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Comparative analysis of two chemostat models including substrate and biomass inhibitions

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Abstract: We consider the acetogenesis and hydrogenotrophic methanogenesis phases of the anaerobic digestion model and we include the inhibition of methanogenics, first by volatile fatty acids (VFAs) then by acetogenics. We investigate mathematically the dynamics of two chemostat models described by systems of four nonlinear ordinary differential equations. We established the conditions of existence and stability of equilibrium points in each of the models with respect to the dilution rate. The operating diagrams allowed to reveal the similarities and the differences between regions of stability of the two models and to present the consequent transcritical bifurcations between boundary and positive equilibrium. Models are equivalent for low inlet substrate concentration and significantly different for high concentration. When inhibition is by acetogens and for high concentrations of inlet substrate, the upstream species tends to eliminate the downstream species from the vessel.

Keywords: synthetic microbial community, chemostat, anaerobic digestion, inhibition, stability, operating diagram, bifurcation

I. INTRODUCTION

In recent years, concerns about the availability and sustainability of traditional energy sources, particularly fossil fuels, have become more pronounced which led to invest in diverse energy sources [1]. Methane production can indeed be part of the solution to address energy shortages, particularly in situations where there is an abundance of organic waste or where natural gas resources are not accessible. Methane, the primary component of natural gas, is a potent energy source that can be produced through anaerobic digestion of organic materials in controlled environmental conditions such as the chemostat. This bioreactor allows for precise control over environmental factors such as temperature, pH, nutrient availability, and substrate concentration, to promote the growth and activity of methanogenic species [2–6].

The microorganisms responsible for producing methane through anaerobic digestion are primarily methanogenic archaea, these methanogens often engage in syntrophic interactions with acetogenic bacteria or hydrogen-producing microorganisms. In a chemostat, these syntrophic relationships can be facilitated by coculturing methanogens with suitable partner microorganisms, the acetogenic bacteria, that produce acetate or hydrogen, which can serve as substrates for methanogenesis by methanogens. These latter microorganisms, utilize acetate, hydrogen, carbon dioxide, and formate as substrates to produce methane (CH₄) and carbon dioxide (CO₂) through several biochemical pathways such as hydrogenotrophic methanogenesis [7–9].

The growth of methanogens can be inhibited, either directly by the excess of acids in the mixture, or indirectly by competitive growth on the same substrates.

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Indeed, acetogens and methanogens compete for common substrates such as hydrogen and carbon dioxide. Acetogenic bacteria convert these substrates into acetate, thereby reducing the availability of hydrogen and carbon dioxide to methanogens, which use them to produce methane through hydrogenotrophic methanogenesis. Furthermore, acetogenic bacteria produce a variety of metabolites, including short-chain fatty acids (SCFAs) such as acetate, butyrate and propionate. The accumulation of these acids can lower the pH of the environment, creating sub-optimal conditions for methanogens, which generally prefer a pH between 6.7 and 7.2. When acid production exceeds its consumption in the environment, the resulting pH drop inhibits acetoclastic methanogens from utilizing acetic acid, leading to its accumulation. Although acetoclastic methanogens depend on acetic acid for growth, their activity is significantly constrained by pH lowered levels. In the case of hydrogenotrophic methanogens, pH inhibition primarily manifests through elevated levels of acetic acid rather than through the accumulation of all volatile fatty acids (VFAs), [8, 10].

In a chemostat, several operating parameters can be controlled and adjusted to maintain the growth of microbial populations under desired conditions. Some key operating parameters in a chemostat include the dilution rate D which represents the rate at which fresh medium is continuously added to the chemostat vessel and the rate at which the culture is diluted and removed from the system and it is typically expressed as the flow rate of medium (in volume per unit time) divided by the volume of the vessel, [11]. The other operating parameters are nutrient concentrations defined as the concentrations of essential nutrients, such as volatile fatty acids (VFAs) or hydrogen, in the culture medium. These parameters can be controlled to support microbial growth [2, 3, 10, 12–14].

In this work, we focus on the acetogenesis and hydrogenotrophic methanogenesis phases and we investigate analytically two chemostat models studied experimentally by Di and Yang, [15]. In the first model, the evolution of the methanogenic and hydrogenotrophic bacteria is inhibited by the volatile fatty acids VFA, that's what we call later inhibition by substrate. The second model deals with inhibition by biomass. That is, the growth of the methanogenics is inhibited by acetogens. We refer to biomass inhibition, the process whereby the density of acetogens negatively influences the growth or survival of methanogens within the chemostat, and thereby limiting their growth, which can slow down or inhibit methane production. We will represent the operating diagrams for each case and determine the consequent bifurcations of stabilities of steady states. This study will be useful for engineering purposes in order to ensure the quantitative comprehension needed to balance bioreactor stability with the costs and benefits of introducing innovative organisms into the chemostat.

This paper is organized as follows: In Section II, we present the mathematical model that describes both cases of inhibition and we specify the assumptions for each of the two cases and we give some properties of both of models like existence, positivity, uniqueness, and boundedness of solutions. In Section III we present the existence and local stability conditions of the steady states of each of the models, with respect to the dilution rate D and the concentrations of substrates at the entrance of the chemostat. In Section IV we illustrate the operating diagrams, for given growth functions, and for different values of operating parameters and biological parameters used in the literature.

In Section V, we depict the transcritical bifurcations and stability exchange between positive equilibrium and the boundary ones for each operating diagram. The study of bifurcations permits to determine especially stability of its equilibrium points, as the operating and/or biological parameters are varied. It helps to understand how small changes in these parameters can have a significant impact on the behavior of the system, and can be used to predict and control system behavior in real-world applications and to predict its long-term behavior [16]. In Section VI, we identify the similarities and differences between the two models and we present discussion and conclusion.

The Appendix contains proofs of propositions given in previous sections. In Table 21 we have consolidated all the symbols and notations used throughout the text to facilitate easier reference and navigation.

II. MATHEMATICAL MODEL

We consider the model of chemostat with two-species x_0 , the acetogenic bacteria, and x_1 , the methanogenic hydrogenotrophic bacteria, and two substrates s_0 , and s_1 , the VFAs and hydrogen, respectively. The substrate s_0 , the VFA, is consumed by acetogens x_0 to produce a second substrate s_1 , the H_2 , which in turn, feeds the methanogens x_1 . In the first model, methanogens x_1 are inhibited by the VFAs s_0 , see Figure 1a. In the second model the methanogens x_1 are inhibited by the acetogens x_0 , see Figure 1b.

The dynamical system of the models of Figures 1a and 1b takes the form of system (1):



Fig. 1: Two-species model with inhibition: (a) inhibition of x_1 by s_0 , (b) inhibition of x_1 by x_0 . The dashed line represents inhibition.

$$\begin{cases} \frac{ds_0}{dt} = D\left(s_0^{in} - s_0\right) - \varphi_0(s_0)x_0\\ \frac{dx_0}{dt} = \left(\varphi_0(s_0) - D\right)x_0\\ \frac{ds_1}{dt} = D\left(s_1^{in} - s_1\right) - \varphi_1(s_1, r_0)x_1 + \varphi_0(s_0)x_0\\ \frac{dx_1}{dt} = \left(\varphi_1(s_1, r_0) - D\right)x_1 \end{cases}$$
(1)

where D > 0 denotes the dilution rate, $s_0^{in} \ge 0$ and $s_1^{in} \ge 0$ denote the input substrate concentrations, x_0 and x_1 denote the biomass concentrations, φ_0 and φ_1 their growth rates respectively and r_0 is such that: $r_0 =$ s_0 when the specie x_1 is inhibited by substrate s_0^{in} in the model of Figure 1a and $r_0 = x_0$ when the specie x_1 is inhibited by the biomass x_0 in the model of Figure 1b.

This model is characterized by the following properties: the system (1) has a solution for any initial condition, the species x_0 grows and develops only if the nutriment s_0 is available. The downstream species x_1 develops only if s_1 is available, and its growth is inhibited by the inlet substrate s_0 or by the upstream species x_0 . This permits to consider general kinetic functions φ_0 and φ_1 which are of class C^1 on \mathbb{R}_+ and \mathbb{R}^2_+ respectively, and that satisfy the following hypothesis:

- **H**₁: $\varphi_0(s_0) > 0$, $\varphi_1(s_1, r_0) > 0$ for $s_0 > 0$, $s_1 > 0$ and $r_0 > 0$.
- **H**₂: $\varphi_0(0) = 0$ and for all $s_0 > 0$: $\varphi_0(s_0) < +\infty$.
- **H**₃: $\varphi_1(0, r_0) = 0$ and for all $s_1 > 0$ and $r_0 > 0$: $\varphi_1(s_1, r_0) < +\infty.$
- $\begin{array}{l} \mathbf{H}_4 \text{: For all } s_0 > 0 \text{: } \varphi_0'(s_0) > 0 \text{.} \\ \mathbf{H}_5 \text{: For all } s_1 > 0 \text{ and } r_0 > 0 \text{: } \frac{\partial \varphi_1}{\partial s_1}(s_1, r_0) > 0 \text{ and } \end{array}$ $\frac{\partial \varphi_1}{\partial r_0}(s_1, r_0) < 0.$

We state the following proposition.

Proposition 1. Assume that hypothesis H_1 to H_5 hold, system (1) has a unique solution for any positive initial condition $(s_0(0), x_0(0), s_1(0), x_1(0))$ and any positive time, \mathbb{R}^4_+ is positively invariant by (1), the solution of (1) is positively bounded, and the set $\Gamma = \{(s_0, x_0, s_1, x_1) \in \mathbb{R}^4_+ : s_0 = s_0^{in} - x_0 \text{ and } s_1 =$ $s_1^{in} + x_0 - x_1$ is positively invariant and is a global attractor for (1).

Based on the theory of asymptotically autonomous dynamical systems, the existence of Γ , allows reducing the original model (1) to the two dimensional system (2), called a limiting system of (1), since the solutions of both systems (1) and (2) have the same behaviour after sufficiently large time, [17]. Thereafter, the study of system (2), lets to analysis the asymptotic behaviour of the solution of system (1) on Γ , since the system (2) is the restriction of system (1) on the projection of set Γ on the plane (x_0, x_1) :

$$\begin{cases} \frac{dx_0}{dt} = \left(\varphi_0(s_0^{in} - x_0) - D\right) x_0 \\ \frac{dx_1}{dt} = \left(\varphi_1(s_1^{in} + x_0 - x_1, r_0) - D\right) x_1 \end{cases}$$
(2)

Notice that, in the case of Figure 1a, $r_0 = s_0 = s_0^{in} - x_0$ and in the case of Figure 1b, $r_0 = x_0$.

We also notice that $s_0 > 0$, $s_1 > 0$, and that $0 \leq x_0 \leq s_0^{in}$ and $0 \leq x_1 \leq s_1^{in} + x_0$, all this allows to say that (x_0, x_1) is always in the set $\sigma =$ $\{(x_0, x_1) \in \mathbb{R}^2_+ : 0 \le x_0 \le s_0^{in}, 0 \le x_1 \le s_1^{in} + x_0\}.$

In what follows, we will study the existence and the stability of equilibrium points of system (2) in each of cases $r_0 = s_0^{in} - x_0$ and $r_0 = x_0$.

We consider in Table 1, some functions and notations used in this paper. We will say that the graph of a function f crosses the graph of a function g at a point with x-axis x_0 if and only if $f(x_0) = g(x_0)$ and there exists a neighborhood V of x_0 such that for any real number x in $V \{x_0\}$, one has f'(x) < g'(x) or g'(x) < g'f'(x). In any other case, we will say that the graph of the function f does not cross the graph of the function g.

Table 1: Functions, their graphs, and definitions of some notations used in this paper.

Function	Definition domain	Graph
$\psi_0^0(x_0) = \varphi_0(s_0^{in} - x_0)$ $\psi_1^0(x_0) = \varphi_1(s_1^{in} + x_0, r_0)$	$0 \leq x_0 \leq s_0^{in} \ 0 \leq r_0 \leq s_0^{in}$	$\Gamma^0_0\\\Gamma^0_1$

Notation	Definition	Existence
$D_0(s_0^{in})$	$D_0(s_0^{in}) = \psi_0^0(0)$	Always exists
$D_1(s_0^{in}, s_1^{in})$	$D_1(s_0^{in}, s_1^{in}) = \psi_1^0(0)$	Exists when
		$r_0 = s_0^{in} - x_0$
$D_1(s_1^{in})$	$D_1(s_1^{in}) = \psi_1^0(0)$	Exists when
		$r_0 = x_0$

III. EXISTENCE AND STABILITY OF EQUILIBRIUM POINTS

A. Equilibrium points of the reduced model (2)

The equilibrium points of model (2) are solutions of the following nonlinear algebraic system by setting in the latter the right-hand sides equal to zero:

$$\begin{cases} \left(\varphi_0(s_0^{in} - x_0) - D\right) x_0 = 0\\ \left(\varphi_1(s_1^{in} + x_0 - x_1, r_0) - D\right) x_1 = 0 \end{cases}$$
(3)

thus

$$\begin{cases} x_0 = 0 \text{ or } \varphi_0(s_0^{in} - x_0) = D\\ x_1 = 0 \text{ or } \varphi_1(s_1^{in} + x_0 - x_1, r_0) = D \end{cases}$$
(4)

Hence, system (2) has at most the four equilibrium points E_0 , E_1 , E_2 , and E_3 defined as follows:

- $E_0 = (0, 0)$, the washout equilibrium.
- $E_1 = (\tilde{x}_0, 0)$ where $\tilde{x}_0 \in (0, s_0^{in}]$ is solution, if it exists, of $\varphi_0(s_0^{in} - x_0) = D$.
- $E_2 = (0, \tilde{x}_1)$ where $\tilde{x}_1 \in (0, s_1^{in}]$ is solution, if it exists, of $\varphi_1(s_1^{in} - x_1, \delta s_0^{in}) = D$, where $\delta = 1$ if $r_0 = s_0^{in} - x_0$ and $\delta = 0$ if $r_0 = x_0$.
- $E_3 = (x_0^*, x_1^*)$ such that (x_0^*, x_1^*) \in $(0, s_0^{in}] \mathbf{x}(0, s_1^{in}]$ is solution, if it exists, of $\varphi_0(s_0^{in} - x_0) = D$ and $\varphi_1(s_1^{in} + x_0 - x_1, r_0) = D$.

Remark 1. If $r_0 = s_0^{in} - x_0$, we notice, according to \mathbf{H}_4 and \mathbf{H}_5 , that function ψ_0^0 is decreasing and function ψ_1^0 is increasing, therefore curve Γ_0^0 crosses Γ_1^0 if and only if $D_1(s_0^{in}, s_1^{in}) < D_0(s_0^{in})$, let's assume in this case that x_2^1 is the solution, in $(0, s_0^{in}]$, of equation $\psi_0^0(x) =$ $\psi_1^0(x)$ and let $D_2^1(s_0^{in}, s_1^{in}) = \psi_0^0(x_2^1) = \psi_1^0(x_2^1).$

If $r_0 = x_0$, let us assume that the graph Γ_0^0 crosses Γ_1^0 p times, $p \ge 1$, for $x \in (0, s_0^{in}]$, one has $x_2^1, x_2^2, ..., x_2^p$ are the solutions, in $(0, s_0^{in}]$, of equation $\psi_0^0(x) = \psi_1^0(x)$ such that $x_2^1 < x_2^2 < \ldots < x_2^p$.

One denotes $D_2^j(s_0^{in}, s_1^{in}) = \psi_0^0(x_2^j) = \psi_1^0(x_2^j), \ j =$ $1,\ldots,p$. Since the function ψ_0^0 is decreasing, we get $D_2^1(s_0^{in}, s_1^{in}) > D_2^2(s_0^{in}, s_1^{in}) > \ldots > D_2^p(s_0^{in}, s_1^{in}) \ge$ 0. Since $\psi_0^0(s_0^{in}) = 0$ and $\psi_1^0(s_0^{in}) > 0$, we notice that:

- p is even if and only if $D_0(s_0^{in}) < D_1(s_1^{in})$,
- p is odd if and only if $D_1(s_1^{in}) < D_0(s_0^{in})$.

B. Existence and stability of equilibrium points of system (2) when s_0 inhibits the growth of x_1

Assume that hypothesis \mathbf{H}_1 to \mathbf{H}_5 hold, the following proposition gives the conditions of existence and stability of the equilibrium points of (2) when x_1 is inhibited by s_0 . The proofs are in the Appendix.

Proposition 2. The washout equilibrium E_0 always exists and is locally asymptotically stable if and only if $D > \max(D_0(s_0^{in}), D_1(s_0^{in}, s_1^{in})).$

Equilibrium E_1 exists if and only if $D < D_0(s_0^{in})$, and is locally asymptotically stable if $D_1(s_0^{in}, s_1^{in}) <$ $D_2^1(s_0^{in}, s_1^{in}) < D < D_0(s_0^{in}).$

Equilibrium E_2 exists if and only if D < $D_1(s_0^{in}, s_1^{in})$, and is locally asymptotically stable if $D_0(s_0^{in}) < D < D_1(s_0^{in}, s_1^{in}).$

The positive equilibrium E_3 exists if and only if $D < \min(D_0(s_0^{in}), D_1(s_0^{in}, s_1^{in})) \text{ or } D_1(s_0^{in}, s_1^{in}) < 0$ $D < D_2^1(s_0^{in}, s_1^{in}) < D_0(s_0^{in})$, and is locally asymptotically stable whenever it exists.

C. Existence and stability of equilibrium points of system (2) when x_0 inhibits the growth of x_1

Assume that hypothesis \mathbf{H}_1 to \mathbf{H}_5 hold, we state the following propositions of existence and stability of the equilibrium points of (2) when x_1 is inhibited by x_0 . The proofs are in the Appendix.

Proposition 3. The existence conditions of E_0 , E_1 , E_2 , and E_3 of system (2) are provided in Table 2.

Proposition 4. The stability conditions of E_0 , E_1 , E_2 , and E_3 are given in Table 3.

D. Equilibrium points of model (1)

System (1) has four equilibrium points E_0^* , E_1^* , E_2^* , and E_3^* , they correspond, respectively, to the equilibrium points E_0 , E_1 , E_2 , and E_3 of system (2) defined in Section III-A:

- $E_0^* = (s_0^{in}, 0, s_1^{in}, 0)$, the washout equilibrium. $E_1^* = (s_0^{in} \tilde{x}_0, \tilde{x}_0, s_1^{in} + \tilde{x}_0, 0)$ where $\tilde{x}_0 \in$ $(0, s_0^{in}].$
- $E_2^* = (s_0^{in}, 0, s_1^{in} \tilde{x}_1, \tilde{x}_1)$ where $\tilde{x}_1 \in (0, s_1^{in}]$.
- $E_3^{\tilde{*}} = (s_0^{in} x_0^*, x_0^*, s_1^{in} + x_0^* x_1^*, x_1^*)$ where $(x_0^*, x_1^*) \in (0, s_0^{in}] \mathbf{x}(0, s_1^{in}]$, the coexistence equilibrium.

Proposition 5. The existence and stability conditions of equilibrium points E_0^* , E_1^* , E_2^* , and E_3^* of model (1) are the same of those of system (2) and they are given in Propositions 2, 3, and 4.

IV. OPERATING DIAGRAMS

In this section, we summarize the results of Propositions 2, 3 and 4 in Tables 4, 5 and 7 that provide the conditions of existence and stability of each steady state, in respect to the dilution rate D, the inlet substrate concentrations s_0^{in} and s_1^{in} , and according to the number of intersection points of curves Γ_0^0 and Γ_1^0 , then we plot some operating diagrams (ODs) corresponding to several values of biological and operating parameters. These ODs allow us to distinguish the differences between the two cases of inhibition: inhibition by substrate $r_0 = s_0$ and inhibition by biomass $r_0 = x_0$.

For seek of simplicity, we will denote $D_0(s_0^{in})$ and $D_2^i(s_0^{in}, s_1^{in})$, i = 1, 2 by D_0 and D_2^i , i = 1, 2, respectively. D_1 denotes $D_1(s_1^{in})$ or $D_1(s_0^{in}, s_1^{in})$ if $r_0 = s_0$ or $r_0 = x_0$, respectively. S denotes locally asymptotically stable. U denotes unstable. No letter means that the steady state doesn't exist.

In what follows, we will consider the kinetics functions φ_0 of Monod type and φ_1 of Monod type with inhibition satisfying properties assumed in hypothesis \mathbf{H}_1 to \mathbf{H}_5 :

$$\varphi_{0}: s_{0} \mapsto \frac{m_{0}s_{0}}{K_{0} + s_{0}}$$

$$\varphi_{1}: (s_{1}, x_{0}) \mapsto \frac{m_{1}s_{1}}{K_{1} + s_{1}} \frac{1}{1 + r_{0}/L_{0}}$$
(5)

where m_i and K_i , i = 1, 2 denote the maximum specific growth rate and the half-saturation constant of species x_i , respectively. In the case $r_0 = s_0$, L_0 denotes the inhibition constant of species x_1 by s_0 and in the case $r_0 = x_0$, L_0 denotes the inhibition constant of species x_1 by x_0 .

Figures 2 and 3 illustrate some possible intersections of curves Γ_0^0 and Γ_1^0 in the cases $r_0 = s_0^{in} - x_0$ and $r_0 = x_0$, with respect to values of D_0 , D_1 , and D_2^i , i = 1, 2, 3.

The biological parameters are fixed depending on substrates and micro-organisms that will be introduced continuously in the chemostat. Since it is not easy to interpret the operating diagrams in three-dimensional space (D, s_0^{in}, s_1^{in}) , [18], we will simply plot the ODs by considering cuts in two-dimensional planes (s_0^{in}, D) and (s_1^{in}, D) . For this end, we will firstly fix s_1^{in} and plot the diagrams in the (s_0^{in}, D) plane then we will fix s_0^{in} and plot the operating diagrams in the (s_1^{in}, D)

plane. We define in Tables 10 and 15, the boundaries of the areas in the ODs of Figures 11, 8, and 6. The colors used to paint the different regions of the ODs are given in Table 9.

A. Operating diagrams in the (s_0^{in}, D) plane

Table 8 holds the values of operating and biological parameters used to draw the boundaries and the ODs of Figures 4, ..., 11.

Tables 11, 12, and 13 summarize the conditions of stability of each steady state in ODs of Figures 4b, 6, 8b, and 11, respectively. \bar{s}^1 and \bar{s}^2 denote the concentrations of s_0^{in} for which Γ_0^0 and Γ_1^0 are tangent. s^0 denotes the concentration of s_0^{in} for which $D_0 = D_1$ and s^1 denotes the concentration of s_0^{in} such that $D_2^1 = D_1$.

B. Operating diagrams in the (s_1^{in}, D) plane

In order to visualize the effect of the inlet substrate s_1^{in} on the ODs, we will, in what follows, fix the value of operating parameter s_0^{in} and plot the operating diagrams in the (s_1^{in}, D) plane. The values of the biological parameters and those of the operating parameters used to represent the diagrams are given in Table 14. The curves γ_i^* , i = 0, 1, 2 and γ_3^{i*} , i = 1, 2 the boundaries of the regions of the ODs of Figures 12b, 13b, 14b, 15b, and 17 and are defined in Table 15. The regions are colored according to Table 9.

The results are summarized in Tables 16, 17, 18 and 9. Notice that s^0 is defined in Section IV-A. \bar{s}^{i*} , i = 1, 2 represent the concentrations of s_1^{in} such that $D_2^1(s_0^{in}, s_1^{in}) = D_2^2(s_0^{in}, s_1^{in})$ which means that Γ_1^0 and Γ_0^0 are tangent.

V. BIFURCATIONS

Bifurcation study permits to analyze, identify and describe the qualitative changes in the behavior of the model as its operating parameters change. It helps in understanding the dynamic evolution of microbial populations in the chemostat, which permits to optimize the behavior of chemostat systems, leading to improved control, efficiency, and productivity in microbial bioprocesses [16].

Proposition 6. All bifurcations of the equilibrium points of system (1) are transcritical and they are given in Table 20.

Table 2: Existence conditions of equilibrium points of (2).

E_0	Always exists
E_1	$D < D_0(s_0^{in})$
E_2	$D < D_1(s_1^{in})$
	Case 1: Curve Γ_0^0 doesn't cross Γ_1^0 : $\left(D < D_0(s_0^{in}) < D_1(s_1^{in})\right)$.
	Case 2: Curve Γ_0^0 crosses Γ_1^0 only one time: $(D_1(s_1^{in}) < D_0(s_0^{in}))$ and $(D < D_2^1(s_0^{in}, s_1^{in}))$.
	Case 3: Curve Γ_0^0 crosses Γ_1^0 two times: $(D_0(s_0^{in}) < D_1(s_1^{in}))$
-	and $(D_2^1(s_0^{in}, s_1^{in}) < D < D_0(s_0^{in}) \text{ or } D < D_2^2(s_0^{in}, s_1^{in})).$
E_3	Case 4: Curve Γ_0^0 crosses $\Gamma_1^0 p$ times, $p \ge 3$:
	• $(D_1(s_1^{in}) < D_0(s_0^{in}))$ and
	$(D < D_2^p(s_0^{in}, s_1^{in}) \text{ or } D_2^{2j+2}(s_0^{in}, s_1^{in}) < D < D_2^{2j+1}(s_0^{in}, s_1^{in})), j = 0, \dots, (p-3)/2.$
	• $(D_0(s_0^{in}) < D_1(s_1^{in}))$ and
	$(D_2^1 < D < D_0(s_0^{in}))$ or $D < D_2^p(s_0^{in}, s_1^{in})$ or $D_2^{2j+1}(s_0^{in}, s_1^{in}) < D < D_2^{2j}(s_0^{in}, s_1^{in})), j = 1, \dots, (p-2)/2.$
1	

E_0	$D > \max\left\{D_0(s_0^{in}), D_1(s_1^{in})\right\}$
	Case 1: Curve Γ_0^0 crosses Γ_1^0 only one time: $(D_1(s_1^{in}) < D_0(s_0^{in}))$ and $(D_2^1(s_0^{in}, s_1^{in}) < D < D_0(s_0^{in}))$.
	Case 2: Curve Γ_0^0 crosses $\Gamma_1^0 p$ times, $p \ge 2$:
E_1	$igstarrow \left(D_0(s_0^{in}) < D_1(s_1^{in}) ight)$ and
21	(there exists $j = 0, \ldots, (p-2)/2$ such that $D_2^{2j+2}(s_0^{in}, s_1^{in}) < D < D_2^{2j+1}(s_0^{in}, s_1^{in})$).
	• $(D_1(s_1^{in}) < D_0(s_0^{in}))$ and
	$(D_2^1 < D < D_0(s_0^{in}))$ or there exists $j = 1, \dots, (p-1)/2$ such that $D_2^{2j+1}(s_0^{in}, s_1^{in}) < D < D_2^{2j}(s_0^{in}, s_1^{in}))$.
E_2	$D_0(s_0^{in}) < D$
E_3	Locally asymptotically stable if it exists

Table 4: Existence and stability of steady states in respect of dilution rate D when $D_0 < D_1$ and Γ_0^0 does not cross Γ_1^0 . The results in this Table are common to both of cases $r_0 = s_0$ and $r_0 = x_0$.

D	E_0	E_1	E_2	E_3
$D_0 < D_1 < D$	S			
$D_0 < D < D_1$	U		S	
$D < D_0 < D_1$	U	U	U	S

Table 5: Existence and stability of steady states in respect of dilution rate D when $D_1 < D_0$ and Γ_0^0 crosses Γ_1^0 once. The results are common to both cases $r_0 = s_0$ and $r_0 = x_0$.

D	$ E_0 $	E_1	E_2	E_3
$D_1 < D_0 < D$	S			
$\max\left\{D_1, D_2^1\right\} < D < D_0$	U	S		
$D_1 < D < D_2^1 < D_0$	U	U		S
$D < \min\left\{D_2^1, D_1\right\} < D_0$	U	U	U	S

Table 6: Existence and stability of steady states in respect of dilution rate D when $D_1 < D_0$ and Γ_0^0 crosses Γ_1^0 once. This result is specific to the case $r_0 = x_0$.

Table 7: Existence and stability of steady states in respect of dilution rate D when $D_0 < D_1$ and Γ_0^0 crosses Γ_1^0 twice in the case of inhibition by biomass $(r_0 = x_0)$.

D	$ E_0 $	E_1	E_2	E_3
$D_0 < D_1 < D$	S			
$D_0 < D < D_1$	U		S	
$D_2^2 < D < D_2^1 < D_0$	U	S	U	
$D_2^1 < D < D_0$ or $D < D_2^2 < D_2^1$	U	U	U	S

Table 8: Values of parameters used in Figures 4,...,11.

m_0	m_1	K_0	K_1	L_0	s_1^{in}	Figure
8	10	2	1	1	0.51	4, 5, and 6
8	10	2	1	1	0.7	7
8	10	2	1	1	2	8
8	10	2	1	1	4	9
8	15	2	1	1	0.54	10, 11

Table 9: Definitions of regions $R_1 \dots R_6$ and $J_1 \dots J_6$ of ODs and their associated colors.

Region	E_0	E_1	E_2	E_3	Color
R_1 and J_1	S				Red
R_2 and J_2	U	S			Yellow
R_3 and J_3	U	S	U		Yellow
R_4 and J_4	U		S		Magenta
R_5 and J_5	U	U	U	S	Green
R_6 and J_6	U	U		S	Green

Table 10: Boundaries of the ODs and their plot colors in Figures 4,...,11. s_1^{in} is fixed according to table 8. $\delta = 1$ if $r_0 = s_0 = s_0^{in} - x_0$, and $\delta = -1$ if $r_0 = x_0$. Notice that x_2^1 , x_2^2 and x_2^3 are defined in Remark 1.

Color
Blue
Red
Magenta
Green
Blue



Fig. 4: OD in the (s_0^{in}, D) plane when $r_0 = s_0^{in} - x_0$ in the case where Γ_0^0 crosses Γ_1^0 at most one time. (a) The boundaries γ_i , i = 0, 1 and γ_2^1 with parameters set in Table 8. (b) OD corresponding to Figure 4a.



Fig. 2: Intersections of Γ_0^0 and Γ_1^0 . (a) and (b): case where $r_0 = s_0^{in} - x_0$. (c) and (d): case where $r_0 = x_0$.



Fig. 3: Case where $r_0 = x_0$. (a) Γ_0^0 crosses Γ_1^0 two times. (b) Γ_0^0 crosses Γ_1^0 three times.



Fig. 5: Boundaries of the OD in the (s_0^{in}, D) plane when $r_0 = x_0$, case where Γ_0^0 crosses Γ_1^0 at most one time. (a) The boundaries γ_i , i = 0, 1 and γ_2^1 with parameters set in Table 8. (b) Zoom in of Figure 5a where $s_0^{in} \in [s^0, s^1]$.



Fig. 6: (a) The OD associated to Figure 5a. (b) The OD associated to Figure 5b.



Fig. 7: The OD in the (s_0^{in}, D) plane when $r_0 = x_0$ in the case where Γ_0^0 crosses Γ_1^0 at most one time. (a) The boundaries γ_i , i = 0, 1 and γ_2^1 with parameters set in Table 8. (b) The corresponding OD.



Fig. 8: OD in the (s_0^{in}, D) plane when $r_0 = x_0$ in the case where Γ_0^0 crosses Γ_1^0 at most two times. (a) The boundaries γ_i , i = 0, 1 and γ_2^i , i = 1, 2 with parameters set in Table 8. (b) The corresponding OD.



Fig. 9: OD in the (s_0^{in}, D) plane when $r_0 = x_0$ in the case where Γ_0^0 does not cross Γ_1^0 or croses it twice. (a) The boundaries γ_i , i = 0, 1 and γ_2^i , i = 1, 2 with parameters set in Table 8. (b) The corresponding OD.



Fig. 10: Boundaries of the OD in the (s_0^{in}, D) plane when $r_0 = x_0$ in the case where Γ_0^0 crosses Γ_1^0 at most three times. (a) The boundaries γ_i , i = 0, 1, 2 and γ_2^i , i = 1, 2, 3 with parameters set in Table 8. (b) Zoom in of Figure 11a where $s_0^{in} \in [3.6, 4.4]$.



Fig. 11: (a) The OD in the (s_0^{in}, D) plane, corresponding to Figure 10a. (b) The OD corresponding to Figure 10b.

Table 11: Regions of the ODs of Figures 4 and 6 in respect of s_0^{in} and D. It should be noted that regions $R_1, R_2, R_4 \dots R_6$ appear in both cases of inhibitions $r_0 = s_0$ and $r_0 = x_0$. The region R_3 appears only in the case $r_0 = x_0$, when the downstream species x_1 is inhibited by upstream species x_0 .

Region	s_0^{in}	D
R_1		$D > \max\{D_0, D_1\}$
R_2	$s_0^{in} > s^0$	$\max\{D_1, D_2^1\} < D < D_0$
R_3	$s^1 < s_0^{in}$	$D_2^1 < D < D_1$
R_4	$s_0^{in} < s^0$	$D_0 < D < D_1$
R_5	$s_0^{in} < s^0$	$D < D_0 < D_1$
	$s_0^{in} > s^0$	$D < \min\{D_2^1, D_1\}$
R_6	$s^0 < s^{in}_0 < \tilde{s}^0$	$D_1 < D < D_2^1$

Table 12: Regions of the ODs of Figure 8 and 9 ($r_0 = x_0$) in respect of s_0^{in} and D.

Region	s_0^{in}	D
R_1		$D > \max\left\{D_0, D_1\right\}$
R_2	$s_0^{in} > s^0$	$D_1 < D < D_0$
R_3	$\bar{s}^1 < s_0^{in} < s^0$	$D_2^2 < D < D_2^1$
	$s_0^{in} > s^0$	$D < D_{2}^{1}$
R_4	$s_0^{in} < s^0$	$D_0 < D < D_1$
	$s_0^{in} < \bar{s}^1$	$D < D_0 < D_1$
R_5	$\bar{s}^1 < s_0^{in} < s^0$	$D_2^1 < D < D_0$ or $D < D_2^2$
	$s_0^{in} > s^0$	$D < D_{2}^{1}$

Region	s_0^{in}	D
R_1		$D > \max\{D_0, D_1\}$
D	$s_0^{in} > \bar{s}^1$	$D_1 < D < D_0$
n_2	$s^0 < s^{in}_0 < \bar{s}^1$	$D_1 < D_2^1 < D < D_0$
	$\bar{s}^2 < s_0^{in} < s^0$	$D < D_2^2 < D < D_2^1$
R_3	$s^0 < s^{in}_0 < \bar{s}^1$	$D_2^3 < D < \min\{D_2^2, D_1\}$
	$s_0^{in} > \bar{s}^1$	$D < D_2^1$
R_4	$s_0^{in} < s^0$	$D_0 < D < D_1$
	$s_0^{in} < \bar{s}^2$	$D < D_0 < D_1$
R_5	$\bar{s}^2 < s_0^{in} < s^0$	$D_2^1 < D < D_0 \text{ or } D < D_2^2$
	$s^0 < s^{in}_0 < \bar{s}^1$	$D_2^2 < D < D_1$ or $D < D_2^3$
	$s_0^{in} > \bar{s}^1$	$D < D_2^1$
R_6	$s^0 < s^{in}_0 < \bar{s}^1$	$\max\{D_2^2, D_1\} < D < D_2^1$

Table 13: Regions of the OD of Figure 11 ($r_0 = x_0$) in respect of s_0^{in} and D.

Table 14: Values of parameters used in ODs of Figures12,...,17.

m_0	m_1	K_0	K_1	L_0	s_0^{in}	Figure
5	4	1	2	2	0.3	13
8	5	2	1	1	4	14
8	10	2	1	1	4	15
8	15	2	1	1	4	12, 16, and 17

Table 15: Boundaries of the ODs and their plot colors in Figures 12,...,17. s_0^{in} is fixed according to Table 14. Notice that x_2^1 , x_2^2 and x_2^3 are defined in Remark 1. $\delta = 1$ if $r_0 = s_0 = s_0^{in} - x_0$, and $\delta = -1$ if $r_0 = x_0$.

$$\label{eq:sphere:sphe$$



Fig. 12: OD in the (s_1^{in}, D) plane when $r_0 = s_0^{in} - x_0$, case where Γ_0^0 crosses Γ_1^0 only one time. (a) The boundaries γ_0^* , γ_1^* , and γ_2^{1*} with parameters set in Table 14. (b) The corresponding OD.



Fig. 13: OD in the (s_1^{in}, D) plane when $r_0 = s_0^{in} - x_0$, case where Γ_0^0 crosses Γ_1^0 at most one time. (a) The boundaries γ_0^* , γ_1^* , and γ_2^{1*} with parameters set in Table 14. (b) The corresponding OD.



Fig. 14: OD in the (s_1^{in}, D) plane when $r_0 = x_0$, case where Γ_0^0 crosses Γ_1^0 only one time. (a) The boundaries γ_0^*, γ_1^* , and γ_2^{1*} with parameters set in Table 14. (b) The corresponding OD.



Fig. 15: OD in the (s_1^{in}, D) plane when $r_0 = x_0$, case where Γ_0^0 crosses Γ_1^0 one or two times. (a) The boundaries γ_i^* , i = 1, 2 and γ_2^{i*} , i = 1, 2 with parameters set in Table 14. (b) The corresponding OD.



Fig. 16: The boundaries of OD in the (s_1^{in}, D) plane when $r_0 = x_0$, case where Γ_0^0 crosses Γ_1^0 at most three times. (a) The boundaries γ_i^* , i = 0, 1 and γ_2^{i*} , i = 1, 2 with parameters set in Table 14. (b) Zoom in of the framed area of Figure 16a.



Fig. 17: a) OD corresponding to Figure 16a. b) OD corresponding to Figure 16b.

Table 16: Definitions of the regions of the ODs of Figures 13b and 14b in respect of s_1^{in} and D. BC means that the definition of the region is the same in both cases of inhibition: $r_0 = s_0$ and $r_0 = x_0$.

Reg.	s_1^{in}	D	Inhib.
J_1		$D > \max\{D_0, D_1\}$	BC
In	$s_1^{in} < s^0$	$\max\{D_1, D_1^1\} < D < D_0$	BC
J_2	$s_1^{in} > s^0$	$\max\{D_1, D_2\} \leq D \leq D_0$	$r_0 = x_0$
J_3	$s_1^{in} > s^{1*}$	$D_2^1 < D < D_1$	$r_0 = x_0$
J_4	$s_1^{in} > s^{1*}$	$D_0 < D < D_1$	$r_0 = s_0$
J_5		$D < \min\{D_1, D_2^1\} < D_0$	BC
J_6	$s_1^{in} < s^{1*}$	$D_1 < D < D_2^1$	BC

Table 17: Definitions of the regions of the OD of Figure 15b in respect of s_1^{in} and D.

Region	s_1^{in}	D
J_1		$D > \max\{D_0, D_1\}$
J_2	$s_1^{in} < s^0$	$\max\{D_1, D_2^1\} < D < D_0$
J_3	$s^{1*} < s^{in}_1 < s^0$	$D_2^1 < D < D_1$
	$s_1^{in} > s^0$	$D_2^2 < D < D_2^1$
J_4	$s_1^{in} > s^0$	$D_0 < D < D_1$
J_5	$s_1^{in} < s^0$	$D < \min\{D_2^1, D_1\}$
	$s_1^{in} > s^0$	$D < D_2^2 \text{ or } D_2^1 < D < D_0$
J_6	$s_1^{in} < s^{1*}$	$D_1 < D < D_2^1$



Fig. 18: Boundaries of ODs for growing values of s_0^{in} when $m_0 = 5$, $m_1 = 4$, $K_0 = 1$, $K_1 = 2$, and $L_0 = 2$. a) Case where $r_0 = s_0^{in} - x_0$ and $s_0^{in} = 0.3$. b), c,) d), e), and f): Cases where $r_0 = x_0$ and $s_0^{in} = 0.3, 1, 1.5, 2$, and 2.2 respectively.

Table 18: Definitions of the regions of the OD of Figure 17 in respect of s_1^{in} and D.

Reg.	s_1^{in}	D
J_1		$D > \max\{D_0, D_1\}$
	$s_1^{in} < \bar{s}^{1*}$	$\max\{D_1, D_2^1\} < D < D_0$
J_2	$\bar{s}^{1*} < s_1^{in} < s^0$	$D_1 < D < D_2^2$ or
		$D_2^1 < D < D_0$
	$s^{1*} < s_1^{in} < \bar{s}^{1*}$	$D_2^1 < D < D_1$
	$\overline{a}^{1*} < a^{in} < a^0$	$D_2^3 < D < \max\{D_2^2, D_1\}$
J_3	$s < s_1 < s$	or $D_2^1 < D < D_0$
	$s^0 < s_1^{in}$	$D_2^2 < D < D_2^1$
	$<\bar{s}^{2*} < s^0$	
J_4	$s_1^{in} > s^0$	$D_0 < D < D_1$
	$s_1^{in} < \bar{s}^{1*}$	$D < \min\{D_2^1, D_1\}$
J_5	$\bar{s}^{1*} < s_1^{in} < s^0$	$D < D_2^3 \text{ or } D_2^2 < D < D_1$
	$s^0 < s_1^{in} < \bar{s}^{2*}$	$D < D_2^2 \text{ or } D_2^1 < D < D_0$
	$\bar{s}^{2*} < s_1^{in}$	$D < D_0$
	$s_1^{in} < s^{1*}$	$D_1 < D < D_2^1$
J_6	$\bar{s}^{1*} < s_1^{in} < s^0$	$\max\{D_1, D_2^2\} < D < D_2^1$

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Fig. 19: Boundaries of ODs for growing values of s_0^{in} when $r_0 = x_0$, $m_0 = 9$, $m_1 = 20$, $K_0 = 2$, $K_1 = 1$, and $L_0 = 1$. a) Case where $s_0^{in} = 4.3$. b) Case where $s_0^{in} = 4.1$. c) Case where $s_0^{in} = 4$. d) Case where $s_0^{in} = 3.5$.

Table 20: Transcritical bifurc	ations acc	ording to	regions	of
operating diagrams of Figures	s 11 and 17	7. TB den	otes Tran	ıs-
critical bifurcations.				

Bound-	Conditions	Transition	Bifurcation	
ary				
γ_0	$s_0^{in} > s^0$	R_1 to R_2	$TB \cdot E_0 = E_1$	
γ_0^*	$s_1^{in} < s^0$	J_1 to J_2	1D . $E_0 = E_1$	
γ_0	$s_0^{in} < s^0$	R_4 to R_5	$TB \cdot E_2 = E_2$	
γ_0^*	$s_1^{in} > s^0$	J_4 to J_5	$[1D, D_2 = D_3]$	
γ_1	$s_0^{in} < s^0$	R_1 to R_2	$TB \cdot E_0 - E_0$	
γ_1^*	$s_1^{in} > s^0$	J_1 to J_4	1D. $E_0 = E_2$	
	$s^0 < s^{in}_0 < \bar{s}^1$	P to P		
\sim^1	$s^1 < s^{in}_0 < \bar{s}^1$	$n_2 \text{ to } n_6$		
/2	$\bar{s}^2 < s_0^{in} < s^1$	R_3 to R_5		
	$s_0^{in} > \bar{s}^1$			
γ_2^2	$s^0 < s^{in}_0 < \bar{s}^1$			
	$\bar{s}^2 < s_0^{in} < s^0$			
γ_2^3	$s^0 < s^{in}_0 < \bar{s}^1$		TD, $F_{\perp} = F_{\perp}$	
γ_2^{2*}	$\bar{s}^{1*} < s_1^{in} < s^{2*}$		1D. $E_1 = E_3$	
	$\bar{s}^{1*} < s_1^{in} < s^0$	J_2 to J_6	J_2 to J_6	
γ_2^{1*}	$s_1^{in} < s^{1*}$			
	$s^0 < s_1^{in} < \bar{s}^{2*}$			
	$s^{1*} < s_1^{in} < \bar{s}^{1*}$			
γ_2^{2*}	$\bar{s}^{2*} < s_1^{in} < s^0$	J_3 to J_5		
	$s^0 < s_1^{in} < \bar{s}^{2*}$			
γ_2^{3*}	$\bar{s}^{1*} < s_1^{in} < s^0$	1		

$< s^0$ > s^0	R_1 to R_2 R_1 to R_4	TB: $E_0 = E_2$
$> s^{0}$	R_1 to R_4	
0		$\Box \mathbf{R} \cdot E_{\alpha} - E_{\alpha}$
$\langle s^0 $	J_1 to J_4	1D . $L_0 = L_1$
$> s^0$	R_4 to R_5	$TB \cdot E_1 - E_2$
$< s^0$	J_4 to J_5	1D. $E_1 = E_3$
	Ro to Ro	
$> s^0$	113 10 116	$TB \cdot E_0 - E_0$
$> \bar{s}^1$	La to La	1D : $L_2 = L_3$
$\frac{in}{1} < s^0$	53 10 56	
	$ < s^{0} $ $ > s^{0} $ $ > \overline{s^{1}} $ $ in < s^{0} $	$ \begin{array}{c c} < s^0 & J_4 \text{ to } J_5 \\ \hline \\ \hline \\ > s^0 \\ \hline \\ > \overline{s^1} \\ 1^n < s^0 \\ \end{array} \begin{array}{c} R_3 \text{ to } R_6 \\ \hline \\ J_3 \text{ to } J_6 \\ \hline \end{array} $

Table 19: Transcritical bifurcations according to regions of OD of Figures 8 and 15. TB denotes Transcritical bifurcations.

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Notation	Definition	First occurrence
D	Dilution rate $[h^{-1}]$	Model 1, p.3
s_0 and s_1	Substrate concentrations [mg/L]	Model 1, p.3
s_0^{in} and s_1^{in}	Inlet substrate concentrations [mg/L]	Model 1, p.3
x_0 and x_1	Biomass concentrations [mg/L]	Model 1, p.3
φ_0 and φ_1	Growth rates of biomass	Model 1, p.3
Γ	$\Gamma = \{(s_0, x_0, s_1, x_1) \in \mathbb{R}^4_+ : s_0 = s_0^{in} - x_0 \text{ and } s_1 = s_1^{in} + x_0 - x_1\}$	Proposition 1, p.3
σ	$\sigma = \left\{ (x_0, x_1) \in \mathbb{R}^2_+ : 0 \le x_0 \le s_0^{in}, 0 \le x_1 \le s_1^{in} + x_0 \right\}$	p.3
ψ_0^0	$\psi^0_0(x_0): x_0\mapsto \varphi_0(s_0^{in}-x_0)$	Table 1, p.4
ψ_1^0	$\psi_1^0(x_0): x_0 \mapsto \varphi_1(s_1^{in} + x_0, r_0), r_0 = x_0 \text{ or } r_0 = s_0$	Table 1, p.4
Γ_0^0 and Γ_1^0	Graphs of functions ψ_0^0 and ψ_1^0 , respectively	Table 1, p.4
$D_0 := D_0(s_0^{in})$	$D_0(s_0^{in}) = \psi_0^0(0)$	Table 1, p.4
$D_1 := D_1(s_1^{in})$	$D_1(s_1^{in}) = \psi_1^0(0)$ for $r_0 = x_0$	Table 1, p.4
$D_1 := D_1(s_0^{in}, s_1^{in})$	$D_1(s_0^{in}, s_1^{in}) = \psi_1^0(0)$ for $r_0 = s_0 = s_0^{in} - x_0$	Table 1, p.4
$ ilde{x}_0$	Solution, if it exists in $(0, s_0^{in}]$, of $\varphi_0(s_0^{in} - x_0) = D$	p.4
$ ilde{x}_1$	Solution, if it exists in $(0, s_1^{in}]$, of $\varphi_1(s_1^{in} - x_1, \delta s_0^{in}) = D$, $\delta = 1$ if $r_0 = s_0^{in} - x_0$ and $\delta = 0$ if $r_0 = x_0$	p. 4
(x_{0}^{*},x_{1}^{*})	Solution, if it exists in σ , of $\varphi_0(s_0^{in} - x_0) = D$ and $\varphi_1(s_1^{in} + x_0 - x_1, r_0) = D$	p .4
$x_2^i, i=1,\ldots,p$	Solutions, in $(0, s_0^{in}]$, of equation $\psi_0^0(x) = \psi_1^0(x)$	Remark 1, p.4
$D_2^i := D_2^i(s_0^{in}, s_1^{in}), i = 1, \dots, p$	$D_2^i(s_0^{in}, s_1^{in}) = \psi_0^0(x_2^i)$	Remark 1, p.4
K_0 and K_1	Half-saturation constants [mg/L]	p.5
L_0	Inhibition constant [mg/L]	p.5
m_0 and m_1	Maximum specific growth rate of cells $[h^{-1}]$	p.5
$\gamma_0, \gamma_1, \text{ and } \gamma_2^i, i = 1, 2, 3$	Boundaries of the ODs in the (s_0^{in}, D) plane	Table 10, p.7
$J_i \ i=1,\ldots,6$	Regions of the ODs in the (s_1^{in}, D) plane	Table 9, p.6
$R_i \ i=1,\ldots,6$	Regions of the ODs in the (s_0^{in}, D) plane	Table 9, p.6
s^0	The value of s_0^{in} for which $D_0 = D_1$	p.5
s^1	The value of s_0^{in} for which $D_2^1 = D_1$	p.5
$ar{s}^1$ and $ar{s}^2$	The values of s_0^{in} for which Γ_0^0 and Γ_1^0 are tangent	p.5
$\gamma_{0}^{*}, \gamma_{1}^{*}, \text{ and } \gamma_{2}^{i*}, i = 1, 2, 3$	Boundaries of the ODs in the (s_1^{in}, D) plane	Table 15, p.9
\overline{s}^{1*}	The concentrations of s_1^{in} for which Γ_0^0 and Γ_1^0 are tangent	p.5
G	$G: x_0 \mapsto x_0 + s_1^{in} - M_1(D, r_0), \text{ for } x_0, \ r_0 \in [0, s_0^{in}]$	p.13
Γ_G	The graph of G in the (x_0, x_1) plane	p.13
Γ_F	The vertical line $x_0 = \tilde{x}_0$ in the (x_0, x_1) plane	p.13

Table 21: The different notations used in this paper, their definitions, and their first occurrences.

VI. DISCUSSION AND CONCLUSION

In this paper, we presented a complete mathematical analysis of two chemostat models where two species are continuously fed by two nutriments in such a way that the downstream biomass x_1 , (methanogenic hydrogenotrophic bacteria), is inhibited by the volatile fatty acids used as a nutriment of biomass x_0 in the first model, and it is inhibited by the upstream biomass x_0 , (acetogenic bacteria), in the second model. Both models are presented by a four-dimensional system of nonlinear ordinary differential equations involving a large class kinetics.

We established the conditions of existence and stability of steady states, of each model, in respect to the dilution rate D and the input substrate concentrations s_0^{in} and s_1^{in} .

We proved that in both models we have four steady states, the washout equilibrium E_0 , where the two species vanish, two boundary steady states E_1 and E_2 where only one species persists, and a fourth steady state E_3 , which we have named the positive equilibrium and in which the two biomasses coexist.

We proved that, in the case of inhibition by biomass x_0 , the conditions of existence of the steady state E_3 and those of stability of E_1 depend closely on the kinetic functions of the model.

The representation of operating diagrams of both of models revealed that the effects of the two inhibitions on stability of steady states, and consequently on the quantity of methane generated, are equivalent for low concentrations of inlet substrate s_0^{in} (see Figure 18), and these effects are extremely different for highest concentrations of s_0^{in} (see Figures 4, 5, and 19).

Indeed, for $s_0^{in} = 0.3$, the regions of stability in both models are almost identical, and for $s_0^{in} \ge 1$ we noticed that, in the case of inhibition by biomass, the coexistence regions (the green ones) are becoming increasingly narrow when s_0^{in} becomes more and more larger, and the yellow region, corresponding to the existence of only the upstream biomass, becomes more and more large, which mean that the more concentration of VFAs s_0^{in} is great, the more concentration of acetogens x_0 is important and the more its inhibition effect on methanogens x_1 is important.

This leads to the diminution of concentration of x_1 and allows x_0 to grow freely and this is why we need to compensate the decrease in methanogens x_1 by introducing s_1^{in} at a higher concentration.

The investigation of the model where the downstream species x_1 is inhibited by both substrate s_0^{in} and upstream species x_0 is underway. The perspective of this

work is to highlight the effect of pH increases, due to VFAs, and H_2 level decreases, consumed by acetogens, on the growth of methanogens and therefore on the production of methane.

VII. APPENDIX

Remark 2. According to \mathbf{H}_5 , there exists a function M_1 such that for $s_1 \ge 0$, $r_0 \ge 0$ and $y \in [0, \varphi_1(+\infty, r_0)]$, $y = \varphi(s_1, r_0)$ is equivalent to $s_1 = M_1(y, r_0)$. When $s_1 = s_1^{in} + x_0 - x_1$, $\varphi_1(s_1^{in} + x_0 - x_1, r_0) = D$ is equivalent to $x_1 = x_0 + s_1^{in} - M_1(D, r_0)$ for $x_0, r_0 \in [0, s_0^{in}]$ and $D \in [0, \varphi_1(+\infty, r_0)]$.

Let $G: x_0 \mapsto x_0 + s_1^{in} - M_1(D, r_0)$, for $x_0, r_0 \in [0, s_0^{in}]$.

If
$$r_0 = s_0 = s_0^{in} - x_0$$
 then

$$\varphi_1(s_1^{in} + x_0 - G(x_0), s_0^{in} - x_0) = D$$

and this implies that

$$(1 - G'(x_0))\frac{\partial \varphi_1}{\partial s_1}(s_1^{in} + x_0 - G(x_0), s_0^{in} - x_0) - \frac{\partial \varphi_1}{\partial s_0}(s_0^{in} + x_0 - G(x_0), s_0^{in} - x_0) = 0$$

which implies that

$$G'(x_0) = 1 - \frac{\frac{\partial \varphi_1}{\partial s_0}(s_1^{in} + x_0 - G(x_0), s_0^{in} - x_0)}{\frac{\partial \varphi_1}{\partial s_1}(s_1^{in} + x_0 - G(x_0), s_0^{in} - x_0)}.$$

According to \mathbf{H}_5 , one gets: for all x_0 in $]0, s_0^{in}[, G'(x_0) > 1.$

If $r_0 = x_0$ then $\varphi_1(s_1^{in} + x_0 - G(x_0), x_0) = D$ and this implies that

$$1 - G'(x_0))\frac{\partial \varphi_1}{\partial s_1}(s_1^{in} + x_0 - G(x_0), x_0) + \frac{\partial \varphi_1}{\partial x_0}(s_0^{in} + x_0 - G(x_0), x_0) = 0$$

which implies that

$$G'(x_0) = \frac{\frac{\partial \varphi_1}{\partial x_0} (s_1^{in} + x_0 - G(x_0), x_0)}{\frac{\partial \varphi_1}{\partial s_1} (s_1^{in} + x_0 - G(x_0), x_0)} + 1.$$

According to \mathbf{H}_5 , one gets: for all x_0 in $]0, s_0^{in}[, G'(x_0) < 1.$

We denote Γ_G the graph of G in the plane (x_0, x_1) . On another hand one denotes Γ_F the vertical line Γ_F : $x_0 = \tilde{x}_0$.

Lemma 1. If a steady state E_3 exists, then steady state E_1 exists too.

Proof: If steady state E_3 exists the system 3 has a solution (x_0^*, x_1^*) in σ . One remarks that the first equation of 3 implies that $E_1(x_0^*, 0)$ exists.

Lemma 2. We denote O(0,0), $B(s_0^{in}, s_0^{in} + s_1^{in})$, and $C(0, s_1^{in})$ in the set σ defined previously. The graph Γ_G of G does not cross the boundary [BC].

Proof: Let $M(x_0, s_1^{in} + x_0)$ a point on [BC], if M belongs to the graph Γ_G then $s_1^{in} + x_0 = G(x_0) = x_0 + s_1^{in} - M_1(D, r_0)$ which is equivalent to $M_1(D, r_0) = 0$ which is to say $D = \varphi_1(0, r_0) = 0$ but this is in contradiction with the fact that D > 0.

Lemma 3. $E_1(\tilde{x}_0, 0)$ exists and $G(\tilde{x}_0) > 0$ is equivalent to $D = \psi_0^0(\tilde{x}_0) < \psi_1^0(\tilde{x}_0)$

Proof: We denote $\tilde{r}_0 = s_0^{in} - \tilde{x}_0$ in the case where $r_0 = s_0$ and we denote $\tilde{r}_0 = \tilde{x}_0$ in the case where $r_0 = x_0$. E_1 exists if and only if $D = \psi_0^0(\tilde{x}_0)$.

 $G(\tilde{x}_0) > 0$ is equivalent to $s_1^{in} + \tilde{x}_0 - M_1(D, \tilde{r}_0)) > 0$ which is to say $s_1^{in} + \tilde{x}_0 > M_1(D, \tilde{r}_0))$ and which means, according to \mathbf{H}_5 , that $\varphi_1(s_1^{in} + \tilde{x}_0, \tilde{r}_0) > D$ and this is equivalent, when E_1 exists, to say $\psi_1^0(\tilde{x}_0) > D = \psi_0^0(\tilde{x}_0)$.

Proof of Proposition 2: E_0 always exists, since (0,0) is a trivial solution of system (2).

 E_1 exists if and only if equation $\psi_0^0(x) = D$ has a solution in $[0, s_0^{in}]$ and this is means that line y = D intercepts the curve Γ_0^0 of function ψ_0^0 , That is possible if and only if $D < \psi_0^0(0) = D_0(s_0^{in})$, since, according to \mathbf{H}_4 , function ψ_0^0 is decreasing on $[0, s_0^{in}]$ and $\psi_0^0(s_0^{in}) = 0$.

The same idea applied on the function $x_1 \mapsto \varphi_1(s_1^{in}-x_1,s_0^{in})$, which is decreasing on $[0,s_1^{in}]$, allows to say that steady state E_1 exists, which is to say that equation $\varphi_1(s_1^{in}-x_1,s_0^{in}) = D$ has a solution, if and only if $D < D_1(s_0^{in},s_1^{in})$.

Steady state $E_3 = (x_0^*, x_1^*)$ exists if and only if system 3 has a solution (x_0^*, x_1^*) in σ , which means, according to Remark 2 and Lemma 2, that Γ_G crosses Γ_F inside σ and this is equivalent to say that steady state $E_1(\tilde{x}_0, 0)$ exists and $G(\tilde{x}_0) > 0$, this means, according to Lemma 3, that curve of function ψ_1^0 is above that of function ψ_0^0 .

In the context of Remark 1, one distinguishes the following cases:

Case 1: The curve Γ_0^0 does not cross the curve Γ_1^0 : this is possible only if $D_0(s_0^{in}) < D_1(s_0^{in}, s_1^{in})$, see Figure 2b. In this case, Γ_1^0 is always above Γ_1^0 , which means that E_3 exists for any value of D such that $D < D_0(s_0^{in}) < D_1(s_0^{in}, s_1^{in})$.

Case 2: The curve Γ_0^0 crosses the curve Γ_1^0 once: Since ψ_0^0 is decreasing and ψ_1^0 is increasing in $[0, s_0^{in}]$, Γ_1^0 lies over Γ_0^0 if and only if $D < D_2^1(s_0^{in}, s_1^{in})$.

In what follows, we will discuss conditions of sta-

bility of steady states. The local asymptotic stability of an equilibrium point will be determined by the signs of the real parts of the eigenvalues of the Jacobian matrix J_i , (i = 0, ..., 3) evaluated at this equilibrium.

For $r_0 = s_0^{in} - x_0$, the Jacobian matrix of system (2) is given by:

$$J = \begin{bmatrix} -\varphi_0' x_0 + \varphi_0 - D & 0\\ \frac{\partial \varphi_1}{\partial s_1} x_1 - \frac{\partial \varphi_1}{\partial s_0} x_1 & -\frac{\partial \varphi_1}{\partial s_1} x_1 + \varphi_1 - D \end{bmatrix}$$

where φ_0, φ'_0 and φ_1 denote $\varphi_0(s_0^{in} - x_0), \varphi'_0(s_0^{in} - x_0)$ and $\varphi_1(s_1^{in} + x_0 - x_1, s_0^{in} - x_0)$ respectively.

The Jacobian matrix at $E_0(0,0)$ is:

$$J_{0} = \begin{bmatrix} \varphi_{0}(s_{0}^{in}) - D & 0 \\ 0 & \varphi_{1}(s_{1}^{in}, s_{0}^{in}) - D \end{bmatrix}$$
$$= \begin{bmatrix} D_{0}(s_{0}^{in}) - D & 0 \\ 0 & D_{1}(s_{0}^{in}, s_{1}^{in}) - D \end{bmatrix}$$

The eigenvalues are $\lambda_0 = D_0(s_0^{in}) - D$ and $\lambda_1 = D_1(s_0^{in}, s_1^{in}) - D$. Steady state E_0 is asymptotically stable if and only if $D > \max(D_0(s_0^{in}), D_1(s_0^{in}, s_1^{in}))$.

The Jacobian matrix at $E_1 = (\tilde{x}_0, 0)$ is given by:

$$J_{1} = \begin{bmatrix} -\varphi_{0}'(s_{0}^{in} - \tilde{x}_{0})\tilde{x}_{0} & 0 \\ 0 & \varphi_{1}(s_{1}^{in} + \tilde{x}_{0}, s_{0}^{in} - \tilde{x}_{0}) - D \end{bmatrix}$$

Its eigenvalues are $\lambda_0 = -\varphi'_0(s_0^{in} - \tilde{x}_0)\tilde{x}_0$ and $\lambda_1 = \varphi_1(s_1^{in} + \tilde{x}_0, s_0^{in} - \tilde{x}_0) - D = \psi_1^0(\tilde{x}_0) - D$. According to hypothesis **H**₄, one has $\lambda_0 < 0$. On an other hand, one has $\lambda_1 = \psi_1^0(\tilde{x}_0) - D = \psi_1^0(\tilde{x}_0) - \psi_0^0(\tilde{x}_0)$, thus, the sign of λ_1 is determined by the position of curve Γ_0^0 relative to curve Γ_1^0 , that is to say, $\lambda_1 < 0$ and therefore steady state E_1 is stable, if and only if curve Γ_0^0 is above Γ_1^0 for x_0 in $[0, s_0^{in}]$. According to Remark 1, this is possible only if $D_1(s_0^{in}, s_1^{in}) < D_2^1(s_0^{in}, s_1^{in}) < D < D_0(s_0^{in})$, see Figure 2a.

The Jacobian matrix at E_2 is:

$$J_{2} = \begin{bmatrix} \varphi_{0} - D & 0\\ \frac{\partial \varphi_{1}}{\partial s_{1}} \tilde{x}_{1} - \frac{\partial \varphi_{1}}{\partial s_{0}} \tilde{x}_{1} & -\frac{\partial \varphi_{1}}{\partial s_{1}} \tilde{x}_{1} \end{bmatrix}$$

where φ_0 and φ_1 denote $\varphi_0(s_0^{in})$ and $\varphi_1(s_1^{in} - \tilde{x}_1, s_0^{in})$ respectively.

Its eigenvalues are $\lambda_0 = \varphi_0(s_0^{in}) - D = D_0(s_0^{in}) - D$ and $\lambda_1 = -\frac{\partial \varphi_1}{\partial s_1}(s_1^{in} - \tilde{x}_1, 0)\tilde{x}_1$. According to hypothesis **H**₅, $\lambda_1 < 0$. $\lambda_0 < 0$ if and only if $D > D_0(s_0^{in})$. Thus, steady state E_2 is stable if and only if $D_0(s_0^{in}) < D < D_1(s_0^{in}, s_1^{in})$.

The Jacobian matrix at $E_3 = (x_0^*, x_1^*)$ is given by:

$$J_{3} = \left[\begin{array}{cc} -\varphi_{0}' x_{0}^{*} & 0 \\ \frac{\partial \varphi_{1}}{\partial s_{1}} x_{1}^{*} - \frac{\partial \varphi_{1}}{\partial s_{0}} x_{1}^{*} & -\frac{\partial \varphi_{1}}{\partial s_{1}} x_{1}^{*} \end{array} \right]$$

where φ'_0 and φ_1 denote $\varphi'_0(s_0^{in} - x_0^*)$ and $\varphi_1(s_1^{in} + x_0^* - x_1^*, s_0^{in} - x_0^*)$ respectively.

Its eigenvalues are $\lambda_0 = -\varphi'_0 x_0^*$ and $\lambda_1 = -\frac{\partial \varphi_1}{\partial s_1} x_1^*$. According to hypothesis \mathbf{H}_4 and \mathbf{H}_5 , both of eigenvalues are negative, therefore, the steady state E_3 is locally asymptotically stable when it exists.

Proof of Proposition 3: The existence of equilibrium points E_0 and E_1 is proved in the proof of proposition 2.

For the steady state E2, the existence condition is the same used in proof of proposition 2, applied on the function $x_1 \mapsto \varphi_1(s_1^{in} - x_1, 0)$.

Steady state $E_3 = (x_0^*, x_1^*)$ exists if and only if system 3 has a solution (x_0^*, x_1^*) in σ , which means, according to Remark 2 and Lemma 2, that Γ_G crosses Γ_F inside σ and this is equivalent to say that steady state $E_1(\tilde{x}_0, 0)$ exists and $G(\tilde{x}_0) > 0$, this means, according to Lemma 3, that curve of function ψ_1^0 is above that of function ψ_0^0 .

In the context of Remark 1, one distinguishes the following cases:

Case 1: The curve Γ_0^0 does not cross the curve Γ_1^0 : this is possible only if $D_0(s_0^{in}) < D_1(s_1^{in})$ and Γ_1^0 is always above Γ_1^0 in $[0, s_0^{in}]$, this means that E_3 exists for any value of D such that $D < D_0(s_0^{in}) < D_1(s_1^{in})$, see Figure 2c.

Case 2: The curve Γ_0^0 crosses the curve Γ_1^0 once, see Figure 2d: According to Remark 1, we should have $D_1(s_1^{in}) < D_0(s_0^{in})$ and in this case, E_3 exists if and only if $D < D_2^1(s_0^{in}, s_1^{in})$.

Case 3: The curve Γ_0^0 crosses the curve Γ_1^0 two times: This is possible only if $D_0(s_0^{in}) < D_1(s_1^{in})$ and in this case, E_3 exists if and only if $D_2^1(s_0^{in}, s_1^{in}) < D < D_0(s_0^{in})$ or $D < D_2^2(s_0^{in}, s_1^{in})$, see Figure 3a.

Case 4: The curve Γ_0^0 crosses the curve $\Gamma_1^0 p$ times, where $p \ge 3$: The existence of steady state E_3 depends on $D_0(s_0^{in})$ relative to $D_1(s_1^{in})$. In fact, if $D_1(s_1^{in}) < D_0(s_0^{in})$ then Γ_1^0 is above Γ_0^0 if and only if $D < D_2^p(s_0^{in}, s_1^{in})$) or $D_2^{2j+2}(s_0^{in}, s_1^{in}) < D < D_2^{2j+1}(s_0^{in}, s_1^{in})$ where $j = 0, \dots, (p-3)/2$ and if $D_0(s_0^{in}) < D_1(s_1^{in})$ then E_3 exists if and only if $D_2^1(s_0^{in}, s_1^{in}) < D < D_0(s_0^{in})$ or $D < D_2^p(s_0^{in}, s_1^{in})$ or $D_2^{2j+1}(s_0^{in}, s_1^{in}) < D < D_2^{2j}(s_0^{in}, s_1^{in})$ where $j = 1, \dots, (p-2)/2$, see Figure 3. **Proof of Proposition** 4: Let φ_0 , φ'_0 and φ_1 designate $\varphi_0(s_0^{in} - x_0)$, $\varphi'_0(s_0^{in} - x_0)$ and $\varphi_1(s_1^{in} + x_0 - x_1, x_0)$ respectively. The Jacobian matrix of system (2) is given by:

$$J = \begin{bmatrix} -\varphi_0' x_0 + \varphi_0 - D & 0\\ \frac{\partial \varphi_1}{\partial s_1} x_1 + \frac{\partial \varphi_1}{\partial x_0} x_1 & -\frac{\partial \varphi_1}{\partial s_1} x_1 + \varphi_1 - D \end{bmatrix}$$

The Jacobian matrix at $E_0(0,0)$ is:

$$J_{0} = \begin{bmatrix} \varphi_{0}(s_{0}^{in}) - D & 0 \\ 0 & \varphi_{1}(s_{1}^{in}, 0) - D \end{bmatrix}$$
$$= \begin{bmatrix} D_{0}(s_{0}^{in}) - D & 0 \\ 0 & D_{1}(s_{1}^{in}) - D \end{bmatrix}$$

The eigenvalues are $\lambda_0 = D_0(s_0^{in}) - D$ and $\lambda_1 = D_1(s_1^{in}) - D$. If $D > \max(D_0(s_0^{in}), D_1(s_1^{in}))$ then $\lambda_0 < 0$ and $\lambda_1 < 0$, therefore, E_0 is asymptotically stable. If $D < \min(D_0(s_0^{in}), D_1(s_1^{in}))$ then $\lambda_0 > 0$ and $\lambda_1 > 0$, hence, E_0 is an unstable node. If $D_0(s_0^{in}) < D < D_1(s_1^{in})$ or $D_1(s_1^{in}) < D < D_0(s_0^{in})$ then $det J_0 < 0$, so E_0 is a saddle point.

The Jacobian matrix at $E_1 = (\tilde{x}_0, 0)$ is given by:

$$J_{1} = \begin{bmatrix} -\varphi_{0}'(s_{0}^{in} - \tilde{x}_{0})\tilde{x}_{0} & 0\\ 0 & \varphi_{1}(s_{1}^{in} + \tilde{x}_{0}, \tilde{x}_{0}) - D \end{bmatrix}$$

Its eigenvalues are $\lambda_0 = -\varphi'_0(s_0^{in} - \tilde{x}_0)\tilde{x}_0$ and $\lambda_1 = \varphi_1(s_1^{in} + \tilde{x}_0, \tilde{x}_0) - D = \psi_1^0(\tilde{x}_0) - D$. According to hypothesis \mathbf{H}_4 , $\lambda_0 < 0$. One has $\lambda_1 = \psi_1^0(\tilde{x}_0) - D = \psi_1^0(\tilde{x}_0) - \psi_0^0(\tilde{x}_0)$, therefore, the sign of λ_1 is determined by the position of curve Γ_0^0 relative to curve Γ_1^0 , indeed, $\lambda_1 < 0$ if and only if curve Γ_0^0 is over Γ_1^0 for x_0 in $[0, s_0^{in}]$, hence, one distinguishes the following cases:

Case 1: Γ_0^0 crosses Γ_1^0 once. It is straightforward to see that $\lambda_1 < 0$ if and only if $(D_1(s_1^{in}) < D_0(s_0^{in}))$ and $(D_2^1(s_0^{in}, s_1^{in}) < D < D_0(s_0^{in}))$.

Case 2: Γ_0^0 crosses $\Gamma_1^0 p$ times, $p \ge 2$, therefore $\lambda_1 < 0$ if and only if one of the following sub-cases is satisfied:

First sub-case: $(D_0(s_0^{in}) < D_1(s_1^{in}))$ and (there exists $j = 0, \dots, (p-2)/2$ such that $D_2^{2j+2}(s_0^{in}, s_1^{in}) < D < D_2^{2j+1}(s_0^{in}, s_1^{in}))$.

Second sub-case: $(D_1(s_1^{in}) < D_0(s_0^{in}))$ and $(D_2^1 < D < D_0(s_0^{in}))$ or there exists $j = 1, \ldots, (p-1)/2$ such that $D_2^{2j+1}(s_0^{in}, s_1^{in}) < D < D_2^{2j}(s_0^{in}, s_1^{in}))$.

The Jacobian matrix at E_2 is:

$$J_{2} = \left[\begin{array}{cc} \varphi_{0} - D & 0 \\ \frac{\partial \varphi_{1}}{\partial s_{1}} \tilde{x}_{1} + \frac{\partial \varphi_{1}}{\partial x_{0}} \tilde{x}_{1} & -\frac{\partial \varphi_{1}}{\partial s_{1}} \tilde{x}_{1} \end{array} \right]$$

where φ_0 and φ_1 designate $\varphi_0(s_0^{in})$ and $\varphi_1(s_1^{in} - \tilde{x}_1, 0)$ respectively.

Its eigenvalues are λ_0 and λ_1 such that:

$$\lambda_0 = \varphi_0(s_0^{in}) - D = D_0(s_0^{in}) - L$$

hence, $\lambda_0 < 0$ if and only if $D > D_0(s_0^{in})$ and $\lambda_1 = -\frac{\partial \varphi_1}{\partial s_1}(s_1^{in} - \tilde{x}_1, 0)\tilde{x}_1 < 0$, according to hypothesis **H**₅. Thus, steady state E_2 exists and it is stable if and only if $D_0(s_0^{in}) < D < D_1(s_1^{in})$.

The Jacobian matrix at $E_3 = (x_0^*, x_1^*)$ is given by:

$$J_3 = \begin{bmatrix} -\varphi_0' x_0^* & 0\\ \frac{\partial \varphi_1}{\partial s_1} x_1^* + \frac{\partial \varphi_1}{\partial x_0} x_1^* & -\frac{\partial \varphi_1}{\partial s_1} x_1^* \end{bmatrix}$$

where φ'_0 and φ_1 denote $\varphi'_0(s_0^{in} - x_0^*)$ and $\varphi_1(s_1^{in} + x_0^* - x_1^*, x_0^*)$ respectively.

According to hypothesis \mathbf{H}_4 and \mathbf{H}_5 , both of eigenvalues are negative, therefore, the steady state E_3 is locally asymptotically stable when it exists.

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REFERENCES

- O. Bernard, Z. Hadj-Sadock, D. Dochain, A. Genovesi, J.-P. Steyer, Dynamical model development and parameter identification for an anaerobic wastewater treatment process, *Biotechnology and Bioengineering*, 75:424–438, 2001.
- [2] N. Abdellatif, R. Fekih-Salem, T. Sari, Competition for a single resource and coexistence of several species in the chemostat, *Mathematical Biosciences and Engineering*, 13:631–652, 2016.
- [3] Y. Daoud, N. Abdellatif, T. Sari, J. Harmand, Steady state analysis of a syntrophic model: The effect of a new input substrate concentration, *Mathematical Modelling of Natural Phenomena*, 13:31, 2018.

- [4] R. Fekih-Salem, N. Abdellatif, T. Sari, J. Harmand, Analyse mathématique d'un modèle de digestion anaérobie à trois étapes, *ARIMA Journal*, 17:53–71, 2014.
- [5] M. Hanaki, J. Harmand, Z. Mghazli, T. Sari, A. Rapaport, P. Ugalde, Mathematical study of a two-stage anaerobic model when the hydrolysis is the limiting step, *Processes*, 9:2050, 2021.
- [6] M. El Hajji, F. Mazenc, J. Harmand, A mathematical study of a syntrophic relationship of a model of anaerobic digestion process, *Mathematical Biosciences and Engineering*, 7:641– 656, 2010.
- [7] J. F. Andrews, A mathematical model for continuous culture of microorganisms utilizing inhibitory substrates, *Biotechnology* and *Bioengineering*, 10:707–723, 1968.
- [8] A. Detman, M. Bucha, L. Treu, A. Chojnacka, Ł. Pleśniak, A. Salamon, et al., Evaluation of acidogenesis products' effect on biogas production performed with metagenomics and isotopic approaches, *Biotechnology for Biofuels*, 14:125, 2021.
- [9] Z. Khedim, B. Benyahia, B. Cherki, T. Sari, J. Harmand, Effect of control parameters on biogas production during the anaerobic digestion of protein-rich substrates, *Applied Mathematical Modelling*, 61:351–376, 2018.
- [10] M. Weedermann, G. Seo, G. Wolkowicz, Mathematical model of anaerobic digestion in a chemostat: effects of syntrophy and inhibition, *Journal of Biological Dynamics*, 7:59–85, 2013.
- [11] N. Dimitrova, Dynamical analysis of a chemostat model for 4-chlorophenol and sodium salicylate mixture biodegradation, *Biomath*, 12:2311027, 2023.
- [12] J. Harmand, C. Lobry, A. Rapaport, T. Sari, *The Chemostat: Mathematical Theory of Microorganism Cultures*, John Wiley & Sons, 2017.
- [13] T. Sari, J. Harmand, A model of a syntrophic relationship between two microbial species in a chemostat including maintenance, *Mathematical Biosciences*, 275:1–9, 2016.
- [14] H. L. Smith, P. Waltman, *The Theory of the Chemostat: Dynamics of Microbial Competition*, Cambridge University Press, 1995.
- [15] S. Di, A. Yang, Analysis of productivity and stability of synthetic microbial communities, *Journal of the Royal Society Interface*, 16:20180859, 2019.
- [16] B. Benyahia, T. Sari, B. Cherki, J. Harmand, Bifurcation and stability analysis of a two-step model for monitoring anaerobic digestion processes, *Journal of Process Control*, 22:1008–1019, 2012.
- [17] T. Sari, M. El Hajji, J. Harmand: The mathematical analysis of a syntrophic relationship between two microbial species in a chemostat, *Mathematical Biosciences*, 9:627–645, 2012.
- [18] B. Bar, T. Sari, The operating diagram for a model of competition in a chemostat with an external lethal inhibitor, *Discrete* and Continuous Dynamical Systems – Series B, 25:2093–2120, 2020.