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Abstract

In this work, we consider a mathematical model of syntrophic relationship between two microbial species of the anaerobic digestion process including mortality (or decay) terms. We focus on the acetogenesis and hydrogenotrophic methanogenesis phases. Our study gives a quite comprehensive analysis of a syntrophic model by analyzing the joined effects of syntrophy relationship, mortality, substrate inhibition and input concentrations that were neglected in previous studies. Using a general class of growth rates, the necessary and sufficient conditions of existence and local stability of all steady states of the four-dimensional system are determined according to the operating parameters. This general model exhibits a rich behavior with the coexistence of two microbial species, the bi-stability, the multiplicity of coexistence steady states, and the existence of two steady states of extinction of the first species. The operating diagram shows how the model behaves by varying the control parameters and illustrates the effect of the inhibition and the new input substrate concentration (hydrogen) on the reduction of the coexistence region and the emergence of a bi-stability region. Similarly to the classical chemostat model, including the substrate inhibition can destabilize a two-tiered microbial 'food chain' where the stability depends on the initial condition.

Keywords: anaerobic digestion, chemostat, syntrophy, inhibition, bi-stability, operating diagram

1. Introduction

Anaerobic Digestion (AD) is a process used for the biological treatment of municipal, agricultural and industrial wastes with the additional benefit of producing energy in the form of biogas. During this process, the waste is first partially transformed into volatile fatty acids and then converted into methane and carbon dioxide. AD process is too complex with difficulty to collect informative experimental data which complicated the model validation and the parameter identification [8]. The generic AD Model No.1 (ADM1) of the IWA Task Group for Mathematical Modeling of AD Processes is characterized by its extreme complexity with 32 dynamic concentration state variables and a large number of parameters [1].

Many mathematical models describing the whole process or some key steps have been considered in the last three decades; see [2, 3, 5, 10, 16, 26, 27, 28, 35, 36, 37, 38]. A synthetic and unified vision of many models involving two or three cross-feeding species and various types of inhibition has been proposed by Di and Yang [7]. Using specific growth functions, the numerical simulations reveal the reduction in both productivity and stability due to inhibitions with the occurrence of stable periodic orbits owing to the presence of negative and positive feedback loops. In Khedim et al. [16], a mathematical analysis of the protein-rich Microalgae AD model (the so-called MAD) shows the process behavior according to the control parameters where the operating diagram illustrates the ideal conditions to optimize biogas yield and ammonia toxicity. In fact, the MAD model has been proposed by Mairet et al. [21] and was validated from experimental data of an AD process of chlorella vulgaris microalgae involving four substrates and three microbial species with three reactions and two steps (methanogenesis and hydrolysis-acetogenesis). Considering syntrophy and inhibition effects, Weedermann et al. [37, 38] have analyzed an eight-dimensional mathematical model describing three of the four main stages of AD: acidogenesis, acetogenesis, and methanogenesis. Following [36] and using general functional responses, Sari and Wade [27] have studied a three-tiered microbial food-web model discovering the emergence of coexistence region in the operating diagram where a stable limit cycle is born via the Hopf bifurcation, which has not been reported by [36].

Using a step by step parameter identification procedure, Bernard et al. [3] have proposed and have validated a reduced two-step model (the so-called AM2) from experimental data of AD process. This model has a cascade structure and has been

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widely applied for control and optimization of AD process [12, 29, 30], as well as, for mathematical analysis [2, 26, 28]. Using a maximum likelihood principal component analysis [20] and generated data built from ADM1 model, the appropriate number of reactions is determined by a systematic data driven-approach followed by a parameter identification procedure [13]. The resulting low-order model is the two-tiered microbial 'food chain' leading to perfectible direct and cross-validation results.

The two-tiered microbial model we consider here describes the next two biological reactions:

$$s_0 \xrightarrow{\mu_0} x_0 + s_1, \qquad s_1 \xrightarrow{\mu_1} x_1$$

where a substrate s_0 (Volatile Fatty Acid) is consumed by a biomass x_0 (the acetogenic bacteria) to produce a product s_1 (the hydrogen). The substrate s_1 is consumed in the second reaction by another biomass x_1 (the hydrogenotrophic methanogenic bacteria). μ_0 and μ_1 are the bacterial growth rates, depending eventually on one or both substrates. The substrates s_0 and s_1 are introduced in the reactor with the inflowing concentrations s_0^{in} and s_1^{in}, respectively, and a dilution rate D. These reactions are described by the following system of differential equations

$$\left\{\begin{array}{lcl} \dot{s}_0 &=& D\left(s_0^{in} - s_0\right) - \mu_0(\cdot)x_0, \\ \dot{x}_0 &=& \left(\mu_0(\cdot) - D_0\right)x_0, \\ \dot{s}_1 &=& D\left(s_1^{in} - s_1\right) + \mu_0(\cdot)x_0 - \mu_1(\cdot)x_1, \\ \dot{x}_1 &=& \left(\mu_1(\cdot) - D_1\right)x_1, \end{array}\right. \tag{1}$$

where D_0 and D_1 represent, respectively, the disappearance rates of acetogenic and methanogenic bacteria. In this study, the two-tiered model (1) is analyzed where D_i can be modeled as in [22, 31] by

$$D_i = \alpha_i D + a_i, \quad i = 0, 1, \tag{2}$$

where the nonnegative death (or decay) rate parameters a_0 and a_1 are taken into consideration. These decay terms included in model (1) are related to consumption of energy, other than growth; see for instance [14] or [23]. The coefficients α_0 and α_1 belong to $[0, 1]$ and represent, respectively, the first and the second biomass proportion that leaves the reactor. For example, in [3] these coefficients are proposed to model a biomass reactor attached to the support or to decouple the residence time of solids and the hydraulic residence time $(1/D)$. Thus, the study will not be restricted to the case $\alpha_i = 1$, $i = 0, 1$, as in [6, 9, 11, 26], and the case $0 \leq \alpha_i \leq 1$, which is of biological interest, will be investigated.

If the growth rate μ_0 depends only on substrate s_0 and μ_1 depends only on s_1, that is,

$$\mu_0(\cdot) = \mu_0(s_0), \quad \mu_1(\cdot) = \mu_1(s_1), \tag{3}$$

then system (1) has a cascade structure and describes a commensalistic relationship where the commensal species x_1 needs the first species x_0 to grow, while x_0 can grow without x_1 and it is not affected by the growth of this commensal species x_1. If μ_0 depends on both substrates s_0 and s_1, and μ_1 depends on substrate s_1, that is,

$$\mu_0(\cdot) = \mu_0(s_0, s_1), \quad \mu_1(\cdot) = \mu_1(s_1), \tag{4}$$

then system (1) describes a syntrophic relationship where two microbial species depend on each other for survival by the production of a required substrate s_1. In this case, the two populations exhibit mutualism by increasing their productivity while one of the population can grow without the other. Tables 1 and 2 summarize the modeling assumptions made in the literature on two-tiered model (1) describing the commensalistic and the syntrophic relationships, respectively, according to the input concentration s_1^{in}, the removal rates D_i, and the choice of the growth functions.

Table 1: Literature examples of commensalistic relationship of two-tiered model (1), the modeling assumptions and the description of the growth rates (3).

References	s_1^{in}	D_i	$\mu_0(s_0)$	$\mu_1(s_1)$
Reilly [24], Simeonov et al. [33]	0	D	Monod	Monod
Stephanopoulos et al. [34]	0	D	Monotonic	Monotonic or Nonmonotonic
Bernard et al. [3]	> 0	αD	Monod	Haldane
Simeonov et al. [32]	0	D	Monod or Contois	Haldane
Sbarciog et al. [28]	> 0	D	Monotonic	Nonmonotonic
Benyahia et al. [2]	> 0	αD	Monotonic	Nonmonotonic

Table 2: Literature examples of syntrophic relationship of two-tiered model (1), the modeling assumptions and the description of the growth rates (4).

References	s_1^{in}	D_i	$\mu_0(s_0, s_1)$	$\mu_1(s_1)$ or $\mu_1(s_0, s_1)$
Kreikenbohm and Bohl [18]	0	D	Monod in s_0, decreasing in s_1	Monod
Kreikenbohm and Bohl [19]	0	D	Monod in s_0, decreasing in s_1	Decreasing in s_0, Monod in s_1
Burchard [5], El-Hajji et al. [9]	0	D	Increasing in s_0, decreasing in s_1	Increasing
Volcke et al. [35]	0	D	Nonmonotonic in s_0, decreasing in s_1	Decreasing in s_0, nonmonotonic in s_1
Xu et al. [39]	0	$D + a_i$	Increasing in s_0, decreasing in s_1	Monod
Sari et al. [25]	> 0	D	Increasing in s_0, decreasing in s_1	Decreasing in s_0, increasing in s_1
Harvey et al. [15]	0	D	Increasing in s_0, decreasing in s_1	Nonmonotonic
Sari and Harmand [26]	0	$D + a_i$	Increasing in s_0, decreasing in s_1	Increasing
Fekih et al. [11]	0	$D + a_i$	Increasing in s_0, decreasing in s_1	Nonmonotonic
Daoud et al. [6]	> 0	$D + a_i$	Increasing in s_0, decreasing in s_1	Increasing

However, the joined effects of syntrophy, mortality of two microbial species, substrate inhibition on their growth and inflowing substrate concentration of the second species has not been studied in the literature. Thus, the goal of the present work is to give a complete analysis of syntrophic model (1) involving these joined effects. Here, we do not specify kinetics but we assume qualitative properties on the growth functions. Using a general function with the same properties as a Haldane function, we assume that the second species is inhibited when the concentration becomes significant. The case $s_1^{in} = 0$ was considered in [11]. The case where μ_1 does not present inhibition was considered in [6]. In this paper, we generalize [15], by allowing a larger class of growth functions, and by considering distinct removal rates. Therefore, the mathematical analysis of the model cannot be reduced to a two dimensional system as in [15].

On the other hand, our study provides an important tool for the experimentation which is the operating diagram showing the behavior of the syntrophic model (1) according to the control parameters D, s_0^{in} and s_1^{in}, when all biological parameters are fixed.

This paper is organized as follows: in Section 2, we present the assumptions made on the growth functions and give some preliminary results. Section 3 is devoted to the analysis of steady states and their local stability. In Section 4, we present the operating diagrams which depict the different outcomes of the model according to control parameters. These diagrams show the regions of stability and bi-stability of the steady states and demonstrate that inhibition has a significant impact on the long-term survival of the micro-organisms and thus the biogas production. Finally, some conclusions are drawn in Section 5. The proof of all results are reported in Appendix A. The definition domains of the functions which correspond to the change of behavior of the system are given in Appendix B. With specific growth rates satisfying the general assumptions, the maximal number of solutions of an equation which determines these definition domains are given in Appendix C where these functions are calculated explicitly. For the numerical simulations, the parameter values used in all figures with the specific growth rates are provided in Appendix D.

2. Mathematical model and assumptions

In what follows, we study model (1) where the growth rates μ_i and the removal rates D_i, $i = 0, 1$, are given by (4) and (2), respectively. Thus, the syntrophic model can be written as follows

$$\left\{\begin{array}{lcl} \dot{s}_0 & = & D\left(s_0^{in} - s_0\right) - \mu_0(s_0, s_1)x_0, \\ \dot{x}_0 & = & \left(\mu_0(s_0, s_1) - D_0\right)x_0, \\ \dot{s}_1 & = & D\left(s_1^{in} - s_1\right) + \mu_0(s_0, s_1)x_0 - \mu_1(s_1)x_1, \\ \dot{x}_1 & = & \left(\mu_1(s_1) - D_1\right)x_1. \end{array}\right. \tag{5}$$

We first make the following general assumptions on the bacterial growth rates μ_0 and μ_1.

(H1) $\mu_0(0, s_1) = 0$, $\mu_0(s_0, s_1) > 0$, $\sup_{s_0 \geq 0} \mu_0(s_0, s_1) < +\infty$, for all $s_0 > 0$ and $s_1 \geq 0$.

(H2) $\mu_1(0) = 0$ and $\mu_1(s_1) > 0$, for all $s_1 > 0$.

(H3) $\frac{\partial \mu_0}{\partial s_0}(s_0, s_1) > 0$ and $\frac{\partial \mu_0}{\partial s_1}(s_0, s_1) < 0$, for all $s_0 > 0$ and $s_1 > 0$.

(H4) $\mu_1(s_1)$ reaches a maximum value $\mu_1^{max} := \mu_1\left(s_1^{max}\right)$ at $s_1 = s_1^{max}$ and satisfies $\mu_1'(s_1) > 0$, for all $s_1 \in \left[0, s_1^{max}\right)$, $\mu_1'(s_1) < 0$, for all $\left(s_1^{max}, +\infty\right)$ and $\mu_1(+\infty) = 0$.

(H5) For all $s_1 > 0$, $\bar{\mu}_0'(s_1) < 0$ where $\bar{\mu}_0(s_1) := \sup_{s_0 \geq 0} \mu_0(s_0, s_1)$.

Hypotheses (H1) and (H2) mean that the growth can take place if, and only if, the substrate is present. Hypothesis (H3) means that the growth rate of the species x_0 increases with the concentration of substrate s_0 and is inhibited by the substrate s_1. Hypothesis (H4) means that the nonmonotonic growth function takes into account the growth-limiting for low concentrations of substrate and the growth-inhibiting for high concentrations. Hypothesis (H5) means that the maximum growth rate of the species x_0 decreases with the concentration of substrate s_1. These assumptions are satisfied by the following growth rates of Monod-type with hydrogen inhibition and of Haldane-type, respectively,

$$\mu_0(s_0, s_1) = \frac{m_0 s_0}{K_0 + s_0} \frac{1}{1 + s_1/K_i}, \quad \mu_1(s_1) = \frac{m_1 s_1}{K_1 + s_1 + s_1^2/K_I}, \tag{6}$$

where m_j and K_j, $j = 0, 1$, denote the maximum growth rates and the Michaelis-Menten constants; K_i and K_I represent the inhibition factor due to s_1 for the growth of the species x_0 and x_1, respectively.

The following result proves that syntrophic model (5) preserves the biological significance where all solutions of the system are nonnegative and bounded for any nonnegative initial condition.

Proposition 2.1. *For any nonnegative initial condition, the solutions of (5) remain nonnegative and are positively bounded. In addition, the set*

$$\Omega = \left\{(s_0, x_0, s_1, x_1) \in \mathbb{R}_+^4 : 2s_0 + x_0 + s_1 + x_1 \leq \frac{D}{D_{\min}}\left(2s_0^{in} + s_1^{in}\right)\right\}, \quad \text{where} \quad D_{\min} = \min(D, D_0, D_1),$$

is positively invariant and a global attractor for (5).

3. Analysis of the syntrophic model

3.1. Existence of steady states

The steady states of (5) are the solutions of the following system

$$\begin{cases} 0 = D\left(s_0^{in} - s_0\right) - \mu_0(s_0, s_1)x_0, \\ 0 = (\mu_0(s_0, s_1) - D_0)\, x_0, \\ 0 = D\left(s_1^{in} - s_1\right) + \mu_0(s_0, s_1)x_0 - \mu_1(s_1)x_1, \\ 0 = (\mu_1(s_1) - D_1)\, x_1. \end{cases} \tag{7}$$

From the second equation of (7), it follows that

$$x_0 = 0 \quad \text{or} \quad \mu_0(s_0, s_1) = D_0,$$

and from the last equation of (7), we deduce that:

$$x_1 = 0 \quad \text{or} \quad \mu_1(s_1) = D_1.$$

Thus, the system can have at most four types of steady states:

- SS_0: $x_0 = x_1 = 0$, called the washout, where both species are extinct.
- SS_1: $x_1 = 0$ and $x_0 > 0$, where species x_1 is extinct while species x_0 survives.
- SS_2: $x_0 > 0$, $x_1 > 0$, where both species are maintained.
- SS_3: $x_0 = 0$ and $x_1 > 0$, where species x_0 is extinct while species x_1 survives.

We show below that the steady states SS_0 and SS_1 are unique if they exist and generically, the system can have two steady states SS_2^1 and SS_2^2 of type SS_2 and two steady states SS_3^1 and SS_3^2 of type SS_3. For the description of the steady states, we need the following notations:

Since the function $s_1 \mapsto \mu_1(s_1)$ is increasing on $\left[0, s_1^{max}\right]$, it has an inverse function $y \mapsto M_1^1(y)$ which is increasing such that,

$$s_1 = M_1^1(y) \iff y = \mu_1(s_1), \quad \text{for all} \quad s_1 \in \left[0, s_1^{max}\right] \quad \text{and} \quad y \in \left[0, \mu_1^{max}\right]. \tag{8}$$

Since the function $s_1 \mapsto \mu_1(s_1)$ is decreasing on $\left[s_1^{max}, +\infty\right)$, it has an inverse function $y \mapsto M_1^2(y)$ which is decreasing such that,

$$s_1 = M_1^2(y) \iff y = \mu_1(s_1), \quad \text{for all} \quad s_1 \in \left[s_1^{max}, +\infty\right) \quad \text{and} \quad y \in \left(0, \mu_1^{max}\right]. \tag{9}$$

Let s_1 be fixed. Since the function $s_0 \mapsto \mu_0(s_0, s_1)$ is increasing, it has an inverse function $y \mapsto M_0(y, s_1)$, such that

$$s_0 = M_0(y, s_1) \iff y = \mu_0(s_0, s_1), \quad \text{for all} \quad s_0, s_1 \geq 0 \quad \text{and} \quad y \in [0, \bar{\mu}_0(s_1)). \tag{10}$$

The following result shows that M_0 is increasing in the first and second variables.

Lemma 3.1. *Under assumption (H3), we have for all $y \in [0, \bar{\mu}_0(s_1))$ and $s_1 \geq 0$,*

$$\frac{\partial M_0}{\partial y}(y, s_1) > 0 \quad \text{and} \quad \frac{\partial M_0}{\partial s_1}(y, s_1) > 0.$$

The following result gives the necessary and sufficient conditions of existence of all steady states of (5).

Proposition 3.1. *Assume that assumptions (H1)–(H4) hold. Then, (5) has at most six steady states:*

- $SS_0 = \left(s_0^{in}, 0, s_1^{in}, 0\right)$*, that always exists.*
- $SS_1 = (s_0, x_0, s_1, 0)$*, with s_0 is the solution of equation: $\mu_0\left(s_0, s_0^{in} + s_1^{in} - s_0\right) = D_0$,*

$$x_0 = \frac{D}{D_0}\left(s_0^{in} - s_0\right) \quad \text{and} \quad s_1 = s_0^{in} + s_1^{in} - s_0.$$

It exists if, and only if,

$$s_0^{in} > F_0\left(D, s_1^{in}\right) \quad \text{with} \quad D \in I_0 := \left[0, \bar{D}_0\left(s_1^{in}\right)\right), \tag{11}$$

where F_0 and $\bar{D}_0\left(s_1^{in}\right)$ are defined by

$$F_0\left(D, s_1^{in}\right) := M_0\left(\alpha_0 D + a_0, s_1^{in}\right), \quad \bar{D}_0\left(s_1^{in}\right) := \frac{\bar{\mu}_0\left(s_1^{in}\right) - a_0}{\alpha_0}. \tag{12}$$

- $SS_2^j = \left(s_0^j, x_0^j, s_1^j, x_1^j\right)$*, $j = 1, 2$, with*

$$s_0^j = F_1^j(D), \quad x_0^j = \frac{D}{D_0}\left(s_0^{in} - s_0^j\right), \quad s_1^j = F_2^j(D) - F_1^j(D), \quad x_1^j = \frac{D}{D_1}\left(s_0^{in} + s_1^{in} - s_0^j - s_1^j\right),$$

where F_i^j, $i = 1, 2$, are defined by

$$F_1^j(D) := M_0\left(\alpha_0 D + a_0, M_1^j(\alpha_1 D + a_1)\right) \quad \text{and} \quad F_2^j(D) := M_1^j(\alpha_1 D + a_1) + F_1^j(D). \tag{13}$$

It exists if, and only if,

$$s_0^{in} > F_1^j(D) \quad \text{and} \quad s_0^{in} + s_1^{in} > F_2^j(D), \quad \text{with} \quad D \in I_j, \tag{14}$$

where I_j is the definition domain of the function F_i^j, $i = 1, 2$, which is defined by

$$I_j := \left\{D \in \bar{I}_j : \Phi_j(D) > 0\right\}, \tag{15}$$

where $\bar{D}_1 := (\mu_1^{max} - a_1)/\alpha_1$, $\bar{I}_1 := \left[0, \bar{D}_1\right]$, $\bar{I}_2 := \bar{I}_1$ when $a_1 > 0$, $\bar{I}_2 := \left(0, \bar{D}_1\right]$ when $a_1 = 0$, and the function $\Phi_j(\cdot)$ is defined by

$$\Phi_j(D) := \bar{\mu}_0\left(M_1^j(D_1)\right) - D_0, \quad j = 1, 2. \tag{16}$$

- $SS_3^j = \left(s_0^{in}, 0, s_1^j, x_1^j\right)$*, $j = 1, 2$, with*

$$s_1^j = M_1^j(D_1) \quad \text{and} \quad x_1^j = \frac{D}{D_1}\left(s_1^{in} - M_1^j(D_1)\right).$$

It exists if, and only if,

$$s_1^{in} > M_1^j(D_1) \quad \text{with} \quad D \in \bar{I}_j. \tag{17}$$

In the case of the specific growth rates (6), the function F_i^j can be calculated explicitly (see Appendix C). We shall allow $\bar{D}_0\left(s_1^{in}\right)$ and/or $\bar{D}_1$ to be equal to $+\infty$ when $\alpha_0 = 0$ and/or $\alpha_1 = 0$.

In the particular case $s_1^{in} = 0$, the existence condition (17) of the steady state SS_3^j, $j = 1, 2$, is not satisfied. Thus, we obtain the same result as in [11, 26] when SS_3^j does not exist. The main change in the existence of steady states of our model (5) compared to [26] is the appearance of a second positive steady state of type SS_2 and two steady states of type SS_3.

3.2. Stability of the steady states

The local asymptotic stability of each steady state of syntrophic model (5) is determined by the sign of the real part of eigenvalues of the corresponding Jacobian matrix or by the Routh-Hurwitz criterion (in the case of SS_2^j, $j = 1, 2$). In the following, we use the abbreviation LES for locally exponentially stable steady state.

Proposition 3.2. *Assume that assumptions (H1)–(H4) hold, we have*

- *SS_0 is LES if, and only if, $D \notin I_0$, or*
$$s_0^{in} < F_0\left(D, s_1^{in}\right) \quad with \quad D \in I_0 \tag{18}$$
and $D > \bar{D}_1$ or
$$s_1^{in} < M_1^1(D_1) \quad with \quad D \in \bar{I}_1 \quad or \quad s_1^{in} > M_1^2(D_1) \quad with \quad D \in \bar{I}_2. \tag{19}$$
- *SS_1 is LES if, and only if, $D \in I_0 \backslash I_1$ or*
$$s_0^{in} + s_1^{in} < F_2^1(D) \quad with \quad D \in I_0 \cap I_1 \quad or \quad s_0^{in} + s_1^{in} > F_2^2(D) \quad with \quad D \in I_0 \cap I_2. \tag{20}$$
- *SS_2^1 is LES for all $D \in I_1 \backslash \bar{D}_1$.*
- *SS_2^2 is unstable for all $D \in I_2 \backslash \bar{D}_1$.*
- *SS_3^1 is LES if, and only if, $D \in \bar{I}_1 \backslash \left(I_1 \cup \left\{\bar{D}_1\right\}\right)$ or for all $D \in I_1 \backslash \left\{\bar{D}_1\right\}$, $s_0^{in} < F_1^1(D)$.*
- *SS_3^2 is unstable for all $D \in \bar{I}_2 \backslash \bar{D}_1$.*

The results of Propositions 3.1 and 3.2 can be summarized in Table 3. All critical values of D are summarized in Table 4.

Table 3: Conditions of existence and local stability of steady states of model (5) where $\bar{D}_1$ is defined in Table 4 and I_0, I_j, and $\bar{I}_j$, $j = 1, 2$, are defined in Table 5.

Steady state	Existence interval	Existence condition	Stability condition
SS_0	for all D	always exists	($D \notin I_0$ or (18) holds) and $\left(D > \bar{D}_1 \text{ or (19) holds}\right)$
SS_1	$D \in I_0$	$s_0^{in} > F_0\left(D, s_1^{in}\right)$	$D \in I_0 \backslash I_1$ or (20) holds
SS_2^1	$D \in I_1$	$s_0^{in} > \max\left(F_1^1(D), F_2^1(D) - s_1^{in}\right)$	LES for all $D \in I_1 \backslash \bar{D}_1$
SS_2^2	$D \in I_2$	$s_0^{in} > \max\left(F_1^2(D), F_2^2(D) - s_1^{in}\right)$	unstable for all $D \in I_2 \backslash \bar{D}_1$.
SS_3^1	$D \in \bar{I}_1$	$s_1^{in} > M_1^1(D_1)$	$D \in \bar{I}_1 \backslash (I_1 \cup \left\{\bar{D}_1\right\})$ or for all $D \in I_1 \backslash \left\{\bar{D}_1\right\}$, $s_0^{in} < F_1^1(D)$
SS_3^2	$D \in \bar{I}_2$	$s_1^{in} > M_1^2(D_1)$	unstable for all $D \in \bar{I}_2 \backslash \bar{D}_1$.

Table 4: Summary of the various critical values of D. Note that $\widetilde{D}_i > \widetilde{D}_j$ for all $i < j$, where $\bar{\mu}_0$ is defined in (H5), μ_1^{max} is defined in (H4), $\Phi_1(\cdot)$ and $\Phi_2(\cdot)$ are defined by (16), and $M_1^1(\cdot)$ and $M_1^2(\cdot)$ are defined by (8) and (9), respectively.

Critical values	Expression
D_i, $i = 1, 2$	$\alpha_i D + a_i$
$\bar{D}_0\left(s_1^{in}\right)$	$(\bar{\mu}_0\left(s_1^{in}\right) - a_0)/\alpha_0$
$\bar{D}_1$	$(\mu_1^{max} - a_1)/\alpha_1$
$\hat{D}_1$	solution of $\Phi_1(D) = 0$
$\widetilde{D}_i$, $i = 1, \ldots, n$	solutions of $\Phi_2(D) = 0$
D_j^*, $j = 1, 2$	solutions of $M_1^j(D_1) = s_1^{in}$

In fact, the following two cases must be distinguished:

$$\text{case 1: } \Phi_j(\bar{D}_1) > 0 \quad \Leftrightarrow \quad \frac{\bar{\mu}_0\left(s_1^{max}\right) - a_0}{\alpha_0} > \bar{D}_1, \quad \text{case 2: } \Phi_j(\bar{D}_1) \leq 0 \quad \Leftrightarrow \quad \frac{\bar{\mu}_0\left(s_1^{max}\right) - a_0}{\alpha_0} \leq \bar{D}_1. \tag{21}$$

The definition domains I_0, I_j and $\bar{I}_j$ of functions $F_0\left(\cdot, s_1^{in}\right)$, $F_i^j(\cdot)$ and $D \mapsto M_1^j(\alpha_1 D + a_1)$, $i, j = 1, 2$, respectively, are summarized in Table 5. Some comments and details on the definition domains I_0 and I_j are given in Appendix B where the interval I_1 is given by (B.3) and the interval I_2 is given by (B.8) if $\alpha_0 = 0$ or $\alpha_1 = 0$ and (B.11) otherwise.

Table 5: Summary of the definition domains I_0, I_j and $\bar{I}_j$, $j = 1,2$, where $\bar{D}_0(\cdot)$ and $\bar{D}_1$ are defined in Table 4, and $\Phi_1(\cdot)$ and $\Phi_2(\cdot)$ are defined by (16).

Function	Interval
$F_0\left(\cdot, s_1^{in}\right)$	$I_0 = \left[0, \bar{D}_0\left(s_1^{in}\right)\right)$
$M_1^1(\alpha_1 D + a_1)$	$\bar{I}_1 = \left[0, \bar{D}_1\right]$ when $\alpha_1 > 0$ and $\bar{I}_1 = [0, +\infty)$ when $\alpha_1 = 0$
$M_1^2(\alpha_1 D + a_1)$	$\bar{I}_2 = \bar{I}_1$ when $a_1 > 0$ and $\bar{I}_2 = \bar{I}_1 \backslash \{0\}$ when $a_1 = 0$
$F_i^j(D)$, $i, j = 1,2$	$I_j = \left\{D \in \bar{I}_j : \Phi_j(D) > 0\right\}$

4. Operating diagrams

The operating diagrams show the asymptotic behavior of the system when the control parameters D, s_0^{in} and s_1^{in} vary, as they are the most easily parameters to manipulate in a chemostat. All other parameters are fixed since they have biological meaning and cannot be easily manipulated by the biologist. In what follows, we define the curve γ_0 of equation $s_0^{in} = F_0\left(D, s_1^{in}\right)$, the curve γ_1^j of equation $s_0^{in} = F_1^j(D)$ and the curve γ_2^j of equation $s_0^{in} = F_2^j(D) - s_1^{in}$, $j = 1,2$. The following result describes the properties of the functions F_0 and F_i^j, $i, j = 1,2$, according to the control parameter s_1^{in}.

Proposition 4.1. *We have $I_2 \subset I_1$ and $F_i^1(D) \le F_i^2(D)$, $i = 1,2$, for all $D \in I_2$. For all s_1^{in},*

$$\lim_{D \to \bar{D}_0^-} F_0\left(D, s_1^{in}\right) = +\infty.$$

When case 1 of (21) holds, we have

$$F_i^1\left(\bar{D}_1\right) = F_i^2\left(\bar{D}_1\right), \quad i = 1,2.$$

When the critical values $\hat{D}_1$ and $\widetilde{D}_i$ exist, respectively, we have

$$\lim_{D \to \hat{D}_1^-} F_i^1(D) = +\infty, \qquad \lim_{D \to \widetilde{D}_i^-} F_i^2(D) = +\infty.$$

Proposition 4.2. *Assume that $s_1^{in} < s_1^{max}$. We have, $I_2 \subset I_0$ such that $\bar{D}_1 < \bar{D}_0\left(s_1^{in}\right)$ when case 1 of (21) holds and $\max_i\left(\widetilde{D}_i\right) < \bar{D}_0\left(s_1^{in}\right)$ when case 2 of (21). In addition,*

$$F_0\left(D, s_1^{in}\right) < F_1^2(D) < F_2^2(D) - s_1^{in}, \quad \textit{for all} \quad D \in I_2.$$

Moreover, there exists a solution $D = D_1^* \in \left(0, \bar{D}_1\right)$ of equation $s_1^{in} = M_1^1\left(\alpha_1 D + a_1\right)$ if, and only if,

$$M_1^1(a_1) < s_1^{in}. \tag{22}$$

It is unique if it exists. When condition (22) holds such that $D_1^* \in I_1$, the three curves γ_0, γ_1^1 and γ_2^1 intersect at the same point $D = D_1^*$ (see Figs. 1- 2(b)) such that for all $D \in \left[0, D_1^*\right)$,

$$F_0\left(D, s_1^{in}\right) > F_1^1(D) > F_2^1(D) - s_1^{in} \tag{23}$$

and for all $D \in \left(D_1^*, \bar{D}_1\right) \cap I_0 \cap I_1$,

$$F_0\left(D, s_1^{in}\right) < F_1^1(D) < F_2^1(D) - s_1^{in}. \tag{24}$$

When condition (22) holds such that $D_1^* \in \left[\hat{D}_1, \bar{D}_1\right]$, the three curves γ_0, γ_1^1 and γ_2^1 do not intersect such that for all $D \in I_0$, (23) holds where $\bar{D}_0\left(s_1^{in}\right) < \hat{D}_1$ (see Fig. 2(c)). When condition (22) is not fulfilled, the three curves γ_0, γ_1^1 and γ_2^1 do not intersect such that for all $D \in I_1$, (24) holds where $\min\left(\hat{D}_1, \bar{D}_1\right) < \bar{D}_0\left(s_1^{in}\right)$ (see Figs. 1-2(a)). In case 1 of (21), we have $\bar{D}_1 < \bar{D}_0\left(s_1^{in}\right)$.

Proposition 4.3. *Assume that $s_1^{in} = s_1^{max}$. We have, for all $D \in I_2 \backslash \left\{\bar{D}_1\right\}$,*

$$F_0\left(D, s_1^{in}\right) < F_1^2(D) < F_2^2(D) - s_1^{in},$$

and for all $D \in I_0 \cap I_1 \setminus \{\bar{D}_1\}$,

$$F_2^1(D) - s_1^{in} < F_1^1(D) < F_0\left(D, s_1^{in}\right).$$

In case 1 of (21), there exists a unique solution $D = \bar{D}_1$ *of equations* $s_1^{in} = M_1^j(\alpha_1 D + a_1)$, $j = 1, 2$, *such that the five curves* γ_0 *and* γ_i^j, $i, j = 1, 2$, *intersect at the same point* $D = \bar{D}_1$ *(see Fig. 3(a)) and for all* $D \in [0, \bar{D}_1)$,

$$F_2^1(D) - s_1^{in} < F_1^1(D) < F_0\left(D, s_1^{in}\right) < F_1^2(D) < F_2^2(D) - s_1^{in}. \tag{25}$$

In case 2 of (21), the condition (25) holds for all $D \in I_2$.

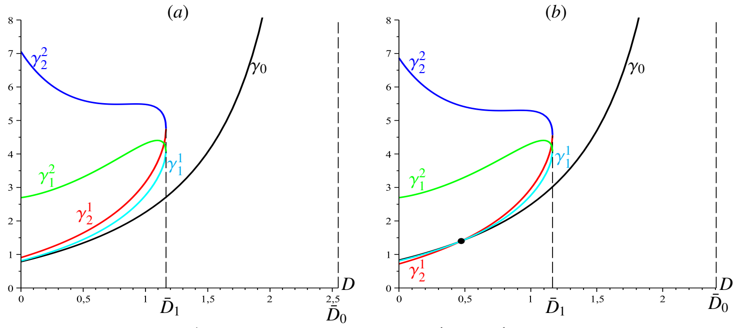

Fig. 1: Case $s_1^{in} < s_1^{max} \simeq 0.689$ and case 1 of (21): (a) $s_1^{in} = 0$, the three curves γ_0, γ_1^1 and γ_2^1 do not intersect where (24) holds, (b) $s_1^{in} = 0.2$, they intersect in D_1^*.

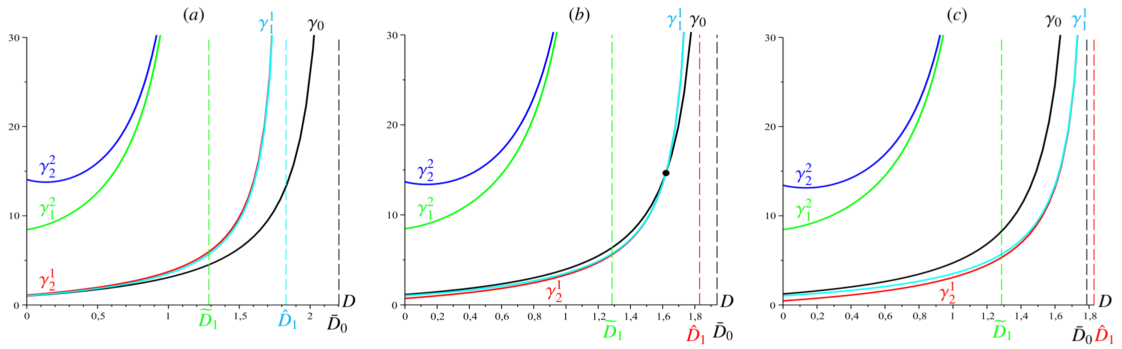

Fig. 2: Case $s_1^{in} < s_1^{max} \simeq 0.689$ and case 2 of (21): (a) $s_1^{in} = 0.01$, the three curves γ_0, γ_1^1 and γ_2^1 do not intersect where (24) holds, (b) $s_1^{in} = 0.4$, they intersect in D_1^*, (c) $s_1^{in} = 0.65$, they do not intersect where (23) holds.

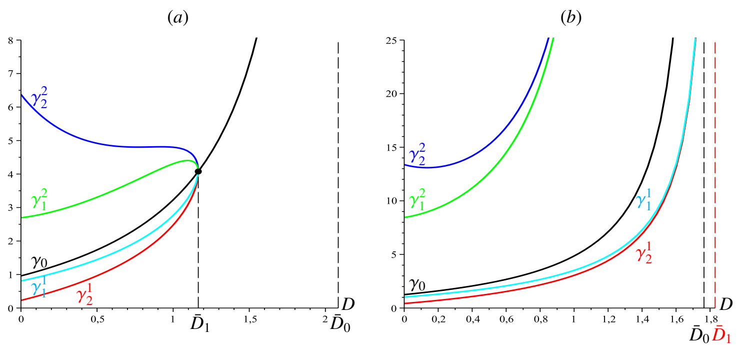

Fig. 3: Case $s_1^{in} = s_1^{max} \simeq 0.689$: (a) case 1 of (21) where all curves intersect in $\bar{D}_1$ and (25) holds, (b) case 2 of (21) where all curves do not intersect.

Proposition 4.4. *Assume that* $s_1^{in} > s_1^{max}$. *If case 2 of (21) holds, then* $I_0 \subset I_1$ *such that* $\bar{D}_0\left(s_1^{in}\right) < \hat{D}_1$. *For all* $D \in I_0 \cap I_1$, *we have*

$$F_2^1(D) - s_1^{in} < F_1^1(D) < F_0\left(D, s_1^{in}\right).$$

Moreover, there exists a solution $D = D_2^* \in \left(0, \bar{D}_1\right)$ *of equation* $s_1^{in} = M_1^2(\alpha_1 D + a_1)$ *if, and only if,*

$$M_1^2(a_1) > s_1^{in}. \tag{26}$$

It is unique if it exists. When condition (26) holds such that $D_2^* \in I_2$, the three curves γ_0, γ_1^2 and γ_2^2 intersect at the same point $D = D_2^*$ (see Figs. 4-5(b)) such that for all $D \in \left[0, D_2^*\right) \cap I_2$,

$$F_0\left(D, s_1^{in}\right) < F_1^2(D) < F_2^2(D) - s_1^{in}, \tag{27}$$

and for all $D \in \left(D_2^*, \bar{D}_1\right) \cap I_0 \cap I_2$,

$$F_0\left(D, s_1^{in}\right) > F_1^2(D) > F_2^2(D) - s_1^{in}. \tag{28}$$

When condition (26) holds such that $D_2^* \notin I_2$, the three curves γ_0, γ_1^2 and γ_2^2 do not intersect. When condition (26) is not fulfilled, (28) holds for all $D \in I_0 \cap I_2$ such that the three curves γ_0, γ_1^2 and γ_2^2 do not intersect (see Figs. 4(a)-5(c)).

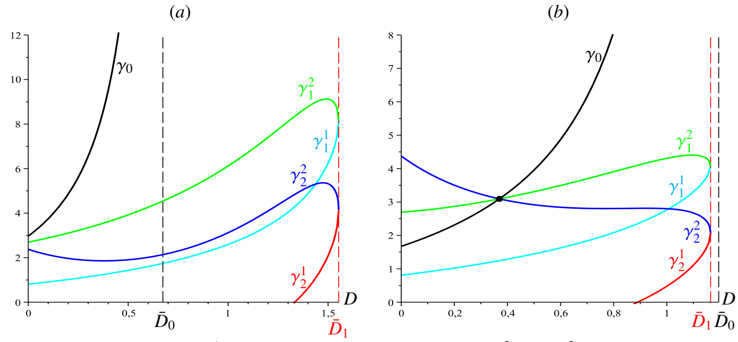

Fig. 4: Case $s_1^{in} > s_1^{max} \simeq 0.689$ and case 1 of (21): (a) $s_1^{in} = 2.689$, the three curves γ_0, γ_1^2 and γ_2^2 do not intersect and (28) holds, (b) $s_1^{in} = 4.689$, they intersect in D_2^*.

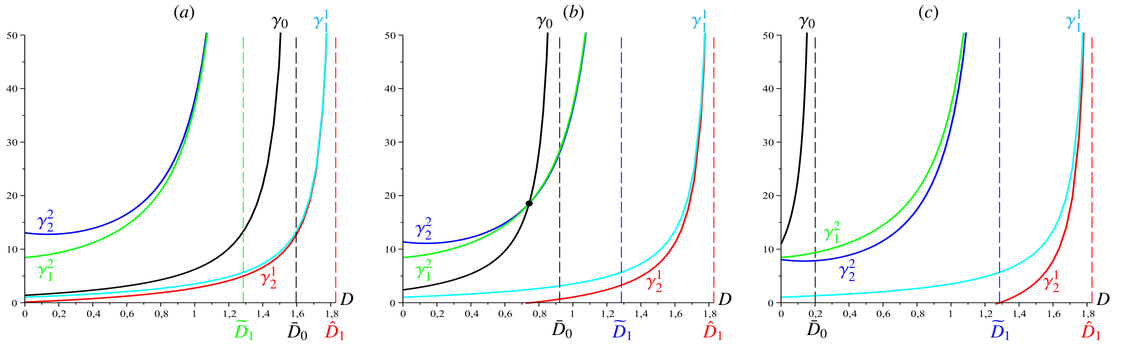

Fig. 5: Case $s_1^{in} > s_1^{max} \simeq 0.689$ and case 2 of (21): (a) $s_1^{in} = 1$, the three curves γ_0, γ_1^2 and γ_2^2 do not intersect and (27) holds, (b) $s_1^{in} = 2.69$, they intersect in D_2^*, (c) $s_1^{in} = 6$, they do not intersect where (28) holds.

4.1. Operating diagrams in the plane $\left(D, s_0^{in}\right)$ when s_1^{in} fixed

To better understand the effect of the second input substrate concentration s_1^{in}, we illustrate the operating diagrams in the $\left(D, s_0^{in}\right)$ plane first when $s_1^{in} = 0$ and then for different values of s_1^{in}. In the following tables, the letter S (resp. U) means that the corresponding steady state is LES (resp. unstable). No letter means that the steady state does not exist.

Proposition 4.5. *When $s_1^{in} = 0$, Table 6 shows the existence and stability of the steady states SS_0, SS_1, SS_2^1 and SS_2^2 in the regions of the operating diagrams in Figs. 6 and 7.*

Table 6: Existence and stability of steady states in the regions of the operating diagrams in Figs. 6 and 7, when $s_1^{in} = 0$.

Condition	Region	SS_0	SS_1	SS_2^1	SS_2^2
$s_0^{in} < F_0(D, 0)$	$\left(D, s_0^{in}\right) \in \mathcal{J}_1$	S			
$F_0(D, 0) < s_0^{in} < F_2^1(D)$	$\left(D, s_0^{in}\right) \in \mathcal{J}_2$	U	S		
$F_2^1(D) < s_0^{in} < F_2^2(D)$	$\left(D, s_0^{in}\right) \in \mathcal{J}_3$	U	U	S	
$s_0^{in} > F_2^2(D)$	$\left(D, s_0^{in}\right) \in \mathcal{J}_4$	U	S	S	U

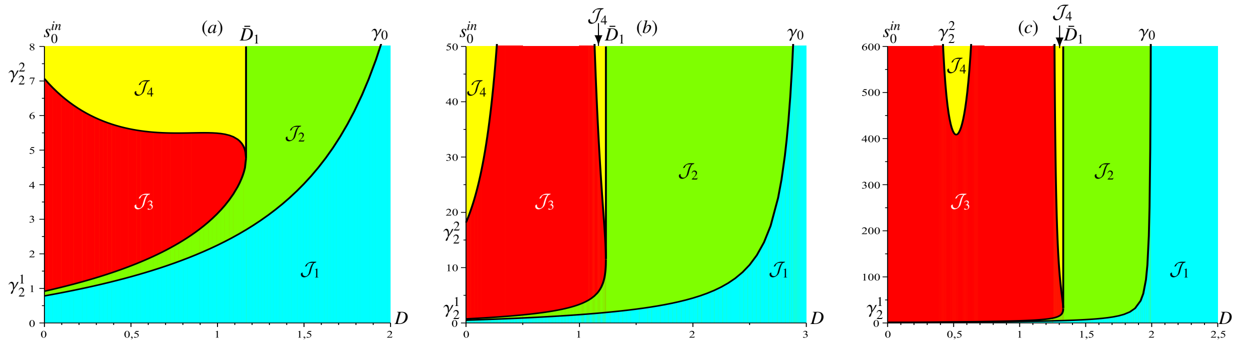

Fig. 6: Operating diagrams of (5) when $s_1^{in} = 0$ and case 1 of (21) holds: (a) equation $\Phi_2(D) = 0$ has no solution, (b) has two solutions and (c) has three solutions.

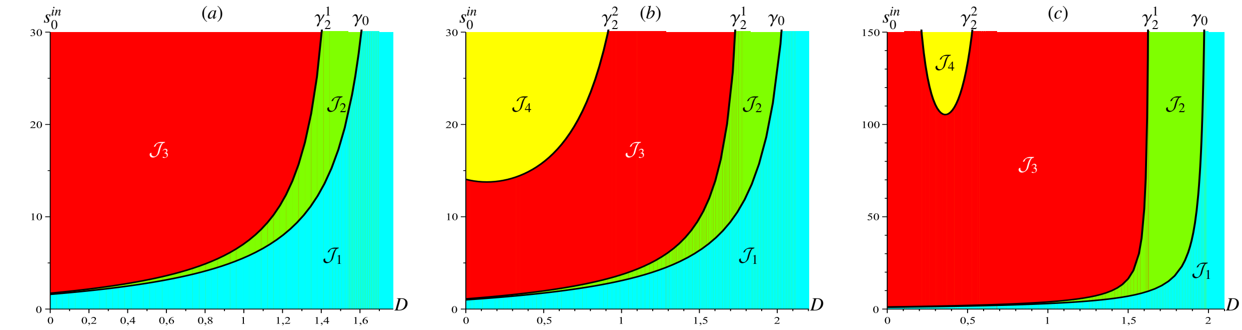

Fig. 7: Operating diagrams of (5) when $s_1^{in} = 0$ and case 2 of (21) holds: (a) equation $\Phi_2(D) = 0$ has no solution (b) has one solution and (c) two solutions.

When $s_1^{in} = 0$, it follows from Table 3 that SS_3^1 and SS_3^2 do not exist and the operating diagram is divided into at most four regions, as shown in Figs. 6 and 7. When equation $\Phi_2(D) = 0$ has one solution, the region $\mathcal{J}_4$ at the left-hand end of Fig. 6(c) is empty in the case where $s_1^{in} = 0$ and the case 1 of (21) holds. In all operating diagrams, the cyan region $\mathcal{J}_1$ corresponds to the washout steady state SS_0 is LES. The green region $\mathcal{J}_2$ corresponds to the exclusion of the species x_1 (SS_1 is LES). The red region $\mathcal{J}_3$ corresponds to the existence of both species (SS_2^1 is LES) and the yellow region $\mathcal{J}_4$ corresponds to the bi-stability (SS_1 and SS_2^1 are LES).

The transition from the region $\mathcal{J}_1$ to the region $\mathcal{J}_2$ by the curve γ_0 corresponds to a transcritical bifurcation making the steady state SS_0 unstable with the appearance of the LES steady state SS_1. The transition from the region $\mathcal{J}_2$ to the region $\mathcal{J}_3$ by the curve γ_2^1 corresponds to a transcritical bifurcation making the steady state SS_1 unstable with the appearance of the LES steady state SS_2^1. The transition from the region $\mathcal{J}_3$ to the region $\mathcal{J}_4$ by the curve γ_2^2 corresponds to a transcritical bifurcation making the steady state SS_1 stable with the appearance of the unstable steady state SS_2^2. However the transition from the region $\mathcal{J}_2$ to the region $\mathcal{J}_4$ by the line of equation $D = \bar{D}_1$ in case 1 of (21) corresponds to a saddle-node bifurcation with the appearance of two positive steady states SS_2^1 and SS_2^2 which are LES and unstable, respectively.

Thus, the operating diagrams in Figs. 6 and 7 show the effect of substrate inhibition on the emergence of the bi-stability region $\mathcal{J}_4$ which is empty in the case where the growth rate μ_1 is monotone increasing [6, 26]. When the inhibition factor K_I in the growth function μ_1 given by (6) decreases, the operating diagrams show the occurrence of $\mathcal{J}_4$ first and then its disappearance and that of the coexistence region $\mathcal{J}_3$ for small enough value of K_I [11]. In the following, we study the operating diagram when $0 < s_1^{in} < s_1^{max}$ in order to show the effect of this control parameter s_1^{in} on the emergence of new regions and on their size. Indeed, by increasing the input concentration s_1^{in} from zero to s_1^{max}, steady state SS_3^1 appears but SS_3^2 does not.

Proposition 4.6. *When $0 < s_1^{in} < s_1^{max}$, Tables 7 and 8 show the existence and stability of the steady states SS_0, SS_1, SS_2^1, SS_2^2 and SS_3^1 in the regions of the operating diagrams in Figs. 8-9(a), respectively.*

Note that the blue region $\mathcal{J}_5$ corresponds to the bi-stability of SS_1 and SS_2^1 while SS_0, SS_2^2 and SS_3^1 are unstable. The pink region $\mathcal{J}_6$ corresponds to the case where SS_2^1 is LES while SS_0, SS_1 and SS_3^1 are unstable. The gray region $\mathcal{J}_7$ corresponds to the case where SS_2^1 is LES while SS_0 and SS_3^1 are unstable. The magenta region $\mathcal{J}_8$ corresponds to the case where the steady state SS_3^1 of exclusion of the species x_0 is LES while SS_0 is unstable.

The transition from the region $\mathcal{J}_4$ to the region $\mathcal{J}_5$ by the line of equation $D = D_1^*$ corresponds to a transcritical bifurcation of SS_0 and SS_3^1 which appears unstable. The transition from the region $\mathcal{J}_5$ to the region $\mathcal{J}_6$ by the curve γ_2^2 corresponds to a transcritical bifurcation making SS_1 unstable with the disappearance of SS_2^2. The transition from the region $\mathcal{J}_6$ to the region $\mathcal{J}_7$ by the curve γ_0 corresponds to a transcritical bifurcation of SS_0 and SS_1 which disappear. The transition from the region $\mathcal{J}_7$ to the region $\mathcal{J}_8$ by the curve γ_1^1 corresponds to a transcritical bifurcation making SS_3^1 LES while SS_2^1 disappears.

Table 7: Existence and stability of steady states in the regions of the operating diagram in Fig. 8, when $0 < s_1^{in} < s_1^{max}$ and case 1 of (21) holds. Note that $\mathcal{I}_1 = \left(0, D_1^*\right)$, $\mathcal{I}_2 = \left(D_1^*, \bar{D}_1\right)$ and $\mathcal{I}_3 = \left(D_1^*, +\infty\right)$ where D_1^* and $\bar{D}_1$ are defined in Table 4.

Condition	Interval	Region	SS_0	SS_1	SS_2^1	SS_2^2	SS_3^1
$s_0^{in} < F_0\left(D, s_1^{in}\right)$	$D \in \mathcal{I}_3$	$\left(D, s_0^{in}\right) \in \mathcal{J}_1$	S				
$F_0\left(D, s_1^{in}\right) < s_0^{in} < F_2^1(D) - s_1^{in}$	$D \in \mathcal{I}_3$	$\left(D, s_0^{in}\right) \in \mathcal{J}_2$	U	S			
$F_2^1(D) - s_1^{in} < s_0^{in} < F_2^2(D) - s_1^{in}$	$D \in \mathcal{I}_2$	$\left(D, s_0^{in}\right) \in \mathcal{J}_3$	U	U	S		
$s_0^{in} > F_2^2(D) - s_1^{in}$	$D \in \mathcal{I}_2$	$\left(D, s_0^{in}\right) \in \mathcal{J}_4$	U	S	S	U	
$s_0^{in} > F_2^2(D) - s_1^{in}$	$D \in \mathcal{I}_1$	$\left(D, s_0^{in}\right) \in \mathcal{J}_5$	U	S	S	U	U
$F_0\left(D, s_1^{in}\right) < s_0^{in} < F_2^2(D) - s_1^{in}$	$D \in \mathcal{I}_1$	$\left(D, s_0^{in}\right) \in \mathcal{J}_6$	U	U	S		U
$F_1^1(D) < s_0^{in} < F_0\left(D, s_1^{in}\right)$	$D \in \mathcal{I}_1$	$\left(D, s_0^{in}\right) \in \mathcal{J}_7$	U		S		U
$s_0^{in} < F_1^1(D)$	$D \in \mathcal{I}_1$	$\left(D, s_0^{in}\right) \in \mathcal{J}_8$	U				S

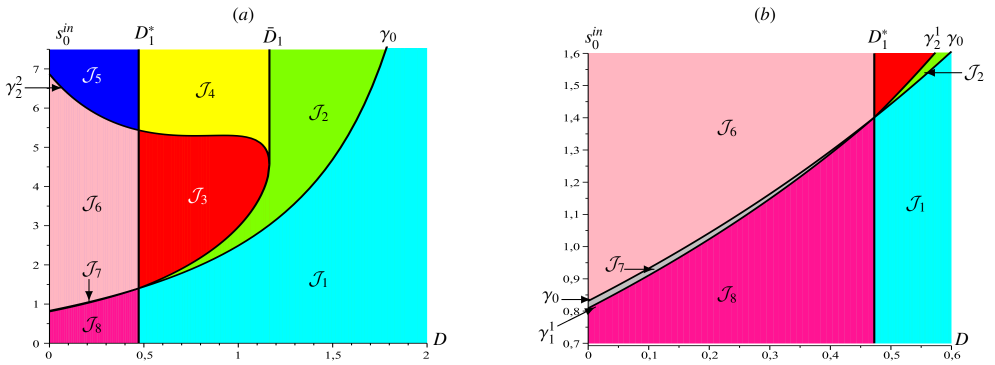

Fig. 8: Operating diagram of (5): $s_1^{in} = 0.2 < s_1^{max} \simeq 0.689$ and case 1 of (21) holds when equation $\Phi_2(D) = 0$ has no solution. (b) A magnification showing region $\mathcal{J}_7$.

Fig. 8 shows that the regions $\mathcal{J}_1$–$\mathcal{J}_4$ are identical to those of the operating diagram in Fig. 6(a) when $s_1^{in} = 0$. However, increasing the control parameter s_1^{in} leads to the occurrence of four other regions $\mathcal{J}_5$–$\mathcal{J}_8$ in Figs. 8 and 9(a) in which at most one steady state corresponding to the exclusion of species x_0 is stable. When $s_1^{in} < s_1^{max}$ and case 1 of (21) holds, the operating diagram is divided into at most eight regions. In case 2 of (21), the operating diagram in Fig. 9(a) shows the disappearance of three regions $\mathcal{J}_2 - \mathcal{J}_4$; the operating diagram is then divided into at most five regions. As in the operating diagrams in Figs. 6 and 7, we can find the same number of regions when equation $\Phi_2(D) = 0$ has several roots.

Table 8: Existence and stability of steady states in the regions of the operating diagram in Fig. 9(a), when $0 < s_1^{in} < s_1^{max}$ and case 2 of (21) holds. Note that $\mathcal{I}_1 = \left(0, D_1^*\right)$ and $\mathcal{I}_3 = \left(D_1^*, +\infty\right)$ where D_1^* is defined in Table 4.

Condition	Interval	Region	SS_0	SS_1	SS_2^1	SS_2^2	SS_3^1
For all s_0^{in}	$D \in \mathcal{I}_3$	$\left(D, s_0^{in}\right) \in \mathcal{J}_1$	S				
$s_0^{in} < F_1^1(D)$	$D \in \mathcal{I}_1$	$\left(D, s_0^{in}\right) \in \mathcal{J}_8$	U				S
$F_1^1(D) < s_0^{in} < F_0\left(D, s_1^{in}\right)$	$D \in \mathcal{I}_1$	$\left(D, s_0^{in}\right) \in \mathcal{J}_7$	U		S		U
$F_0\left(D, s_1^{in}\right) < s_0^{in} < F_2^2(D) - s_1^{in}$	$D \in \mathcal{I}_1$	$\left(D, s_0^{in}\right) \in \mathcal{J}_6$	U	U	S		U
$s_0^{in} > F_2^2(D) - s_1^{in}$	$D \in \mathcal{I}_1$	$\left(D, s_0^{in}\right) \in \mathcal{J}_5$	U	S	S	U	U

Proposition 4.7. *When $s_1^{in} = s_1^{max}$, Table 9 shows the existence and stability of the steady states SS_0, SS_1, SS_2^1, SS_2^2 and SS_3^1 in the regions of the operating diagram in Fig. 9(b) where case 1 of (21) holds. Moreover, when case 2 of (21) holds, the operating diagram is similar to that in Table 8 and Fig. 9(a) where it is divided into five regions with $D_1^* = \bar{D}_1$.*

When $s_1^{in} = s_1^{max}$, the operating diagram in case 1 of (21) is similar to that of case $s_1^{in} < s_1^{max}$ by eliminating the regions $\mathcal{J}_3$ and $\mathcal{J}_4$ (see Table 7 and Fig. 8), where it is divided into six regions.

Proposition 4.8. *When $s_1^{in} > s_1^{max}$, Table 10 shows the existence and stability of the steady states SS_0, SS_1, SS_2^1, SS_2^2, SS_3^1 and SS_3^2 in the regions of the operating diagram in Fig. 10(a) where case 1 of (21) holds and equation $\Phi_2(D) = 0$ has no solution. In addition, when case 2 of (21) holds, the operating diagram in Fig. 11(b) is similar to that in Table 10 where the region $\mathcal{J}_2$ is empty.*

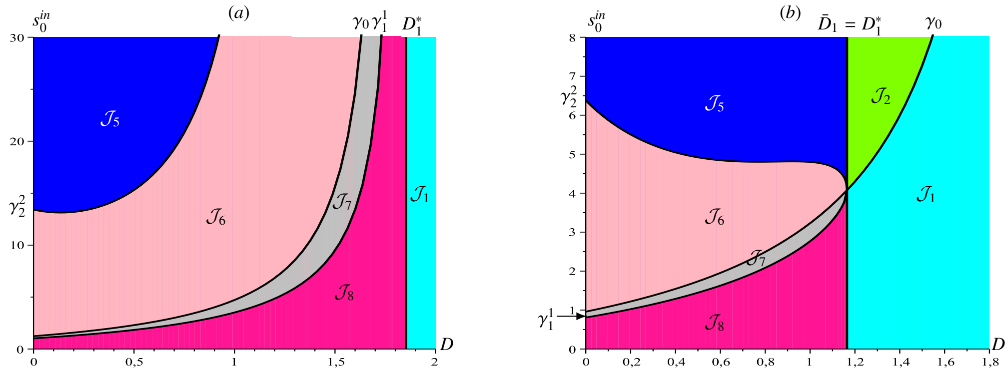

Fig. 9: Operating diagrams of (5): (a) $s_1^{in} = 0.65 < s_1^{max} \simeq 0.689$ and case 2 of (21) holds when equation $\Phi_2(D) = 0$ has one solution. (b) $s_1^{in} = s_1^{max} \simeq 0.689$ and case 1 of (21) holds when equation $\Phi_2(D) = 0$ has no solution.

Table 9: Existence and stability of steady states in the regions of the operating diagram in Fig. 9(b), when $s_1^{in} = s_1^{max}$ and case 1 of (21) holds. Note that $\mathcal{I}_1 = \left(0, D_1^*\right)$ and $\mathcal{I}_3 = \left(D_1^*, +\infty\right)$ where D_1^* is defined in Table 4.

Condition	Interval	Region	SS_0	SS_1	SS_2^1	SS_2^2	SS_3^1
$s_0^{in} < F_0\left(D, s_1^{in}\right)$	$D \in \mathcal{I}_3$	$\left(D, s_0^{in}\right) \in \mathcal{J}_1$	S				
$s_0^{in} > F_0\left(D, s_1^{in}\right)$	$D \in \mathcal{I}_3$	$\left(D, s_0^{in}\right) \in \mathcal{J}_2$	U	S			
$s_0^{in} > F_2^2(D) - s_1^{in}$	$D \in \mathcal{I}_1$	$\left(D, s_0^{in}\right) \in \mathcal{J}_5$	U	S	S	U	U
$F_0\left(D, s_1^{in}\right) < s_0^{in} < F_2^2(D) - s_1^{in}$	$D \in \mathcal{I}_1$	$\left(D, s_0^{in}\right) \in \mathcal{J}_6$	U	U	S		U
$F_1^1(D) < s_0^{in} < F_0\left(D, s_1^{in}\right)$	$D \in \mathcal{I}_1$	$\left(D, s_0^{in}\right) \in \mathcal{J}_7$	U		S		U
$s_0^{in} < F_1^1(D)$	$D \in \mathcal{I}_1$	$\left(D, s_0^{in}\right) \in \mathcal{J}_8$	U				S

Table 10: Existence and stability of steady states in the regions of the operating diagram in Fig. 10(a), when $s_1^{in} > s_1^{max}$ and case 1 of (21) holds. Note that $\mathcal{I}_4 = \left(0, D_2^*\right)$, $\mathcal{I}_5 = \left(D_2^*, \bar{D}_1\right)$ and $\mathcal{I}_6 = \left(\bar{D}_1, +\infty\right)$ where D_2^* and $\bar{D}_1$ are defined in Table 4.

Condition	Interval	Region	SS_0	SS_1	SS_2^1	SS_2^2	SS_3^1	SS_3^2
$s_0^{in} < F_0\left(D, s_1^{in}\right)$	$D \in \mathcal{I}_6$	$\left(D, s_0^{in}\right) \in \mathcal{J}_1$	S					
$s_0^{in} > F_0\left(D, s_1^{in}\right)$	$D \in \mathcal{I}_6$	$\left(D, s_0^{in}\right) \in \mathcal{J}_2$	U	S				
$s_0^{in} < F_1^1(D)$	$D \in \mathcal{I}_5$	$\left(D, s_0^{in}\right) \in \mathcal{J}_9$	S				S	U
$F_1^1(D) < s_0^{in} < F_1^2(D)$	$D \in \mathcal{I}_5$	$\left(D, s_0^{in}\right) \in \mathcal{J}_{10}$	S		S		U	U
$F_1^2(D) < s_0^{in} < F_0\left(D, s_1^{in}\right)$	$D \in \mathcal{I}_5$	$\left(D, s_0^{in}\right) \in \mathcal{J}_{11}$	S		S	U	U	U
$s_0^{in} > F_0\left(D, s_1^{in}\right)$	$D \in \mathcal{I}_5$	$\left(D, s_0^{in}\right) \in \mathcal{J}_{12}$	U	S	S	U	U	U
$s_0^{in} > F_2^2(D) - s_1^{in}$	$D \in \mathcal{I}_4$	$\left(D, s_0^{in}\right) \in \mathcal{J}_5$	U	S	S	U	U	
$F_0\left(D, s_1^{in}\right) < s_0^{in} < F_2^2(D) - s_1^{in}$	$D \in \mathcal{I}_4$	$\left(D, s_0^{in}\right) \in \mathcal{J}_6$	U	U	S		U	
$F_1^1(D) < s_0^{in} < F_0\left(D, s_1^{in}\right)$	$D \in \mathcal{I}_4$	$\left(D, s_0^{in}\right) \in \mathcal{J}_7$	U		S		U	
$s_0^{in} < F_1^1(D)$	$D \in \mathcal{I}_4$	$\left(D, s_0^{in}\right) \in \mathcal{J}_8$	U				S	

When $s_1^{in} > s_1^{max}$, we have the occurrence of the following new regions: $\mathcal{J}_9$ (represented in khaki) where the system exhibits bi-stability of SS_0 and SS_3^1 where SS_3^2 is unstable; $\mathcal{J}_{10}$ (in maroon) with bi-stability of SS_0 and SS_2^1 where SS_3^1 and SS_3^2 are unstable; $\mathcal{J}_{11}$ (in violet) with bi-stability of SS_0 and SS_2^1 where SS_2^2, SS_3^1 and SS_3^2 are unstable; $\mathcal{J}_{12}$ (in orange) with bi-stability of SS_1 and SS_2^1 where SS_0, SS_2^2, SS_3^1 and SS_3^2 are unstable.

When case 1 of (21) holds, the region $\mathcal{J}_2$ of the operating diagram in Fig. 10(a) can disappear when $\bar{D}_0\left(s_1^{in}\right) < \bar{D}_1$. In addition, it follows from Proposition 4.4 that when condition (26) is not fulfilled (see Fig. 4(a)) the operating diagram is provided by Table 10 and Fig. 10(a) when the four regions $\mathcal{J}_5$–$\mathcal{J}_8$ are empty. However, it follows from Proposition 4.4 that the region $\mathcal{J}_2$ is always empty in case 2 since $\bar{D}_0\left(s_1^{in}\right) < \hat{D}_1 < \bar{D}_1$. In addition, when condition (26) is not fulfilled (see Fig. 5(c)) the operating diagram is provided by Table 10 and Fig. 11(b) when the five regions $\mathcal{J}_2$ and $\mathcal{J}_5$–$\mathcal{J}_8$ are empty. As in the

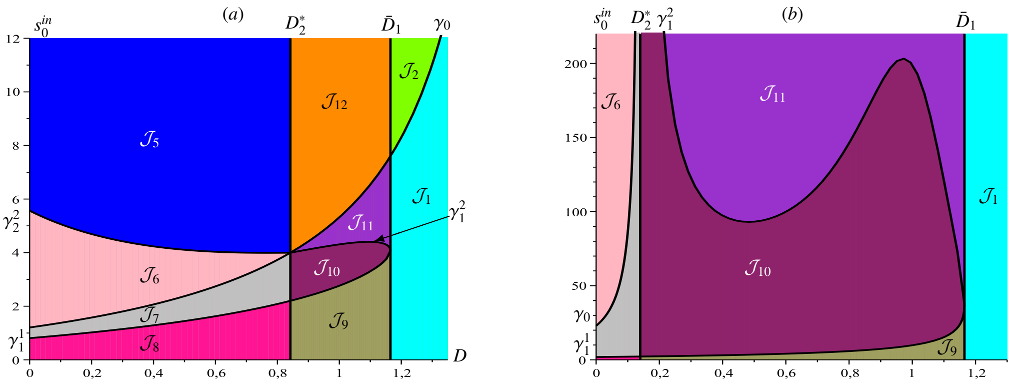

Fig. 10: Operating diagrams of (5) in case 1 of (21): (a) $s_1^{in} = 1.5 > s_1^{max} \simeq 0.689$, equation $\Phi_2(D) = 0$ has no solution. (b) $s_1^{in} = 3.6 > s_1^{max}$, equation $\Phi_2(D) = 0$ has one solution.

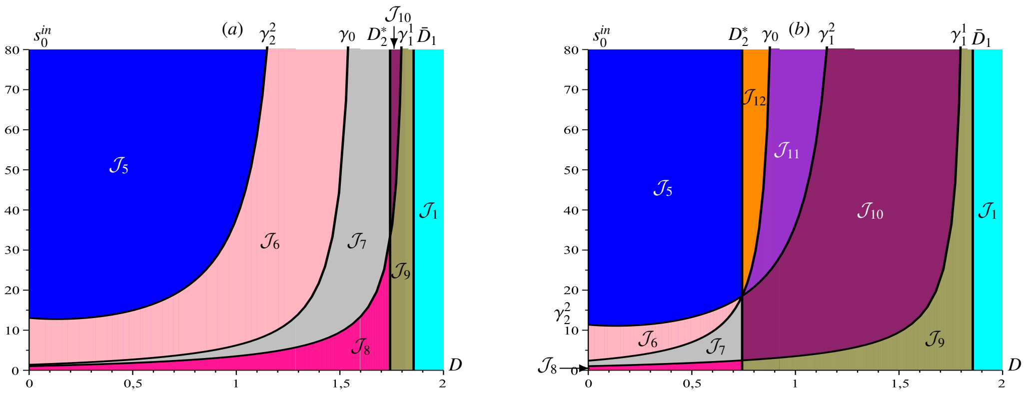

Fig. 11: Operating diagrams of (5) in case 2 of (21) when equation $\Phi_2(D) = 0$ has one solution: (a) $s_1^{in} = 1 > s_1^{max} \simeq 0.689$, the three curves γ_0, γ_1^2 and γ_2^2 do not intersect where $D_2^* \in \left[\widetilde{D}_1, \bar{D}_1\right)$, (b) $s_1^{in} = 2.69 > s_1^{max}$, they intersect where $D_2^* \in \left(0, \widetilde{D}_1\right)$.

case $s_1^{in} = 0$ (see Fig. 6(a-c)), when equation $\Phi_2(D) = 0$ has more than one solution, the operating diagrams are similar to that in Figs. 10 and 11 where we can have at most ten regions but with a change in the shape of the curves γ_1^2 and γ_2^2 as well as the connectivity of the regions $\mathcal{I}_5$ or $\mathcal{I}_{11}$.

4.2. *Operating diagrams in the plane* $\left(s_1^{in}, s_0^{in}\right)$ *when D fixed*

In what follows, we analyze the operating diagrams in the plane $\left(s_1^{in}, s_0^{in}\right)$ according to the position of D relatively to the following critical values $\widetilde{D}_i$, $\hat{D}_1$, $\bar{D}_1$ and $\bar{D}_0(0)$ which are defined in Table 4. The following proposition determines the intervals on which the function $s_1^{in} \mapsto F_0\left(D, s_1^{in}\right)$ is defined according to the control parameter D.

Proposition 4.9. *Let D be fixed. If* $D \leq \bar{D}_0(+\infty)$*, then the function* $s_1^{in} \mapsto F_0\left(D, s_1^{in}\right)$ *is defined on the interval* $[0, +\infty)$*. If* $\bar{D}_0(+\infty) < D < \bar{D}_0(0)$*, then the function* $s_1^{in} \mapsto F_0\left(D, s_1^{in}\right)$ *is defined on the interval* $\left[0, \bar{s}_1^{in}\right)$ *where* $\bar{s}_1^{in}$ *is the unique solution of equation* $\bar{D}_0(s_1^{in}) = D$*. If* $D \geq \bar{D}_0(0)$*, then the function* $s_1^{in} \mapsto F_0\left(D, s_1^{in}\right)$ *is not defined. Moreover, one has*

$$\lim_{s_1^{in} \to \bar{s}_1^{in-}} F_0\left(D, s_1^{in}\right) = +\infty$$

with $\bar{D}_1 < \bar{D}_0(0)$ *in case 1 of (21) and* $\hat{D}_1 < \bar{D}_0(0)$ *in case 2 of (21).*

The existence of critical parameter values of s_1^{in} corresponding to the passage from one region to another in the operating diagram is given by the following result.

Proposition 4.10. *For all* $D \in \bar{I}_j$, $j = 1, 2$*, there exists a unique nonnegative solution* s_{1j}^{in*} *of equation* $M_1^j(D_1) = s_1^{in}$ *such that* $s_{11}^{in*} < s_{12}^{in*}$*. In addition,*

1. *For all* $D \in I_1$*, the three curves* γ_0*,* γ_1^1 *and* γ_2^1 *intersect at the same point* $s_1^{in} = s_{11}^{in*}$ *(see Figs. 12 and 13(c)) such that* $s_{11}^{in*} < \bar{s}_1^{in}$*, condition (24) holds for all* $s_1^{in} \in \left[0, s_{11}^{in*}\right)$ *and condition (23) holds for all* $s_1^{in} \in \left(s_{11}^{in*}, \bar{s}_1^{in}\right)$*.*
2. *For all* $D \in I_2$*, three curves* γ_0*,* γ_1^2 *and* γ_2^2 *intersect at the same point* $s_1^{in} = s_{12}^{in*}$ *(see Fig. 12) such that* $s_{12}^{in*} < \bar{s}_1^{in}$*, condition (27) holds for all* $s_1^{in} \in \left[0, s_{12}^{in*}\right)$ *and condition (28) holds for all* $s_1^{in} \in \left(s_{12}^{in*}, \bar{s}_1^{in}\right)$*.*

Proposition 4.11. *Table 11 shows the existence and stability of the steady states SS_0, SS_1, SS_2^1, SS_2^2, SS_3^1 and SS_3^2 in the regions of the operating diagrams in Figs. 12 and 13 where cases 1 and 2 of (21) hold, respectively.*

Table 11: Existence and stability of steady states in the regions of the operating diagram in Fig. 12, when case 1 of (21) holds. Note that $\mathcal{I}_7 = \left(0, s_{11}^{in*}\right)$, $\mathcal{I}_8 = \left(s_{11}^{in*}, s_{12}^{in*}\right)$ and $\mathcal{I}_9 = \left(s_{12}^{in*}, +\infty\right)$ where s_{11}^{in*} and s_{12}^{in*} are defined in Proposition 4.10.

Condition	Interval	Region	SS_0	SS_1	SS_2^1	SS_2^2	SS_3^1	SS_3^2
$s_0^{in} < F_0\left(D, s_1^{in}\right)$	$s_1^{in} \in \mathcal{I}_7$	$(s_1^{in}, s_0^{in}) \in \mathcal{J}_1$	S					
$F_0\left(D, s_1^{in}\right) < s_0^{in} < F_2^1(D) - s_1^{in}$	$s_1^{in} \in \mathcal{I}_7$	$(s_1^{in}, s_0^{in}) \in \mathcal{J}_2$	U	S				
$F_2^1(D) - s_1^{in} < s_0^{in} < F_2^2(D) - s_1^{in}$	$s_1^{in} \in \mathcal{I}_7$	$(s_1^{in}, s_0^{in}) \in \mathcal{J}_3$	U	U	S			
$s_0^{in} > F_2^2(D) - s_1^{in}$	$s_1^{in} \in \mathcal{I}_7$	$(s_1^{in}, s_0^{in}) \in \mathcal{J}_4$	U	S	S	U		
$s_0^{in} > F_2^2(D) - s_1^{in}$	$s_1^{in} \in \mathcal{I}_8$	$(s_1^{in}, s_0^{in}) \in \mathcal{J}_5$	U	S	S	U	U	
$F_0\left(D, s_1^{in}\right) < s_0^{in} < F_2^2(D) - s_1^{in}$	$s_1^{in} \in \mathcal{I}_8$	$(s_1^{in}, s_0^{in}) \in \mathcal{J}_6$	U	U	S		U	
$F_1^1(D) < s_0^{in} < F_0\left(D, s_1^{in}\right)$	$s_1^{in} \in \mathcal{I}_8$	$(s_1^{in}, s_0^{in}) \in \mathcal{J}_7$	U		S		U	
$s_0^{in} < F_1^1(D)$	$s_1^{in} \in \mathcal{I}_8$	$(s_1^{in}, s_0^{in}) \in \mathcal{J}_8$	U				S	
$s_0^{in} < F_1^1(D)$	$s_1^{in} \in \mathcal{I}_9$	$(s_1^{in}, s_0^{in}) \in \mathcal{J}_9$	S				S	U
$F_1^1(D) < s_0^{in} < F_1^2(D)$	$s_1^{in} \in \mathcal{I}_9$	$(s_1^{in}, s_0^{in}) \in \mathcal{J}_{10}$	S		S		U	U
$F_1^2(D) < s_0^{in} < F_0\left(D, s_1^{in}\right)$	$s_1^{in} \in \mathcal{I}_9$	$(s_1^{in}, s_0^{in}) \in \mathcal{J}_{11}$	S		S	U	U	U
$s_0^{in} > F_0\left(D, s_1^{in}\right)$	$s_1^{in} \in \mathcal{I}_9$	$(s_1^{in}, s_0^{in}) \in \mathcal{J}_{12}$	U	S	S	U	U	U

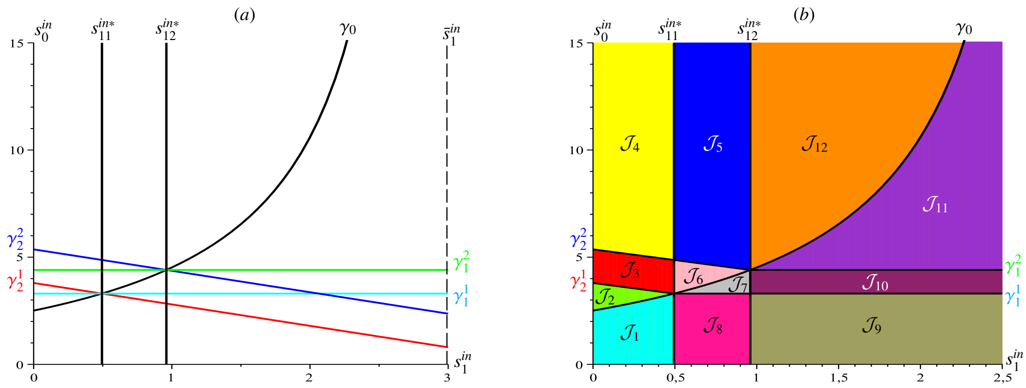

Fig. 12: Case 1 of (21) holds when $D = 1.1 < \min\left(\bar{D}_1 \simeq 1.165, \bar{D}_0(0) \simeq 2.556\right)$ which corresponds to Figs. 1 and 4(a) where the curves γ_0, γ_i^j, $i, j = 1, 2$, are defined and the lines $s_1^{in} = s_{11}^{in*} \simeq 0.495$ and $s_1^{in} = s_{12}^{in*} \simeq 0.961$ exist. (b) The corresponding operating diagram in the plane $\left(s_1^{in}, s_0^{in}\right)$.

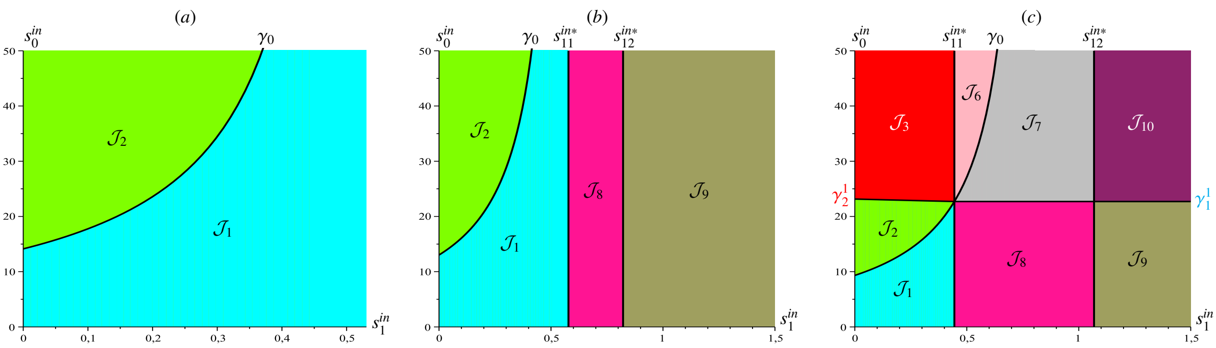

Fig. 13: Operating diagrams corresponding to Figs. 2 and 5 when D is fixed and case 2 of (21) holds: (a) $\bar{D}_1 \simeq 1.856 < D = 1.857 < \bar{D}_0(0) \simeq 2.21$ (b) $\hat{D}_1 \simeq 1.829 < D = 1.83 < \bar{D}_1$ (c) $\widetilde{D}_1 \simeq 1.285 < D = 1.7 < \hat{D}_1$.

From Propositions 4.9 and 4.10, there exist six cases that must be distinguished:

- $D > \max\left(\bar{D}_1, \bar{D}_0(0)\right)$: the five functions F_0 and F_i^j, $i, j = 1, 2$, are not defined and the two lines $s_1^{in} = s_{11}^{in*}$ and $s_1^{in} = s_{12}^{in*}$ do not appear in the positive quadrant. In this case, the operating diagram contains only one region $\mathcal{J}_1$ (see Fig. 13(a) when $\mathcal{J}_2$ is empty).

- $\bar{D}_1 < D < \bar{D}_0(0)$: only the function F_0 is defined and the operating diagram is illustrated in Fig. 13(a).
- $\bar{D}_0(0) < D < \bar{D}_1$: only the two lines $s_1^{in} = s_{11}^{in*}$ and $s_1^{in} = s_{12}^{in*}$ exist and the operating diagram is given by Fig. 13(b) where $\mathcal{J}_2$ is empty.
- $\hat{D}_1 < D < \min\left(\bar{D}_1, \bar{D}_0(0)\right)$ and case 2 of (21) holds: only the function F_0 is defined and the two lines $s_1^{in} = s_{11}^{in*}$ and $s_1^{in} = s_{12}^{in*}$ exist, the corresponding operating diagram is illustrated in Fig. 13(b).
- $D < \min\left(\hat{D}_1, \bar{D}_1\right)$ and $D \notin I_2$: only the two functions F_i^2, $i = 1, 2$, are not defined (see Fig. 13(c)).
- $D \in I_2$: all the functions F_0 and F_i^j, $i, j = 1, 2$, are defined and the two lines $s_1^{in} = s_{11}^{in*}$ and $s_1^{in} = s_{12}^{in*}$ exist (see Fig. 12).

5. Discussion and conclusion

In this paper, we have generalized the mathematical analysis of a simplified model of anaerobic digestion in the form of a two-tiered microbial food chain describing a syntrophic relationship between two microbial species in a chemostat. In order to give a complete analysis of this syntrophic model (5), we allow a large class of growth functions with distinct disappearance rates. The main contribution of this study is to bring out the common effects of the syntrophy relationship, the decay of the two microbial species, the substrate inhibition on the growth of the second species and a new inflowing concentration (the hydrogen) which is neglected in previous studies. First, we have determined the necessary and sufficient conditions of existence and local stability of all steady states of syntrophic model (5) according to the operating parameters D, s_0^{in} and s_1^{in}. We have shown that substrate inhibition has a significant impact on the behavior of the syntrophic relationship system. For a general class of nonmonotonic growth rates including the Haldane kinetics, we proved that, depending on the initial conditions, the system can exhibit a bi-stability with presence of two coexistence steady states which can bifurcate through saddle-node bifurcations or transcritical bifurcations. These two features cannot occur in the syntrophic relationship model with monotonic growth functions for both species and only one input substrate concentration (the fatty acids) [26], or two substrate inflow concentrations (the fatty acids and the hydrogen) [6]. Our findings on the destabilization of a two-tiered microbial 'food chain' by substrate inhibition are similar to those in [11, 15] where the behavior of system depends on the initial condition. However, mortality can destabilize a trophic chain (prey-predator) in a chemostat by the occurrence of stable limit cycles and multiple chaotic attractors [17]. Furthermore, low as well as high concentration of input substrate can cause destabilization by the extinction of the highest trophic level of a tri-trophic food chain model in the chemostat [4]. This result is similar to our process of a two-tiered microbial food chain by varying an inhibition factor by the hydrogen.

The second contribution is the mathematical analysis of the operating diagram in order to determine the behavior of the system according to the control parameters and to choose appropriate inputs and initial states to achieve a good operation of the process. To protect the coexistence of two microbial species in the process, the operating parameter values should be chosen in the regions $\mathcal{J}_i$, $i = 3, 6, 7$, where there exists a unique stable steady state of coexistence. Indeed, the new input substrate concentration can exhibit two steady states of extinction of the first species and leads to the emergence of new regions where one of them is stable ($\mathcal{J}_i$, $i = 8, 9$). These steady states do not exist in the case of the syntrophic relationship model, [11], with only one input substrate concentration. Furthermore, the operating diagrams show that the system can have a unique stable steady state: either of coexistence ($\mathcal{J}_i, i = 3, 6, 7$) or washout ($\mathcal{J}_1$) or exclusion of one of two microbial species ($\mathcal{J}_i, i = 2, 8$). It can also exhibit a bi-stability between coexistence and washout ($\mathcal{J}_i, i = 10, 11$) or exclusion of the second species ($\mathcal{J}_i, i = 4, 5, 12$) or between washout and exclusion of the first species ($\mathcal{J}_9$).

These theoretical messages explain the joined effect of syntrophy, mortality, substrate inhibition and input substrates on the maintenance of coexistence and the protection of microbial ecosystems. Finally, the results in this contribution may also serve for optimal experimental design by studying the biogas production and the process performance with respect to operating parameters. This is an important question that deserves further attention and will be the object of future work.

Appendix A. Proofs

Proof of Proposition 2.1. Since

$$s_0 = 0 \quad \Rightarrow \quad \dot{s}_0 = D s_0^{in} > 0\,,$$

then no trajectory can leave the positive octant $\mathbb{R}_+^4$ by crossing the boundary face $s_0 = 0$. On the other hand, since the quadrant $\Theta_i := \{s_0 \geq 0, s_1 \geq 0, x_i = 0\}$, $i = 0, 1$, is invariant under the system (5) because the function

$$t \to (s_0(t), x_0(t), s_1(t), x_1(t)) = \left(s_0^{in} + \left(s_0(0) - s_0^{in}\right)e^{-Dt}, 0, s_1^{in} + \left(s_1(0) - s_1^{in}\right)e^{-Dt}, 0\right)$$

is a solution of (5). By uniqueness of solutions, Θ_i cannot be reached in finite time by trajectories for which $x_i > 0$, $i = 0, 1$. Finally, since $s_0 \geq 0$ and $x_0 \geq 0$, it follows that

$$s_1 = 0 \quad \Rightarrow \quad \dot{s}_1 = Ds_1^{in} + \mu_0(s_0, 0)\, x_0 > 0\,.$$

Thus, no trajectory can leave the positive octant $\mathbb{R}_+^4$ by crossing the boundary face $s_1 = 0$. Therefore, all solutions of (5) remain nonnegative.

Let $z = 2s_0 + x_0 + s_1 + x_1$. From (5), it follows that

$$\dot{z} = D\left(2s_0^{in} + s_1^{in} - 2s_0 - s_1\right) - D_0 x_0 - D_1 x_1.$$

Consequently,

$$\dot{z} \leq D_{\min}\left[\frac{D}{D_{\min}}\left(2s_0^{in} + s_1^{in}\right) - z\right].$$

Introducing the variable

$$v = z - \frac{D}{D_{\min}}\left(2s_0^{in} + s_1^{in}\right),$$

the last inequality can be expressed as $\dot{v} \leq -D_{\min} v$. By applying Gronwall lemma, we obtain $v(t) \leq v(0)e^{-D_{\min}t}$ and consequently,

$$z(t) \leq \frac{D}{D_{\min}}\left(2s_0^{in} + s_1^{in}\right) + \left(z(0) - \frac{D}{D_{\min}}\left(2s_0^{in} + s_1^{in}\right)\right)e^{-D_{\min}t}, \quad \text{for all} \quad t \geq 0. \tag{A.1}$$

We deduce that

$$z(t) \leq \max\left(z(0), \frac{D}{D_{\min}}\left(2s_0^{in} + s_1^{in}\right)\right), \quad \text{for all} \quad t \geq 0.$$

Consequently, the solutions of (5) are bounded for all $t \geq 0$. Inequality (A.1) implies that the set Ω is positively invariant and is a global attractor for (5). □

Proof of Lemma 3.1. From equivalence (10), we have

$$\mu_0\left(M_0(y, s_1), s_1\right) = y, \quad \text{for all} \quad y \in [0, \bar{\mu}_0(s_1)) \quad \text{and} \quad s_1 \geq 0. \tag{A.2}$$

Using (H3), after taking the derivative of (A.2) according to y first and to s_1 then, we can prove that:

$$\frac{\partial M_0}{\partial y}(y, s_1) = \left[\frac{\partial \mu_0}{\partial s_0}\left(M_0(y, s_1), s_1\right)\right]^{-1} > 0,$$

$$\frac{\partial M_0}{\partial s_1}(y, s_1) = -\left[\frac{\partial \mu_0}{\partial s_1}\left(M_0(y, s_1), s_1\right)\right]\left[\frac{\partial \mu_0}{\partial s_0}\left(M_0(y, s_1), s_1\right)\right]^{-1} > 0.$$

□

Proof of Proposition 3.1. The steady states of (5) are the solutions of the set of equations (7).

- At the steady state SS_0, one has $x_0 = 0$, $x_1 = 0$. From the first and third equations of (7), it follows that $s_0 = s_0^{in}$ and $s_1 = s_1^{in}$. Thus, the washout steady state $SS_0 = \left(s_0^{in}, 0, s_1^{in}, 0\right)$ always exists.
- For SS_1, one has $x_0 > 0$, $x_1 = 0$. From the second equation of (7), we deduce that

$$\mu_0(s_0, s_1) = D_0. \tag{A.3}$$

From the first and third equations of (7), one has

$$D\left(s_0^{in} - s_0\right) = \mu_0(s_0, s_1)x_0 \quad \text{and} \quad D\left(s_1 - s_1^{in}\right) = \mu_0(s_0, s_1)x_0.$$

Then, $x_0 = \frac{D}{D_0}\left(s_0^{in} - s_0\right)$ which is positive if, and only if, $s_0 < s_0^{in}$. Moreover, $D(s_0^{in} - s_0) = D(s_1 - s_1^{in})$, that is, $s_1 = s_0^{in} + s_1^{in} - s_0$ which is positive if, and only if, $s_0 < s_0^{in} + s_1^{in}$. Using (A.3), we see that s_0 must be a solution of the equation

$$\mu_0(s_0, s_0^{in} + s_1^{in} - s_0) = D_0. \tag{A.4}$$

Thus, the steady state SS_1 exists if, and only if, (A.4) has a solution in $\left(0, s_0^{in}\right)$. We define the function

$$s_0 \mapsto \psi(s_0) := \mu_0\left(s_0, s_0^{in} + s_1^{in} - s_0\right).$$

From (H3), it follows that ψ is strictly increasing since its derivative

$$\psi'(s_0) = \frac{\partial \mu_0}{\partial s_0}(s_0, s_1) - \frac{\partial \mu_0}{\partial s_1}(s_0, s_1)$$

is positive. Since $\psi(0) = 0$ and $\psi\left(s_0^{in}\right) = \mu_0\left(s_0^{in}, 0\right)$, (A.4) has a solution in $\left(0, s_0^{in}\right)$ if, and only if,

$$\psi\left(s_0^{in}\right) = \mu_0\left(s_0^{in}, s_1^{in}\right) > D_0. \tag{A.5}$$

If such a solution exists then it is unique. If $D \geq \bar{D}_0\left(s_1^{in}\right)$, that is, $D_0 \geq \bar{\mu}_0\left(s_1^{in}\right)$, the condition (A.5) is not satisfied. However, if $D < \bar{D}_0\left(s_1^{in}\right)$, then the function $D \mapsto M_0\left(\alpha_0 D + a_0, s_1^{in}\right)$ is defined. From Lemma 3.1 and using (10), it follows that the condition (A.5) is equivalent to

$$s_0^{in} > M_0\left(\alpha_0 D + a_0, s_1^{in}\right) \quad \text{with} \quad D < \bar{D}_0\left(s_1^{in}\right)$$

which is the same as (11) by using definition (12) of the function F_0.

- For SS_2, one has $x_0 > 0$ and $x_1 > 0$. From the last equation of (7), s_1 satisfies:

$$\mu_1(s_1) = D_1. \tag{A.6}$$

From hypothesis (H4), the function $s_1 \mapsto \mu_1(s_1)$ is increasing from $\mu_1(0) = 0$ to $\mu_1\left(s_1^{max}\right) = \mu_1^{max}$. Thus, there exists a solution $s_1^1 \in \left[0, s_1^{max}\right]$ of (A.6) if, and only if, $D_1 \leq \mu_1^{max}$, that is, $D \leq \bar{D}_1$. In addition, the function $s_1 \mapsto \mu_1(s_1)$ is decreasing from $\mu_1\left(s_1^{max}\right) = \mu_1^{max}$ to $\mu_1(+\infty) = 0$. Thus, there exists a solution $s_1^2 \in \left[s_1^{max}, +\infty\right)$ of (A.6) if, and only if, $0 < D_1 \leq \mu_1^{max}$, that is, $D \in \left[0, \bar{D}_1\right]$ if $a_1 > 0$ and $D \in \left(0, \bar{D}_1\right]$ if $a_1 = 0$ (or equivalently $D \in \bar{I}_2$). If such a solution s_1^j, $j = 1, 2$, exists then it is unique. Using (8) and (9), we see that $s_1^j = M_1^j(D_1)$. From the second equation of (7), it follows that

$$\mu_0\left(s_0, M_1^j(D_1)\right) = D_0, \quad j = 1, 2. \tag{A.7}$$

From hypotheses (H1), (H3) and (H5), the function $s_0 \mapsto \mu_0\left(s_0, M_1^j(D_1)\right)$ is increasing from $\mu_0\left(0, M_1^j(D_1)\right) = 0$ to $\mu_0\left(+\infty, M_1^j(D_1)\right) = \bar{\mu}_0\left(M_1^j(D_1)\right)$. From definition (16) of the function Φ_j, it follows that (A.7) has a solution $s_0^j \geq 0$ if, and only if,

$$\bar{\mu}_0\left(M_1^j(D_1)\right) - D_0 = \Phi_j(D) > 0, \quad \text{with} \quad D \in \bar{I}_j,$$

or equivalently, $D \in I_j$ which is defined by (15). If such a solution s_0^j, $j = 1, 2$, exists then it is unique. Using definition (13) of F_i^j, one sees that F_2^j is defined on the same domain as F_1^j for $j = 1, 2$. For $i, j = 1, 2$, the function F_i^j is defined if, and only if, $M_1^j(D_1)$ and $M_0\left(D_0, M_1^j(D_1)\right)$ are defined. From (8-10), one can see that the function F_i^j is defined if, and only if,

$$D_0 < \bar{\mu}_0\left(M_1^j(D_1)\right) \quad \text{and} \quad D_1 \leq \mu_1^{max}, \ \text{with} \ D \neq 0 \quad \text{when} \quad a_1 = 0 \ \text{and} \ j = 2,$$

that is, for all $D \in I_j$. Using notation (13), it follows that

$$s_1^j = M_1^j(D_1) = F_2^j(D) - F_1^j(D), \quad \text{for all} \quad D \in I_j.$$

From (10) and definition (13) of F_1^j, (A.7) is equivalent to

$$s_0^j = M_0\left(D_0, M_1^j(D_1)\right) = F_1^j(D), \quad \text{for all} \quad D \in I_j.$$

As a consequence of the first and third equations of (7), we have

$$x_0^j = \frac{D}{D_0}\left(s_0^{in} - s_0^j\right), \qquad x_1^j = \frac{D}{D_1}\left(s_0^{in} + s_1^{in} - s_0^j - s_1^j\right).$$

Thus, one can conclude that SS_2^j exists if, and only if, $s_0^{in} + s_1^{in} > s_0^j + s_1^j$ and $s_0^{in} > s_0^j$, that is, condition (14) is satisfied with $D \in I_j$.

- For SS_3, one has $x_0 = 0$ and $x_1 > 0$. From the first and last equations of (7), we obtain $s_0 = s_0^{in}$ and s_1 satisfies (A.6). Similar arguments applied to SS_2 show that there exists a solution $s_1^1 \in \left[0, s_1^{max}\right]$ of (A.6) if, and only if, $D \in \left[0, \bar{D}_1\right] = \bar{I}_1$. Furthermore, there exists a solution $s_1^2 \in \left[s_1^{max}, +\infty\right)$ of (A.6) if, and only if, $D \in \bar{I}_2 = \bar{I}_1$ if $a_1 > 0$ and $D \in \bar{I}_2 = \left(0, \bar{D}_1\right]$ if $a_1 = 0$. Thus, $s_1^j = M_1^j(D_1)$, $j = 1, 2$. The third equation of (7) implies

$$x_1^j = \frac{D}{D_1}\left(s_1^{in} - M_1^j(D_1)\right).$$

  Thus, we conclude that SS_3^j exists if, and only if, condition (17) holds.

□

Proof of Proposition 3.2. Let J be the Jacobian matrix of (5) at a steady state (s_0, x_0, s_1, x_1), that is given by

$$J = \begin{bmatrix} -D - Ex_0 & -\mu_0 & Fx_0 & 0 \\ Ex_0 & \mu_0 - D_0 & -Fx_0 & 0 \\ Ex_0 & \mu_0 & -D - Fx_0 - G_j x_1 & -\mu_1 \\ 0 & 0 & Gx_1 & \mu_1 - D_1 \end{bmatrix}, \tag{A.8}$$

where

$$E = \frac{\partial \mu_0}{\partial s_0}(s_0, s_1) > 0, \quad F = -\frac{\partial \mu_0}{\partial s_1}(s_0, s_1) > 0 \quad \text{and} \quad G = \mu_1'(s_1).$$

- At $SS_0 = \left(s_0^{in}, 0, s_1^{in}, 0\right)$, the Jacobian matrix (A.8) is written as follows:

$$J = \begin{bmatrix} -D & -\mu_0\left(s_0^{in}, s_1^{in}\right) & 0 & 0 \\ 0 & \mu_0\left(s_0^{in}, s_1^{in}\right) - D_0 & 0 & 0 \\ 0 & \mu_0\left(s_0^{in}, s_1^{in}\right) & -D & -\mu_1\left(s_1^{in}\right) \\ 0 & 0 & 0 & \mu_1\left(s_1^{in}\right) - D_1 \end{bmatrix}.$$

  The eigenvalues are

$$\lambda_1 = \mu_0\left(s_0^{in}, s_1^{in}\right) - D_0, \quad \lambda_2 = \mu_1\left(s_1^{in}\right) - D_1, \quad \lambda_3 = \lambda_4 = -D.$$

  Thus, SS_0 is stable if, and only if,

$$\mu_0\left(s_0^{in}, s_1^{in}\right) < D_0 \quad \text{and} \quad \mu_1\left(s_1^{in}\right) < D_1. \tag{A.9}$$

  If $D \geq \bar{D}_0\left(s_1^{in}\right)$, that is, $D_0 \geq \bar{\mu}_0\left(s_1^{in}\right)$, then the first condition of (A.9) is satisfied. If $D < \bar{D}_0\left(s_1^{in}\right)$, then the function $D \mapsto F_0\left(D, s_1^{in}\right)$ is defined. From Lemma 3.1 and using (10), we deduce that the first condition of (A.9) is equivalent to

$$s_0^{in} < M_0\left(D_0, s_1^{in}\right) = F_0\left(D, s_1^{in}\right), \quad \text{for all} \quad D < \bar{D}_0\left(s_1^{in}\right).$$

  If $D > \bar{D}_1$, that is, $D_1 > \mu_1^{max}$, then the second condition of (A.9) is satisfied. In the particular case $a_1 = 0$ and $D = 0$, the second condition of (A.9) is not satisfied. Thus, if $D \in \bar{I}_j$ which is defined by (15) where $D \leq \bar{D}_1$, then the function $D \mapsto M_1^j(\alpha_1 D + a_1)$, $j = 1, 2$, is defined. Since the function $s_1 \mapsto \mu_1(s_1)$ is increasing on $\left[0, s_1^{max}\right]$ and is decreasing on $\left[s_1^{max}, +\infty\right)$, and using (8) and (9), the second condition of (A.9) is equivalent to (19).

- At $SS_1 = (s_0, x_0, s_1, 0)$, the Jacobian matrix is given by

$$J = \begin{bmatrix} -D - Ex_0 & -D_0 & Fx_0 & 0 \\ Ex_0 & 0 & -Fx_0 & 0 \\ Ex_0 & D_0 & -D - Fx_0 & -\mu_1 \\ 0 & 0 & 0 & \mu_1 - D_1 \end{bmatrix}.$$

  The characteristic polynomial is given by $P(\lambda) = \det(J - \lambda I)$, where I is the 4×4 identity matrix. Denote C_i and L_i the columns and lines of the matrix $J - \lambda I$. The replacements of L_1 by $L_1 + L_3$ and then C_3 by $C_3 - C_1$ preserve the determinant and lead to

$$P(\lambda) = (\mu_1 - D_1 - \lambda)(-D - \lambda)\begin{vmatrix} -\lambda & -(E+F)x_0 \\ D_0 & -(E+F)x_0 - D - \lambda \end{vmatrix}.$$

The eigenvalues of J are $\lambda_1 = \mu_1 - D_1$, $\lambda_2 = -D$, λ_3 and λ_4 such that

$$\lambda_3 + \lambda_4 = -[D + (E + F)x_0] < 0, \quad \lambda_3\lambda_4 = D_0(E + F)x_0 > 0.$$

Hence, the real parts of λ_3 and λ_4 are negative. Therefore, SS_1 is LES if, and only if,

$$\mu_1\left(s_0^{in} + s_1^{in} - s_0\right) < D_1. \tag{A.10}$$

If $D > \bar{D}_1$, then condition (A.10) is satisfied. If $D \in \bar{I}_j$ where $D \leq \bar{D}_1$, then condition (A.10) is equivalent to

$$s_0 > s_0^{in} + s_1^{in} - M_1^1(D_1) \quad \text{or} \quad s_0 < s_0^{in} + s_1^{in} - M_1^2(D_1). \tag{A.11}$$

Recall that the function $s_0 \mapsto \psi(s_0) = \mu_0\left(s_0, s_0^{in} + s_1^{in} - s_0\right)$ is increasing. Hence, condition (A.11) of stability of SS_1 is equivalent to

$$\psi(s_0) > \psi\left(s_0^{in} + s_1^{in} - M_1^1(D_1)\right) \quad \text{or} \quad \psi(s_0) < \psi\left(s_0^{in} + s_1^{in} - M_1^2(D_1)\right).$$

At SS_1, one has $\psi(s_0) = \mu_0\left(s_0, s_0^{in} + s_1^{in} - s_0\right) = D_0$. Thus, condition (A.11) is equivalent to

$$D_0 > \mu_0\left(s_0^{in} + s_1^{in} - M_1^1(D_1), M_1^1(D_1)\right) \quad \text{or} \quad D_0 < \mu_0\left(s_0^{in} + s_1^{in} - M_1^2(D_1), M_1^2(D_1)\right). \tag{A.12}$$

If $D \in \left[0, \bar{D}_1\right] \backslash I_1$, that is, $\Phi_1(D) < 0$ (or equivalently $\bar{\mu}_0\left(M_1^1(D_1)\right) < D_0$) then the first condition of (A.12) is satisfied. If $D \in I_1$, then $F_1^1(\cdot)$ is defined and the first condition of (A.12) is equivalent to

$$s_0^{in} + s_1^{in} < M_0\left(D_0, M_1^1(D_1)\right) + M_1^1(D_1),$$

because $M_0\left(\cdot, M_1^1(D_1)\right)$ is increasing (see Lemma 3.1). If $D \in \left[0, \bar{D}_1\right] \backslash I_2$, that is, $\bar{\mu}_0\left(M_1^2(D_1)\right) < D_0$, then the second condition of (A.12) is not satisfied. If $D \in I_2$, then $F_1^2(\cdot)$ is defined and the second condition of (A.12) is equivalent to

$$s_0^{in} + s_1^{in} > M_0\left(D_0, M_1^2(D_1)\right) + M_1^2(D_1).$$

Thus, we can conclude that SS_1 is LES if, and only if, $D \in I_0 \backslash I_1$ or condition (20) holds.

- At $SS_2^j = \left(s_0^j, x_0^j, s_1^j, x_1^j\right)$, the Jacobian matrix is given by the following matrix:

$$J_2^j = \begin{bmatrix} -D - Ex_0 & -D_0 & Fx_0 & 0 \\ Ex_0 & 0 & -Fx_0 & 0 \\ Ex_0 & D_0 & -D - Fx_0 - G_jx_1 & -D_1 \\ 0 & 0 & G_jx_1 & 0 \end{bmatrix}.$$

If SS_2^j exists with $D \neq \bar{D}_1$, then $G_1 := \mu_1'\left(s_1^1\right) > 0$ since $s_1^1 < s_1^{max}$ and $G_2 := \mu_1'\left(s_1^2\right) < 0$ since $s_1^2 > s_1^{max}$. It will be convenient to use the notation $H = E + F$ in order to shorten the following notation. The characteristic polynomial is given by

$$\det(J - \lambda I) = \lambda^4 + c_1\lambda^3 + c_2\lambda^2 + c_3\lambda + c_4,$$

with

$$c_1 = G_jx_1 + Hx_0 + 2D, \quad c_2 = EG_jx_0x_1 + (D + D_0)Hx_0 + (D + D_1)G_jx_1 + D^2,$$

$$c_3 = (D_0 + D_1)EG_jx_0x_1 + DD_0Hx_0 + DD_1G_jx_1, \quad c_4 = D_0D_1EG_jx_0x_1.$$

According to the Routh–Hurwitz criterion, SS_2^j is LES if, and only if,

$$c_i > 0, \quad i = 1, \dots, 4, \quad c_1c_2 - c_3 > 0 \quad \text{and} \quad c_1c_2c_3 - c_1^2c_4 - c_3^2 > 0. \tag{A.13}$$

Since E, F, H are positive, it follows that $c_i > 0$, for all $i = 1, \dots, 4$. We have

$$c_1c_2 - c_3 = 2D^3 + \beta_2D^2 + \beta_1D + \beta_0,$$

$$c_1c_2c_3 - c_1^2c_4 - c_3^2 = \gamma_5D^5 + \gamma_4D^4 + \gamma_3D^3 + \gamma_2D^2 + \gamma_1D + \gamma_0.$$

Following [26], where the case $\alpha_0 = \alpha_1 = 1$ were considered, we can write the coefficients β_i, $i = 0, 1, 2$, and γ_j, $j = 0, \ldots, 5$, as follows:

$$\beta_2 = (3 + \alpha_0) Hx_0 + (3 + \alpha_1) G_1 x_1,$$
$$\beta_1 = a_0 Hx_0 + a_1 G_1 x_1 + (1 + \alpha_0) H^2 x_0^2 + (1 + \alpha_1) G_1^2 x_1^2 + [(\alpha_0 + \alpha_1 + 2) F + 4E] G_1 x_0 x_1,$$
$$\beta_0 = (a_0 + a_1) FG_1 x_0 x_1 + a_0 H^2 x_0^2 + a_1 G_1^2 x_1^2 + EHG_1 x_0^2 x_1 + EG_1^2 x_0 x_1^2,$$
$$\gamma_5 = 2 (\alpha_0 Hx_0 + \alpha_1 G_1 x_1),$$
$$\gamma_4 = 2a_0 Hx_0 + 2a_1 G_1 x_1 + 2 [\alpha_0 (1 - \alpha_1) + \alpha_1 (1 - \alpha_0)] EG_1 x_0 x_1 + \beta_2 (\alpha_0 Hx_0 + \alpha_1 G_1 x_1),$$
$$\begin{aligned}\gamma_3 = {} & [2 (\alpha_1 a_0 + \alpha_0 a_1) H + (a_0 (5 - 4\alpha_1) + a_1 (5 - 4\alpha_0)) E + 3 (a_0 + a_1) F)] G_1 x_0 x_1 + (3 + 2\alpha_0) a_0 H^2 x_0^2 \\ & + (3 + 2\alpha_1) a_1 G_1^2 x_1^2 + \left[\alpha_1 (\alpha_0 + 1) H + \left((7 - 3\alpha_1) \alpha_0 + 3\alpha_1 + \alpha_0^2\right) E + \alpha_0 (\alpha_0 + \alpha_1 + 2) F\right] HG_1 x_0^2 x_1 \\ & + \left[\alpha_0 (\alpha_1 + 1) H + \left((7 - 3\alpha_0) \alpha_1 + 3\alpha_0 + \alpha_1^2\right) E + \alpha_1 (\alpha_0 + \alpha_1 + 2) F\right] G_1^2 x_0 x_1^2 + \alpha_0 (\alpha_0 + 1) H^3 x_0^3 \\ & + \alpha_1 (\alpha_1 + 1) G_1^3 x_1^3,\end{aligned}$$
$$\begin{aligned}\gamma_2 = {} & [a_0 ((2\alpha_0 + \alpha_1 + 2) H + (5 - 3\alpha_1) E + \alpha_1 F) + a_1 ((\alpha_0 + 1) H + 3 (1 - \alpha_0) E + \alpha_0 F)] HG_1 x_0^2 x_1 \\ & + [a_0 ((\alpha_1 + 1) H + 3 (1 - \alpha_1) E + \alpha_1 F) + a_1 (2 (\alpha_0 + \alpha_1 + 1) H + (5 - 4\alpha_0) E)] G_1^2 x_0 x_1^2 \\ & + \left(\alpha_0^2 + 2\alpha_0 + \alpha_1\right) EH^2 G_1 x_0^3 x_1 + \left(\alpha_1^2 + 2\alpha_1 + \alpha_0\right) EG_1^3 x_0 x_1^3 + (1 + 2\alpha_0) a_0 H^3 x_0^3 + (1 + 2\alpha_1) a_1 G_1^3 x_1^3 \\ & + \left[((3 - 2\alpha_1) \alpha_0 + \alpha_1) H + 2 (\alpha_0 + 2\alpha_1) E + \left((\alpha_0 + \alpha_1)^2 + 2\alpha_1\right) F\right] EG_1^2 x_0^2 x_1^2 \\ & + (a_0 Hx_0 - a_1 G_1 x_1)^2 + 4a_0 a_1 FG_1 x_0 x_1,\end{aligned}$$
$$\begin{aligned}\gamma_1 = {} & (Hx_0 + G_1 x_1) (a_0 Hx_0 - a_1 G_1 x_1)^2 + 4a_0 a_1 FHG_1 x_0^2 x_1 + 4a_0 a_1 FG_1^2 x_0 x_1^2 + (2 (\alpha_0 + 1) a_0 + a_1) EH^2 G_1 x_0^3 x_1 \\ & + (a_0 + 2 (\alpha_1 + 1) a_1) EG_1^3 x_0 x_1^3 + [a_0 ((5 - 2\alpha_1) E + (2\alpha_0 + 3) F) + a_1 ((5 - 2\alpha_0) E + (2\alpha_1 + 3) F)] EG_1^2 x_0^2 x_1^2 \\ & + (\alpha_0 + \alpha_1) (Hx_0 + G_1 x_1) E^2 G_1^2 x_0^2 x_1^2,\end{aligned}$$
$$\gamma_0 = (a_0 + a_1) (Hx_0 + G_1 x_1) E^2 G_1^2 x_0^2 x_1^2 + (a_0 + a_1)^2 EFG_1^2 x_0^2 x_1^2 + (a_0 Hx_0 - a_1 G_1 x_1)^2 EG_1 x_0 x_1.$$

Since α_0 and α_1 are in $[0, 1]$, then $\beta_i > 0$ for $i = 0, 1, 2$ and $\gamma_j > 0$ for $j = 0, \ldots, 5$. Thus, the conditions of the Routh–Hurwitz criterion (A.13) are satisfied for the steady state SS_2^1 which is LES as long as it exists with $D \neq \bar{D}_1$. However, the steady state SS_2^2 is unstable as long as it exists with $D \neq \bar{D}_1$ because the condition $c_4 > 0$ of the Routh–Hurwitz criterion (A.13) is unfulfilled as $G_2 < 0$.

- At $\mathrm{SS}_3^j = \left(s_0^{in}, 0, M_1^j(D_1), \frac{D}{D_1}\left(s_1^{in} - M_1^j(D_1)\right)\right)$, $j = 1, 2$, the Jacobian matrix is given by

$$J_3^j = \begin{bmatrix} -D & -\mu_0 & 0 & 0 \\ 0 & \mu_0 - D_0 & 0 & 0 \\ 0 & \mu_0 & -D - G_j x_1 & -D_1 \\ 0 & 0 & G_j x_1 & 0 \end{bmatrix}.$$

Its eigenvalues are $\lambda_1 = -D$, $\lambda_2 = \mu_0\left(s_0^{in}, M_1^j(D_1)\right) - D_0$, λ_3 and λ_4 such that

$$\lambda_3 \lambda_4 = D_1 G_j x_1 \quad \text{and} \quad \lambda_3 + \lambda_4 = -(D + G_j x_1).$$

At SS_3^1 with $D \neq \bar{D}_1$, $\lambda_3 \lambda_4 > 0$ and $\lambda_3 + \lambda_4 < 0$ because $G_1 > 0$. Therefore, SS_3^1 is LES if, and only if,

$$\mu_0\left(s_0^{in}, M_1^1(D_1)\right) < D_0 \quad \text{with} \quad D \in \bar{I}_1 \setminus \left\{\bar{D}_1\right\}. \tag{A.14}$$

If $D \notin I_1 \cup \left\{\bar{D}_1\right\}$, then condition (A.14) holds. If $D \in I_1 \setminus \left\{\bar{D}_1\right\}$, then condition (A.14) is the same as

$$s_0^{in} < M_0\left(D_0, M_1^1(D_1)\right),$$

since $M_0\left(\cdot, M_1^1(D_1)\right)$ is increasing. At SS_3^2 with $D \neq \bar{D}_1$, $\lambda_3 \lambda_4 = D_1 G_2 x_1 < 0$ since $G_2 < 0$. Therefore, λ_3 and λ_4 are real and have opposite signs. Consequently, if SS_3^2 exists with $D \neq \bar{D}_1$, it is unstable.

□

Proof of Proposition 4.1. For all $D \in \bar{I}_2$, given that $M_1^1(D_1) \leq M_1^2(D_1)$, we can write

$$\Phi_1(D) = \bar{\mu}_0\left(M_1^1(D_1)\right) - D_0 \geq \bar{\mu}_0\left(M_1^2(D_1)\right) - D_0 = \Phi_2(D), \tag{A.15}$$

since the function $\bar{\mu}_0(\cdot)$ is decreasing (H5). If $D \in I_2$, that is, $D \in \bar{I}_2$ such that $\Phi_2(D) > 0$, then $D \in \bar{I}_1$ and $\Phi_1(D) \geq \Phi_2(D) > 0$, that is, $D \in I_1$. Thus, $I_2 \subset I_1$.

For all $D \in I_2$, since the function $M_0\left(D_0, \cdot\right)$ is increasing according to Lemma 3.1, we have

$$F_1^1(D) = M_0\left(D_0, M_1^1(D_1)\right) \leq M_0\left(D_0, M_1^2(D_1)\right) = F_1^2(D).$$

Similarly, using definition (13) of F_2^j, $j = 1, 2$, we deduce that

$$F_2^1(D) = M_0\left(D_0, M_1^1(D_1)\right) + M_1^1(D_1) \leq M_0\left(D_0, M_1^2(D_1)\right) + M_1^2(D_1) = F_2^2(D), \quad \text{for all} \quad D \in I_2.$$

From (10), we have $M_0\left(\mu_0\left(+\infty, s_1^{in}\right), s_1^{in}\right) = +\infty$. Using definition (12) of the function F_0 and $\bar{D}_0\left(s_1^{in}\right)$, it follows that

$$F_0\left(\bar{D}_0\left(s_1^{in}\right), s_1^{in}\right) = M_0\left(\alpha_0\bar{D}_0\left(s_1^{in}\right) + a_0, s_1^{in}\right) = M_0\left(\bar{\mu}_0\left(s_1^{in}\right), s_1^{in}\right) = +\infty.$$

When case 1 of (21) holds, the function F_i^j, $i, j = 1, 2$, is defined for $D = \bar{D}_1$. Using assumption (H4) and the definition of $\bar{D}_1$ in Table 4, it follows that

$$M_1^1\left(\alpha_1\bar{D}_1 + a_1\right) = M_1^1\left(\mu_1^{max}\right) = M_1^2\left(\mu_1^{max}\right) = s_1^{max}. \tag{A.16}$$

Consequently,

$$F_1^1\left(\bar{D}_1\right) = M_0\left(\alpha_0\bar{D}_1 + a_0, M_1^1\left(\mu_1^{max}\right)\right) = M_0\left(\alpha_0\bar{D}_1 + a_0, M_1^2\left(\mu_1^{max}\right)\right) = F_1^2\left(\bar{D}_1\right).$$

Similarly,

$$F_2^1\left(\bar{D}_1\right) = M_0\left(\alpha_0\bar{D}_1 + a_0, s_1^{max}\right) + s_1^{max} = F_2^2\left(\bar{D}_1\right).$$

Let $\hat{D}_1$ be a solution of the equation $\Phi_1(D) = 0$. From definition (16) of the function Φ_1, we obtain

$$\bar{\mu}_0\left(M_1^1\left(\alpha_1\hat{D}_1 + a_1\right)\right) = \alpha_0\hat{D}_1 + a_0.$$

Therefore,

$$F_1^1\left(\hat{D}_1\right) = M_0\left(\bar{\mu}_0\left(M_1^1\left(\alpha_1\hat{D}_1 + a_1\right)\right), M_1^1\left(\alpha_1\hat{D}_1 + a_1\right)\right) = +\infty.$$

Consequently,

$$F_2^1\left(\hat{D}_1\right) = F_1^1\left(\hat{D}_1\right) + M_1^1\left(\alpha_1\hat{D}_1 + a_1\right) = +\infty.$$

The last limit follows similarly. □

Proof of Proposition 4.2. In the case $s_1^{in} < s_1^{max}$, it follows from (A.16) that

$$F_0\left(\bar{D}_1, s_1^{in}\right) = M_0\left(\alpha_0\bar{D}_1 + a_0, s_1^{in}\right) < M_0\left(\alpha_0\bar{D}_1 + a_0, M_1^1\left(\alpha_1\bar{D}_1 + a_1\right)\right) = F_1^1\left(\bar{D}_1\right),$$

since the function $M_0\left(D_0, \cdot\right)$ is increasing (see Lemma 3.1). Noting that $F_1^1\left(\bar{D}_1\right) < +\infty$ in case 1 of (21), it follows that

$$F_0\left(\bar{D}_1, s_1^{in}\right) < +\infty = F_0\left(\bar{D}_0\left(s_1^{in}\right), s_1^{in}\right).$$

Therefore, $\bar{D}_1 < \bar{D}_0\left(s_1^{in}\right)$ because the function $D \mapsto F_0\left(D, s_1^{in}\right)$ is increasing on I_0. Thus, $I_2 \subset I_0$ in case 1.

In case 2, we have $\Phi_2\left(\bar{D}_1\right) \leq \Phi_1\left(\bar{D}_1\right) < 0$ by using inequality (A.15). If equation $\Phi_2(D) = 0$ has no solution then $\Phi_2(D) < 0$, for all $D \in \bar{I}_2$ and consequently the set I_2 is empty. Thus, it is included in I_0. If equation $\Phi_2(D) = 0$ has n solutions which are denoted $\widetilde{D}_i$, $i = 1, \ldots, n$, (see Table 4), then $\widetilde{D}_i \in I_1 \cap \bar{I}_2$ where $I_1 = [0, \hat{D}_1)$ in this case 2. Since the function $D \mapsto f_2(D) := M_1^2(\alpha_1 D + a_1) - s_1^{in}$ is decreasing from $f_2(0)$ to $f_2(\bar{D}_1) = s_1^{max} - s_1^{in}$ which is positive in the case $s_1^{in} < s_1^{max}$. Thus, $f_2(D) > 0$, for all $D \in \bar{I}_2$. Thus,

$$F_0\left(\widetilde{D}_i, s_1^{in}\right) = M_0\left(\alpha_0\widetilde{D}_i + a_0, s_1^{in}\right) < M_0\left(\alpha_0\widetilde{D}_i + a_0, M_1^2\left(\alpha_1\widetilde{D}_i + a_1\right)\right) = F_1^2(\widetilde{D}_i) = +\infty.$$

Then,

$$F_0\left(\widetilde{D}_i, s_1^{in}\right) < +\infty = F_0\left(\bar{D}_0\left(s_1^{in}\right), s_1^{in}\right).$$

Since the function $D \mapsto F_0\left(D, s_1^{in}\right)$ is increasing, $\max_i\left(\widetilde{D}_i\right) < \bar{D}_0\left(s_1^{in}\right)$. We conclude that $I_2 \subset I_0$ in case 2. The positivity of $f_2(D)$ for all $D \in \bar{I}_2$ implies that

$$F_1^2(D) = M_0\left(D_0, M_1^2(D_1)\right) < M_0\left(D_0, M_1^2(D_1)\right) + M_1^2(D_1) - s_1^{in} = F_2^2(D) - s_1^{in}, \qquad \text{for all } D \in I_2.$$

Since M_0 is increasing in the second variable, we have

$$F_0\left(D, s_1^{in}\right) = M_0\left(D_0, s_1^{in}\right) < M_0\left(D_0, M_1^2(D_1)\right) = F_1^2(D), \qquad \text{for all } D \in I_2.$$

Since the function $D \mapsto f_1(D) := M_1^1(\alpha_1 D + a_1) - s_1^{in}$ is increasing from $f_1(0) = M_1^1(a_1) - s_1^{in}$ to $f_1(\bar{D}_1) = s_1^{max} - s_1^{in}$, it follows that there exists a solution $D_1^* \in \left(0, \bar{D}_1\right)$ of equation $f_1(D) = 0$ if, and only if, $f_1(0) < 0$, that is, (22) holds. If such D_1^* exists then it is unique. When (22) holds such that $D_1^* \in I_1$, the function F_i^1 is defined for $D = D_1^*$ and it follows that,

$$F_2^1(D_1^*) - s_1^{in} = M_0\left(\alpha_0 D_1^* + a_0, M_1^1(\alpha_1 D_1^* + a_1)\right) = F_1^1(D_1^*) = M_0\left(\alpha_0 D_1^* + a_0, s_1^{in}\right) = F_0\left(D_1^*, s_1^{in}\right).$$

Since $M_1^1(D_1) < s_1^{in}$ when $D < D_1^*$ and M_0 is increasing in the second variable, it follows that

$$F_2^1(D) - s_1^{in} = M_0\left(D_0, M_1^1(D_1)\right) + M_1^1(D_1) - s_1^{in} < M_0\left(D_0, M_1^1(D_1)\right) = F_1^1(D) < M_0\left(D_0, s_1^{in}\right) = F_0\left(D, s_1^{in}\right),$$

that is, (23) holds. Similarly, (24) holds when $D > D_1^*$ because $M_1^1(D_1) > s_1^{in}$. When (22) holds such that $D_1^* \in \left[\hat{D}_1, \bar{D}_1\right]$, $f_1(D) < 0$ for all $D \in \left[0, \hat{D}_1\right)$. Therefore, (23) holds where $\bar{D}_0\left(s_1^{in}\right) < \hat{D}_1$. When condition (22) is not fulfilled, $f_1(D) > 0$ for all $D \in I_1$ and consequently (24) holds. □

Proof of Propositions 4.3-4.4. The results are proved in a similar manner to that in the proof of the previous Proposition 4.2. □

Proof of Proposition 4.5. From Proposition 4.2, when $s_1^{in} = 0$, we have

$$F_0(D, 0) < F_1^2(D) < F_2^2(D), \qquad \text{for all} \quad D \in I_2,$$

where $I_2 \subset I_1 \subset I_0$. In addition, since $M_1^1(a_1) \geq 0$, that is, condition (22) is not satisfied, then the three curves γ_0, γ_1^1 and γ_2^1 do not intersect and we have

$$F_0(D, 0) < F_1^1(D) < F_2^1(D), \qquad \text{for all} \quad D \in I_1.$$

From Proposition 4.1, we have $F_2^1(D) \leq F_2^2(D)$. Consequently, $F_0(D, 0) < F_2^1(D) \leq F_2^2(D)$ for all $D \in I_2$. Using Table 3, we can prove the following results:

- $\mathcal{J}_1$ is defined by $D \geq \bar{D}_0(0)$ or $D \in I_0$ such that $s_0^{in} < F_0(D, 0)$. Consequently, SS_0 is the only existing steady state in this region which is LES.
- $\mathcal{J}_2$ is defined by $D \in I_0 \backslash I_1$ such that $s_0^{in} > F_0(D, 0)$ or $D \in I_1$ such that $F_0(D, 0) < s_0^{in} < F_2^1(D)$. Hence, SS_0 is unstable and SS_1 is LES.
- $\mathcal{J}_3$ is defined by $D \in I_1 \backslash I_2$ such that $s_0^{in} > F_2^1(D)$ or $D \in I_2$ such that $F_2^1(D) < s_0^{in} < F_2^2(D)$. Thus, SS_0 and SS_1 are unstable while SS_2^1 is LES.
- $\mathcal{J}_4$ is defined by $D \in I_2$ such that $s_0^{in} > F_2^2(D)$. Thereby, SS_0 and SS_2^2 are unstable while SS_1 and SS_2^1 are LES.

□

Proof of Propositions 4.6,4.7,4.8,4.11. The results follow from Table 3 where the details are as in the proof of the previous Proposition 4.5 and are left to the reader. □

Proof of Proposition 4.9. From (10), the function $s_1^{in} \mapsto F_0\left(D, s_1^{in}\right) = M_0\left(D_0, s_1^{in}\right)$ is defined if, and only if,

$$D_0 < \bar{\mu}_0\left(s_1^{in}\right) \iff D < \bar{D}_0\left(s_1^{in}\right) = \frac{\bar{\mu}_0\left(s_1^{in}\right) - a_0}{\alpha_0}. \tag{A.17}$$

From hypothesis (H5), the function $s_1^{in} \mapsto \bar{D}_0\left(s_1^{in}\right)$ is decreasing from $\bar{D}_0(0)$ to $\bar{D}_0(+\infty)$. If $D \leq \bar{D}_0(+\infty)$, then condition (A.17) is satisfied for all $s_1^{in} \geq 0$, that is the function $s_1^{in} \mapsto F_0\left(D, s_1^{in}\right)$ is defined on $[0, +\infty)$. If $\bar{D}_0(+\infty) < D < \bar{D}_0(0)$, then there exists a solution $\bar{s}_1^{in}$ of equation $D = \bar{D}_0(s_1^{in})$. It is unique if it exists. Moreover, condition (A.17) holds for all $s_1^{in} \in \left[0, \bar{s}_1^{in}\right)$. If $D \geq \bar{D}_0(0)$, condition (A.17) does not hold for all $s_1^{in} \geq 0$. Since $\bar{s}_1^{in}$ satisfies $D_0 = \mu_0\left(+\infty, \bar{s}_1^{in}\right)$, it follows that

$$F_0\left(D, \bar{s}_1^{in}\right) = M_0\left(\mu_0\left(+\infty, \bar{s}_1^{in}\right), \bar{s}_1^{in}\right) = +\infty.$$

When case 1 of (21) holds, we have

$$\alpha_0\bar{D}_1 + a_0 < \bar{\mu}_0\left(M_1^1(\alpha_1\bar{D}_1 + a_1)\right) < \bar{\mu}_0\left(0\right)),$$

because the function $\bar{\mu}_0(\cdot)$ is decreasing (see assumption (H5)). Thus, $\bar{D}_1 < \bar{D}_0(0)$. Moreover, when case 2 of (21) holds and $\hat{D}_1$ is a solution of equation $\Phi_1(D) = 0$, we have

$$\alpha_0\hat{D}_1 + a_0 = \bar{\mu}_0\left(M_1^1(\alpha_1\hat{D}_1 + a_1)\right) < \bar{\mu}_0\left(0\right)).$$

We conclude that, $\hat{D}_1 < \bar{D}_0(0)$. □

Proof of Proposition 4.10. Since the function $D \mapsto M_1^j(\alpha_1 D + a_1)$, $j = 1, 2$, is defined for all $D \in I_j$, then the function $s_1^{in} \mapsto f_j\left(s_1^{in}\right) = M_1^j(D_1) - s_1^{in}$ is decreasing from $f_j(0) = M_1^j(D_1) \geq 0$ to $f_j(+\infty) = -\infty$. Therefore, there exists a unique solution $s_{1j}^{in*} \geq 0$ of equation $f_j\left(s_1^{in}\right) = 0$. Since $M_1^1(D_1) \leq M_1^2(D_1)$, for all $D \in \bar{I}_2$, then $s_{11}^{in*} \leq s_{12}^{in*}$.

Let $D \in I_1$. The function $F_i^1(\cdot)$, $i = 1, 2$, is defined on I_1. Using Proposition 4.9 yields $D < \bar{D}_0(0)$, for all $D \in I_1$, because $D \leq \bar{D}_1 < \bar{D}_0(0)$ if case 1 of (21) holds and $D < \hat{D}_1 < \bar{D}_0(0)$ when case 2 of (21) holds.

From Proposition 4.9, we deduce that the function $s_1^{in} \mapsto F_0\left(D, s_1^{in}\right)$ is defined on the interval $\left[0, \bar{s}_1^{in}\right)$ where we put $\bar{s}_1^{in} = +\infty$ if $D \leq \bar{D}_0(+\infty)$. Since $D \in \bar{I}_1$, then $s_{11}^{in*} = M_1^1(D_1)$ and consequently

$$F_0\left(D, s_{11}^{in*}\right) = M_0\left(D_0, M_1^1(D_1)\right) = F_1^1(D) < +\infty = F_0\left(D, \bar{s}_1^{in}\right), \quad \text{for all} \quad D \in I_1.$$

As the function $F_0(D, \cdot)$ is increasing, we obtain $s_{11}^{in*} < \bar{s}_1^{in}$. Moreover, we have

$$F_1^1(D) = F_1^1(D) + M_1^1(D_1) - s_{11}^{in*} = F_2^1(D) - s_{11}^{in*}, \quad \text{for all} \quad D \in I_1,$$

that is, the three curves γ_0, γ_1^1 and γ_2^1 intersect at the same point $s_1^{in} = s_{11}^{in*}$. For all $s_1^{in} \in \left[0, s_{11}^{in*}\right)$, $f_1\left(s_1^{in}\right) > 0$ implies that

$$M_0\left(D_0, s_1^{in}\right) < M_0\left(D_0, M_1^1(D_1)\right) < M_0\left(D_0, M_1^1(D_1)\right) + M_1^1(D_1) - s_1^{in},$$

that is, condition (24) holds. Similarly, for all $s_1^{in} \in \left(s_{11}^{in*}, \bar{s}_1^{in}\right)$, $f_1\left(s_1^{in}\right) < 0$ yields condition (23). The second assertion is proved in a similar manner. □

Appendix B. Definition domains of functions F_0 and F_i^j

The following lemma analyzes the monotonicity of the function Φ_1, and determines the interval where the function Φ_1 is positive according to the coefficients α_0 and α_1.

Lemma B1. *Assume that*

$$a_1 < \mu_1^{max}. \tag{B.1}$$

When $\alpha_0 = \alpha_1 = 0$, the function Φ_1 is defined and constant for all D. It is positive if, and only if,

$$\bar{\mu}_0\left(M_1^1(a_1)\right) > a_0. \tag{B.2}$$

When $\alpha_0 > 0$ or $\alpha_1 > 0$, the function Φ_1 is decreasing on $\bar{I}_1$ which is defined in Table 5. In addition, if (B.2) holds, then $\Phi_1(D) > 0$, for all $D \in I_1$ which is defined by

$$I_1 = \begin{cases} [0, +\infty), & \text{when} \quad \alpha_0 = 0 \quad \text{and} \quad \alpha_1 = 0, \\ \left[0, \frac{\bar{\mu}_0\left(M_1^1(a_1)\right) - a_0}{\alpha_0}\right), & \text{when} \quad \alpha_0 > 0 \quad \text{and} \quad \alpha_1 = 0, \\ \left[0, \bar{D}_1\right], & \text{when} \quad \alpha_0 \geq 0 \quad \text{and} \quad \alpha_1 > 0, \quad \text{and case 1 of (21) holds}, \\ \left[0, \hat{D}_1\right), & \text{when} \quad \alpha_0 \geq 0 \quad \text{and} \quad \alpha_1 > 0, \quad \text{and case 2 of (21) holds}, \end{cases} \tag{B.3}$$

where $\hat{D}_1$ is the solution of equation $\Phi_1(D) = 0$.

Proof. When $\alpha_0 = \alpha_1 = 0$, it follows from definition (16) of Φ_j that,

$$\Phi_j(D) = \bar{\mu}_0\left(M_1^j(a_1)\right) - a_0, \quad j = 1, 2, \tag{B.4}$$

that is, the function Φ_j is constant for all D. Hence, it is positive if, and only if, condition (B.2) holds.

When $\alpha_0 > 0$ and $\alpha_1 = 0$, using definition (16), we obtain $\Phi_j'(D) = -\alpha_0 < 0$. Assume that condition (B.2) holds, that is, $\Phi_1(0) > 0$. Thus, the equation $\Phi_1(D) = 0$ has a unique solution

$$\hat{D}_1 = \frac{\bar{\mu}_0(M_1^1(a_1)) - a_0}{\alpha_0},$$

such that $\Phi_1(D) > 0$ for all $D \in [0, \hat{D}_1)$.

When $\alpha_0 \geq 0$ and $\alpha_1 > 0$, straightforward calculation shows that

$$\Phi_j'(D) = \alpha_1 \bar{\mu}_0'\left(M_1^j(D_1)\right) M_1^{j\prime}(D_1) - \alpha_0. \tag{B.5}$$

Recall that the function M_1^1 is increasing. From assumption (H5), it follows that $\Phi_1'(D) < 0$ for all $D \in \bar{I}_1$. Therefore, $\Phi_1(D) > 0$ for all $D \in [0, \bar{D}_1]$ since $\Phi_1(\bar{D}_1) > 0$ when case 1 of (21) holds and $\Phi_1(D) > 0$ for all $D \in [0, \hat{D}_1)$ since $\Phi_1(\bar{D}_1) \leq 0$ when case 2 of (21) holds. □

The following result determines the definition domains I_0 and I_1, respectively, of the functions $F_0\left(\cdot, s_1^{in}\right)$ and $F_i^1(\cdot)$, $i = 1, 2$.

Proposition B1. *For all $s_1^{in} \geq 0$, the function $F_0(\cdot, s_1^{in})$ is defined on $I_0 = [0, \bar{D}_0(s_1^{in}))$. Notice that this interval is not empty if, and only if, $\bar{\mu}_0(s_1^{in}) > a_0$.*

Assume that (B.1) and (B.2) hold. The function F_i^1 is defined on the interval I_1 defined by (B.3).

Proof. From (10) and (12), it follows that the function $D \mapsto F_0\left(D, s_1^{in}\right) = M_0\left(D_0, s_1^{in}\right)$ is defined if, and only if,

$$D_0 < \bar{\mu}_0\left(s_1^{in}\right), \quad \text{or equivalently} \quad D < \bar{D}_0\left(s_1^{in}\right) = \frac{\bar{\mu}_0\left(s_1^{in}\right) - a_0}{\alpha_0}.$$

Note that $\bar{D}_0\left(s_1^{in}\right)$ is positive if, and only if, $\bar{\mu}_0(s_1^{in}) > a_0$ and in the particular case $\alpha_0 = 0$, we put $\bar{D}_0\left(s_1^{in}\right) = +\infty$.

From (10) and (13), the function F_i^1, $i = 1, 2$, is defined if, and only if,

$$D_1 < \mu_1^{max} \quad \text{and} \quad D_0 < \bar{\mu}_0\left(M_1^1(D_1)\right) \iff D < \frac{\mu_1^{max} - a_1}{\alpha_1} = \bar{D}_1 \quad \text{and} \quad \Phi_1(D) > 0. \tag{B.6}$$

Note that $\bar{D}_1$ is positive if, and only if, (B.1) holds and in the particular case $\alpha_1 = 0$, we put $\bar{D}_1 = +\infty$ where the condition (B.6) is equivalent to (B.1) and $\Phi_1(D) > 0$. The result will then follow from Lemma B1. □

The next lemma studies the monotonicity of the function Φ_2, and determines the interval where the function Φ_2 is positive when the coefficients $\alpha_0 = 0$ or $\alpha_1 = 0$.

Lemma B2. *Assume that (B.1) holds. When $\alpha_0 = \alpha_1 = 0$, the function Φ_2 is defined and constant for all D. It is positive if, and only if,*

$$\bar{\mu}_0\left(M_1^2(a_1)\right) > a_0. \tag{B.7}$$

When $\alpha_0 > 0$ and $\alpha_1 = 0$, the function Φ_2 is decreasing on $\bar{I}_2$ which is defined in Table 5. When $\alpha_0 = 0$ and $\alpha_1 > 0$, the function Φ_2 is increasing on $\bar{I}_2$. We have $\Phi_2(D) > 0$, for all $D \in I_2$ which is defined by

$$I_2 = \begin{cases} [0, +\infty), & \textit{when} \quad \alpha_0 = 0 \quad \textit{and} \quad \alpha_1 = 0, \quad \textit{and (B.7) holds}, \\ \left[0, \frac{\bar{\mu}_0\left(M_1^2(a_1)\right) - a_0}{\alpha_0}\right), & \textit{when} \quad \alpha_0 > 0 \quad \textit{and} \quad \alpha_1 = 0, \quad \textit{and (B.7) holds}, \\ \left[0, \bar{D}_1\right], & \textit{when} \quad \alpha_0 = 0 \quad \textit{and} \quad \alpha_1 > 0, \quad \Phi_2(\bar{D}_1) > 0 \quad \textit{and (B.7) holds}, \\ \left(\widetilde{D}_1, \bar{D}_1\right], & \textit{when} \quad \alpha_0 = 0 \quad \textit{and} \quad \alpha_1 > 0, \quad \Phi_2(\bar{D}_1) > 0 \quad \textit{and (B.7) is not fulfilled}, \end{cases} \tag{B.8}$$

where $\widetilde{D}_1$ is the unique solution of the equation $\Phi_2(D) = 0$. Note that the function Φ_2 is not defined for $D = 0$ in the particular case $a_1 = 0$.

Proof. When $\alpha_0 = \alpha_1 = 0$, it follows from (B.4) that the function Φ_2 is constant for all D and is positive if, and only if, condition (B.7) holds. When $\alpha_0 > 0$ and $\alpha_1 = 0$, we have shown that $\Phi_j'(D) = -\alpha_0 < 0$ for all $\bar{I}_2$. Thus, when condition (B.7) holds, that is, $\Phi_2(0) > 0$, the equation $\Phi_2(D) = 0$ has a unique solution

$$\widetilde{D}_1 = \frac{\bar{\mu}_0(M_1^2(a_1)) - a_0}{\alpha_0},$$

such that $\Phi_2(D) > 0$ for all $D \in [0, \widetilde{D}_1)$. Recall that the function M_1^2 is decreasing. Using (B.5), it follows that $\Phi_2'(D) > 0$ for all $\bar{I}_2$ in the case where $\alpha_0 = 0$ and $\alpha_1 > 0$. Hence, when $\Phi_2(\bar{D}_1) > 0$, the equation $\Phi_2(D) = 0$ has no solution where $\Phi_2(D) > 0$ for all $D \in [0, \bar{D}_1]$ if (B.7) holds; otherwise the equation $\Phi_2(D) = 0$ has a unique solution $\widetilde{D}_1 \in [0, \bar{D}_1]$ such that $\Phi_2(D) > 0$ for all $D \in (\widetilde{D}_1, \bar{D}_1]$. □

In what follows, we study the definition domain of the function F_i^2, $i = 1, 2$ in the remaining case $\alpha_0 > 0$ and $\alpha_1 > 0$. When the growth functions are given by (6), we show, see Proposition C1, that the equation $\Phi_2(D) = 0$ has at most three solutions in the case 1 of (21) with

$$\frac{a_1}{\alpha_1} \neq \frac{a_0}{\alpha_0}, \tag{B.9}$$

and at most two solutions in the case 2 of (21). However, the equation $\Phi_2(D) = 0$ has at most two solutions in the case 1 of (21) with

$$\frac{a_1}{\alpha_1} = \frac{a_0}{\alpha_0}, \tag{B.10}$$

in particular without decay ($a_0 = a_1 = 0$) and at most one solution in the case 2 of (21), (see Figs. C.14-C.15(b-d)).

Therefore, for simplicity, we assume that the equation $\Phi_2(D) = 0$ has at most three solutions in the case 1 of (21) and two solutions in the case 2 of (21) where condition (B.9) holds. The general case can be treated similarly, without added difficulty. In this particular case, the definition domain I_2 of function F_i^2, $i = 1, 2$, is given as follows.

Proposition B2. *Assume that $\alpha_0 > 0$ and $\alpha_1 > 0$ and condition (B.1) holds. The function Φ_2 is nonmonotonic on $\bar{I}_2$ (see Figs. C.14-C.15(b-d)). The function F_i^2 is defined on I_2 which is defined by*

$$I_2 = \begin{cases} \left[0, \bar{D}_1\right], & \text{when case 1 of (21) holds and } n = 0, \\ \left(\widetilde{D}_1, \bar{D}_1\right], & \text{when case 1 of (21) holds and } n = 1, \\ \left[0, \widetilde{D}_2\right) \bigcup \left(\widetilde{D}_1, \bar{D}_1\right], & \text{when case 1 of (21) holds and } n = 2, \\ \left(\widetilde{D}_3, \widetilde{D}_2\right) \bigcup \left(\widetilde{D}_1, \bar{D}_1\right], & \text{when case 1 of (21) holds and } n = 3, \\ \left[0, \widetilde{D}_1\right), & \text{when case 2 of (21) holds and } n = 1, \\ \left(\widetilde{D}_2, \widetilde{D}_1\right) & \text{when case 2 of (21) holds and } n = 2, \end{cases} \tag{B.11}$$

where $\widetilde{D}_i$, $i = 1, \dots, n$, are the solutions of the equation $\Phi_2(D) = 0$ and n denotes the number of solutions such that $\widetilde{D}_i > \widetilde{D}_j$, for all $i < j$. Note that the function F_i^2 is not defined for $D = 0$ in the particular case $a_1 = 0$.

Proof. Let $\alpha_0 > 0$ and $\alpha_1 > 0$. Recall that the function M_1^2 is decreasing. From (B.5), the sign of $\Phi_2'(D)$ can change at $D \in \bar{I}_2$ since the function $\bar{\mu}_0$ is decreasing. Thus, the function Φ_2 can be nonmonotonic on $\bar{I}_2$ (see Figs. C.14-C.15(b-d)). The function F_i^2 is defined if, and only if,

$$0 < D_1 < \mu_1^{max} \quad \text{and} \quad D_0 < \bar{\mu}_0\left(M_1^2(D_1)\right) \quad \Longleftrightarrow \quad -\frac{a_1}{\alpha_1} < D < \frac{\mu_1^{max} - a_1}{\alpha_1} = \bar{D}_1 \quad \text{and} \quad \Phi_2(D) > 0.$$

When case 1 holds and $n = 0$, we have $\Phi_2(\bar{D}_1) > 0$ and the equation $\Phi_2(D) = 0$ has no solution. Consequently, $\Phi_2(D) > 0$ for all $D \in [0, \bar{D}_1]$. Hence, the function F_i^2 is defined on $I_2 = \bar{I}_2$ where $\bar{I}_2 = [0, \bar{D}_1]$ when $a_1 > 0$ and $\bar{I}_2 = (0, \bar{D}_1]$ when $a_1 = 0$. When case 1 holds and $n = 1$, the equation $\Phi_2(D) = 0$ has a unique solution $\widetilde{D}_1 \in [0, \bar{D}_1]$. Thus, the function F_i^2 is defined on $(\widetilde{D}_1, \bar{D}_1]$ since $\Phi_2(D) > 0$ for all $D \in (\widetilde{D}_1, \bar{D}_1]$. The other cases can be treated similarly (see Fig. C.15(b-d)). □

Appendix C. The particular case for growth functions (6)

The following result determines the maximal number of solutions of the equation $\Phi_2(D) = 0$ in the particular case of growth functions (6) when $\alpha_0 > 0$ and $\alpha_1 > 0$.

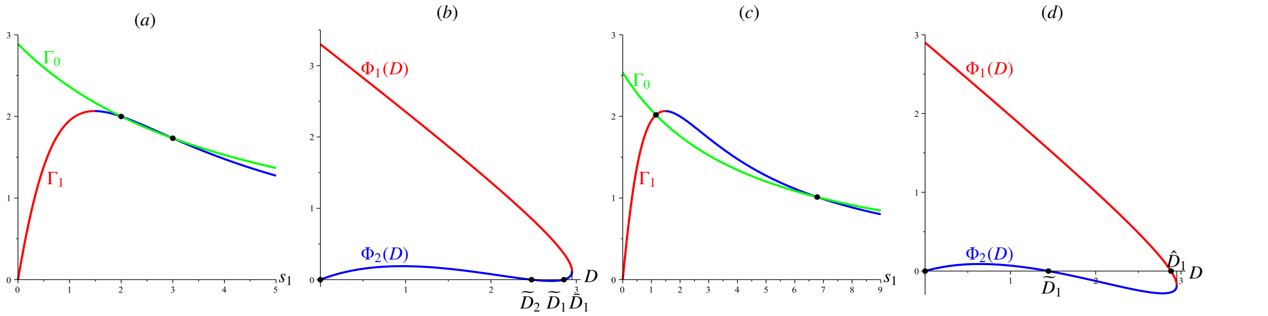

Fig. C.14: Case (B.10), in particular without decay: (a,c) number of intersections of the curves Γ_0 and Γ_1 of the functions $\widetilde{\mu}_0$ and μ_1, respectively, and (b,d) the corresponding number of solutions of equation $\Phi_j(D) = 0$. (a-b) In case 1 of (21), the equation $\Phi_2(D) = 0$ has two solutions on $[0, \bar{D}_1]$. (c-d) In case 2 of (21), the equation $\Phi_2(D) = 0$ has one solution on $[0, \bar{D}_1]$.

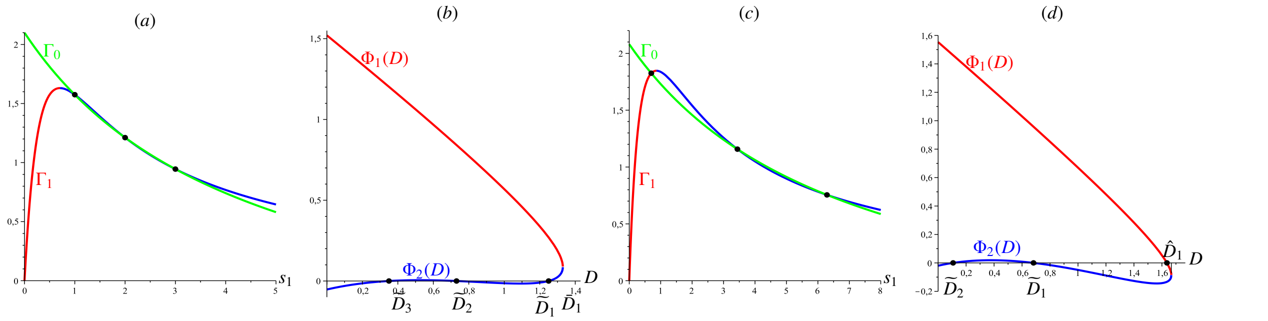

Fig. C.15: Case (B.9): (a,c) number of intersections of the curves Γ_0 and Γ_1 and (b,d) the corresponding number of solutions of equation $\Phi_j(D) = 0$. (a-b) In case 1 of (21), the equation $\Phi_2(D) = 0$ has three solutions on $[0, \bar{D}_1]$. (c-d) In case 2 of (21), the equation $\Phi_2(D) = 0$ has two solutions on $[0, \bar{D}_1]$.

Proposition C1. *Assume that $\alpha_0 > 0$ and $\alpha_1 > 0$. Let*

$$y = \bar{\mu}_0^{-1}(D_0). \tag{C.1}$$

We have

$$\Phi_1(D) = 0 \quad \text{and} \quad \Phi_2(D) = 0 \quad \Longleftrightarrow \quad \mu_1(y) = \widetilde{\mu}_0(y) := \frac{\alpha_1}{\alpha_0}\bar{\mu}_0(y) + a_1 - \frac{\alpha_1}{\alpha_0}a_0. \tag{C.2}$$

When the growth functions μ_0 and μ_1 are of type (6), the equation $\Phi_2(D) = 0$ has at most three solutions in the case 1 of (21) when condition (B.9) holds, and at most two solutions in the case 2 of (21). It has at most two solutions in the case 1 of (21) when condition (B.10) holds, and at most one solution in the case 2 of (21).

Proof. From definition (16) of the function Φ_j and as the function $\bar{\mu}_0$ is decreasing according to (H5), we have

$$\Phi_j(D) = 0 \quad \Longleftrightarrow \quad M_1^j(D_1) = \bar{\mu}_0^{-1}(D_0), \quad j = 1, 2.$$

According to (9), it follows that

$$\mu_1\left(\bar{\mu}_0^{-1}(D_0)\right) = D_1.$$

Using (C.1), we obtain $D_0 = \bar{\mu}_0(y)$. From definition (2) of D_i, $i = 0, 1$, we have

$$D_1 = \alpha_1 \frac{D_0 - a_0}{\alpha_0} + a_1.$$

Using (C.1), it can be deduced that equation (C.2) holds. When the growth functions μ_0 and μ_1 are of type (6), we obtain

$$\frac{m_1 y}{K_1 + y + y^2/K_I} = \frac{K_i m_0 \alpha_1/\alpha_0 + (K_i + y)(a_1 - a_0\alpha_1/\alpha_0)}{K_i + y}.$$

When condition (B.9) holds, we obtain an algebraic equation of degree three in y and consequently the equation (C.2) has at most three solutions. Hence, if case 1 of (21) holds, that is, the equation $\Phi_1(D) = 0$ has no solution, then the equation $\Phi_2(D) = 0$ has at most three solutions. However, if case 2 of (21) holds, that is, the equation $\Phi_1(D) = 0$ has one solution, then the equation $\Phi_2(D) = 0$ has at most two solutions. When condition (B.10) holds, we obtain an algebraic equation of degree two in y. Thus, the rest of the results follows similarly. □

When the growth functions are given by (6), we succeeded in finding a set of parameters such that we show the maximum number of intersections of the curves Γ_0 and Γ_1 and the corresponding number of solutions of the equation $\Phi_j(D) = 0$ (see Figs. C.14 and C.15).

For growth functions (6), straightforward computations show that the inverse functions $M_1^j(\cdot)$, $j = 1, 2$, and $M_0(\cdot, s_1)$ are explicitly given by

$$M_1^j(y) = \frac{(m_1 - y) \pm \sqrt{(m_1 - y)^2 - 4\frac{K_1}{K_I}y^2}}{\frac{2y}{K_I}}, \quad \text{for all} \quad y \in \left(0, \frac{m_1\sqrt{k_I}}{\sqrt{k_I} + 2\sqrt{K_1}}\right),$$

$$M_0(y, s_1) = \frac{K_0 y}{\frac{m_0}{1+s_1/K_i} - y}, \quad \text{for all} \quad y \in \left[0, \frac{m_0}{1 + s_1/K_i}\right).$$

The functions F_0 and F_i^j, $i, j = 1, 2$, are given explicitly by

$$F_0\left(D, s_1^{in}\right) = \frac{K_0 D_0\left(1 + \frac{s_1^{in}}{K_i}\right)}{m_0 - D_0\left(1 + \frac{s_1^{in}}{K_i}\right)}, \quad F_1^j(D) = \frac{K_0 D_0\left(1 + \frac{M_1^j(D_1)}{K_i}\right)}{m_0 - D_0\left(1 + \frac{M_1^j(D_1)}{K_i}\right)} \quad \text{and} \quad F_2^j(D) = M_1^j(D_1) + F_1^j(D).$$

Appendix D. Parameter values used for numerical simulations

For the numerical simulations, we have used the growth functions given by (6). All the values of the parameters used in the figures are provided in Table D.12.

Table D.12: The nominal values used for (5) and growth functions given by (6).

Parameter	m_0 (d^{-1})	K_0 (kg COD/m^3)	K_i (kg COD/m^3)	m_1 (d^{-1})	K_1 (kg COD/m^3)	K_I (kg COD/m^3)	α_0	a_0 (d^{-1})	α_1	a_1 (d^{-1})
Figs. 1-3(a)-4(a)-6(a)-8-9(b)- 10(a)-12	3.5	1.5	5	4	0.5	0.95	0.9	1.2	0.8	0.7
Figs. 2-3(b)-5-7(b)-9(a)-11 -13	3.5	1.5	5	3.5	0.5	0.95	0.95	1.4	0.5	0.5
Fig. 4(b)	3.5	1.5	5	4	0.5	0.95	0.9	1.2	0.6	0.7
Fig. 7(a)	3.5	1.5	5	3.5	0.5	0.95	0.95	1.8	0.5	0.5
Fig. 10(b)	3.5	1.5	5	4	0.5	0.95	0.9	1.91	0.8	0.7
Fig. 6(b)	4	1.5	1.2	5.32	0.5	0.95	1	1	0.8	1.18
Figs. C.14(a-b)	3.3	1	4.5	8.21	2.21	1	0.8	0	0.7	0
Figs. C.14(c-d)	2.9	1	4.5	8.21	2.21	1	0.8	0	0.7	0
Figs. C.15(a-b)-6(c)	3.3	1	4.5	3.94	0.5	1	0.8	1.7	0.7	0.7
Figs. C.15(c-d)-7(c)	3.2	1	7	3.98	0.5	1.5	0.8	1.6	0.7	0.68

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References

[1] D. Batstone, J. Keller, I. Angelidaki, S. Kalyhuzhnyi, S. Pavlosthathis, A. Rozzi, W. Sanders, H. Siegrist, and V. Vavilin, *The IWA Anaerobic Digestion Model No 1 (ADM1)*, Water Sci Technol., 45 (2002), pp. 66–73, https://doi.org/10.2166/wst.2002.0292.

[2] B. Benyahia, T. Sari, B. Cherki, and J. Harmand, *Bifurcation and stability analysis of a two step model for monitoring anaerobic digestion processes*, J. Process Control, 22 (2012), pp. 1008–1019, https://doi.org/10.1016/j.jprocont.2012.04.012.

[3] 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 (2001), pp. 424–438, https://doi.org/10.1002/bit.10036.

[4] M. Boer, B. Kooi, and S. Kooijman, *Food chain dynamics in the chemostat*, Math. Biosci., 150 (1998), pp. 43–62, https://doi.org/10.1016/S0025-5564(98)00010-8.

[5] A. Burchard, *Substrate degradation by a mutualistic association of two species in the chemostat*, J. Math. Biol., 32 (1994), pp. 465–489, https://doi.org/10.1007/BF00160169.

[6] 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 (2018), pp. 1–22, https://doi.org/10.1051/mmnp/2018037.

[7] S. Di and A. Yang, *Analysis of productivity and stability of synthetic microbial communities*, J. R. Soc. Interface, 16 (2019), pp. 1–19, https://doi.org/10.1098/rsif.2018.0859.

[8] A. Donoso-Bravo, J. Mailier, C. Martin, J. Rodríguez, C. A. Aceves-Lara, and A. Vande Wouwer, *Model selection, identification and validation in anaerobic digestion: A review*, Water Research, 45 (2011), pp. 5347–5364, https://doi.org/10.1016/j.watres.2011.08.059.

[9] M. El-Hajji, F. Mazenc, and J. Harmand, *A mathematical study of a syntrophic relationship of a model of anaerobic digestion process*, Math. Biosci. Eng, 7 (2010), pp. 641–656, https://doi.org/10.3934/mbe.2010.7.641.

[10] R. Fekih-Salem, N. Abdellatif, T. Sari, and J. Harmand, *Analyse mathématique d'un modèle de digestion anaérobie à trois étapes*, ARIMA Journal, 17 (2014), pp. 53–71, http://arima.inria.fr/017/017003.html.

[11] R. Fekih-Salem, N. Abdellatif, and A. Yahmadi, *Effect of inhibition on a syntrophic relationship model in the anaerobic digestion process*, in Proceedings of the 8th conference on Trends in Applied Mathematics in Tunisia, Algeria, Morocco, 2017, pp. 391–396, https://indico.math.cnrs.fr/event/1335.

[12] A. Ghouali, T. Sari, and J. Harmand, *Maximizing biogas production from the anaerobic digestion*, J. Process Control, 36 (2015), pp. 79–88, https://doi.org/10.1016/j.jprocont.2015.09.007.

[13] G. Giovannini, M. Sbarciog, J.-P. Steyer, R. Chamy, and A. Vande Wouwer, *On the derivation of a simple dynamic model of anaerobic digestion including the evolution of hydrogen*, Water Research, 134 (2018), pp. 209–225, https://doi.org/10.1016/j.watres.2018.01.036.

[14] J. Harmand, C. Lobry, A. Rapaport, and T. Sari, *The Chemostat: Mathematical Theory of Microorganism Cultures*, vol. 1, Chemical Eng. Ser., Chemostat Bioprocesses Set, Wiley, New York, 2017, https://doi.org/10.1002/9781119437215.

[15] E. Harvey, J. Heys, and T. Gedeon, *Quantifying the effects of the division of labor in metabolic pathways*, J. Theor. Biol., 360 (2014), pp. 222–242, https://doi.org/10.1016/j.jtbi.2014.07.011.

[16] 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 (2018), pp. 351–376, https://doi.org/10.1016/j.apm.2018.04.020.

[17] B. W. Kooi and M. P. Boer, *Chaotic behaviour of a predator–prey system in the chemostat*, Dyn. Contin. Discrete Impulse Syst. Ser. B App. Algorithms, 10 (2003), pp. 259–272.

[18] R. Kreikenbohm and E. Bohl, *A mathematical model of syntrophic cocultures in the chemostat: (anaerobic degradation; H2-producing acetogenic bacteria; methanogens; interspecies H2-transfer; continuous culture; growth rate expressions)*, FEMS Microbiol. Ecol., 2 (1986), pp. 131–140, https://doi.org/10.1111/j.1574-6968.1986.tb01722.x.

[19] R. Kreikenbohm and E. Bohl, *Bistability in the chemostat*, Ecological Modelling, 43 (1988), pp. 287–301, https://doi.org/10.1016/0304-3800(88)90009-9.

[20] J. Mailier, M. Remy, and A. Vande Wouwer, *Stoichiometric identification with maximum likelihood principal component analysis*, J. Math. Biol., 67 (2013), pp. 739–765, https://doi.org/10.1007/s00285-012-0559-0.

[21] F. Mairet, O. Bernard, E. Cameron, M. Ras, L. Lardon, J.-P. Steyer, and B. Chachuat, *Three-reaction model for the anaerobic digestion of microalgae*, Biotechnol. Bioeng., 109 (2012), pp. 415–425, https://doi.org/10.1002/bit.23350.

[22] S. Marsili-Libelli and S. Beni, *Shock load modelling in the anaerobic digestion process*, Ecol. Model., 84 (1996), pp. 215–232, https://doi.org/10.1016/0304-3800(94)00125-1.

[23] B.-J. Ni, G.-P. Sheng, and H.-Q. Yu, *Model-based characterization of endogenous maintenance, cell death and predation processes of activated sludge in sequencing batch reactors*, Chem. Eng. Sci., 66 (2011), pp. 747–754, https://doi.org/10.1016/j.ces.2010.11.033.

[24] P. J. Reilly, *Stability of commensalistic systems*, Biotechnol. Bioeng., 16 (1974), pp. 1373–1392, https://doi.org/10.1002/bit.260161006.

[25] T. Sari, M. E. Hajji, and J. Harmand, *The mathematical analysis of a syntrophic relationship between two microbial species in a chemostat*, Math. Biosci. Eng., 9 (2012), pp. 627–645, https://doi.org/10.3934/mbe.2012.9.627.

[26] T. Sari and J. Harmand, *A model of a syntrophic relationship between two microbial species in a chemostat including maintenance*, Math. Biosci., 275 (2016), pp. 1–9, https://doi.org/10.1016/j.mbs.2016.02.008.

[27] T. Sari and M. Wade, *Generalised approach to modelling a three-tiered microbial food-web*, Math. Biosci., 291 (2017), pp. 21–37, https://doi.org/10.1016/j.mbs.2017.07.005.

[28] M. Sbarciog, M. Loccufier, and E. Noldus, *Determination of appropriate operating strategies for anaerobic digestion systems*, Biochem. Eng. J., 51 (2010), pp. 180–188, https://doi.org/10.1016/j.bej.2010.06.016.

[29] M. Sbarciog, M. Loccufier, and A. Vande Wouwer, *An optimizing start-up strategy for a bio-methanator*, Bioprocess Biosyst Eng, 35 (2012), pp. 565–578, https://doi.org/10.1007/s00449-011-0629-5.

[30] M. Sbarciog, J. A. Moreno, and A. Vande Wouwer, *A biogas-based switching control policy for anaerobic digestion systems*, IFAC Proceedings Volumes, 45 (2012), pp. 603–608, https://doi.org/10.3182/20120710-4-SG-2026.00056.

[31] S. Shen, G. C. Premier, A. Guwy, and R. Dinsdale, *Bifurcation and stability analysis of an anaerobic digestion model*, Nonlinear Dynam., 48 (2007), pp. 391–408, https://doi.org/10.1007/s11071-006-9093-1.

[32] I. Simeonov and S. Diop, *Stability analysis of some nonlinear anaerobic digestion models*, Int. J. Bioautomation, 14 (2010), pp. 37–48.

[33] I. Simeonov and S. Stoyanov, *Modelling and dynamic compensator control of the anaerobic digestion of organic wastes*, Chem. Biochem. Eng. Q., 17 (2003), pp. 285–292.

[34] G. Stephanopoulos, *The dynamics of commensalism*, Math. Biosci., 23 (1981), pp. 2243–2255, https://doi.org/10.1002/bit.260231008.

[35] E. Volcke, M. Sbarciog, E. Noldus, B. D. Baets, and M. Loccufier, *Steady state multiplicity of two-step biological conversion systems with general kinetics*, Math. Biosci., 228 (2010), pp. 160–170, https://doi.org/10.1016/j.mbs.2010.09.004.

[36] M. Wade, R. Pattinson, N. Parker, and J. Dolfing, *Emergent behaviour in a chlorophenol-mineralising three-tiered microbial 'food web'*, J. Theor. Biol., 389 (2016), pp. 171–186, https://doi.org/10.1016/j.jtbi.2015.10.032.

[37] M. Weedermann, G. Seo, and G. S. Wolkowicz, *Mathematical model of anaerobic digestion in a chemostat: effects of syntrophy and inhibition*, J. Biol. Dyn., 7 (2013), pp. 59–85, https://doi.org/10.1080/17513758.2012.755573.

[38] M. Weedermann, G. S. Wolkowicz, and J. Sasara, *Optimal biogas production in a model for anaerobic digestion*, Nonlinear Dyn, 81 (2015), pp. 1097–1112, https://doi.org/10.1007/s11071-015-2051-z.

[39] A. Xu, J. Dolfing, T. Curtis, G. Montague, and E. Martin, *Maintenance affects the stability of a two-tiered microbial 'food chain'?*, J. Theor. Biol., 276 (2011), pp. 35–41, https://doi.org/10.1016/j.jtbi.2011.01.026.