Equilibria and stability analysis of a branched metabolic network with feedback inhibition

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Abstract:

This paper deals with the analysis of a metabolic network with feedback inhibition. The considered system is an acyclic network of mono-molecular enzymatic reactions in which metabolites can act as feedback regulators on enzymes "at the beginning" of their own pathway, and in which one metabolite is the root of the whole network. We show, under mild assumptions, the uniqueness of the equilibrium. We then show that this equilibrium is globally attractive if we impose conditions on the kinetic parameters of the metabolic reactions.

1 Introduction

The cellular metabolism is defined as the (huge) set of biochemical reactions that occur inside a living cell for growth and reproduction. It is usually represented by an intricate network connecting the involved biochemical species (called "metabolites"). The pathways of the network are called "metabolic pathways". In the metabolic engineering literature, it is widely accepted that "despite their immense complexity, metabolic systems are characterized by their ability to reach stable steady states" (quoted from [8], Chapter 4). It should however be fair to recognize that a mathematical analysis of this fundamental stability property is a difficult question which was not much investigated. We shall limit ourselves to simple metabolic pathways which are made up of sequences of mono-molecular enzyme-catalysed reactions in the form

$$X_s \to X_p$$

whose velocity can be written as

$$\varphi_{sp}(x_s, x^{[p]})$$

where x_s is the concentration of the metabolite X_s in the cell and $x^{[p]}$ is a vector containing the concentrations of the metabolites inhibiting the reaction $X_s \to X_p$. The stability analysis of metabolic networks without this inhibition is straightforward because the corresponding mass-balance models are cooperative; we will therefore concentrate on networks with inhibition, like the one given by the aspartate amino-acid pathways [9], see Figure 1. In the network of Figure 1, each produced amino-acid inhibits an enzyme of its own pathway. This action can be seen as a negative feedback, that regulates the behavior of the network. Indeed, if we, for example, consider a large excess of isoleucine (X_{20}), the reaction $X_{16} \longrightarrow X_{17}$ is shut down, so that the concentration of isoleucine is progressively reduced. In this paper, we will give conditions for the stability of the equilibrium of such a network and show that inhibition, which is intuitively seen as regulation, can also lead to the destabilization of the system.

The structure of the paper is as follows: in Section 2, a mass balance dynamical model of metabolic networks such as the one of Figure 1 will be presented. The equilibria of these models will then be studied in Section 3, followed by a stability analysis in Section 4, where global attractivity of a unique equilibrium is shown under some assumptions on the kinetic parameters. The non-genericity of the stability of the equilibrium is then illustrated in Section 5 on a metabolic chain with *sequential feedback inhibition* (cf. [1])

$$X_1 \to X_2 \to \cdots \to X_n.$$

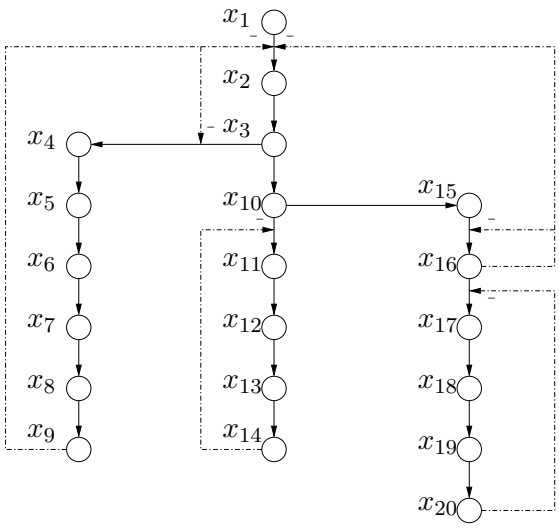

Figure 1: Metabolic network representing the aspartate amino-acid pathways: the solid lines represent the reactions and the dash-dotted lines the inhibition produced by the state at the start of the arrow onto the reaction that lies at the end of the arrow. The root of the metabolic pathway is x_1 (aspartate), and the products are the corresponding amino acids: lysine (x_9), methionine (x_{14}), threonine(x_{16}), and isoleucine (x_{20}).

with the last metabolite X_n acting as an inhibitor of the first reaction X_1.

2 Model of a metabolic network

In our model of a metabolic network made of enzyme-catalysed reactions in the form

$$X_s \to X_p$$

which are inhibited by other metabolites of the network, we will denote the inhibition factor by $x^{[p]}$, a n_p dimensional vector containing the molar fractions of all the metabolites inhibiting the reaction $X_s \to X_p$. This reaction is characterized by a velocity

$$\varphi_{sp}(x_s, x^{[p]})$$

Those reactions are not the only ones that we should incorporate in our model. Indeed, some metabolites of the considered network are used in stages of the metabolism that are not modelled in the considered network. Therefore, we must include consumption terms in the model for those reactions in the form $X_s \to ...$ We will generically denote those terms $\varphi_{s0}(x_s)$.

Because it is natural to think that the larger the concentration of X_s is, the faster the reaction will take place, and the larger the concentration of any given inhibitor is, the slower the reaction will take place, we impose the following assumption for the reaction velocities:

Assumption 1 • *For all s, p such that the reaction $X_s \to X_p$ belongs to the metabolic network, the function*

$$\varphi_{sp}(x_s, x^{[p]})$$

is locally Lipschitz on $\mathbb{R}_+ \times \mathbb{R}_+^{n_p}$, satisfies $\varphi_{sp}(0) = 0$, is non-decreasing in x_s for $x_s \geq 0$ and non-increasing in $x_j^{[p]}$ for $x_j^{[p]} \geq 0$. Note that this must also be valid when the value of p is 0.

We will represent the networks as graphs; we therefore need the following definition from graph theory to precisely define the class of metabolic networks that we consider:

Definition 1 *A directed graph is called an arborescence if, from a given node x, known as the root node, there is exactly one elementary path from this node to any other node y.*

which leads to the following assumption that we impose on the metabolic networks that we consider

Assumption 2 a) *the involved species are denoted $X_1, X_2, \cdots, X_n$*

b) *the graphic representation of the network (with the different metabolites as nodes and the different reactions as oriented edges) is an arborescence with X_1 as root*

c) *the inhibition acting on a reaction $X_s \to X_p$ only results from the action of metabolites from the (sub)-arborescence rooted in X_p*

d) *a reaction $X_s \to X_p$ can only be inhibited if $s = 1$.*

Note that the aspartate-amino acids network that is represented as a graph on Figure 1 satisfies the points a)-b) and c) of this assumption.

Stemming from the definition and known properties of arborescences, Assumption 2b has the following consequence on the class of metabolic networks that we consider:

(i) Each metabolite is produced by a single other metabolite;

(ii) There is no cycle of reactions;

With these definitions and notations, we shall now define a mass-balance dynamical model in the form

$$\dot{x} = \Phi(x) - \mu x + ce_1$$

where $x = (x_1, \cdots, x_n)^T \in \mathbb{R}^n_+$, and x_i denotes the molar fraction of the metabolite X_i inside the cell. The factor $\mu \geq 0$ represents the specific growth rate of the cell: we assume that the cell metabolism is analyzed during a period of exponential cell growth with a constant specific growth rate μ. The vector $e_1 = (1, 0, \cdots, 0)^T$ and the scalar c denote the constant supply rate of the metabolite X_1 at the root of the network. The function Φ includes all the reaction velocities, whether they correspond to reactions inside the network or reactions consuming the metabolites of the network for use in subsequent stages of the metabolism.

In order to specify $\Phi(x)$, we introduce the following notations:

Notation 1

- $\mathcal{P}(j) = \{k|$ *the reaction* $X_j \to X_k$ *belongs to the network* $\}$. $\mathcal{P}(j)$ *defines the set of all metabolites that are produced by reactions having* X_j *as substrate. If there is a consumption term in the form* $\varphi_{j0}(x_j)$ *in the derivative of* x_j*, the index 0 is included in* $\mathcal{P}(j)$.
- $\mathcal{A}(j) = \{k|X_k$ *belongs to the arborescence with its root in* $X_j\}$. *"0" is not included in* $\mathcal{A}(j)$.

It can easily be seen that $\mathcal{P}(j) \setminus \{0\}$ is a subset of $\mathcal{A}(j)$ because of Assumption 2c, and that for all $k \notin \mathcal{P}(1)$, $x^{[k]} = \emptyset$ because of Assumption 2d.

From the arborescence structure, it is clear that we can separate the metabolites into three different families:

- the root X_1: noting that there is a constant supply rate c of X_1, the corresponding mass-balance equation is the following:

$$\dot{x}_1 = c - \sum_{k \in \mathcal{P}(1)} \varphi_{1k}(x_1, x^{[k]}) - \mu x_1 \tag{1}$$

- the intermediate metabolite X_j, which is the result of the reaction $X_i \to X_j$:

$$\dot{x}_j = \varphi_{ij}(x_i, x^{[j]}) - \sum_{k \in \mathcal{P}(j)} \varphi_{jk}(x_j) - \mu x_j \tag{2}$$

- the boundary metabolite X_j (such that $\mathcal{P}(j) = \{0\}$ or $\emptyset$), which is the product of a reaction $X_i \to X_j$:

$$\dot{x}_j = \varphi_{ij}(x_i, x^{[j]}) - \varphi_{j0}(x_j) - \mu x_j \tag{3}$$

Under Assumption 2, we can only have $x^{[j]} = x_j$ (only if $i = 1$) or $x^{[j]} = \emptyset$.

A particular case of this network is the metabolic chain with *sequential feedback inhibition* (cf. [1]) that we presented in the Introduction and that we will develop in Section 5.

3 Equilibrium of a metabolic network

The first step in our analysis of the behavior of the network consists in the search of equilibria for our model. Based on equations (1)-(2)-(3), we can compute the mass-balance of the whole arborescence:

$$\frac{d}{dt}(\sum_{l=1}^{n} x_l) = c - \mu \sum_{l=1}^{n} x_l - \sum_{\{k|0 \in \mathcal{P}(k)\}} \varphi_{k0}(x_k) \tag{4}$$

and of the arborescence that has its root in X_j

$$\frac{d}{dt}(\sum_{l \in \mathcal{A}(j)} x_l) = \varphi_{ij}(x_i, x^{[j]}) - \mu \sum_{l \in \mathcal{A}(j)} x_l - \sum_{\{k|0 \in \mathcal{P}(k) \text{ and } k \in \mathcal{A}(j)\}} \varphi_{k0}(x_k) \tag{5}$$

Those expressions will be critical in the proof of the following proposition:

Proposition 1 *If Assumptions 1 and 2 are satisfied, then:*

(A) *the system (1)-(2)-(3) is positive;*

(B) *if all $\varphi_{s,p}(x_s, x^{[p]})$ (where p can be 0) are increasing in x_s then there is at most one equilibrium $\bar{x} = (\bar{x}_1, \cdots, \bar{x}_n)$ of (1)-(2)-(3) in $\mathbb{R}^n_+$;*

(C) *if $\mu > 0$, then system (1)-(2)-(3) has a unique equilibrium $\bar{x} = (\bar{x}_1, \cdots, \bar{x}_n)$ in $\mathbb{R}^n_+$. Moreover, the solutions of (1)-(2)-(3) are bounded for any initial condition in $\mathbb{R}^n_+$.*

Proof: (A) is easily seen by considering the system on the boundaries of the positive orthant.

The proofs of (B) and (C) are very similar. We write the proof for (B) and highlight the differences that arise for the proof of (C) .

We will first consider system (2)-(3) with $x_1 = \bar{x}_1$ as constant input. For any value of $\bar{x}_1$, we will denote by $(\bar{x}_2, \cdots, \bar{x}_n)$ the equilibrium of (2)-(3); this equilibrium is a function of $\bar{x}_1$, so that we will state that it is $(\bar{x}_2, \cdots, \bar{x}_n)(\bar{x}_1)$. We will now show, by induction, that every element $\bar{x}_i$ is an increasing function of $\bar{x}_1$ (resp. non-decreasing in case (C)).

The initial step of the proof considers the equilibrium of (3), an equation characterizing the evolution of the concentration of a boundary metabolite, with $x_i = \bar{x}_i$ as constant input

$$\varphi_{ij}(\bar{x}_i, \bar{x}^{[j]}) - \varphi_{j0}(\bar{x}_j) - \mu \bar{x}_j = 0$$

where $\bar{x}^{[j]} = \bar{x}_j$ or $x^{[j]} = \emptyset$. When $\bar{x}_i = 0$, $\bar{x}_j = 0$ is the only solution. Also, the left-hand side of this equation is an increasing function of $\bar{x}_i$ (resp. non-decreasing in case (C)) and a decreasing function of $\bar{x}_j$. It is then easily seen that, if we increase $\bar{x}_i$, $\bar{x}_j$ needs also to be increased (resp.increased or kept constant) to keep this equality satisfied. We then have that, in this case, $\bar{x}_j(\bar{x}_i)$ is an increasing function such that $\bar{x}_j(0) = 0$ (resp. non-decreasing function such that $\bar{x}_j(0) = 0$). When $\mu = 0$ (which can only happen in case (B)), the definition of $\bar{x}_j(.)$ could be limited to an interval $[0, \bar{x}_i^m)$ with $\lim_{\bar{x}_i \to \bar{x}_i^m} \bar{x}_j(\bar{x}_i) = +\infty$. Indeed, in the case where $\varphi_{j0}(x_j) < B_j$ for all $x_j \geq 0$ and for some $B_j > 0$, there might exist some $\bar{x}_i^m$ such that $\varphi(\bar{x}_i, \bar{x}_j) > B_j$ for all $\bar{x}_i > \bar{x}_i^m$ and all $\bar{x}_j \geq 0$.

Let us now make the following induction hypothesis: for a given j, the functions $\bar{x}_k(\bar{x}_j)$ are increasing (resp. non-decreasing) functions for all $k \in \mathcal{A}(j)$ with $\bar{x}_k(0) = 0$. We then study the equilibrium of the mass-balance of the arborescence that has its root in X_j. From (5):

$$\varphi_{ij}(\bar{x}_i, \bar{x}^{[j]}(\bar{x}_j)) - \mu \sum_{l \in \mathcal{A}(j)} \bar{x}_l(\bar{x}_j) - \sum_{\{k | 0 \in \mathcal{P}(k) \text{ and } k \in \mathcal{A}(j)\}} \varphi_{k0}(\bar{x}_k(\bar{x}_j)) = 0$$

With a similar argument to that of the initial step, we see that $\bar{x}_j$ is an increasing (resp. non-decreasing) function of $\bar{x}_i$ and that $\bar{x}_j(0) = 0$. The same can be said for all $\bar{x}_k$ with $k \in \mathcal{A}_j$ because they are already increasing (resp. non-decreasing) functions of x_j.

By induction, we then have that every $\bar{x}_k(\bar{x}_1)$ is an increasing function of $\bar{x}_1$ defined on the interval $[0, \bar{x}_1^m)$ (resp. non decreasing function defined for all $\bar{x}_1$). An equilibrium of the whole system then has to satisfy the equilibrium of the total mass-balance. From (4), this comes to:

$$\mu \sum_{l=1}^{n} \bar{x}_l(\bar{x}_1) + \sum_{\{k | 0 \in \mathcal{P}(k)\}} \varphi_{k0}(\bar{x}_k(\bar{x}_1)) = c$$

The system admits as many equilibria as this equation has roots. In case (B), the left-hand side is increasing from 0 when $\bar{x}_1$ increases from 0 to $\bar{x}_1^m$ (because of the second term). Therefore, if there exists an equilibrium, it is unique. In case (C), the left-hand side is increasing from 0 to $+\infty$ when $\bar{x}_1$ increases from 0 to $+\infty$ (because of the $\mu \bar{x}_1$ term), so that the equilibrium exists and it is unique.

The final point of (C) is a direct consequence of (4); this implies

$$\frac{d}{dt}(\sum_{l=1}^{n} x_l) \leq c - \mu \sum_{l=1}^{n} x_l$$

which clearly implies boundedness of the solutions when $\mu > 0$. □

We have shown in [4] that this result is valid for a larger class of metabolic networks, namely networks that do not satisfy Assumption 2d, where inhibiting metabolites are not restricted to act

on reactions that have X_1 as substrate, but can act on any reactions that are upstream in their own pathway (so that it is valid for the aspartate-amino acids pathways). Note that uniqueness of this equilibrium is also a consequence of the results of [3, 5, 7], which state that at least a positive feedback loop is necessary in the system in order to have multiple equilibria; the absence of positive feedback loop can be shown in our model.

Uniqueness of the equilibrium, especially when it is coupled with boundedness of solutions, gives hope of having some general result about the structural global asymptotic stability of the equilibrium. In the next section, we will impose constraints on the velocities so that we will be able to prove global attractivity of the single equilibrium.

4 Stability of a class of metabolic networks

In this section, we will study the stability of the equilibrium of the class of metabolic networks that was described in Section 2. In order to do that, we will first present a technical lemma and a useful corollary.

4.1 Technical lemma

In order to analyze the stability of the considered metabolic networks, we will use the small-gain theorem of [2] for the interconnection of monotone systems. This states:

Theorem 1 *Consider the following two systems*

$$\dot{x}_1 = f_1(x_1, u_1), \qquad y_1 = h_1(x_1) \tag{6}$$

$$\dot{x}_2 = f_2(x_2, u_2), \qquad y_2 = h_2(x_2) \tag{7}$$

where $x_i \in \mathbb{R}^{n_i}_+$, $u_1, y_2 \in \mathbb{R}^m$, *and* $u_2, y_1 \in \mathbb{R}^p$, f_i *and* h_i *are smooth (with* h_i *increasing). Assume that:*

(i) *for any fixed* u_1, *system (6) is cooperative;*

(ii) $\frac{\partial f_{1j}}{\partial u_{1k}} \geq 0$ *for* $j \in \{1, \cdots, n_1\}, k \in \{1, \cdots, m\}$;

(iii) *for any fixed* u_1, *system (6) has a unique equilibrium* $k_{x_1}(u_1)$, *which is globally attractive. At the equilibrium, the output takes the value* $k_{y_1}(u_1) = h_1(k_{x_1}(u_1))$, *which is called the static input/output characteristic.*

Assumptions on (7) are similar to that on (6).

If both systems are interconnected through the negative feedback loop

$$u_2 = y_1, \;\; u_1 = -y_2$$

and all forward solutions of the feedback system are bounded then the feedback system possesses a globally attractive fixed point if the discrete-time system

$$u_{k+1} = -(k_{y_2} \circ k_{y_1})(u_k)$$

with $u_k \in \mathbb{R}^m$, possesses a unique globally attractive fixed point.

The application of this theorem presents two challenges: showing that, for u_1 constant, the system $\dot{x}_1 = f_1(x_1, u_1)$ presents a unique equilibrium, and showing the convergence of $u_{k+1} = -(k_{y_2} \circ k_{y_1})(u_k)$. Both those challenges will be answered by Banach fixed-point theorem that states that, if a mapping T is a contraction, that is, if there exists $0 \leq q < 1$ such that for all u, v

$$\|T(u) - T(v)\| \leq q\|u - v\|$$

then the mapping presents a unique fixed-point u^* such that $u^* = T(u^*)$ and the sequence $u_{k+1} = T(u_k)$ globally converges to u^*.

In our main stability theorem, we will have to analyze the stability of a system

$$\dot{x} = f(x) \quad x \in \mathbb{R}^n_+ \tag{8}$$

which is not cooperative. However, we will make the following assumption:

Assumption 3 *Each off-diagonal element of the Jacobian $\mathcal{J}$ of $f(x)$ is sign-definite (the sign is independent of x).*

We then build a new system $\dot{x} = F(x, -v)$ according to the following construction: for all i, j with $j \neq i$, if $\mathcal{J}_{ij} < 0$, then replace x_j in $f_i(x)$ with the (negative) constant $-v_j$. Define u_1 as the vector that contains all the constants v_j that are necessary for the preceding construction (not necessarily all j have been required); we denote those constants by $v_{s_1}, \cdots, v_{s_l}$ so that $u_1 \in \mathbb{R}^l_-$. From this construction, it directly appears that assumptions (i) and (ii) of Theorem 1 on equation (6) are satisfied by the system

$$\dot{x} = F(x, -u_1) \tag{9}$$

We then define the output of (9) as $y_1 = (x_{s_1}, \cdots, x_{s_l})^T$. The interconnection of system (9) with the trivial system

$$y_2 = u_2 \tag{10}$$

through the feedback connections $u_1 = -y_2$ and $u_2 = y_1$ results in the original system (8). All the assumptions of Theorem 1 are satisfied by system (10), so that the main difficulty for the application of Theorem 1 is therefore concentrated on

- the uniqueness and global attractivity of the equilibrium of system (9) with constant u_1 ($\in \mathbb{R}^l_-$)
- the analysis of convergence of system $u_{k+1} = -k_{y_1}(u_k)$.

This is solved through the following lemma:

Lemma 1 Suppose that the solutions of (8) are bounded and that system (9) has a unique globally attractive equilibrium for any constant input $u_1 \in \mathbb{R}^l_-$ (this equilibrium defines an input output characteristic $k_{y_1}(u_1)$ for system (9)). Then system (8) has a unique globally attractive equilibrium if there exists a vector norm $\|.\|$ (whose definition is readily extended to a matrix-norm) such that

$$\sup_{u_1 \in \mathbb{R}^l_-} \|\frac{\partial k_{y_1}}{\partial u_1}\| < 1 \tag{11}$$

Proof: The assumption of existence of a unique globally asymptotically stable equilibrium for system (9) with constant input implies that Assumption (iii) of Theorem 1 is satisfied with system (9) (as well as Assumptions (i) and (ii) which were already satisfied). We therefore have to concentrate on the analysis of $u_{k+1} = -k_{y_1}(u_k)$. We will now show that it is a contraction. Let us now use the norm that was defined in the statement of the lemma. We have

$$\begin{aligned} \|k_{y_1}(v) - k_{y_1}(u)\| &= \|\int_0^1 \frac{\partial k_{y_1}}{\partial u_1}(v-u)ds\| \\ &\leq \|\int_0^1 \frac{\partial k_{y_1}}{\partial u_1}ds\|\|v-u\| \\ &\leq \int_0^1 \|\frac{\partial k_{y_1}}{\partial u_1}\|ds\|v-u\| \\ &\leq \sup_{u_1 \in \mathbb{R}^l_-} \|\frac{\partial k_{y_1}}{\partial u_1}\|\|v-u\| \end{aligned}$$

so that $-k_{y_1}$ is a contraction with constant $q = \sup_{u_1 \in \mathbb{R}^l_-} \|\frac{\partial k_{y_1}}{\partial u_1}\| < 1$, and u_k globally converges to a single equilibrium, so that Theorem 1 can be applied to prove global attractivity of a single equilibrium of $\dot{x} = f(x)$. □

In the following corollary, we show that we do not need to know the exact expression of $k_{y_1}(u_1)$ to be able to apply Theorem 1:

Corollary 1 *Suppose that the expression of $y_1 = k_{y_1}(u_1)$ is unknown, but that it is known that it satisfies an equation in the form*

$$y_1 = G(y_1, u_1)$$

and that there exists a norm $\|.\|$ such that

$$\sup_{u_1 \in \mathbb{R}^l_-,\, y_1 \in \mathbb{R}^l_+} \|\frac{\partial G}{\partial y_1}\| + \|\frac{\partial G}{\partial u_1}\| < 1 \tag{12}$$

then system (9) has a unique equilibrium for any constant $u_1 \in \mathbb{R}^l_-$. Moreover, if this equilibrium is globally attractive for all $u_1 \in \mathbb{R}^l_-$, then system (8) has a unique globally attractive equilibrium.

Proof: From the definition of G, it is not clear that system (9) with u_1 fixed has a single equilibrium. However, condition (12) indicates that, for u_1 fixed, $G(y_1, u_1)$ is a contraction (because $\sup_{u_1 \in \mathbb{R}^l_-,\, y_1 \in \mathbb{R}^l_+} \|\frac{\partial G}{\partial y_1}\| < 1$). Therefore, $y_1 = G(y_1, u_1)$ has a single fixed-point for u_1 fixed (which corresponds to a single equilibrium of (9)). We can denote this equilibrium $k_{y_1}(u_1)$. We will now try to obtain an upper bound for the norm of the Jacobian of k_{y_1}. Differentiating $k_{y_1}(u_1) = G(k_{y_1}(u_1), u_1)$ with respect to u_1, we obtain

$$\frac{\partial k_{y_1}}{\partial u_1} = \frac{\partial G}{\partial y_1}\frac{\partial k_{y_1}}{\partial u_1} + \frac{\partial G}{\partial u_1}$$

so that we have

$$\begin{aligned} \|\tfrac{\partial k_{y_1}}{\partial u_1}\| &= \|\tfrac{\partial G}{\partial y_1}\tfrac{\partial k_{y_1}}{\partial u_1} + \tfrac{\partial G}{\partial u_1}\| \\ &\leq \|\tfrac{\partial G}{\partial y_1}\|\,\|\tfrac{\partial k_{y_1}}{\partial u_1}\| + \|\tfrac{\partial G}{\partial u_1}\| \end{aligned}$$

which results in

$$(1 - \|\frac{\partial G}{\partial y_1}\|)\,\|\frac{\partial k_{y_1}}{\partial u_1}\| \leq \|\frac{\partial G}{\partial u_1}\|$$

and

$$\|\frac{\partial k_{y_1}}{\partial u_1}\| \leq \frac{\|\frac{\partial G}{\partial u_1}\|}{1 - \|\frac{\partial G}{\partial y_1}\|} < 1$$

where the last inequality results from (12). The proof is completed through the use of Lemma 1. □

4.2 Stability analysis

We will now use Corollary 1 in order to prove the stability of the unique equilibrium of the networks that were presented in Section 2 under some assumptions on the reaction velocities. For simplification of notations, we will denote the elements of $\mathcal{P}(1)$ as $\{k_1, \cdots, k_r\}$.

A mass-balance model for such a network has a unique equilibrium, as was shown in Section 3. In order to show the uniqueness of the equilibrium, we had separated the metabolites into three families: the root, the intermediate, and the boundary metabolites, which resulted in (1)- (2)-(3); in order to prove the stability of this equilibrium, it is needed to split the metabolites differently:

- The first equation, (1), is unchanged

$$\dot{x}_1 = c - \sum_{i=1}^{r} \varphi_{1k_i}(x_1, x^{[k_i]}) - \mu x_1 \tag{13}$$

- For the products of x_1 (that we now denote x_{k_i}), the general equation, (2), is rewritten as

$$\dot{x}_{k_i} = \varphi_{1k_i}(x_1, x^{[k_i]}) - \sum_{j \in \mathcal{P}(k_i)} \varphi_{k_i j}(x_{k_i}) - \mu x_{k_i} \tag{14}$$

- When x_k is a product of $x_l \neq x_1$, the general equation (2) becomes

$$\dot{x}_k = \varphi_{lk}(x_l) - \sum_{j \in \mathcal{P}(k)} \varphi_{kj}(x_k) - \mu x_k \tag{15}$$

We impose the boundedness of the partial derivatives of φ_{ij} in the following assumption:

Assumption 4 *The growth rate satisfies*

$$\mu > 0$$

and there exist $d_{ij} \geq 0$, $\alpha_b^{[k_j]} \geq 0$ *such that*

$$\begin{array}{rcccl} 0 & \leq & \frac{\partial \varphi_{ij}}{\partial x_i} & \leq & d_{ij} \quad \textit{for all } i,j \\ -\alpha_b^{[k_j]} & \leq & \frac{\partial \varphi_{1k_j}}{\partial x_b^{[k_j]}} & \leq & 0 \quad \textit{for all } j \leq r,\ b \leq n_{k_j} \end{array}$$

where $x_b^{[k_j]}$ *is the inhibitor of the reaction* $X_1 \to X_{k_j}$*, which lies at the index* b *in the vector* $x^{[k_j]}$*.*

We then need the definition of the following function:

$$\begin{array}{rccl} z_1: & \mathbb{R}_+^{\sum_{i=1}^r n_{k_i}} & \to & \mathbb{R}_+ \\ & (x^{[k_1]}, \cdots, x^{[k_r]}) & \to & z_1(x^{[k_1]}, \cdots, x^{[k_r]}) \end{array}$$

defined as the solution of the equation $\dot{x}_1 = 0$:

$$\sum_{i=1}^{r} \varphi_{1k_i}(z_1, x^{[k_i]}) + \mu z_1 = c$$

From this we see that $z_1 \in (0, \frac{c}{\mu})$ for all values of the vectors $x^{[k_i]}$. Differentiating this last expression with respect to one of the inhibitors $x_b^{[k_j]}$ gives:

$$\sum_{k_i} \frac{\partial \varphi_{1k_i}}{\partial x_1} \frac{\partial z_1}{\partial x_b^{[k_j]}} + \frac{\partial \varphi_{1k_j}}{\partial x_b^{[k_j]}} + \mu \frac{\partial z_1}{\partial x_b^{[k_j]}} = 0$$

We then isolate

$$\frac{\partial z_1}{\partial x_b^{[k_j]}} = -\frac{\frac{\partial \varphi_{1k_j}}{\partial x_b^{[k_j]}}}{\mu + \sum_{k_i} \frac{\partial \varphi_{1k_i}}{\partial x_1}} \geq 0$$

from which we can find the upper bound

$$\frac{\partial z_1}{\partial x_b^{[k_j]}} \leq \frac{\alpha_b^{[k_j]}}{\mu}$$

We now define a new notation

Notation 2 *From the arborescence structure, we know that there exists a unique path from X_1 to any metabolite X_s. This path takes the form $X_1 \to X_{k_j} \to \cdots X_k \to X_l \to \cdots \to X_w \to X_s$; if X_s is an arbitrary metabolite, we will store the indices of this path (without 1 and s) in C_s; alternatively, if x_s corresponds to some $x_b^{[k_j]}$, we will also denote this path $C_b^{[k_j]}$. Similarly, we denote $g_s(k)$ or $g_b^{[k_j]}(k)$ the index of the metabolite that follows X_k in the path that connects X_1 to X_s.*

This allows for the following theorem

Theorem 2 *If Assumptions 1, 2, and 4 are satisfied and*

$$\sum_{j=1}^{r} \sum_{b=1}^{n_{k_j}} \left[\left(\frac{d_{1,k_j}}{\mu} + 1 \right) \prod_{k \in C_b^{[k_j]}} \frac{d_{kg_b^{[k_j]}(k)}}{\mu + d_{kg_b^{[k_j]}(k)}} \right] < \frac{\mu}{\max_{k_s,c} \alpha_c^{[k_s]}} \tag{16}$$

and φ_{1k_i} is bounded for all $i \in \{1, \cdots, r\}$ ($0 \leq \varphi_{1k_i} \leq B_{k_i}$), then the equilibrium of system (13)-(14)-(15) is globally attractive in the positive orthant.

Proof: We already know that the solutions of (13)-(14)-(15) are bounded and that there is a single equilibrium because $\mu > 0$ (see Proposition 2). In order to apply Theorem 1, we will decompose system (13)-(14)-(15) into two subsystems as was done for Lemma 1 and Corollary 1:

- the first subsystem is made of equations (13) and (15) in an unchanged form, with, for all k_i in $\mathcal{P}(1)$, the set of equations

$$\dot{x}_{k_i} = \varphi_{1k_i}(x_1, -v^{[k_i]}) - \sum_{j \in \mathcal{P}(k_i)} \varphi_{k_i j}(x_{k_i}) - \mu x_{k_i} \tag{17}$$

  The input is defined as

$$u_1 = \left((v^{[k_1]})^T, \cdots, (v^{[k_r]})^T \right)^T$$

and the output as

$$y_1 = \left((x^{[k_1]})^T, \cdots, (x^{[k_r]})^T \right)^T$$

- The second subsystem is

$$y_2 = u_2 \tag{18}$$

The interconnection of those two cooperative subsystems through negative feedback as in Theorem 1, Lemma 1 or Corollary 1 results in (13)-(14)-(15), as needed. The structure of this interconnection is such that we can apply Corollary 1 directly. In order to do that, we have to show that (13)-(15)-(17) has a unique globally attractive equilibrium, and that some condition in the form of (12) is satisfied.

We first study the equilibrium of (13)-(15)-(17) for $u_1 \leq 0$ constant (every entry of the vector u_1 is non positive). Let us consider a given $x_b^{[k_j]}$; from the structure of the network, we know that there exists a unique pathway in the arborescence that connects X_{k_j} to $X_b^{[k_j]}$. We can write the differential equations corresponding to the metabolites of this path as

$$\left\{ \begin{array}{lcl} \dot{x}_{k_j} & = & \varphi_{1k_j}(x_1, -v^{[k_j]}) - \sum_{i \in \mathcal{P}(k_j)} \varphi_{k_j i}(x_{k_j}) - \mu x_{k_j} \\ \vdots & \vdots & \vdots \\ \dot{x}_k & = & \varphi_{tk}(x_t) - \sum_{i \in \mathcal{P}(k)} \varphi_{ki}(x_k) - \mu x_k \\ \dot{x}_l & = & \varphi_{kl}(x_k) - \sum_{i \in \mathcal{P}(l)} \varphi_{li}(x_l) - \mu x_l \\ \vdots & \vdots & \vdots \\ \dot{x}_s & = & \varphi_{ws}(x_w) - \sum_{i \in \mathcal{P}(s)} \varphi_{si}(x_s) - \mu x_s \end{array} \right. \tag{19}$$

where $x_s \equiv x_b^{[k_j]}$ (we will sometimes use x_s in the following equations to simplify the notations).

Let us now define $f_l = \sum_{i \in \mathcal{P}(l)} \varphi_{li}(x_l) + \mu x_l$. For $\bar{x}_1$ fixed, (19) has a unique equilibrium that has $\bar{x}_s$ following

$$\begin{array}{rcl} \bar{x}_b^{[k_j]} = \bar{x}_s & = & f_s^{-1} \circ \varphi_{ws} \circ f_w^{-1} \cdots \circ f_l^{-1} \circ \varphi_{kl} \circ f_k^{-1} \circ \cdots \circ f_{k_j}^{-1} \circ \varphi_{1k_j}(\bar{x}_1, -v^{[k_j]}) \\ & = & M_b^{[k_j]} \left(\varphi_{1k_j}(\bar{x}_1, -v^{[k_j]}) \right) \end{array} \tag{20}$$

which can easily be seen as an increasing function of $\bar{x}_1$ whose derivative is

$$\begin{array}{rcl} \frac{dM_b^{[k_j]}\left(\varphi_{1k_j}(\bar{x}_1, -v^{[k_j]})\right)}{dx_1} & = & \frac{\frac{\partial \varphi_{1k_j}}{\partial x_1}}{\sum_{i \in \mathcal{P}(s)} \varphi'_{si} + \mu} \prod_{k \in C_b^{[k_j]}} \frac{\varphi'_{k, g_b^{[k_j]}(k)}}{\sum_{i \in \mathcal{P}(k)} \varphi'_{ki} + \mu} \\ & \leq & \frac{d_{1k_j}}{\mu} \prod_{k \in C_b^{[k_j]}} \frac{d_{k, g_b^{[k_j]}(k)}}{\mu + d_{k, g_b^{[k_j]}(k)}} \end{array}$$

because $\varphi'_{si} = \frac{d\varphi_{si}}{dx_s} \geq 0$ implies that

$$\frac{1}{\sum_{i\in\mathcal{P}(s)}\varphi'_{si}+\mu} < \frac{1}{\mu}$$

and $0 \leq \varphi'_{k,g_b^{[k_j]}(k)} \leq d_{k,g_b^{[k_j]}(k)}$ implies that

$$\frac{\varphi'_{k,g_b^{[k_j]}(k)}}{\sum_{i\in\mathcal{P}(k)}\varphi'_{ki}+\mu} \leq \frac{\varphi'_{k,g_b^{[k_j]}(k)}}{\varphi'_{k,g_b^{[k_j]}(k)}+\mu} \leq \frac{d_{k,g_b^{[k_j]}(k)}}{\mu+d_{k,g_b^{[k_j]}(k)}}$$

(note that

$$\begin{aligned}\frac{dM_b^{[k_j]}\left(\varphi_{1k_j}(\bar{x}_1,-v^{[k_j]})\right)}{dv_c^{[k_j]}} &= \frac{-\frac{\partial\varphi_{1k_j}}{\partial x_c^{[k_j]}}}{\sum_{i\in\mathcal{P}(s)}\varphi'_{si}+\mu}\prod_{k\in C_b^{[k_j]}}\frac{\varphi'_{k,g_b^{[k_j]}(k)}}{\sum_{i\in\mathcal{P}(k)}\varphi'_{ki}+\mu} \\ &\leq \frac{\alpha_c^{[k_j]}}{\mu}\prod_{k\in C_b^{[k_j]}}\frac{d_{k,g_b^{[k_j]}(k)}}{\mu+d_{k,g_b^{[k_j]}(k)}}\end{aligned}$$

This is a result that we will need later).

If we now introduce the requirement of $\dot{x}_1 = 0$, we must have

$$\bar{x}_1 = z_1(\bar{x}^{[k_1]},\cdots,\bar{x}^{[k_r]})$$

in equation (20), so that $\bar{x}_b^{[k_j]}$ must satisfy

$$\bar{x}_b^{[k_j]} = M_b^{[k_j]}\left(\varphi_{1k_j}\left(z_1(\bar{x}^{[k_1]},\cdots,\bar{x}^{[k_r]}),-v^{[k_j]}\right)\right) \tag{21}$$

for all inhibitors of the first equations. (21) then represents a large number of equations to be satisfied by the equilibrium of (13)-(15)-(17). This is a set of equations $y_1 = G(y_1,u_1)$ that defines the equilibrium of (9), as in Corollary 1. We are then left with condition (12) to prove for this G:

$$\frac{\partial G}{\partial y_1} = \begin{pmatrix} \frac{dM_1^{[k_1]}(\varphi_{1k_1}(\cdots))}{dx_1}\frac{dz_1}{du} \\ \vdots \\ \frac{dM_{n_{k_1}}^{[k_1]}(\varphi_{1k_1}(\cdots))}{dx_1}\frac{dz_1}{du} \\ \vdots \\ \frac{dM_1^{[k_r]}(\varphi_{1k_r}(\cdots))}{dx_1}\frac{dz_1}{du} \\ \vdots \\ \frac{dM_{n_{k_r}}^{[k_r]}(\varphi_{1k_r}(\cdots))}{dx_1}\frac{dz_1}{du} \end{pmatrix}$$

and a generic element of this matrix has the form

$$-\frac{\frac{\partial\varphi_{1k_j}}{\partial x_1}}{\sum_{i\in\mathcal{P}(s)}\varphi'_{si}+\mu}\prod_{k\in C_b^{[k_j]}}\frac{\varphi'_{k,g_b^{[k_j]}(k)}}{\sum_{i\in\mathcal{P}(k)}\varphi'_{ki}+\mu}\frac{\frac{\partial\varphi_{1k_s}}{\partial x_c^{[k_s]}}}{\mu+\sum_{k_i}\frac{\partial\varphi_{1k_i}}{\partial x_1}} \leq \frac{d_{1k_j}}{\mu}\prod_{k\in C_b^{[k_j]}}\frac{d_{k,g_b^{[k_j]}(k)}}{\mu+d_{k,g_b^{[k_j]}(k)}}\frac{\alpha_c^{[k_s]}}{\mu}$$

where the pair (k_j, b) indicates the line of interest and the pair (k_s, c) the column of interest.

$$\frac{\partial G}{\partial u_1} = \begin{pmatrix} \frac{dM_1^{[k_1]}(\varphi_{1k_1}(\cdots))}{dv_1^{[k_1]}} & \cdots & \frac{dM_1^{[k_1]}(\varphi_{1k_1}(\cdots))}{dv_{n_{k_1}}^{[k_1]}} & 0 & \cdots & 0 & 0 & \cdots & 0 \\ \vdots & \vdots & \vdots & \vdots & \vdots & \vdots & \vdots & \vdots & \vdots \\ \frac{dM_{n_{k_1}}^{[k_1]}(\varphi_{1k_1}(\cdots))}{dv_1^{[k_1]}} & \cdots & \frac{dM_{n_{k_1}}^{[k_1]}(\varphi_{1k_1}(\cdots))}{dv_{n_{k_1}}^{[k_1]}} & 0 & \cdots & 0 & 0 & \cdots & 0 \\ \vdots & \vdots & \vdots & \vdots & \vdots & \vdots & \vdots & \vdots & \vdots \\ 0 & \cdots & 0 & 0 & \cdots & 0 & \frac{dM_1^{[k_r]}(\varphi_{1k_r}(\cdots))}{dv_1^{[k_r]}} & \cdots & \frac{dM_1^{[k_r]}(\varphi_{1k_r}(\cdots))}{dv_{n_{k_r}}^{[k_r]}} \\ \vdots & \vdots & \vdots & \vdots & \vdots & \vdots & \vdots & \vdots & \vdots \\ 0 & \cdots & 0 & 0 & \cdots & 0 & \frac{dM_{n_{k_r}}^{[k_r]}(\varphi_{1k_r}(\cdots))}{dv_1^{[k_r]}} & \cdots & \frac{dM_{n_{k_r}}^{[k_r]}(\varphi_{1k_r}(\cdots))}{dv_{n_{k_r}}^{[k_r]}} \end{pmatrix}$$

and a generic element of this matrix has the form

$$\frac{-\frac{\partial \varphi_{1k_j}}{\partial x_c^{[k_j]}}}{\sum_{i\in\mathcal{P}(s)}\varphi'_{si}+\mu}\prod_{k\in C_b^{[k_j]}}\frac{\varphi'_{k,g_b^{[k_j]}(k)}}{\sum_{i\in\mathcal{P}(k)}\varphi'_{ki}+\mu} \leq \frac{\alpha_c^{[k_j]}}{\mu}\prod_{k\in C_b^{[k_j]}}\frac{d_{k,g_b^{[k_j]}(k)}}{\mu+d_{k,g_b^{[k_j]}(k)}}$$

where the pair (k_j, b) indicates the line of interest and the pair (k_j, c) the column of interest.

Forcing $\|\frac{\partial G}{\partial y_1}\|_1 + \|\frac{\partial G}{\partial u_1}\|_1 < 1$ for all y_1, u_1 results in

$$\frac{\max_{k_s,c}\alpha_c^{[k_s]}}{\mu}\sum_{k_j}\sum_{b=1}^{n_{k_j}}\frac{d_{1k_j}}{\mu}\prod_{k\in C_b^{[k_j]}}\frac{d_{k,g_b^{[k_j]}(k)}}{\mu+d_{k,g_b^{[k_j]}(k)}}+\frac{1}{\mu}\max_{k_j,c}\left\{\sum_{b=1}^{n_{k_j}}\alpha_c^{[k_j]}\prod_{k\in C_b^{[k_j]}}\frac{d_{k,g_b^{[k_j]}(k)}}{\mu+d_{k,g_b^{[k_j]}(k)}}\right\}<1$$

which can be (conservatively) simplified as follows

$$\frac{\max_{k_s,c}\alpha_c^{[k_s]}}{\mu}\sum_{k_j}\sum_{b=1}^{n_{k_j}}\left(\frac{d_{1,k_j}}{\mu}+1\right)\prod_{k\in C_b^{[k_j]}}\frac{d_{kg_b^{[k_j]}(k)}}{\mu+d_{kg_b^{[k_j]}(k)}}<1$$

that is (16). Corollary 1 then ensures global attractivity, if we show that the unique equilibrium of system (13)-(15)-(17) is globally attractive. This system is cooperative and can be decomposed into the cascade of an irreducible cooperative system Σ_1 (containing x_1 and all the inhibition terms) and several small metabolic networks without inhibition (neither inside the small network, nor towards Σ_1). It is easily seen from [6] that the solutions of Σ_1 globally converge to its single equilibrium. Subsequently, the other small networks also globally converge, which results in global attractivity of the unique equilibrium of (13)-(15)-(17). The proof is then completed by using Corollary 1 □

We see that, despite the fact that the inhibition is classically presented as a negative feedback that regulates the system, we have only been able to prove global attractivity under the restrictive condition (16), while stability is easily seen in the absence of inhibition. This condition is very strong, especially if the specific growth rate is small. We will analyze this condition further in the next section.

5 Limit cycles in metabolic networks

Having obtained the sufficient result for global attractivity of Theorem 2, it is relevant to ask two questions: is condition (16) necessary (to add to the sufficiency that we have shown), on the one hand, and is Theorem 2 still valid without condition (16) on the other hand? The answer to the first question is easily seen to be "no": the stability of the equilibrium is retained even if condition (16) is slightly violated (a few simulations of simple systems is already convincing).

In this section, we will show that the answer to the second question is also "no": without condition (16), the stability can be lost, so that we see that the stability of the metabolic networks is not a simple consequence of the structure of the models. Indeed, in this section, we shall exhibit an example where the equilibrium becomes unstable with a limit cycle (Hopf bifurcation) when condition (16) is not satisfied. We will concentrate on the stability of the equilibrium of a simple sequential pathway of n metabolites without branching and with sequential feedback inhibition (that was presented at the end of the Introduction). Each metabolite produces a single other metabolite, and $X_1 \longrightarrow X_2$ is inhibited by the last metabolite, X_n. We can directly apply Theorem 2 to this system:

$$\left\{\begin{array}{lcccc} \dot{x}_1 &=& & -\varphi_1(x_1,x_n) & -\mu x_1 \quad +c \\ \dot{x}_2 &=& \varphi_1(x_1,x_n) & -\varphi_2(x_2) & -\mu x_2 \\ \dot{x}_3 &=& \varphi_2(x_2) & -\varphi_3(x_3) & -\mu x_3 \\ \vdots & \vdots & \vdots & \vdots & \vdots \\ \dot{x}_n &=& \varphi_{n-1}(x_{n-1}) & -\varphi_n(x_n) & -\mu x_n \end{array}\right. \tag{22}$$

and condition (16) becomes

$$\frac{\alpha}{\mu}\left(\frac{d_1}{\mu}+1\right)\prod_{k=2}^{n-1}\frac{d_k}{\mu+d_k} < 1 \tag{23}$$

which is a condition very similar to what was obtained in [1]. We will study the local stability of the equilibrium of (22) when we do not impose condition (16), and see if it is generic. The Jacobian linearization of (22) has the following form:

$$\mathcal{A} = \begin{pmatrix} -\frac{\partial\varphi_1(x_1,x_n)}{\partial x_1}-\mu & 0 & \cdots & 0 & 0 & -\frac{\partial\varphi_1(x_1,x_n)}{\partial x_n} \\ \frac{\partial\varphi_1(x_1,x_n)}{\partial x_1} & -\varphi_2'(x_2)-\mu & \cdots & 0 & 0 & \frac{\partial\varphi_1(x_1,x_n)}{\partial x_n} \\ 0 & \varphi_2'(x_2) & \cdots & 0 & 0 & 0 \\ \vdots & \vdots & \vdots & \vdots & \vdots & \vdots \\ 0 & 0 & \cdots & \varphi_{n-2}'(x_{n-2}) & -\varphi_{n-1}'(x_{n-1})-\mu & 0 \\ 0 & 0 & \cdots & 0 & \varphi_{n-1}'(x_{n-1}) & -\varphi_n'(x_n)-\mu \end{pmatrix}$$

so that we can compute $\det(sI-\mathcal{A})$ by working on the first line

$$\begin{aligned} det(sI-\mathcal{A}) &= \left(s+\tfrac{\partial\varphi_1(x_1,x_n)}{\partial x_1}+\mu\right)\left[\prod_{i=2}^{n}(s+\varphi_i'(x_i)+\mu)+(-1)^n(-\tfrac{\partial\varphi_1(x_1,x_n)}{\partial x_n})\prod_{i=2}^{n-1}(-\varphi_i'(x_i))\right] \\ &\quad +(-1)^{n+1}\tfrac{\partial\varphi_1(x_1,x_n)}{\partial x_n}\tfrac{\partial\varphi_1(x_1,x_n)}{\partial x_1}\prod_{i=2}^{n-1}(-\varphi_i'(x_i)) \\ &= \left(s+\tfrac{\partial\varphi_1(x_1,x_n)}{\partial x_1}+\mu\right)\prod_{i=2}^{n}(s+\varphi_i'(x_i)+\mu) \\ &\quad -\left(s+\tfrac{\partial\varphi_1(x_1,x_n)}{\partial x_1}+\mu\right)\tfrac{\partial\varphi_1(x_1,x_n)}{\partial x_n}\prod_{i=2}^{n-1}\varphi_i'(x_i)+\tfrac{\partial\varphi_1(x_1,x_n)}{\partial x_n}\tfrac{\partial\varphi_1(x_1,x_n)}{\partial x_1}\prod_{i=2}^{n-1}\varphi_i'(x_i) \end{aligned}$$

It is easily seen that the last term compensates the term containing $\frac{\partial\varphi_1(x_1,x_n)}{\partial x_1}$ in the second term so that

$$\begin{aligned} det(sI-\mathcal{A}) &= \left(s+\tfrac{\partial\varphi_1(x_1,x_n)}{\partial x_1}+\mu\right)\prod_{i=2}^{n}(s+\varphi_i'(x_i)+\mu)-(s+\mu)\tfrac{\partial\varphi_1(x_1,x_n)}{\partial x_n}\prod_{i=2}^{n-1}\varphi_i'(x_i) \\ &= \left(s+\tfrac{\partial\varphi_1(x_1,x_n)}{\partial x_1}+\mu\right)\prod_{i=2}^{n}(s+\varphi_i'(x_i)+\mu)+(s+\mu)\left|\tfrac{\partial\varphi_1(x_1,x_n)}{\partial x_n}\right|\prod_{i=2}^{n-1}\varphi_i'(x_i) \end{aligned}$$

In order to evaluate the local stability of the equilibrium $\bar{x}$, we evaluate this polynomial in $x=\bar{x}$. The application of the Routh-Hurwitz criterion for $n=3$ shows that $\mathcal{A}$ is Hurwitz if $\frac{\partial\varphi_1(x_1,x_n)}{\partial x_1}+\mu>0$, $\varphi_i'(x_i)+\mu>0$, and $\frac{\partial\varphi_1(x_1,x_n)}{\partial x_n}\leq 0$ at the equilibrium, so that the system is locally exponentially stable independently of condition (16). We then concentrate on dimension 4. The application of the Routh-Hurwitz criterion shows that, if everything else stays the same, at least one root of $det(sI-\mathcal{A})$ goes into the right-half plane if $\left|\frac{\partial\varphi_1(x_1,x_n)}{\partial x_n}\right|$ becomes large.

We have then built an example exhibiting such a property: when the parameter present in the inhibition factor (p) is modified, only $\left|\frac{\partial\varphi_1(x_1,x_n)}{\partial x_n}\right|$ is modified in $det(sI-\mathcal{A})$ at the equilibrium: we consider

$$\begin{cases} \dot{x}_1 = & & -\frac{1}{1+(x_4/19)^p}\frac{3.2x_1}{1+x_1} & -0.01x_1 & +1.71 \\ \dot{x}_2 = & \frac{1}{1+(x_4/19)^p}\frac{3.2x_1}{1+x_1} & -\frac{1.4x_2}{1+x_2} & -0.01x_2 & \\ \dot{x}_3 = & \frac{1.4x_2}{1+x_2} & -\frac{1.2x_3}{1+x_3} & -0.01x_3 & \\ \dot{x}_4 = & \frac{1.2x_3}{1+x_3} & -\frac{x_4}{1+x_4} & -0.01x_4 & \end{cases} \tag{24}$$

which has the structure of (22) with $n=4$, and where we take $\varphi_1(x_1,x_4)=\frac{1}{1+(x_4/19)^p}\frac{3.2x_1}{1+x_1}$ so that we can easily see that condition (23) becomes

$$\frac{\alpha}{\mu}\left(\frac{d_1}{\mu}+1\right)\frac{d_2}{\mu+d_2}\frac{d_3}{\mu+d_3}<1\equiv p<3.16\ 10^{-5}$$

The different forms of the inhibiting factor $\frac{1}{1+(x_4/19)^p}$ are illustrated on Figure 2 where we see that p mainly influences the maximal slope of the inhibition function. System (24) has a single equilibrium in $x=(19,19,19,19)^T$, and, at the equilibrium, we have

$$\det(sI-\mathcal{A})=(s+0.0140)(s+0.0135)(s+0.0130)(s+0.0125)+4.2\,10^{-7}p(s+0.01)$$

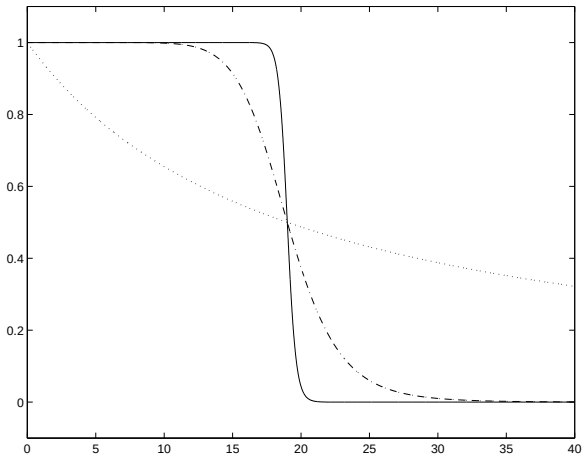

Figure 2: Form of the inhibiting factor $\frac{1}{1+(x_4/19)^p}$ for $p = 1$ (dotted line), p=10 (dash-dotted line) and $p = 60$ (solid line)

which is hurwitz for $p < 56.4519$, and is not Hurwitz for p larger than that value. This transition from a stable to an unstable equilibrium is illustrated on Figure 3, where the time responses of the four states is illustrated for the value of $p = 0$ (no inhibition), $p = 10$ (weak inhibition), and $p = 60$ (strong inhibition). In the latter case, oscillations appear. This corresponds to a limit cycle in the state-space. A Hopf bifurcation has taken place in $p = 56.4519$. Despite this oscillation, the reaction rates for the three reactions $X_2 \longrightarrow X_3$, $X_3 \longrightarrow X_4$, and $X_4 \longrightarrow ...$ are close to their maximum after the transient. The only limiting reaction is $X_1 \longrightarrow X_2$ which, due to the inhibition is far from its maximum reaction rate.

The local stability of the equilibrium for $p < 56.4519$ does not ensure global attractivity, but we have not noticed other behaviors than convergence towards the equilibrium when $p < 56.4519$. The sufficient condition $p < 3.16\ 10^{-5}$ is therefore very conservative. This is due to the fact that it comes from a small-gain analysis.

6 Conclusion

In this paper, we have shown that a large class of models of metabolic system only has a single equilibrium. We then have proved that, under a small gain condition, this equilibrium is globally attractive. Finally, we have shown that stability of this equilibrium is not a generic property of the metabolic systems: a condition needs to be imposed on the parameters to have stability (similar to the small gain condition that we found). This last point disproves the common belief that inhibitions

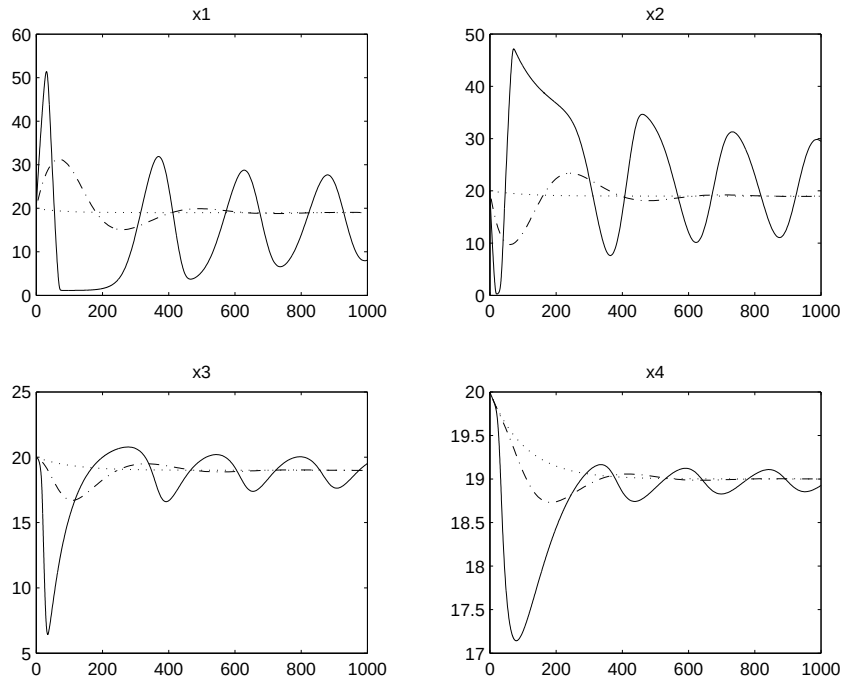

Figure 3: Evolution of the states of system (24) for $p = 0$ (dotted line), p=10 (dash-dotted line) and $p = 60$ (solid line).

have a stabilizing effect.

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