Effect of a new variable integration on steady states of a two-step Anaerobic Digestion Model

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Abstract

This paper deals with a mathematical analysis of two-steps model of anaerobic digestion process, including dynamics of soluble microbial products (SMP). We propose to investigate effects of the new variable SMP on qualitative properties of the process in different generic cases. Equilibria of the model are graphically established considering qualitative properties of the kinetics and, their stability are proved theoretically and/or verified by numerical simulations. It will shown that the model has a rich qualitative behavior as equilibria bifurcation and multi-stability according to the considered parameter bifurcation.

Keywords: Anaerobic digestion; Equilibria bifurcation; Modeling; Stability; Steady state analysis; Wastewater treatment plant.

1. Introduction

Mathematical modeling of bioprocesses is a powerful tool to i) explain observed phenomena, ii) understand some mechanisms of the system and predict its evolution, iii) better control the process operations and iv) build the "roots" for dialogue and discussion with biologists. In recent years, many studies was carried out on mathematical analysis of biological ecosystems using chemostat. A number of mathematical modeling methods that are relevant to the field of microbial ecology and bioprocesses was presented in [1]. Di and Yang [2], evaluated how structures and parametrization of synthetic microbial communities with two or three species could affect their productivity and stability.

The technology of Anaerobic Digestion becomes very interesting this last time, considering its high efficiency in wastewater treatment and biogas production. Anaerobic digestion is a complex process, which is widely described by the most phenomenological model ADM1 (Anaerobic Digestion Model n.1) [3]. In the literature, a number of studies have been made on equilibria and the nature of their stability of reduced and simplified models of anaerobic digestion processes, using operating diagram analysis. Khedim et al. [4], investigated how operating parameters (dilution rate and substrate inflow concentration) could ensure an optimal production of biogas in a Microalgae Anaerobic Digestion process. As regards [5], authors showed that the stability of the positive equilibrium of a two-tiered microbial food-chain is not affected when maintenance is included in the model and for a large class of kinetics. A generalised form of a three-tiered microbial food-web was proposed in [6] ; when maintenance is not considered in the model, it was shown that one can explicitly determine the stability of the system and, boundaries between the different stability regions are characterized by analytical expressions.

Even if modeling of anaerobic digestion is increasing in complexity and new challenges should be addressed [7], for a simplified modeling, the biological process may be described mainly by two-steps reactional framework as given in [8]: in the first step (acidogenesis), the acidogenic bacteria consume the organic substrate and produce Volatile Fatty Acids (VFA) and CO2, while in the second step (methanogenesis), the

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methanogenic population consumes VFA and produces methane and CO2. A well known model for such process is the AM2 model [8] which has four main variables (two substrates and two microbial populations). Many mathematical studies were carried out on the qualitative behavior of the AM2 model in generic cases [9], [10], or in particular cases [11], [12], [13]. It is shown that the AM2 model can have at most six equilibria and it can have a monostability or a bistability behavior, according to the functioning conditions. A model for anaerobic membrane digesters has been proposed in [14] for control design purposes. This model named AM2b is based on the modification of the two step model AM2 and integrates the dynamic of a new variable (SMP: Soluble Microbial Products) in the system. It is shown in [14] that the AM2b model can have a rich qualitative with respect to the maximum growth rate of acidogenic bacteria on SMP (which is considered as a bifurcation parameter).

This paper is complementary to [14] and it proposes a detailed mathematical analysis of the qualitative behavior of the model AM2b. It reports briefly some results which presented in [14] and, gives pertinently their mathematical backgrounds. The paper is structured as follows: first, we recall the AM2b model and we prove positivity and boundedness of its variables. Then, we characterize equilibria in some generic cases and we explain the background of their graphical determination. Finally, we investigate through numerical simulation equilibria and their stability of the system, before conclusions and perspectives are formulated.

2. Mathematical model

2.1. Mass balance equations

In Fig. 1, we give a schematic representation of the anaerobic membrane bioreactor for which the model (5-9) is proposed below and, where the membrane retention of soluble and particulate components is illustrated.

We consider the anaerobic mathematical model AM2b presented in [14], where we have four reaction networks:

$$k_1 S_1 \xrightarrow{\mu_1(.)X_1} X_1 + k_2 S_2 + b_3 S + k_4 CO_2 \tag{1}$$

$$k_3 S_2 \xrightarrow{\mu_2(.)X_2} X_2 + b_4 S + k_5 CO_2 + k_6 CH_4 \tag{2}$$

$$b_1 S \xrightarrow{\mu(.)X_1} X_1 + b_2 S_2 + k_7 CO_2 \tag{3}$$

$$D_0 X_1 \longrightarrow D_0 S, \qquad D_0 X_2 \longrightarrow D_0 S \tag{4}$$

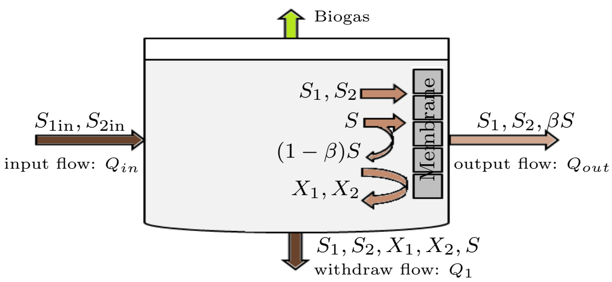


Figure 1: Schematic representation of the compartment bioreactor-membrane

In the first reaction, the substrate S_1 (organic matter) is degraded into substrates S_2 (Volatile Fatty Acids) and S (SMP) by acidogenic bacteria X_1 and then in the second reaction, S_2 is converted into S by methanogenic bacteria X_2. The third reaction network consist in degrading S into S_2 by the consortium X_1. A part of S is produced from biomasses mortality. During reactions (1), (2) and (3), there is a production of biogas.

Mass balance equations are given by:

$$\dot{S}_1 = D(S_{1\text{in}} - S_1) - k_1\mu_1(S_1)X_1, \tag{5}$$
$$\dot{X}_1 = \Big(\mu_1(S_1) + \mu(S) - D_0 - D_1\Big)X_1, \tag{6}$$
$$\dot{S}_2 = D(S_{2\text{in}} - S_2) - k_3\mu_2(S_2)X_2 + \Big(k_2\mu_1(S_1) + b_2\mu(S)\Big)X_1, \tag{7}$$
$$\dot{X}_2 = \Big(\mu_2(S_2) - D_0 - D_1\Big)X_2, \tag{8}$$
$$\dot{S} = \Big(b_3\mu_1(S_1) + D_0 - b_1\mu(S)\Big)X_1 + \Big(b_4\mu_2(S_2) + D_0\Big)X_2 - MS, \tag{9}$$

where $S_{1\text{in}}$ and $S_{2\text{in}}$ are input substrate concentrations, D, D_0 and D_1 are the dilution rate, the mortality biomass and the withdraw rates respectively. Parameters k_i and b_i are pseudo-stoichiometric coefficients associated to the bioreactions and $M = [\beta D + (1-\beta)D_1]$, where $\beta \in [0,1]$ represents the fraction of S leaving the bioreactor (see [14] for more detail on the model development).

We make the following matter conservation principles:

- over a given period of time, the quantity of biomass (or products) produced is always smaller than the quantity of substrate degraded. Thus, from (1-3) one has:

$$k_1 \geq 1 + b_3 + k_2, \tag{10}$$
$$k_3 \geq 1 + b_4, \tag{11}$$
$$b_1 \geq 1 + b_2. \tag{12}$$

- the quantity S_2 produced from S_1 is higher than the quantity produced from the SMP (see (1) and (3)):

$$k_2 > b_2. \tag{13}$$

The kinetics μ_1, μ_2 and μ are assumed to be dependent on S_1, S_2 and S respectively, satisfying the following hypotheses:

Hypothesis 2.1. *$\mu_1(S_1)$ and $\mu(S)$ are of class $\mathcal{C}^1$ and satisfy the following properties:*

- $\mu_1(0) = \mu(0) = 0$,
- $\mu_1'(S_1) > 0$ *and* $\mu'(S) > 0$ *for* $S_1 > 0$ *and* $S > 0$ *respectively,*
- $\mu_1(\infty) = m_1$ *and* $\mu(\infty) = m$.

Hypothesis 2.2. *$\mu_2(S_2)$ is of class $\mathcal{C}^1$ and satisfies the following properties:*

- $\mu_2(0) = \mu_2(\infty) = 0$,
- $\mu_2(S_2)$ *has a maximum* $\mu_2\left(S_2^M\right) > 0$ *for* $S_2 = S_2^M$,
- $\mu_2'(S_2) > 0$ *for* $0 < S_2 < S_2^M$,
- $\mu_2'(S_2) < 0$ *for* $S_2 > S_2^M$.

The model analysis given in this paper, is valid for all functions verifying the hypothesises (2.1) and (2.2). Examples of functions satisfying these assumptions are (see appendix 1 of [15]):

- The Monod kinetics $\mu(\xi) = m\frac{\xi}{\xi+K}$, the Tessier kinetics $\mu(\xi) = m\Big(1 - e^{\frac{-\xi}{K}}\Big)$, the Moser or the Ming et al. kinetics $\mu(\xi) = \frac{m\xi^2}{K+\xi^2}$ (with m and K are constants), which all satisfy hypothesis 2.1.

- The Haldane kinetics $\mu(\xi) = m\frac{\xi}{\frac{\xi^2}{K_i}+\xi+K}$, or the function $\mu(\xi) = K\left(e^{-\alpha_1\xi} - e^{-\alpha_2\xi}\right)$ (with m, K, K_i and $\alpha_2 > \alpha_1$ are constants), which satisfy hypothesis 2.2.

Positivity and boundedness are very important properties for biological systems. We have to check that for zero or positive initial conditions, all variables of system (5-9) are non-negative and bounded for all time.

Proposition 2.3. *The variables (S_1, X_1, S_2, X_2, S) of system (5-9) are positive and bounded.*

Proof 1. *The proof is given in Appendix Appendix A.1.*

3. Equilibria of model

The equilibria of system are solutions of the following nonlinear algebraic system:

$$\begin{aligned}
0 &= D(S_{1\text{in}} - S_1) - k_1\mu_1(S_1)X_1 && (14)\\
0 &= [\mu_1(S_1) + \mu(S) - D_0 - D_1]X_1 && (15)\\
0 &= D(S_{2\text{in}} - S_2) - k_3\mu_2(S_2)X_2 + [k_2\mu_1(S_1) + b_2\mu(S)]X_1 && (16)\\
0 &= [\mu_2(S_2) - D_0 - D_1]X_2 && (17)\\
0 &= [b_3\mu_1(S_1) + D_0 - b_1\mu(S)]X_1 + [b_4\mu_2(S_2) + D_0]X_2 - MS && (18)
\end{aligned}$$

We use the following notations:

$$A = \frac{b_4(D_0 + D_1) + D_0}{k_3(D_0 + D_1)}, \qquad B = \frac{M}{D} = \left[\beta + (1-\beta)\frac{D_1}{D}\right]. \tag{19}$$

If $D_0 + D_1 < \mu_2(S_2^M)$ then $S_2^{1*} < S_2^M < S_2^{2*}$ are the roots of equation $\mu_2(S_2) = D_0 + D_1$ and, we note:

$$\alpha_i := \frac{A}{B}\left(S_{2\text{in}} - S_2^{i*}\right), \quad \beta_i = \frac{D}{k_3(D_0 + D_1)}\left(S_{2\text{in}} - S_2^{i*}\right), \qquad i = 1, 2 \tag{20}$$

From equation (15) one deduce that $X_1 = 0$ or $\mu_1(S_1) + \mu(S) = D_0 + D_1$. The following lemma describes the equilibria points for which $X_1 = 0$, that is to say, there is a washout of X_1.

Lemma 3.1. *The equilibria $(S_1^*, 0, S_2^*, X_2^*, S^*)$ of the system (5-9) are given by:*

- *the washout equilibrium of X_1 and X_2, $E_0^0 = (S_{1\text{in}}, 0, S_{2\text{in}}, 0, 0)$, which always exists,*
- *the washout equilibrium of X_1 but not of X_2,*

$$E_1^i = (S_{1\text{in}}, 0, S_2^{i*}, X_2^{i*}, S^{i*}), \qquad i = 1, 2$$

where S_2^{i*} are the roots of equation $\mu_2(S_2) = D_0 + D_1$, X_2^{i*} and S^{i*} are given by the formulas:

$$X_2^{i*} = \beta_i, \quad S^{i*} = \alpha_i, \quad i = 1, 2.$$

The equilibrium E_1^i exists if and only if:

$$S_{2\text{in}} > S_2^{i*}. \tag{21}$$

Proof 2. *The proof is given in Appendix Appendix A.2.1.*

Now, we consider equilibria for which there is no washout of X_1 but washout of X_2. We introduce the following notations:

$$\begin{aligned}
F(S) &:= \mu_1^{-1}\left(D_0 + D_1 - \mu(S)\right), && (22)\\
G(S_1) &:= (S_{1\text{in}} - S_1)\left(B_1 - \frac{B_2}{\mu_1(S_1)}\right), && (23)
\end{aligned}$$

where:
$$B_1 = \frac{b_1 + b_3}{k_1\beta}, \quad B_2 = \frac{b_1(D_0 + D_1) - D_0}{k_1\beta}, \tag{24}$$

Lemma 3.2. *Let $E_2^0 = (S_1^*, X_1^*, S_2^*, 0, S^*)$ an equilibrium point of the system (5-9), such that $X_1^* > 0$. Then S_1^* and S^* are solutions of the system of equations:*
$$\begin{cases} S_1 = F(S) \\ S = G(S_1) \end{cases} \tag{25}$$
and X_1^* and S_2^* are given by the formulas:
$$X_1^* = \frac{D}{k_1\mu_1(S_1^*)}\left(S_{1\text{in}} - S_1^*\right), \quad S_2^* = S_{2\text{in}} + \frac{k_2\mu_1(S_1^*) + b_2\mu(S^*)}{k_1\mu_1(S_1^*)}\left(S_{1\text{in}} - S_1^*\right).$$
The equilibrium E^* exists if and only if:
$$S_{1\text{in}} > S_1^*. \tag{26}$$

Proof 3. *The proof is given in Appendix Appendix A.2.2.*

Now, we consider equilibria for which there is no washout of X_1 nor X_2. We introduce the following notations:
$$H(S_1) \;:=\; (S_{1\text{in}} - S_1)\left(C_1 - \frac{C_2}{\mu_1(S_1)}\right), \tag{27}$$
$$H_i(S_1) \;:=\; \alpha_i + H(S_1), \qquad i = 1, 2. \tag{28}$$

where:
$$C_1 = B_1 + \frac{A(k_2 - b_2)}{k_1\beta}, \quad C_2 = B_2 - \frac{Ab_2}{k_1\beta}, \tag{29}$$

Lemma 3.3. *Let $E_2^i = (S_1^*, X_1^*, S_2^{i*}, X_2^{i*}, S^*)$, $i = 1, 2$ an equilibrium point of the system (5-9) such that $X_1^* > 0$ and $X_2^* > 0$. Then one has S_2^{i*}, $i = 1, 2$ are the roots of equation $\mu_2(S_2) = D_0 + D_1$, and S_1^* and S^* are solutions of the system of equations:*
$$\begin{cases} S_1 = F(S), \\ S = H_i(S_1), \quad i = 1, 2. \end{cases} \tag{30}$$
and X_1^* and X_2^{i*} are given by the formulas:
$$X_1^* = \frac{D}{k_1\mu_1(S_1^*)}\left(S_{1\text{in}} - S_1^*\right), \quad X_2^{i*} = \beta_i + \frac{D}{k_3(D_0 + D_1)}\frac{k_2\mu_1(S_1^*) + b_2\mu(S^*)}{k_1\mu_1(S_1^*)}\left(S_{1\text{in}} - S_1^*\right)$$
The equilibrium E^* exists if and only if the following conditions hold:
$$S_{1\text{in}} > S_1^* \text{ and } H_i(S_1^*) > G(S_1^*), \quad i = 1, 2. \tag{31}$$

Proof 4. *The proof is given in Appendix Appendix A.2.3.*

Remark 1. *When the system (25) or the system (30) has several solutions (S_{1j}^*, S_j^*), one notes E_{2j}^0 (respectively E_{2j}^1 and E_{2j}^2), $j = 1, 2$ the corresponding equilibria (see section 6.3).*

4. Graphical determination of equilibria

Equilibria of system (5-9) are determined, by finding graphically solutions of system (25) and (30). Values of S_1^* and S^* should be positive and satisfy conditions (26) and (31). Thus, we should study sign of functions $G(S_1)$ and $H_i(S_1)$ and, specify the domain where they are positive.

First, let us give the following lemma:

Lemma 4.1. *We have $\lambda_H < \lambda_G < \lambda_1$ where λ_H, λ_G and λ_1 are defined by:*

$$\lambda_1 = \mu_1^{-1}(D_0 + D_1), \quad \lambda_G = \mu_1^{-1}(D_G), \quad \lambda_H = \mu_1^{-1}(D_H),$$

with $D_G = B_2/B_1$ and $D_H = C_2/C_1$.

Proof 5. *The proof is given in Appendix Appendix A.3.*

In Fig. 2 on the left, we illustrate positions of λ_H, λ_G and λ_1. On the right, we show solutions S_2^{i*}, $i = 1, 2$ of equation $\mu_2(S_2) = D_0 + D_1$.

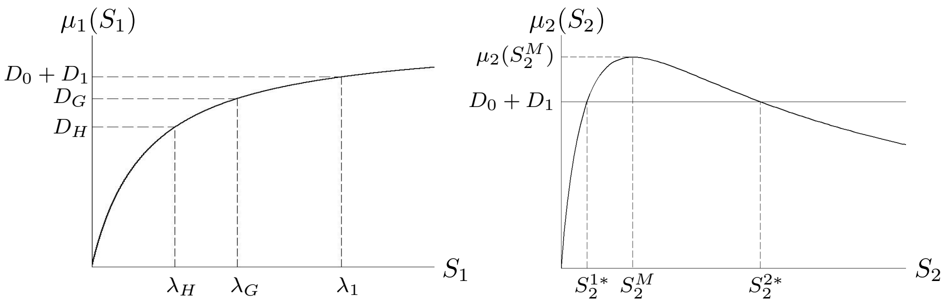


Figure 2: Positions of values λ_1, λ_G and λ_H (left). Solutions $S_2^{i*}, i = 1, 2$ of $\mu_2(S_2) = D_0 + D_1$ (right).

The function $G(S_1)$ defined by (23) is positive for S_1 between $S_{1\text{in}}$ and λ_G, the root of:

$$g(S_1) = B_1 - \frac{B_2}{\mu_1(S_1)}$$

We have two cases (see Fig. 3):

- $D_G > m_1$, where $g(S_1)$ is always negative for $S_1 < S_{1\text{in}}$ (Fig 3, left) and values of $S_1 > S_{1\text{in}}$ do not satisfy the condition (26), or
- $D_G < m_1$, where $g(S_1) > 0$ if and only if $S_1 > \lambda_G$ (Fig 3, right).

The case corresponding to Fig 3, center, is not considered since it does not satisfy the condition (26).

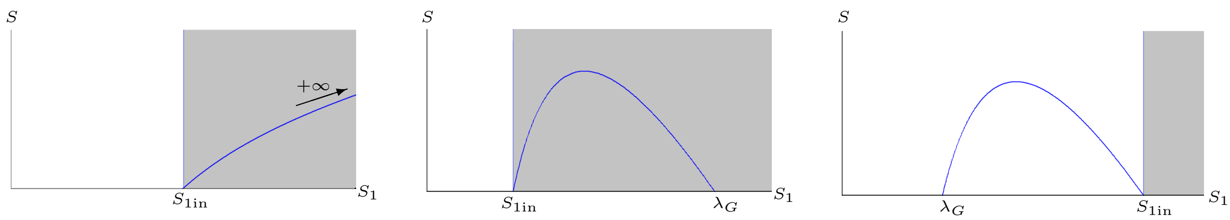


Figure 3: Graphical representation of $G(S_1)$, left: if $D_G > m_1$, center: if $D_G < m_1$ and $S_{1\text{in}} < \lambda_G$, right: if $D_G < m_1$ and $S_{1\text{in}} > \lambda_G$. Gray area represents zone where (26) is not satisfied.

Proposition 4.2. *A necessary condition for (25) to have positive solutions is $\lambda_G < S_{1\text{in}}$ that is to say $\mu_1(S_{1\text{in}}) > D_G$.*

Proof 6. *The proof is given in Appendix Appendix A.4.*

The function $H(S_1)$ defined by (27) is positive for S_1 between $S_{1\text{in}}$ and λ_H, the root of:

$$H(S_1) = C_1 - \frac{C_2}{\mu_1(S_1)}$$

Two cases can be distinguished:

- $D_H > m_1$, where $H(S_1)$ is always negative for $S_1 < S_{1\text{in}}$, or
- $D_H < m_1$, where $H(S_1) > 0$ if and only if $S_{1\text{in}} > S_1 > \lambda_H$ (see Fig. 4).

In the following, we assume that $\mu_1(S_{1\text{in}}) > D_G$. Let us notice that:

- $H(S_1)$ is positive if and only if $\lambda_H < S_1 < S_{1\text{in}}$,
- $G(S_1)$ is positive if and only if $\lambda_G < S_1 < S_{1\text{in}}$,
- $H(S_1) > G(S_1)$ for all $\lambda_H < S_1 < S_{1\text{in}}$.

Proposition 4.3. *The equilibrium E_2^i, $i = 1, 2$ exists if and only if the graph of $H_i(S_1)$ intersects the axis of S_1 on the right of $S_{1\text{in}}$.*

Proof 7. *The proof is given in Appendix Appendix A.5.*

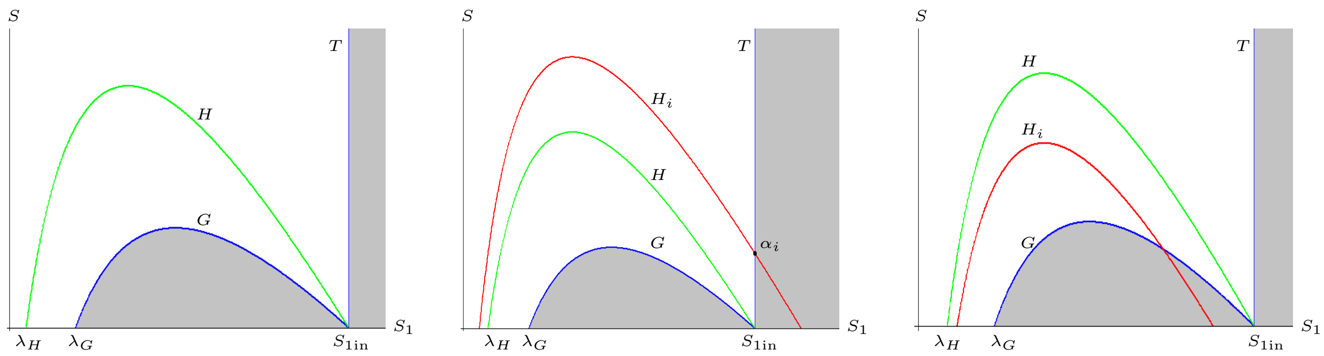


Figure 4: Graphical representation of $H(S_1)$, $H_i(S_1)$ $i = 1, 2$ and $G(S_1)$, left: $H(S_1)$ and $G(S_1)$, center: $\alpha_i > 0$ thus $H_i(S_1) > H(S_1)$, right: $\alpha_i < 0$ thus $H_i(S_1) < H(S_1)$. Gray area represents zone where (26) is not satisfied. Character T is to say *Trivial Equilibria* given by Lemma 3.1.

5. Necessary conditions of existence of equilibria

The existence of equilibria depend on the relative positions of the value of $S_{1\text{in}}$ and the values of λ_H, λ_G and λ_1 (see Fig. 2). We have four cases:

- $S_{1\text{in}} < \lambda_H < \lambda_G < \lambda_1$
- $\lambda_H < S_{1\text{in}} < \lambda_G < \lambda_1$
- $\lambda_H < \lambda_G < S_{1\text{in}} < \lambda_1$
- $\lambda_H < \lambda_G < \lambda_1 < S_{1\text{in}}$

Recall that an equilibrium exists if and only if the conditions (31) are satisfied. We list in the Table 1 the possible existence of equilibria in the four above cases. In all the following figures, the doted vertical line represents $\lambda_1 = F(0)$, the blue graph represents $G(S_1)$, the green one represents $H(S_1)$ and those in red represent $H_1(S_1)$ (top red graph) and $H_2(S_1)$ (bottom red graph).

Remark 2. . *The function $F(S)$ depends on $\mu(S)$, but functions $G(S_1)$ and $H_i(S_1)$, $i = 1, 2$ do not depend on it. For $\mu(0) = 0$, intersections of $F(0) = \lambda_1$ with $G(S)$ and $H_i(S)$ correspond to cases of [9] and [14] as mentioned in the last column of Table 1 and seen on Fig. 8.*

Table 1: Existence of equilibria in the four cases. The symbol 'X' indicates that the equilibrium can exist. If there is no symbol, that indicates that equilibrium does not exist.

Case	Figure	$F\cap G$	$F\cap H_1$	$F\cap H_2$	Corresponding cases in [9] and/or in [14]
$S_{1\text{in}}<\lambda_H<\lambda_G<\lambda_1$	Fig. 5, left				1.1 of [9]
	Fig. 5, center		X		1.2 of [9]
	Fig. 5, right		X	X	1.3 of [9]
$\lambda_H<S_{1\text{in}}<\lambda_G<\lambda_1$	Fig. 6, left		X	X	1.1 of [9]
	Fig. 6, center		X	X	2.1 of [9]
	Fig. 6, right		X	X	1.3 of [9]
$\lambda_H<\lambda_G<S_{1\text{in}}<\lambda_1$	Fig. 7, left	X	X	X	1.1 of [9]
	Fig. 7, center	X	X	X	1.2 of [9] C of [14]
	Fig. 7, right	X	X	X	1.3 of [9] B of [14]
$\lambda_H<\lambda_G<\lambda_1<S_{1\text{in}}$	Fig. 8, top left	X	X	X	2.1 of [9]
	Fig. 8, top center	X	X	X	2.2 of [9]
	Fig. 8, top right	X	X	X	2.3 of [9]
	Fig. 8, bottom left	X	X	X	2.4 of [9]
	Fig. 8, bottom center	X	X	X	2.5 of [9]
	Fig. 8, bottom right	X	X	X	2.6 of [9] A of [14]

Case : $S_{1\text{in}}<\lambda_H<\lambda_G<\lambda_1$

In the case 1, illustrated by Fig. 5 on the left, we have $H>H_1>H_2$ and, intersections $F\cap H_i$ and $F\cap G$ do not give any positive equilibria, because it does not satisfy the condition (26). In the case 2, illustrated by Fig. 5 on the center, we have $H_1>H>H_2$. The equilibrium of $F\cap H_1$ can exist, but there are no equilibria of $F\cap H_2$ and $F\cap G$. The last case 3, represented by Fig. 5 on the right, we have $H_1>H_2>H$. There is a possibility of existence of equilibria $F\cap H_1$ and $F\cap H_2$ for values of m enough high, but equilibria of $F\cap G$ do not exist.

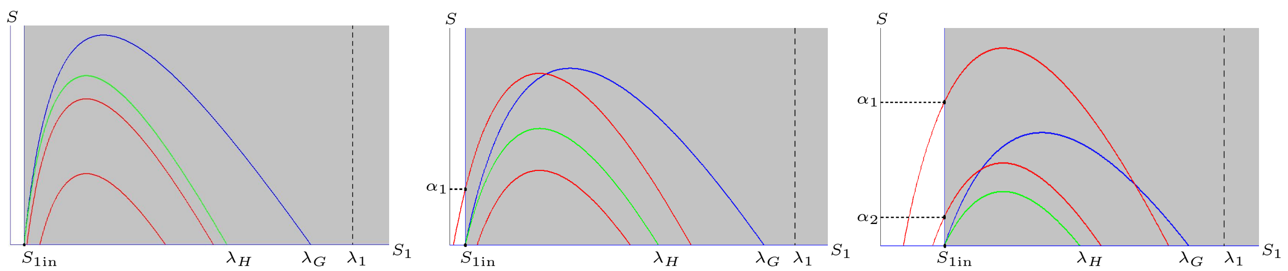


Figure 5: Different graphical representations corresponding to the case $S_{1\text{in}}<\lambda_H$, blue: G, top red: H_1, bottom red: H_2, green: H.

Remark 3. . *When we have intersection of the function $F(S)$ with functions $H_1(S_1)$ and $H_2(S_1)$ at $S_1=S_{1\text{in}}$, then we obtain equilibria $E_1^i=(S_{1\text{in}},0,S_2^{i*},X_2^{i*},S^{i*})$ where $S^{i*}=\alpha_i=H_i(S_{1\text{in}})$, $i=1,2$ as it can be seen on Fig. 5, center, for E_1^1 and on Fig. 5, right, for E_1^i, $i=1,2$.*

Case : $\lambda_H<S_{1\text{in}}<\lambda_G<\lambda_1$

This case is illustrated by Fig. 6, left, center and right for $H>H_1>H_2$, $H_1>H>H_2$ and $H_1>H_2>H$ respectively. Equilibria of $F\cap H_i$, $i=1,2$ can exist for higher values of m, but not those of $F\cap G$ for all values of m.

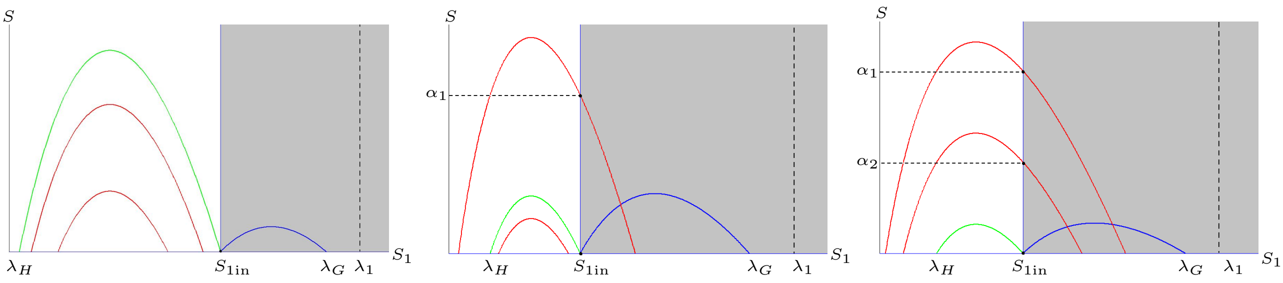


Figure 6: Different graphical representations corresponding to the case $\lambda_H < S_{1\text{in}} < \lambda_G$, blue: G, top red: H_1, bottom red: H_2, green: H.

Case : $\lambda_H < \lambda_G < S_{1\text{in}} < \lambda_1$

We represents this case by Fig. 7, where all equilibria of $F \cap H_i$, $i = 1, 2$ and $F \cap G$ can exist since condition (26) is satisfied. Also, some equilibria bifurcations can occur for higher values of m.

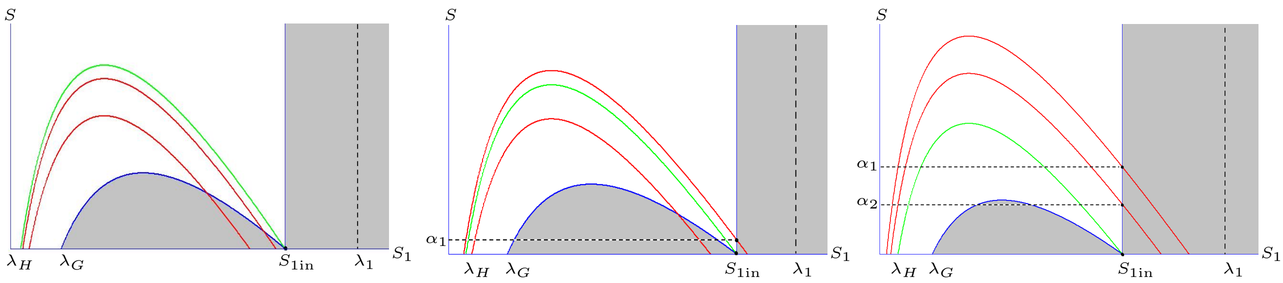


Figure 7: Different graphical representations corresponding to the case $\lambda_G < S_{1\text{in}} < \lambda_1$, blue: G, top red: H_1, bottom red: H_2, green: H.

Case : $\lambda_H < \lambda_G < \lambda_1 < S_{1\text{in}}$

Here we have rich situations, equilibria for $F \cap G$ exist always, while $F \cap H_1$ and $F \cap H_2$ may give both equilibria for all m (see Fig. 8, top-right, bottom-center and bottom-right), only $F \cap H_1$ gives always equilibria (see Fig. 8, top-center and bottom-left) or there is equilibria bifurcations for large values of m for $F \cap H_1$ and/or $F \cap H_2$ (see Fig. 8, top-left for $F \cap H_1$, top-center and bottom-left for $F \cap H_2$).

6. Existence and stability of equilibria

6.1. Stability of equilibria with washout of X_1

For trivial equilibria given by lemma 3.1, the results on their stability are summarized in Theorem 6.1.

Theorem 6.1. *Existence and stability of washout equilibria of X_1 are as follows:*

1. *The equilirium E_0^0 exists always and it is stable if and only if:*

$$\mu_1(S_{1\text{in}}) < D_0 + D_1 \text{ and, } \mu_2(S_{2\text{in}}) < D_0 + D_1 \tag{32}$$

2. *The equilibrium E_1^2 exist if and only if $S_{2\text{in}} > S_2^{2*}$ and it is always unstable.*
3. *The equilibrium E_1^1 exist if and only if $S_{2\text{in}} > S_2^{1*}$ and it is stable if and only if:*

$$\mu_1 \left(S_{1\text{in}}\right) + \mu \left(S^{1*}\right) < D_0 + D_1 \tag{33}$$

Proof 8. *The proof is given in Appendix Appendix A.6.*

The condition (33) my be graphically explained on the Fig. 9. The graph $f(S_1, S) = \mu_1 (S_1) + \mu (S) - D_0 - D_1 = 0$ separates the plane (S_1, S) into two zones:

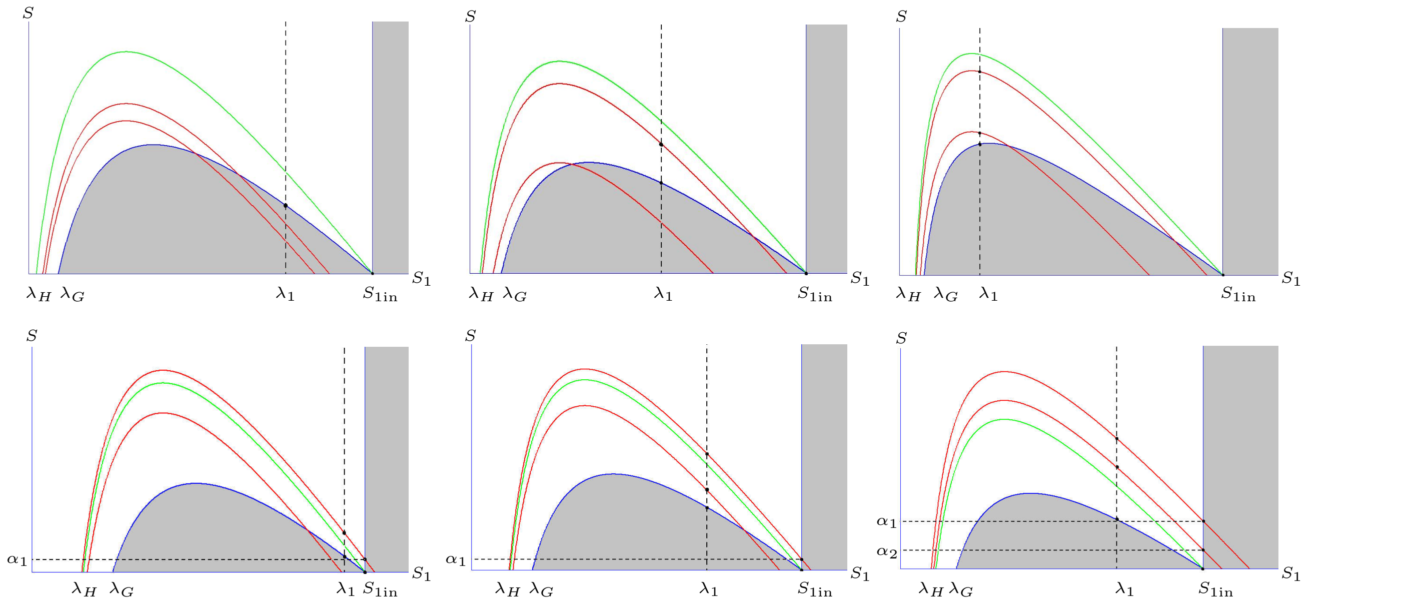


Figure 8: Different graphical representation corresponding to the case $\lambda_1 < S_{1\text{in}}$, blue: G, top red: H_1, bottom red: H_2, green: H.

- Zone Z_0: where $f(S_1, S) > 0$,
- Zone Z_1: where $f(S_1, S) < 0$.

According to the condition (33), the equilibrium E_1^1 is stable if and only if: $E_1^1 \in Z_1$ (the case represented on left in Fig. 9).

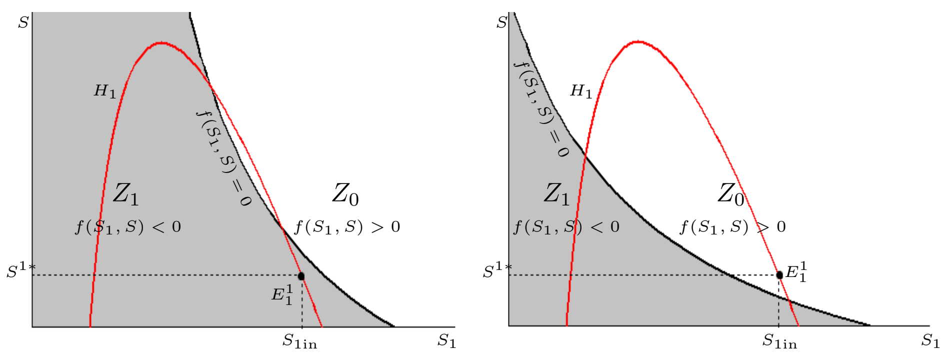


Figure 9: Condition of stability of the equilibrium E_1 (Left: $E_1^1 \in Z_1$, stable. Right: $E_1^1 \in Z_0$, unstable).

6.2. Stability of equilibria with washout of X_2 and stability of equilibria with $X_1 > 0$ and $X_2 > 0$

Here, we improve numerical simulations to check the system stability. Values of model parameters are chosen as in Tables 3 and 4 except the parameter m. According to the considered generic case 1, 2 or 3 represented by Fig. 10, 11 and 12 respectively, the value of the parameter m is varying in specific intervals for which we could have all possible equilibria bifurcations. Stability nature does not depend on values of m in those intervals (see the column *Condition* in Table 5 for values of m). Then, we proceed as follows:

- Develop the Jacobian matrix J of system (5-9) as given by (34),
- Evaluate this matrix for each equilibrium characterized by lemma 3.2 or 3.3,
- Develop the characteristic equation of the evaluated matrix,

- Use Routh-Herwitz criterion to analyze the system stability by numerical simulations (plot the coefficients of the first column of the Routh Table 2 according to m).

The jacobian matrix of system (5-9) evaluated at equilibria is given by (34).

$$J=\begin{bmatrix} -D-k_1\mu_1'(S_1^*)X_1^* & -k_1\mu_1(S_1^*) & 0 & 0 & 0 \\ \mu_1'(S_1^*)X_1^* & \mu_1(S_1^*)+\mu(S^*)-D_0-D_1 & 0 & 0 & \mu'(S^*)X_1^* \\ k_2\mu_1'(S_1^*)X_1^* & k_2\mu_1(S_1^*)+b_2\mu(S^*) & -D-k_3\mu_2'(S_2^*)X_2^* & -k_3\mu_2(S_2^*) & b_2\mu'(S^*)X_1^* \\ 0 & 0 & \mu_2'(S_2^*)X_2^* & \mu_2(S_2^*)-D_0-D_1 & 0 \\ b_3\mu_1'(S_1^*)X_1^* & b_3\mu_1(S_1^*)+D_0-b_1\mu(S^*) & b_4\mu_2'(S_2^*)X_2^* & b_4\mu_2(S_2^*)+D_0 & -M-b_1\mu'(S^*)X_1^* \end{bmatrix} \tag{34}$$

Which can be symbolized as follows:

$$J=\begin{bmatrix} j_{11} & j_{12} & 0 & 0 & 0 \\ j_{21} & j_{22} & 0 & 0 & j_{25} \\ j_{31} & j_{32} & j_{33} & j_{34} & j_{35} \\ 0 & 0 & j_{43} & j_{44} & 0 \\ j_{51} & j_{52} & j_{53} & j_{54} & j_{55} \end{bmatrix} \tag{35}$$

We can distinguish two cases according to lemma 3.2 where $X_1 > 0$ and $X_2 = 0$ or, lemma 3.3 where $X_1 > 0$ and $X_2 > 0$.

- In the case $X_2 = 0$, one has: $j_{33} = -D$ and $j_{43} = j_{53} = 0$.
- In the case $X_1 > 0$ and $X_2 > 0$, one has: $j_{22} = j_{44} = 0$ (from (15) and (17)).

The characteristic equation of the linearized system of (5-9) is:

$$|\lambda.I - J| = 0 \Leftrightarrow \lambda^5 + a_1\lambda^4 + a_2\lambda^3 + a_3\lambda^2 + a_4\lambda + a_5 = 0. \tag{36}$$

where a_i are coefficients depending on j_{ik}, $(i, k = 1..5)$ given by (35). Now, one establishes the following Routh table:

Table 2: Table of Routh for the linearized system of (5-9).

λ^5	1	a_2	a_4	0
λ^4	a_1	a_3	a_5	0
λ^3	n_1	n_2	0	0
λ^2	l_1	a_5	0	0
λ^1	r_1	0	0	0
λ^0	a_5	0	0	0
	0	0	0	0

with:

$$n_1 = \frac{a_1a_2 - a_3}{a_1}, \quad n_2 = \frac{a_1a_4 - a_5}{a_1}, \quad l_1 = \frac{n_1a_3 - n_2a_1}{n_1}, \quad r_1 = \frac{l_1n_2 - a_5n_1}{l_1}$$

The Routh-Herwitz criterion imposes that all coefficients of the first column of the Table 2 must have the same sign, i.e they must be positive (because the first element of the column is positive).

$$a_1 > 0, \quad n_1 > 0, \quad l_1 > 0, \quad r_1 > 0, \quad a_5 > 0 \tag{37}$$

6.3. Numerical simulations

To illustrate our approach, we improve numerical simulations. We present three generic cases illustrated by Figs. 10, 11 and 12, which are obtained for the biological parameters values given in Tables 3 and 4 and, kinetics functions (38), satisfying hypotheses 2.1 and 2.2.

$$\mu_1(S_1) = m_1 \frac{S_1}{S_1 + K_1}, \qquad \mu(S) = m \frac{S}{S + K}, \qquad \mu_2(S_2) = m_2 \frac{S_2}{\frac{S_2^2}{K_i} + S_2 + K_2}. \tag{38}$$

Table 3: Nominal values for the parameters of the AM2b model [14]

Parameter	Value	Parameter	Value	Parameter	Value
m_1	1.2	β	0.6	b_2	0.6
m_2	1.5	k_2	15	b_4	5
K_2	0.3	k_3	16.08	m	varying
K_I	0.9	b_1	5	K	3
D	1	D_0	0.25		

Table 4: Values for adjustable parameters K_1, $S_{1\text{in}}$, $S_{2\text{in}}$ and D_1 for each figure.

Parameter	Generic case 1 (Fig. 10)	Generic case 2 (Fig. 11)	Generic case 3 (Fig. 12)
K_1	10	16	18
$S_{1\text{in}}$	15	15	10
$S_{2\text{in}}$	1	1	0.6
D_1	0.4	0.4	0.25

If they exist, equilibria are noted on figures by:

- E_{2j}^1: equilibria given by the intersection of $F(S)$ with $H_1(S_1)$, $j = 1, 2$,
- E_{2j}^2: equilibria given by the intersection of $F(S)$ with $H_2(S_1)$, $j = 1, 2$,
- E_{2j}^0: equilibria given by the intersection of $F(S)$ with $G(S_1)$, $j = 1, 2$.

The form of $F(S)$ changes according to the value of the parameter m, the maximum growth rate of $\mu(S)$ (see (22)). Consequently, $F(S)$ can have one or two intersections with each one of functions $H_1(S_1)$, $H_2(S_1)$ or $G(S_1)$ as illustrated in Figs. 10, 11 and 12.

In Fig. 10 corresponding to the generic case 1, we have only one equilibrium noted E_1^1, E_1^2 and E_1^0 for each intersection of F with H_1, H_2 and G respectively.

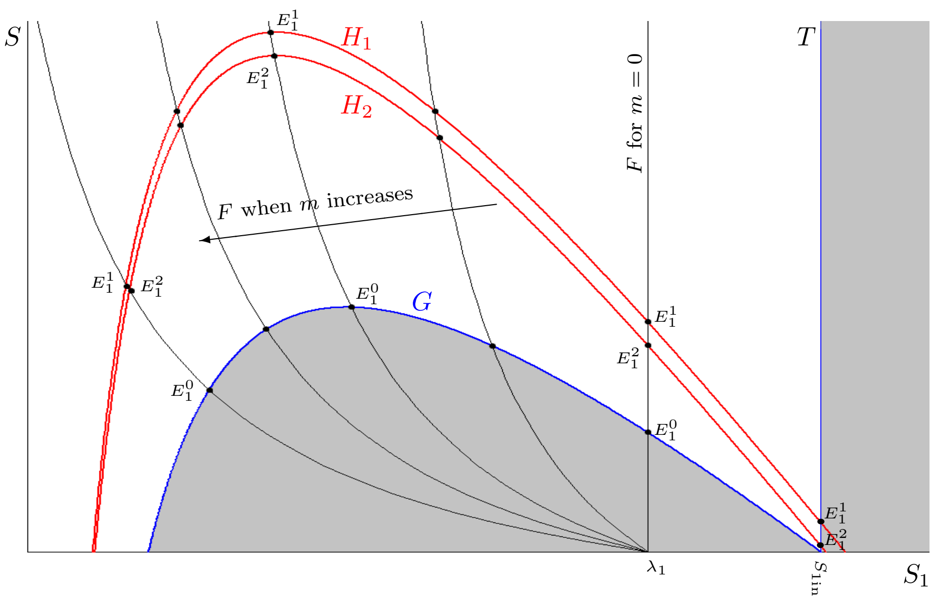


Figure 10: Intersection of the graph of $F(S)$ with the graphs $G(S_1)$ and $H_i(S_1)$ in the Case 1.

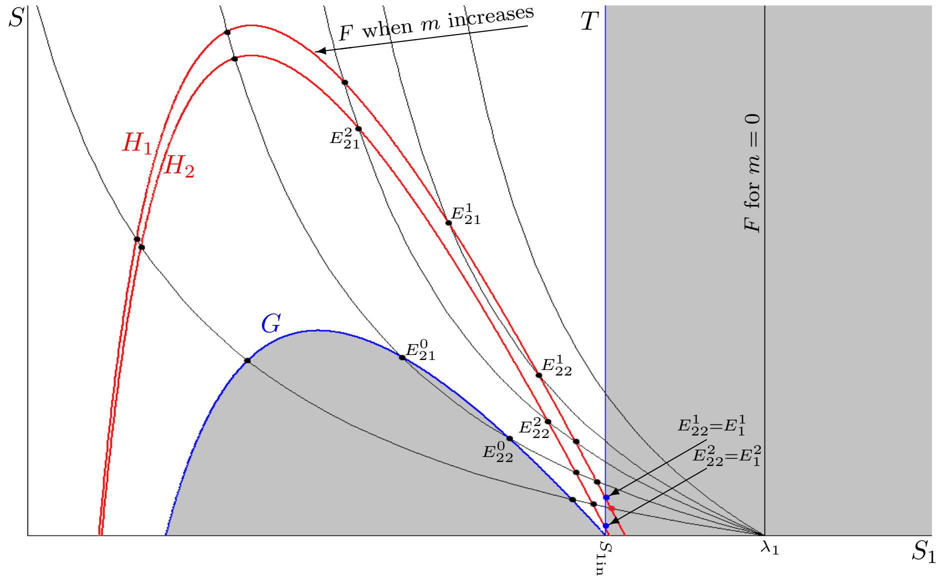


Figure 11: Intersection of the graph of $F(S)$ with the graphs $G(S_1)$ and $H_i(S_1)$ in the Case 2.

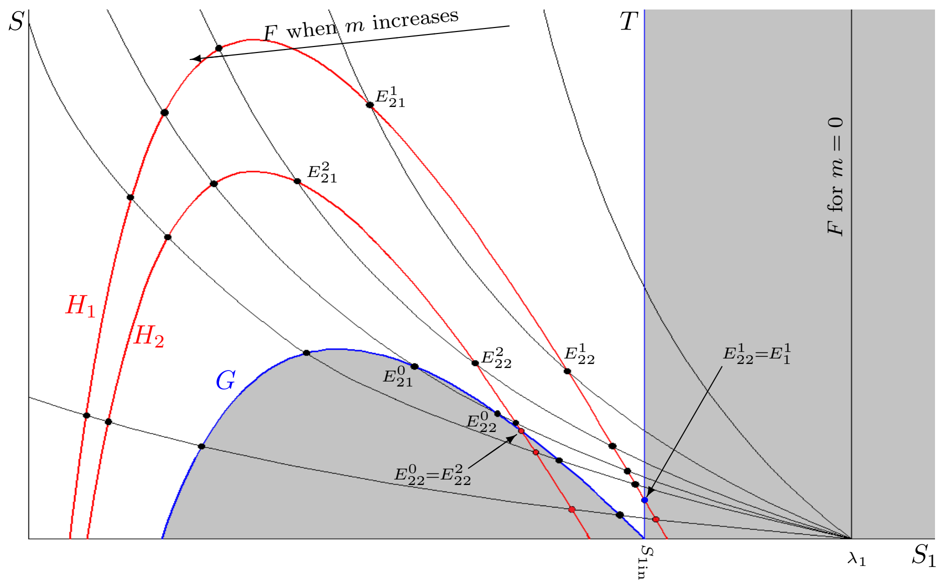


Figure 12: Intersection of the graph of $F(S)$ with the graphs $G(S_1)$ and $H_i(S_1)$ in the Case 3.

In generic cases 2 and 3, we have equilibria bifurcation when m varies. For some values c_i, $i = 1, .., 5$ of m, (of course, they are different between cases 2 and 3), the graph of $F(S)$ intersects graphs of $H_i(S_1)$ and $G(S_1)$ (see Figs. 13 and 14), leading to the apparition of new equilibria. The reader can refer to [14], [16] for more details.

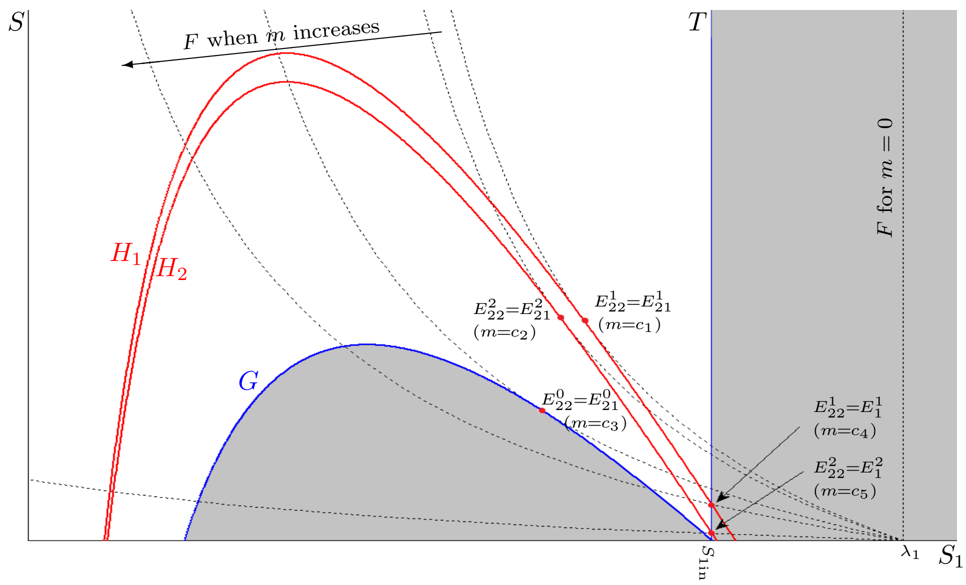


Figure 13: Values c_i, $i = 1..5$ of m giving equilibria bifurcation in the generic case 2.

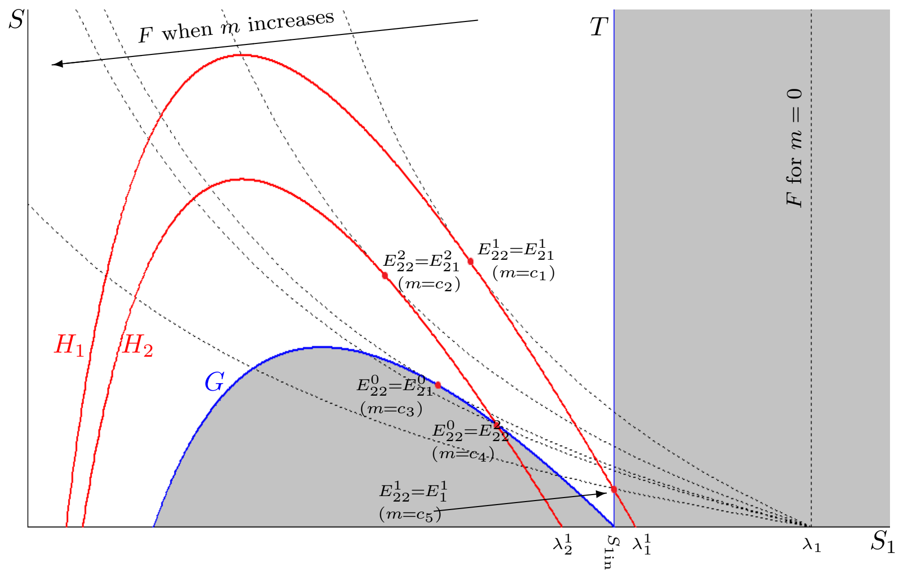


Figure 14: Values c_i, $i = 1..5$ of m giving equilibria bifurcation in the generic case 3.

Equilibria of system and their nature according to m in the three generic cases are summarized in Table 5, where T stands for *Trivial Equilibria* E^0_0, E^1_1 *and* E^2_1, $F \cap H_1$, $F \cap H_2$ and $F \cap G$ stand for *Equilibria obtained by the intersections of the graph* F *with graphs* H_1, H_2 *and* G, respectively, S and U stand for *Stable* and *Unstable* Equilibrium, respectively. If there is no symbol, then it means that the equilibrium does not exist. Stability nature of equilibria corresponding to the washout of X_2 ($F \cap G$) and the existence of both X_1 and X_2 ($F \cap H_1$ and $F \cap H_2$) is checked by using the Routh-Herwitz criterion as detailed in the section 6.2.

On Figs. 15, 16, and 17, we represent the coefficients of the first column of Table 2 with different colors: a_1 in black, n_1 in blue, l_1 in red, r_1 in magenta and a_5 in green. On Figs. 16, and 17, vertical lines represent bifurcation values c_i, $i = 1..5$ of the parameter m (they are different between the two figures). According to the considered case, coefficients are represented only for values of m, for which equilibria may exist. For instance, Routh coefficients for the equilibrium E^1_{22} are represented on Fig. 16, bottom-left, only for $c_1 \leq m \leq c_4$. If equilibrium is stable, then all coefficients must be positive in the corresponding interval of m.

Table 5: Equilibria and their nature in generic cases represented in Figs. 10, 11 and 12. Values of c_i, $i = 1..5$ of the case 2 are different from those of the case 3.

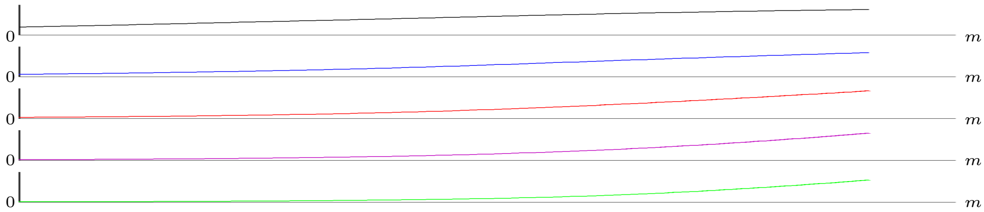
Cases	**Condition**	**Equilibria and nature**								
			$F \cap T$		$F \cap G$		$F \cap H_1$		$F \cap H_2$	
		E_0^0	E_1^1	E_1^2	E_{21}^0	E_{22}^0	E_{21}^1	E_{22}^1	E_{21}^2	E_{22}^2
Case 1 (Fig. 10)	$m \geq 0$	U	U	U	S		U		S	
Case 2 (Fig. 11)										
2.1	$0 \leq m < c_1$	S	S	U						
2.2	$c_1 < m < c_2$	S	S	U			S	U		
2.3	$c_2 < m < c_3$	S	S	U			S	U	U	U
2.4	$c_3 < m < c_4$	S	S	U	S	U	S	U	U	U
2.5	$c_4 < m < c_5$	S	U	U	S	U	S		U	U
2.6	$c_5 < m$	S	U	U	S	U	S		U	
Case 3 (Fig. 12)										
3.1	$0 \leq m < c_1$	U	S							
3.2	$c_1 < m < c_2$	U	S				S	U		
3.3	$c_2 < m < c_3$	U	S				S	U	U	U
3.4	$c_3 < m < c_4$	U	S		S	U	S	U	U	U
3.5	$c_4 < m < c_5$	U	S		S	U	S	U	U	
3.6	$c_5 < m$	U	U		S	U	S		U	

Remark 4. . *Stability nature of equilibria E_0^0, E_1^1 and E_1^2 of the case 1 in Table 5, can be seen on Fig. 10 as follows:*

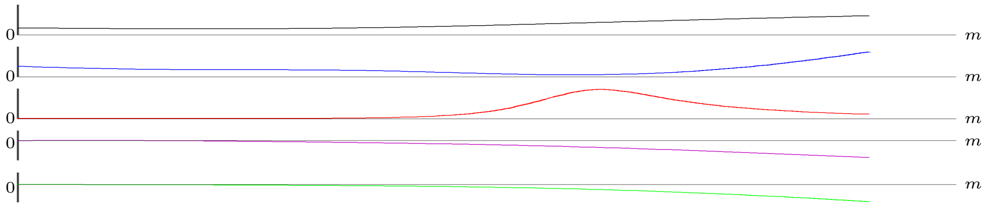
- $\lambda_1 < S_{1\text{in}} \Rightarrow \mu_1(\lambda_1) < \mu_1(S_{1\text{in}})$, *that is to say* $D_0 + D_1 < \mu_1(S_{1\text{in}}) \Rightarrow E_0^0$ *is unstable according to theorem 6.1.1*
- $(S_{1\text{in}}, S^{1*}) \in Z_0 \Rightarrow E_1^1$ *is unstable according to condition (33) of theorem 6.1.3 and, Fig. 9.*
- E_1^2 *does exist according to proposition 4.3 and, is unstable thanks to theorem 6.1.2.*

Remark 5. . *Stability nature of the equilibrium E_0^0 of the cases 2 and 3 in Table 5, can be analyzed as follows:*

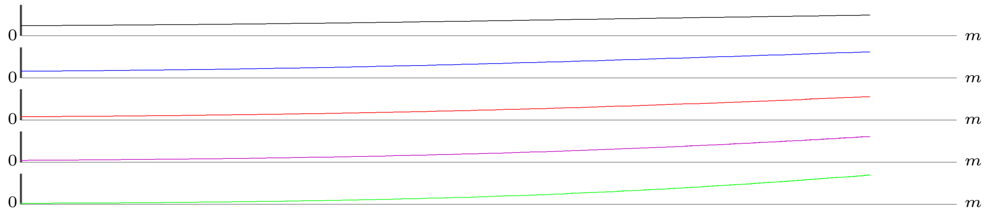
- $\mu_1(S_{1\text{in}}) < D_0 + D_1$ *as it is seen on Fig. 11 and 12 for both cases.*
- *From parameters values in Tables 3 and 4 and, according to condition (32) of theorem 6.1 we have:*
  $\mu_2(S_{2\text{in}}) < D_0 + D_1$ *for the case 2, thus the equilibrium E_0^0 is Stable.*
  $\mu_2(S_{2\text{in}}) > D_0 + D_1$ *for the case 3, thus the equilibrium E_0^0 is Unstable.*



Equilibrium of $F \cap H_1$

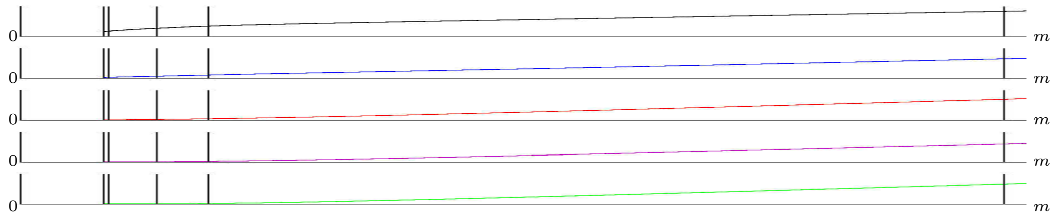


Equilibrium of $F \cap H_2$



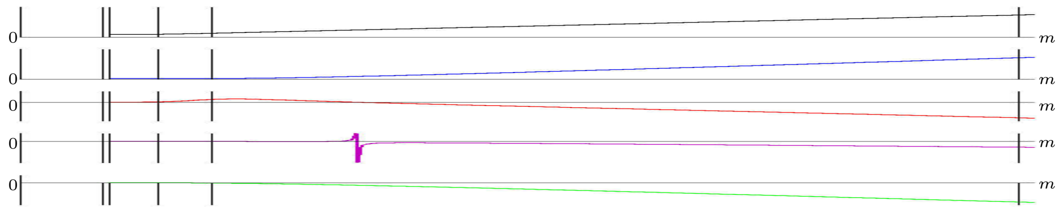
Equilibrium of $F \cap G$

Figure 15: The coefficients of the first column of the Routh Table 2 in the generic case 1: a_1 (——), n_1 (——), l_1 (——), r_1 (——) and a_5 (——).

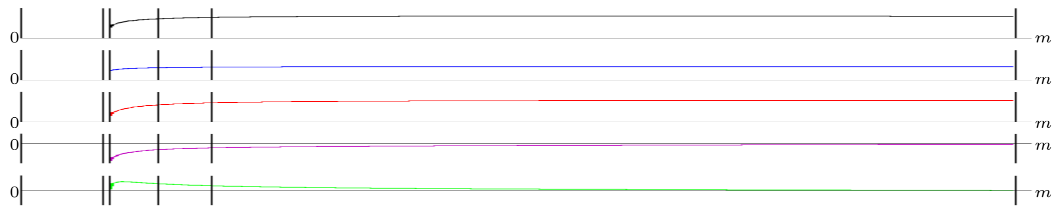


First equilibrium E^1_{21} of $F \cap H_1$

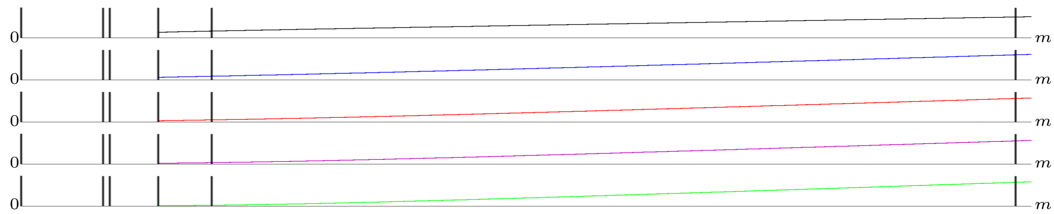
Second equilibrium E^1_{22} of $F \cap H_1$



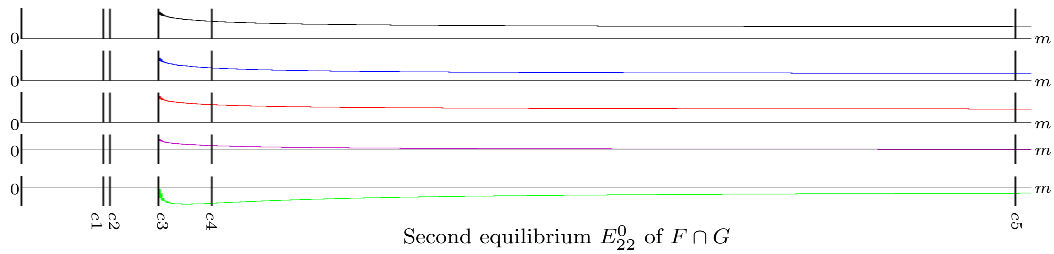
First equilibrium E^2_{21} of $F \cap H_2$



Second equilibrium E^2_{22} of $F \cap H_2$

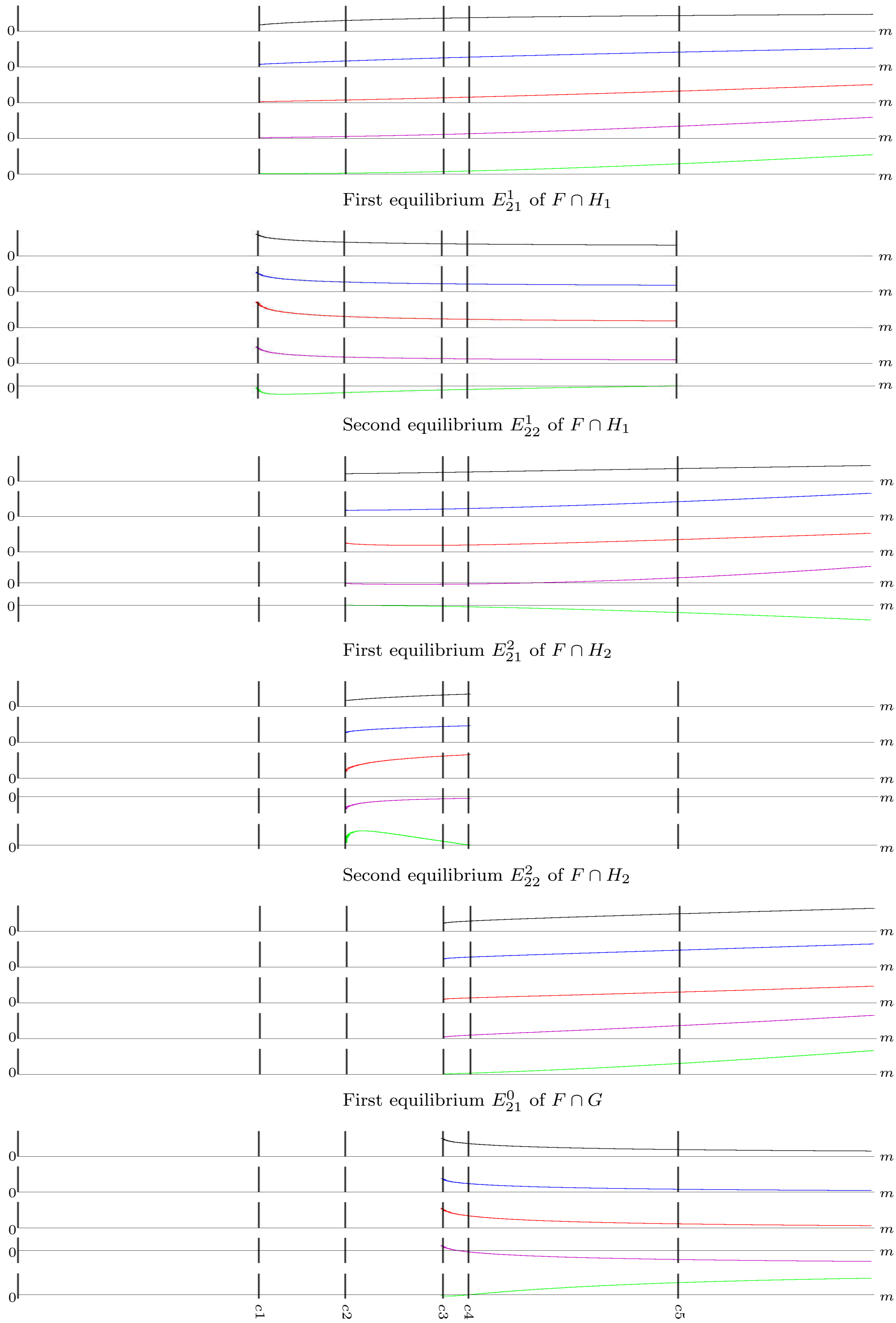


First equilibrium E^0_{21} of $F \cap G$



Second equilibrium E^0_{22} of $F \cap G$

Figure 16: The coefficients of the first column of the Routh Table 2 in the generic case 2, (coordinates $m_{ci}, i = 1..5$ on the X-axis are the same for all the sub-figures): a_1 (——), n_1 (——), l_1 (——), r_1 (——) and a_5 (——)



First equilibrium E^1_{21} of $F \cap H_1$

Second equilibrium E^1_{22} of $F \cap H_1$

First equilibrium E^2_{21} of $F \cap H_2$

Second equilibrium E^2_{22} of $F \cap H_2$

First equilibrium E^0_{21} of $F \cap G$

Second equilibrium E^0_{22} of $F \cap G$

Figure 17: The coefficients of the first column of the Routh Table 2 in the generic case 3, (coordinates $m_{ci}, i = 1..5$ on the X-axis are the same for all the sub-figures): a_1 (——), n_1 (——), l_1 (——), r_1 (——) and a_5 (——).

As can be seen on figures, if they exist:

- The first equilibrium E^1_{21} of $F \cap H_1$ and the first equilibrium E^0_{21} of $F \cap G$ are always stable. Coefficients given by (37) of the first column of Routh table are always positive.
- The second equilibrium E^1_{22} of $F \cap H_1$ and the second equilibrium E^0_{22} of $F \cap G$ are always unstable. Some coefficients given by (37) are (or become) negative (for instance a_5 on Fig. 16, second sub-Fig

from top, is always negative, or r_1 in magenta on Fig. 17, last sub-Fig, becomes negative).

- both equilibria E_{21}^2 and E_{22}^2 of $F \cap H_2$ are unstable. Some coefficients of (37) are always negative (for instance r_1 in magenta and a_5 in green on Fig. 15).

At this stage of discussion about stability nature of possible equilibria, we give a conjecture on positive ones which are obtained for $F \cap H_i$, $i = 1, 2$.

Conjecture 6.2. .

- *Equilibria E_{2j}^2, $j = 1, 2$, resulting resulting from $F \cap H_2$ are unstable if they exist.*
- *The only stable equilibrium E_{21}^1 resulting from $F \cap H_1$, is the one which corresponds to the smallest value of S_1^*.*

7. Conclusion

In this paper we investigated the effect of new variable integration on steady states of a two-step Anaerobic Digestion model. This later consider dynamics of five variables: two bacteria populations and three substrates including the new variable. Model equilibria and their stability was analyzed analytically and using numerical simulations in different generic cases accordingly to the maximum growth rate (bifurcation parameter) of the first bacteria population on the new substrate. We have highlighted that the system displays rich qualitative behavior in terms of bifurcation equilibria and multistability. We have defined a set of system parameters values, especially for the maximum growth rate on the new variable, for which we obtained nine equilibria where four of theme are stable (multistability). Perspective of this work consists of establishing of complete operating diagram of the considered model with respect of operating parameters, especially the maximum growth rate of the first bacteria on the new variable.

Appendix A. Proofs

Appendix A.1. Proof of propositions 2.3

Appendix A.1.1. Positivity

We have the following solutions for equations (6) and (8):

$$X_1(t) = X_1(0)e^{\int_0^t [\mu_1(S_1(\tau))+\mu(S(\tau))-D_0-D_1]d\tau},$$

$$X_2(t) = X_2(0)e^{\int_0^t [\mu_2(S_2(\tau))-D_0-D_1]d\tau}.$$

Thus, we have:

- $X_1(0) = 0 \Rightarrow X_1(t) = 0$ and $X_2(0) = 0 \Rightarrow X_2(t) = 0$,
- $X_1(0) > 0 \Rightarrow X_1(t) > 0$ and $X_2(0) > 0 \Rightarrow X_2(t) > 0$.

To prove the positivity of S_1, S_2 and S, we set these variables equal to zero in (5), (7) and (9) respectively and, we verify if their derivatives are positives:

- $\dot{S}_1 = DS_{1\text{in}} > 0$,
- $\dot{S}_2 = DS_{2\text{in}} > 0$,
- $\dot{S} = D_0X_1 + D_0X_2 > 0$, if $X_1 > 0$ and $X_2 > 0$.

Notice that $\dot{S}_1$, $\dot{S}_2$ and $\dot{S}$ are positives. All vector fields at bounds are inside directed. Consequently, the variables S_1, S_2 and S remain positives for positive initial conditions.

Appendix A.1.2. Boundedness

Let us define the quantity:

$$\Sigma = S_1 + S_2 + X_1 + X_2 + S.$$

The dynamic of Σ is written as follows:

$$\dot{\Sigma} = D(S_{1\text{in}} + S_{2\text{in}}) - D(S_1 + S_2) - D_1(X_1 + X_2) - MS$$

$$-(k_1 - 1 - b_3 - k_2)\mu_1(S_1)X_1 - (k_3 - 1 - b_4)\mu_2(S_2)X_2 - (b_1 - 1 - b_2)\mu(S)X_1.$$

We have three dilution rates: D, D_1 and M which is a combination of D and D_1. Let us set $D_{min} = min(D, D_1)$, which allows to write:

$$\dot{\Sigma} \leq D(S_{1\text{in}} + S_{2\text{in}}) - D_{min}\Sigma - (k_1 - 1 - b_3 - k_2)\mu_1X_1 - (k_3 - 1 - b_4)\mu_2X_2 - (b_1 - 1 - b_2)\mu X_1. \quad \text{(A.1)}$$

By using inequalities (10), (11) and (12), we can write:

$$\dot{\Sigma} \leq D(S_{1\text{in}} + S_{2\text{in}}) - D_{min}\Sigma.$$

Since the solution of the equation $\dot{\Sigma}_0 = D(S_{1\text{in}} + S_{2\text{in}}) - D_{min}\Sigma_0$ is:

$$\Sigma_0(t) = \frac{D(S_{1\text{in}} + S_{2\text{in}})}{D_{min}} + Ce^{-D_{min}t}, \quad \text{with } C \text{ is constant},$$

then, we have $\Sigma(t) \leq \Sigma_0(t)$, i.e:

$$\Sigma(t) \leq \frac{D(S_{1\text{in}} + S_{2\text{in}})}{D_{min}} + Ce^{-D_{min}t} \Longrightarrow \lim_{t\to+\infty} \Sigma(t) \leq \frac{D(S_{1\text{in}} + S_{2\text{in}})}{D_{min}}.$$

Consequently, the variables of system (5-9) remain bounded.

Appendix A.2. Proof of Lemma 3.1, 3.2 and 3.3

The equilibrium points are solutions of the nonlinear algebraic system obtained from (5-9) by setting the right-hand sides equal to zero.

Appendix A.2.1. Proof of Lemma 3.1 ($X_1^* = 0$)

From (15), we can have a trivial solution $X_1^* = 0$, which if replaced in (14), then we obtain $S_1^* = S_{1\text{in}}$. From equation (17), we can have two cases:

- A trivial solution $X_2^* = 0$: which if replaced in (16) and (18), then we have $S_2^* = S_{2\text{in}}$ and $S^* = 0$ respectively. This the equilibrium E_0^0.
- A nontrivial solution $S_2^* = \mu_2^{-1}(D_0 + D_1) = S_2^{i*}$, $i = 1, 2$: which if replaced in (16) and (18), then we deduce corresponding values of X_2^{i*} and S^{i*} respectively. These are equilibria E_1^i, $i = 1, 2$.

Appendix A.2.2. Proof of Lemma 3.2 ($X_1^* > 0$ and $X_2^* = 0$)

Let $(S_1^*, X_1^*, S_2^*, X_2^*, S^*)$ a solution of system (14)-(18).
Since $X_1^* > 0$, from (15) we have $\mu_1(S_1^*) + \mu(S^*) = D_0 + D_1$, i.e:

$$S_1^* = \mu_1^{-1}(D_0 + D_1 - \mu(S^*)) = F(S^*).$$

From (14), we deduce:

$$X_1^* = D\frac{S_{1\text{in}} - S_1^*}{k_1\mu_1(S_1^*)},$$

which is positive and bounded if $S_1^* < S_{1\text{in}}$. By replacing $X_2^* = 0$ and X_1^* in (16) we obtain:

$$S_2^* = S_{2\text{in}} + [k_2\mu_1(S_1^*) + b_2\mu(S^*)]\frac{S_{1\text{in}} - S_1^*}{k_1\mu_1(S_1^*)}.$$

Finally, if we replace X_2^*, X_1^* and $\mu(S^*) = D_0 + D_1 - \mu_1(S_1^*)$ in (18), then we have after simplification:

$$S^* = (S_{1\text{in}} - S_1^*)\left(B_1 + \frac{B_2}{\mu_1(S_1^*)}\right) = G(S_1^*),$$

with:

$$B_1 = \frac{b_3 + b_1}{k_1 B}, \qquad B_2 = \frac{D_0 - b_1(D_0 + D_1)}{k_1 B}, \qquad B = \beta + (1 - \beta)\frac{D_1}{D}.$$

Then S_1^* and S^* are solutions of the system of equations (25).

Appendix A.2.3. Proof of Lemma 3.3 ($X_1^* > 0$ and $X_2^* > 0$)

Let $(S_1^*, X_1^*, S_2^*, X_2^*, S^*)$ a solution of system (14)-(18).
Since $X_2^* > 0$, from (17) we have the nontrivial solution:

$$S_2^* = \mu_2^{-1}(D_0 + D_1) = S_2^{i*}, \quad i = 1, 2.$$

Since $X_1^* > 0$, from (15) we have:

$$S_1^* = \mu_1^{-1}(D_0 + D_1 - \mu(S^*)) = F(S^*),$$

and thus, if $0 < S_1^* < S_{1\text{in}}$ then, we deduce from (14):

$$X_1^* = \frac{D[S_{1\text{in}} - S_1^*]}{k_1\mu_1(S_1^*)}.$$

By replacing X_1^* in (16), we obtain:

$$X_2^{i*} = \beta_i + \frac{D}{k_3(D_0 + D_1)} \frac{k_2\mu_1(S_1^*) + b_2\mu(S^*)}{k_1\mu_1(S_1^*)} \left(S_{1\text{in}} - S_1^*\right),$$

with : $\beta_i = \frac{D}{k_3(D_0+D_1)} \left(S_{2\text{in}} - S_2^{i*}\right), \quad i = 1, 2$
Finally, from (18) we have after simplification:

$$S^* = \alpha_i + (S_{1\text{in}} - S_1^*) \left(C_1 - \frac{C_2}{\mu_1(S_1^*)}\right) = H_i(S_1^*), i = 1, 2,$$

with:

$$\alpha_i = \frac{A}{B}(S_{2\text{in}} - S_2^{i*}), \qquad C_1 = B_1 + \frac{A(k_2 - b_2)}{k_1\beta}, \qquad C_2 = B_2 - \frac{Ab_2}{k_1\beta}.$$

$$A = \frac{b_4(D_0 + D_1) + D_0}{k_3(D_0 + D_1)}, \qquad B = \beta + (1 - \beta)\frac{D_1}{D}.$$

Then S_1^* and S^* are solutions of the system of equations (30).
The function $H_i(S_1^*)$ can be written as:

$$H_i(S_1) = G(S_1) + \frac{A}{B}\Big[S_{2\text{in}} - S_2^{i*} + (k_2\mu_1(S_1) + b_2\mu(S))\frac{S_{1\text{in}} - S_1}{k_1\mu_1(S_1)}\Big].$$

The condition for which $X_2^{i*} > 0$ is:

$$S_{2\text{in}} - S_2^{i*} + (k_2\mu_1(S_1^*) + b_2\mu(S^*))\frac{S_{1\text{in}} - S_1^*}{k_1\mu_1(S_1^*)} > 0,$$

which is equivalent to: $H_i(S_1^*) > G(S_1^*), \quad i = 1, 2$. (condition of lemma 3.3).

Appendix A.3. Proof of Lemma 4.1

Using (13), we have $C_1 > B_1$ and $C_2 > B_2 > 0$, consequently: $D_H < D_G < D_0 + D_1$.
Using the fact that mu_1 is increasing, we deduce that:

$$\lambda_H < \lambda_G < \lambda_1 \tag{A.2}$$

with: $\lambda_1 = \mu_1^{-1}(D_0 + D_1)$, $\lambda_G = \mu_1^{-1}(D_G)$ and $\lambda_H = \mu_1^{-1}(D_H)$, where $D_G = B_2/B_1$ and $D_H = C_2/C_1$.

Appendix A.4. Proof of the proposition 4.2

The function $G(S_1)$ given by (23) is positive between $S_{1\text{in}} > S_1 > \lambda_G$ and, solutions of the system (25) must satisfy $S_{1\text{in}} > S_1^*$, then $S_{1\text{in}} > \lambda_G$ that is to say $\mu_1(S_{1\text{in}}) > D_G$.

Appendix A.5. Proof of the proposition 4.3

Functions $H_i(S_1)$, $i = 1, 2$ given by (28) are translations of the function $H(S_1)$ with quantities α_i given by (20). The sign of this later indicates if the equilibrium E_2^i, $i = 1, 2$ does exist or not (see Fig. 4).

Appendix A.6. Proof of Theorem 6.1

The study of the local stability of trivial equilibria follows easily from the study of the Jacobian matrix of system (5-9), which has a block-diagonal structure:

$$J = \begin{bmatrix} A & 0 & 0 \\ C & B & 0 \\ M_1 & M_2 & -M \end{bmatrix},$$

Hence, the eigenvalues of J are the eigenvalues of A, the eigenvalues of B and $-M$ (which is always negative).

For the X_1 and X_2 washout equilibrium $E_0^0 = [S_{1\text{in}}, 0, S_{2\text{in}}, 0, 0]$:
one has:

$$A = \begin{bmatrix} -D & -k_1\mu_1(S_{1\text{in}}) \\ 0 & \mu_1(S_{1\text{in}}) - D_0 - D_1 \end{bmatrix},$$

$$B = \begin{bmatrix} -D & -k_3\mu_2(S_{2\text{in}}) \\ 0 & \mu_2(S_{2\text{in}}) - D_0 - D_1 \end{bmatrix}.$$

Conditions of stability are: $tr(A) < 0$, $tr(B) < 0$, $det(A) > 0$ and $det(B) > 0$. Thus, E_0^0 is stable if and only if:

$$\mu_1(S_{1\text{in}}) < D_0 + D_1 \text{ and, } \mu_2(S_{2\text{in}}) < D_0 + D_1$$

For the X_1 washout equilibria $E_1^i = [S_{1\text{in}}, 0, S_2^{i*}, X_2^{i*}, S^{i*}]$, $i = 1, 2$:
one has:

$$A = \begin{bmatrix} -D & -k_1\mu_1(S_{1\text{in}}) \\ 0 & \mu_1(S_{1\text{in}}) + \mu\left(S^{i*}\right) - D_0 - D_1 \end{bmatrix},$$

$$B = \begin{bmatrix} -D - k_3\mu_2'\left(S_2^{i*}\right)X_2^{i*} & -k_3\mu_2\left(S_2^{i*}\right) \\ \mu_2'\left(S_2^{i*}\right)X_2^{i*} & 0 \end{bmatrix}.$$

One can easily deduce that if E_1^2 exists, then it is unstable because $det(B) < 0$, since $\mu_2'\left(S_2^{2*}\right) < 0$.
On the other hand, stability of E_1^1 depends on $\mu(S^{1*})$. Indeed, E_1^1 is stable if and only if:

$$\mu_1(S_{1\text{in}}) + \mu\left(S^{1*}\right) < D_0 + D_1$$

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Conflict of interest

The authors declare that they have no competing interests.

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