



**HAL**  
open science

## Analysis of anaerobic digestion Model with two serial interconnected chemostats \*

Thamer Hmidhi, Radhouane Fekih-Salem, Jérôme Harmand

### ► To cite this version:

Thamer Hmidhi, Radhouane Fekih-Salem, Jérôme Harmand. Analysis of anaerobic digestion Model with two serial interconnected chemostats \*. 2024. hal-04667969

**HAL Id: hal-04667969**

**<https://hal.science/hal-04667969>**

Preprint submitted on 5 Aug 2024

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

# Analysis of anaerobic digestion Model with two serial interconnected chemostats \*

THAMER HMIDHI <sup>†</sup>, RADHOUANE FEKIH-SALEM <sup>\*†‡</sup>, and JÉRÔME HARMAND <sup>§</sup>

**Abstract.** In this paper, we study a well known two-step anaerobic digestion model in a configuration of two chemostats in series. This model is an eight-dimensional system of ordinary differential equations. Since the reaction system has a cascade structure, we show that the eight-order model can be reduced to a four-dimensional one. Using general growth rates, we provide an in-depth mathematical analysis of the asymptotic behavior of the system. First, we determine all the steady states of the model where there can be more than fifteen equilibria with a non-monotonic growth rate. Then, the necessary and sufficient conditions of existence and local stability of all steady states are established according to the operating parameters: the dilution rate, the input concentrations of the two nutrients, and the distribution of the total process volume considered. The operating diagrams are then analyzed theoretically to describe the asymptotic behavior of the process according to the four control parameters. There can be seventy regions with rich behavior where the system may exhibit bistability or tristability with the coexistence of both microbial species in the two bioreactors.

**Key words.** Anaerobic digestion, Bifurcation diagram, Coexistence, Interconnected chemostats, Stability.

**MSC codes.** 34A34, 34D20, 37N25, 92B05

**1. Introduction.** Anaerobic Digestion (AD) is a biological process in which organic matter is transformed by a complex microbial ecosystem into biogas in the absence of oxygen. It is used for the treatment of wastewater and the production of energy in the form of biogas containing methane. Under specific conditions, it may also be used to produce hydrogen from waste: the process is then named ‘black fermentation’. It is thus a fundamental process in the field of circular economy and green energy production from waste, [48].

On the one hand, biological are attractive: based on natural processes, they are relatively easy and simple to manage. On the other hand, they are usually considered as low yield and rate processes when compared to other energy producing processes. This why it is particularly important to optimize their design. In particular, in (bio)chemical engineering, one very important and actually quite old question is whether a biological process must be implemented in a homogeneous medium, or not. To study this question and characterize the mixing characteristics of the medium in a reactor, chemical engineers have proposed formal representations of chemical and biochemical devices known as ‘ideal’ (or ‘perfect’) reactors. On the one side of the scale on which reactors would be classified according to their homogeneity, there are the Completely Stirred Tank Reactors: they are completely mixed systems where the medium is considered as being perfectly homogeneous (CSTR). On the other side, we define the Plug Flow Reactors (PFR) which are nonhomogeneous systems in which small volumes of matter progress from the input to the output of the process independently of what happens around them. Practically, we can represent this situation with a long pipe of small diameter in which slices of little volumes of medium progress at a given speed without any exchange with those which go before and those which come after, the bioreaction taking place only inside each slice of liquid. Notice that a PFR may be approximated by an infinite number of CSTR in series. A limited number of CSTR is thus suited to represent situations between these two ideal cases, see for instance [31].

---

\*Submitted to the editors 2024-08-05.

**Funding:** This work was supported by the Euro-Mediterranean research network TREASURE (<http://www.inra.fr/treasure>).

<sup>†</sup>University of Tunis El Manar, National Engineering School of Tunis, LAMSIN, Tunisia ([thamer.hmidhi@enit.utm.tn](mailto:thamer.hmidhi@enit.utm.tn)).

<sup>‡</sup>University of Monastir, Higher Institute of Computer Science of Mahdia, Tunisia ([radhouane.fekih-saleme@enit.utm.tn](mailto:radhouane.fekih-saleme@enit.utm.tn)).

<sup>§</sup>LBE, University of Montpellier, INRAE, Narbonne, France ([jerome.harmand@inrae.fr](mailto:jerome.harmand@inrae.fr)).

In microbiology/biotechnology, a CSTR is named ‘chemostat’. It consists in a tank filled with an aqueous solution in which microorganisms, generically named ‘biomass’, grow on one or more resources, named ‘substrates’. The first introduction of the chemostat dates back to 1950 by its inventors Novick and Szilard [38] simultaneously with Monod [32]. More than a common laboratory apparatus used for the continuous cultivation of microorganisms, its model has been studied as a mathematical object since its origins in the fifties [27, 46]. As such, it plays an important role as a model to study the growth of microbes and their interactions in mathematical biology and mathematical ecology. It has been long time used as a model of wastewater biological treatment processes and has been widely used to model industrial bioreactors.

Following the increasing knowledge accumulated over time about the anaerobic microbial ecosystems, the AD process is schematically represented as a set of bioreactions in cascade realized by several groups of microbes, the output of a reaction step being considered to be the input of the next one. Depending on the nature of its inputs, the AD is represented either as a one, two, three, or even four-step process, *cf.* for instance [51]. It is recognized that the most complete actual model is the ADM1 developed initially proposed by Batstone *et al.* [3, 29]. Of course, greater the complexity, lesser the possibility of analytically analyzing the properties of the associated model. Indeed, because of its complexity, and notably its relatively high dimension, the ADM1 is not easy to study from a mathematical viewpoint: very few studies were able to investigate its properties. From the best of our knowledge, there exists only one paper studying its steady states and their stability (see [7]). The AM2 model proposed by [6] is a two-step AD model. It is particularly interesting because it is of medium complexity. It has been shown to be able to represent real data in capturing the main dynamical features of different full-scale AD processes and is thus well suited for control design. During the last decade, many papers in the literature have studied the AM2 model and some of its extensions (see [4, 5, 19, 41]).

The ‘optimal design’ of a biological system consists in finding the ‘best configuration’ - in the sense of a given performance criterion - of this system. In particular, a major question in process engineering is to determine which type of reactor or interconnection of reactors - typically perfect reactors - is the most suitable to maximize such a performance for a given biological process, [21]. By ‘type or reactor’ or ‘interconnection of reactors’, we essentially mean determining whether it is important to favor a homogeneous medium (and thus use a chemostat), or not (and thus rather use an interconnection of chemostats, or even a PFR). Notice that there exist many alternative configurations, such as several chemostats working in parallel with or without convective or diffusive connections. Several mathematical studies have addressed these issues in considering what is named the ‘minimal chemostat model’, that is a single-step biological reaction and different biomass growth rates, see [9, 10, 11, 12, 23, 24, 25] for configurations involving chemostats in series, and [23, 31] for configurations of chemostats in parallel. For instance, considering the maximization of conversion yield, Haidar *et al.* [23] have shown that there exists a threshold on the concentration of nutrient supply for which the series and parallel configurations are better than a single chemostat at steady state. In [10], authors have compared the performances between the two serial interconnected chemostats and of a single chemostat and showed the most effective serial configuration of two chemostats against one to maximize the conversion of the substrate. Notice that if the objective is to maximize the productivity of biomass or, equivalently the production of biogas, the results may be different. And indeed, in the most general case, the results highly depend on both the growth rate considered, the input substrate concentration, and on the objective pursued. The papers cited hereabove are very general studies that did not consider specifically the AD process and only concentrated on the optimal design of single-step biological reactional systems. What about the optimal design of multiple-step processes as it is the case for the AD? In particular, do the results establish for a one-step system hold for a two-step process? In addition, one may further question whether a configuration in which only the first reaction would take place in one reactor (in which case the first reactor may be used to produce hydrogen) and the second reaction in another one

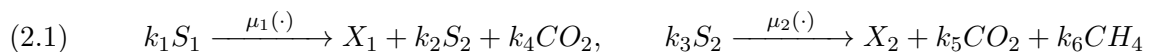
would be better than any other configuration of reactors, or not. Several authors have begun to address these questions either experimentally (*cf.* for instance [45]) or theoretically in using the minimal chemostat model in each stage (*cf.* for instance [8, 18, 39]). However, before addressing the problem of optimally designing multi-step biological processes, it is essential to study the properties of such a process in a cascade of chemostats, that are the existence and the steady states properties of the steady states.

In this work, we propose an original model describing the two-stage AD process in two series-connected chemostats. Using the same removal rates of two species in the two bioreactors, the eight-dimensional system with a cascade structure can be reduced to a four-dimensional system. Using monotonic growth rates for the first species and non-monotonic growth rates for the second species, we investigate the existence and stability of all steady states according to the control parameters. Moreover, we theoretically describe the operating diagrams and show that the model exhibits a very rich behavior, where there can be bistability and tristability with the coexistence of two populations of micro-organisms in each bioreactor.

Indeed, the operating diagram is a very useful tool for engineers, enabling them to graphically synthesize the asymptotic behavior of the process as a function of the control parameters. It was established in the book [27] for the classical chemostat model with Monod and Haldane growth rates. Three methods of analysis and construction of the operating diagram in the existing literature have been reported and summarized in Mtar et al [34]: purely numerical point-by-point [49, 50], by the numerical continuation of bifurcations through software packages such as MatCont (see [17] and the reference therein) and theoretical from the existence and stability conditions. We can cite the following papers based on the numerical study of the operating diagram as in [22, 30, 44, 50, 52, 53]. Several other recent papers have been based on the theoretical study of the operating diagram with various biological and ecological applications: anaerobic digestion [13, 19, 40, 41, 42, 51], microbial food webs [35, 36, 37, 43, 47] serially interconnected chemostats [9, 10, 11, 12], competition with inhibition and allelopathy effects [2, 14, 15, 16, 54] and density-dependence models [20, 33, 34].

This paper is organized as follows: in section 2, we propose a original model describing the AD process with a serial configuration of two chemostats. In section 3, we show that the study of the eight-dimension system can be reduced to a four-dimension one. Subsection 3.1 is devoted to analyzing the existence conditions and multiplicity of all steady states of the reduced model. Subsection 3.2 is dedicated to establishing the local stability conditions of all steady states. Then, in section 4, we establish theoretically the operating diagram from the existence and stability conditions. In section 5, we discuss our results by comparing them with those of the existing literature. The proofs of all results are reported in Appendix A. The parameter values used in all figures and the tables defining the various regions of the operating diagram are provided in section SM1.

**2. Modeling the configuration of two interconnected chemostats.** We first recall the general two-step model in a single chemostat with a cascade of the following two biological reactions (see [6]):



where the organic substrate  $S_1$  is consumed by acidogenic bacteria  $X_1$  to produce a product  $S_2$  (Volatile Fatty Acid). In the second reaction, the methanogenic bacteria  $X_2$  consumes the substrate  $S_2$  and produces biogas.  $\mu_1$  and  $\mu_2$  are the kinetics of the two reactions,  $k_i$ ,  $i = 1, \dots, 6$  are the pseudo-stoichiometric coefficients. The substrates  $S_1$  and  $S_2$  are introduced in the reactor with the inflowing concentrations  $S_1^{in}$  and  $S_2^{in}$ , respectively, with the input flow rate  $Q$  so that the dilution rate  $D = Q/V$ . Thus, we obtain the so-called AM2 model which is described by

the following ordinary differential equations:

$$(2.2) \quad \begin{cases} \dot{S}_1 &= D(S_1^{in} - S_1) - k_1\mu_1(\cdot)X_1, \\ \dot{X}_1 &= (\mu_1(\cdot) - \alpha D)X_1, \\ \dot{S}_2 &= D(S_2^{in} - S_2) + k_2\mu_1(\cdot)X_1 - k_3\mu_2(\cdot)X_2, \\ \dot{X}_2 &= (\mu_2(\cdot) - \alpha D)X_2, \end{cases}$$

where only a fraction  $\alpha$  of the biomass is in the liquid phase. More precisely,  $\alpha \in [0, 1]$  is a parameter allowing us to decouple the Hydraulic Retention Time (HRT) and the Solid Retention Time (SRT) (see [6] for more detail on the model development).

The AM2 model (2.2) has been studied in [5, 41, 44] when the growth rate  $\mu_i$  for  $i = 1, 2$  depends only on substrate  $S_i$  ( $\mu_i(\cdot) = \mu_i(S_i)$ ), that is, (2.2) has a cascade-connected subsystems describing a commensalistic relationship. In [19], an in-depth mathematical study of model (2.2) has been established by considering a syntrophic relationship between the species, that is,  $\mu_1(\cdot)$  depends on both substrates  $S_1$  and  $S_2$  while  $\mu_2(\cdot)$  depends on the substrate  $S_2$  ( $\mu_1(\cdot) = \mu_1(S_1, S_2)$ ,  $\mu_2(\cdot) = \mu_2(S_2)$ ). A summary review of the AM2 model for commensalistic and syntrophic relationships describing the modeling assumptions and the growth rates is provided in [19]. Recently, the maximization of biogas production of the AM2 model (2.2) was established theoretically according to the bioreactor control parameters [40].

In this paper, we consider a serial configuration of two interconnected chemostats where the volume  $V$  is divided into two volumes,  $r_1V = rV$  and  $r_2V = (1 - r)V$  with  $r \in (0, 1)$  (see Figure 1). The two-tiered model we consider here describes the same biological reactions (2.1). However, the concentrations of substrate  $S_1$  and  $S_2$  and biomass  $X_1$  and  $X_2$  are different in each bioreactor, which we'll note as  $S_i^j$  and  $X_i^j$ ,  $i, j = 1, 2$  in each bioreactor  $j$ . Moreover,  $S_1^{in}$  and  $S_2^{in}$  are the concentrations of input substrate  $S_1^1$  and  $S_2^1$  in the first chemostat at a flow rate  $Q$ . The substrates  $S_1^2$  and  $S_2^2$  are introduced in the second chemostat with the inflowing concentrations  $S_1^1$  and  $S_2^1$ , respectively, and a flow rate  $Q$ .

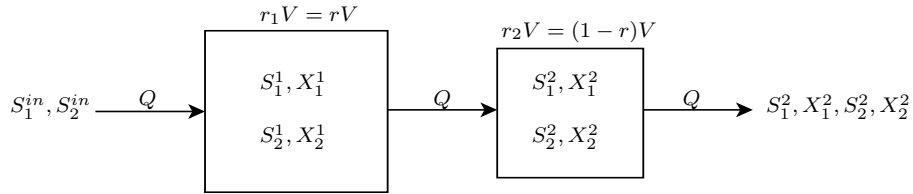


Figure 1. The serial configuration of two interconnected chemostats.

Using Antoine Lavoisier's law: "Nothing is lost, nothing is created, everything is transformed" and establishing the mass balance between the instants  $t$  and  $t + dt$ , we obtain the mathematical model given by the following eight-dimensional system of ordinary differential equations:

$$(2.3) \quad \begin{cases} \dot{S}_1^1 &= D_1(S_1^{in} - S_1^1) - k_1\mu_1(S_1^1)X_1^1, \\ \dot{X}_1^1 &= (\mu_1(S_1^1) - D_1)X_1^1, \\ \dot{S}_2^1 &= D_1(S_2^{in} - S_2^1) + k_2\mu_1(S_1^1)X_1^1 - k_3\mu_2(S_2^1)X_2^1, \\ \dot{X}_2^1 &= (\mu_2(S_2^1) - D_1)X_2^1, \\ \dot{S}_1^2 &= D_2(S_1^1 - S_1^2) - k_1\mu_1(S_1^2)X_1^2, \\ \dot{X}_1^2 &= D_2(X_1^1 - X_1^2) + \mu_1(S_1^2)X_1^2, \\ \dot{S}_2^2 &= D_2(S_2^1 - S_2^2) + k_2\mu_1(S_1^2)X_1^2 - k_3\mu_2(S_2^2)X_2^2, \\ \dot{X}_2^2 &= D_2(X_2^1 - X_2^2) + \mu_2(S_2^2)X_2^2, \end{cases}$$

where  $D_1 = D/r_1$  and  $D_2 = D/r_2$  are the dilution rates corresponding to the first and second chemostats, respectively. In this paper, we assume that the growth functions  $\mu_1(S_1)$  and  $\mu_2(S_2)$  belongs to  $\mathcal{C}^1(R_+)$  and satisfy the following hypotheses.

*Hypothesis 2.1.*  $\mu_1(0) = 0$ ,  $\mu_1(+\infty) = m_1$ , and  $\mu_1'(S_1) > 0$  for all  $S_1 > 0$ .

*Hypothesis 2.2.*  $\mu_2(0) = 0$ ,  $\mu_2(+\infty) = 0$ , and there exists  $S_2^m > 0$  such that  $\mu_2'(S_2) > 0$ , for all  $0 < S_2 < S_2^m$  and  $\mu_2'(S_2) < 0$ , for all  $S_2 > S_2^m$ .

*Hypothesis 2.1* means that the substrate is necessary for the growth of the first species. In addition, the growth rate of the first species is favored by the concentration of the substrate  $S_1$ . *Hypothesis 2.2* means that the growth of the second species takes into account the growth-limiting for low concentrations of substrate  $S_2$  and the growth-inhibiting for high concentrations.

To preserve the biological meaning of our model (2.3), we prove the following result. Since the proof is classical, it is left to the reader.

**Proposition 2.3.** *All solutions of model (2.3) with nonnegative initial condition remain non-negative and bounded for all positive times. Let  $\xi = (S_1^1, X_1^1, S_2^1, X_2^1, S_1^2, X_1^2, S_2^2, X_2^2)$  be the state vector. The set*

$$\Omega = \{ \xi \in \mathbb{R}_+^8 : S_1^i + k_1 X_1^i = S_1^{in} \text{ and } S_2^i + k_3 X_2^i - k_2 X_1^i = S_2^{in}, i = 1, 2 \}$$

*is positively invariant and is a global attractor for (2.3).*

**3. Reduced model.** In the following, we show that the existence and asymptotic stability of the steady states of the eight-dimensional system (2.3) can be deduced from that of the four-dimensional system (3.2). The change of variable

$$Z_1^i = S_1^i + k_1 X_1^i \quad \text{and} \quad Z_2^i = S_2^i + k_3 X_2^i - k_2 X_1^i, \quad i = 1, 2,$$

transforms system (2.3) into a system having the same structure, namely a system of the form

$$(3.1) \quad \begin{cases} \dot{Z}_1^1 &= D_1 (S_1^{in} - Z_1^1), \\ \dot{Z}_2^1 &= D_1 (S_2^{in} - Z_2^1), \\ \dot{Z}_1^2 &= D_2 (Z_1^1 - Z_1^2), \\ \dot{Z}_2^2 &= D_2 (Z_2^1 - Z_2^2), \\ \dot{X}_1^1 &= (\mu_1 (Z_1^1 - k_1 X_1^1) - D_1) X_1^1, \\ \dot{X}_2^1 &= (\mu_2 (Z_2^1 + k_2 X_1^1 - k_3 X_2^1) - D_1) X_2^1, \\ \dot{X}_1^2 &= D_2 (X_1^1 - X_1^2) + \mu_1 (Z_1^2 - k_1 X_1^2) X_1^2, \\ \dot{X}_2^2 &= D_2 (X_2^1 - X_2^2) + \mu_2 (Z_2^2 + k_2 X_1^2 - k_3 X_2^2) X_2^2. \end{cases}$$

Thus, the steady states of the eight-dimension system (2.3) are given by the steady state of (3.1). From the first four equations of (3.1), we see that a steady state of (3.1) must satisfy  $Z_i^j = S_i^{in}$ ,  $i, j = 1, 2$ . Setting  $Z_i^j = S_i^{in}$  in the lower subsystem of (3.1), we obtain the following reduced system

$$(3.2) \quad \begin{cases} \dot{X}_1^1 &= (\mu_1 (S_1^{in} - k_1 X_1^1) - D_1) X_1^1, \\ \dot{X}_2^1 &= (\mu_2 (S_2^{in} + k_2 X_1^1 - k_3 X_2^1) - D_1) X_2^1, \\ \dot{X}_1^2 &= D_2 (X_1^1 - X_1^2) + \mu_1 (S_1^{in} - k_1 X_1^2) X_1^2, \\ \dot{X}_2^2 &= D_2 (X_2^1 - X_2^2) + \mu_2 (S_2^{in} + k_2 X_1^2 - k_3 X_2^2) X_2^2. \end{cases}$$

Hence, the last four components of a steady state of the eight-dimensional system (3.1) are given by putting the left-hand side of the reduced system (3.2) equal to zero. Thus, for each steady state of the four-dimensional system (3.2) denoted by

$$\mathcal{E}_{ij}^{kl} (X_1^1, X_2^1, X_1^2, X_2^2), \quad \{i, j, k, l\} \in \{0, 1, 2\}$$

corresponds a steady state of the eight-dimensional system (2.3) denoted by

$$\mathcal{F}_{ij}^{kl} (S_1^1, X_1^1, S_2^1, X_2^1, S_1^2, X_1^2, S_2^2, X_2^2)$$

where

$$S_1^i = S_1^{in} - k_1 X_1^i \quad \text{and} \quad S_2^i = S_2^{in} - k_3 X_2^i + k_2 X_1^i, \quad i = 1, 2.$$

To analyze the local stability of each steady state, system (3.1) possesses a pair of cascade-connected subsystems in the  $(\xi_1, \xi_2)$  coordinates of the form (see e.g. [28])

$$\begin{cases} \dot{\xi}_1 = f(\xi_1) \\ \dot{\xi}_2 = g(\xi_2, \xi_1), \end{cases}$$

where

$$f(\xi_1) = \begin{bmatrix} D_1 (S_1^{in} - Z_1^1) \\ D_1 (S_2^{in} - Z_2^1) \\ D_2 (Z_1^1 - Z_1^2) \\ D_2 (Z_2^1 - Z_2^2) \end{bmatrix}, \quad g(\xi_2, \xi_1) = \begin{bmatrix} (\mu_1 (Z_1^1 - k_1 X_1^1) - D_1) X_1^1 \\ (\mu_2 (Z_2^1 + k_2 X_1^1 - k_3 X_2^1) - D_1) X_2^1 \\ D_2 (X_1^1 - X_1^2) + \mu_1 (Z_1^2 - k_1 X_1^2) X_1^2, \\ D_2 (X_2^1 - X_2^2) + \mu_2 (Z_2^2 + k_2 X_1^2 - k_3 X_2^2) X_2^2 \end{bmatrix}$$

where  $\xi_1 = (Z_1^1, Z_2^1, Z_1^2, Z_2^2) \in \mathbb{R}_+^4$  and  $\xi_2 = (X_1^1, X_2^1, X_1^2, X_2^2) \in \mathbb{R}_+^4$ . So that

$$g(\xi_1^*) = 0, \quad g(\xi_2^*, \xi_1^*) = 0,$$

Note that  $\xi_1^*$  is LES of the upper subsystem of (3.1) since at  $\xi_1^*$ , the characteristic polynomial is

$$P(\lambda) = (D_1 + \lambda)^2 (D_2 + \lambda)^2.$$

Using [28, Corollary 10.3.2], we show that the local stability of  $\xi_2^*$  of the lower subsystem  $\dot{\xi}_2 = g(\xi_2, \xi_1^*)$  is the same as the one of the cascade (3.1) at  $(\xi_1, \xi_2) = (\xi_1^*, \xi_2^*)$ .

Since the components  $S_1^1, S_2^1, S_1^2$  and  $S_2^2$  are nonnegative, system (3.2) is defined on the set:

$$(3.3) \quad M = \left\{ (X_1^1, X_2^1, X_1^2, X_2^2) \in \mathbb{R}_+^4 : X_1^i \leq \frac{S_1^{in}}{k_1} \text{ and } X_2^i \leq \frac{S_2^{in} + k_2 X_1^i}{k_3}, i = 1, 2 \right\}.$$

**3.1. Existence and multiplicity of the steady states.** A steady state exists if and only if all its components are nonnegative. Each steady state is preceded by a subscript and an exponent, indicating which population of microorganisms survives in the first and the second chemostat, respectively. For example, the subscript in  $\mathcal{E}_{0i}^{11}$ ,  $i = 1, 2$  implies that  $X_1^1 = 0$  and  $X_2^1 > 0$  with the possibility of two different concentrations of  $X_2^1$  at the steady state. However, the exponent in  $\mathcal{E}_{0i}^{11}$  implies that  $X_1^2 > 0$  and  $X_2^2 > 0$ . Indeed, system (3.2) has the following nine types of steady states with  $i = 1, 2$ .

- $\mathcal{E}_{00}^{00}$  ( $X_1^1 = 0, X_2^1 = 0, X_1^2 = 0, X_2^2 = 0$ ) : washout of the two species in each bioreactor.
- $\mathcal{E}_{00}^{0i}$  ( $X_1^1 = 0, X_2^1 = 0, X_1^2 = 0, X_2^2 > 0$ ) : only the second species is in the second bioreactor.
- $\mathcal{E}_{00}^{10}$  ( $X_1^1 = 0, X_2^1 = 0, X_1^2 > 0, X_2^2 = 0$ ) : only the first species is in the second bioreactor.
- $\mathcal{E}_{00}^{1i}$  ( $X_1^1 = 0, X_2^1 = 0, X_1^2 > 0, X_2^2 > 0$ ) : only the two species is in the second bioreactor.
- $\mathcal{E}_{10}^{10}$  ( $X_1^1 > 0, X_2^1 = 0, X_1^2 > 0, X_2^2 = 0$ ) : only the first species is in each bioreactor.
- $\mathcal{E}_{10}^{1i}$  ( $X_1^1 > 0, X_2^1 = 0, X_1^2 > 0, X_2^2 > 0$ ) : exclusion of the second species in the first bioreactor.
- $\mathcal{E}_{0i}^{01}$  ( $X_1^1 = 0, X_2^1 > 0, X_1^2 = 0, X_2^2 > 0$ ) : only the second species is in each bioreactor.
- $\mathcal{E}_{0i}^{11}$  ( $X_1^1 = 0, X_2^1 > 0, X_1^2 > 0, X_2^2 > 0$ ) : exclusion of the first species in the first bioreactor.
- $\mathcal{E}_{1i}^{11}$  ( $X_1^1 > 0, X_2^1 > 0, X_1^2 > 0, X_2^2 > 0$ ) : the two species in each bioreactor are maintained.

To describe the steady states of (3.2), we define in Table 1 the following auxiliary functions depending on the operating parameters  $D, r, S_1^{in}$ , and  $S_2^{in}$ . To simplify the notations, we will denote in the following

$$\lambda_1^i = \lambda_1^i(D, r) \quad \text{and} \quad \lambda_2^{ij} = \lambda_2^{ij}(D, r), \quad i, j = 1, 2.$$

On the other hand, to establish the existence and multiplicity of steady states of (3.2), we also need to define some auxiliary functions listed in Table 2. In the following proposition, we provide the necessary and sufficient conditions for the existence of all steady states of model (3.2).

Table 1

Auxiliary functions depending on  $D$ ,  $r$ ,  $S_1^{in}$ , and  $S_2^{in}$  for  $i, j = 1, 2$  where  $D_i := D/r_i$ ,  $D_i^m := r_i \mu_2(S_2^m)$ ,  $r_1 = r$ ,  $r_2 = 1 - r$ , and  $X_1^{2*}$  is the unique solution of equation  $f_1(x) = g_1(x)$  with the functions  $f_1$  and  $g_1$  are defined in Table 2.

	Definition
$\lambda_1^i(D, r)$	$\lambda_1^i(D, r)$ is the unique solution of equation $\mu_1(S_1) = D_i$ . It is defined for $0 < D < r_i m_1$ . If $D \geq r_i m_1$ , by convention we let $\lambda_1^i(D, r) = +\infty$ .
$\lambda_2^{ij}(D, r)$	$\lambda_2^{i1}(D, r) < \lambda_2^{i2}(D, r)$ are the two solutions of equation $\mu_2(S_2) = D_i$ . They are defined for $0 < D \leq D_i^m$ . If $D > D_i^m$ , by convention we let $\lambda_2^{ij}(D, r) = +\infty$ .
$F_{1j}(D, r, S_2^{in})$	$F_{1j}(D, r, S_2^{in}) = \lambda_1^1(D, r) + \frac{k_1}{k_2} (\lambda_2^{1j}(D, r) - S_2^{in})$ . It is defined for $0 < D < \min(r_1 m_1, D_1^m)$ .
$F_{2j}(D, r, S_2^{in})$	$F_{2j}(D, r, S_2^{in}) = \lambda_1^2(D, r) + \frac{k_1}{k_2} (\lambda_2^{2j}(D, r) - S_2^{in})$ . It is defined for $0 < D < \min(r_2 m_1, D_2^m)$ .
$\phi_j(D, r, S_1^{in}, S_2^{in})$	$\phi_j(D, r, S_1^{in}, S_2^{in}) = S_2^{in} + k_2 X_1^{2*}(D, r, S_1^{in}) - \lambda_2^{2j}(D, r)$ . It is defined for $0 < D \leq D_2^m$ and $S_1^{in} > \lambda_1^1$ .

Table 2

Auxiliary functions and their domains of definition. Note that  $X_1^{1*}$ ,  $X_2^{1*}$ ,  $X_1^{2*}$  are the components of different steady state in Table 3, and  $k_i, i = 1, 2, 3$  are the pseudo-stoichiometric coefficients.

Auxiliary functions	Domains of definition
$g_1(x) := D_2 \left( \frac{x - X_1^{1*}}{x} \right)$	$x \in (0, +\infty)$
$g_2(x) := D_2 \left( \frac{x - X_2^{1*}}{x} \right)$	$x \in (0, +\infty)$
$f_1(x) := \mu_1(S_1^{in} - k_1 x)$	$x \in [0, S_1^{in}/k_1]$
$f_2(x) := \mu_2(S_2^{in} - k_3 x)$	$x \in [0, S_2^{in}/k_3]$
$f_3(x) := \mu_2(S_2^{in} + k_2 X_1^{2*} - k_3 x)$	$x \in [0, (S_2^{in} + k_2 X_1^{2*})/k_3]$

**Proposition 3.1.** Assume that *Hypotheses 2.1* and *2.2* hold. Then, the nine types of steady states of (3.2) are given in Table 3. Their existence conditions are given in Table 4.

The following proposition determines the existence and multiplicity of steady states of (3.2).

**Proposition 3.2.** Assume that *Hypotheses 2.1* and *2.2*, and the existence conditions in Table 4 hold for the steady states  $\mathcal{E}_{00}^{kl}$ ,  $\mathcal{E}_{10}^{1l}$ ,  $\mathcal{E}_{0i}^{k1}$  and  $\mathcal{E}_{1i}^{11}$ ,  $i = 1, 2$ ,  $k = 0, 1$ , and  $l = 0, 1, 2$ .

- The steady states  $\mathcal{E}_{00}^{kl}$ ,  $\mathcal{E}_{10}^{1l}$  and  $\mathcal{E}_{01}^{01}$  are unique.
- There exists at least one steady state of the form  $\mathcal{E}_{02}^{01}$ . Generically, the system has an odd number of steady states of the form  $\mathcal{E}_{02}^{01}$ .
- If  $X_2^{1*} > x_2^m$ , the steady state  $\mathcal{E}_{0i}^{11}$  and  $\mathcal{E}_{1i}^{11}$  are unique. If  $X_2^{1*} < x_2^m$ , then there exists at least one steady state of the form  $\mathcal{E}_{0i}^{11}$  and  $\mathcal{E}_{1i}^{11}$ . Generically, the system has an odd number of steady states of the form  $\mathcal{E}_{0i}^{11}$  and  $\mathcal{E}_{1i}^{11}$ ,  $i = 1, 2$ .

**3.2. Local stability of steady states.** The following result provides the local stability condition of each steady state of system (3.2). For convenience, we shall use the abbreviation LES for Locally Exponentially Stable.

**Proposition 3.3.** Assume that *Hypotheses 2.1* and *2.2* hold. The steady states  $\mathcal{E}_{00}^{02}$ ,  $\mathcal{E}_{00}^{12}$ ,  $\mathcal{E}_{10}^{12}$ ,  $\mathcal{E}_{02}^{01}$ ,  $\mathcal{E}_{02}^{11}$  and  $\mathcal{E}_{12}^{11}$  of system (3.2) are unstable when they exist. The conditions stability of all other steady states are given in Table 5.

**4. Operating diagrams.** In this section, we study theoretically the operating diagrams that describe the asymptotic behavior of the process according to four control parameters  $D$ ,  $S_1^{in}$ ,  $S_2^{in}$ ,



**Table 3**

Components of all steady states of (3.2). The functions  $\lambda_1^i, \lambda_2^i, \lambda_2^{2i}, F_{ij}$  and  $\phi_i, i, j = 1, 2$  are defined in Table 1 while the functions  $f_1, f_2, f_3, g_1$  and  $g_2$  are defined in Table 2.

	$(X_1^{1*}, X_2^{1*})$	$(X_1^{2*}, X_2^{2*})$
$\mathcal{E}_{00}^{00}$	(0, 0)	(0, 0)
$\mathcal{E}_{00}^{0i}$	(0, 0)	$(0, (S_2^{in} - \lambda_2^{2i}) / k_3)$
$\mathcal{E}_{00}^{10}$	(0, 0)	$(\frac{1}{k_1} (S_1^{in} - \lambda_1^2), 0)$
$\mathcal{E}_{00}^{1i}$	(0, 0)	$((S_1^{in} - \lambda_1^2) / k_1, k_2 (S_1^{in} - F_{2i}) / (k_1 k_3))$
$\mathcal{E}_{10}^{10}$	$((S_1^{in} - \lambda_1^1) / k_1, 0)$	$(X_1^{2*}, 0)$ $X_1^{2*}$ is the unique solution of $f_1(x) = g_1(x)$
$\mathcal{E}_{10}^{1i}$	$((S_1^{in} - \lambda_1^1) / k_1, 0)$	$(X_1^{2*}, \phi_i / k_3)$ $X_1^{2*}$ is the unique solution of $f_1(x) = g_1(x)$
$\mathcal{E}_{0i}^{01}$	$(0, (S_2^{in} - \lambda_2^{1i}) / k_3)$	$(0, X_2^{2*})$ $X_2^{2*}$ is a solution of $f_2(X_2^2) = g_2(X_2^2)$
$\mathcal{E}_{0i}^{11}$	$(0, (S_2^{in} - \lambda_2^{1i}) / k_3)$	$((S_1^{in} - \lambda_1^2) / k_1, X_2^{2*})$ $X_2^{2*}$ is a solution of $f_3(x) = g_2(x)$
$\mathcal{E}_{1i}^{11}$	$(\frac{1}{k_1} (S_1^{in} - \lambda_1^1), \frac{k_2}{k_1 k_3} (S_1^{in} - F_{1i}))$	$X_1^{2*}$ is a unique solution of $f_1(x) = g_1(x)$ $X_2^{2*}$ is a solution of $f_3(x) = g_2(x)$

**Table 4**

Existence condition of steady states of system (3.2) where  $X_1^{2*}$  is a unique solution of equation  $f_1(x) = g_1(x)$ . The functions  $\lambda_1^i, \lambda_2^i, \lambda_2^{2i}, F_{ij}$  and  $\phi_i, i, j = 1, 2$  are defined in Table 1 and the functions  $f_1$  and  $g_1$  are defined in Table 2.

	Existence conditions
$\mathcal{E}_{00}^{00}$	Always exists
$\mathcal{E}_{00}^{0i}$	$S_2^{in} > \lambda_2^{2i}$
$\mathcal{E}_{00}^{10}$	$S_1^{in} > \lambda_1^2$
$\mathcal{E}_{00}^{1i}$	$S_1^{in} > \max(\lambda_1^2, F_{2i})$
$\mathcal{E}_{10}^{10}$	$S_1^{in} > \lambda_1^1$
$\mathcal{E}_{10}^{1i}$	$S_1^{in} > \lambda_1^1$ and $\phi_i > 0$
$\mathcal{E}_{0i}^{01}$	$S_2^{in} > \lambda_2^{1i}$
$\mathcal{E}_{0i}^{11}$	$S_1^{in} > \lambda_1^2$ and $S_2^{in} > \lambda_2^{1i}$
$\mathcal{E}_{1i}^{11}$	$S_1^{in} > \max(\lambda_1^1, F_{1i})$

**Table 5**

The stability condition of steady states of system (3.2) except for  $\mathcal{E}_{00}^{02}, \mathcal{E}_{00}^{12}, \mathcal{E}_{10}^{12}, \mathcal{E}_{02}^{01}, \mathcal{E}_{02}^{11}$  and  $\mathcal{E}_{12}^{11}$  which are unstable. The functions  $f_1, f_3, g_1$  and  $g_2$  are defined in Table 2 while  $X_1^{2*}$  is a unique solution of equation  $f_1(x) = g_1(x)$  and  $X_2^{2*}$  is a solution of  $f_3(x) = g_2(x)$ .

	Stability conditions
$\mathcal{E}_{00}^{00}$	$S_1^{in} < \min(\lambda_1^1, \lambda_1^2)$ and $S_2^{in} \notin [\min(\lambda_2^{11}, \lambda_2^{21}), \max(\lambda_2^{12}, \lambda_2^{22})]$
$\mathcal{E}_{00}^{01}$	$S_1^{in} < \min(\lambda_1^1, \lambda_1^2)$ and $S_2^{in} \notin [\lambda_2^{11}, \lambda_2^{12}]$
$\mathcal{E}_{00}^{10}$	$S_1^{in} < \lambda_1^1, S_2^{in} \notin [\lambda_2^{11}, \lambda_2^{12}]$ and $S_2^{in} + \frac{k_2}{k_1} (S_1^{in} - \lambda_1^2) \notin [\lambda_2^{21}, \lambda_2^{22}]$
$\mathcal{E}_{00}^{11}$	$S_1^{in} < \lambda_1^1$ and $S_2^{in} \notin [\lambda_2^{11}, \lambda_2^{12}]$
$\mathcal{E}_{10}^{10}$	$S_2^{in} + \frac{k_2}{k_1} (S_1^{in} - \lambda_1^1) \notin [\lambda_2^{11}, \lambda_2^{12}]$ and $\phi_1 < 0$ or $\phi_2 > 0$
$\mathcal{E}_{10}^{11}$	$S_2^{in} + \frac{k_2}{k_1} (S_1^{in} - \lambda_1^1) \notin [\lambda_2^{11}, \lambda_2^{12}]$
$\mathcal{E}_{01}^{01}$	$S_1^{in} < \min(\lambda_1^1, \lambda_1^2)$
$\mathcal{E}_{01}^{11}$	$S_1^{in} < \lambda_1^1$ and $g_2'(X_2^{2*}) > f_3'(X_2^{2*})$
$\mathcal{E}_{11}^{11}$	$g_2'(X_2^{2*}) > f_3'(X_2^{2*})$

and  $r$  which are the easiest parameters to manipulate in a chemostat. It is the best tool presented for the biologist to understand the asymptotic behavior of the process according to the operating parameters. However, since it is difficult to visualize all the regions of the operating diagrams in the four-dimensional space, we fix two parameters and release two others to illustrate these diagrams in the plan. In [subsection 4.1](#), it is analyzed in the two-dimensional plane  $(D, S_1^{in})$  by fixing the parameter  $S_2^{in}$  and  $r$ . In [subsection 4.2](#), we fix  $D$  and  $r$ , and we determine the operating diagrams in the plane  $(S_2^{in}, S_1^{in})$ . The other cases can be treated similarly. To determine the different regions in the operating diagram, we must define the surfaces  $\gamma_i$ ,  $i = 1, \dots, 15$ , listed in [Table 6](#). They are obtained from the existence and stability conditions given in [Tables 4](#) and [5](#). They correspond to the boundaries of the change in the number of steady states in the positive octant and/or their local asymptotic behavior.

**Table 6**

Definitions of the surfaces  $\gamma_i$ ,  $i = 1, \dots, 15$ . The functions  $F_{ij}$  and  $\phi_j$ ,  $i, j = 1, 2$  are defined in [Table 1](#) and  $X_1^{2*}$  is the unique solution of equation  $f_1(x) = g_1(x)$ .

Curve $\gamma_i$
$\gamma_0 = \{(D, S_1^{in}, S_2^{in}, r) : S_1^{in} = \lambda_1^1(D, r), D \in [0, r_1 m_1)\}$
$\gamma_1 = \{(D, S_1^{in}, S_2^{in}, r) : S_1^{in} = \lambda_1^2(D, r), D \in [0, r_2 m_1)\}$
$\gamma_2 = \{(D, S_1^{in}, S_2^{in}, r) : S_1^{in} = F_{21}(D, r, S_2^{in}), D \in (0, r_2 \min(m_1, \mu_2(S_2^m)))\}$
$\gamma_3 = \{(D, S_1^{in}, S_2^{in}, r) : S_1^{in} = F_{22}(D, r, S_2^{in}), D \in (0, r_2 \min(m_1, \mu_2(S_2^m)))\}$
$\gamma_4 = \{(D, S_1^{in}, S_2^{in}, r) : S_1^{in} = F_{11}(D, r, S_2^{in}), D \in (0, r_1 \min(m_1, \mu_2(S_2^m)))\}$
$\gamma_5 = \{(D, S_1^{in}, S_2^{in}, r) : S_1^{in} = F_{12}(D, r, S_2^{in}), D \in (0, r_1 \min(m_1, \mu_2(S_2^m)))\}$
$\gamma_6 = \{(D, S_1^{in}, S_2^{in}, r) : D = D_1^* := r_1 \mu_2(S_2^{in})\}$
$\gamma_7 = \{(D, S_1^{in}, S_2^{in}, r) : D = D_2^* := r_2 \mu_2(S_2^{in})\}$
$\gamma_8 = \{(D, S_1^{in}, S_2^{in}, r) : D = D_1^m := r_1 \mu_2(S_2^m)\}$
$\gamma_9 = \{(D, S_1^{in}, S_2^{in}, r) : D = D_2^m := r_2 \mu_2(S_2^m)\}$
$\gamma_{10} = \{(D, S_1^{in}, S_2^{in}, r) : S_2^{in} = S_{21}^{in*} = \lambda_2^{11}(D, r), D < D_1^m\}$
$\gamma_{11} = \{(D, S_1^{in}, S_2^{in}, r) : S_2^{in} = S_{22}^{in*} = \lambda_2^{21}(D, r), D < D_2^m\}$
$\gamma_{12} = \{(D, S_1^{in}, S_2^{in}, r) : S_2^{in} = S_{23}^{in*} = \lambda_2^{12}(D, r), D < D_1^m\}$
$\gamma_{13} = \{(D, S_1^{in}, S_2^{in}, r) : S_2^{in} = S_{24}^{in*} = \lambda_2^{22}(D, r), D < D_2^m\}$
$\gamma_{14} = \{(D, S_1^{in}, S_2^{in}, r) : \phi_1(D, r, S_1^{in}, S_2^{in}) = 0, D \leq D_2^m \text{ and } S_1^{in} > \lambda_1^1\}$
$\gamma_{15} = \{(D, S_1^{in}, S_2^{in}, r) : \phi_2(D, r, S_1^{in}, S_2^{in}) = 0, D \leq D_2^m \text{ and } S_1^{in} > \lambda_1^1\}$

**Remark 4.1.** 1. Note that for all  $D < D_1^m$ , (or equivalently  $D < r_1 \mu_2(S_2^m)$ ), the equation  $\mu_2(S_2^{in}) = D_1 := D/r_1$  is equivalent to  $S_2^{in} = \lambda_2^{11}$  or  $S_2^{in} = \lambda_2^{12}$ . From [Table 6](#), we see that  $\gamma_6 = \gamma_{10} \cup \gamma_{12}$ . Similarly, for all  $D < D_2^m$ , (or equivalently  $D < r_2 \mu_2(S_2^m)$ ), the equation  $\mu_2(S_2^{in}) = D_2 := D/r_2$  is equivalent to  $S_2^{in} = \lambda_2^{21}$  or  $S_2^{in} = \lambda_2^{22}$ . From [Table 6](#), we see that  $\gamma_7 = \gamma_{11} \cup \gamma_{13}$ .

2. Let  $r$  be fixed. When  $S_2^{in}$  is fixed, the curves  $\gamma_6$  and  $\gamma_7$  are vertical lines in the plan  $(D, S_1^{in})$  with equation  $D = D_1^* := r_1 \mu_2(S_2^{in})$  and  $D = D_2^* := r_2 \mu_2(S_2^{in})$ , respectively. When  $D$  is fixed, the curve  $\gamma_i$ ,  $i = 10, \dots, 13$  is a vertical line in the plan  $(S_2^{in}, S_1^{in})$  with equation  $S_2^{in} = S_{2j}^{in*}$ ,  $j = 1, \dots, 4$ .

Using the definitions of the break-even concentrations  $\lambda_1^i$  and  $\lambda_2^{ij}$ ,  $i, j = 1, 2$ , the following proposition determines the relative positions of these critical values according to the distribution of the total volume  $r \in (0, 1)$ .

**Lemma 4.2.** Assume that [Hypotheses 2.1](#) and [2.2](#) hold.

- If  $r \in (0, 1/2)$ , then  $\lambda_1^2(D, r) < \lambda_1^1(D, r)$ ,  $\lambda_2^{21}(D, r) < \lambda_2^{11}(D, r) < \lambda_2^{12}(D, r) < \lambda_2^{22}(D, r)$ .
- If  $r \in (1/2, 1)$ , then  $\lambda_1^1(D, r) < \lambda_1^2(D, r)$ ,  $\lambda_2^{11}(D, r) < \lambda_2^{21}(D, r) < \lambda_2^{22}(D, r) < \lambda_2^{12}(D, r)$ .
- If  $r = 1/2$ , then  $\lambda_1^1(D, r) = \lambda_1^2(D, r)$ ,  $\lambda_2^{11}(D, r) = \lambda_2^{21}(D, r)$ ,  $\lambda_2^{12}(D, r) = \lambda_2^{22}(D, r)$ .

**4.1.** . Operating diagram in the plane  $(D, S_1^{in})$  when  $S_2^{in}$  and  $r$  are fixed. Fix  $r$  and  $S_2^{in}$ . In this case, the surfaces  $\gamma_i$  correspond to curves for  $i = 0, \dots, 5$  and vertical lines for  $i = 6, \dots, 9$ . Depending on the critical values  $m_1$ ,  $\mu_2(S_2^{in})$ , and  $\mu_2(S_2^m)$ , the following three cases should be distinguished in the operating diagram study when  $r \in (0, 1/2)$  or  $r \in (1/2, 1)$ :

$$(4.1) \quad \text{case 1: } m_1 > \mu_2(S_2^m), \quad \text{case 2: } \mu_2(S_2^{in}) > m_1, \quad \text{case 3: } \mu_2(S_2^m) > m_1 > \mu_2(S_2^{in}).$$

Note that for each case in (4.1),  $S_2^{in}$  may be less than or greater than  $S_2^m$ . In this section, we present cases 1 and 2 for  $r \in (0, 1/2)$  and case 1 for  $r \in (1/2, 1)$ . However, the study is similar in the other cases.

From the definitions of the auxiliary functions in Table 1, the following proposition establishes the relative positions of surfaces  $\gamma_i$ ,  $i = 0, \dots, 5$  according to the critical values  $D_1^*$  and  $D_2^*$  defined in Table 6.

**Proposition 4.3.** *Let  $r \in (0, 1)$ . If  $m_1 > \mu_2(S_2^{in})$ , then the curve  $\gamma_0$  and the vertical line  $\gamma_6$  intersect at the point  $P_1(S_2^{in}, r) = (D_1^*, \lambda_1^1(D_1^*, r))$  in the plane  $(D, S_1^{in})$  (see Figure 2 and Figure 10(a)). If, in addition,  $S_2^{in} \geq S_2^m$ , then  $\gamma_0$ ,  $\gamma_5$ , and  $\gamma_6$  intersect at the same point  $P_1$ . Otherwise, when  $S_2^{in} < S_2^m$ , then  $\gamma_0$ ,  $\gamma_4$ , and  $\gamma_6$  intersect at the same point  $P_1$ . Moreover:*

- If  $D < D_1^*$ , then  $F_{11}(D, r, S_2^{in}) < \lambda_1^1(D, r) < F_{12}(D, r, S_2^{in})$ .
- If  $D > D_1^*$  and  $S_2^{in} \geq S_2^m$ , then  $F_{11}(D, r, S_2^{in}) < F_{12}(D, r, S_2^{in}) < \lambda_1^1(D, r)$ .
- If  $D > D_1^*$  and  $S_2^{in} < S_2^m$ , then  $\lambda_1^1(D, r) < F_{11}(D, r, S_2^{in}) < F_{12}(D, r, S_2^{in})$ .

If  $m_1 > \mu_2(S_2^{in})$ , the curve  $\gamma_1$  and the vertical line  $\gamma_7$  intersect at  $P_2(S_2^{in}, r) = (D_2^*, \lambda_1^2(D_2^*, r))$  in the plane  $(D, S_1^{in})$  (see Figure 2 and Figure 10(a)). If, in addition,  $S_2^{in} \geq S_2^m$ , then  $\gamma_1$ ,  $\gamma_3$ , and  $\gamma_7$  intersect at the same point  $P_2$ . Otherwise, when  $S_2^{in} < S_2^m$ , then  $\gamma_1$ ,  $\gamma_2$ , and  $\gamma_7$  intersect at the same point  $P_2$ . Moreover:

- If  $D < D_2^*$ , then  $F_{21}(D, r, S_2^{in}) < \lambda_1^2(D, r) < F_{22}(D, r, S_2^{in})$ .
- If  $D > D_2^*$  and  $S_2^{in} \geq S_2^m$ , then  $F_{21}(D, r, S_2^{in}) < F_{22}(D, r, S_2^{in}) < \lambda_1^2(D, r)$ .
- If  $D > D_2^*$  and  $S_2^{in} < S_2^m$ , then  $\lambda_1^2(D, r) < F_{21}(D, r, S_2^{in}) < F_{22}(D, r, S_2^{in})$ .

The asymptotic behavior of system (2.3) varies according to the value of  $r$  in the intervals  $(0, 1/2)$  and  $(1/2, 1)$  such that the position of the various curves changes with  $r$ . The following proposition establishes the relative positions of the curves  $\gamma_2$  and  $\gamma_4$  when  $S_2^{in} > S_2^m$ .

**Proposition 4.4.** *Let  $S_2^{in} > S_2^m$ .*

- If  $r \in (0, 1/2)$ , then  $F_{21}(D, r, S_2^{in}) < F_{11}(D, r, S_2^{in})$ .
- If  $r \in (1/2, 1)$ , then  $F_{21}(D, r, S_2^{in}) > F_{11}(D, r, S_2^{in})$ .

Now to construct the operating diagrams, the growth rates  $\mu_1$  and  $\mu_2$  are chosen of Monod and Haldane type which are written:

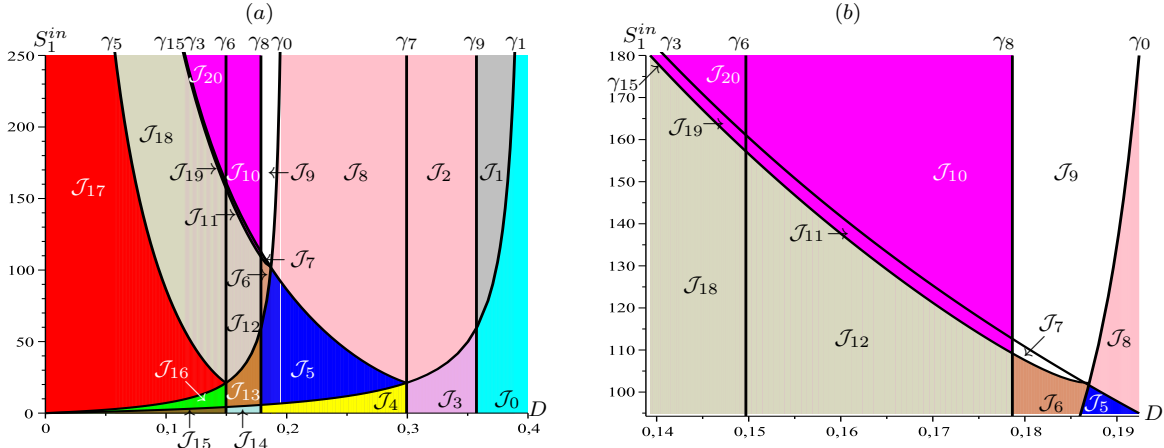
$$(4.2) \quad \mu_1(S_1) = \frac{m_1 S_1}{k_{S_1} + S_1} \quad \text{and} \quad \mu_2(S_2) = \frac{m_2 S_2}{k_{S_2} + S_2 + \frac{(S_2)^2}{k_I}},$$

where  $m_i$  is the maximum growth rate and  $k_{S_i}$  is the half-saturation (or Michaelis-Menten) constant associated to  $S_i$ , for  $i = 1, 2$ . However, the construction can be applied to any growth rate verifying Hypotheses 2.1 and 2.2. The biological parameter values used in all figures are provided in Table SM1. Note that we have used the same specific growth rates of Monod and Haldane types (4.2) and parameter values as in [6], with the exception of the maximum growth rate  $m_1$  to have a richer process behavior.

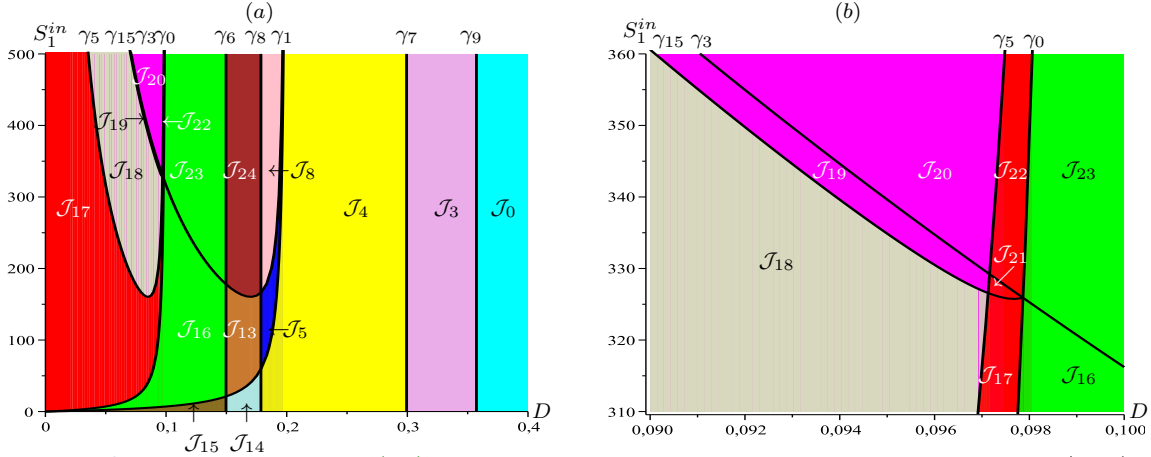
**Proposition 4.5.** *Assume that Hypotheses 2.1 and 2.2 hold. Let  $r$  and  $S_2^{in}$  be fixed. Let  $\mu_1$  and  $\mu_2$  be the specific growth rates defined in (4.2) and the set of the biological parameter values in Table SM1. Let  $\mathcal{J}_i$ ,  $i = 0, \dots, 69$  be the various regions defined in Table 7. The existence and the local stability properties of steady states of the AM2 model with two interconnected chemostats (2.3) in the operating diagram are defined as follows:*

1. If  $r \in (0, 1/2)$ ,  $S_2^{in} > S_2^m$  and Case 1 of (4.1) holds ( $m_1 > \mu_2(S_2^m)$ ), there are twenty-one regions  $\mathcal{J}_i$ ,  $i = 0 - 20$  shown in Figure 2.
2. If  $r \in (0, 1/2)$ ,  $S_2^{in} > S_2^m$  and Case 2 of (4.1) holds ( $m_1 < \mu_2(S_2^{in})$ ), there are seventeen regions  $\mathcal{J}_i$ ,  $i = 0, 3 - 5, 8, 13 - 24$  shown in Figure 3.
3. If  $r \in (0, 1/2)$ ,  $S_2^{in} < S_2^m$  and Case 1 of (4.1) holds, there are twenty-one regions  $\mathcal{J}_i$ ,  $i = 0, 1, 4 - 9, 15 - 20, 25 - 31$  shown in Figure 4.
4. If  $r \in (1/2, 1)$ ,  $S_2^{in} > S_2^m$  and Case 1 of (4.1) holds, there are twenty regions  $\mathcal{J}_i$ ,  $i = 0, 15, 20 - 22, 32 - 46$  shown in Figure 5.

The conditions on the operating parameters defining the various boundaries of the regions are described in Table SM2 [resp. Table SM3] for the first three items [last item].



**Figure 2.** Operating diagram of (2.3) with twenty-one regions  $\mathcal{J}_i$ ,  $i = 0 - 20$  when  $r = 1/3 \in (0, 1/2)$  and  $S_2^{in} = 150 > S_2^m \approx 48.740$ . Case 1 of (4.1):  $m_1 = 0.6 > \mu_2(S_2^m) \approx 0.535$ .



**Figure 3.** Operating diagram of (2.3) with seventeen regions  $\mathcal{J}_i$ ,  $i = 0, 3 - 5, 8, 13 - 24$  when  $r = 1/3 \in (0, 1/2)$  and  $S_2^{in} = 150 > S_2^m \approx 48.740$ . Case 2 of (4.1):  $m_1 = 0.3 < \mu_2(S_2^{in}) \approx 0.449$ .

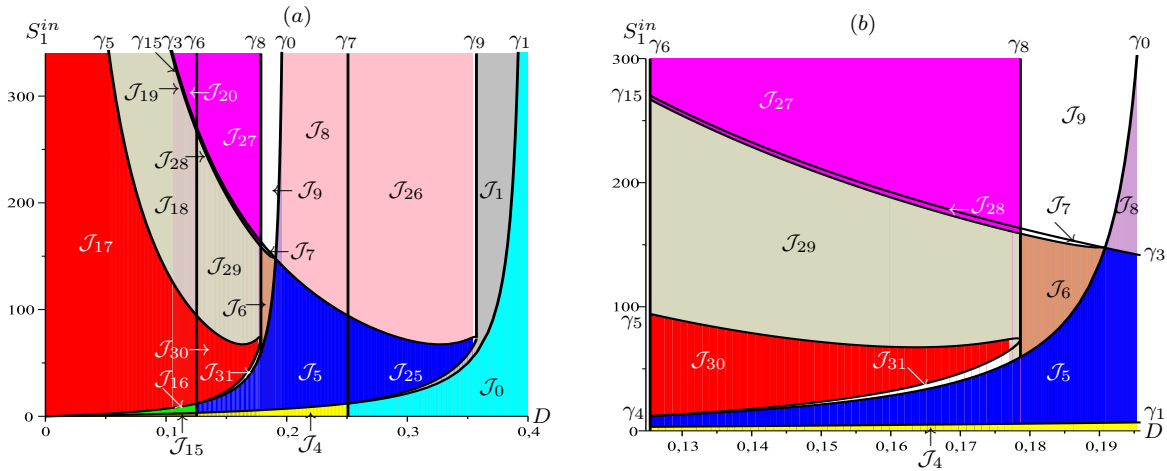
Note that we have chosen the same color for regions with the same number of LES steady states. For example, the regions  $\mathcal{J}_2$  and  $\mathcal{J}_8$  don't have the same number of unstable steady states, but they are colored in pink because both regions have the same behavior with the bistability between  $\mathcal{E}_{00}^{10}$  and  $\mathcal{E}_{00}^{11}$  (see Table 7). In summary, there are three types of behavior of the process: either the stability of only one steady state while all others are unstable if they exist; or the system exhibits bistability or the tristability of only two or three steady states while all others are unstable if they exist.

More precisely, the regions of existence of only one steady state LES (while all others are unstable if they exist) with the color are the following:  $\mathcal{E}_{00}^{00}$  in cyan ( $\mathcal{J}_0$ ),  $\mathcal{E}_{00}^{10}$  in grey ( $\mathcal{J}_1$ ),  $\mathcal{E}_{00}^{01}$  in yellow ( $\mathcal{J}_4$ ),  $\mathcal{E}_{00}^{11}$  in blue ( $\mathcal{J}_5$ ,  $\mathcal{J}_{25}$ ,  $\mathcal{J}_{64}$ ),  $\mathcal{E}_{10}^{11}$  in tan ( $\mathcal{J}_6$ ,  $\mathcal{J}_{63}$ ,  $\mathcal{J}_{65}$ ),  $\mathcal{E}_{01}^{01}$  in sienna ( $\mathcal{J}_{15}$ ,  $\mathcal{J}_{35}$ ,

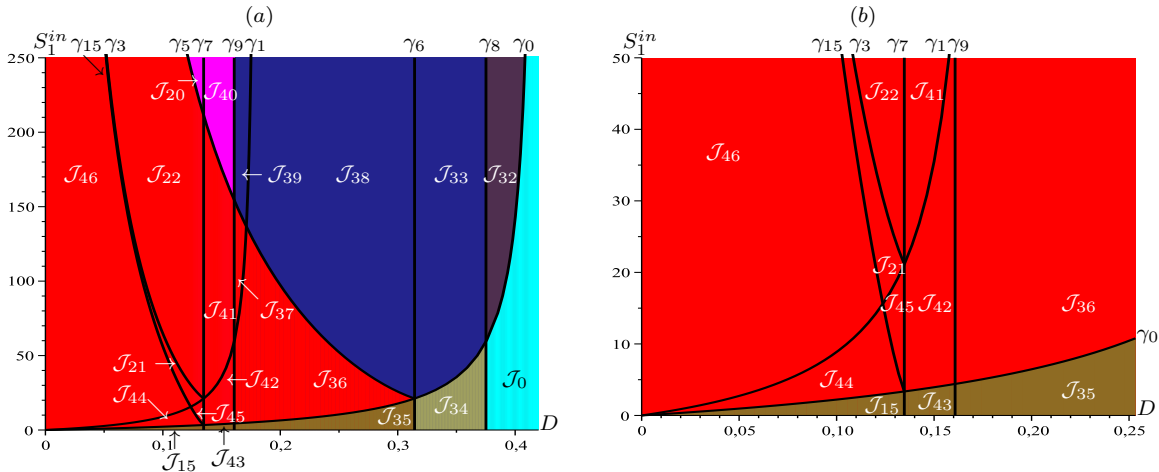
Table 7

Existence and stability of steady states in the regions  $\mathcal{J}_i$ ,  $i = 0, \dots, 69$  of the operating diagrams in Figures 2 to 6.

	$\mathcal{E}_{00}^{00}$	$\mathcal{E}_{00}^{01}$	$\mathcal{E}_{00}^{02}$	$\mathcal{E}_{00}^{10}$	$\mathcal{E}_{00}^{11}$	$\mathcal{E}_{00}^{12}$	$\mathcal{E}_{10}^{10}$	$\mathcal{E}_{10}^{11}$	$\mathcal{E}_{10}^{12}$	$\mathcal{E}_{01}^{01}$	$\mathcal{E}_{02}^{01}$	$\mathcal{E}_{01}^{11}$	$\mathcal{E}_{02}^{11}$	$\mathcal{E}_{11}^{11}$	$\mathcal{E}_{12}^{11}$	Color
$\mathcal{J}_0$	S															Cyan
$\mathcal{J}_1$	U			S												Grey
$\mathcal{J}_2$	U	U	U	S	S	U										Pink
$\mathcal{J}_3$	S	S	U													Plum
$\mathcal{J}_4$	U	S														Yellow
$\mathcal{J}_5$	U	U		U	S											Blue
$\mathcal{J}_6$	U	U		U	U		U	S								Tan
$\mathcal{J}_7$	U	U		U	U		S	S	U							White
$\mathcal{J}_8$	U	U		S	S	U										Pink
$\mathcal{J}_9$	U	U		U	U	U	S	S	U							White
$\mathcal{J}_{10}$	U	U		U	U	U	S	S	U	U	U	U	S	U		Magenta
$\mathcal{J}_{11}$	U	U		U	U		S	S	U	U	U	U	S	U		Magenta
$\mathcal{J}_{12}$	U	U		U	U		U	S		U	U	U	S	U		Wheat
$\mathcal{J}_{13}$	U	U		U	S					U	U	S	U			Gold
$\mathcal{J}_{14}$	U	S								S	U					Turquoise
$\mathcal{J}_{15}$	U	U								S						Sienna
$\mathcal{J}_{16}$	U	U		U	U					U		S				Green
$\mathcal{J}_{17}$	U	U		U	U		U	U		U		U		S		Red
$\mathcal{J}_{18}$	U	U		U	U		U	S		U		U		S	U	Wheat
$\mathcal{J}_{19}$	U	U		U	U		S	S	U	U		U		S	U	Magenta
$\mathcal{J}_{20}$	U	U		U	U	U	S	S	U	U		U		S	U	Magenta
$\mathcal{J}_{21}$	U	U		U	U		U	U	U	U		U		S		Red
$\mathcal{J}_{22}$	U	U		U	U	U	U	U	U	U		U		S		Red
$\mathcal{J}_{23}$	U	U		U	U	U				U		S				Green
$\mathcal{J}_{24}$	U	U		S	S	U				U	U	S	U			Brown
$\mathcal{J}_{25}$	U			U	S											Blue
$\mathcal{J}_{26}$	U			S	S	U										Pink
$\mathcal{J}_{27}$	U	U		U	U	U	S	S	U					S	U	Magenta
$\mathcal{J}_{28}$	U	U		U	U		S	S	U		U		U	S	U	Magenta
$\mathcal{J}_{29}$	U	U		U	U		U	S						S	U	Wheat
$\mathcal{J}_{30}$	U	U		U	U		U	U						S		Red
$\mathcal{J}_{31}$	U	U		U	U		S	S								White
$\mathcal{J}_{32}$	U						S									Violet
$\mathcal{J}_{33}$	U						S							S	U	Navy
$\mathcal{J}_{34}$	S									S	U					Khaki
$\mathcal{J}_{35}$	U									S						Sienna
$\mathcal{J}_{36}$	U						U			U				S		Red
$\mathcal{J}_{37}$	U			U	U	U	U			U		U		S		Red
$\mathcal{J}_{38}$	U						S			U				S	U	Navy
$\mathcal{J}_{39}$	U			U	U	U	S			U		U		S	U	Navy
$\mathcal{J}_{40}$	U	U	U	U	U	U	S	S	U	U		U		S	U	Magenta
$\mathcal{J}_{41}$	U	U	U	U	U	U	U	U	U	U		U		S		Red
$\mathcal{J}_{42}$	U	U	U				U	U	U	U				S		Red
$\mathcal{J}_{43}$	U	U	U							S						Sienna
$\mathcal{J}_{44}$	U	U					U	U		U				S		Red
$\mathcal{J}_{45}$	U	U					U	U	U	U				S		Red
$\mathcal{J}_{46}$	U	U		U	U		U	U		U		U		S		Red
$\mathcal{J}_{47}$	S	S	U							S	U					Black
$\mathcal{J}_{48}$	U	U	U	U	S	U				U	U	S	U			Gold
$\mathcal{J}_{49}$	U	U	U	U	U	U	S	S	U	U	U	U	U	S	U	Magenta
$\mathcal{J}_{50}$	U	U		U	U	U	S	S	U	U	U	U	U	S	U	Magenta
$\mathcal{J}_{51}$	U	U		U	U		S	S	U	U	U	U	U	S	U	Magenta
$\mathcal{J}_{52}$	U	U		U	U		U	S		U	U	U	U	S	U	Wheat
$\mathcal{J}_{53}$	U	U		S	S	U				U	U	S	U			Coral
$\mathcal{J}_{54}$	U	U		U	S					U	U	S	U			Gold
$\mathcal{J}_{55}$	U	S								S	U					Turquoise
$\mathcal{J}_{56}$	U	U		U	U					U		S				Green
$\mathcal{J}_{57}$	U	U		U	U		U	S		U		U		S	U	Wheat
$\mathcal{J}_{58}$	U	U		U	U		S	S	U	U		U		S	U	Magenta
$\mathcal{J}_{59}$	U	U		U	U	U	S	S	U	U		U		S	U	Magenta
$\mathcal{J}_{60}$	U	U		U	U		S	S	U					S	U	Magenta
$\mathcal{J}_{61}$	U	U		U	U		U	S						S	U	Wheat
$\mathcal{J}_{62}$	U	U		U	U		U	U						S		Red
$\mathcal{J}_{63}$	U	U		U	U		U	S								Tan
$\mathcal{J}_{64}$	U			U	S											Blue
$\mathcal{J}_{65}$	U			U	U		U	S								Tan
$\mathcal{J}_{66}$	U			U	U		U	U						S		Red
$\mathcal{J}_{67}$	U			U	U		U	S						S	U	Wheat
$\mathcal{J}_{68}$	U			U	U		S	S	U					S	U	Magenta
$\mathcal{J}_{69}$	U			U	U	U	S	S	U					S	U	Magenta



**Figure 4.** Operating diagram of (2.3) with twenty-one regions  $\mathcal{J}_i$ ,  $i = 0, 1, 4 - 9, 15 - 20, 25 - 31$  when  $r = 1/3 \in (0, 1/2)$ , and  $S_2^{in} = 10 < S_2^m \approx 48.740$ . Case 1 of (4.1):  $m_1 = 0.6 > \mu_2(S_2^m) \approx 0.535$ .



**Figure 5.** Operating diagram of (2.3) with twenty regions  $\mathcal{J}_i$ ,  $i = 0, 15, 20 - 22, 32 - 46$  when  $r = 0.7 \in (1/2, 1)$  and  $S_2^{in} = 150 > S_2^m \approx 48.740$ . Case 1 of (4.1):  $m_1 = 0.6 > \mu_2(S_2^m) \approx 0.535$ .

$\mathcal{J}_{43}$ ,  $\mathcal{E}_{01}^{11}$  in green ( $\mathcal{J}_{16}, \mathcal{J}_{23}, \mathcal{J}_{56}$ ),  $\mathcal{E}_{11}^{11}$  in red ( $\mathcal{J}_i$ ,  $i = 17, 21, 22, 30, 36, 37, 41, 42, 44 - 46, 62, 66$ ),  $\mathcal{E}_{10}^{10}$  in violet ( $\mathcal{J}_{32}$ ).

The bistability regions with the corresponding two steady states LES (while all others are unstable if they exist) and color are the following:  $\mathcal{E}_{00}^{10}$  and  $\mathcal{E}_{00}^{11}$  in pink ( $\mathcal{J}_2, \mathcal{J}_8, \mathcal{J}_{26}$ ),  $\mathcal{E}_{00}^{00}$  and  $\mathcal{E}_{00}^{01}$  in plum ( $\mathcal{J}_3$ ),  $\mathcal{E}_{10}^{10}$  and  $\mathcal{E}_{10}^{11}$  in white ( $\mathcal{J}_7, \mathcal{J}_9, \mathcal{J}_{31}$ ),  $\mathcal{E}_{10}^{11}$  and  $\mathcal{E}_{11}^{11}$  in wheat ( $\mathcal{J}_i$ ,  $i = 12, 18, 29, 52, 57, 61, 67$ ),  $\mathcal{E}_{00}^{11}$  and  $\mathcal{E}_{01}^{11}$  in gold ( $\mathcal{J}_{13}, \mathcal{J}_{48}, \mathcal{J}_{54}$ ),  $\mathcal{E}_{00}^{01}$  and  $\mathcal{E}_{01}^{01}$  in turquoise ( $\mathcal{J}_{14}, \mathcal{J}_{55}$ ),  $\mathcal{E}_{10}^{10}$  and  $\mathcal{E}_{11}^{11}$  in navy ( $\mathcal{J}_{33}, \mathcal{J}_{38}, \mathcal{J}_{39}$ ),  $\mathcal{E}_{00}^{00}$  and  $\mathcal{E}_{01}^{01}$  in khaki ( $\mathcal{J}_{34}$ ).

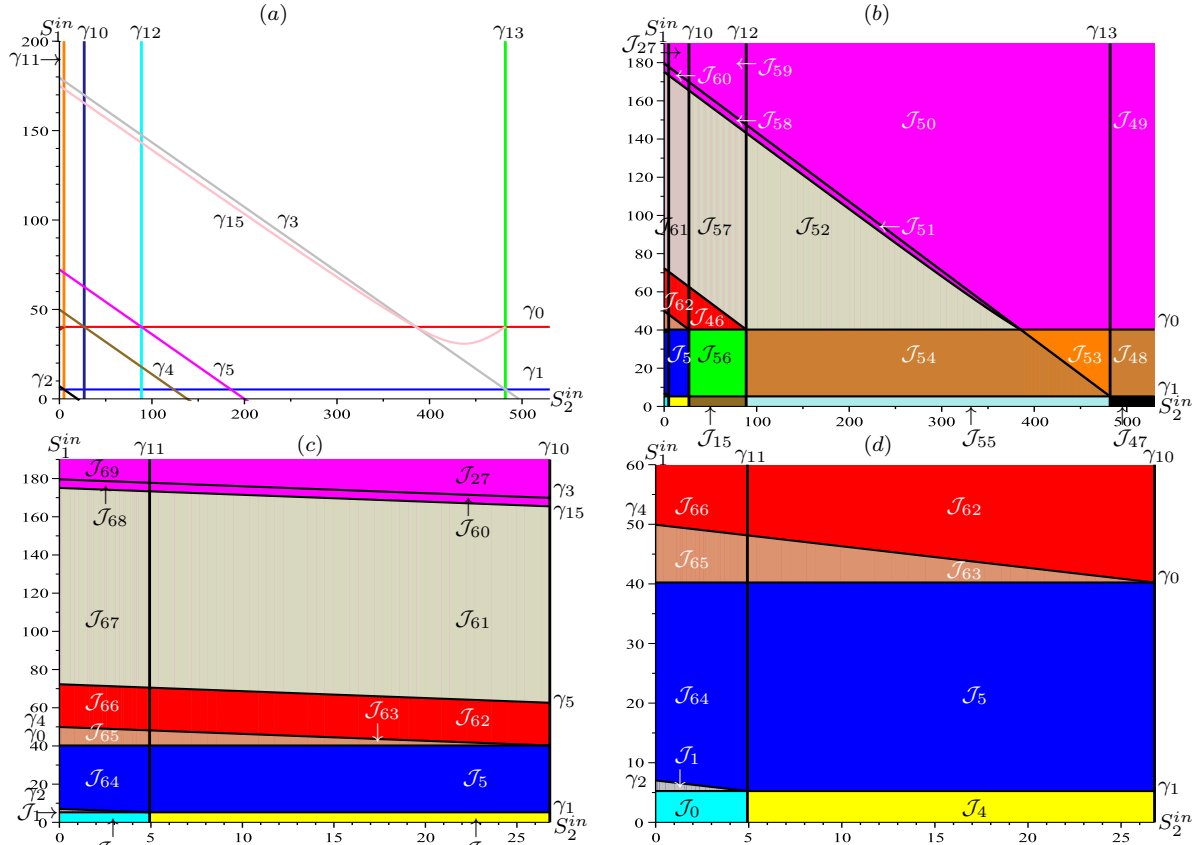
The tristability regions with the corresponding three steady states LES and color are the following:  $\mathcal{E}_{10}^{10}$ ,  $\mathcal{E}_{10}^{11}$  and  $\mathcal{E}_{11}^{11}$  in magenta ( $\mathcal{J}_i$ ,  $i = 10, 11, 19, 20, 27, 28, 40, 49 - 51, 58 - 60, 68, 69$ ),  $\mathcal{E}_{00}^{10}$ ,  $\mathcal{E}_{00}^{11}$  and  $\mathcal{E}_{01}^{11}$  in brown ( $\mathcal{J}_{24}$ ),  $\mathcal{E}_{00}^{00}$ ,  $\mathcal{E}_{00}^{01}$  and  $\mathcal{E}_{01}^{01}$  in black ( $\mathcal{J}_{47}$ ),  $\mathcal{E}_{00}^{10}$ ,  $\mathcal{E}_{00}^{11}$  and  $\mathcal{E}_{01}^{11}$  in coral ( $\mathcal{J}_{53}$ ).

#### 4.2. . Operating diagrams in the plane $(S_2^{in}, S_1^{in})$ when $D$ and $r$ are fixed.

**Proposition 4.6.** For  $D$  fixed in  $(0, r\mu_2(S_2^m))$  and  $r \in (0, 1)$ , the lines  $\gamma_0, \gamma_4$  and  $\gamma_{10}$  intersect at the same point  $(S_{21}^{in*}, \lambda_1^1(D, r))$ . In addition, the lines  $\gamma_0, \gamma_5$  and  $\gamma_{12}$  intersect at the same point  $(S_{23}^{in*}, \lambda_1^1(D, r))$  in the plane  $(S_2^{in}, S_1^{in})$ , where  $S_{21}^{in*} = \lambda_2^{11}(D, r)$  and  $S_{23}^{in*} = \lambda_2^{12}(D, r)$  (see Figure 6). Moreover:

- If  $S_2^{in} < S_{21}^{in*}$ , then  $\lambda_1^1(D, r) < F_{11}(D, r, S_2^{in}) < F_{12}(D, r, S_2^{in})$ .
- If  $S_{21}^{in*} < S_2^{in} < S_{23}^{in*}$ , then  $F_{11}(D, r, S_2^{in}) < \lambda_1^1(D, r) < F_{12}(D, r, S_2^{in})$ .
- If  $S_2^{in} > S_{23}^{in*}$ , then  $F_{11}(D, r, S_2^{in}) < F_{12}(D, r, S_2^{in}) < \lambda_1^1(D, r)$ .

For  $D$  fixed in  $(0, r_2\mu_2(S_2^m))$  and  $r \in (0, 1)$ , the lines  $\gamma_1, \gamma_2, \gamma_{11}$  intersect at the same point  $(S_{22}^{in*}, \lambda_1^2(D, r))$  and the lines  $\gamma_1, \gamma_3, \gamma_{13}$  intersect at the same point  $(S_{24}^{in*}, \lambda_1^2(D, r))$  in the plane



**Figure 6.** Operating diagram of (2.3) in the plane  $(S_2^{in}, S_1^{in})$  with thirty regions  $\mathcal{J}_i$ ,  $i = 0, 1, 4, 5, 15, 27, 46 - 69$  when  $D = 0.17$  and  $r = 0.3 \in (0, 1/2)$ .

$(S_2^{in}, S_1^{in})$ , where  $S_{22}^{in*} = \lambda_2^{21}(D, r)$  and  $S_{24}^{in*} = \lambda_2^{22}(D, r)$  (see Figure 6). Moreover:

- If  $S_2^{in} < S_{22}^{in*}$ , then  $\lambda_1^2(D, r) < F_{21}(D, r, S_2^{in}) < F_{22}(D, r, S_2^{in})$ .
- If  $S_{24}^{in*} < S_2^{in} < S_{22}^{in*}$ , then  $F_{21}(D, r, S_2^{in}) < \lambda_1^2(D, r) < F_{22}(D, r, S_2^{in})$ .
- If  $S_2^{in} > S_{24}^{in*}$ , then  $F_{21}(D, r, S_2^{in}) < F_{22}(D, r, S_2^{in}) < \lambda_1^2(D, r)$ .

**Proposition 4.7.** Assume that *Hypotheses 2.1* and *2.2* hold. Let  $r$  and  $D$  be fixed so that  $0 < r < 1/2$  and  $D \in (0, \mu_2(S_2^m))$ . For the specific growth rates  $\mu_1$  and  $\mu_2$  defined in (4.2) and the set of the biological parameter values in Table SM1, the existence and the local stability properties of steady states of system (2.3) in the thirty regions  $\mathcal{J}_i$ ,  $i = 0, 1, 4, 5, 15, 27, 46 - 69$  of the operating diagram shown in Figure 6 are defined in Table 7, where the conditions on the operating parameters defining the various boundaries of the regions are described in Table SM4.

**5. Discussion and conclusion.** In this paper, we have introduced the original AM2 model (2.3) with a serial configuration of two chemostats, thanks to the mass conservation principle. The mathematical model considered is a differential system in eight dimensions. The analysis of this model is original and has not been studied in the literature. Using the same dilution rate with a large class of monotonic and nonmonotonic growth functions for the first and the second species, we have presented an in-depth mathematical study of system (2.3). Since this model has a cascade structure, we show that it can be reduced to a four-dimensional system (3.2). The existence and the stability of each steady state of the complete model (2.3) can be deduced from the reduced model (3.2). The necessary and sufficient conditions of existence and local stability of all steady states of (3.2) are established according to the four operating parameters  $D$ ,  $r$ ,  $S_1^{in}$  and  $S_2^{in}$ . Generically, there can be nine types of steady states, six of which can be doubled for non-monotonic growth rates, giving a total of fifteen steady states.

To describe the behavior of the process when these four control parameters are varied, we have determined theoretically the operating diagram from the existence and local stability

conditions. Fixing the biological parameter set provided in [Table SM1](#), there can be seventy regions with three different behaviors: either the stability of a single LES steady state, or bistability or tristability, with the existence of two or three stable steady states, while all other steady states are unstable when they exist.

It is interesting to note that this general study includes in fact a number of already known results. In other words, the actual results comprise results that were already established for specific functioning conditions. In particular, it is the case when the functioning conditions are such that both biomasses are washed in the first reactor (i.e. when  $X_1^1 = 0$  and  $X_2^1 = 0$ ): the results obtained are then equivalent to those obtained for the analysis of the AM2 model considered in a single chemostat [5, 41]. It is logically the case when the first reactor is smaller than the first one, in which case, for a given too high dilution rate, the washout of the biomasses arises in the first reactor. The other singular case is that one in which the first biomass is washed out in both reactors: in such a case the analysis is equivalent to the study of a single bioreaction i) having as bioreactional scheme  $S_2 \rightarrow X_2 + \text{biogas}$  and ii) taking place in a system of two chemostats in series [9, 11]. Following the same idea, we also find more recent results established in [26]. These authors had shown that under certain circumstances, well characterized as a function of operating conditions and system parameters by Sari and Benyahia (cf. [41]), a multi-step system (in the sense that the substrate feed to a reaction  $i$  is the reaction output of step  $i - 1$ , which is the case of the AM2 reaction scheme), in a chemostat could exhibit highly original behavior. In particular, it was shown that in these particular circumstances, the system can be stabilized by increasing the feed rate, rather than decreasing it as is traditionally the case. It's interesting to note that this is the case here for two chemostats in series, and it may happen when the value of  $r$  is smaller than  $1/2$ . In fact it appears that the operating diagrams shown in [Figures 3](#) and [4](#), were drawn up with values that allow these results to be found. Consider [Figure 3](#) where  $S_2^{in} > S_2^m$  and Case 1 of (4.1) ( $m_1 > \mu_2(S_2^m)$ ) holds: the red zone rises slightly to the right of the lowest point of the wheat zone. Let's start from a point in the wheat zone, very close to its low point. In this zone (denoted  $\mathcal{J}_{18}$  in [Figure 3](#)), there are two stable steady states. Suppose that the initial conditions are such that the system converges to the steady state  $\mathcal{E}_{10}^{11}$  characterized by  $X_2^1 = 0$ . We can imagine that on an operational level, it is not desirable to have one of the biomasses of the first reactor which is zero. By increasing  $D$  very slightly, we will find ourselves in the part of the red zone ( $\mathcal{J}_{17}$ ) which goes up to the right of the low point of the wheat zone. In other words, we find ourselves in a zone where the only stable steady state is now the positive steady state  $\mathcal{E}_{11}^{11}$ , having increased rather than decreased the feed rate of the system!

Now, when  $r < 1/2$ ,  $S_2^{in} < S_2^m$  and Case 2 of (4.1) ( $m_1 < \mu_2(S_2^{in})$ ) holds, one has exactly the same phenomenon that can be highlighted from the operating diagram plotted in [Figure 4](#).

To enhance and extend the results of the mathematical study of this anaerobic digestion process with a series configuration of interconnected chemostats, a forthcoming paper will analyse performance criteria by comparing with the single chemostat.

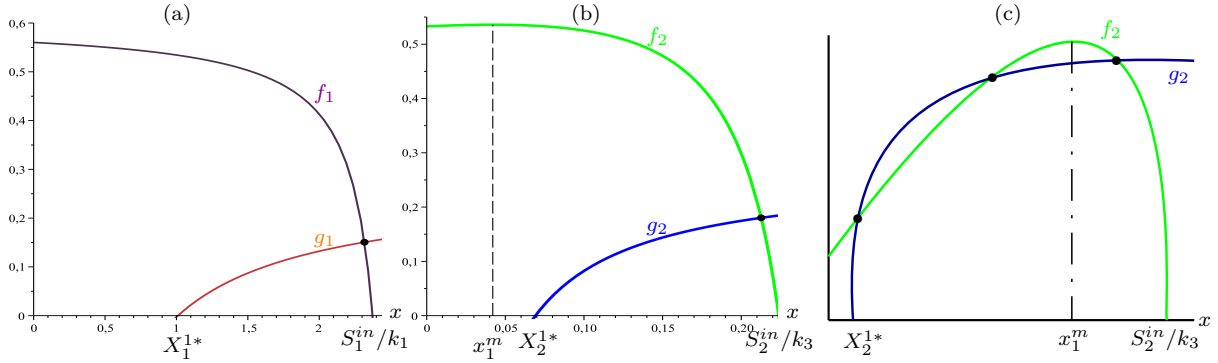
**Appendix A. Proofs.** First, we need the following Lemmas to establish the existence and multiplicity of all steady states of (3.2).

**Lemma A.1.** *Let  $f_1$  and  $g_1$  be the functions defined in [Table 2](#). Let  $X_1^{1*} < S_1^{in}/k_1$ . Assume that [Hypothesis 2.1](#) holds. The equation  $f_1(x) = g_1(x)$  has a unique solution in  $(X_1^{1*}, S_1^{in}/k_1)$  (see [Figure 7\(a\)](#)).*

**Proof of Lemma A.1.** Under [Hypothesis 2.1](#), the function  $x \mapsto f_1(x)$  is nonnegative, decreasing and continuous on  $[0, S_1^{in}/k_1]$ . The function  $x \mapsto g_1(x)$  is nonnegative, increasing hyperbola and continuous on  $[X_1^{1*}, +\infty)$ . Indeed,

$$g_1'(x) = D_2 \frac{X_1^{1*}}{x^2} > 0.$$





**Figure 7.** Number of positive solutions of equations : (a)  $f_1(x) = g_1(x)$  (unique solution); (b)  $f_2(x) = g_2(x)$  (unique solution when  $X_2^{1*} \geq x_1^m$ ); (c)  $f_2(x) = g_2(x)$  (an odd number of intersections when  $X_2^{1*} < x_1^m$ ).

Let  $X_1^{1*} < S_1^{in}/k_1$  and  $F_1$  be the function defined by  $F_1(x) := f_1(x) - g_1(x)$ . Therefore,  $F_1$  is decreasing continuous on  $[X_1^{1*}, S_1^{in}/k_1]$ . In addition, we have

$$F_1(X_1^{1*}) = \mu_1(S_1^{in} - k_1 X_1^{1*}) > 0 \quad \text{and} \quad F_1\left(\frac{S_1^{in}}{k_1}\right) = -g_1\left(\frac{S_1^{in}}{k_1}\right) < 0.$$

Using the Intermediate Value Theorem, it follows that the equation  $F_1(x) = 0$  has a unique solution in  $(X_1^{1*}, S_1^{in}/k_1)$  (see [Figure 7\(a\)](#)). ■

**Lemma A.2.** Let  $f_2$  and  $g_2$  be the functions defined in [Table 2](#). Let  $X_2^{1*}$  be a positive constant so that  $X_2^{1*} < S_2^{in}/k_3$  and  $x_1^m := (S_2^{in} - S_2^m)/k_3$  be the maximum value of  $f_2$  on  $[0, S_2^{in}/k_3]$ . Assume that [Hypothesis 2.2](#) holds.

1. When  $X_2^{1*} \geq x_1^m$ , then the equation  $f_2(x) = g_2(x)$  has a unique solution in  $(X_2^{1*}, S_2^{in}/k_3)$ , (see [Figure 7\(b\)](#)).
2. When  $X_2^{1*} < x_1^m$  then the equation  $f_2(x) = g_2(x)$  has at least one solution in  $(X_2^{1*}, S_2^{in}/k_3)$ . Generically, there exist an odd number of solutions in  $(X_2^{1*}, S_2^{in}/k_3)$ , (see [Figure 7\(c\)](#)).

**Proof of Lemma A.2.** Under [Hypothesis 2.2](#),  $f_2$  is nonnegative and continuous on the interval  $[0, S_2^{in}/k_3]$ . In addition,  $f_2$  is increasing on  $[0, x_1^m]$  and is decreasing on  $[x_1^m, S_2^{in}/k_3]$ . The function  $g_2$  is nonnegative and increasing hyperbola continuous on  $[X_2^{1*}, +\infty)$ . Indeed,

$$(A.1) \quad g_2'(x) = D_2 \frac{X_2^{1*}}{x^2} > 0.$$

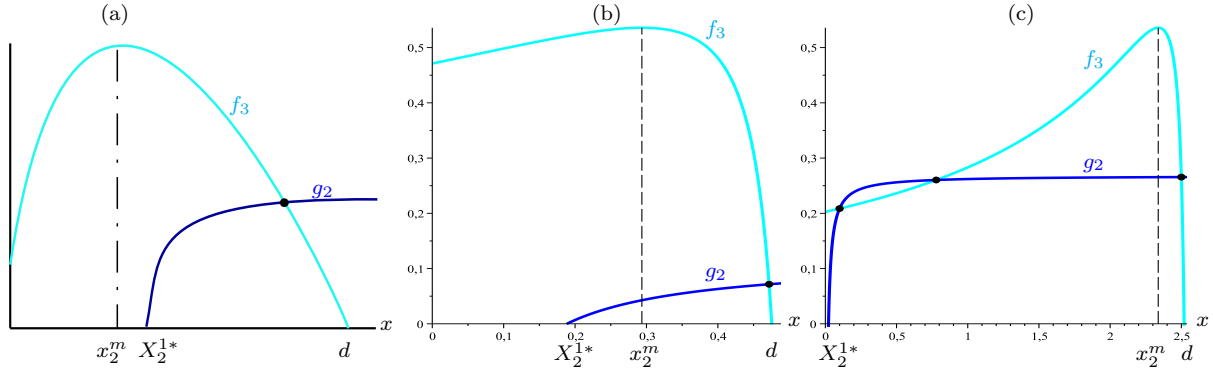
Let  $X_2^{1*} < S_2^{in}/k_3$  and let  $F_2$  be the function defined by  $F_2(x) := f_2(x) - g_2(x)$ . Therefore,  $F_2$  is continuous on  $[X_2^{1*}, S_2^{in}/k_3]$ . In addition, we have

$$F_2(X_2^{1*}) = \mu_2(S_2^{in} - k_3 X_2^{1*}) > 0 \quad \text{and} \quad F_2\left(\frac{S_2^{in}}{k_3}\right) = -g_2\left(\frac{S_2^{in}}{k_3}\right) < 0.$$

If  $X_2^{1*} > x_1^m$  then  $F_2$  is decreasing on  $[X_2^{1*}, S_2^{in}/k_3]$ , because  $f_2$  is decreasing and  $g_2$  is increasing on the same interval. Consequently, the equation  $F_2(x) = 0$  has a unique solution on  $(X_2^{1*}, S_2^{in}/k_3)$ , (see [Figure 7\(b\)](#)). If  $X_2^{1*} < x_1^m$ , then  $F_2$  can be nonmonotonic. We complete the proof by using the Intermediate Value Theorem. ■

**Lemma A.3.** Let  $f_3$  and  $g_2$  be the functions defined in [Table 2](#). Let  $d := (S_2^{in} + k_2 X_1^{2*})/k_3$  be the root of  $f_3(x) = 0$  and  $x_2^m := (S_2^{in} + k_2 X_1^{2*} - S_2^m)/k_3$  be the maximum value of  $f_3$ . Assume that  $X_2^{1*} < d$  and [Hypothesis 2.2](#) holds.

1. When  $X_2^{1*} \geq x_2^m$  then the equation  $f_3(x) = g_2(x)$  has a unique solution in  $(X_2^{1*}, d)$  (see [Figure 8\(a\)](#)).
2. When  $X_2^{1*} < x_2^m$  then the equation  $f_3(x) = g_2(x)$  has at least one solution in  $(X_2^{1*}, d)$ . Generically, there exist an odd number of solutions in  $(X_2^{1*}, d)$ , (see [Figure 8\(b-c\)](#)).



**Figure 8.** Number of positive solutions of equation  $f_3(x) = g_2(x)$  on  $(0, d)$  where  $d$  is defined in Lemma A.3: (a) unique solution when  $X_2^{1*} \geq x_2^m$ ; (b-c) an odd number of intersections when  $X_2^{1*} < x_2^m$ .

**Proof of Lemma A.3.** Under Hypothesis 2.2,  $f_3$  is nonnegative and continuous on  $[0, d]$  with  $d := (S_2^{in} + k_2 X_1^{2*})/k_3$ . In addition,  $f_3$  is increasing on  $[0, x_2^m]$  and is decreasing on  $[x_2^m, d]$ . Let  $X_2^{1*} < d$  and  $F_3$  be the function defined by  $F_3(x) := f_3(x) - g_2(x)$ . Therefore,  $F_3$  is continuous on  $[X_2^{1*}, d]$ . In addition, we have

$$F_3(X_2^{1*}) = \mu_2 (S_2^{in} + k_2 X_1^{2*} - k_3 X_2^{1*}) > 0, \quad F_3\left(\frac{S_2^{in} + k_2 X_1^{2*}}{k_3}\right) = -g_2\left(\frac{S_2^{in} + k_2 X_1^{2*}}{k_3}\right) < 0.$$

Recall from (A.1) that  $g_2$  is increasing on  $[X_2^{1*}, +\infty)$ . If  $X_2^{1*} \geq x_2^m$  then  $F_3$  is decreasing on  $[X_2^{1*}, d]$ , because  $f_3$  is decreasing on the same interval. Consequently, the equation  $F_3(x) = 0$  has a unique solution in  $(X_2^{1*}, d)$ . If  $X_2^{1*} < x_2^m$  then  $F_3$  can be nonmonotonic. Using the Intermediate Value Theorem, the proof is completed. ■

**Proof of Proposition 3.1.** Similarly to system (2.2), subsystem (3.2) has a cascade structure. Thus, for each steady state of the upper two-dimensional system of (3.2) denoted by

$$E_{ij}(X_1^1, X_2^1), \quad \{i, j\} \in \{0, 1, 2\}$$

corresponds a steady state of the lower two-dimensional system of (3.2) denoted by

$$E_{ij}^{kl}(X_1^2, X_2^2).$$

This steady state is given by putting the left-hand side of the lower two-dimensional subsystem of (3.2) equal to zero when the two components  $X_1^1$  and  $X_2^1$  are at the steady state  $E_{ij}$ . Then, we can deduce the corresponding steady state of the four-dimensional system (3.2) denoted by  $\mathcal{E}_{ij}^{kl}(X_1^1, X_2^1, X_1^2, X_2^2)$  as well as their existence condition by checking those of  $E_{ij}$  and  $E_{ij}^{kl}$  simultaneously. Indeed, the upper two-dimensional system of (3.2) corresponds to the classic AM2 model where the steady states are given by the solutions of the following equations:

$$(A.2) \quad [\mu_1 (S_1^{in} - k_1 X_1^1) - D_1] X_1^1 = 0,$$

$$(A.3) \quad [\mu_2 (S_2^{in} + k_2 X_1^1 - k_3 X_2^1) - D_1] X_2^1 = 0.$$

- For  $E_{00}$ ,  $X_1^1 = X_2^1 = 0$ . It always exists.
- For  $E_{10}$ ,  $X_1^1 > 0$  and  $X_2^1 = 0$ . Thus, (A.2) results in  $\mu_1 (S_1^{in} - k_1 X_1^1) = D_1/r$ . Using Hypothesis 2.1, we obtain  $X_1^1$  and the existence condition in Table 8.
- For  $E_{0i}$ ,  $i = 1, 2$ ,  $X_1^1 = 0$  and  $X_2^1 > 0$ . Hence, (A.3) results in  $\mu_2 (S_2^{in} - k_3 X_2^1) = D_1/r$ . Using Hypothesis 2.2, we obtain  $X_2^1$  and the existence condition in Table 8.
- For  $E_{1i}$ ,  $i = 1, 2$ ,  $X_1^1 > 0$  and  $X_2^1 > 0$ . From (A.2) and (A.3), it follows that

$$\mu_1 (S_1^{in} - k_1 X_1^1) = D_1 \quad \text{and} \quad \mu_2 (S_2^{in} + k_2 X_1^1 - k_3 X_2^1) = D_1.$$

Table 8

Two components  $(X_1^1, X_2^1)$  of all steady states and their existence condition for the upper two-dimensional system of (3.2). The functions  $\lambda_1^1, \lambda_2^1$ , and  $F_{1i}$ ,  $i = 1, 2$  are defined in Table 1.

	$X_1^1$	$X_2^1$	Existence conditions
$E_{00}$	0	0	Always exists
$E_{10}$	$\frac{1}{k_1} (S_1^{in} - \lambda_1^1)$	0	$S_1^{in} > \lambda_1^1$
$E_{0i}$	0	$\frac{1}{k_3} (S_2^{in} - \lambda_2^{1i})$	$S_2^{in} > \lambda_2^{1i}$
$E_{1i}$	$\frac{1}{k_1} (S_1^{in} - \lambda_1^1)$	$\frac{k_2}{k_1 k_3} (S_1^{in} - F_{1i})$	$S_1^{in} > \lambda_1^1$ and $S_1^{in} > F_{1i}$

Using the expressions of  $\lambda_1^1, \lambda_2^1$ , and  $F_{1i}$ ,  $i = 1, 2$  in Table 1, we obtain the two components of  $E_{1i}$  and the two existence conditions in Table 8.

The steady states of the lower two-dimensional system of (3.2) are given by the solutions of the following equations:

$$(A.4) \quad \mu_1 (S_1^{in} - k_1 X_1^2) X_1^2 + D_2 (X_1^1 - X_1^2) = 0,$$

$$(A.5) \quad \mu_2 (S_2^{in} + k_2 X_1^2 - k_3 X_2^2) X_2^2 + D_2 (X_2^1 - X_2^2) = 0.$$

Table 9

Two components  $(X_1^2, X_2^2)$  and existence condition of all steady states of the lower two-dimensional system of (3.2). The functions  $\lambda_1^2, \lambda_2^2$ , and  $F_{2i}$ ,  $i = 1, 2$  are defined in Table 1. The functions  $f_1, f_2, f_3, g_1$  and  $g_2$  are defined in Table 2 while  $X_1^{2*}$  is the unique solution of equation  $f_1(x) = g_1(x)$ .

	$X_1^2$	$X_2^2$	Existence conditions
$E_{00}^{00}$	0	0	Always exists
$E_{00}^{10}$	$\frac{1}{k_1} (S_1^{in} - \lambda_1^2)$	0	$S_1^{in} > \lambda_1^2$
$E_{00}^{0i}$	0	$\frac{1}{k_3} (S_2^{in} - \lambda_2^{2i})$	$S_2^{in} > \lambda_2^{2i}$
$E_{00}^{1i}$	$\frac{1}{k_1} (S_1^{in} - \lambda_1^2)$	$\frac{k_2}{k_1 k_3} (S_1^{in} - F_{2i})$	$S_1^{in} > \lambda_1^2$ and $S_1^{in} > F_{2i}$
$E_{10}^{10}$	$X_1^{2*}$	0	Always exists
$E_{10}^{1i}$	$X_1^{2*}$	$\frac{1}{k_3} (S_2^{in} + k_2 X_1^{2*} - \lambda_2^{2i})$	$S_2^{in} + k_2 X_1^{2*} > \lambda_2^{2i}$
$E_{0i}^{01}$	0	a solution of $f_2(X_2^2) = g_2(X_2^2)$	Always exists
$E_{0i}^{11}$	$\frac{1}{k_1} (S_1^{in} - \lambda_1^2)$	a solution of $f_3(x) = g_2(x)$	$S_1^{in} > \lambda_1^2$
$E_{1i}^{11}$	$X_1^{2*}$	a solution of $f_3(x) = g_2(x)$	Always exists

- For  $E_{00}$ , we have  $X_1^1 = X_2^1 = 0$ . Hence, (A.4) and (A.5) result in

$$(A.6) \quad [\mu_1 (S_1^{in} - k_1 X_1^2) - D_2] X_1^2 = 0,$$

$$(A.7) \quad [\mu_2 (S_2^{in} + k_2 X_1^2 - k_3 X_2^2) - D_2] X_2^2 = 0.$$

- For  $E_{00}^{00}$ ,  $X_1^2 = X_2^2 = 0$ . It always exists.
- For  $E_{00}^{10}$ ,  $X_1^2 > 0$  and  $X_2^2 = 0$ . Thus, (A.6) results in  $\mu_1 (S_1^{in} - k_1 X_1^2) = D_2/(1-r)$ . Using Hypothesis 2.1, we obtain  $X_1^2$  and the existence condition in Table 9.
- For  $E_{00}^{0i}$ ,  $i = 1, 2$ ,  $X_1^2 = 0$  and  $X_2^2 > 0$ . Hence, (A.7) results in  $\mu_2 (S_2^{in} - k_3 X_2^2) = D_2/(1-r)$ . Using Hypothesis 2.2, we obtain  $X_2^2$  and the existence condition in Table 9.
- For  $E_{00}^{1i}$ ,  $i = 1, 2$ ,  $X_1^2 > 0$  and  $X_2^2 > 0$ . Thus, (A.6) and (A.7) result in

$$\mu_1 (S_1^{in} - k_1 X_1^2) = D_2 \quad \text{and} \quad \mu_2 (S_2^{in} + k_2 X_1^2 - k_3 X_2^2) = \frac{D_2}{1-r}.$$

Using Hypotheses 2.1 and 2.2 and the expressions of  $\lambda_1^2, \lambda_2^2$ , and  $F_{2i}$  in Table 1, we obtain two components  $(X_1^2, X_2^2)$  and the two existence conditions in Table 9.

- For  $E_{10}$ , we have  $X_1^1 > 0$  and  $X_2^1 = 0$ . Hence, (A.4) and (A.5) result in

$$(A.8) \quad \mu_1 (S_1^{in} - k_1 X_1^2) X_1^2 + D_2 (X_1^1 - X_1^2) = 0,$$

$$(A.9) \quad [\mu_2 (S_2^{in} + k_2 X_1^2 - k_3 X_2^2) - D_2] X_2^2 = 0.$$

- For  $E_{10}^{00}$ ,  $X_1^2 = X_2^2 = 0$ . Then (A.8) implies that  $X_1^1 = 0$ , which is in contradiction with the existence condition of  $E_{10}$  where  $X_1^1 > 0$ . Hence,  $E_{10}^{00}$  does not exist.
- For  $E_{10}^{10}$ ,  $X_1^2 > 0$  and  $X_2^2 = 0$ . Thus, (A.8) results in

$$(A.10) \quad \mu_1 (S_1^{in} - k_1 X_1^2) = D_2 \left( \frac{X_1^2 - X_1^1}{X_1^2} \right),$$

which is equivalent to  $f_1(x) = g_1(x)$  (see Table 2). From the component  $X_1^1$  of  $E_{10}$  in Table 8, it follows that  $X_1^1 < S_1^{in}/k_1$ . From Lemma A.1, it follows that the equation  $f_1(x) = g_1(x)$  has a unique solution  $X_1^{2*} \in (X_1^1, S_1^{in}/k_1)$  (see Figure 7(a)). Thus, we obtain the components of steady state  $E_{10}^{10}$  in Table 9, which always exists.

- For  $E_{10}^{0i}$ ,  $i = 1, 2$ ,  $X_1^2 = 0$  and  $X_2^2 > 0$ . Then (A.8) implies that  $X_1^1 = 0$ , which is in contradiction with the existence condition of  $E_{10}$  where  $X_1^1 > 0$ . Hence,  $E_{10}^{0i}$  does not exist.
- For  $E_{10}^{1i}$ ,  $i = 1, 2$ ,  $X_1^2 > 0$  and  $X_2^2 > 0$ . Thus, (A.8) is equivalent to (A.10). Similarly to the steady state  $E_{10}^{10}$ , equation (A.10) has a unique solution  $X_1^{2*} \in (X_1^{1*}, S_1^{in}/k_1)$ . In addition, (A.9) implies that

$$\mu_2 (S_2^{in} + k_2 X_1^{2*} - k_3 X_2^2) = D_2.$$

From Hypothesis 2.2 and the expression of  $\lambda_2^{2i}$  in Table 1, we obtain the component  $X_2^2$  of the steady state  $E_{10}^{1i}$  in Table 9. Moreover, the existence condition in Table 9 follows.

- For  $E_{0i}$ , we have  $X_1^1 = 0$  and  $X_2^{1i} > 0$ ,  $i = 1, 2$ . Hence, (A.4) and (A.5) result in

$$(A.11) \quad [\mu_1 (S_1^{in} - k_1 X_1^2) - D_2] X_1^2 = 0,$$

$$(A.12) \quad \mu_2 (S_2^{in} + k_2 X_1^2 - k_3 X_2^2) X_2^2 + D_2 (X_2^{1i} - X_2^2) = 0.$$

- For  $E_{0i}^{00}$  [resp.  $E_{0i}^{10}$ ],  $X_1^2 = X_2^2 = 0$  [resp.  $X_1^2 > 0$  and  $X_2^2 = 0$ ]. Then (A.12) implies that  $X_2^{1i} = 0$ , which is in contradiction with  $X_2^{1i} > 0$ . Hence,  $E_{0i}^{00}$  and  $E_{0i}^{10}$  does not exist.
- For  $E_{0i}^{01}$ , we have  $X_1^2 = 0$  and  $X_2^2 > 0$ . Then, (A.12) results in  $X_2^2$  must be a solution of equation

$$\mu_2 (S_2^{in} - k_3 X_2^2) = D_2 \left( \frac{X_2^2 - X_2^{1i}}{X_2^2} \right),$$

which is equivalent to  $f_2(X_2^2) = g_2(X_2^2)$  (see Table 2). From the component  $X_2^{1i}$  of  $E_{0i}$  in Table 8, we have  $X_2^{1i} < S_2^{in}/k_3$ . From Lemma A.2, it follows that the equation  $f_2(X_2^2) = g_2(X_2^2)$  has at least one solution  $X_2^{2*} \in (X_2^{1i}, S_2^{in}/k_3)$ . Hence, we obtain the components of steady state  $E_{0i}^{01}$  in Table 9, where there exists at least one.

- For  $E_{0i}^{11}$ , we have  $X_1^2 > 0$  and  $X_2^2 > 0$ . Then, the component  $X_2^2$  in Table 9 follows from equation (A.11) and Hypothesis 2.1. In addition, (A.12) implies that  $X_2^2$  must be a solution of equation

$$(A.13) \quad \mu_2 (S_2^{in} + k_2 X_1^2 - k_3 X_2^2) = D_2 \left( \frac{X_2^2 - X_2^{1i}}{X_2^2} \right),$$

which is equivalent to  $f_3(X_2^2) = g_2(X_2^2)$  (see Table 2). Using the expression of  $d$  in Lemma A.3 and the component of  $X_2^{1i}$  in Table 8, we see that  $X_2^{1i} < d$ . From Lemma A.3, it follows that the equation  $f_3(X_2^2) = g_2(X_2^2)$  has at least one solution  $X_2^{2*} \in (X_2^{1i}, d)$  (see Figure 8). Consequently, we obtain the two components and the existence condition of the steady state  $E_{0i}^{11}$  in Table 9.

- For  $E_{1i}$ , we have  $X_1^1 > 0$  and  $X_2^{1i} > 0$ ,  $i = 1, 2$ .
  - For  $E_{1i}^{00}$  [resp.  $E_{1i}^{10}$ , and  $E_{1i}^{01}$ ],  $X_1^2 = X_2^2 = 0$  [resp.  $(X_1^2 > 0$  and  $X_2^2 = 0)$  and  $(X_1^2 = 0$  and  $X_2^2 > 0)$ ]. Then, (A.4) or (A.5) implies that  $X_1^1 = 0$  or  $X_2^{1i} = 0$ , which is a contradiction. Hence, these three types of steady states do not exist.
  - For  $E_{1i}^{11}$ , we have  $X_1^2 > 0$  and  $X_2^2 > 0$ . Then, (A.4) and (A.5) are equivalent to (A.10) and (A.13), or, equivalently,  $f_1(x) = g_1(x)$  and  $f_3(x) = g_2(x)$ , respectively. Similarly to the steady state  $E_{10}^{10}$ , we obtain the component  $X_1^2$  which is the unique solution of equation  $f_1(x) = g_1(x)$ . Using the expression of  $X_1^{1*}$  and  $X_2^{1i*}$  in Table 8 and the expressions of  $F_{1i}$  in Table 1, it follows that

$$X_2^{1i*} = \frac{1}{k_3} (k_2 X_1^{1*} - \lambda_2^{1i} + S_2^{in}).$$

From the expressions of  $d$  in Lemma A.3, we have

$$X_2^{1i*} - d = \frac{1}{k_3} (k_2 (X_1^{1*} - X_1^{2*}) - \lambda_2^{1i}),$$

which is negative since  $X_1^{1*} < X_1^{2*}$  (see Lemma A.1). From Lemma A.3, it follows that the equation  $f_3(x) = g_2(x)$  has at least one solution in  $(X_2^{1i*}, d)$ . Therefore, we obtain the two components of the steady state  $E_{1i}^{11}$  in Table 9, which always exists. ■

**Proof of Proposition 3.2.** For  $k = 0, 1$  and  $l = 0, 1, 2$ , the uniqueness of the six first steady states  $\mathcal{E}_{00}^{kl}$  and  $\mathcal{E}_{10}^{ll}$ , follows from Table 3. For  $\mathcal{E}_{01}^{01}$ , we have  $\lambda_2^{11} < S_2^m$ , that is,  $X_2^{1*} > (S_2^{in} - S_2^m)/k_3 := x_1^m$  where  $X_2^{1*}$  is defined in Table 3 and  $x_1^m$  in Lemma A.2, implying that the equation  $f_2(X_2^2) = g_2(X_2^2)$  has a unique solution in  $(X_2^{1*}, S_2^{in}/k_3)$  (see Figure 7(b)). That is,  $\mathcal{E}_{01}^{01}$  is unique when it exists. Inversely, for  $\mathcal{E}_{02}^{01}$ , we have  $\lambda_2^{12} > S_2^m$ , that is,  $X_2^{1*} < x_1^m$ . From Lemma A.2, the equation  $f_2(X_2^2) = g_2(X_2^2)$  has at least one solution on  $(X_2^{1*}, S_2^{in}/k_3)$  (see Figure 7(b-c)). For  $\mathcal{E}_{0i}^{11}$ ,  $i = 1, 2$ , the  $X_2^2$ -component is a solution of  $f_3(x) = g_2(x)$ . Using the expressions of  $X_2^{1*}$  and  $X_1^{2*}$  in Table 3 and the expressions of  $x_2^m$  in Lemma A.3, a straightforward calculation shows that

$$X_2^{1*} - x_2^m = \frac{1}{k_3} \left( S_2^m - \lambda_2^{11} - \frac{k_2}{k_1} (S_1^{in} - \lambda_1^2) \right).$$

As  $S_2^m > \lambda_2^{11}$  and  $S_1^{in} > \lambda_1^2$ , the two case  $X_2^{1*} \geq x_2^m$  and  $X_2^{1*} < x_2^m$  can be hold. From Lemma A.3, the equation  $f_3(x) = g_2(x)$  has at least one solution in  $(X_2^{1*}, d)$  (see Figure 8). The multiplicity of the other steady states  $\mathcal{E}_{11}^{11}$  and  $\mathcal{E}_{12}^{12}$  follows similar arguments. ■

**Proof of Proposition 3.3.** Let  $J$  be the Jacobian matrix of (3.2) at  $(X_1^1, X_2^1, X_1^2, X_2^2)$ , which is given by the following lower block triangular matrix of the form

$$J = \begin{bmatrix} J_1 & 0 \\ J_2 & J_3 \end{bmatrix}; \quad J_1 = \begin{bmatrix} a_{11} & 0 \\ a_{21} & a_{22} \end{bmatrix}, \quad J_2 = \begin{bmatrix} a_{31} & 0 \\ 0 & a_{42} \end{bmatrix}, \quad J_3 = \begin{bmatrix} a_{33} & 0 \\ a_{43} & a_{44} \end{bmatrix}$$

where

$$\begin{aligned} a_{11} &= \mu_1 (S_1^{in} - k_1 X_1^1) - D_1 - k_1 \mu_1' (S_1^{in} - k_1 X_1^1) X_1^1, & a_{21} &= k_2 \mu_2' (S_2^{in} + k_2 X_1^1 - k_3 X_2^1) X_2^1, \\ a_{22} &= \mu_2 (S_2^{in} + k_2 X_1^1 - k_3 X_2^1) - D_1 - k_3 \mu_2' (S_2^{in} + k_2 X_1^1 - k_3 X_2^1) X_2^1, & a_{31} &= D_2, \\ a_{33} &= \mu_1 (S_1^{in} - k_1 X_1^2) - D_2 - k_1 \mu_1' (S_1^{in} - k_1 X_1^2) X_1^2, & a_{43} &= k_2 \mu_2' (S_2^{in} + k_2 X_1^2 - k_3 X_2^2) X_2^2, \\ a_{44} &= \mu_2 (S_2^{in} + k_2 X_1^2 - k_3 X_2^2) - D_2 - k_3 \mu_2' (S_2^{in} + k_2 X_1^2 - k_3 X_2^2) X_2^2, & a_{42} &= D_2. \end{aligned}$$

Note that,  $J_1$  is the Jacobian matrix of the upper two-dimensional subsystem of (3.2) evaluated at  $(X_1^1, X_2^1)$ , and  $J_3$  is the Jacobian matrix of the lower two-dimensional subsystem of (3.2) evaluated at  $(X_1^2, X_2^2)$ . Thus, the stability of the steady state is determined by the sign

of the real parts of the eigenvalues of  $J_1$  and  $J_3$ . A steady state is LES if the real parts of the eigenvalues of  $J_1$  and  $J_3$  at this steady state are negative. Since they are lower triangular matrices, a steady state is LES if the corresponding eigenvalues  $a_{ii}$ ,  $i = 1, \dots, 4$ .

To simplify the presentation of the proof, we first determine the stability conditions of the steady states of the upper two-dimensional subsystem of (3.2) which corresponds to the AM2 model. Then, we will establish the stability conditions of the steady states of the lower two-dimensional subsystem of (3.2). Finally, the stability of the complete system (3.2) follows by combining all conditions.

**Table 10**

The stability conditions of all steady states of the upper two-dimensional subsystem of (3.2), where the existence condition are defined in Table 8 and the functions  $\lambda_1^1$  and  $\lambda_2^{1i}$  are defined in Table 1.

	Stability conditions
$E_{00}$	$S_1^{in} < \lambda_1^1$ and $S_2^{in} \notin [\lambda_2^{11}, \lambda_2^{12}]$
$E_{10}$	$S_2^{in} + \frac{k_2}{k_1} (S_1^{in} - \lambda_1^1) \notin [\lambda_2^{11}, \lambda_2^{12}]$
$E_{01}$	$S_1^{in} < \lambda_1^1$
$E_{02}$	Always unstable
$E_{11}$	LES when it exists
$E_{12}$	Always unstable

- For  $E_{00} = (0, 0)$ , the two eigenvalues of  $J_1$  are

$$a_{11} = \mu_1 (S_1^{in}) - D_1 \quad \text{and} \quad a_{22} = \mu_2 (S_2^{in}) - D_1.$$

From Hypotheses 2.1 and 2.2 and the expressions of  $\lambda_1^1$ ,  $\lambda_2^{11}$  and  $\lambda_2^{12}$  in Table 1,  $E_{00}$  is LES if and only if  $a_{11}$  and  $a_{22}$  are negative, that is, the stability conditions of  $E_{00}$  in Table 10 hold.

- For  $E_{10} = (X_1^{1*}, 0)$ , where  $X_1^{1*} = (S_1^{in} - \lambda_1^1)/k_1$ , the two eigenvalues of  $J_1$  are

$$a_{11} = -k_1 \mu_1' (\lambda_1^1) X_1^{1*} \quad \text{and} \quad a_{22} = \mu_2 \left( S_2^{in} + \frac{k_2}{k_1} (S_1^{in} - \lambda_1^1) \right) - D_1.$$

From Hypothesis 2.1,  $a_{11}$  is negative. From Hypothesis 2.2,  $a_{22}$  is negative if and only if the stability condition of  $E_{10}$  in Table 10 holds.

- For  $E_{0i} = (0, X_2^{1i*})$ ,  $i = 1, 2$ , where  $X_2^{1i*} = (S_2^{in} - \lambda_2^{1i})/k_3$ , the two eigenvalues of  $J_1$  are

$$a_{11} = \mu_1 (S_1^{in}) - D_1 \quad \text{and} \quad a_{22} = -k_3 \mu_2' (\lambda_2^{1i}) X_2^{1i*}.$$

Using Hypothesis 2.2,  $E_{02}$  is unstable when it exists since  $a_{22}$  is positive. However,  $E_{01}$  is LES if and only if  $S_1^{in} < \lambda_1^1$  since  $a_{22}$  is negative.

- For  $E_{1i} = (X_1^{1*}, X_2^{1i*})$ ,  $i = 1, 2$ , where  $X_1^{1*} = (S_1^{in} - \lambda_1^1)/k_1$  and  $X_2^{1i*} = (S_2^{in} - \lambda_2^{1i})/k_3$ , the two eigenvalues of  $J_1$  are

$$a_{11} = -k_1 \mu_1' (\lambda_1^1) X_1^{1*} \quad \text{and} \quad a_{22} = -k_3 \mu_2' (\lambda_2^{1i}) X_2^{1i*}.$$

From Hypothesis 2.2,  $E_{12}$  is unstable when it exists since  $a_{22}$  is positive. However,  $E_{11}$  is LES when it exists since  $a_{11}$  and  $a_{22}$  are negative.

Let us now consider the lower two-dimensional system of (3.2). From Table 10, it follows that the steady states  $E_{02}^{01}$ ,  $E_{02}^{11}$  and  $E_{12}^{11}$  are unstable.

- For  $E_{00}^{00} = (0, 0)$ , the two eigenvalues of  $J_3$  are

$$a_{33} = \mu_1 (S_1^{in}) - D_2 \quad \text{and} \quad a_{44} = \mu_2 (S_2^{in}) - D_2,$$

which are negative if and only if the two stability conditions in Table 11 hold.

Table 11

The stability conditions of all steady states of the lower two-dimensional system of (3.2). The functions  $f_1$ ,  $f_3$ ,  $g_1$  and  $g_2$  are defined in Table 2 while  $X_1^{2*}$  is a unique solution of equation  $f_1(x) = g_1(x)$  and  $X_2^{2*}$  is a solution of equation  $f_3(x) = g_2(x)$ .

Stability conditions	
$E_{00}^{00}$	$S_1^{in} < \lambda_1^2$ and $S_2^{in} \notin [\lambda_2^{21}, \lambda_2^{22}]$
$E_{00}^{01}$	$S_1^{in} < \lambda_1^2$
$E_{00}^{02}$	Always unstable
$E_{00}^{10}$	$S_2^{in} + \frac{k_2}{k_1} (S_1^{in} - \lambda_1^2) \notin [\lambda_2^{21}, \lambda_2^{22}]$
$E_{00}^{11}$	LES when it exists
$E_{00}^{12}$	Always unstable
$E_{10}^{10}$	$S_2^{in} + k_2 X_1^{2*} \notin [\lambda_2^{21}, \lambda_2^{22}]$
$E_{10}^{11}$	LES when it exists
$E_{10}^{12}$	Always unstable
$E_{01}^{01}$	$S_1^{in} < \lambda_1^2$
$E_{01}^{11}$	$g_2'(X_2^{2*}) > f_3'(X_2^{2*})$
$E_{11}^{11}$	$g_2'(X_2^{2*}) > f_3'(X_2^{2*})$

- For  $E_{00}^{0i} = (0, X_2^{2i*})$ ,  $i = 1, 2$ , where  $X_2^{2i*} = (S_2^{in} - \lambda_2^{2i})/k_3$ , the eigenvalues of  $J_3$  are written as follows:

$$a_{33} = \mu_1 (S_1^{in}) - D_2 \quad \text{and} \quad a_{44} = -k_3 \mu_2' (\lambda_2^{2i}) X_2^{2i*}.$$

From Hypothesis 2.2,  $E_{00}^{02}$  is unstable when it exists since  $a_{44}$  is positive. However, from Hypothesis 2.1,  $E_{00}^{01}$  is LES if and only if  $S_1^{in} < \lambda_1^2$ , since  $a_{44}$  is negative.

- For  $E_{00}^{10} = (X_1^{2*}, 0)$ , where  $X_1^{2*} = (S_1^{in} - \lambda_1^2)/k_1$ , the eigenvalues of  $J_3$  are

$$a_{33} = -k_1 \mu_1' (\lambda_1^2) X_1^{2*} \quad \text{and} \quad a_{44} = \mu_2 \left( S_2^{in} + \frac{k_2}{k_1} (S_1^{in} - \lambda_1^2) \right) - D_2.$$

From Hypothesis 2.1,  $a_{33}$  is negative. From Hypothesis 2.2, it follows that  $E_{00}^{10}$  is LES if and only if the stability condition in Table 11 holds.

- For  $E_{00}^{1i} = (X_1^{2*}, X_2^{2i*})$ , where  $X_1^{2*} = \frac{S_1^{in} - \lambda_1^2}{k_1}$  and  $X_2^{2i*} = \frac{1}{k_3} \left( S_2^{in} + \frac{k_2}{k_1} (S_1^{in} - \lambda_1^2) - \lambda_2^{2i} \right)$ , the eigenvalues of  $J_3$  are

$$a_{33} = -k_1 \mu_1' (\lambda_1^2) X_1^{2*} \quad \text{and} \quad a_{44} = -k_3 \mu_2' (\lambda_2^{2i}) X_2^{2i*}, \quad i = 1, 2.$$

From Hypothesis 2.1,  $a_{33}$  is negative. From Hypothesis 2.2 and the expressions of  $X_2^{2j*}$ , the steady state  $E_{00}^{12}$  is unstable when it exists since  $a_{44}$  is positive. However,  $E_{00}^{11}$  is LES since  $a_{44}$  is negative.

- For  $E_{10}^{10} = (X_1^{2*}, 0)$ , where  $X_1^{2*}$  is the unique solution of equation  $f_1(X_1^2) = g_1(X_1^2)$ , the eigenvalues of  $J_3$  are

$$a_{33} = -D_2 \frac{X_1^{1*}}{X_1^{2*}} - k_1 \mu_1' (S_1^{in} - k_1 X_1^{2*}) X_1^{2*} \quad \text{and} \quad a_{44} = \mu_2 (S_2^{in} + k_2 X_1^{2*}) - D_2.$$

Using the definitions of  $f_1$  and  $g_1$  in Table 2, a straightforward calculation shows that

$$a_{33} = -[g_1'(X_1^{2*}) - f_1'(X_1^{2*})] X_1^{2*}.$$

Recall, from the proof of Lemma A.1, that the function  $x \mapsto g_1(x) - f_1(x)$  is increasing. Hence,  $a_{33}$  is negative. Using Hypothesis 2.2,  $E_{10}^{10}$  is LES if and only if  $a_{44}$  is negative, that is, the stability condition in Table 11 holds.

- For  $E_{10}^{1i} = (X_1^{2*}, X_2^{2i*})$ ,  $i = 1, 2$ , where  $X_1^{2*}$  is the unique solution of equation  $f_1(x) = g_1(x)$  and  $X_2^{2i*} = \frac{1}{k_3} (S_2^{in} + k_2 X_1^{2*} - \lambda_2^{2i})$ , the eigenvalues of  $J_3$  are

$$a_{33} = - [g_1'(X_1^{2*}) - f_1'(X_1^{2*})] X_1^{2*} \quad \text{and} \quad a_{44} = -k_3 \mu_2' (\lambda_2^{2i}) X_2^{2i*}.$$

Similarly to the steady state  $E_{10}^{10}$ , the term  $a_{33}$  is negative. Thus,  $E_{10}^{12}$  is unstable when it exists since  $a_{44}$  is positive, while  $E_{10}^{11}$  is LES when it exists since  $a_{44}$  is negative.

- For  $E_{01}^{01} = (0, X_2^{2*})$ , where  $X_2^{2*}$  is the unique solution of equation  $f_2(X_2^2) = g_2(X_2^2)$  (see [Proposition 3.2](#)), the eigenvalues of  $J_3$  are given by

$$a_{33} = \mu_1(S_1^{in}) - D_2 \quad \text{and} \quad a_{44} = -D_2 \frac{X_2^{1*}}{X_2^{2*}} - k_3 \mu_2'(S_2^{in} - k_3 X_2^{2*}) X_2^{2*},$$

where  $X_2^{1*} = (S_2^{in} - \lambda_2^{11})/k_3$ . From [Hypothesis 2.1](#),  $a_{33}$  is negative if and only if  $S_1^{in} < \lambda_1^2$ . Using the definitions of  $f_2$  and  $g_2$  in [Table 2](#), we can easily calculate

$$a_{44} = - [g_2'(X_2^{2*}) - f_2'(X_2^{2*})] X_2^{2*}.$$

Recall that  $X_2^{1*} > x_1^m := (S_2^{in} - S_2^m)/k_3$  so that the function  $x \mapsto g_2(x) - f_2(x)$  is increasing (see the proof of [Lemma A.2](#)). Hence,  $a_{44}$  is negative. Therefore, the stability condition of  $E_{01}^{01}$  in [Table 11](#) hold.

- For  $E_{01}^{11} = (X_1^{2*}, X_2^{2*})$ , where  $X_1^{2*} = \frac{1}{k_1} (S_1^{in} - \lambda_1^2)$  and  $X_2^{2*}$  is a solution of  $f_3(x) = g_2(x)$ , the eigenvalues of  $J_3$  are given by

$$a_{33} = -k_1 \mu_1'(\lambda_1^2) X_1^{2*} \quad \text{and} \quad a_{44} = - [g_2'(X_2^{2*}) - f_3'(X_2^{2*})] X_2^{2*},$$

From [Hypothesis 2.1](#),  $a_{33}$  is negative. Consequently,  $E_{10}^{11}$  is LES if and only if  $a_{44}$  is negative, that is, the stability condition in [Table 11](#) holds.

- For  $E_{11}^{11} = (X_1^{2*}, X_2^{2*})$ , where  $X_1^{2*}$  is a solution of equation  $f_1(x) = g_1(x)$  and  $X_2^{2*}$  is a solution of equation  $f_3(x) = g_2(x)$ , the eigenvalues of  $J_3$  are given by

$$a_{33} = - [g_1'(X_1^{2*}) - f_1'(X_1^{2*})] X_1^{2*} \quad \text{and} \quad a_{44} = - [g_2'(X_2^{2*}) - f_3'(X_2^{2*})] X_2^{2*},$$

Similarly to  $E_{10}^{10}$ ,  $a_{33}$  is negative. As for  $E_{01}^{11}$ ,  $E_{11}^{11}$  is LES if and only if  $a_{44}$  is negative, that is, the stability condition in [Table 11](#) holds. ■

**Proof of Proposition 4.3.** The function  $D \mapsto \lambda_1^1(D)$  is defined, positive and increasing on the interval  $[0, rm_1)$ . It tends to infinity as  $D$  tends to  $rm_1$ . From [Table 6](#), it follows that  $\gamma_0$  and  $\gamma_6$  intersect if  $rm_1 > D_1^*$ , that is,  $m_1 > \mu_2(S_2^{in})$ . In this case, we have  $D = D_1^* = r\mu_2(S_2^{in})$  and  $S_1^{in} = \lambda_1^1(D_1^*, r)$ , that is,  $\gamma_0 \cap \gamma_6 = P_1$ . Since  $D_1^* < \min(D_1^m, rm_1)$ , then the functions  $F_{11}$  and  $F_{12}$  are defined in  $D_1^*$ . At this values, we have  $\mu_1(S_1^{in}) = \mu_2(S_2^{in}) = D_1$ . If  $S_2^{in} < S_2^m$ , we obtain  $S_2^{in} = \lambda_2^{11}$ ; otherwise  $S_2^{in} = \lambda_2^{12}$ . From the definition of the functions  $F_{11}$  and  $F_{12}$  in [Table 1](#), it follows that  $S_1^{in} = \lambda_1^1$  in these two cases. Consequently,  $\gamma_0 \cap \gamma_6 \cap \gamma_4 = P_1$  if  $S_2^{in} < S_2^m$  and  $\gamma_0 \cap \gamma_6 \cap \gamma_5 = P_1$ , otherwise. Similar arguments apply to the second part of the proposition. ■

**Proof of Proposition 4.4.** Under [Lemma 4.2](#), we have for all  $r \in (0, 1/2)$

$$\lambda_1^2(D, r) < \lambda_1^1(D, r) \quad \text{and} \quad \lambda_2^{21}(D, r) < \lambda_2^{11}(D, r).$$

From the definition of  $F_{11}$  and  $F_{21}$  in [Table 1](#),  $F_{21}(D, r, S_2^{in}) < F_{11}(D, r, S_2^{in})$ . Similarly,  $F_{21}(D, r, S_2^{in}) > F_{11}(D, r, S_2^{in})$ , for all  $r \in (1/2, 1)$ . ■

Let  $\phi_i$ ,  $i = 1, 2$  be the function defined in [Table 1](#). To describe the existence condition  $\phi_i > 0$  of the steady state  $\mathcal{E}_{10}^{1i}$  in [Table 4](#) and the stability condition  $\phi_1 < 0$  or  $\phi_2 > 0$  of the steady state  $\mathcal{E}_{10}^{10}$  in [Proposition 3.3](#), we need the following lemma showing the positivity of  $\phi_i$  according to the values of  $S_2^{in}$  and  $D$ .

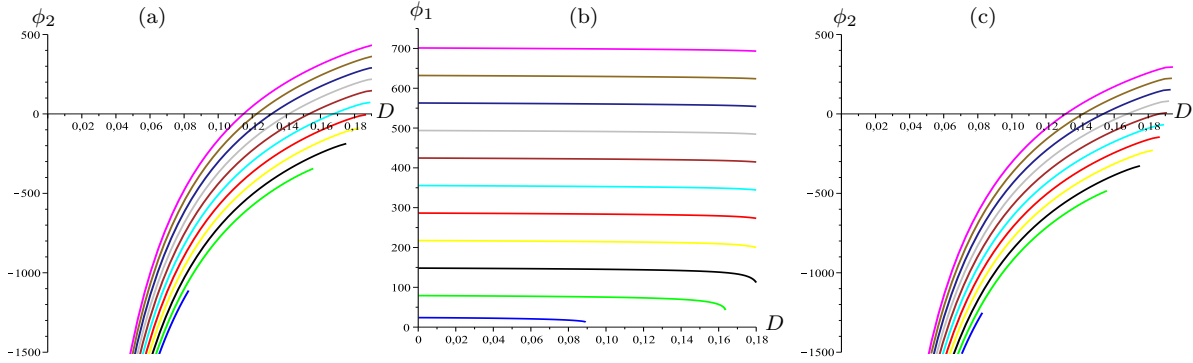


**Lemma A.4.** Let  $D \in [0, \min(rm_1, (1-r)\mu_2(S_2^m))]$  and  $\phi_j(D)$  be the function defined in Table 1. For all  $S_2^{in} > S_2^m$ , we have  $\phi_1(D) > 0$ . In addition, when  $D > D_2^* = (1-r)\mu_2(S_2^{in})$ , then  $\phi_2(D) > 0$ .

**Proof of Lemma A.4.** For all  $S_2^{in} > S_2^m$ , we have  $S_2^{in} > \lambda_2^{21}(D, r)$ , so that  $\phi_1(D) > 0$  holds. In addition, from the definition of  $D_2^*$  in Table 6, we have  $\lambda_2^{22}(D, r) < \lambda_2^{22}(D_2^*, r) = S_2^{in}$  for all  $D > D_2^*$ , so that  $\phi_2(D) > 0$  holds. ■

In the next remark, we show that the existence condition of  $\mathcal{E}_{10}^{1i}$  and the stability condition of  $\mathcal{E}_{10}^{10}$  are not always satisfied where the function  $\phi_i(D)$  can change their sign as we will see in Figure 9.

**Remark A.5.** For the set of parameters in Table SM1, the condition  $\phi_2(D) > 0$  is not always verified when  $D < D_2^*$  (see Figure 9(a)). For the set of parameters in Figure 4 where  $S_2^{in} < S_2^m$ , condition  $\phi_1(D) > 0$  holds (see Figure 9(b)) and the condition  $\phi_2(D) > 0$  is not always verified (see Figure 9(c)).



**Figure 9.** Curves of the functions  $\phi_1(D)$  and  $\phi_2(D)$ , for several fixed values of  $S_1^{in} = 5, 25, 50, 75, 100, 125, 150, 175, 200, 225, 250$  and  $S_2^m \approx 48.74$ : (a)  $\phi_2(D)$  when  $S_2^{in} = 150 > S_2^m$  (b)  $\phi_1(D)$  when  $S_2^{in} = 10 < S_2^m$ ; (c)  $\phi_2(D)$  when  $S_2^{in} = 10 < S_2^m$ .

**Proof of Proposition 4.5.** We determine the operating diagram of Figure 2 in the case  $r \in (0, 1/2)$ ,  $S_2^{in} > S_2^m$ , and  $m_1 > \mu_2(S_2^m)$ . However, the cases in items (2) and (3) can be treated similarly and are left to the reader using Figures 10 and 11, respectively. In addition, the only difference between items (4) and (1) is the stability of the steady states  $\mathcal{E}_{00}^{00}$ ,  $\mathcal{E}_{00}^{01}$ , and  $\mathcal{E}_{01}^{01}$ , where the minimum of the break-even concentrations  $\lambda_1^i$  and  $\lambda_2^{ij}$  is given by

$$\min(\lambda_1^1, \lambda_1^2) = \lambda_1^1, \quad \min(\lambda_2^{11}, \lambda_2^{21}) = \lambda_2^{11} \quad \text{and} \quad \max(\lambda_2^{12}, \lambda_2^{22}) = \lambda_2^{12},$$

using Lemma 4.2 for  $r > 1/2$ . Inversely, in item (1) for  $r < 1/2$ , we have

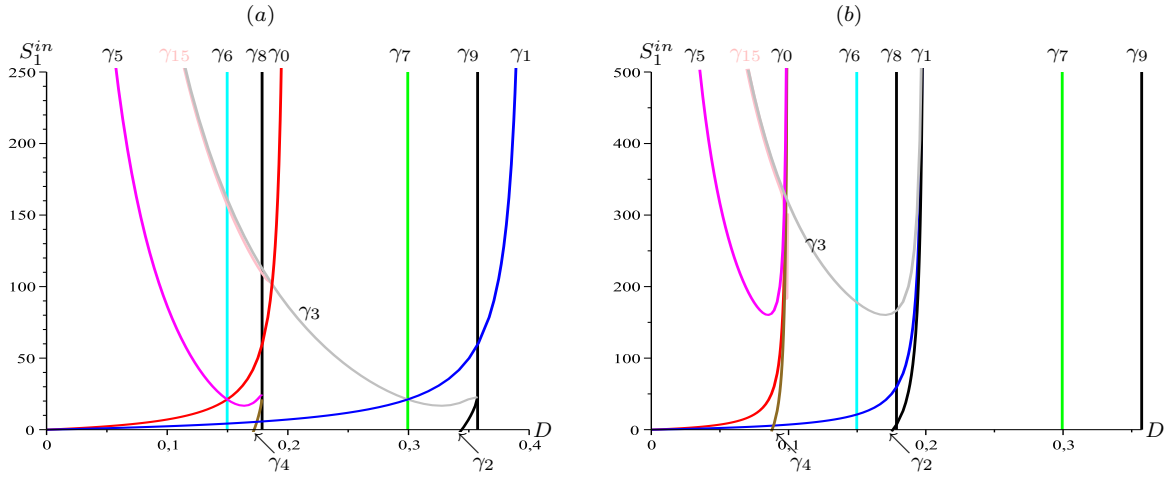
$$(A.14) \quad \min(\lambda_1^1, \lambda_1^2) = \lambda_1^2, \quad \min(\lambda_2^{11}, \lambda_2^{21}) = \lambda_2^{21} \quad \text{and} \quad \max(\lambda_2^{12}, \lambda_2^{22}) = \lambda_2^{22}.$$

From Proposition 3.3, the six steady states  $\mathcal{E}_{00}^{02}$ ,  $\mathcal{E}_{00}^{12}$ ,  $\mathcal{E}_{10}^{12}$ ,  $\mathcal{E}_{02}^{01}$ ,  $\mathcal{E}_{02}^{11}$  and  $\mathcal{E}_{12}^{11}$  are unstable, when they exist. Next, we establish the existence and stability of each steady state in the various regions of the operating diagram in the case of item (1).

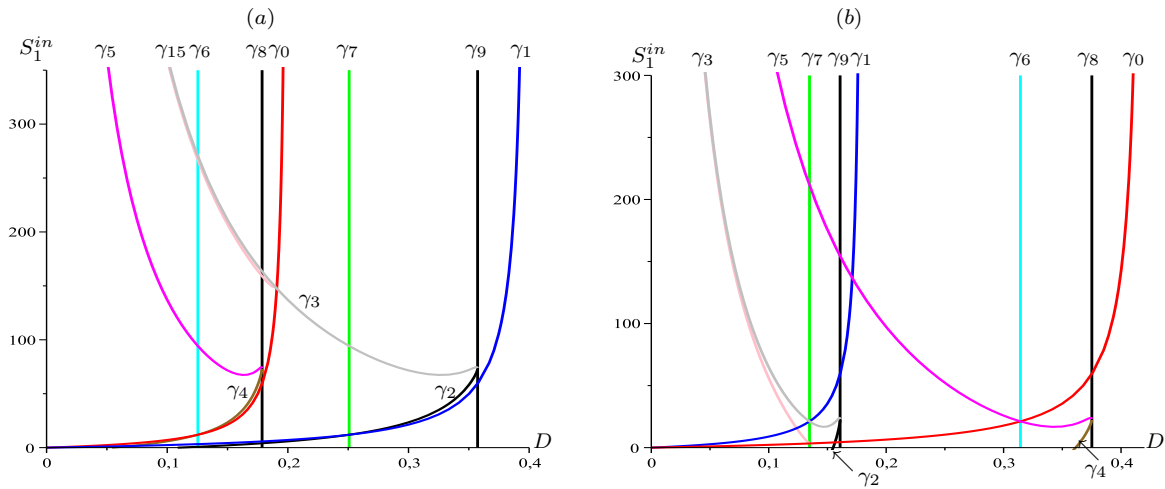
- $\mathcal{E}_{00}^{00}$  always exists. From Table 5 and (A.14), it is LES if and only if,

$$S_1^{in} < \lambda_1^2 \quad \text{and} \quad (S_2^{in} < \lambda_2^{21} \quad \text{or} \quad S_2^{in} > \lambda_2^{22}).$$

From Table 6, the first stability condition of  $\mathcal{E}_{00}^{00}$  holds, for all  $(D, S_1^{in})$  in the region bounded by the curve  $\gamma_1$  and located below this curve. The second stability condition is equivalent to  $D_2^* := (1-r)\mu_2(S_2^{in}) < D$ , that is, this condition holds in the regions at the right of the vertical line  $\gamma_8$ . Using the definition of regions in Table SM2,  $\mathcal{E}_{00}^{00}$  is LES in  $\mathcal{J}_0$  and  $\mathcal{J}_3$  (see Figure 2). Hence, we obtain the first column in Table 7 corresponding to  $\mathcal{E}_{00}^{00}$ .



**Figure 10.** The curves  $\gamma_i, i = 1 - 9$  and  $\gamma_{15}$ , when  $r = 1/3 \in (0, 1/2)$ ,  $S_2^{in} = 150 > S_2^m \approx 48.740$ : (a)  $m_1 = 0.6 > \mu_2(S_2^m) \approx 0.535$ ; (b)  $m_1 = 0.3 < \mu_2(S_2^m) \approx 0.535$ .



**Figure 11.** The curves  $\gamma_i, i = 1 - 9$  and  $\gamma_{15}$ : (a) case when  $r = 1/3 \in (0, 1/2)$ ,  $S_2^{in} = 10 < S_2^m \approx 48.740$  and  $m_1 = 0.6 > \mu_2(S_2^m) \approx 0.535$ ; (b) case when  $r = 0.7 \in (0, 1/2)$ ,  $S_2^{in} = 150 > S_2^m \approx 48.740$  and  $m_1 = 0.6 > \mu_2(S_2^m) \approx 0.535$ .

- For  $\mathcal{E}_{00}^{01}$ , the existence condition is given in Table 4 by  $S_2^{in} > \lambda_2^{21}$ . Since  $S_2^{in} > S_2^m$ , then  $S_2^{in} > \lambda_2^{21}$  for all  $D < D_2^m = (1 - r)\mu_2(S_2^m)$ , that is, for all  $(D, S_1^{in})$  in the regions bounded by the vertical line  $\gamma_9$  and located to the left of this line. Using Table SM2,  $\mathcal{E}_{00}^{01}$  exists in  $\mathcal{J}_i$ ,  $i = 2 - 20$  (see Figure 2). From Table 5 and (A.14),  $\mathcal{E}_{00}^{01}$  is LES if and only if,

$$S_1^{in} < \lambda_1^2 \quad \text{and} \quad (S_2^{in} < \lambda_2^{11} \quad \text{or} \quad S_2^{in} > \lambda_2^{12}).$$

Thus, the first stability condition holds in the region below the curve  $\gamma_1$ . The second stability condition is equivalent to  $D_1^* := r\mu_2(S_2^{in}) < D$ , that is, this condition holds in the regions at the right of the vertical line  $\gamma_6$ . Using Table SM2,  $\mathcal{E}_{00}^{01}$  is LES in  $\mathcal{J}_3, \mathcal{J}_4$  and  $\mathcal{J}_{14}$  (see Figure 2). Consequently, we obtain the second column in Table 7 corresponding to  $\mathcal{E}_{00}^{01}$ .

- $\mathcal{E}_{00}^{02}$  exists if and only if  $S_2^{in} > \lambda_2^{22}$ , that is, for all  $D_2^* < D < D_2^m$ . Thus, the existence condition of  $\mathcal{E}_{00}^{02}$  holds in the regions at the left of the vertical line  $\gamma_9$  and the right of the vertical line  $\gamma_8$ . Using Table SM2,  $\mathcal{E}_{00}^{02}$  exists in  $\mathcal{J}_2$  and  $\mathcal{J}_3$ .
- $\mathcal{E}_{00}^{10}$  exists in the regions above the curve  $\gamma_1$  (see Figure 2) as the existence condition is  $S_1^{in} > \lambda_1^2$ . Using the definition of regions in Table SM2,  $\mathcal{E}_{00}^{10}$  exists in  $\mathcal{J}_i, i = 1, 2, 5 - 13, 16 - 20$ . From Table 5, the inequalities (A.14) and the functions  $F_{21}$  and  $F_{21}$  defined in Table 1, the stability

conditions of  $\mathcal{E}_{00}^{10}$  become

$$S_1^{in} < \lambda_1^1 \quad \text{and} \quad (S_2^{in} < \lambda_2^{11} \quad \text{or} \quad S_2^{in} > \lambda_2^{12}) \quad \text{and} \quad (S_1^{in} < F_{21} \quad \text{or} \quad S_1^{in} > F_{22}).$$

Thus, the first stability condition of  $\mathcal{E}_{00}^{10}$  holds in the regions below the curve  $\gamma_0$ . Similarly to  $\mathcal{E}_{00}^{01}$ , the second stability condition holds in the regions at the right of the vertical line  $\gamma_6$ . The third stability condition can be written as

$$\mu_2 \left( S_2^{in} + \frac{k_2}{k_1} (S_1^{in} - \lambda_1^2) \right) < \frac{D}{1-r}$$

or equivalently

$$D > D_2^m := (1-r)\mu_2(S_2^m) \quad \text{or} \quad S_2^{in} + \frac{k_2}{k_1} (S_1^{in} - \lambda_1^2) > \lambda_2^{22} \quad (\text{or also } S_1^{in} > F_{22})$$

since  $S_2^{in} > S_2^m$ . Therefore, the third stability condition holds in the region at the right of the vertical line  $\gamma_9$  or above the curve  $\gamma_3$  (see [Figure 2](#)). From [Table SM2](#), it follows that  $\mathcal{E}_{00}^{10}$  is LES in  $\mathcal{J}_i, i = 1, 2, 8$ .

- For  $\mathcal{E}_{00}^{1i}, i = 1, 2$ , the existence conditions become

$$S_1^{in} > \max(\lambda_1^2, F_{2i}),$$

where  $F_{2i}$  is defined for all  $D < D_2^m := (1-r)\mu_2(S_2^m)$  (see [Table 1](#)), that is, this condition holds for all  $(D, S_1^{in})$  in the regions at the left of the vertical line  $\gamma_9$ . From [Proposition 4.3](#), we have  $F_{21} < \lambda_1^2$  and  $\lambda_1^2 < F_{22}$ , for all  $D < D_2^*$  and inversely  $\lambda_1^2 > F_{22}$ , for all  $D > D_2^*$ . Hence,  $\mathcal{E}_{00}^{11}$  exists in the regions above the curve  $\gamma_1$  and at the left of the vertical line  $\gamma_9$ . However,  $\mathcal{E}_{00}^{12}$  exists either in the regions above the curve  $\gamma_3$  when  $(D, S_1^{in})$  is on the left of the vertical line  $\gamma_8$  either in the regions above the curve  $\gamma_1$  when  $(D, S_1^{in})$  is on the right of the vertical line  $\gamma_8$  and the left of the vertical line  $\gamma_9$  (see [Figure 2](#)). From [Table SM2](#),  $\mathcal{E}_{00}^{12}$  exists in  $\mathcal{J}_i, i = 2, 8 - 10, 20$  while  $\mathcal{E}_{00}^{11}$  exists in  $\mathcal{J}_i, i = 2, 5 - 13, 16 - 20$ . The stability conditions of  $\mathcal{E}_{00}^{11}$  are given by

$$S_1^{in} < \lambda_1^1 \quad \text{and} \quad (S_2^{in} < \lambda_2^{11} \quad \text{or} \quad S_2^{in} > \lambda_2^{12}).$$

Thus, the first stability condition of  $\mathcal{E}_{00}^{10}$  holds in the regions below the curve  $\gamma_0$ . Similarly to  $\mathcal{E}_{00}^{01}$ , the second stability condition of  $\mathcal{E}_{00}^{11}$  holds in the regions at the right of the vertical line  $\gamma_6$ . From [Table SM2](#),  $\mathcal{E}_{00}^{11}$  is LES in  $\mathcal{J}_i, i = 2, 5, 8, 13$ .

- For  $\mathcal{E}_{10}^{10}$ , the existence conditions become  $S_1^{in} > \lambda_1^1$ . Thus,  $\mathcal{E}_{10}^{10}$  exists in the regions above the curve  $\gamma_0$  (see [Figure 2](#)). Using the definition of regions in [Table SM2](#),  $\mathcal{E}_{10}^{10}$  exists in  $\mathcal{J}_i, i = 6, 7, 9 - 12, 17 - 20$ . The stability conditions of  $\mathcal{E}_{10}^{10}$  become

$$[D_1 > \mu_2(S_2^m) \quad \text{or} \quad (D_1 < \mu_2(S_2^m) \quad \text{and} \quad (S_1^{in} < F_{11} \quad \text{or} \quad S_1^{in} > F_{12}))] \quad \text{and} \quad [\phi_1 < 0 \quad \text{or} \quad \phi_2 > 0].$$

From [Proposition 4.3](#), we have  $\lambda_1^1 > F_{11}$  so that the condition  $S_1^{in} < F_{11}$  does not hold since the existence condition of  $\mathcal{E}_{10}^{10}$  is  $S_1^{in} > \lambda_1^1$ . Hence, the first stability condition of  $\mathcal{E}_{10}^{10}$  holds in the regions at the right of the vertical line  $\gamma_8$  or on the left of  $\gamma_8$  and above the maximum of the two curves  $\gamma_0$  and  $\gamma_5$  (see [Figure 10\(a\)](#)). According to [Lemma A.4](#), condition  $\phi_1 < 0$  is not satisfied. [Figure 9\(a\)](#) shows that the condition  $\phi_2(D) > 0$  holds in the right of the root of equation  $\phi_2(D) = 0$  for several values of  $S_1^{in}$ . Hence, the second stability condition of  $\mathcal{E}_{10}^{10}$  holds in the regions at the right of the curve  $\gamma_{15}$ . From [Table SM2](#),  $\mathcal{E}_{10}^{10}$  is LES in  $\mathcal{J}_i, i = 7, 9, 10, 11, 19, 20$ .

- For  $\mathcal{E}_{10}^{1i}, i = 1, 2$ , the existence conditions become

$$S_1^{in} > \lambda_1^1 \quad \text{and} \quad \phi_i > 0.$$

From Lemma A.4, condition  $\phi_1 > 0$  is always verified. Thus,  $\mathcal{E}_{10}^{11}$  exists in the regions above the curve  $\gamma_0$  while  $\mathcal{E}_{10}^{12}$  exists in the regions on the left of the curve  $\gamma_0$  and the right of the curve  $\gamma_{15}$ . From Table SM2,  $\mathcal{E}_{10}^{11}$  exists in  $\mathcal{J}_i, i = 6, 7, 9 - 12, 17 - 20$  while  $\mathcal{E}_{10}^{12}$  exists in  $\mathcal{J}_i, i = 7, 9, 11, 19, 20$ . The stability condition of  $\mathcal{E}_{10}^{11}$  becomes

$$S_1^{in} < F_{11} \quad \text{or} \quad S_1^{in} > F_{12}.$$

Similarly to  $\mathcal{E}_{10}^{10}$ , the stability conditions of  $\mathcal{E}_{10}^{11}$  holds in the regions at the right of the vertical line  $\gamma_8$  or on the left of  $\gamma_8$  and above the maximum of the two curves  $\gamma_0$  and  $\gamma_5$ . From Table SM2,  $\mathcal{E}_{10}^{11}$  is LES in  $\mathcal{J}_i, i = 6, 7, 9 - 12, 18, 19, 20$ .

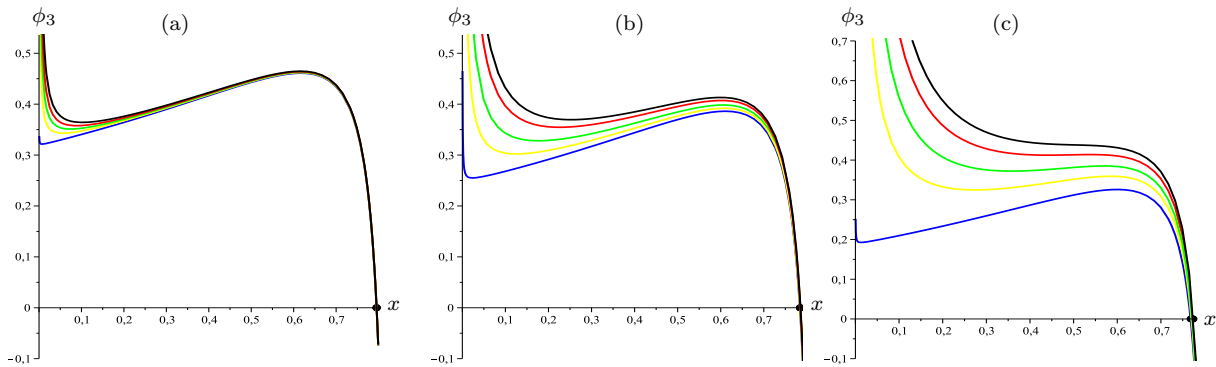
- For  $\mathcal{E}_{0i}^{01}, i = 1, 2$ , the existence conditions become  $S_2^{in} > \lambda_2^{1i}$ . Since  $S_2^{in} > S_2^m$ , then the condition  $S_2^{in} > \lambda_2^{21}$  holds for all  $D < D_1^m$ . Thus, the existence condition of  $\mathcal{E}_{01}^{01}$  holds in the regions at the left of the vertical line  $\gamma_8$ . However, the existence condition of  $\mathcal{E}_{02}^{01}$  holds in the regions on the left of the curve  $\gamma_8$  and the right of the curve  $\gamma_6$ . From Table SM2,  $\mathcal{E}_{01}^{01}$  exists in  $\mathcal{J}_i, i = 10 - 20$  while  $\mathcal{E}_{02}^{01}$  exists in  $\mathcal{J}_i, i = 10 - 14$ . The stability condition of  $\mathcal{E}_{01}^{01}$  becomes  $S_1^{in} < \lambda_1^2$ , that is, this condition holds in the regions below the curve  $\gamma_1$ . From Table SM2, it follows that  $\mathcal{E}_{01}^{01}$  is LES in  $\mathcal{J}_{14}$  and  $\mathcal{J}_{15}$ .
- For  $\mathcal{E}_{0i}^{11}, i = 1, 2$ , the existence conditions become

$$S_2^{in} > \lambda_2^{1i} \quad \text{and} \quad S_1^{in} > \lambda_1^2.$$

The second existence condition of  $\mathcal{E}_{0i}^{11}$  holds in the regions above the curve  $\gamma_1$ . Since  $S_2^{in} > S_2^m > \lambda_2^{11}$  for all  $D < D_1^m$ , the first existence condition of  $\mathcal{E}_{01}^{11}$  holds in the regions at the left of the vertical line  $\gamma_8$ . The first existence condition of  $\mathcal{E}_{02}^{11}$  holds in the regions on the left of the curve  $\gamma_8$  and the right of the curve  $\gamma_6$ . From Table SM2, it follows that  $\mathcal{E}_{01}^{11}$  exists in  $\mathcal{J}_i, i = 10 - 13, 16 - 20$  while  $\mathcal{E}_{02}^{11}$  exists in  $\mathcal{J}_i, i = 10 - 13$ . The stability conditions of  $\mathcal{E}_{01}^{11}$  is given by

$$S_1^{in} < \lambda_1^1 \quad \text{and} \quad g_2'(X_2^{2*}) > f_3'(X_2^{2*})$$

The first stability condition holds in the region below the curve  $\gamma_0$ . For the set of parameters in Table SM1 corresponding to the operating diagram in Figure 2, the equation  $\phi_3(x) := f_3(x) - g_2(x) = 0$  has unique solution, that is,  $g_2'(X_2^{2*}) > f_3'(X_2^{2*})$  holds. Since the operating parameters  $D$  and  $S_1^{in}$  are variable, the numerical simulations show the uniqueness of the solution of equation  $\phi_3(x) = 0$  for several values of  $D$  and  $S_1^{in}$  as shown in Figure 12. From Table SM2, it follows that  $\mathcal{E}_{01}^{11}$  is LES in  $\mathcal{J}_{13}$  and  $\mathcal{J}_{16}$ .



**Figure 12.** Number of positive solutions of equation  $\phi_3(x) = 0$  corresponding to the steady state  $\mathcal{E}_{01}^{11}$ , when  $S_2^{in} = 150 > S_2^m \approx 48.740$ , and for various values of  $D < D_1^* \approx 0.149$  and  $S_1^{in} \in [\lambda_1^2, \lambda_1^1]$ : (a)  $D = 0.05$  and  $S_1^{in} \in \{1.02, 1.4, 1.7, 2, 2.35\}$  where  $\lambda_1^2 = 1.014$  and  $\lambda_1^1 = 2.366$ ; (b)  $D = 0.1$  and  $S_1^{in} \in \{2.4, 3.4, 4.5, 6, 7\}$  where  $\lambda_1^2 = 2.366$  and  $\lambda_1^1 = 7.10$ ; (c)  $D = 0.14$  and  $S_1^{in} \in \{3.83, 7.8, 10.8, 14, 16.5\}$  where  $\lambda_1^2 = 3.82$  and  $\lambda_1^1 = 16.56$ .

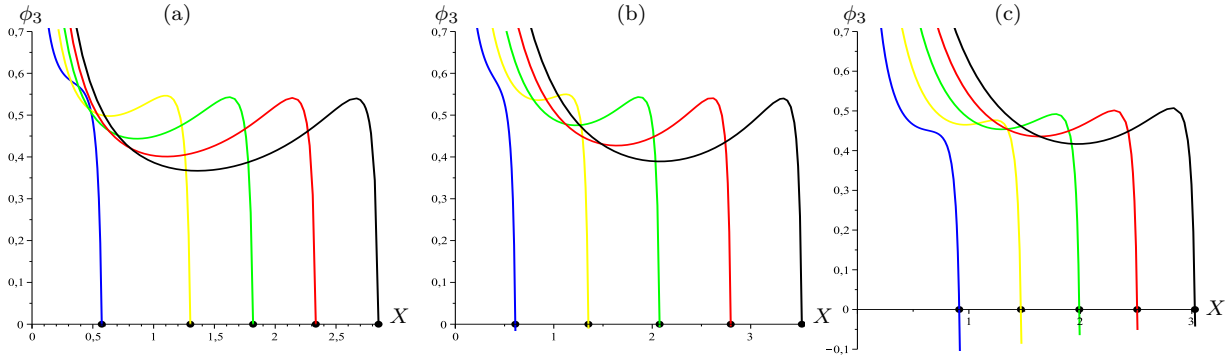
- For  $\mathcal{E}_{1i}^{11}, i = 1, 2$ , the existence conditions become

$$S_1^{in} > \max(\lambda_1^1, F_{1i}),$$

where  $F_{1i}$  is defined for all  $D < \min(r_1 m_1, D_1^m) = D_1^m$  (see Table 1), that is, this last condition holds for all  $(D, S_1^{in})$  in the regions at the left of the vertical line  $\gamma_8$ . From Proposition 4.3, we have  $\lambda_1^1 > F_{11}$  so that the existence condition of  $\mathcal{E}_{11}^{11}$  holds in the regions above the curve  $\gamma_0$ . However, the existence condition of  $\mathcal{E}_{12}^{11}$  holds in the regions on the left of  $\gamma_8$  and above the maximum of the two curves  $\gamma_0$  and  $\gamma_5$  (see Figure 10(a)). From Table SM2, we see that  $\mathcal{E}_{11}^{11}$  exists in  $\mathcal{J}_i, i = 10, 11, 12, 17 - 20$  while  $\mathcal{E}_{02}^{01}$  exists in  $\mathcal{J}_i, i = 10 - 13$ . Recall that the stability condition of  $\mathcal{E}_{11}^{11}$  is

$$g_2'(X_2^{2*}) > f_3'(X_2^{2*}).$$

Similarly to the steady state  $\mathcal{E}_{01}^{11}$ , the equation  $\phi_3(x) := f_3(x) - g_2(x) = 0$  has unique solution (that is,  $g_2'(X_2^{2*}) > f_3'(X_2^{2*})$ ) as we see in Figure 13 for the set of parameters in Table SM1 corresponding to the operating diagram in Figure 2. Thus,  $\mathcal{E}_{11}^{11}$  is LES when it exists. ■



**Figure 13.** Number of positive solutions of equation  $\phi_3(x) = 0$  corresponding to the steady state  $\mathcal{E}_{11}^{11}$  when  $S_2^{in} = 150 > S_2^m \approx 48.740$ , and for various values of  $S_1^{in} > \lambda_1^1$  and  $D \in \{0.05, 0.1, 0.17\}$ : (a)  $D = 0.05$  and  $S_1^{in} \in \{2.37, 72, 122, 172, 222\}$  where  $\lambda_1^1 \approx 2.366$ ; (b)  $D = 0.1$  and  $S_1^{in} \in \{7.2, 77, 147, 217, 287\}$  where  $\lambda_1^1 \approx 7.1$ ; (c)  $D = 0.17$  and  $S_1^{in} \in \{40.24, 90, 140, 190, 240\}$  where  $\lambda_1^1 \approx 40.233$ .

**Proof of Proposition 4.6.** For  $D$  fixed in  $(0, r\mu_2(S_2^m))$  and  $r \in (0, 1)$ , we have  $S_{21}^{in*} = \lambda_2^{11}(D, r)$ . Therefore,

$$\lambda_1^1(D, r) + \frac{k_1}{k_2} (\lambda_2^{11}(D, r) - S_{21}^{in*}) = \lambda_1^1(D, r).$$

Using Table 6, we see that the curves  $\gamma_0, \gamma_4, \gamma_{10}$  intersect at the same point. In the same way, as  $S_{23}^{in*} = \lambda_2^{12}(D, r)$ . Therefore,

$$\lambda_1^1(D, r) + \frac{k_1}{k_2} (\lambda_2^{12}(D, r) - S_{23}^{in*}) = \lambda_1^1(D, r).$$

Consequently, the curves  $\gamma_0, \gamma_5, \gamma_{12}$  intersect at the same point. For  $S_2^{in} < S_{21}^{in*}$ , we have  $\lambda_2^{11}(D, r) < S_2^{in}$  and  $\lambda_2^{12}(D, r) > S_2^{in}$ . Then, the first item holds. Similarly, the result holds when  $S_{21}^{in*} < S_2^{in} < S_{23}^{in*}$  and  $S_2^{in} > S_{23}^{in*}$ . ■

Using the definitions of the auxiliary functions in Table 6, we determine in the following proposition the relative positions of  $\gamma_i, i = 6, 7$  and the curve  $\gamma_5$ .

**Lemma A.6.** Fix  $r \in (0, 1)$  and  $D \in (0, (1-r)\mu_2(S_2^m))$ . Let  $S_{2i}^{in*}, i = 1, \dots, 4$  be the critical values of  $S_2^{in}$  defined in Table 6. Let  $\phi_j(D, r, S_1^{in}, S_2^{in}), j = 1, 2$  be the functions defined in Table 1. If  $S_2^{in} > S_{22}^{in*}$ , then  $\phi_1 > 0$ . In addition, if  $S_2^{in} > S_{24}^{in*}$ , then  $\phi_2 > 0$ .

**Proof of Lemma A.6.** From the expression of  $S_{22}^{in*}$  in Table 6, if  $S_2^{in} > S_{22}^{in*}$ , it follows that  $S_2^{in} > \lambda_2^{21}(D, r)$  so that  $\phi_1 > 0$  holds. For  $S_2^{in} > S_{24}^{in*}$ . Similarly, if  $S_2^{in} > \lambda_2^{22}(D, r)$ , then  $\phi_2 > 0$  holds. ■

**Proof of Proposition 4.7.** Similarly to the proof of the operating diagram in Proposition 4.5 and using Proposition 4.6 and Lemma A.6, we obtain the result. ■

## REFERENCES

- [1] N. Abdellatif, R. Fekih-Salem and T. Sari, Competition for a single resource and coexistence of several species in the chemostat, *Math. Biosci. Eng.*, **13**, 631–652 (2016).
- [2] B. Bar and T. Sari, The operating diagram for a model of competition in a chemostat with an external lethal inhibitor, *Discrete and Continuous Dyn. Syst. - B*, **25**, 2093–2120 (2020).
- [3] D.J. Batstone, J. Keller, I. Angelidaki, S.V. Kalyuzhnyi, S.G. Pavlosthathis, A. Rozzi, W.T.M Sanders, H. Siegrist and V.A. Vavilin, The IWA Anaerobic Digestion Model No 1 (ADM1), *Water Sci Technol.*, **45**, 66–73 (2002).
- [4] B. Benyahia and T. Sari, Effect of a new variable integration on steady states of a two-step anaerobic digestion model, *Math. Biosci. Eng.*, **17**, 5504–5533 (2020).
- [5] B. Benyahia, T. Sari, B. Cherki and J. Harmand, Bifurcation and stability analysis of a two step model for monitoring anaerobic digestion processes, *J. Proc. Control*, **22** 1008–1019 (2012).
- [6] O. Bernard, Z. Hadj-Sadok, D. Dochain, A. Genovesi and J-P. Steyer, Dynamical model development and parameter identification for an anaerobic wastewater treatment process, *Biotechnol. Bioeng.* **75**, 424–438 (2001).
- [7] A. Bornhft, R. Hanke-Rauschenbach and K. Sundmacher, Steady-state analysis of the anaerobic digestion model no. 1 (ADM1), *Nonlinear Dyn.*, **73**, 535–549 (2013).
- [8] E. Chorukova, I. Simeonov and L. Kabaivanova, Volumes ratio optimization in a cascade anaerobic digestion system producing hydrogen and methane, *Ecol. Chem. Eng. S*, **28**, 183–200 (2021).
- [9] M. Dali-Youcef, J. Harmand, A. Rapaport and T. Sari, Some non-intuitive properties of serial chemostats with and without mortality, *IFAC-PapersOnLine* **55**, 475–480 (2022) .
- [10] M. Dali-Youcef, A. Rapaport and T. Sari, Study of performance criteria of serial configuration of two chemostats, *Math. Biosci. Eng.* **17**, 6278–6309 (2020) .
- [11] M. Dali-Youcef, A. Rapaport and T. Sari, Performance study of two serial interconnected chemostats with mortality, *Bull. Math. Biol.* **84**, 110 (2022).
- [12] M. Dali-Youcef and T. Sari, The productivity of two serial chemostats, *Int. J. Biomath.*, **16**, 2250113 (2023).
- [13] Y. Daoud, N. Abdellatif, T. Sari and J. Harmand, Steady state analysis of a syntrophic model: The effect of a new input substrate concentration, *Math. Model. Nat. Phenom.*, **13**, 1–22 (2018).
- [14] M. Dellal and B. Bar, Global analysis of a model of competition in the chemostat with internal inhibitor, *Discrete and Continuous Dyn. Syst. - B*, **26**, 1129–1148 (2021).
- [15] M. Dellal, B. Bar and M. Lakrib, A competition model in the chemostat with allelopathy and substrate inhibition, *Discrete and Continuous Dyn. Syst. - B*, **27**, 2025–2050 (2022).
- [16] M. Dellal, M. Lakrib and T. Sari, The operating diagram of a model of two competitors in a chemostat with an external inhibitor, *Math. Biosci.*, **302**, 27–45 (2018).
- [17] A. Dhooge, W. Govaerts, Yu. A. Kuznetsov, H. G. E. Meijer and B. Sautois, New features of the software MatCont for bifurcation analysis of dynamical systems, *Math. Comput. Model. Dyn.*, **14**, 147–175 (2008).
- [18] A. Donoso-Bravo, P. Gajardo, M. Sebbah and D. Vicencio, Comparison of performance in an anaerobic digestion process: one-reactor vs two-reactor configurations, *Math. Biosci. Eng.*, **16**, 2447–2465 (2019).
- [19] R. Fekih-Salem, Y. Daoud, N. Abdellatifi and T. Sari, A mathematical model of anaerobic digestion with syntrophic relationship, substrate inhibition and distinct removal rates, *SIAM J. Appl. Dyn. Syst. (SIADS)*, **20**, 1621–1654 (2021).
- [20] R. Fekih-Salem, C. Lobry and T. Sari, A density-dependent model of competition for one resource in the chemostat, *Math. Biosci.*, **286**, 104–122 (2017).
- [21] C.D. de Gooijer, W.A.M. Bakker, H.H. Beftink and J. Tramper, Bioreactors in series: An overview of design procedures and practical applications, *Enzyme Microb. Technol.*, **18**, 202–219 (1996).
- [22] M. Hanaki, J. Harmand, Z. Mghazli, A. Rapaport, T. Sari and P. Ugalde, Mathematical study of a two-stage anaerobic model when the hydrolysis is the limiting step, *Processes*, **9**, 2050 (2021).
- [23] I. Haidar, A. Rapaport, F. Gérard, Effects of spatial structure and diffusion on the performances of the chemostat, *Math. Biosci. Eng.*, **8**, 953–971 (2011).
- [24] J. Harmand, A. Rapaport and A. Trofino, Optimal design of two interconnected bioreactors some new results, *AIChE J.*, **49** (2003), 1433–1450.
- [25] J. Harmand, A. Rapaport and A. Dramé, Optimal design of two interconnected enzymatic reactors, *J. Proc. Control*, **14**, 785–794 (2004).
- [26] J. Harmand, A. Rapaport and D. Dochain, Increasing the dilution rate can globally stabilize two-step biological systems, *J. Proc. Control*, **95**, 67–74 (2020).

- [27] J. Harmand, C. Lobry, A. Rapaport, and T. Sari, *The Chemostat: Mathematical Theory of Microorganism Cultures*, **Chemical Eng. Ser.**, ISTE-Wiley, New York (2017).
- [28] A. Isidori, *Nonlinear Control Systems II*, Springer-Verlag, London (1999).
- [29] IWA Publishing, IWA task group for mathematical modelling of anaerobic digestion processes, Anaerobic Digestion Model No.1 (ADM1). Scientific and Technical Report No. 13, IWA Publishing, London (2002).
- [30] Z. Khedim, B. Benyahia, B. Cherki, T. Sari and J. Harmand, Effect of control parameters on biogas production during the anaerobic digestion of protein-rich substrates, **Appl. Math. Model.**, **61**, 351–376 (2018).
- [31] O. Levenspiel, *Chemical reaction engineering*, 3rd Edition, Wiley, New York, New York (1998).
- [32] J. Monod, The technique of a continuous culture. Theory and applications, **Ann. Inst. Pasteur**, **79**, 390–410 (1950).
- [33] T. Mtar, R. Fekih-Salem and T. Sari, Interspecific density-dependent model of predator-prey relationship in the chemostat, **Int. J. Biomath.**, **14**, 2050086 (2021).
- [34] T. Mtar, R. Fekih-Salem and T. Sari, Mortality can produce limit cycles in density-dependent models with a predator-prey relationship, **Discrete and Continuous Dyn. Syst. - B**, **27**, 7445–7467 (2022).
- [35] S. Nouaoura, N. Abdellatif, R. Fekih-Salem and T. Sari, Mathematical analysis of a three-tiered model of anaerobic digestion, **SIAM J. Appl. Math.**, **81**, 1264–1286 (2021).
- [36] S. Nouaoura, R. Fekih-Salem, N. Abdellatif and T. Sari, Mathematical analysis of a three-tiered food-web in the chemostat, **Discrete and Continuous Dyn. Syst. - B**, **26**, 5601–5625 (2021).
- [37] S. Nouaoura, R. Fekih-Salem, N. Abdellatif, T. Sari, Operating diagrams for a three-tiered microbial food web in the chemostat, **J. Math. Biol.**, **85**, 7445–7467 (2022).
- [38] A. Novick and L. Szilard, Description of the chemostat, **Science**, **112**, 715–716 (1950).
- [39] N. Pan, H. Wang, Y. Tian, E. Chorukova, I. Simeonov and N. Christov, Comparison Study of Dynamic Models for One-stage and Two-stage Anaerobic Digestion Processes, **IFAC-PapersOnLine**, **55**, 667–672 (2022).
- [40] T. Sari, Best operating conditions for biogas production in some simple anaerobic digestion models, **Processes**, **10**, 258 (2022).
- [41] T. Sari and B. Benyahia, The operating diagram for a two-step anaerobic digestion model, **Nonlinear Dyn.**, **105**, 2711–2737 (2021).
- [42] T. Sari and J. Harmand, A model of a syntrophic relationship between two microbial species in a chemostat including maintenance, **Math. Biosci.**, **275**, 1–9 (2016).
- [43] T. Sari and M.J. Wade, Generalised approach to modelling a three-tiered microbial food-web, **Math. Biosci.**, **291**, 21–37 (2017).
- [44] M. Sbarciog, M. Loccufier and E. Noldus, Determination of appropriate operating strategies for anaerobic digestion systems, **Biochem. Eng. J.**, **51**, 180–188 (2010).
- [45] A. Schievano, A. Tenca, S. Lonati, E. Manzini and F. Adani, Can two-stage instead of one-stage anaerobic digestion really increase energy recovery from biomass?, **Appl. Energy**, **124**, 335–342 (2014).
- [46] H. L. Smith and P. Waltman, *The Theory of the Chemostat: Dynamics of Microbial Competition*, Cambridge University Press, Cambridge, UK (1995).
- [47] S. Sobieszek, M.J. Wade and G.S.K. Wolkowicz, Rich dynamics of a three-tiered anaerobic food-web in a chemostat with multiple substrate inflow, **Math. Biosci. Eng.**, **17**, 7045–7073 (2020).
- [48] P.M.V. Subbarao, T.C. D’Silva, K. Adlak, S. Kumar, R. Chandra and V. K. Vijay, Anaerobic digestion as a sustainable technology for efficiently utilizing biomass in the context of carbon neutrality and circular economy, **Environ. Res.**, **234**, 116286 (2023).
- [49] M. J. Wade, J. Oakley, S. Harbisher, N. G. Parker and J. Dolfing, MI-Sim: A MATLAB package for the numerical analysis of microbial ecological interactions, **PLoS ONE.**, **12**, e0173249 (2017).
- [50] M.J. Wade, R.W. Pattinson, N.G. Parker and J. Dolfing, Emergent behaviour in a chlorophenol-mineralising three-tiered microbial ‘food web’, **J. Theor. Biol.**, **389**, 171–186 (2016).
- [51] M. Weederma, G. Seo and G.S.K. Wolkowicz, Mathematical model of anaerobic digestion in a chemostat: effects of syntrophy and inhibition, **J. Biol. Dyn.**, **7**, 59–85 (2013).
- [52] M. Weederma, G.S.K. Wolkowicz and J. Sasarag, Optimal biogas production in a model for anaerobic digestion, **Nonlinear Dyn.**, **81**, 1097–1112 (2015).
- [53] A. Xu, J. Dolfing, T.P. Curtis, G. Montague, E. Martin, Maintenance affects the stability of a two-tiered microbial ‘food chain’?, **J. Theor. Biol.**, **276**, 35–41 (2011).
- [54] N.E.H. Zitouni, M. Dellal and M. Lakrib, Substrate inhibition can produce coexistence and limit cycles in the chemostat model with allelopathy, **J. Math. Biol.**, **87:7**, 35–41 (2023).

# SUPPLEMENTARY MATERIALS: Analysis of anaerobic digestion Model with two serial interconnected chemostats \*

THAMER HMIDHI <sup>†</sup>, RADHOUANE FEKIH-SALEM <sup>\*†‡</sup>, and JÉRÔME HARMAND <sup>§</sup>

**SM1. Tables.** In this supplementary material, we first provide in [Table SM1](#) all the values of the parameters used in the numerical simulations.

**Table SM1**

*Parameter values used for systems (2.3) and (3.2) when the growth rates  $\mu_1$  and  $\mu_2$  are given by (4.2).*

Parameter	$m_1$ ( $d^{-1}$ )	$k_{S_1}$ ( $g/l$ )	$m_2$ ( $d^{-1}$ )	$k_{S_2}$ ( $mmol/l$ )	$k_I$ ( $mmol/l$ )	$k_1$ ( $mmol/g$ )	$k_2$ ( $mmol/g$ )	$k_3$ ( $mmol/g$ )
Figures 2, 4 to 6, 7, and 8	0.6	7.1	0.74	9.28	256	42.14	116.5	268
Figure 3	0.3	7.1	0.74	9.28	256	42.14	116.5	268

Next, we first define in [Table SM2](#) the regions  $\mathcal{J}_i$ ,  $i = 0, \dots, 31$  of the operating diagram of [Figures 2 to 4](#) in the two-dimensional plane  $(D, S_1^{in})$  when  $r$  and  $S_2^{in}$  are fixed so that  $r \in (0, 1/2)$ . Then, we define in [Table SM3](#) the regions  $\mathcal{J}_i$ ,  $i = 32, \dots, 46$  of the operating diagram of [Figure 5](#) in the two-dimensional plane  $(D, S_1^{in})$  when  $r$  and  $S_2^{in}$  are fixed so that  $r \in (1/2, 1)$ . Then, we define in [Table SM4](#) the regions  $\mathcal{J}_i$ ,  $i = 46, \dots, 69$  of the operating diagram of [Figure 6](#) in the two-dimensional plane  $(S_2^{in}, S_1^{in})$  when  $r$  and  $D$  are fixed so that  $r \in (0, 1/2)$  and  $D \in (0, \mu_2(S_2^m))$ .

\*Submitted to the editors 2024-08-05.

**Funding:** This work was supported by the Euro-Mediterranean research network TREASURE (<http://www.inra.fr/treasure>).

<sup>†</sup>University of Tunis El Manar, National Engineering School of Tunis, LAMSIN, Tunisia ([thamer.hmidhi@enit.utm.tn](mailto:thamer.hmidhi@enit.utm.tn)).

<sup>‡</sup>University of Monastir, Higher Institute of Computer Science of Mahdia, Tunisia ([radhouane.fekih-saleh@enit.utm.tn](mailto:radhouane.fekih-saleh@enit.utm.tn)).

<sup>§</sup>LBE, University of Montpellier, INRAE, Narbonne, France ([jerome.harmand@inrae.fr](mailto:jerome.harmand@inrae.fr)).



Table SM2

Definitions of the regions  $\mathcal{J}_i$ ,  $i = 0, \dots, 31$  of the operating diagram of Figures 2 to 4 when  $r \in (0, 1/2)$ . For  $i, j = 1, 2$ , the functions  $D_i^m$  and  $D_i^*$  are defined in Table 6 while  $F_{ij}$  and  $\phi_2$  are defined in Table 1.

Condition on			
$S_2^{in}$	$S_1^{in}$ and $\phi_2$	$D$	Region
[0, +∞[	$(\lambda_1^1, F_{22})$ and $\phi_2 < 0$	$(D_1^m, \min(r_1 m_1, D_2^m))$	$\mathcal{J}_6$
	$(\lambda_1^1, F_{22})$ and $\phi_2 > 0$	$(D_1^m, \min(r_1 m_1, D_2^m))$	$\mathcal{J}_7$
	$(F_{22}, \lambda_1^1)$	$(D_1^m, \min(r_2 m_1, D_2^*, D_2^m))$	$\mathcal{J}_8$
	$(\max(\lambda_1^1, F_{22}), +\infty)$	$(D_1^m, \min(r_1 m_1, r_2 m_1, D_2^m))$	$\mathcal{J}_9$
	$[0, \lambda_1^2)$	$(0, D_1^*)$	$\mathcal{J}_{15}$
	$(\lambda_1^2, \lambda_1^1)$	$(0, \min(r_2 m_1, D_1^*))$	$\mathcal{J}_{16}$
	$(\lambda_1^1, \min(F_{12}, F_{22}))$ and $\phi_2 < 0$	$(0, \min(r_1 m_1, D_1^*, D_2^m))$	$\mathcal{J}_{17}$
	$(F_{12}, F_{22})$ and $\phi_2 < 0$	$(0, \min(r_1 m_1, D_1^*, D_1^m, D_2^m))$	$\mathcal{J}_{18}$
	$(F_{12}, F_{22})$ and $\phi_2 > 0$	$(0, \min(r_1 m_1, D_1^*, D_1^m, D_2^m))$	$\mathcal{J}_{19}$
	$(\max(F_{12}, F_{22}), +\infty)$	$(0, \min(r_1 m_1, r_2 m_2, D_1^*, D_1^m, D_2^m))$	$\mathcal{J}_{20}$
$(S_2^m, +\infty)$	$[0, \lambda_1^2)$	$(D_2^m, +\infty)$	$\mathcal{J}_0$
	$(\lambda_1^2, +\infty)$	$(D_2^m, +\infty)$	$\mathcal{J}_1$
	$(\lambda_1^2, +\infty)$	$(D_2^*, D_2^m)$	$\mathcal{J}_2$
	$[0, \lambda_1^2)$	$(D_2^*, D_2^m)$	$\mathcal{J}_3$
	$[0, \lambda_1^2)$	$(D_1^m, D_2^*)$	$\mathcal{J}_4$
	$(\lambda_1^2, \min(\lambda_1^1, F_{22}))$	$(D_1^m, r_1 m_1)$	$\mathcal{J}_5$
	$(F_{22}, +\infty)$	$(D_1^*, \min(r_2 m_1, D_1^m, D_2^m))$	$\mathcal{J}_{10}$
	$(\lambda_1^1, F_{22})$ and $\phi_2 > 0$	$(D_1^*, \min(r_1 m_1, D_1^m, D_2^m))$	$\mathcal{J}_{11}$
	$(\lambda_1^1, F_{22})$ and $\phi_2 < 0$	$(D_1^*, \min(r_1 m_1, D_1^m, D_2^m))$	$\mathcal{J}_{12}$
	$(\lambda_1^2, \max(\lambda_1^1, F_{22}))$	$(D_1^*, \min(r_2 m_1, D_1^m))$	$\mathcal{J}_{13}$
	$[0, \lambda_1^2)$	$(D_1^*, D_1^m)$	$\mathcal{J}_{14}$
	$(\max(\lambda_1^1, \lambda_1^2), \min(F_{12}, F_{22}))$ and $\phi_2 > 0$	$(0, \min(r_1 m_1, r_2 m_1, D_2^m))$	$\mathcal{J}_{21}$
	$(\max(\lambda_1^1, F_{22}), F_{12})$	$(0, \min(r_1 m_1, r_2 m_1, D_2^m))$	$\mathcal{J}_{22}$
	$(F_{22}, \lambda_1^1)$	$(0, \min(r_2 m_1, D_1^*, D_2^m))$	$\mathcal{J}_{23}$
$(F_{22}, +\infty)$	$(D_1^*, \min(r_2 m_1, D_1^m, D_2^m))$	$\mathcal{J}_{24}$	
[0, $S_2^m$ )	$[0, \lambda_1^2)$	$(D_2^*, +\infty)$	$\mathcal{J}_0$
	$(\lambda_1^2, F_{21}) \cup [\lambda_1^2, +\infty)$	$(D_2^m, +\infty) \cup (D_2^*, +\infty)$	$\mathcal{J}_1$
	$(F_{21}, F_{22})$	$(D_2^*, \min(r_2 m_1, D_2^m))$	$\mathcal{J}_{25}$
	$(F_{22}, +\infty)$	$(D_2^*, \min(r_2 m_1, D_2^m))$	$\mathcal{J}_{26}$
	$(F_{22}, +\infty)$	$(D_1^*, \min(r_2 m_1, D_1^m, D_2^m))$	$\mathcal{J}_{27}$
	$(F_{12}, F_{22})$ and $\phi_2 > 0$	$(D_1^*, \min(r_1 m_1, D_1^m))$	$\mathcal{J}_{28}$
	$(F_{12}, F_{22})$ and $\phi_2 < 0$	$(D_1^*, \min(r_1 m_1, D_1^m))$	$\mathcal{J}_{29}$
	$(F_{11}, F_{12})$	$(D_1^*, \min(r_1 m_1, D_1^m))$	$\mathcal{J}_{30}$
	$(\lambda_1^1, F_{11})$	$(D_1^*, \min(r_1 m_1, D_1^m))$	$\mathcal{J}_{31}$
	$(\lambda_1^2, \min(\lambda_1^1, F_{22}))$	$(D_1^*, \min(r_2 m_1, D_2^*))$	$\mathcal{J}_5$
	$[0, \lambda_1^2)$	$(D_1^*, D_2^*)$	$\mathcal{J}_4$

**Table SM3**

Definitions of the regions  $\mathcal{J}_i$ ,  $i = 0, 20-22, 32-46$  of the operating diagram of *Figure 5* in the case  $r \in (1/2, 1)$  and  $S_2^n \in (S_2^m, +\infty)$ . The functions  $D_i^m$  and  $D_i^*$ ,  $i = 1, 2$  are defined in *Table 6* while the functions  $F_{ij}$  and  $\phi_2$ ,  $i, j = 1, 2$  are defined in *Table 1*.

$S_1^n$ and $\phi_2$	Condition on		Region
	$D$		
$[0, \lambda_1^1)$	$(D_1^m, +\infty)$		$\mathcal{J}_0$
$[\lambda_1^1, +\infty)$	$(D_1^m, r_1 m_1)$		$\mathcal{J}_{32}$
$[\lambda_1^1, +\infty)$	$(D_1^*, \min(r_1 m_1, D_1^m))$		$\mathcal{J}_{33}$
$[0, \lambda_1^1)$	$(D_1^*, D_1^m)$		$\mathcal{J}_{34}$
$[0, \lambda_1^1)$	$(D_2^m, D_1^*)$		$\mathcal{J}_{35}$
$(\lambda_1^1, \min(\lambda_1^2, F_{12}))$	$(D_2^m, r_1 m_1)$		$\mathcal{J}_{36}$
$(\lambda_1^2, F_{12})$	$(D_2^m, r_2 m_1)$		$\mathcal{J}_{37}$
$(F_{12}, \lambda_1^2)$	$[0, \min(r_1 m_1, D_1^*, D_1^m))$		$\mathcal{J}_{38}$
$(\max(\lambda_1^2, F_{12}), +\infty)$	$(D_2^m, \min(r_1 m_1, r_2 m_1, D_1^m))$		$\mathcal{J}_{39}$
$(F_{12}, +\infty)$	$(D_2^*, \min(r_1 m_1, D_1^m, D_2^m))$		$\mathcal{J}_{40}$
$(\lambda_1^2, F_{12})$	$(D_2^*, \min(r_2 m_1, D_2^m))$		$\mathcal{J}_{41}$
$(\lambda_1^1, \lambda_1^2)$	$(D_2^*, \min(r_1 m_1, D_2^m))$		$\mathcal{J}_{42}$
$[0, \lambda_1^1)$	$(D_2^*, D_2^m)$		$\mathcal{J}_{43}$
$[0, \lambda_1^1)$	$(0, D_2^*)$		$\mathcal{J}_{15}$
$(\lambda_1^1, \lambda_1^2)$ and $\phi_2 < 0$	$(0, \min(r_1 m_1, D_2^*, D_2^m))$		$\mathcal{J}_{44}$
$(\lambda_1^1, \lambda_1^2)$ and $\phi_2 > 0$	$(0, \min(r_1 m_1, D_2^*, D_2^m))$		$\mathcal{J}_{45}$
$(\lambda_1^2, F_{22})$ and $\phi_2 < 0$	$(0, \min(r_1 m_1, r_2 m_1, D_2^m))$		$\mathcal{J}_{46}$
$(\lambda_1^2, F_{22})$ and $\phi_2 > 0$	$(0, \min(r_1 m_1, r_2 m_1, D_2^m))$		$\mathcal{J}_{21}$
$(F_{22}, F_{12})$	$(0, \min(r_2 m_1, D_2^*, D_2^m))$		$\mathcal{J}_{22}$
$(F_{12}, +\infty)$	$(0, \min(r_1 m_1, D_2^*, D_1^m))$		$\mathcal{J}_{20}$

**Table SM4**

Definitions of the regions  $\mathcal{J}_i$ ,  $i = 0, 1, 4, 5, 15, 27, 46 - 69$  of the operating diagram of [Figure 6](#) in the plane  $(S_2^{in}, S_1^{in})$  when  $r \in (0, 1/2)$  and  $D \in (0, \mu_2(S_2^m))$ . For  $i, j = 1, 2$ , the functions  $D_i^m$  and  $D_i^*$  are defined in [Table 6](#) while the functions  $F_{ij}$  and  $\phi_2$  in [Table 1](#).

$S_2^{in}$	Condition on $S_1^{in}$ and $\phi_2$	Region
$[0, \lambda_2^{21})$	$[0, \lambda_1^2)$	$\mathcal{J}_0$
	$(\lambda_1^2, F_{21})$	$\mathcal{J}_1$
	$(F_{21}, \lambda_1^1)$	$\mathcal{J}_{64}$
	$(\lambda_1^1, F_{11})$	$\mathcal{J}_{65}$
	$(F_{11}, F_{12})$	$\mathcal{J}_{66}$
	$(F_{12}, F_{22})$ and $\phi_2 < 0$	$\mathcal{J}_{67}$
	$(F_{12}, F_{22})$ and $\phi_2 > 0$	$\mathcal{J}_{68}$
	$(F_{22}, +\infty)$	$\mathcal{J}_{69}$
	$(\lambda_2^{21}, \lambda_2^{11})$	$[0, \lambda_1^2)$
$(\lambda_1^2, \lambda_1^1)$		$\mathcal{J}_5$
$(\lambda_1^1, F_{11})$		$\mathcal{J}_{63}$
$(F_{11}, F_{12})$		$\mathcal{J}_{62}$
$(F_{12}, F_{22})$ and $\phi_2 < 0$		$\mathcal{J}_{61}$
$(F_{12}, F_{22})$ and $\phi_2 > 0$		$\mathcal{J}_{60}$
$(F_{22}, +\infty)$		$\mathcal{J}_{27}$
$(\lambda_2^{11}, \lambda_2^{12})$	$[0, \lambda_1^2)$	$\mathcal{J}_{15}$
	$(\lambda_1^2, \lambda_1^1)$	$\mathcal{J}_{56}$
	$(\lambda_1^1, F_{12})$	$\mathcal{J}_{46}$
	$(F_{12}, F_{22})$ and $\phi_2 < 0$	$\mathcal{J}_{57}$
	$(F_{12}, F_{22})$ and $\phi_2 > 0$	$\mathcal{J}_{58}$
$(\lambda_2^{12}, \lambda_2^{22})$	$(F_{22}, +\infty)$	$\mathcal{J}_{59}$
	$(\max(\lambda_1^1, F_{22}), +\infty)$	$\mathcal{J}_{50}$
	$(\lambda_1^1, F_{22})$ and $\phi_2 > 0$	$\mathcal{J}_{51}$
	$(\lambda_1^1, F_{22})$ and $\phi_2 < 0$	$\mathcal{J}_{52}$
	$(F_{22}, \lambda_1^1)$	$\mathcal{J}_{53}$
	$(\lambda_1^2, \min(\lambda_1^1, F_{22}))$	$\mathcal{J}_{54}$
$(\lambda_2^{22}, +\infty)$	$[0, \lambda_1^2)$	$\mathcal{J}_{55}$
	$[0, \lambda_1^2)$	$\mathcal{J}_{47}$
	$(\lambda_1^2, \lambda_1^1)$	$\mathcal{J}_{48}$
	$(\lambda_1^1, +\infty)$	$\mathcal{J}_{49}$