^{ND}$ stands for the computed product at final time in the simulation layer for the given value of **E** and **ΔT**.

Sensitivity computation

For the solution with a NLP it is favorably to calculate the sensitivities of Eqs. (27) - (29) with respect to **E** and **ΔT.** These can be easily derived by simple differentiation. The sensitivities of Eq. (28) are based on the residual Eq. (20) for all metabolites in the n'th element:

$$\mathbf{R}_n(\mathbf{X}_{n,0},\mathbf{X}_n,\mathbf{E}_n,\Delta t_n) = 0. \tag{30}$$

where the initial conditions are defined by

$$\mathbf{X}_{n,0} = \mathbf{D}\mathbf{X}_{n-1} \tag{31}$$

with the mapping matrix **D**. The first Taylor expansion of Eq. (30) reads

$$\begin{aligned}\frac{d\mathbf{R}_n}{d\mathbf{X}_{n,0}}\cdot\Delta\mathbf{X}_{n,0} + \frac{d\mathbf{R}_n}{d\mathbf{X}_n}\cdot\Delta\mathbf{X}_n \\ + \frac{d\mathbf{R}_n}{d\mathbf{E}_n}\cdot\Delta\mathbf{E}_n + \frac{d\mathbf{R}_n}{d(\Delta t_n)}\cdot\Delta(\Delta t_n) = \mathbf{0}.\end{aligned} \tag{32}$$

Therefore we have the following sensitivities of metabolites with respect to enzyme levels, to element lengths and initial conditions in each element:

$$\begin{aligned}\frac{d\mathbf{X}_n}{d\mathbf{E}_n} &= -\left[\frac{d\mathbf{R}_n}{d\mathbf{X}_n}\right]^{-1}\cdot\frac{d\mathbf{R}_n}{d\mathbf{E}_n} \\ \frac{d\mathbf{X}_n}{d(\Delta t_n)} &= -\left[\frac{d\mathbf{R}_n}{d\mathbf{X}_n}\right]^{-1}\cdot\frac{d\mathbf{R}_n}{d(\Delta t_n)} \\ \frac{d\mathbf{X}_n}{d\mathbf{X}_{n,0}} &= -\left[\frac{d\mathbf{R}_n}{d\mathbf{X}_n}\right]^{-1}\cdot\frac{d\mathbf{R}_n}{d\mathbf{X}_{n,0}}.\end{aligned} \tag{33}$$

The continuity of the state profiles allows to transfer the sensitivities from element to element by using the chain rule [6]. Thus the sensitivities of the states in the n'th element with respect to the enzyme levels or element lengths of the i=1…n-1 can be computed by

$$\frac{d\mathbf{X}_n}{d\mathbf{E}_i} = \frac{d\mathbf{X}_n}{d\mathbf{D}\mathbf{X}_{n-1}}\cdot\frac{d\mathbf{D}\mathbf{X}_{n-1}}{d\mathbf{E}_i} = \frac{d\mathbf{X}_n}{d\mathbf{X}_{n,0}}\mathbf{D}\frac{d\mathbf{X}_{n-1}}{d\mathbf{E}_i} \tag{34}$$

$$\frac{d\mathbf{X}_n}{d(\Delta t_i)} = \frac{d\mathbf{X}_n}{d\mathbf{D}\mathbf{X}_{n-1}}\cdot\frac{d\mathbf{D}\mathbf{X}_{n-1}}{d(\Delta t_i)} = \frac{d\mathbf{X}_n}{d\mathbf{X}_{n,0}}\mathbf{D}\frac{d\mathbf{X}_{n-1}}{d(\Delta t_i)}\,. \tag{35}$$

This is done until the last finite element is reached and provides the sensitivities of Eq. (28).

4. CASE STUDIES

In previous studies the activations of unbranched metabolic pathways were identified based on a time-dependent distribution of an available protein amount. The transition time [3] or the formation time [4] from substrate to product was minimized with indirect optimization approaches (analytical methods).

In this section we show the main behaviors of the just-in-time activations of a glycolysis inspired metabolic pathway under different enzyme synthesis constraints (Fig. 2-5). Additionally we consider buffered and unbuffered substrate to study the impact on the enzyme profiles. The theoretical case studies are based on the chosen values of $s_0 = 1$, $p_{tf} = 0.75 s_0$ and all synthesis rate values to -0.1 or 0.1. The optimization problems are solved with the quasi-sequential approach presented in section 3. Due to space limitations we do not show the optimal metabolite profiles and element lengths. However, a discussion of osmotic pressure is nevertheless presented at the end of this section.

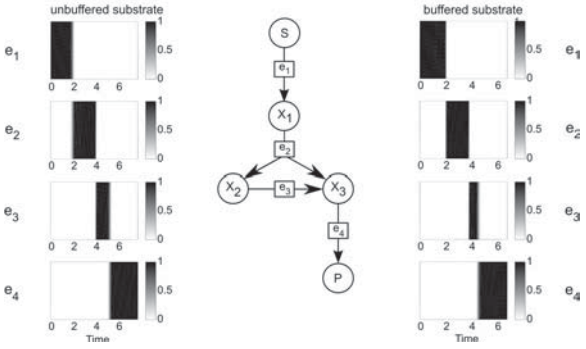

Figure 2: Optimal enzyme profiles for unbuffered (left) and buffered substrate (right) with constraints only on the total protein amount. Zero enzyme amounts are shown in white and highest enzyme amounts in black. Additionally the network structure of Fig. 1 is represented for a better illustration.

At first we consider the just-in-time activation of a glycolysis inspired metabolic network with the time depended distribution of a total protein amount to minimize the formation time (see Eq. (6) and as was done in principle in [4]). The results are shown in Fig. 2, where we use the similar plots as in [5]. Here a typical bang-bang solution in the enzyme profiles can be seen for both substrate variants. This is in line with the sequential activation of the unbranched pathway obtained in [3, 4]. Interestingly, in the case of a buffered substrate the activation time of the first enzyme is a bit longer and a clearly shorter period of the third enzyme is obtained (and of course the formation time is also reduced).

In the study of metabolic pathway activation we found three different principle behaviors in literature: first, sequential activation showing protein

degradation to synthesize another protein [9, 10] (similar to Fig. 2), second, sequential synthesis of enzymes without regression of available enzymes [5], and third, simultaneously expression of enzyme groups [14]. In all cases, limits to the rates of the enzyme synthesis were found. Therefore we add Eq. (7) as a constraint in the next case study to the problem formulation and in the second part of the case study we remove the constraint of total protein amount for cases where only the rates are the limiting factors.

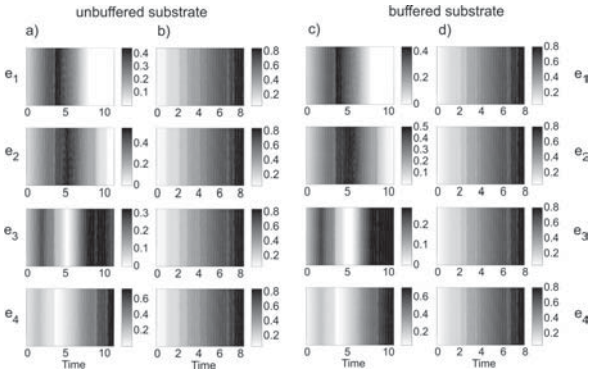

Figure 3: Optimization results of enzyme profiles for unbuffered (left) and buffered (right) substrate (zero to highest enzyme amount is shown in gray scale). Simultaneous constraints on total protein amount and the individual synthesis are presented in a) and c). In contrast only the individual synthesis rate of each enzyme is considered in b) and d).

The results obtained are shown in Fig 3. Here in each case enzyme profiles with smoothness in the enzyme synthesis are identified. From the results of a) and c) a sequential activation with protein degradation to synthesize another protein (see [9, 10]) can be seen. However, a deviation from a sequential activation can be found in the first phase by the explicit activation of all enzymes. From the results without constrained total protein amount in b) and d) simultaneous activations can be seen (similar results are observed in [14]). In all cases no noticeable difference between the enzyme profiles for buffered or unbuffered substrate can be found, only the formation time is slightly reduced.

In summary, the assumption of individual enzyme synthesis rate constraints is not consistent with the observed just-in-time activations in [5, 9, 10]. Therefore we replace the constraints on individual enzyme synthesis rate with the overall enzyme synthesis rate from Eq. (8). Additionally, the influence of total protein amount is studied.

The optimal enzyme profiles obtained are shown in Fig. 4. In the cases of a) and c) we obtain sequential activations with protein degradation to synthesize another protein (observed in [9, 10]). The degradation can be clearly seen by the first and second enzyme profiles. The sequential activation without degradation (from [5]) is obtained by b) and d) without boundaries on the total protein amount.

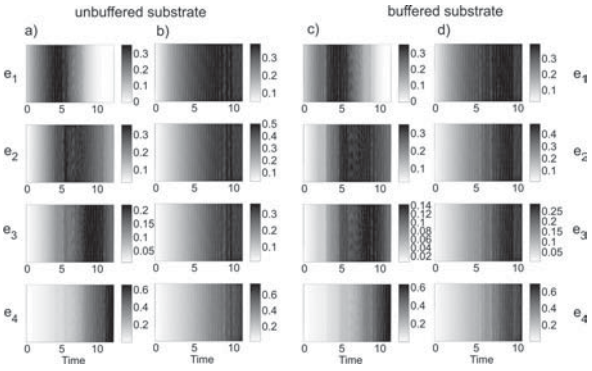

Figure 4: Optimal enzyme profiles (for unbuffered and buffered substrate) with simultaneous constraints on total protein amount and overall enzyme synthesis rate are shown in a) and c). In contrast, b) and d) show the optimal enzyme profiles without the constraints on total protein amount.

The difference of results between unbuffered and buffered substrate can be described by the following two aspects. First, in the case of a buffered substrate a higher concentration of the first enzyme is reached, which is consistent with the longer activation shown in Fig. 1, i.e. for a) 0.3603 to c) 0.3613 and for b) 0.3851 to d) 0.3990. Second, in the case of a buffered substrate a lower concentration of the third enzyme is obtained, which is consistent with a shorter activation presented in Fig 1, i.e. for a) 0.2214 to c) 0.1450 and for b) 0.3683 to d) 0.2906. Nevertheless, the formation time is shorter in the case of buffered substrate.

Next we study the influence of the recycling rate from Eqs. (9) and (10). Again the influence of the total protein amount is also studied (see Fig. 5).

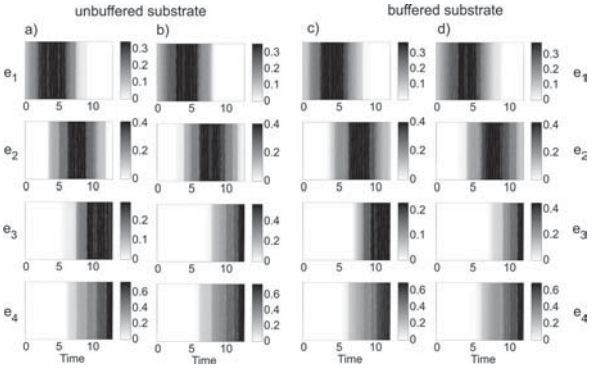

Figure 5: Optimal results of enzyme profiles for unbuffered (left) and buffered substrate (right) with boundaries on total protein amount, on overall enzyme synthesis rate and on individual recycling rate in a) and c). The constraint on total protein amount is removed in b) and d).

Interestingly in all cases a sequential activations with protein degradation to synthesize another protein can be obtained (observed in [9, 10]). This is in contrast to the case where these activations are influenced by the constrained total protein amount. However, the activation profiles observed in [5, 14] cannot be obtained. The difference of the results between

unbuffered and buffered substrate are similar to that of Fig. 4.

Here we present results of enzyme synthesis rate constraints in metabolic pathway activation and indicate that experimental observations can be explained or even modeled with different constraints. However, further considerations for constraints are needed in the regulation of metabolic pathways. The accumulation of metabolite intermediates increases the osmotic pressure which could be harmful to the cell. This need to be considers because only in the situations presented in Fig. 3, a) and Fig. 4, a) no accumulation occur (not shown), whereas in the cases with buffered substrate the accumulation is higher.

5. CONCLUSIONS

In this paper we extend the previous studies by considering different constraints on enzyme synthesis and their impact on the optimal enzyme profiles.

The sequential activations with protein degradation to synthesize another protein observed in [9, 10] can be modeled by constraints of the overall enzyme synthesis rate with total protein amount boundaries and by the recycling of available protein amounts. The sequential activations found by Zaslaver et al. [5] can only be obtained by constraints of the overall enzyme synthesis rate without total protein amount boundaries. The simultaneous activations (similar to that observed in [14]) can be obtained by constraints of the individual enzyme synthesis rate without total protein amount boundaries. In the case of constrained enzyme synthesis rates, the impact of buffered or unbuffered substrate can only be seen in the adaption of the enzyme concentrations.

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