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On the Local Stability of Irreversible and Reversible Linear Metabolic Pathways with Allosteric and Genetic Regulation

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Abstract: This work gives mathematical conditions that guarantee the local stability of the equilibrium regimen of two classes of cell metabolism. In fact, we have analyzed reversible and irreversible linear bacterial metabolic pathways that integrate both genetic and enzymatic control. Moreover, due to these conditions, we can state that: regardless the size of a doubly controlled linear metabolic pathway, the local stability of its steady state depends only on the dynamics of its input and output flux with respect to the concentration of its end product. These results are proved theoretically using some properties of the cooperative matrices.

Keywords: Linear metabolic pathways, genetic regulation, end-product control structure, nonlinear systems, cooperative systems, local stability.

1. INTRODUCTION

Stability analysis is recognized as an issue of great importance in order to deduce key properties associated with dynamical models of biological systems. For example, thanks to the stability analysis, we can better understand how the bacteria during the growth phase adapt the concentration of enzymes to ensure that the *de novo* biosynthesis is sufficients to complete or to fully provide the flux demand for metabolites. Moreover, even though the metabolic network is a complex system, it can be broken down into subsystems with particular control structures which leads bacteria to be able to manage efficiently the control of its metabolic pathways. So, the goal of this work is to analyze the local stability of an *end-product control structure*, shared by a large number of bacteria and yeasts biosynthesis pathways. In this structure, both genetic and enzymatic controls involve the last product as metabolic effector (Tyson and Othmer, 1978), (Goelzer et al., 2008). In the literature, the stability of bacterial metabolic pathways that integrate only the allosteric regulation is well investigated. For instance, one can apply diagonal stability results for cyclic dynamical systems (Arcak and Sontag, 2006) to prove the global stability of the irreversible pathway. As well, one can use some result concerning monotone tridiagonal systems with negative feedback (Wang et al., 2008), (Wang et al., 2010) to show the global stability of the reversible pathways. However, to the best of our knowledge, stability issue of bacterial metabolic pathways that integrate both genetic and enzymatic control has not been fully studied. In some previous work, we have presented some results about global attractivity of such biological systems (Meslem et al., 2010). Likewise, recently we have built a Lyapunov function for the irreversible metabolic pathways (Meslem and Fromion, 2011).

Currently, we attempt to prove the global stability of both irreversible and reversible linear metabolic pathways, under nonrestrictive, easy-to-check conditions. To reach this goal, We will tackle this issue in two stages. The first stage is devoted to improve and to soften the conditions that ensure the global attractivity of the unique equilibrium of linear metabolic pathways given in (Meslem et al., 2010). In the second stage, we will show the local stability of the globally attractive steady states. Thus, this work is dedicated to accomplish the second stage of this proposed methodology. In fact, we will show that for the type of systems, the local stability of the equilibrum point is guaranteed if the dynamics of the input flux is more important than that of the output flux.

This paper is organized as follows. In Section 2, we exhibit realistic models for reversible and irreversible linear bacterial metabolic pathways. Of course, these models highlight both genetic and enzymatic control. The existence and the unicity of equilibrium regimens of these complex biological systems is proved in Section 3. Then, the main findings of this paper about the local stability are stated and proved in section 4.

2. LINEAR METABOLIC PATHWAYS

As aforementioned in the introduction, here we will study the same *end-product control structures* in metabolic pathways introduced in (Meslem et al., 2010). For this reason, we have prefered to keep the same statements and definitions as that given in the previous work with slight modifications.

Let us consider the linear biosynthesis pathways with n metabolites involved in enzymatic reactions with an input flux v_0 and an output flux v_n as depicted in Figure 1.

We assume that the pool X_1 of the first metabolite is maintained by the input flux v_0 which corresponds to a supply flux, hence its concentration $\bar{x}_1$ is strictly positive and constant. The output of the pathway is the flux v_n which corresponds to the bacterium requirement for the metabolite X_n. In fact, if the cell requires large amounts of X_n, the value of v_n will be high and if small amounts of X_n are required the value of v_n will be low. Furthermore, as has been experimentally observed and

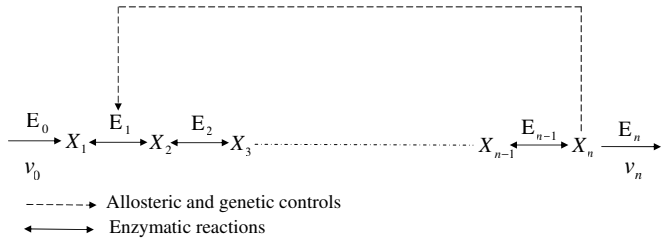

Fig. 1. Linear *end-product control structure*. X_i $(i = 1,\ldots,n)$ represent the metabolic pools and E_i $(i = 1,\ldots,n)$ represent the enzymes that acting in the pathway.

theoretically rationalized (Goelzer et al., 2008), the end product X_n inhibits the first reaction via two control levels: (*i*) regulation of enzymatic activity and (*ii*) regulation of enzyme synthesis, herein referred as *allosteric control* and *genetic control* respectively. Henceforward, for each $i \in \{2,\ldots,n\}$ we denote by x_i the nonnegative concentration of the metabolite X_i, and by e_i we the assumed constant concentration of the enzyme E_i. In the literature, the dynamical behavior of the three biochemical phenomena *enzymatic reactions*, *allosteric control* and *genetic control* as presented in Figure 1 is usually modeled by a set of interconnected nonlinear differential equations (Tyson and Othmer, 1978),(Goelzer et al., 2008). As aforementioned, the aim of this study is to analyze the local asymptotic stability of the kind of biological systems. Hereafter, nonlinear models for both reversible and irreversible *end-product control structure* are presented.

2.1 Reversible metabolic pathways

Systematic analysis of bacterial metabolic networks shows that almost all reversible pathways have at least one irreversible step and in most cases the irreversible step corresponds to the first enzyme of the pathways. Then, the common *end-product control structure* of linear reversible metabolic pathways which integrates allosteric and genetic control is described by the following dynamical system:

$$\begin{cases} \dot{x}_2 &= e_1 f_1(\bar{x}_1, x_n) - e_2 f_2(x_2, x_3) \\ \dot{x}_3 &= e_2 f_2(x_2, x_3) - e_3 f_3(x_3, x_4) \\ \vdots \quad \vdots & \qquad \vdots \qquad\qquad \vdots \\ \dot{x}_n &= e_{n-1} f_{n-1}(x_{n-1}, x_n) - e_n f_n(x_n) \\ \dot{e}_1 &= g(x_n) - \mu e_1 \end{cases} \tag{1}$$

where the functions f_i denote the reaction rates of the enzymes E_i. Remember, in this work, we consider that the concentration $\bar{x}_1$ of the first metabolite is constant and except for e_1 all other enzyme concentrations are assumed constant. Note that, in this structure, the growth rate functions of all intermediate reactions depend on product and substrate concentration and have the following properties.

- *Activity of the first enzyme*: it is well-known that the end product X_n modulates the activity of the enzyme E_1 through an allosteric effect. So, the function $f_1(x_1, x_n)$ is strictly increasing in its first argument and strictly decreasing with respect to its second argument. Moreover, one has for any
$$x_1 > 0, \;\; x_n \geq 0 \;\; f_1(x_1, x_n) > 0$$
and for any
$$x_n \geq 0, \;\; f_1(0, x_n) = 0.$$
In addition, there exists a constant $M_1 > 0$ such that for any
$$x_1 > 0, \;\; x_n \geq 0 \;\; f_1(x_1, x_n) \in [0, \; M_1)$$
and for all
$$x_1 > 0 \;\text{ one has, }\; \lim_{x_n \to +\infty} f_1(x_1, x_n) = 0.$$
- *Activity of the intermediate enzymes*: each reaction rate $f_i(.,.)$, $i \in \{2,\ldots,n-1\}$, is strictly increasing in x_i and strictly decreasing in x_{i+1} with the following signs,
$$\forall x_i > 0, \;\; f_i(x_i, 0) > 0, \;\; \forall x_{i+1} > 0, \; f_i(0, x_{i+1}) < 0$$
$$\text{and } f_i(0,0) = 0.$$
Moreover, there exist $M_i > 0$ and $M_i' \geq 0$ such that
$$\forall x_i > 0, \;\; \forall x_{i+1} \geq 0, \;\; f_i(x_i, x_{i+1}) \in (-M_i', \; M_i).$$
Finally, for any $x_i > 0$ there exists $x_{i+1}' > 0$ such that
$$f_i(x_i, x_{i+1}') = 0$$
and for any constant concentration $\bar{x}_{i+1}$, one has
$$\lim_{x_i \to +\infty} f_i(x_i, \bar{x}_{i+1}) = M_i.$$
- *Activity of the final enzyme*: E_n describes the properties of the remainder parts of the metabolic network which are supplied by the end product X_n. Then, the properties of f_n mainly depends on the properties of the next modules, and generally f_n is a strictly increasing, positive and bounded function in x_n such that
$$f_n(0) = 0 \;\text{ and }\; \lim_{x_n \to +\infty} f_n(x_n) = M_n.$$
where M_n is a positive constant.

Now, consider the enzyme synthesis phenomenon. It is well-known that the variation of the first enzyme concentration during the exponential growth phase is mostly the result of two phenomena: *(i)* the *de novo* production, and *(ii)* the *dilution* effect caused by the increase of the cell volume. The two biochemical observable facts are described by the last differential equation of (1). The continuous, positive and strictly decreasing function $g(.)$ represents the instantaneous production of the enzyme E_1 modulated by the concentration of the end product x_n (implicitly through a transcription factor) with,
$$g(0) = g_{max}, \text{ where } g_{max} > 0 \;\text{ and }\; \lim_{x \to +\infty} g(x) = 0$$
and μ is the growth rate of the bacterium assumed to be in the exponential growth phase.

2.2 Irreversible metabolic pathways

The main difference between irreversible and reversible end-product control structure in metabolic pathways is the nature of the reaction rates f_i for the intermediate enzymes. Indeed, in the case of irreversible reactions the activities of enzymes depend only on the substrate concentrations and have the following properties:

- *Activity of the intermediate enzymes*: each reaction rate $f_i(.)$, $i \in \{2,\ldots,n-1\}$ is positive and strictly increasing in x_i and $f_i(0) = 0$. Moreover, there exists $M_i > 0$ such that
$$\lim_{x_i \to +\infty} f_i(x_i) = M_i.$$

Then, one can describe the dynamical behavior of the end-product control structure in the case of irreversible linear metabolic pathways by the following dynamical system

$$\begin{cases} \dot{x}_2 &= e_1 f_1(\bar{x}_1, x_n) - e_2 f_2(x_2) \\ \dot{x}_3 &= e_2 f_2(x_2) - e_3 f_3(x_3) \\ \vdots \quad \vdots & \qquad \vdots \qquad\qquad \vdots \\ \dot{x}_n &= e_{n-1} f_{n-1}(x_{n-1}) - e_n f_n(x_n) \\ \dot{e}_1 &= g(x_n) - \mu e_1. \end{cases} \tag{2}$$

Remark: *In the case of irreversible reactions, a classical representation of the function $f_i(.)$ $i = 2,\ldots,n-1$ associated with the enzyme E_i which catalyzes the reaction between the substrate X_i and the product X_{i+1} is given by the so-called Michaelis-Menten function, see (Tyson and Othmer, 1978), (Mulquiney and Kuchel, 2003) for more explanations.*

After the detailed description of the nonlinear models for both reversible and irreversible linear metabolic pathways that integrate allosteric and genetic control, in the next section we give conditions that guarantee the existence and the uniqueness of equilibrium regimens of the nonlinear dynamical systems (1) and (2).

3. EXISTENCE AND UNIQUENESS OF STEADY STATES

Let us start our proof by showing the boundedness of the first enzyme concentration e_1, which is governed by the last differential equation in (1) or in (2). By definition, the function $g(.)$ is positive and bounded,

$$\forall x_n \geq 0,\ g(x_n) \in (0, g_{max}]$$

Then, we can deduce that for any $x_n \geq 0$ the time evolution of the first enzyme $e_1(t)$ is framed between

$$\check{e}_1(t) \leq e_1(t) \leq \hat{e}_1(t),$$

where $\check{e}_1(t)$ and $\hat{e}_1(t)$ are respectively the solutions of the following stable first-order linear systems

$$\begin{aligned} \dot{\check{e}}_1 &= -\mu\check{e}_1, \\ \dot{\hat{e}}_1 &= -\mu\hat{e}_1 + g_{max}. \end{aligned}$$

with initial conditions $\check{e}_1(t_0) = \hat{e}_1(t_0) = e_1(t_0)$. Consequently, we can conclude that there exists a positive constant $\bar{e}_1 > 0$ such that

$$\forall t \geq 0,\ e_1(t) \leq \bar{e}_1.$$

Once the boundedness of the first enzyme concentration has been proved, the following proposition gives sufficient conditions, which guarantee in the same time the existence of the equilibrium regimen and its uniqueness for both irreversible and reversible linear metabolic pathways (2), (1).

Proposition 1. If for each $i \in \{2,\ldots,n-1\}$ the following inequality holds

$$\bar{e}_1 M_1 < e_i M_i$$

both irreversible and reversible linear metabolic pathways (2), (1) have a unique equilibrium point.

Proof: First, let us consider the irreversible case. Thus, the nonlinear system (2) reaches an equilibrium regimen if and only if the following set of nonlinear equations are satisfied

$$\left\{\begin{array}{rcl} g(x_n) &=& \mu e_1 \\ e_1 f_1(\bar{x}_1, x_n) &=& e_n f_n(x_n) \\ &=& e_{n-1} f_{n-1}(x_{n-1}) \\ &\vdots& \quad\vdots \\ &=& e_2 f_2(x_2). \end{array}\right. \tag{3}$$

So, consider the first equation of (3). It is clear that at the steady state regimen, from this equation we obtain

$$e_1^* = \frac{g(x_n^*)}{\mu}$$

Now consider the second equation of (3). By construction, we know that the left side of this equation is a strictly decreasing function in x_n and for $x_n \in [0,+\infty)$ it takes its values in the interval $[0, \bar{e}_1 f_1(\bar{x}_1, 0)]$. On the other hand, the right side of this equation is a strictly increasing function in x_n and for $x_n \in [0,+\infty)$ it belongs in the interval $[0, M_n)$. Therefore, we can claim that there exists a unique x_n^* such that

$$e_1^* f_1(\bar{x}_1, x_n^*) = e_n f_n(x_n^*)$$

For the remainder equations of the system (3), we must verify that for each $i \in \{2,\ldots,n-1\}$ there exists a unique x_i^* such that

$$e_i f_i(x_i^*) = e_1^* f_1(\bar{x}_1, x_n^*)$$

Since the functions $f_i(.)$ are strictly increasing in x_i and bounded with known bound M_i, the above equation is satisfied for all $i \in \{2,\ldots,n-1\}$ if

$$\bar{e}_1 M_1 < e_i M_i$$

This completes the proof of Proposition 1 in the case of irreversible linear metabolic pathways (2).

Now, let us consider the case of reversible metabolic pathways. The equilibrium conditions of the nonlinear system (1) are given by the following set of nonlinear equations

$$\left\{\begin{array}{rcl} g(x_n) &=& \mu e_1 \\ e_1 f_1(\bar{x}_1, x_n) &=& e_n f_n(x_n) \\ &=& e_{n-1} f_{n-1}(x_{n-1}, x_n) \\ &\vdots& \quad\vdots \\ &=& e_2 f_2(x_2, x_3). \end{array}\right. \tag{4}$$

Note that, the first and the second equation of the above set of nonlinear equations (4) are the same as in the case of irreversible pathways. Then, we can claim that, each of these two equations has a unique solution x_n^* and e_1^* respectively. That means, by definition there exists a unique value x_n^* such that

$$\begin{aligned} e_1^* &= \frac{g(x_n^*)}{\mu} \\ \frac{g(x_n^*)}{\mu} f_1(\bar{x}_1, x_n^*) &= e_n f_n(x_n^*) \end{aligned}$$

Now consider the third equation of (4), namely

$$e_{n-1} f_{n-1}(x_{n-1}, x_n^*) = e_1^* f_1(\bar{x}_1, x_n^*)$$

We know that, by construction, the function $f_{n-1}(.,.)$ is strictly increasing in x_{n-1} and for any $x_n* \in [0,+\infty)$ we get

$$\lim_{x_{n-1} \to +\infty} f_{n-1}(x_{n-1}, x_n^*) = M_{n-1}.$$

Thus, according to these properties, we can claim that if the following inequality holds

$$\bar{e}_1 M_1 < e_{n-1} M_{n-1}$$

then there exists a unique solution x_{n-1}^* for the third equation of (4). In the same way, since the functions $f_i(.,.)$ have the same properties, we can state that the following inequalities

$$\bar{e}_1 M_1 < e_i M_i \ \ i = 2,\ldots,n-2$$

guarantee both existence and uniqueness of equilibrium points x_i^* for the remainder equations of (4). This completes the proof of Proposition 1. □

4. LOCAL STABILITY

In this section, we state and prove the main contribution of this study concerning the local stability of the equilibrium regimens of both reversible and irreversible linear metabolic pathways (1), (2).

Proposition 2. Denote by f_1', f_n' and g' the derivatives of the functions f_1, f_n and g with respect to x_n. Then, if the inequality is satisfied

$$\mu > \frac{f_1(\bar{x}_1, x_n^*)|g'(x_n^*)|}{e_n f_n'(x_n^*) - e_1^* |f_1'(\bar{x}_1, x_n^*)|} \tag{5}$$

the equilibrium regimens of the reversible and irreversible linear metabolic pathways are locally stables. Furthermore, it is clear that the condition (5) is always fulfilled if

$$\frac{e_1^*|f_1'(\bar{x}_1,x_n^*)|}{e_n f_n'(x_n^*)} > 1 \tag{6}$$

That is, the local stability of these biological systems is guaranteed if the dynamics of the input flux with respect to the concentration of the end product is strictly greater than the dynamics of the output flux with respect to the concentration of the same metabolite.

The next subsections are devoted to give the proof of Proposition 2.

4.1 Proof of Proposition 2 (irreversible case)

First, denote by A the Jacobian matrix of the nonlinear system (2) evaluated at its equilibrium point,

$$A = \begin{bmatrix} -e_2 f_2' & 0 & \dots & \dots & 0 & e_1 f_1' & f_1 \\ e_2 f_2' & -e_3 f_3' & \ddots & \vdots & \vdots & 0 & 0 \\ 0 & e_3 f_3' & -e_4 f_4' & \ddots & \vdots & \vdots & \vdots \\ \vdots & \ddots & \ddots & \ddots & \ddots & \vdots & \vdots \\ \vdots & \vdots & \ddots & \ddots & \ddots & \ddots & \vdots \\ \vdots & & & & & & \\ \vdots & \dots & \dots & 0 & e_{n-1} f_{n-1}' & -e_n f_n' & 0 \\ 0 & \dots & \dots & \dots & 0 & g' & -\mu \end{bmatrix}$$

where each f_i' represents the derivative of the function f_i with respect to x_i computed for $x_i = x_i^*$. Recall that, by definition, except f_1' and g' which are negative all the derivatives f_i' are positive. Then, according to the monotonicity of the functions $f_i(.)$ with respect to their arguments x_i, we can easily determine from the matrix A a cooperative matrix $\mathscr{C}$. In fact, to obtain $\mathscr{C}$ we just substitute in A the negative entries f_1' and g' by their absolute values.

$$\mathscr{C} = \begin{bmatrix} -e_2 f_2' & 0 & \dots & \dots & 0 & e_1|f_1'| & f_1 \\ e_2 f_2' & -e_3 f_3' & \ddots & \vdots & \vdots & 0 & 0 \\ 0 & e_3 f_3' & -e_4 f_4' & \ddots & \vdots & \vdots & \vdots \\ \vdots & \ddots & \ddots & \ddots & \ddots & \vdots & \vdots \\ \vdots & \vdots & \ddots & \ddots & \ddots & \ddots & \vdots \\ \vdots & & & & & & \\ \vdots & \dots & \dots & 0 & e_{n-1} f_{n-1}' & -e_n f_n' & 0 \\ 0 & \dots & \dots & \dots & 0 & |g'| & -\mu \end{bmatrix}$$

Now, consider the linearized system (7) of the nonlinear system (2),

$$\delta\dot{\mathbf{y}} = A\delta\mathbf{y} \tag{7}$$

where $\delta y_i = x_i - x_i^*$ for $i = 2,\dots n$ and $\delta y_{n+1} = e_1 - e_1^*$. Then, according to the Lemma 16 of (Wang et al., 2010) or by using the well known properties of nonnegative matrices gathered in (Berman and Plemmons, 1994), we can state that the origin ($\delta\mathbf{y} = 0$) of the linearized system is asymptotically stable if there exist two positive componentwise vectors D and B such that

$$\mathscr{C}D \leq -B \tag{8}$$

which is equivalent to say that there exists a positive componentwise vector D such that

$$-\mathscr{C}D > 0 \tag{9}$$

In (8) and (9), the order relations $(\leq, >)$ must be understood component by component. Thus, to prove Proposition 2 in the case of irreversible metabolic pathways, it is enough to check (9). That means, we will show that there exists a positive vector $D^T = [d_1,\dots,d_n]$ such that (9) is true. To do so, let us start from the last element of the vector $-\mathscr{C}D$. Hence, we must choose positive constants d_n and d_{n-1} such that

$$\mu d_n > |g'| d_{n-1}$$

Then, for a given d_n we must take

$$d_{n-1} < \frac{\mu}{|g'|} d_n \tag{10}$$

For the next element of the vector $-\mathscr{C}D$, we should choose a positive constant d_{n-2} such that

$$e_n f_n' d_{n-1} > e_{n-1} f_{n-1}' d_{n-2}$$

which is equivalent to

$$d_{n-2} < \frac{e_n f_n'}{e_{n-1} f_{n-1}'} d_{n-1} \tag{11}$$

Thus, we iterate this procedure from the $(n-2)$th element of the vector $-\mathscr{C}D$ until its 2nd element to obtain the following conditions on the entries of the positive vector D

$$d_i < \frac{e_{i+2} f_{i+2}'}{e_{i+1} f_{i+1}'} d_{i+1},\ i = n-3,\dots,1 \tag{12}$$

Moreover, the first element of vector $-\mathscr{C}D$ must satisfy

$$e_2 f_2' d_1 > e_1 |f_1'| d_{n-1} + f_1 d_n$$

That is,

$$d_1 > \frac{1}{e_2 f_2'} \left(e_1 |f_1'| d_{n-1} + f_1 d_n \right)$$

Furthermore, taking into account (10), the latter equation is true if

$$d_1 \geq \frac{1}{e_2 f_2'} \left(\frac{\mu}{|g'|} e_1 |f_1'| + f_1 \right) d_n \tag{13}$$

Then, from (12) and (13) d_1 must satisfy the following double inequalities

$$\frac{1}{e_2 f_2'} \left(\frac{\mu}{|g'|} e_1 |f_1'| + f_1 \right) d_n \leq d_1 < \frac{e_3 f_3'}{e_2 f_2'} d_2 \tag{14}$$

By direct computation, we can show that the above double inequalities (14) is always satisfied if and only if

$$d_2 > \frac{1}{e_3 f_3'} \left(\frac{\mu}{|g'|} e_1 |f_1'| + f_1 \right) d_n \tag{15}$$

Thus, there exists d_1 satisfying (14). In the same manner, we can show that for $i = 2,\dots,n-2$, there exists d_i belonging to the following interval

$$\frac{1}{e_{i+1} f_{i+1}'} \left(\frac{\mu}{|g'|} e_1 |f_1'| + f_1 \right) d_n < d_i < \frac{e_{i+2} f_{i+2}'}{e_{i+1} f_{i+1}'} d_{i+1} \tag{16}$$

if and only if

$$d_{i+1} > \frac{1}{e_{i+2} f_{i+2}'} \left(\frac{\mu}{|g'|} e_1 |f_1'| + f_1 \right) d_n \tag{17}$$

Hence, the last condition implies that

$$d_{n-1} > \frac{1}{e_n f_n'} \left(\frac{\mu}{|g'|} e_1 |f_1'| + f_1 \right) d_n \tag{18}$$

and according to (10), d_{n-1} must satisfy the following double inequalities

$$\frac{1}{e_n f_n'} \left(\frac{\mu}{|g'|} e_1 |f_1'| + f_1 \right) d_n < d_{n-1} < \frac{\mu}{|g'|} d_n \tag{19}$$

Then, by direct computation we can easily show that (19) is satisfied if

$$\mu > \frac{f_1 |g'|}{e_n f_n' - e_1 |f_1'|}$$

which is exactly the condition (5) of Proposition 2. That means, if (5) holds then there exists a positive componentwise vector D for that (9) is true. This completes the proof of Proposition 2 in the case of irreversible metabolic pathways. In the next subsection, we will prove Proposition 2 for the reversible case.

4.2 Proof of Proposition 2 (reversible case)

Let us consider the Jacobian matrix A of the nonlinear system (1) evaluated at its equilibrium point. By direct computation we get

$$A = \begin{bmatrix} -e_2 f'_{22} & -e_2 f'_{23} & 0 & \dots & 0 & e_1 f'_{1n} & f_1 \\ e_2 f'_{22} & e_2 f'_{23} - e_3 f'_{33} & -e_3 f'_{34} & 0 & \vdots & 0 & 0 \\ 0 & e_3 f'_{33} & e_3 f'_{34} - e_4 f'_{44} & -e_4 f'_{45} & 0 & \vdots & \vdots \\ \vdots & 0 & \ddots & \ddots & \ddots & \vdots & \vdots \\ \vdots & \vdots & \ddots & \ddots & \ddots & \ddots & \vdots \\ \vdots & & & & & & \\ \vdots & \dots & \dots & 0 & e_{n-1} f'_{(n-1)(n-1)} & e_{n-1} f'_{(n-1)n} - e_n f'_n & 0 \\ 0 & \dots & \dots & \dots & 0 & g' & -\mu \end{bmatrix}$$

where each $f'_{i,j}$ represents the partial derivative of the function $f_i(.,.)$ with respect to the argument x_j computed for $x_i = x_i^*$ and $x_j = x_j^*$. Note that, by definition, the partial derivatives f'_{ii} are strictly positive and for $i \neq j$ the partial derivatives f'_{ij} are strictly negative. Then, from the Jacobian matrix A we build the following cooperative matrix

$$\mathscr{C} = \begin{bmatrix} -e_2 f'_{22} & -e_2 f'_{23} & 0 & \dots & 0 & e_1 |f'_{1n}| & f_1 \\ e_2 f'_{22} & e_2 f'_{23} - e_3 f'_{33} & -e_3 f'_{34} & 0 & \vdots & 0 & 0 \\ 0 & e_3 f'_{33} & e_3 f'_{34} - e_4 f'_{44} & -e_4 f'_{45} & 0 & \vdots & \vdots \\ \vdots & 0 & \ddots & \ddots & \ddots & \vdots & \vdots \\ \vdots & \vdots & \ddots & \ddots & \ddots & \ddots & \vdots \\ \vdots & & & & & & \\ \vdots & \dots & \dots & 0 & e_{n-1} f'_{(n-1)(n-1)} & e_{n-1} f'_{(n-1)n} - e_n f'_n & 0 \\ 0 & \dots & \dots & \dots & 0 & |g'| & -\mu \end{bmatrix}$$

Thus, as in the case of irreversible metabolic pathways, the local stability of the nonlinear system (1) is guaranteed if the condition (9) holds. Then, let us show that under (5) this condition is fulfilled.

For the last element of the vector $-\mathscr{C}D$, we should choose positive constants d_n and d_{n-1} such that

$$\mu d_n > |g'| d_{n-1}$$

This implies that for a given d_n we must take

$$d_{n-1} < \frac{\mu}{|g'|} d_n \tag{20}$$

Then, for the next element of the vector $-\mathscr{C}D$, the positive constant d_{n-2} must satisfy

$$(e_n f'_n - e_{n-1} f'_{(n-1)n}) d_{n-1} > e_{n-1} f'_{(n-1)(n-1)} d_{n-2}$$

That is,

$$d_{n-2} < \frac{e_n f'_n - e_{n-1} f'_{(n-1)n}}{e_{n-1} f'_{(n-1)(n-1)}} d_{n-1} \tag{21}$$

In the same way, the $(n-2)$th element of the vector $-\mathscr{C}D$ must satisfy the following inequality

$$\begin{aligned} d_{n-3} < & \frac{e_{(n-1)} f'_{(n-1)(n-1)} - e_{(n-2)} f'_{(n-2)(n-1)}}{e_{(n-2)} f'_{(n-2)(n-2)}} d_{n-2} \\ & + \frac{e_{(n-1)} f'_{(n-1)n}}{e_{(n-2)} f'_{(n-2)(n-2)}} d_{n-1} \end{aligned} \tag{22}$$

Moreover, d_{n-3} must be positive. Then, to guarantee this condition on d_{n-3} we should choose d_{n-2} such that

$$\begin{aligned} \big(e_{n-1} f'_{(n-1)(n-1)} - e_{n-2} f'_{(n-2)(n-1)}\big) d_{n-2} + \\ e_{(n-1)} f'_{(n-1)n}) d_{n-1} > 0 \end{aligned}$$

That implies the following condition on d_{n-2},

$$d_{n-2} > \frac{-e_{n-1} f'_{(n-1)n}}{e_{n-1} f'_{(n-1)(n-1)} - e_{n-2} f'_{(n-2)(n-1)}} d_{n-1}$$

In the next, in order to simplify the demonstration, we take

$$d_{n-2} > \frac{-f'_{(n-1)n}}{f'_{(n-1)(n-1)}} d_{n-1} \tag{23}$$

Thus, from (21) and (23) we get

$$\frac{-f'_{(n-1)n}}{f'_{(n-1)(n-1)}} d_{n-1} < d_{n-2} < \frac{e_n f'_n - e_{n-1} f'_{(n-1)n}}{e_{n-1} f'_{(n-1)(n-1)}} d_{n-1} \tag{24}$$

Now, the $(n-3)$th element of the vector $-\mathscr{C}D$ must verify

$$\begin{aligned} d_{n-4} < & \frac{e_{(n-2)} f'_{(n-2)(n-2)} - e_{(n-3)} f'_{(n-3)(n-2)}}{e_{(n-3)} f'_{(n-3)(n-3)}} d_{n-3} \\ & + \frac{e_{(n-2)} f'_{(n-2)n-1}}{e_{(n-3)} f'_{(n-3)(n-3)}} d_{n-2} \end{aligned} \tag{25}$$

Here also d_{n-4} must be positive. Then to fulfil this condition, we must have

$$d_{n-3} > \frac{-e_{n-2} f'_{(n-2)(n-1)}}{e_{n-2} f'_{(n-2)(n-2)} - e_{n-3} f'_{(n-3)(n-2)}} d_{n-2}$$

For simplicity's sake, in the next we consider

$$d_{n-3} > \frac{-f'_{(n-2)(n-1)}}{f'_{(n-2)(n-2)}} d_{n-2} \tag{26}$$

So, by direct computation we can show that under the condition (23), d_{n-3} must be framed between the bounds defined by (22) and (26).

With the same method, we can show that from the $(n-4)$th element of the real vector $-\mathscr{C}D$ to its 2nd element, condition (9) is satisfied if the entries $d_i,\ i = (n-4), \dots, 1$ are chosen as follows

$$\begin{aligned} d_i < & \frac{e_{(i+2)} f'_{(i+2)(i+2)} - e_{(i+1)} f'_{(i+1)(i+2)}}{e_{(i+1)} f'_{(i+1)(i+1)}} d_{i+1} \\ & + \frac{e_{(i+2)} f'_{(i+2)(i+3)}}{e_{(i+1)} f'_{(i+1)(i+1)}} d_{i+2} \end{aligned} \tag{27}$$

$$d_i > \frac{-f'_{(i+1)(i+2)}}{f'_{(i+1)(i+1)}} d_{i+1} \tag{28}$$

That means d_i must be bracketed between by the known bounds (28) and (27).

Now, consider the first element of the vector $\mathscr{C}D$. Here d_1 must satisfy

$$d_1 > \frac{1}{e_2 f'_{22}} \big(e_1 |f'_{1n}| d_{n-1} + f_1 d_n - e_2 f'_{23} d_2\big) \tag{29}$$

Moreover, d_1 has to be lower than (27). By direct computation, we can show that this condition on d_1 is satisfied if

$$d_2 > \frac{1}{e_3 f'_{33}}\left(e_1|f'_{1n}|d_{n-1} + f_1 d_n - e_3 f'_{34} d_3\right) \tag{30}$$

Following this reasoning, we can show that for all $i = 1,\ldots,n-3$ the double inequalities defined by (28) and (27) are satisfied if

$$d_{n-2} > \frac{\left((e_1|f'_{1n}| - e_{n-1}f'_{(n-1)(n-1)})d_{n-1} + f_1 d_n\right)}{e_{n-1}f'_{(n-1)(n-1)}} \tag{31}$$

In other hand d_{n-2} must be lower than (21). Thus, by direct computation, we can show that this condition is fulfilled if

$$d_{n-1} > \frac{f_1}{e_n f'_n - e_1|f'_{1n}|} d_n \tag{32}$$

Finally, according to the condition (20) we should have

$$\frac{\mu}{|g'|}d_n - \frac{f_1}{e_n f'_n - e_1|f'_{1n}|}d_n > 0$$

So, it is clear that to ensure this inequality the following condition must be true

$$\mu > \frac{|g'|f_1}{e_n f'_n - e_1|f'_{1n}|}$$

which is exactly the condition (5) of Proposition 2. That means if (5) is satisfied then for a given positive constant d_n we can construct a positive vector D such that $-\mathscr{C}D > 0$. This completes the proof of Proposition 2 in the case of reversible metabolic pathways.

Before to conclude, note that one can easily extend the use of Proposition 1 and Proposition 2 to analyse the local stability of more complicated *end-product control structures*. For instance, one can apply these propositions to study the case of *end-product control structures* where all the enzyme concentrations $e_i,\ i = 1,\ldots,n$ are considered variable in time but bounded with known bounds and also the case where the intermediate enzymatic reactions are mixed. That is, metabolic pathways where some enzymatic reactions are reversible and some other ones are irrreversible. For example, the case of the metabolic pathway of Tryptophan synthesis (Goelzer et al., 2008).

5. CONCLUSION

This work proposed mathematical conditions to check the local stability of linear reversible and irreversible metabolic pathways. Moreover, thanks to these conditions, we can claim that regardless of the size of these linear metabolic pathways, only dynamical properties of the activity of the first enzyme can guarantee the local stability of their steady state. This shows the natural capacity of bacteria to cope with increasing of their flux demand by activating only the first enzyme of their metabolic pathway. As well, the mathematical property of the *end-product control structure* helps to simplify the behavior analysis of complex metabolic pathways and to predict the changes of their components (enzyme concentrations, metabolite pools and fluxes). Furthermore, a comparative analysis between the mathematical predicted behavior and biological data may help to detect still undescribed regulations in a systematic way. In fact, unknown regulations can be highlighted by discrepancies. Indeed, in practice, the bacterium always adapts the flux capacity provided by the pathway to satisfy the flux demand and may employ other mechanisms to cope with the flux demand in some situations. Such mechanisms may modify enzyme activity, enzyme production or flux capacity.

For forthcoming work we will use the local stability result to prove the global stability of both reversible and irreversible linear metabolic pathway. In fact, to do so, we will simplify our global attractivity conditions presented in (Meslem et al., 2010) and then we will show that the local stability condition given in this paper allied with the global attractivity conditions guarantee the global stability of the *end-product control structures*. Also, in order to show the merits of this theoretical work, we will compare our mathematical findings to real world problems. For instance, we plan to use measurement data coming from the Tryptophan or Lysine synthesis to validate our theoretical prediction concerning the stability of these types of *end-product control structures*.

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