Preprints of the 8th IFAC Symposium on Advanced Control of Chemical Processes
The International Federation of Automatic Control
Furama Riverfront, Singapore, July 10-13, 2012

Global Stability of Full Open Reversible Michaelis-Menten Reactions ⋆

Ismail Belgacem * **Jean-Luc Gouzé** **

* *INRIA, BIOCORE project-team, 2004 Route des Lucioles, BP 93, 06902 Sophia Antipolis, France; e-mail: ismail.belgacem@inria.fr*
** *INRIA, BIOCORE project-team, 2004 Route des Lucioles, BP 93, 06902 Sophia Antipolis, France; e-mail: gouze@sophia.inria.fr*

Abstract: We consider the full closed and open Michaelis-Menten enzymatic reactions. We study the corresponding dynamical system and show its global stability if the equilibrium exists. If the system is open, the equilibrium may not exist. Then we consider an open chain of reversible metabolic reactions and also prove global stability. Our mathematical tools are monotone systems theory and compartmental systems theory.

Keywords: Biological systems; Dynamical Systems; Nonlinear systems; Stability; Enzymatic systems

1. INTRODUCTION

In the field of biology, metabolic systems are an important class of dynamical systems (Heinrich and Schuster (1996)). They are similar to chemical systems, but the reactions are catalyzed by enzymes. These enzymes are proteins synthesized by genes, and metabolic and genetic systems are coupled by control loops (metabolites can regulate the synthesis of an enzyme). From a biological point of view, the study of this coupled system is of first importance (Yeang (2011)). Its dynamical behavior can be complex and it should be studied with mathematical models (Steuer and Junker (2008)). These models themselves are often large and complex, and tools for study or reduction are necessary, as shown by some cases studies (Bettenbrock et al. (2006); Goelzer et al. (2008)).

The most famous and classical enzymatic system is the (irreversible or reversible) Michaelis-Menten system. In particular, the study of the reduced system of Michaelis-Menten with the QSSA (Quasi-steady state approximation), based on the difference between the time scales of the reactions, is the subject of thousand of studies (Segel and Slemrod (1989); Heinrich and Schuster (1996)). These studies mainly compare the behavior of the full system and that of the reduced one.

Our goal in this work is quite different: we wish to study the dynamical behavior of the full system without any approximation. We are able to show, with simple mathematical tools, the stability of the closed or open reversible Michaelis-Menten systems. But we also show that the open reversible Michaelis-Menten may have no equilibrium, if the input is too large. The mathematical tools we use are known but not so classical: they belong to the theory of monotone systems, and of compartmental systems.

In a second step, we study, with the same tools and methodology, the behavior of reversible metabolic chains. We show that for a "pure" reversible enzyme system (all reactions are reversible enzymatic reactions), then, depending on the input, there is either no steady-state, either a single globally asymptotically stable steady-state.

Many papers have studied Michaelis-Menten approximations, but few are interested by the full system. The paper Fraser and Roussel (1994) studies the full closed reversible Michaelis-Menten (and gives an interesting introduction concerning the QSSA) by reducing it to a two-dimensional system. Then a phase-plane analysis is done. Our tools are more systematic, and work even for higher dimensions (open reversible Michaelis-Menten systems cannot be reduced to two dimensions) when this two-dimensional analysis cannot apply.

There exist other studies of metabolic chains controlled by enzymes, in other contexts (Oyarzún et al. (2012)), but, to our knowledge, none of them with our tools. For a work using monotone systems for chemical chains, see De Leenheer et al. (2007). For a work on a similar problems of metabolic chains, with a linear approach, see Flach and Schnell (2010). We believe that this kind of tools (monotony, positive matrices) are well adapted to biological problems, as remarked by other studies (Sontag (2004)). In spite of the non linear and complicated form of kinetics rates, we are able to study the system in a simple and global way.

The study of the full Michaelis-Menten system stability is of great interest because this system is often use as a fast metabolic system coupled with a slow genetic system. The global stability allows to apply QSSA methods to reduce the whole system.

The mathematical notions and theorems used in this paper are recalled in the appendix. In the first section, we study the reversible closed Michaelis-Menten system, then the open one. In a third section, we study metabolic reversible chains.

Notations: First we give some classical notations (see Perko (2001)). Let the autonomous n-dimensional differential system

$$\dot{x} = f(x) \tag{1}$$

We define the flow as the set of solutions of (1) parametrized by the time t. The notation $\Phi(t, x_1)$ corresponds to the solution starting from the initial condition x_1 parametrized by time $t \geq 0$. Function f is supposed to be continuously differentiable within

⋆ This work was supported by the ANR Gemco project, and INRIA Colage action.

some domain of interest, that will be in our case $X = \mathbb{R}^n_+$. We deduce the existence and uniqueness of solutions on some time interval for the differential equation (1). Throughout the paper, we use the classical notions of Lyapunov stability. The term "global stability" will mean "global asymptotic stability" (inside some domain, depending on the context).

2. THE CLOSED REVERSIBLE MICHAELIS-MENTEN SYSTEM

In 1913, Michaelis and Menten studied the kinetics of a simple enzymatic reaction involving a single enzyme. Consider the reaction consisting of a substrate S , of an enzyme E and a product P. Michaelis and Menten proposed the following description and equations (we refer to Murray (2002) and Edelstein-Keshet (1988)). The enzyme forms a transitory complex C before returning to its original form, giving product P from substrate S. The reactions are described by:

$$S + E \rightleftarrows C \rightarrow P + E$$

However, (see Cornish-Bowden (2004)), in principle all reactions catalyzed by enzymes are **reversible**, and this fact could play a prominent role in biochemistry. It turns out that it is interesting to add a reversible reaction to the last step of the above model. The new model becomes the reversible Michaelis-Menten system:

$$S + E \rightleftarrows C \rightleftarrows P + E$$

The mathematical model, based on classical mass-actions chemical laws, is:

$$\begin{cases} \dfrac{ds}{dt} = -k_1 se + k_{-1}c \\ \dfrac{de}{dt} = -k_1 se + k_{-1}c + k_2 c - k_{-2} pe \\ \dfrac{dc}{dt} = k_1 se - k_{-1}c - k_2 c + k_{-2} pe \\ \dfrac{dp}{dt} = k_2 c - k_{-2} pe \end{cases} \tag{2}$$

This is the model of the full reversible Michaelis-Menten system, that we want to study without any approximation. In a first step, we remark that, for nonnegative initial conditions, the variables stay positive for all time t : it is enough to check that the vector field is repulsive on the boundary of the positive orthant.

The sum of the free form and bound form of the enzyme is constant

$$\dot{e} + \dot{c} = 0$$

or $e + c = E_0$, where E_0 is a positive constant, which is the total concentration of enzyme. Therefore we can eliminate the state variable $e = E_0 - s$. Yet, we have to keep in mind that, first, E_0 depends on the initial conditions $s(0)$ and $e(0)$, and secondly that the constraint $c(t) \leq E_0$ has to be fulfilled.

The reduced system is :

$$\begin{cases} \dfrac{ds}{dt} = -k_1 s(E_0 - c) + k_{-1}c \\ \dfrac{dc}{dt} = k_1 s(E_0 - c) - k_{-1}c - k_2 c + k_{-2} p(E_0 - c) \\ \dfrac{dp}{dt} = k_2 c - k_{-2} p(E_0 - c) \end{cases} \tag{3}$$

The Jacobian matrix is $J(s,c,p)$

$$= \begin{pmatrix} -k_1(E_0 - c) & k_{-1} + k_1 s & 0 \\ k_1(E_0 - c) & -k_{-1} - k_1 s - k_2 - k_{-2} p & k_{-2}(E_0 - c) \\ 0 & k_2 + k_{-2} p & -k_{-2}(E_0 - c) \end{pmatrix} \tag{4}$$

The system (3) is closed, in the sense that $\dot{s} + \dot{c} + \dot{p} = 0$. Moreover, the Jacobian matrix has interesting properties. We recall in the appendix the definition of a compartmental matrix, used to describe compartmental systems Jacquez and Simon (1993).

In our case, we check that the diagonal elements of $J(s,c,p)$ are negative and the off-diagonal elements nonnegative. Remark that it is a consequence of the signs of the variables. Moreover, the matrix is compartmental, the last property A.3 is also verified because the system is closed.

We still need the notion of irreducibility of a matrix, we give one of the possible definition

Definition 1. **Irreducible Matrix**
A matrix is irreducible if its graph is strongly connected (there is a directed path from any compartment to any other compartment).

We can draw the graph of $J(s,c,p)$ and check that it is strongly connected (under the assumption that all parameters are strictly positive). Then a powerful theorem applies:

Theorem 2. Property 5 in Bastin and Guffens (2006)
Let $M(s,c,p) = s + c + p$ the (fixed) total concentration of the closed system.
The Jacobian matrix of the system is irreducible (the system is strongly connected) and compartmental; then for any $M_0 > 0$, hyperplane $H_0 = \{(s,c,p) \in \mathbb{R}^3_+ : M(s,c,p) = M_0 > 0\}$ is forward invariant and contains a unique globally stable equilibrium in H_0.

The results of this theorem are very strong: they say that the equilibrium is unique on the hyperplane and globally stable with respect to this hyperplane. What is interesting is that the assumptions of this theorem are of a qualitative nature. It is even not necessary to compute the equilibrium.

Remark 1. If $c = E_0$, the Jacobian matrix is not irreducible any more, and that could be a problem to apply the theorem. But then $\dot{c} = -k_{-1}E_0 - k_2 E_0$ is negative (this point corresponds to $e = 0$) and the point is never attained.

3. THE OPEN REVERSIBLE MICHAELIS-MENTEN SYSTEM

Now we consider the case when the system is open: there is an input u for substrate, coming from "outside", and an output for the final product, taken as a linear decay rate $-kp$. These two assumptions are classical. We consider that the input u is fixed. The network is

$$\longrightarrow S + E \rightleftarrows C \rightleftarrows E + P \longrightarrow$$

and the system:

$$\begin{cases} \dfrac{ds}{dt} = u - k_1 se + k_{-1} c \\ \dfrac{de}{dt} = -k_1 se + k_{-1} c + k_2 c - k_{-2} pe \\ \dfrac{dc}{dt} = k_1 se - k_{-1} c - k_2 c + k_{-2} pe \\ \dfrac{dp}{dt} = k_2 c - k_{-2} pe - kp \end{cases} \tag{5}$$

The system can be reduced in the same way as before, because the first integral $\dot{e} + \dot{c} = 0$ still holds.

$$\begin{cases} \dfrac{ds}{dt} = u - k_1 s(E_0 - c) + k_{-1} c \\ \dfrac{dc}{dt} = k_1 s(E_0 - c) - k_{-1} c - k_2 c + k_{-2} p(E_0 - c) \\ \dfrac{dp}{dt} = k_2 c - k_{-2} p(E_0 - c) - kp \end{cases} \tag{6}$$

The new Jacobian matrix is $J_1(s,c,p)$

$$= \begin{pmatrix} -k_1(E_0 - c) & k_{-1} + k_1 s & 0 \\ k_1(E_0 - c) & -k_{-1} - k_1 s - k_2 - k_{-2} p & k_{-2}(E_0 - c) \\ 0 & k_2 + k_{-2} p & -k_{-2}(E_0 - c) - k \end{pmatrix} \tag{7}$$

It is still compartmental, because the sum of the last column is $-k$, and therefore negative. It is tempting to apply the following theorem (Jacquez and Simon (1993)):

Proposition 3. If $J(x)$ is a compartmental matrix $\forall x \in \mathbb{R}^n_+$, then all bounded trajectories converge toward an equilibrium in $\mathbb{R}^n_+$.

But, in our case, we will show that unbounded trajectories may exist; it comes from the fact that the system (6) may admit no equilibrium.

Proposition 4. There exists a positive equilibrium (s^*, c^*, p^*) (with $c^* < E_0$) if and only if $u < k_2 E_0$.

The biological interpretation is that there is an equilibrium if the input is not too large with respect to the total quantity of enzymes.

Proof: Summing the three equations at equilibrium, we obtain

$$u = kp^*$$

. The last equation gives:

$$k_2 c^* - k_{-2} p^* (E_0 - c^*) - kp^* = 0$$

therefore

$$c^* = \frac{u + k_{-2} E_0 u/k}{k_2 + k_{-2} u/k}$$

This last function, as a function of u, is increasing from 0 (for $u = 0$) to $E_0 + k/k_{-2}$ when u tends to infinity. This value is greater than E_0, but we have the constraint $c^* < E_0$, so the limit case is when

$$E_0 = \frac{u + k_{-2} E_0 u/k}{k_2 + k_{-2} u/k}$$

giving $u < k_2 E_0$. If this inequality is fulfilled, we can compute the value s^* from the first equation of (6) to obtain:

$$s^* = \frac{u + k_{-1} c^*}{k_1 (E_0 - c^*)}.$$

This function always exists if $0 < c^* < E_0$. ■

Proposition 5. If $u \geq k_2 E_0$, then there is no equilibrium and function $s(t) + c(t)$ is increasing and unbounded as t increases.

Proof: The fact that there is no equilibrium is given by the preceding proposition. We write

$$\dot{s} + \dot{c} = u - k_2 c + k_{-2} p(E_0 - c).$$

Because $u \geq k_2 E_0$, and $0 \leq c(t) \leq E_0$ and $p(t) \geq 0$, then

$$\dot{s} + \dot{c} \geq k_2 E_0 - k_2 E_0 = 0$$

Moreover, if $c = E_0$, $\dot{c}$ is negative, $c(t)$ decreases, and the above inequality for $\dot{s} + \dot{c}$ is strict if $c(0) \neq E_0$. ■

Biologically, it means that, if $u \geq k_2 E_0$ (the input is too large), then the sum $s + c$ will "explode" without bounds: the system is not viable (and has no equilibrium).

Now we explore the case when the system does have one equilibrium (that is $u < k_2 E_0$). We want to obtain the stability of this equilibrium, with a basin of attraction as large as possible. But it has also to fulfill the constraints on the variables (positivity and $C < E_0$). To show that all trajectories are bounded can be quite complex; here we will use a simple property of cooperative (or monotone) systems. The theory of monotone systems has strong links with compartmental systems, but it is more general. The basic facts are recalled in the appendix.

Monotone systems conserve the partial ordering of two solutions; if there is a point greater than the equilibrium point (with respect to the usual partial ordering) where all the derivatives are nonpositive, then the trajectory issued from this point is always decreasing, and converges toward the equilibrium point. Similarly, if there is a point smaller than the equilibrium where the derivatives are nonnegative, then the solution from this point increases until the equilibrium. The whole hyperrectangle built with these two points is invariant, and all the trajectories initiating in this rectangle converge toward the equilibrium.

Thus we have to find the two extreme points vertices of the rectangle. For the lower one, it is easy ; we choose $s = c = p = 0$ and obtain that the vector field is

$$\dot{s} = u; \dot{c} = 0; \dot{p} = 0$$

The vector field is nonnegative.

The case of the upper vertex is more intricate: we want to find a point (s,c,p) as large as possible, such that the vector field is nonpositive. If $c = E_0$, it does not work because $\dot{s} = u + k_{-1} E_0 > 0$. Thus we have to choose a c a bit smaller than E_0. Remember that $e = E_0 - c$. We write the inequality we wish to fulfill:

$$\begin{aligned} & u - k_1 s(E_0 - c) + k_{-1} c \leq 0 \\ & k_1 s(E_0 - c) - k_{-1} c - k_2 c + k_{-2} p(E_0 - c) \leq 0 \\ & k_2 c - k_{-2} p(E_0 - c) - kp \leq 0 \end{aligned} \tag{8}$$

Remark that if we fix $e = E_0 - c$, the system becomes linear with respect to the two other variables s and p. Summing the two first inequalities and replacing c by $E_0 - e$, we obtain:

$$k_{-2} pe < k_2 E_0 - u - k_2 e \tag{9}$$

But we know (because of the equilibrium existence) that $k_2 E_0 - u > 0$, therefore we can choose e very small such that $k_2 E_0 - u - k_2 e > 0$. Then we can choose

$$p = \frac{k_2 E_0 - u - k_2 e}{k_{-2} e}$$

The second inequality of the system is transformed with the help of (9):

$$k_1 se - k_{-1}E_0 + k_{-1}e - k_2 E_0 + k_2 e + k_{-2} pe <$$
$$k_1 se - k_{-1}E_0 + k_{-1}e - u$$

and is fulfilled if

$$s = \frac{k_{-1}E_0 - k_{-1}e + u}{k_1 e} \tag{10}$$

We remark that when $e \longrightarrow 0$ in the equations (9) (10), then the values of s and p tends to infinity. That means that the upper point can be chosen such that c is very close to E_0, and the values of s and p will be very large.

In conclusion we obtain the global stability in the hyperrectangle with the upper and lower vertices given above.

Proposition 6. If $u < k_2 E_0$, then the equilibrium exists ; it is globally stable in the invariant hyperrectangle defined by $(0,0,0)$ as the lower corner, and (s,c,p) as the upper corner, with $e = E_0 - c$ very small, and

$$p = \frac{k_2 E_0 - u - k_2 e}{k_{-2} e}$$
$$s = \frac{k_{-1}E_0 - k_{-1}e + u}{k_1 e}$$

The values of p and s tends to infinity when c tends to E_0.

The theorem says that if the equilibrium exists, it is globally stable in nearly all the possible space.

Applications. The most immediate application concerns the coupled metabolic/genetic systems. The concentrations of enzymes E_0 are slowly varying (they are fabricated by genes), and catalize metabolic reactions which are on a faster time-scale. Very often the QSSA is applied, but it is valid only if the fast system is globally stable: this is what we have shown above.

But the equilibrium may also disappear, if the concentrations E_0 is too weak with respect to the input u. In that case the solution becomes unbounded. If there is a switch between these two regimes (equilibrium exists or not), what is the result? For simplicity, we chose a varying input $u(t)$, having two values corresponding to the existence, or not, of an equilibrium. By varying the frequency of commutation between the two inputs, we may obtain a limit cycle which is globally stable (see Fig. 1). Our techniques could also prove this fact. The solution could also becomes unbounded if the time interval when there is no equilibrium is longer.

4. OPEN ENZYMATIC CHAIN WITH ONE INPUT AND OUTPUT

In this section we wish to apply the same tools to the classical case of a metabolic chains with input and output. Each step of the chain is a reversible enzymatic reaction, but here we will use a reduced Michaelis-Menten model. There is one input on the first metabolite, and one output on the last. The diagram of the chain is as follows:

$$u \rightarrow x_1 \leftrightarrows x_2 \leftrightarrows \dots x_i \leftrightarrows x_{i+1} \dots \leftrightarrows x_n \rightarrow$$

The vector $x \in X = R_+^n$ denotes the concentrations of the n variables. The expression of the velocity of reaction between x_i and x_{i+1} will be the classical (reduced with QSSA arguments) expression for reversible Michaelis-Menten reactions (Cornish-Bowden (2004)) (E_i is the enzyme concentration for the step i):

$$R_i(x_i, x_{i+1}) = E_i \frac{k_{i,i+1}x_i - k_{i+1,i}x_{i+1}}{K_{i,i+1} + k'_{i,i+1}x_i + k'_{i+1,i}x_{i+1}} \tag{11}$$

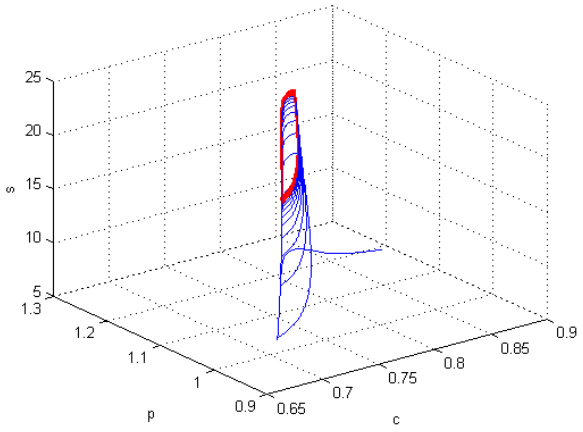

Fig. 1. Convergence of a trajectory toward a limit cycle (red) in the state space, with an input u commuting between $u_1 = 0.2$ (equilibrium exists) during $t_1 = 40$ and $u_2 = 0.8$ (no equilibrium) during $t_2 = 15$. Other parameters $k_{-2} = 0.2, k_{-1} = 0.3, k_1 = 0.2, k_2 = 0.5, E_0 = 1, k = 0.3, s(t_0) = 12, c(t_0) = 0.8, p(t_0) = 1$.

The mathematical model, expressed with a stoichiometric form (A is a matrix with n rows and $(n-1)$ columns), is

$$\dot{x} = AR(x) + U - \Gamma x \tag{12}$$

with $A = \begin{pmatrix} -1 & 0 & 0 & \dots & 0 \\ 1 & -1 & 0 & \dots & 0 \\ \vdots & \ddots & \ddots & \ddots & \vdots \\ 0 & \dots & 0 & 1 & -1 \\ 0 & \dots & 0 & 0 & 1 \end{pmatrix}$

and R as above, U is the input $U = (u, 0, 0, \dots, 0)^T$ and Γx is the output vector $\Gamma = (0, 0, \dots, kx_n)^T$, k is a positive constant standing for the output (or degradation) of x_n.

The first point is that an equilibrium does not always exist.

4.1 Equilibrium

We solve $\dot{x}_i = 0 \;\; for \;\; i = 1, \dots, n$. Then $\sum \dot{x}_i = 0 \;\; \Rightarrow \;\; u = kx_n^*$. Using $\sum_{j=1}^{i} \dot{x}_j = 0, \;\; i = 1, \dots, n-1$, we obtain :

$$u = R_i$$

Then

$$x_i^* = \frac{E_i k_{i+1,i} x_{i+1}^* + u(K_{i,i+1} + k'_{i+1,i} x_{i+1}^*)}{E_i k_{i,i+1} - k'_{i,i+1} u}$$

The constraint is:

$$E_i k_{i,i+1} - k'_{i,i+1} u > 0 \;\; \Rightarrow \;\; u < \frac{k_{i,i+1}}{k'_{i,i+1}} E_i \; \forall i$$

Suppose that this condition is fulfilled, and $u = kx_n^*$.

Then $x_{n-1}^* = \frac{E_{n-1}k_{n,n-1}x_n^* + u(K_{n-1,n} + k'_{n,n-1}x_n^*)}{E_{n-1}k_{n-1,n} - k'_{n-1,n}u}$ also exists, and we proceed iteratively. Moreover, the equilibrium is positive.

Proposition 7. System (12) has a unique positive equilibrium if and only if

$$u < \frac{k_{i,i+1}}{k'_{i,i+1}} E_i \ \forall i \tag{13}$$

Proposition 8. System (12) admits no equilibrium if there is an index i such that $u \geq \frac{k_{i,i+1}}{k'_{i,i+1}} E_i$.

4.2 Stability when $u < \frac{k_{i,i+1}}{k'_{i,i+1}} E_i \quad \forall i$

We are in the case when there is one single equilibrium x^*. First we recall some definitions and properties, see Bastin and Guffens (2006) .

Definition 9. Fully outflow connected network

A compartment x_i is outflow connected if there is a path $x_i \rightarrow x_j \rightarrow ... \rightarrow x_l$ from x_i until a compartment x_l with an outflow. The network is fully outflow connected if all compartments are outflow connected.

Proposition 10. Invertibility and stability of a compartmental matrix: a compartmental matrix is regular and stable if and only if the network is fully outflow connected.

Intuitively, it means that the system has no traps where the flows accumulate (see Jacquez and Simon (1993)).

The property 3 can also be applied because the Jacobian matrix J_3 of model (12) is:

$$J_3 = \begin{pmatrix} -\frac{\partial R_1}{\partial x_1} & -\frac{\partial R_1}{\partial x_2} & 0 & \cdots & \cdots \\ \frac{\partial R_1}{\partial x_1} & \frac{\partial R_1}{\partial x_2} - \frac{\partial R_2}{\partial x_2} & -\frac{\partial R_2}{\partial x_3} & 0 & \cdots \\ \vdots & \ddots & \ddots & \ddots & \vdots \\ 0 & \cdots & \frac{\partial R_{n-2}}{\partial x_{n-2}} & \frac{\partial R_{n-2}}{\partial x_{n-1}} - \frac{\partial R_{n-1}}{\partial x_{n-1}} & -\frac{\partial R_{n-1}}{\partial x_n} \\ 0 & \cdots & \cdots & \frac{\partial R_{n-1}}{\partial x_{n-1}} & -k + \frac{\partial R_{n-1}}{\partial x_n} \end{pmatrix}$$

and it is easy to check it is a compartmental matrix, because of the signs of the derivatives in R_i. We prove that the trajectories are bounded, with the help of a norm-like function V:

$$V(x) = \sum_{i=1}^{n} |x_i - x_i^*| \tag{14}$$

This function is not differentiable at $x_i = x_i^*$, and we use the right Dini derivative with respect to time (Siljak (1978)) and define the operator:

$$\sigma_i = \begin{cases} 1 \ if \ \ x_i(t) > x_i^* \ \ or \ \ if \ \ x_i(t) = x_i^* \ \ and \ \dot{x}_i(t) > 0 \\ 0 \ \ if \ \ x_i(t) = x_i^* \ \ and \ \ \dot{x}_i(t) = 0 \\ -1 \ if \ \ x_i(t) < x_i^* \ \ or \ \ if \ \ x_i(t) = x_i^* \ \ and \ \dot{x}_i(t) < 0 \end{cases} \tag{15}$$

We compute the right derivative of $V(x(t)) = \sum_{i=1}^{n} \sigma_i (x_i - x_i^*)$:

$$\frac{d^+}{dt} V(x(t)) = \sum_{i=1}^{n} \sigma_i (\dot{x}_i - \dot{x}_i^*)$$

$$= -\sigma_1 (R_1 - R_1^*) + \sum_{i=2}^{n-1} \sigma_i (R_{i-1} - R_{i-1}^* - R_i + R_i^*) + \sigma_n (R_{n-1} - R_{n-1}^* - k(x_n - x_n^*)) = \sum_{i=1}^{n-1} (R_i - R_i^*)(\sigma_{i+1} - \sigma_i) - \sigma_n k (x_n - x_n^*)$$

For each term $(R_i - R_i^*)(\sigma_{i+1} - \sigma_i)$, we have two cases (the cases with $\sigma_i = 0$ can be neglected because they correspond to the corners of the level sets of the function, and the value in these points is a consequence of the continuity of the vector field):

- If $\sigma_i = \sigma_{i+1} \Rightarrow (R_i - R_i^*)(\sigma_{i+1} - \sigma_i) = 0$
- If $\sigma_i = -\sigma_{i+1}$ then we compute the sign of
  $R_i - R_i^* = \frac{(x_i - x_i^*)[k_{i,i+1} K_{i,i+1} + (k_{i,i+1} k'_{i+1,i} + k'_{i,i+1} k_{i+1,i}) x_{i+1}^*]}{(K_{i,i+1} + k'_{i,i+1} x_i + k'_{i+1,i} x_{i+1})(K_{i,i+1} + k'_{i,i+1} x_i^* + k'_{i+1,i} x_{i+1}^*)}$
  $- \frac{(x_{i+1} - x_{i+1}^*)[k_{i+1,i} K_{i,i+1} + (k_{i,i+1} k'_{i+1,i} + k'_{i,i+1} k_{i+1,i}) x_i^*]}{(K_{i,i+1} + k'_{i,i+1} x_i + k'_{i+1,i} x_{i+1})(K_{i,i+1} + k'_{i,i+1} x_i^* + k'_{i+1,i} x_{i+1}^*)}$
  and check that if $\sigma_i = \text{sign}(x_i - x_i^*) = -\text{sign}(x_{i+1} - x_{i+1}^*)$ then $(R_i - R_i^*)$ has the same sign as σ_i, and therefore $(R_i - R_i^*)(\sigma_{i+1} - \sigma_i) \leq 0$.
  Therefore all the terms (included the last one $\sigma_n k(x_n - x_n^*)$) are non positive and

$$\frac{d^+}{dt} V(x(t)) \leq 0$$

We deduce that all trajectories are bounded, and apply property 3. We cannot use this function as a Lyapunov function because we were not able to prove easily that the derivative only cancels at the equilibrium.

Proposition 11. The Jacobian matrix J_3 is compartmental and all trajectories are bounded, therefore all trajectories tend to a unique equilibrium in $\mathbb{R}^n_+$ which is globally attractive.

The Jacobian matrix J_3 is strongly connected and because the model has one outflow on the last variable, we know that property 9 is verified (the model is fully outflow connected). Property 10 gives us the global stability.

Proposition 12. Matrix J_3 is regular and stable, therefore the equilibrium is locally asymptotically stable.

Finally, the equilibrium is locally stable and globally attractive, and thus we have finally:

Proposition 13. If $u < \frac{k_{i,i+1}}{k'_{i,i+1}} E_i$ for all i, all trajectories tend to a unique globally stable equilibrium in $\mathbb{R}^n_+$.

4.3 Case $\exists i \ ; u \geq \frac{k_{i,i+1}}{k'_{i,i+1}} E_i$

Consider now the case $u \geq \frac{k_{i,i+1}}{k'_{i,i+1}} E_i$ then the equilibrium does not exist. The equation for x_n still give the result: $u = k x_n^*$, but equation for another coordinate (at least) has no solution. It is easy to see that at least one variable x_i will tend to infinity.

Biologically, it means that the system is not "sustainable" or "viable": one metabolite will grow without bound. We see from the equations that this happens if the input u is too large, or the concentrations in enzymes E_i too small. We remark that an equilibrium may exist for some u and disappear if u increases. As above, these results are useful when the metabolic system is coupled with a genetic/enzymatic system where the E_i are variables.

5. CONCLUSION

We applied tools from monotone systems theory and compartmental systems theory to the study of stability of the full open and closed Michaelis-Menten system, and of metabolic

chains. We were able to show stability in a simple and global way. For the open systems, we showed that if the input u is too large, there is no equilibrium. We think that these kind of tools could be applied to other metabolic systems, and to coupled metabolic/genetic systems. For example, a simple generalization would be to study an open chain of full reversible Michaelis-Menten systems.

REFERENCES

G. Bastin and V. Guffens. Congestion control in compartmental network systems. *Systems and Control Letters*, 55:689–696, 2006.

K. Bettenbrock, S. Fischer, A. Kremling, K. Jahreis, T. Sauter, and E.D. Gilles. A quantitative approach to catabolite repression in escherichia coli. *Journal of Biological Chemistry*, 281 (5):2578, 2006.

A. Cornish-Bowden. *Fundamentals of enzyme kinetics*, volume 3. Portland Press London, 2004.

P. De Leenheer, D. Angeli, and E.D. Sontag. Monotone chemical reaction networks. *Journal of mathematical chemistry*, 41(3):295–314, 2007.

L. Edelstein-Keshet. *Mathematical Models in Biology*. The Random House, 1988.

Edward H. Flach and Santiago Schnell. Stability of open pathways. *Mathematical Biosciences*, 228(2):147 – 152, 2010.

Simon J. Fraser and Marc R. Roussel. Phase-plane geometries in enzyme kinetics. *Canadian Journal of Chemistry*, 72(3): 800–812, 1994.

A. Goelzer, F.B. Brikci, I. Martin-Verstraete, P. Noirot, P. Bessières, S. Aymerich, and V. Fromion. Reconstruction and analysis of the genetic and metabolic regulatory networks of the central metabolism of bacillus subtilis. *BMC systems biology*, 2(1):20, 2008.

R. Heinrich and S. Schuster. *The regulation of cellular systems*. Chapman & Hall. New York. US, 1996.

J. A. Jacquez and C. P. Simon. Qualitative theory of compartmental systems. *SIAM Review*, 35:43–79, 1993.

J.D. Murray. *Mathematical biology*. Springer, 2002.

D.A. Oyarzún, M. Chaves, and M. Hoff-Hoffmeyer-Zlotnik. Multistability and oscillations in genetic control of metabolism. *Journal of Theoretical Biology*, 295:139–153, 2012.

L. Perko. *Differential equations and dynamical systems*. Springer, Berlin, 2001.

L.A. Segel and M. Slemrod. The quasi-steady-state assumption: a case study in perturbation. *SIAM review*, pages 446–477, 1989.

D. Siljak. *Large-scale dynamic systems: stability and structure*. North Holland, 1978.

H. L. Smith. *Monotone Dynamical Systems: An introduction to the theory of competitive and cooperative systems*, volume 41. American Mathematical Soc. Mathematical surveys and monographs, 1995.

E.D. Sontag. Some new directions in control theory inspired by systems biology. *Syst. Biol*, 1(1):10, 2004.

Ralf Steuer and Bjorn H. Junker. Computational models of metabolism: Stability and regulation in metabolic networks. In *Advances in Chemical Physics*, pages 105–251. John Wiley and Sons, 2008.

Chen-Hsiang Yeang. Integration of metabolic reactions and gene regulation. *Molecular Biotechnology*, 47:70–82, 2011.

Appendix A. MONOTONE AND COMPARTMENTAL SYSTEMS

Monotone systems form an important class of dynamical systems, and are particularly well adapted to mathematical models in biology (Sontag (2004)), because they are defined by conditions related to the signs of Jacobian matrix. Such a sign for one element traduces the fact that some variable will contribute positively to the variation of some other variables, and this kind of qualitative dependence is very frequent in biological models. The reader may consult the references Smith (1995) for a review or an exhaustive presentation of the theory of monotone systems.

In summary, if the system is cooperative, then the flow preserves the partial order in $\mathbb{R}^n$ (the flow is monotone). Cooperativity is easy to check by looking at the signs of the elements of the Jacobian matrix, that should verify

$$\frac{\partial f_i}{\partial x_j}(t,x) \geq 0 \quad \forall i \neq j$$

These systems have a strong tendency to converge to the set of their equilibria (Smith (1995)). It can be shown that almost any solution converges to the set of equilibria except a set of zero measure. In particular, there are no stable periodic solutions. For more precise theorems, see Smith (1995). Here we only need a simple proposition, easily deduced from Proposition 2.1 p. 34 of Smith (1995). The system is defined on a convex set X.

Proposition 14. Let us suppose that only one equilibrium x^* exists in X; if moreover it exists two point x^+, x^- in X such that $f(x^+) \leq 0$ and $f(x^-) \geq 0$, with $x^- \leq x^* \leq x^+$, then the hyperrectangle built by the two points x^-, x^+ is invariant, and every solution in this rectangle converges toward the equilibrium point.

Let us now give a few reminders about compartmental systems (see Jacquez and Simon (1993)). This kind of models describes the dynamics of n-compartments interconnected by links with fluxes of matter. The overall equation is written by making a global mass balance between inputs and outputs of each compartment. The definition of a compartmental matrix is the following:

Definition 15. **Compartmental Matrix**
Matrix $f_{(n\times n)}$ is a compartmental matrix if it satisfies the following three properties (Jacquez and Simon (1993)):

$$f_{ii} \leq 0 \quad for \quad all \ i, \tag{A.1}$$

$$f_{ij} \geq 0 \quad for \quad all \ i \neq j, \tag{A.2}$$

$$-f_{jj} \geq \sum_{i \neq j} f_{ij} \quad for \quad all \quad j \tag{A.3}$$

Note that f_{ij} can in general depend on $x_k, k = 1 \ldots n$ which are the concentrations in each compartment. A common case is when f_{ij}, flow of compartment j in the compartment i, depends only on x_j (thus on the concentration of the initial compartment). This is not the case in our systems. There are also some theorems on the stability of linear and nonlinear compartmental systems (see Jacquez and Simon (1993)).