Performances study of two serial interconnected chemostats with mortality

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PERFORMANCES STUDY OF TWO SERIAL INTERCONNECTED CHEMOSTATS WITH MORTALITY.

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Abstract. In this work, a mathematical model representing two series interconnected chemostats where the mortality of the species is taken into consideration, is studied in detail. The study is carried out with different mortalities of the two tanks. The specificity of this study is the intervention of two types of heterogeneities. There is heterogeneity in relation to the distribution of the total volume in both tanks and heterogeneity in relation to the different mortalities of the two tanks. We study the performance of the serial configuration under two different criteria which consists on the substrate concentration leaving the second tank and the biogas flow rate production. A comparison is made with a single chemostat where the mortality rate is considered to be the same in all tanks, i.e. in the single chemostat and in the interconnected tanks. Conditions depending on the mortality rate, on the parameter; defining the distribution of the total volume between the two tanks and on the operating parameters that are the substrate concentration at the entrance of the first tank and the dilution rate, are involved. These conditions allow to have a serial configuration with mortality more efficient than a single chemostat with the same mortality.

1. Introduction

The mathematical model of the chemostat has received a great attention in the literature for many years (see for instance [13] and literature cited inside). This is probably due to its relative simplicity that can explain and predict quite faithfully the dynamics of real bioprocesses exploiting microbial ecosystems. It is today an important tool for decision making in industrial world, such as for dimensioning bioreactors or designing efficient operating conditions [10, 17]. Several extensions of the original model of the chemostat, considering spatial heterogeneity, have been proposed to better cope reality (see for instance [16]). Lovitt and Wimpenny has proposed the "gradostat" experimental device as a collection of chemostats of same volume interconnected in series [19, 20], which has led to the so-called "gradostat model" representing in a more general framework a gradient of concentrations [31, 34]. The gradostat model has been further generalized as the "general gradostat model" representing more general interconnection graphs with tanks of different volumes [32, 33]. Particular interconnection structures have been investigated and compared for the properties in terms of input-output performances (see for instance [5, 6, 12, 25]). It has been notably shown that a series of reactors instead of a single perfectly mixed one can significantly improve the performances of the bioprocess (in terms of matter conversion) while preserving the same residence time, or equivalently that the same performance can be obtained with a smaller residence time considering several tanks in series instead of a single one [11, 14, 21, 22, 40]. On another hand, it is known that in real processes, various growth conditions can be met and that it could be difficult to setup exactly the same perfect conditions in different reactors. These conditions include toxicity levels of culture media, which means more concretely that the consideration of a bacterial mortality, although often neglected compared to the removal rate, might be non avoidable and could also be variable. To the best of our knowledge, the possible impacts of mortality in the design of series of chemostats has not been yet studied in the literature, which is the purpose of the present work. Its contributions also cover interests in theoretical ecology for a better grasp of the interplay between spatial heterogeneity and mortality in resource-consumers models. Indeed, considering different removal rates in the classical chemostat model or more general ones allows to consider additional mortality terms [18, 26, 28, 38]. However, these mathematical studies have mainly concern analyses of equilibria and stability and not the performances of the system in presence of mortality.

In view of providing clear messages to the practitioners, we investigate how the operating diagram of a series of two interconnected chemosats in series is modified when considering different or identical mortality rates in both tanks. Operating diagrams have proven to be a good synthetic tool to summarize the possible operating modes, emphasized in [23] for its importance for bioreactors. Indeed, such diagrams are more and more often constructed both in the biological literature [23, 30, 35, 39] and the mathematical literature [1, 2, 4, 7, 8, 9, 15, 27, 29, 36, 37].

Then, we study the performances in terms of conversion ratio and byproduct production (such as biogas). As we shall see, several aspects are not intuitive, which show that the consideration of mortality can significantly modify the favorable operating conditions.

The paper is organized as follows. Section 2 includes the introduction of the mathematical model corresponding to the serial configuration of two chemostats with mortality rate and is devoted to the study of the existence and stability analysis of the steady states. This is an extension of former results without mortality but that has required to revisit significantly the mathematical proofs. Afterwards, Section 3 presents the richness of the various possible operating diagrams, playing with volumes distribution between tanks and different mortality rates. Then, Section 4 focuses on the study of performances of 1. the output substrate concentration and 2. the biogas production as functions of the input concentration, the flow rate and the volumes distribution. Next, Section 5 is devoted to illustrations and numerical simulations and a conclusion is given in Section 6. Moreover, we set up the single chemostat case with mortality, and give its mathematical analysis in terms of asymptotic behavior and performances at steady state, in Appendix A. Finally, Appendix B and Appendix C contain technical proofs.

2. Presentation of the model and preliminaries

We consider two serial interconnected chemostats where the total volume V is divided into two volumes, $V_1 := rV$ and $V_2 := (1-r)V$ with $r \in (0,1)$, as shown in Figure 1. The substrate and the biomass concentrations in the tank i are respectively denoted S_i and x_i, $i = 1,2$. The input substrate concentration in the first chemostat is designated S^{in}, the flow rate is constant and is designated by Q. The dilution rate of the whole structure denoted D is defined by $D := Q/V$. The dilution rates of tanks i, denoted D_i and defined by $D_i := Q/V_i$, are different. Thus, we consider that the growth environment differs from one tank to another one. This can lead to two different mortality rates in the tanks. We denote by a_i ($a_i > 0$, $i = 1,2$) the mortality rate in the tank i.

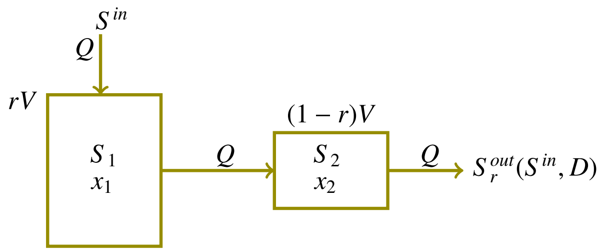


FIGURE 1. The serial configuration of two chemostats.

The particular case when $a_1 = a_2 = 0$ is already studied in-depth in [6]. The mathematical model is given by the following equations:

$$\begin{aligned}
\dot{S}_1 &= \frac{D}{r}(S^{in} - S_1) - f(S_1)x_1 \\
\dot{x}_1 &= -\frac{D}{r}x_1 + f(S_1)x_1 - a_1x_1 \\
\dot{S}_2 &= \frac{D}{1-r}(S_1 - S_2) - f(S_2)x_2 \\
\dot{x}_2 &= \frac{D}{1-r}(x_1 - x_2) + f(S_2)x_2 - a_2x_2.
\end{aligned} \tag{2.1}$$

Note that these equations are not valid for $r = 0$ and $r = 1$, which correspond to a single chemostat. For sake of completeness, this case is treated in Appendix A (see equations A.1 where a denotes the mortality rate). The considered growth function satisfies the following properties.

Assumption 1. *The function f is C^1, with $f(0) = 0$ and $f'(S) > 0$ for all $S > 0$.*

We define

$$m := \sup_{S>0} f(S), \quad (m \text{ may be } +\infty). \tag{2.2}$$

As f is increasing then the *break-even concentration* is defined by

$$\lambda(D) := f^{-1}(D) \quad \text{when} \quad 0 \leq D < m. \tag{2.3}$$

The following result is classical in the mathematical theory of the chemostat and is left to the reader.

Lemma 1. *For any nonnegative initial condition, the solution $(S_1(t), x_1(t), S_2(t), x_2(t))$ is nonnegative for any $t > 0$ and bounded.*

For the description of the steady states, we need to define the auxiliary function h given by:

$$h(S_2) = \frac{D + (1-r)a_2}{1-r} \frac{S_1^* - S_2}{\frac{DS^{in}+ra_1S_1^*}{D+ra_1} - S_2}, \text{ where } S_1^* = \lambda(D/r + a_1) \tag{2.4}$$

Lemma 2. *Assume that $D/r + a_1 < f(S^{in})$. The function h is decreasing from $h(0) = \frac{D+(1-r)a_2}{1-r} \frac{(D+ra_1)S_1^*}{DS^{in}+ra_1S_1^*}$ to $h(S_1^*) = 0$.*

Proof. From the condition $D/r + a_1 < f(S^{in})$ it is deduced that $S_1^* < S^{in}$. Let us denote by $b = \frac{DS^{in}+ra_1S_1^*}{D+ra_1}$. Since b is a convex combination of S^{in} and S_1^*, we have $S_1^* < b < S^{in}$. The function h is written $h(S_2) = \frac{D+(1-r)a_2}{1-r}\frac{S_1^*-S_2}{b-S_2}$. Its derivative is $h'(S_2) = \frac{D+(1-r)a_2}{1-r}\frac{S_1^*-b}{(b-S_2)^2}$. The vertical asymptote $S_2 = b$ is at right of S_1^* and $h'(S_2) < 0$. Therefore, h is defined on the interval $(0, S_1^*)$ and is decreasing from $h(0)$ to $h(S_1^*)$. This ends the proof of the lemma. □

Therefore if $D/r + a_1 < f(S^{in})$, equation $f(S_2) = h(S_2)$ admits a unique solution, denoted by $S_2^*(S^{in}, D, r)$, as shown in Figure 2 (a). Along the paper, we use the abbreviations LES for locally exponentially stable and GAS for globally exponentially stable in the positive orthant. The existence and stability of steady states of (2.1) are given by the following result.

Theorem 1. *Assume that Assumption 1 is satisfied. The steady states of* (2.1) *are:*

- *The washout steady state* $E_0 = (S^{in}, 0, S^{in}, 0)$ *which always exists. It is GAS if and only if*

$$D \geq \max\{r(f(S^{in}) - a_1), (1-r)(f(S^{in}) - a_2)\}. \tag{2.5}$$

  It is LES if and only if $D > \max\{r(f(S^{in}) - a_1), (1-r)(f(S^{in}) - a_2)\}$.
- *The steady state* $E_1 = (S^{in}, 0, \overline{S}_2, \overline{x}_2)$ *of washout in the first chemostat but not in the second one with*

$$\overline{S}_2 = \lambda\left(\frac{D}{1-r} + a_2\right) \text{ and } \overline{x}_2 = \frac{D}{D+(1-r)a_2}\left(S^{in} - \overline{S}_2\right). \tag{2.6}$$

  This steady state exists if and only if $D < (1-r)(f(S^{in}) - a_2)$. *It is GAS if and only if*

$$r(f(S^{in}) - a_1) \leq D \text{ and } D < (1-r)(f(S^{in}) - a_2) \tag{2.7}$$

  and LES if and only if $r(f(S^{in}) - a_1) < D$ *and* $D < (1-r)(f(S^{in}) - a_2)$.
- *The steady state* $E_2 = (S_1^*, x_1^*, S_2^*, x_2^*)$ *of persistence of the species in both chemostats with*

$$S_1^* = \lambda\left(\frac{D}{r} + a_1\right), \quad x_1^* = \frac{D}{D+ra_1}(S^{in} - S_1^*), \tag{2.8}$$

$$x_2^* = \frac{D}{D+(1-r)a_2}\left(\frac{D}{D+ra_1}(S^{in} - S_1^*) + S_1^* - S_2^*\right) \tag{2.9}$$

  and $S_2^* = S_2^*(S^{in}, D, r)$ *is the unique solution of the equation* $h(S_2) = f(S_2)$ *with* h *defined by* (2.4). *This steady state exists and is positive if and only if* $D < r(f(S^{in}) - a_1)$. *It is GAS and LES whenever it exists and is positive.*

Proof. The proof is given in Appendix B. □

Proposition 1. *For* D *and* r *fixed, the function* $S^{in} \mapsto S_2^*(S^{in}, D, r)$ *is decreasing.*

The proof is the same as the proof of Proposition 1 in [6] and is omitted. The result is illustrated in Figure 2 (b). This result means that the effluent steady state concentration of substrate decreases when the influent concentration of substrate increases. This behavior is very different from the single chemostat, where the effluent steady state substrate concentration is independent of the influent substrate concentration.

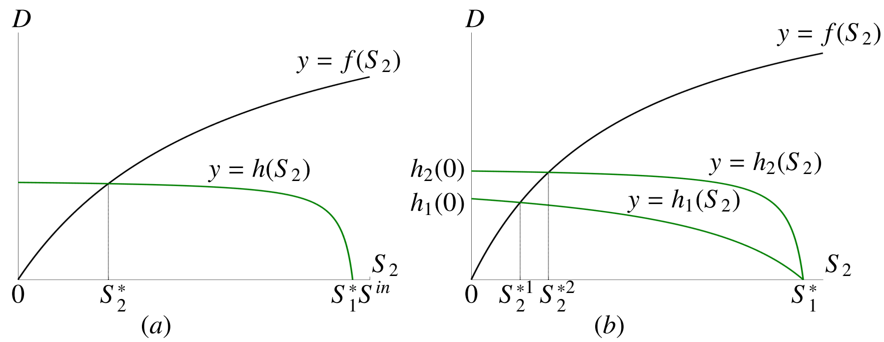


FIGURE 2. (a): Existence and uniqueness of S_2^*, and then of E_2. (b): The result of Proposition 1 with $S_2^{*i} = S_2^*(S^{in,i}, D, r)$, functions h_i are defined by the expression (2.4) of h with $S^{in} = S^{in,i}$, such that $S^{in,1} < S^{in,2}$, and $h_i(0) = \frac{(D+(1-r)a_2)(D+ra_1)S_1^*}{(1-r)(DS^{in,i}+ra_1S_1^*)}$, $i = 1, 2$.

3. OPERATING DIAGRAM

For the chemostat model, the operating diagram has as coordinates the input substrate concentration S^{in} and the dilution rate D, and shows how the solutions of the system behave for different values of these two parameters. The regions constituting the operating diagram correspond to different qualitative asymptotic behaviors. Indeed, the main interest of an operating diagram is to highlight the number and stability of the steady states for a given pair of parameters (S^{in}, D). The input substrate concentration S^{in} and the dilution rate D are the usual parameters manipulated by the experimenter of a chemostat. Apart from these parameters, and the parameter r that can be also chosen by the experimenter but not easily changed as S^{in} and D, all other parameters have biological meaning and are fitted using experimental data from real measurements of concentrations of micro-organisms and substrates. Therefore the operating diagram is a bifurcation diagram, quite useful to understand the possible behaviors of the solutions of the system from both the mathematical and biological points of view.

Here, we fix $r \in (0, 1)$ and we depict in the plane (S^{in}, D) the regions in which the solution of system (2.1) globally converges towards one of the steady state E_0, E_1 or E_2. From the results given in Theorem 1, it is seen that these regions are delimited by the curves Φ_r^1 and Φ_{1-r}^2 defined by:

$$\Phi_r^1 := \left\{(S^{in}, D) \in \mathbb{R}_+^2 : D = r(f(S^{in}) - a_1)\right\}, \tag{3.1}$$

$$\Phi_{1-r}^2 := \left\{(S^{in}, D) \in \mathbb{R}_+^2 : D = (1-r)(f(S^{in}) - a_2)\right\}. \tag{3.2}$$

When $a_1 = a_2 = 0$, as we have shown in [6], these curves meet only at one point (the origin) and merge when $r = 1/2$. Therefore, in this case the curves Φ_r^1 and Φ_{1-r}^2 separate the operating plane (S^{in}, D), in only three regions, see [6, Figure 5]. This property continue to hold when $a_1 = a_2$, that is to say, the curves intersect only at $(\lambda(a_1), 0)$ and merge when $r = 1/2$. In this case the curves Φ_r^1 and Φ_{1-r}^2 separate the operating plane (S^{in}, D), in only three regions, see Figure 3 (c,d). The novelty when a_1 and a_2 is that the intersection of the curves Φ_r^1 and Φ_{1-r}^2 can lie outside the S^{in} axis. Therefore there can be four regions in the operating plane, as depicted in Figure 3 (a,f). For the description of the intersection of the curves Φ^1_r and Φ^2_{1-r}, we need some definitions and notations. Let $\bar{r} \in (0,1)$ be defined by

$$\bar{r} := \frac{m - a_2}{2m - a_1 - a_2}. \tag{3.3}$$

Note that if $a_1 < a_2$ then $\bar{r} < 1/2$, and if $a_1 > a_2$ then $\bar{r} > 1/2$. For $a_1 < a_2$ and $0 < r < \bar{r}$ (or $a_1 > a_2$ and $\bar{r} < r < 1$), we define the point $P = \left(S^{in}_P, D_P\right)$ of the operating plane by:

$$S^{in}_P := \lambda\left(\frac{ra_1 - (1-r)a_2}{2r-1}\right) \quad \text{and} \quad D_P := \frac{r(1-r)(a_2-a_1)}{1-2r}. \tag{3.4}$$

Note that $S^{in}_P > 0$ and $D_P > 0$. With these notations we can state the following result:

Proposition 2. *(1) If $a_1 < a_2$ then for all $r \in (0,\bar{r})$, the curves Φ^1_r and Φ^2_{1-r} intersect at the point P and Φ^1_r is strictly below [resp. above] Φ^2_{1-r} for $S^{in} > S^{in}_P$ [resp. $S^{in} < S^{in}_P$], see Figure 3 (a). For all $r \in (\bar{r}, 1)$, Φ^1_r is strictly above Φ^2_{1-r}, see Figure 3 (b).*

(2) If $a_1 > a_2$ then for all $r \in (\bar{r}, 1)$, the curves Φ^1_r and Φ^2_{1-r} intersect at the point P and Φ^1_r is strictly above [resp. below] Φ^2_{1-r} for $S^{in} > S^{in}_P$ [resp. $S^{in} < S^{in}_P$], see Figure 3 (f). For all $r \in (0,\bar{r})$, Φ^1_r is below Φ^2_{1-r}, see Figure 3 (e).

(3) If $a_1 = a_2$ then, for $r = 1/2$, $\Phi^1_r = \Phi^2_{1-r}$. Moreover, if $r < 1/2$ then Φ^1_r is strictly below Φ^2_{1-r}, see Figure 3 (c) and, if $r > 1/2$ then Φ^1_r is strictly above Φ^2_{1-r}, see Figure 3 (d).

Proof. Let φ_i, $i = 1,2$, be defined, for $S^{in} > \lambda(a_i)$ and $0 < r < 1$, by

$$\varphi_1(S^{in}, r) := r(f(S^{in}) - a_1) \quad \text{and} \quad \varphi_2(S^{in}, r) := (1-r)(f(S^{in}) - a_2). \tag{3.5}$$

The curves Φ^1_r and Φ^2_{1-r}, defined respectively by (3.1) and (3.2), intersect if and only if there exists $r \in (0,1)$ and $S^{in} > \max(\lambda(a_1), \lambda(a_2))$ such that $\varphi_1(S^{in}, r) = \varphi_2(S^{in}, r)$, that is to say

$$f(S^{in}) = A(r), \quad \text{with} \quad A(r) := \frac{ra_1 - (1-r)a_2}{2r-1}. \tag{3.6}$$

This equation has a solution $S^{in} > \max(\lambda(a_1), \lambda(a_2))$ if and only if

$$\max(a_1, a_2) < A(r) < m, \tag{3.7}$$

where m is defined by (2.2). When these conditions are satisfied, the solution of (3.6) is given by $S^{in} = \lambda(A(r))$, where λ is defined by (2.3). Hence, $S^{in} = S^{in}_P$, given in (3.4). The corresponding intersection point of Φ^1_r and Φ^2_{1-r} is given by $D_p = r\left(f(S^{in}_P) - a_1\right)$, which is the value given in (3.4).

Let us determine now for which value of r, the conditions (3.7) are satisfied. The function A is a homographic function. Its graphical representation is a hyperbola, whose vertical asymptote is $r = 1/2$. Its derivative is given by

$$A'(r) = \frac{a_2 - a_1}{(2r-1)^2}. \tag{3.8}$$

Note that $A(r) = m$ if and only if $r = \bar{r}$, where $\bar{r}$ is defined by (3.3). Therefore if $a_1 < a_2$ then, according to (3.8), A is increasing. Since $A(0) = a_2$, $A(\bar{r}) = m$, and $\bar{r} < 1/2$, the condition (3.7) is satisfied if and only if $0 < r < \bar{r}$. Similarly, if $a_1 > a_2$, then, according to (3.8), A is decreasing. Since $A(1) = a_1$, $A(\bar{r}) = m$ and $\bar{r} > 1/2$, the condition (3.7) is satisfied if and only if $\bar{r} < r < 1$. Finally, if $a_1 = a_2$ then $A(r) = a_1$ and the condition (3.7) cannot be satisfied.

Suppose that $a_1 < a_2$. Note that for $0 < r < 1/2$, $f(S^{in}) > A(r)$ [resp. $f(S^{in}) < A(r)$] is equivalent to $\varphi_1(S^{in}, r) < \varphi_2(S^{in}, r)$ [resp. $\varphi_1(S^{in}, r) > \varphi_2(S^{in}, r)$]. Thus:

- If $r \in (0, \bar{r})$, then $f(S^{in}) < A(r)$ if and only if $S^{in} < S^{in}_P$, where S^{in}_P is defined by (3.4). Hence, the curves Φ^1_r and Φ^2_{1-r} intersect at $P = (S^{in}_P, D_P)$ and the curve Φ^1_r is strictly below [resp. above] the curve Φ^2_{1-r}, for all $S^{in} > S^{in}_P$ [resp. $S^{in} < S^{in}_P$].
- If $r \in [\bar{r}, 1/2)$ then $f(S^{in}) < A(r)$ for all $S^{in} > 0$, so that the curve Φ^1_r is strictly above the curve Φ^2_{1-r}.
- If $r \in [1/2, 1)$, then, using $r \geq 1 - r$ and $a_1 < a_2$, one has $\varphi_1(S^{in}, r) > \varphi_2(S^{in}, r)$. Therefore, the curve Φ^1_r is strictly above the curve Φ^2_{1-r}.

If $a_1 > a_2$, the proof is similar to the case $a_1 < a_2$. If $a_1 = a_2$ then $\varphi_1(S^{in}, r) = \varphi_2(S^{in}, r)$ is equivalent to $r(f(S^{in}) - a_1) = (1 - r)(f(S^{in}) - a_1)$. Therefore, $r = 1 - r$, that is $r = 1/2$. In this case the curves Φ^1_r and Φ^2_{1-r} merge. In addition, if $r < 1/2$ [resp. $r > 1/2$] then $r < 1 - r$ [resp. $r > 1 - r$] and the curve Φ^1_r is strictly below [resp. above] the curve Φ^2_{1-r}. This ends the proof of the proposition. □

These curves split the plane (S^{in}, D) in several regions denoted $I_0(r)$, $I_1(r)$, $I_2(r)$ and $I_3(r)$. For all positive (a_1, a_2), these regions are depicted in Figure 3 and are defined by:

$$\begin{aligned}
I_0(r) &:= \Big\{(S^{in}, D) : \max\{r(f(S^{in}) - a_1), (1 - r)(f(S^{in}) - a_2)\} \leq D\Big\},\\
I_1(r) &:= \Big\{(S^{in}, D) : r(f(S^{in}) - a_1) \leq D \text{ and } D < (1 - r)(f(S^{in}) - a_2)\Big\},\\
I_2(r) &:= \Big\{(S^{in}, D) : 0 < D < \min\{r(f(S^{in}) - a_1), (1 - r)(f(S^{in}) - a_2)\}\Big\},\\
I_3(r) &:= \Big\{(S^{in}, D) : (1 - r)(f(S^{in}) - a_2) \leq D \text{ and } D < r(f(S^{in}) - a_1)\Big\}.
\end{aligned}$$

The behavior of the system in each region, when it is not empty, is given in Table 1. Notice that E_1 exists in both regions $I_1(r)$ and $I_2(r)$, but is stable only when (S^{in}, D) is fixed in $I_1(r)$.

	$I_0(r)$	$I_1(r)$	$I_2(r)$	$I_3(r)$
E_0	GAS	U	U	U
E_1		GAS	U	
E_2			GAS	GAS

TABLE 1. Stability of the steady states in the various regions of the operating diagram. The letter U means that the steady state is unstable. The letters GAS mean that the steady state is globally asymptotically stable in the positive orthant. No letter means that the steady state does not exist.

When $a_1 = a_2 = 0$ then $\lambda(a_1) = \lambda(a_2) = 0$ and the curves Φ^1_r and Φ^2_{1-r} of the operating diagram start from the origin of the plane (S^{in}, D) and merge for $r = 1/2$. Therefore, the (a), (b), (c), (d), (e) and (f) diagrams are reduced to only two different cases characterized by $0 < r < 1/2$ and $1/2 < r < 1$, as shown in Figure 5 of [6]. There is no changes in the stability of the steady states and in the number of the regions depicted in the operating diagram. This result reveals an interplay between spatial heterogeneity (the ratio r of volume distribution between tanks) and the mortality heterogeneity (difference between a_1 and a_2). Indeed, cases (a) and (f) bring a particular feature when mortality rates are different: domains $I_1(r)$ and $I_3(r)$ can appear or disappear playing only with the spatial distribution r, a

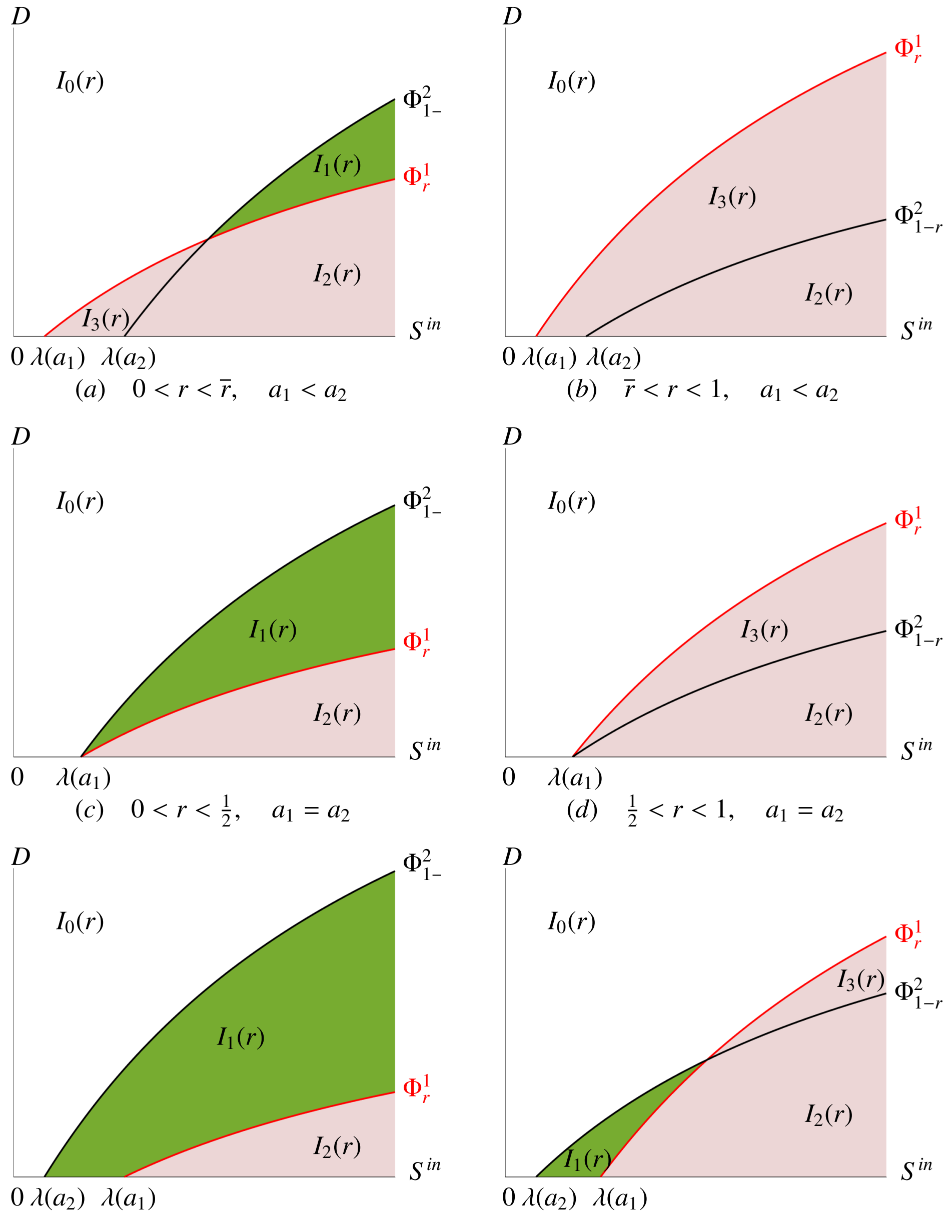


FIGURE 3. The operating diagram of the two serial interconnected chemostats with positive mortality rates depending on the parameter r.

phenomenon which does not happens when mortality is identical in each tank. This shows that the existence of domains $I_1(r)$ and $I_3(r)$ is controlled by a relative toxicity in the tanks, and not only the spatial distribution as it is the case for identical mortality. This feature can have interest when practitioners can adjust pH or other abiotic parameters having impacts on the mortality rate, independently in each tank. Given operating parameters S^{in}, D and

r, pictures (a) and (f) show that it is theoretically possible to pass from domain $I_3(r)$ to $I_2(r)$ when mortality parameter is diminished only in the second tank. In practice, being in domain $I_2(r)$ might be more desirable than $I_3(r)$ with respect to some dysfunctioning of the first tank that can drop suddenly its biomass to zero. Indeed, in $I_2(r)$, the second tank is no conducted to the wash-out differently to the $I_3(r)$ case.

We describe the bifurcations that occur when $a_1 = a_2$ in Remark 1 and the general case i.e. when $a_1 \neq a_2$ is similar.

Remark 1. *Transcritical bifurcations occur in the limit cases $D = (1-r)(f(S^{in}) - a_1)$ and $D = r(f(S^{in}) - a_1)$, for system* (4.1)*. If $0 < r < 1/2$ then, we have a transcritical bifurcation of E_0 and E_1 when $D = (1-r)(f(S^{in}) - a_1)$ and a transcritical bifurcation of E_1 and E_2 when $D = r(f(S^{in}) - a_1)$. If $1/2 < r < 1$ then, we have a transcritical bifurcation of E_0 and E_1 when $D = (1-r)(f(S^{in}) - a_1)$ and a transcritical bifurcation of E_0 and E_2 when $D = r(f(S^{in}) - a_1)$. If $r = 1/2$ and $D = (f(S^{in}) - a_1)/2$ then, we have transcritical bifurcations of E_0 and E_1, and E_0 and E_2, simultaneously.*

4. Performances

The aim of this work is to establish a comparison of the performance of the serial configuration with ones of the single chemostat defined by system (A.1). This is why we shall consider the same mortality rate in each tank with $a = a_1 = a_2$ ($a > 0$). Thus, system (2.1) becomes

$$
\begin{aligned}
\dot{S}_1 &= \frac{D}{r}(S^{in} - S_1) - f(S_1)x_1 \\
\dot{x}_1 &= -\frac{D}{r}x_1 + f(S_1)x_1 - ax_1 \\
\dot{S}_2 &= \frac{D}{1-r}(S_1 - S_2) - f(S_2)x_2 \\
\dot{x}_2 &= \frac{D}{1-r}(x_1 - x_2) + f(S_2)x_2 - ax_2
\end{aligned} \tag{4.1}
$$

and the operating diagram of system (4.1) corresponds to diagrams (c) and (d) of Figure 3. In the following, we compare both structures according to two different criteria; the output substrate concentration and the biogas flow rate.

4.1. **Output substrate concentration.** Let us consider a volume V, a dilution rate D and an input concentration S^{in}. Considering $a = a_1 = a_2$ and according to Theorem 1, for all $r \in (0,1)$, the output substrate concentration, at steady state, of system (4.1), is defined by

$$
S_r^{out}(S^{in}, D) := \begin{cases} S^{in} & \text{if} \quad D \geq \max(r, 1-r)(f(S^{in}) - a) \\ \lambda\,(D/(1-r) + a) & \text{if} \quad r(f(S^{in}) - a) \leq D \text{ and } D \leq (1-r)(f(S^{in}) - a) \\ S_2^*(S^{in}, D, r) & \text{if} \quad D < r(f(S^{in}) - a) \end{cases} \tag{4.2}
$$

with $S_2^*(S^{in}, D, r)$ the unique solution of equation $h(S_2) = f(S_2)$ such that for $a = a_1 = a_2$, the function h is defined by:

$$
h(S_2) := \frac{D + (1-r)a}{1-r} \frac{S_1^* - S_2}{\frac{D}{D+ra}(S^{in} - S_1^*) + S_1^* - S_2} \tag{4.3}
$$

with $S_1^* = \lambda(D/r + a)$.

Lemma 3. *For all $0 \leq D < f(S^{in}) - a$, one has $\lim_{r \to 1} S_2^*(S^{in}, D, r) = \lambda(D + a)$.*

Proof. Let $0 \leq D < f(S^{in}) - a$. Using (4.3), one has $h(S_2) = f(S_2)$ equivalent to

$$(D + (1-r)a)(S_1^* - S_2^*) = (1-r)\left(\frac{D}{D+ra}(S^{in} - S_1^*) + S_1^* - S_2^*\right) f(S_2^*). \tag{4.4}$$

As $S_1^*|_{r=1} = \lambda(D+a)$ and $\lim_{r\to 1} f(S_2^*) < +\infty$ then, (4.4) gives $D(\lambda(D+a) - \lim_{r\to 1} S_2^*(S^{in}, D, r)) = 0$. Consequently, one has $\lim_{r\to 1} S_2^*(S^{in}, D, r)) = \lambda(D + a)$. This ends the proof of the lemma. □

Although $S_r^{out}(S^{in}, D)$ is defined by (4.2) for $0 < r < 1$, as $\overline{S}_2|_{r=0} = \lambda(D + a)$ and according to Lemma 3, we extend it, by continuity, for $r = 0$ and $r = 1$ by

$$S_0^{out}(S^{in}, D) = S_1^{out}(S^{in}, D) = S^{out}(S^{in}, D). \tag{4.5}$$

We have to compare the quantity S_r^{out} defined by (4.2) and (4.5) with S^{out} defined by (A.5).

For a fixed $r \in (0, 1)$, let $g_r : [0, r(m - a)) \mapsto \mathbb{R}$ be defined by

$$g_r(D) := \lambda\left(\frac{D}{r} + a\right) + \frac{r(D + ar)}{(1-r)(D+a)}\left(\lambda\left(\frac{D}{r} + a\right) - \lambda(D + a)\right). \tag{4.6}$$

Theorem 2. *For any $r \in (0, 1)$, one has $S_r^{out}(S^{in}, D) < S^{out}(S^{in}, D)$ if and only if $S^{in} > g_r(D)$. Moreover, $S_r^{out}(S^{in}, D) = S^{out}(S^{in}, D)$ if and only if $S^{in} = g_r(D)$.*

Proof. Recall that $S_2^*(S^{in}, D, r)$ is the unique solution of equation $f(S_2) = h(S_2)$ with h defined by (2.4). Let us first prove that

$$S_2^*(S^{in}, D, r) < \lambda(D + a) \quad \text{if and only if} \quad S^{in} > g_r(D). \tag{4.7}$$

Since f is increasing (see Assumption 1) and h is decreasing then, $S_2^*(S^{in}, D, r) < \lambda(D+a)$ is equivalent to $h(\lambda(D + a)) < f(\lambda(D + a)) = D + a$. Thus, using (4.3) the definition of h, the condition $h(\lambda(D + a)) < D + a$ is written as

$$\frac{D + (1-r)a}{1-r} \frac{\lambda(D/r + a) - \lambda(D + a)}{\frac{D}{D+ra}\left(S^{in} - \lambda\left(\frac{D}{r} + a\right)\right) + \lambda\left(\frac{D}{r} + a\right) - \lambda(D + a)} < D + a,$$

which is equivalent to

$$S^{in} > \lambda\left(\frac{D}{r} + a\right) + \frac{r(D + ar)}{(1-r)(D+a)}\left(\lambda\left(\frac{D}{r} + a\right) - \lambda(D + a)\right).$$

Hence, according to (4.6) the definition of g_r, this last inequality is equivalent to $S^{in} > g_r(D)$. Therefore, one has $g_r(D) > \lambda(D/r + a)$.

Let us go now to the proof of the theorem. Assume that $S^{in} > g_r(D)$. Then, $S^{in} > \lambda(D/r + a) > \lambda(D + a)$, so that, as shown by (4.2) and (A.5), we have

$$S_r^{out}(S^{in}, D) = S_2^*(S^{in}, D, r) \quad \text{and} \quad S^{out}(S^{in}, D) = \lambda(D + a). \tag{4.8}$$

Therefore, using (4.7), we have $S_r^{out}(S^{in}, D) < S^{out}(S^{in}, D)$. Assume now that $S^{in} \leq g_r(D)$. When $r < 1/2$, three cases must be distinguished. First, if $\lambda(D + a) < \lambda(D/r + a) < S^{in} \leq g_r(D)$, then, by (4.2) and (A.5), we obtain (4.8). Hence, using (4.7), we have $S_r^{out}(S^{in}, D) \geq S^{out}(S^{in}, D)$. Secondly, if $\lambda(D + a) < \lambda(D/(1 - r) + a) \leq S^{in}$ and $S^{in} \leq \lambda(D/r + a)$ then, by (4.2) and (A.5), $S_r^{out}(S^{in}, D) = \lambda(D/(1 - r) + a)$ and $S^{out}(S^{in}, D) = \lambda(D + a)$. Therefore, we have $S_r^{out}(S^{in}, D) > S^{out}(S^{in}, D)$. Finally, if $S^{in} \leq \lambda(D + a)$, then $S_r^{out}(S^{in}, D) = S^{out}(S^{in}, D) = S^{in}$. When $r \geq 1/2$, the proof is similar, excepted that we must distinguish only two cases, $\lambda(D + a) < S^{in} \leq \lambda(D/r + a)$ and $S^{in} \leq \lambda(D + a)$. The same calculations show the equivalence if inequalities are replaced by equalities. This ends the proof of the theorem. □

Theorem 2 asserts that the serial configuration respectively of volumes rV and $(1-r)V$ is more efficient than the single chemostat of volume V if and only if $S^{in} > g_r(D)$.

We need the following Assumption that is satisfied by any concave growth function but also by an Hill function, as shown in Section 5.

Assumption 2. *For every $D \in [0, m-a)$, the function $r \in (D/(m-a), 1) \mapsto g_r(D) \in \mathbb{R}$ is decreasing.*

Let the curves Φ_r, Φ_{1-r} and Γ_r be defined by

$$\Phi_{1-r} := \left\{(S^{in}, D) : S^{in} = \lambda(D/(1-r)+a)\right\}, \quad \Phi_r := \left\{(S^{in}, D) : S^{in} = \lambda(D/r+a)\right\}, \tag{4.9}$$

$$\Gamma_r := \left\{(S^{in}, D) : S^{in} = g_r(D)\right\}. \tag{4.10}$$

Lemma 4. *For all $r \in (0,1)$ and $S^{in} > \lambda(a)$, the curve Φ_r is always strictly above the curve Γ_r in the plane (S^{in}, D).*

Proof. As $0 < r < 1$ and λ is an increasing function then, we have $\lambda(D/r+a) > \lambda(D+a)$. Using definition (4.6), we deduce that $g_r(D) > \lambda(D/r+a)$. According to the respective definitions (4.9) and (4.10) of the curves Φ_r and Γ_r, we deduce that the curve Φ_r is always above the curve Γ_r. This ends the proof of the lemma. □

According to Theorem 2, the output substrate concentration at steady state of the serial configuration is smaller than the one of the single chemostat if and only if (S^{in}, D) is strictly below the curve Γ_r depicted in Figure 4.

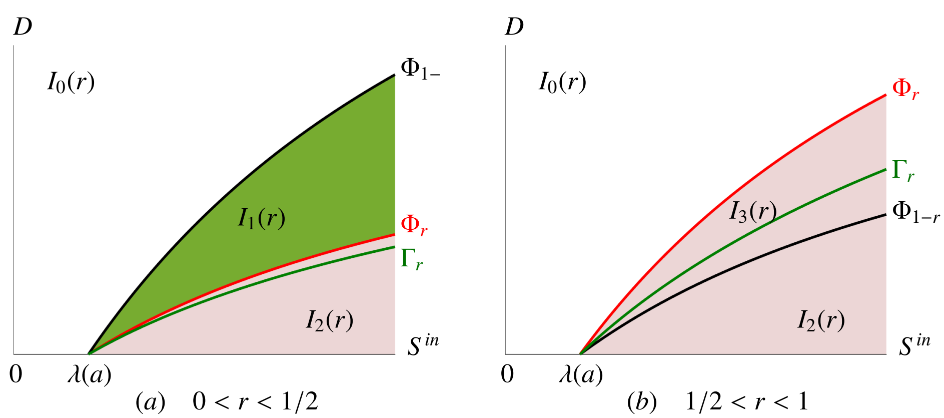


FIGURE 4. Positioning of the curve Γ_r in the operating diagram.

Lemma 5. *Assume that Assumptions 1 and 2 are satisfied. Taken S^{in} and D such that if $S^{in} > g(D)$ then there exists a unique $r = r_1(S^{in}, D) \in (0,1)$ such that $S^{in} = g_r(D)$. One has $r > r_1(S^{in}, D)$ if and only if $S^{in} > g_{r_1}(D)$.*

Proof. Let $D < m-a$. From Assumption 2, for all $r \in (D/(m-a), 1)$, the function $r \mapsto g_r(D)$ is decreasing. From Assumption 1, we have $\lim_{r\to D/(m-a)} = \lambda(D/r+a) = \lambda(m) = +\infty$. Thus, $\lim_{r\to D/(m-a)} g_r(D) = +\infty$. Let calculate $\lim_{r\to 1} g_r(D)$. According to (4.6), the definition of g_r, one remarks that $\lim_{r\to 1} r(D+ar)(\lambda(D/r+a) - \lambda(D+a)) = 0$ and

$\lim_{r\to 1}(1-r)(D+a) = 0$. Therefore, let use L'Hôspital's rule to calculate $\lim_{r\to 1} g_r(D)$. One has

$$\lim_{r\to 1} g_r(D) = \lambda(D+a) + \lim_{r\to 1}\left[-\frac{D+2ar}{D+a}\left(\lambda\left(\frac{D}{r}+a\right)-\lambda(D+a)\right)+\frac{D}{r}\lambda'\left(\frac{D}{r}+a\right)\right].$$

One then obtains

$$\lim_{r\to 1} g_r(D) = \lambda(D+a) + D\lambda'(D+a) = g(D).$$

In addition, the function $r \mapsto g_r(D)$ is continuous, D and S^{in} being fixed then, using Intermediate Value Theorem, we deduce that for $S^{in} > g(D)$ there exists a unique $r = r_1(S^{in}, D)$ in $(0,1)$ such that $S^{in} = g_r(D)$. Since the function $r \mapsto g_r(D)$ is decreasing then, $r > r_1$ if and only if $S^{in} = g_{r_1}(D) > g_r(D)$. This ends the proof of the Lemma. □

Theorem 3. *Assume that Assumptions 1 and 2 are satisfied.*

- *If* $S^{in} \le g(D)$ *then for any* $r \in (0,1)$*,* $S_r^{out}(S^{in}, D) > S^{out}(S^{in}, D)$.
- *If* $S^{in} > g(D)$ *then* $S_r^{out}(S^{in}, D) < S^{out}(S^{in}, D)$ *if and only if* $r_1(S^{in}, D) < r < 1$ *with* r_1 *defined in Lemma 5.*

The function g is defined by (A.9). *In addition,* $S_r^{out}(S^{in}, D) = S^{out}(S^{in}, D)$ *for* $r = 0$*,* $r = r_1(S^{in}, D)$ *and* $r = 1$.

Proof. Recall that g_r is defined by (4.6).

- From Assumptions 1 and 2, the function $r \in (D/m, 1) \mapsto g_r(D)$ is decreasing. Thus, for any $r \in (0,1)$, one has $g(D) < g_r(D)$. If $S^{in} \le g(D)$ then one has $S^{in} < g_r(D)$ and according to Theorem 2 one deduces that $S_r^{out}(S^{in}, D) > S^{out}(S^{in}, D)$.
- If $S^{in} > g(D)$ then according to Lemma 5, there exists a unique $r = r_1(S^{in}, D)$ in $(0,1)$ such that $S^{in} = g_r(D)$, where for all $r > r_1$, one has $S^{in} > g_r(D)$. Thus, according to Theorem 2 one deduces that $S_r^{out}(S^{in}, D) < S^{out}(S^{in}, D)$.

Notice that the equality $S_r^{out}(S^{in}, D) = S^{out}(S^{in}, D)$ for the particular cases $r = 0$ and $r = 1$ is already verified, see (4.5). In addition, if $r = r_1(S^{in}, D)$ then $S^{in} = g_r(D)$, as stated in Lemma 5. Consequently, according to Theorem 2, $S^{in} = g_r(D)$ is equivalent to $S_r^{out}(S^{in}, D) = S^{out}(S^{in}, D)$. This ends the proof of the theorem. □

Proposition 3. *Let* $D > 0$ *and* $S^{in} > 0$.

If $S^{in} \le \lambda(D+a)$ *then for any* $r \in [0,1]$*, one has* $S_r^{out}(S^{in}, D) = S^{out}(S^{in}, D) = S^{in}$.

In the following two cases, we denote by r_0 *the ratio* $r_0 = D/(f(S^{in}) - a)$.

If $\lambda(D+a) < S^{in} < \lambda(2D+a)$ *then one has* $1/2 < r_0 < 1$ *and*

$$S_r^{out}(S^{in}, D) = \begin{cases} \lambda(D/(1-r)+a) & \text{if} \quad 0 \le r \le 1-r_0 \\ S^{in} & \text{if} \quad 1-r_0 \le r \le r_0 \\ S_2^*(S^{in}, D, r) & \text{if} \quad r_0 \le r \le 1, \end{cases} \tag{4.11}$$

If $\lambda(2D+a) \le S^{in}$ *then one has* $0 < r_0 \le 1/2$ *and*

$$S_r^{out}(S^{in}, D) = \begin{cases} \lambda(D/(1-r)+a) & \text{if} \quad 0 \le r \le r_0 \\ S_2^*(S^{in}, D, r) & \text{if} \quad r_0 \le r \le 1. \end{cases} \tag{4.12}$$

Proof. Let $D > 0$ and $S^{in} > 0$.

When $S^{in} \le \lambda(D+a)$ one has, for all $r \in (0,1)$, $\lambda(D+a) \le \min\{\lambda(D/(1-r)+a), \lambda(D/r+a)\}$ i.e. $S^{in} \le \min\{\lambda(D/(1-r)+a), \lambda(D/r+a)\}$. Then, according to (4.2) one has $S_r^{out}(S^{in}, D) = S^{in}$.

In the following two cases, we consider $r_0 = D/(f(S^{in}) - a)$ i.e. $S^{in} = \lambda(D/r_0 + a)$.

When $\lambda(D+a) < S^{in} < \lambda(2D+a)$, one has $r_0 \in (1/2, 1)$. Firstly, if $0 \leq r \leq 1-r_0$ then, one has $\lambda(D/(1-r)+a) \leq \lambda(D/r_0+a) \leq \lambda(D/r+a)$ i.e. $\lambda(D/(1-r)+a) \leq S^{in} \leq \lambda(D/r+a)$. This is equivalent to $r(f(S^{in})-a) \leq D \leq (1-r)(f(S^{in})-a)$. According to (4.2), one has $S_r^{out}(S^{in}, D) = \lambda(D/(1-r)+a)$. Secondly, if $1-r_0 \leq r \leq r_0$, one has $\lambda(D/r_0+a) \leq \min\{\lambda(D/(1-r)+a), \lambda(D/r+a)\}$ i.e. $S^{in} \leq \min\{\lambda(D/(1-r)+a), \lambda(D/r+a)\}$. According to (4.2), one has $S_r^{out}(S^{in}, D) = S^{in}$. Finally, if $r_0 < r \leq 1$, one has $\lambda(D/r+a) \leq \lambda(D/r_0+a)$ i.e. $\lambda(D/r+a) \leq S^{in}$ then, according to (4.2), one has $S_r^{out}(S^{in}, D) = S_2^*(S^{in}, D, r)$. These all prove (4.11).

When $\lambda(2D+a) \leq S^{in}$ one has $r_0 \in (0, 1/2]$. If $0 \leq r \leq r_0$ then $\lambda(D/(1-r)+a) \leq \lambda(D/r_0+a) \leq \lambda(D/r+a)$ i.e. $\lambda(D/(1-r)+a) \leq S^{in} \leq \lambda(D/r+a)$. According to (4.2), one has $S_r^{out}(S^{in}, D) = \lambda(D/(1-r)+a)$. If $r_0 \leq r \leq 1$ then $\lambda(D/r+a) \leq \lambda(D/r_0+a)$ i.e. $\lambda(D/r+a) \leq S^{in}$. According to (4.2), one has $S_r^{out}(S^{in}, D) = S_2^*(S^{in}, D, r)$. These all prove (4.12). □

Let us consider the operating diagram to give a better understanding of the behavior of the function $r \mapsto S_r^{out}(S^{in}, D)$, for different values of the pair (S^{in}, D). Therefore, in the operating plane (S^{in}, D), we consider the curve Γ defined by (A.10) and the curves Φ_1 and $\Phi_{1/2}$ defined by:

$$\Phi_{1/2} = \{(S^{in}, D) : S^{in} = \lambda(2D+a)\}, \quad \Phi_1 = \{(S^{in}, D) : S^{in} = \lambda(D+a)\} \tag{4.13}$$

Curves Γ and $\Phi_{1/2}$ lie below the curve Φ_1. The three curves split the operating plane into at most five regions defined by:

$$\begin{aligned} J_0 &= \left\{(S^{in}, D) : S^{in} \leq \lambda(D+a)\right\}, \\ J_1 &= \left\{(S^{in}, D) : \lambda(D+a) < S^{in} \leq \min(g(D), \lambda(2D+a))\right\}, \\ J_2 &= \left\{(S^{in}, D) : g(D) < S^{in} < \lambda(2D+a)\right\}, \\ J_3 &= \left\{(S^{in}, D) : \max(g(D), \lambda(2D+a) \leq S^{in}\right\}, \\ J_4 &= \left\{(S^{in}, D) : \lambda(2D+a) < S^{in} < g(D)\right\}. \end{aligned} \tag{4.14}$$

Remark 2. *All the domains J_i, $i = 0, \ldots, 4$ are disjoint excepted when $S^{in} = g(D) = \lambda(2D+a)$, which corresponds to the intersection of the curves Γ and $\Phi_{1/2}$ depicted in Figure 5.*

Proposition 4. *Let, J_i, $i = 0, \ldots, 4$ be the regions defined by* (4.14)*. The behavior of the function $r \mapsto S_r^{out}(S^{in}, D)$, according to (S^{in}, D) is as follows:*

1. *If $(S^{in}, D) \in J_0$ then, for all $r \in [0, 1]$, $S_r^{out}(S^{in}, D) = S^{out}(S^{in}, D) = S^{in}$.*
2. *If $(S^{in}, D) \in J_1$ then when $S^{in} < \lambda(2D+a)$, $S_r^{out}(S^{in}, D)$ is given by* (4.11) *and when $S^{in} = \lambda(2D+a)$, $S_r^{out}(S^{in}, D)$ is given by* (4.12)*. In addition, for all $r \in (0, 1)$, $S_r^{out}(S^{in}, D) > S^{out}(S^{in}, D)$. The equality is fulfilled for $r = 0$ and $r = 1$, see Figure 6 (a).*
3. *If $(S^{in}, D) \in J_2$ then $S_r^{out}(S^{in}, D)$ is given by* (4.11) *and $S_r^{out}(S^{in}, D) < S^{out}(S^{in}, D)$ if and only if $r \in (r_1(S^{in}, D), 1)$ where $r = r_1(S^{in}, D)$ is the unique solution of $S^{in} = g_r(D)$. The equality is fulfilled for $r = 0$, $r = r_1(S^{in}, D)$ and $r = 1$, see Figure 6 (b).*
4. *If $(S^{in}, D) \in J_3$ then $S_r^{out}(S^{in}, D)$ is given by* (4.12) *and $S_r^{out}(S^{in}, D) < S^{out}(S^{in}, D)$ if and only if $S^{in} > g(D)$ and $r \in (r_1(S^{in}, D), 1)$ where $r = r_1(S^{in}, D)$ is the unique solution of $S^{in} = g_r(D)$. The equality is fulfilled for $r = 0$, $r = r_1(S^{in}, D)$ and $r = 1$, see Figure* (6) (c)*.*

(5) If $(S^{in}, D) \in J_4$ then $S_r^{out}(S^{in}, D)$ is given by (4.12) and for all $r \in (0, 1)$, $S_r^{out}(S^{in}, D) > S^{out}(S^{in}, D)$. The equality is fulfilled for $r = 0$ and $r = 1$, see Figure 6 (d).

Proof. The result is a direct consequence of Proposition 3 and Theorem 3. □

The regions J_i $(i = 0, \ldots, 4)$ looks typically as in Figure 5.

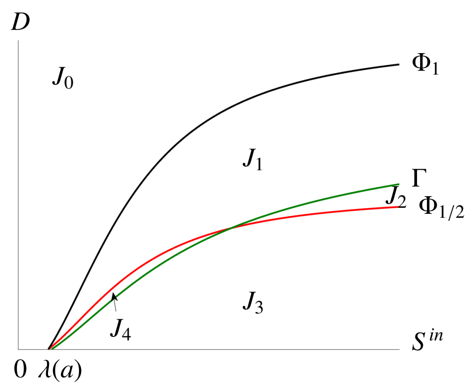


FIGURE 5. In each region, a different behavior of the map $r \mapsto S_r^{out}(S^{in}, D)$ for fixed (S^{in}, D).

The behavior of the map $r \mapsto S_r^{out}(S^{in}, D)$ in the four regions J_i $(i = 1, \ldots, 4)$ is depicted in red in Figure 6. One remarks that the lowest value of the red curve corresponding to lowest output substrate concentration of the serial configuration is obtained for $(S^{in}, D) \in J_2 \cap J_3$ and $r > r_1(S^{in}, D)$. This lowest concentration is obtained from the best possible serial configuration. In addition, notices that the red curve is not always above the blue one but when $r > r_1(S^{in}, D)$, the lowest value of the red curve is always lesser than $\lambda(D + a)$.

We need the following Assumption that is satisfied by any concave growth function but also by an Hill function, as shown in Section 5.

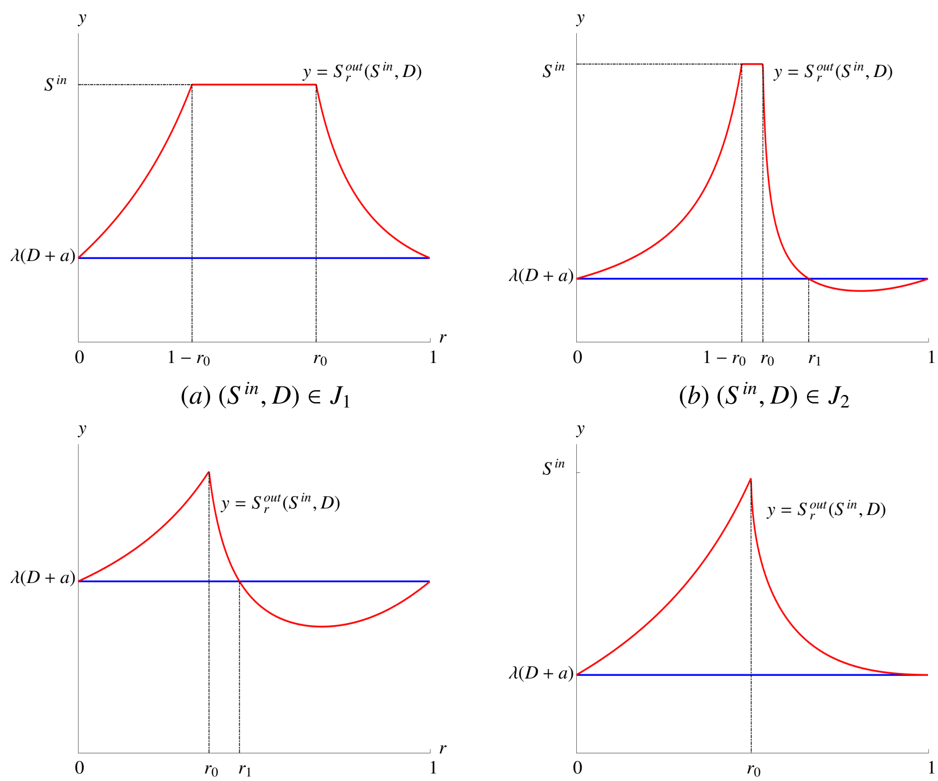
Assumption 3. *For every $r \in (0, 1)$, the function $D \in [0, r(m - a)) \mapsto g_r(D) \in \mathbb{R}$ is increasing.*

Proposition 5. *Assume that Assumptions 1 and 3 are satisfied. For any $r \in (0, 1)$ and $S^{in} > \lambda(a)$, the equation $S^{in} = g_r(D)$ admits a unique solution $D = D_r(S^{in})$ such that $S_r^{out}(S^{in}, D) < S^{out}(S^{in}, D)$ if and only if $0 < D < D_r(S^{in})$.*

Proof. Let $r \in (0, 1)$. From Assumption 3, the function $D \mapsto g_r(D)$ is increasing. From Assumption 1, we have $\lim_{D \to r(m-a)} \lambda(D/r + a) = \lambda(m) = +\infty$. Thus, $\lim_{D \to r(m-a)} g_r(D) = +\infty$ and $g_r(0) = \lambda(a)$. Then, using the Intermediate Value Theorem, we deduce that for $S^{in} > \lambda(a)$ there exists a unique $D = D_r(S^{in})$ in $[0, r(m - a))$ such that $S^{in} = g_r(D)$. Since the function $D \mapsto g_r(D)$ is increasing then the property $0 < D < D_r(S^{in})$ is satisfied if and only if $0 < g_r(D) < g_r(D_r(S^{in})) = S^{in}$. Consequently, according to Theorem 2, $g_r(D) < S^{in}$ if and only if $S_r^{out}(S^{in}, D) < S^{out}(S^{in}, D)$, which ends the proof of the proposition. □

The following Lemmas 6 and 7 provide sufficient conditions for Assumption 2 and 3 to be satisfied. These conditions will be useful for the applications given in Section 5. For this purpose we consider the function γ defined by

$$\gamma(r, D) = g_r(D) \quad \text{where} \quad \text{dom}(\gamma) = \{(r, D) : 0 < r < 1, 0 < D/r + a < m\}, \tag{4.15}$$



(a) $(S^{in}, D) \in J_1$ (b) $(S^{in}, D) \in J_2$

(c) $(S^{in}, D) \in J_3$ (d) $(S^{in}, D) \in J_4$

Figure 6. The output substrate concentrations of the serial configuration in red and the single chemostat in blue. The value $r = r_1 = r_1(S^{in}, D)$ is the unique solution of $S^{in} = g_r(D)$ and $r_0 = D/(f(S^{in}) - a)$.

which consists simply in considering $g_r(D)$, given by (4.6), as a function of both variables r and D. If $\frac{\partial\gamma}{\partial r}(r, D) < 0$ for all $(r, D) \in \mathrm{dom}(\gamma)$, then Assumption 2 is satisfied. Similarly, if $\frac{\partial\gamma}{\partial D}(r, D) > 0$ for all $(r, D) \in \mathrm{dom}(\gamma)$, then Assumption 3 is satisfied. The following Lemmas give sufficient conditions, for partial derivatives of γ to have their signs as indicated above.

Lemma 6. *For $D \in (0, m - a)$, let l_D be defined on $(D/(m - a), 1]$ by $l_D(r) = \lambda(D/r + a)$.*

a: *Assume that for all $D \in (0, m - a)$ and all $r \in (D/(m - a), 1)$ we have*

$$l_D(1) > l_D(r) + (1 - r)l'_D(r) \tag{4.16}$$

then, for all $(r, D) \in \mathrm{dom}(\gamma)$, we have $\frac{\partial\gamma}{\partial r}(r, D) < 0$.

b: *If, for $D \in (0, m - a)$, l_D is strictly convex on $r \in (D/(m - a), 1]$, then the condition (4.16) is satisfied.*

c: *If f is twice derivable, then l_D is twice derivable and the following conditions are equivalent*

  1: *For all $D \in (0, m - a)$ and $r \in (D/(m - a), 1]$, $l''_D(r) > 0$.*

  2: *For all $S > \lambda(a)$, $(f(S) - a)f''(S) < 2\,(f'(S))^2$.*

Proof. Notice first that $\gamma(r,D)$ can be written as follows

$$\gamma(r,D) = g_r(D) = \lambda(D+a) + \left(\frac{1}{1-r} - \frac{ra}{D+a}\right)\left(\lambda\left(\frac{D}{r}+a\right) - \lambda(D+a)\right). \tag{4.17}$$

Using the definition of l_D, $\gamma(r,D)$ is given then by

$$\gamma(r,D) = l_D(1) + \left(\frac{1}{1-r} - \frac{ra}{D+a}\right)(l_D(r) - l_D(1))$$

The partial derivative, with respect to r of γ is given then by

$$\frac{\partial\gamma}{\partial r}(r,D) = \left(\frac{1}{(1-r)^2} - \frac{a}{D+a}\right)\left(l_D(r) - l_D(1) + (1-r)l'_D(r)\right) + \frac{a(1-2r)}{D+a}l'_D(r). \tag{4.18}$$

Notice that $\frac{1}{(1-r)^2} - \frac{a}{D+a} > 0$ for all $r \in (0,1)$. From $l'_D(r) = -\frac{D}{r^2}\lambda'\left(\frac{D}{r}+a\right)$, it is deduced that $l'_D(r) < 0$. Therefore, if the condition (4.16) is satisfied, and, in addition $0 < r \leq 1/2$, then, from (4.18), it is deduced that $\frac{\partial\gamma}{\partial r}(r,D) < 0$.
In the case $r \in (1/2,1)$, we use the following expression of $\gamma(r,D)$ which is deduced from (4.17):

$$\gamma(r,D) = l_D(1) + B(r)\frac{l_D(r)-l_D(1)}{1-r}, \quad \text{where } B(r) = \frac{D+a-ar(1-r)}{D+a}$$

Straightforward computation show that

$$\frac{\partial\gamma}{\partial r}(r,D) = \frac{D+ar(2-r)}{(D+a)(1-r)^2}\left(l_D(r) - l_D(1) + (1-r)C(r)l'_D(r)\right), \quad \text{where } C(r) = \frac{D+a-ar(1-r)}{D+ar(2-r)}. \tag{4.19}$$

We have

$$C'(r) = \frac{a}{(D+ar(2-r))^2}\left(ar^2 + 2(a+2D)r - 3D - 2a\right).$$

Thus $C'(r) = 0$ for $r = r^* := \frac{1}{a}\left(\sqrt{3a^2+7aD+4D^2} - a - 2D\right) \in (1/2,1)$ and $(r-r^*)C'(r) > 0$ for $r \in (1/2,1)$, $r \neq r^*$. Hence, from $C(1/2) = C(1) = 1$, it is deduced that $0 < C(r) < 1$ for all $r \in (1/2,1)$. Now, if we assume that (4.16) is satisfied, we have

$$l_D(1) > l_D(r) + (1-r)l'_D(r) > l_D(r) + (1-r)C(r)l'_D(r) \quad \text{for } 1/2 < r < 1.$$

Hence, from (4.19), it is deduced that $\frac{\partial\gamma}{\partial r}(r,D) < 0$. This proves part **a** of the lemma.

Moreover, if l_D is strictly convex on $(D/m,1]$ then for all s and r in $(D/(m-a),1]$, if $s \neq r$, then

$$l_D(s) > l_D(r) + (s-r)l'_D(r).$$

Taking $s = 1$ and $r \in (D/(m-a),1)$ one obtains the condition (4.16). This proves part **b** of the lemma. Assume now that f, and hence l_D, are twice derivable. Using $\lambda'(D) = 1/f'(\lambda(D))$ and $\lambda''(D) = -f''(\lambda(D))/\left(f'(\lambda(D))\right)^3$, we can write

$$l''_D(r) = \frac{2D}{r^3}\lambda'\left(\frac{D}{r}+a\right) + \frac{D^2}{r^4}\lambda''\left(\frac{D}{r}+a\right) = \frac{D}{r^3\left(f'\left(\lambda\left(\frac{D}{r}+a\right)\right)\right)^3}\left(2\left(f'\left(\lambda\left(\frac{D}{r}+a\right)\right)\right)^2 - \frac{D}{r}f''\left(\lambda\left(\frac{D}{r}+a\right)\right)\right).$$

Therefore, the condition **1** in the lemma is equivalent to the following condition:

$$\text{For all } D \in (0,m-a) \text{ and } r \in (D/(m-a),1],\ \frac{D}{r}f''\left(\lambda\left(\frac{D}{r}+a\right)\right) < 2f'\left(\lambda\left(\frac{D}{r}+a\right)\right)^2. \tag{4.20}$$

Using the notation $S = \lambda\left(\frac{D}{r}+a\right)$, which is the same as $D/r = f(S) - a$, the condition (4.20) is equivalent to : For all $S > 0$, $(f(S)-a)f''(S) < 2\left(f'(S)\right)^2$, which is the condition **2** in the lemma. This proves part **c** of the lemma. □

Lemma 7. *Assume that*

$$f'\left(\lambda\left(\frac{D}{r}+a\right)\right) \leq \frac{1}{r}f'(\lambda(D+a)) \tag{4.21}$$

then $\frac{\partial\gamma}{\partial D}(r,D) > 0$. *If f' is decreasing, then the condition* (4.21) *is satisfied.*

Proof. From (4.17) we deduce that

$$\frac{\partial\gamma}{\partial D}(r,D) = \lambda'(D+a)+\left(\frac{1}{1-r}-\frac{ra}{D+a}\right)\left(\frac{1}{r}\lambda'\left(\frac{D}{r}+a\right)-\lambda'(D+a)\right)+\frac{ra}{(D+a)^2}\left(\lambda\left(\frac{D}{r}+a\right)-\lambda(D+a)\right).$$

Notice that

$$\frac{1}{1-r}-\frac{ra}{D+a}>0,\qquad \lambda'(D+a)>0,\qquad \lambda\left(\frac{D}{r}+a\right)-\lambda(D+a)>0.$$

Therefore the condition

$$\frac{1}{r}\lambda'\left(\frac{D}{r}+a\right)-\lambda'(D+a)\geq 0$$

is sufficient to have $\frac{\partial\gamma}{\partial D}(r,D)>0$. Using $\lambda'(D)=1/f'(\lambda(D))$, this condition is equivalent to (4.21). Note that if f' is decreasing, then this condition is satisfied. Indeed, we have

$$f'\left(\lambda\left(\frac{D}{r}+a\right)\right)\leq f'\left(\lambda\left(D+a\right)\right)\leq \frac{1}{r}f'\left(\lambda\left(D+a\right)\right)$$

which is the condition (4.21). This ends the proof of the lemma. □

Remark 3. *Notice that:*

- **i/:** *The condition **2** in Lemma 6 is equivalent to* $\frac{d^2}{dS^2}\left(\frac{1}{f(S)-a}\right)>0$ *for all* $S>\lambda(a)$.
- **ii/:** *If the increasing growth function f is twice derivable and satisfies* $f''(S)\leq 0$ *for all* $S>0$*, then the condition **b** in Lemma 6 and the condition* (4.21) *in Lemma 7 are satisfied. Thus, Assumptions 2 and 3 are satisfied and our results apply for any concave growth function.*
- **iii/:** *Assume that the increasing growth function f is twice derivable and there exists* $\hat{S}\in(0,+\infty)$ *such that* f'' *is nonnegative on* $(0,\hat{S})$ *and nonpositive on* $(\hat{S},+\infty)$*. If moreover the condition **2** of Lemma 6 is verified for* $a=0$*, then this condition is also verified for any* $a>0$ *and* $S\in(\lambda(a),\hat{S})$*. Therefore, if* $\frac{d^2}{dS^2}\left(\frac{1}{f(S)}\right)>0$ *on* $(0,\hat{S})$ *then Assumption 2 is satisfied.*

The three points of Remark 3 are illustrated in Section 5.

4.2. **Biogas flow rate.** We recall that the biogas flow rate is proportional to the microbial activity, as defined for instance in [3, 24]. We consider here the biogas flow rate as a function of the input substrate concentration S^{in}, the dilution rate D and the parameter r.

For $r(f(S^{in})-a)\leq D$ and $D<(1-r)(f(S^{in})-a)$, the biogas flow rate corresponding to the steady state E_1 is given by the expression

$$G_1(S^{in},D,r)=V_2\overline{x}_2 f(\overline{S}_2), \tag{4.22}$$

with $V_2=(1-r)V$, $\overline{x}_2$ and $\overline{S}_2$ defined in (2.6).

For $D<r(f(S^{in})-a)$, the biogas flow rate corresponding to the positive steady state E_2 is given by the expression

$$G_2(S^{in},D,r)=V_1x_1^*f(S_1^*)+V_2x_2^*f(S_2^*), \tag{4.23}$$

with $V_1=rV$, $V_2=(1-r)V$, x_1^* and S_1^* defined in (2.8), x_2^* defined by (2.9) and S_2^* the unique solution of $h(S_2)=f(S_2)$.

Proposition 6. *For all* $r\in(0,1)$*,*

(1) When $r(f(S^{in})-a)\leq D$ *and* $D<(1-r)(f(S^{in})-a)$ *then* $G_1(S^{in},D,r)=VD(S^{in}-\overline{S}_2)$.
(2) When $D<f(S^{in})-a$ *then* $G_2(S^{in},D,r)=VD(S^{in}-S_2^*)$.

Proof. Let $r\in(0,1)$.

(1) From system (2.1), considering $\dot{S}_2 = 0$ gives $\overline{x}_2 f(\overline{S}_2) = D(S^{in} - \overline{S}_2)/(1-r)$. Thus, one has
$$G_1(S^{in}, D, r) = rV\frac{D}{r}(S^{in} - \overline{S}_2) = VD(S^{in} - \overline{S}_2).$$
(2) From system (2.1), considering $\dot{S}_1 = 0$ and $\dot{S}_2 = 0$ gives respectively $x_1^* f(S_1^*) = D(S^{in} - S_1^*)/r$ and $x_2^* f(S_2^*) = D(S_1^* - S_2^*)/(1-r)$. Thus, one has
$$G_2(S^{in}, D, r) = rV\frac{D}{r}(S^{in} - S_1^*) + (1-r)V\frac{D}{1-r}(S_1^* - S_2^*) = VD(S^{in} - S_2^*).$$

□

One has $\overline{S}_2|_{r=0} = \lambda(D+a)$ then for all $r(f(S^{in}) - a) \leq D$ and $D < (1-r)(f(S^{in}) - a)$, one has $G_1(S^{in}, D, 0) = VD(S^{in} - \lambda(D+a))$. In addition, according to Lemma 3, for all $0 \leq D < f(S^{in}) - a$, one has $\lim_{r\to 1} G_2(S^{in}, D, r) = VD(S^{in} - \lambda(D+a))$. Notices that $G_1(S^{in}, D, 0) = G_{chem}(S^{in}, D)$ and $\lim_{r\to 1} G_2(S^{in}, D, r) = G_{chem}(S^{in}, D)$ where G_{chem} defined in (A.8) represents the biogas flow rate of the single chemostat when $0 < D < f(S^{in}) - a$.

Proposition 7. *For all $r \in (0,1)$ and $0 \leq D < f(S^{in}) - a$, one has*

(1) $G_1(S^{in}, D, r) < G_{chem}(S^{in}, D)$.
(2) $G_2(S^{in}, D, r) > G_{chem}(S^{in}, D)$ if and only if $S^{in} > g_r(D)$.

Proof. According to (A.8), for $0 \leq D < f(S^{in}) - a$, one has $G_{chem}(S^{in}, D) = VD(S^{in} - \lambda(D+a))$.

(1) Recall that $D/(1-r) > D$ and λ is increasing. Thus, one deduces that $\lambda(D/(1-r) + a) > \lambda(D+a)$. This induces the inequality $VD(S^{in} - \lambda(D/(1-r)+a)) < VD(S^{in} - \lambda(D+a))$ which is equivalent to $G_1(S^{in}, D, r) < G_{chem}(S^{in}, D)$.
(2) From Proposition 6, one has $G_2(S^{in}, D, r) = VD(S^{in} - S_2^*(S^{in}, D, r))$. In addition, according to Theorem 2, for any $r \in (0,1)$ one has $S_r^{out}(S^{in}, D) < S^{out}(S^{in}, D)$ if and only if $S^{in} > g_r(D)$ which gives, $S_2^*(S^{in}, D, r) < \lambda(D+a)$ if and only if $S^{in} > g_r(D)$. Consequently, one has $G_2(S^{in}, D, r) > G_{chem}(S^{in}, D)$ if and only if $S^{in} > g_r(D)$.

□

Proposition 7 is illustrated in Figure 7. One remarks that for any $r \in (0,1)$, the green curve of the biogas flow rate corresponding to the steady state E_1 is always below the black curve of the biogas flow rate of the single chemostat. This illustrates the first result of the proposition. In addition, one notices that according to Assumption 3, $D = D_r(S^{in})$ is the unique solution of $S^{in} = g_r(D)$. Therefore, one remarks that for all $0 < D < D_r(S^{in})$ (equivalently, for all $S^{in} > g_r(D)$), the orange curve of the biogas flow rate corresponding to the steady state E_2 is always above the black curve of the biogas flow rate of the single chemostat, which illustrates the second result of the proposition.

In Figure 7 (b) and (c), it seems that we can have the following inequality
$$\max_{D\in(0,f(S^{in})-a)} G_2(S^{in}, D, r) > \max_{D\in(0,f(S^{in})-a)} G_{chem}(S^{in}, D).$$
This is interesting and new because it never happens in the case without mortality. Indeed, in the case without mortality, we proved that for a fixed $S^{in} > 0$, for any $D > 0$ and $r \in (0,1)$, the maximal biogas flow rate of the serial configuration never exceed the maximal biogas flow rate of the single chemostat, a result given in Proposition 6 of [6].

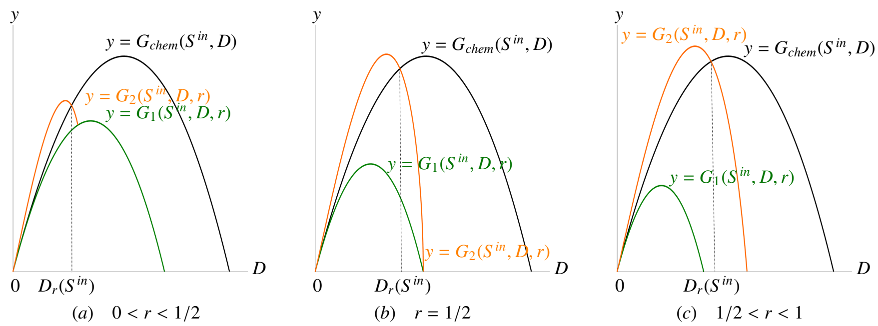


FIGURE 7. The maps $D \mapsto G_{chem}(S^{in}, D)$, $D \mapsto G_1(S^{in}, D, r)$ and $D \mapsto G_2(S^{in}, D, r)$ representing respectively the biogas flow rate of the single chemostat (in black), the biogas flow rate corresponding to the steady state E_1 of the serial configuration (in green) and to the biogas flow rate corresponding to the positive steady state E_2 of the serial configuration (in orange).

Observe that for any fixed $S^{in} > 0$ and $r \in [0,1]$, the continuous function $D \mapsto G_2(S^{in}, D, r)$ is null for $D = 0$ and $D \geq f(S^{in}) - a$, and positive on the interval $(0, f(S^{in}) - a)$. Therefore, it reaches it maximum. For a given $S^{in} > 0$, we then consider the function

$$\overline{G}(r) := \max_{D \geq 0} G_2(S^{in}, D, r). \tag{4.24}$$

Assumption 4. *S^{in} is such that for any $r \leq 1$ in a neighborhood $\mathcal{V}_1$ of 1, the maximum of the function $D \mapsto G_2(S^{in}, D, r)$ is unique, denoted $\overline{D}(r)$. Moreover, we assume that f is C^2 and that $\overline{D}$ is differentiable on $\mathcal{V}_1 \cap \{r < 1\}$ with bounded derivative.*

One can easily check that this assumption is fulfilled for the usual growth functions (linear, Monod, Hill) that we shall consider in Section 5.

Proposition 8. *Under Assumption 4, the function $\overline{G}$ admits left limits of its first and second derivatives at $r = 1$, which are*

$$\overline{G}'(1^-) = 0 \quad \text{and} \quad \overline{G}''(1^-) = \frac{2a\overline{D}(1)}{\overline{D}(1) + a}(S^{in} - \lambda(\overline{D}(1) + a)). \tag{4.25}$$

Proof. The proof is given in Appendix C. □

From Proposition 8, we deduce the remarkable feature about the maximization of the biogas which is reached with a configuration of two non empty chemostats when the mortality rate is non null, differently to the case without mortality (for which the single chemostat is always the best configuration). This is illustrated in Section 5.4.

Proposition 9. *Assume that Assumptions 1 and 2 are satisfied.*

(1) If $S^{in} \leq g(D)$ then for any $r \in (0,1)$, $G_2(S^{in}, D, r) < G(S^{in}, D)$.
(2) If $S^{in} > g(D)$ then $G_2(S^{in}, D, r) > G_{chem}(S^{in}, D)$ if and only if $r_1(S^{in}, D) < r < 1$ with r_1 defined in Lemma 5.

with g defined by (A.9). *The equality is fulfilled for $r = r_1(S^{in}, D)$ and $r = 1$.*

Proof. Recall that G_{chem} and G_2 are respectively defined by (A.8) and (4.23). According to Theorem 3:

(1) If $S^{in} \leq g(D)$ then for any $r \in (0,1)$ one has $S_r^{out}(S^{in}, D) > S^{out}(S^{in}, D)$. Thus, one deduces that if $S^{in} \leq g(D)$ then for any $r \in (0,1)$, $S_2^*(S^{in}, D, r) < \lambda(D+a)$. Consequently, if $S^{in} \leq g(D)$ then for any $r \in (0,1)$, $G_2(S^{in}, D, r) < G_{chem}(S^{in}, D)$.
(2) If $S^{in} > g(D)$ then $S_r^{out}(S^{in}, D) < S^{out}(S^{in}, D)$ if and only if $r_1(S^{in}, D) < r < 1$. Thus, one deduces that if $S^{in} > g(D)$ then $S_2^*(S^{in}, D, r) < \lambda(D+a)$ if and only if $r_1(S^{in}, D) < r < 1$. Consequently, if $S^{in} > g(D)$ then $G_2(S^{in}, D, r) > G_{chem}(S^{in}, D)$ if and only if $r_1(S^{in}, D) < r < 1$.

Finally, according to Theorem 3, for $r = r_1(S^{in}, D)$ and $r = 1$, one has $S_r^{out}(S^{in}, D) = S^{out}(S^{in}, D)$ then $G_2(S^{in}, D, r) = G_{chem}(S^{in}, D)$. This ends the proof of the proposition. □

5. Illustrations and numerical simulations

This section illustrates some of our results using three different growth functions. As concave functions, we choose the linear growth function and the Monod function. As a non concave function, we choose the Hill function which has the special behavior being convex then concave.

5.1. **Linear growth function.**

Let consider a linear function defined by $f(S) = \alpha S$, $\alpha > 0$. As it is concave, according to **ii/** in Remark 3, the linear function verifies Assumptions 2 and 3. Therefore, our results apply for a linear function.

One has $\lambda(2D+a) = g(D) = (2D+a)/\alpha$ then, the curves $\Phi_{1/2}$ and Γ defined respectively in (4.13) and (A.10) constitute the same curve. Consequently, the operating plane (S^{in}, D) consists of three regions J_i, $i = 0, 1, 3$ defined in (4.14) that describe the behavior of the output substrate concentration and the biogas flow rate, see Figure 8.

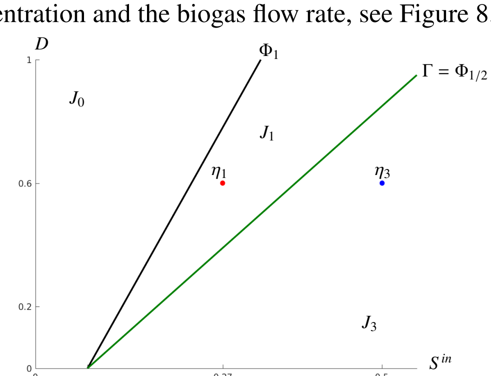
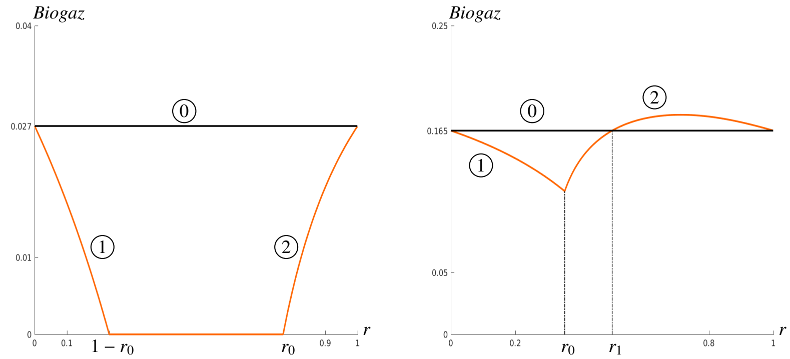


Figure 8. The three regions in the operating plane with $f(S) = 4S$ and $a = 0.3$. The biogas flow rate corresponding to points $\eta_1 = (0.27, 0.6)$ and $\eta_3 = (0.5, 0.6)$ is depicted in Figure 9.

Let η_1 and η_3 be two points fixed respectively in regions J_1 and J_3, as shown in Figure 8. The behavior of the biogas flow rate for these points is depicted in Figure 9. It should be noticed that for any other point $(S^{in}, D) \in J_1$, the curve representing the biogas flow rate with respect to r should be similar to the curve shown in Figure 9 (a), and corresponding to $(S^{in}, D) = \eta_1$. Similarly, for any other point $(S^{in}, D) \in J_3$, it should be similar to the curve shown in Figure 9 (b), and corresponding to $(S^{in}, D) = \eta_3$.



(*a*) $(S^{in}, D) = \eta_1$ (*b*) $(S^{in}, D) = \eta_3$

FIGURE 9. The biogas flow rate of the serial configuration with respect to $r \in [0, 1]$, corresponding to points η_1 and η_3 depicted in Figure 8. The numbered curves ⓪ (in black), and ①, ② (in orange) are respectively defined by $y = G_{chem}(S^{in}, D)$, $y = G_1(S^{in}, D, r)$ and $y = G_2(S^{in}, D, r)$. Recall that $r_0 = D/(f(S^{in}) - a)$ and $r = r_1$ is the solution of $S^{in} = g_r(D)$. In (*a*): $(S^{in}, D) = \eta_1$ and $r_0 \approx 0.77$. In (*b*): $(S^{in}, D) = \eta_3$, $r_0 \approx 0.35$ and $r_1(0.5, 0.6) = 0.5$.

In the linear case, $S^{in} = g_r(D)$ is a second degree algebraic equation in r that gives two solutions, one corresponds to $r_1(S^{in}, D)$ cited in Lemma 5 and the other one is not considered as it does not belong to $(0, 1)$.

Since the point $\eta_3 = (0.5, 0.6)$ satisfies the condition $S^{in} > g(D)$, as stated in Proposition 9, there exists a threshold $r = r_1(0.5, 0.6) \approx 0.5$, solution of $0.5 = g_r(0.6)$ such that, the serial configuration has a higher biogas flow rate production than a single chemostat if and only if $r \in (r_1, 1)$, see Figure 9 (*b*).

5.2. **Monod function.** Let consider the Monod function defined by $f(S) = mS/(K + S)$. As it is concave, according to **ii/** in Remark 3, the Monod function verifies Assumptions 2 and 3. Therefore, our results apply for Monod function.

Lemma 8. *The curve* Γ *is strictly above the curve* $\Phi_{1/2}$.

Proof. The curves $\Phi_{1/2}$ and Γ are respectively defined in (4.13) and (A.10). Let the function $H : [0, (m-a)/2) \mapsto \mathbb{R}$ be defined by

$$H(D) = \lambda(2D + a) - g(D) = \frac{KmD^2}{(m - D - a)^2(m - a - 2D)}.$$

For any $D \in [0, (m-a)/2)$, one has $H(D) > 0$ i.e. $\lambda(2D + a) > g(D)$. Therefore, the curve Γ is always strictly above the curve $\Phi_{1/2}$. □

As a consequence of Lemma 8, the operating plane (S^{in}, D) consists of four regions J_i, $i = 0, 1, 2, 3$ defined in (4.14) that describe the behavior of the output substrate concentration and the biogas flow rate, see Figure 10.

Let η_1, η_2 and η_3 be three points fixed respectively in regions J_1, J_2 and J_3, as shown in Figure 10. The behavior of the biogas flow rate for these points is depicted in Figure 11. It should be noticed that for any other point $(S^{in}, D) \in J_1$ (resp. $(S^{in}, D) \in J_2$ and $(S^{in}, D) \in J_3$), the curve representing the biogas flow rate with respect to r should be similar to the curve shown in Figure 11 (*a*) (resp. (*b*) and (*c*)), and corresponding to $(S^{in}, D) = \eta_1$ (resp. $(S^{in}, D) = \eta_2$ and $(S^{in}, D) = \eta_3$).

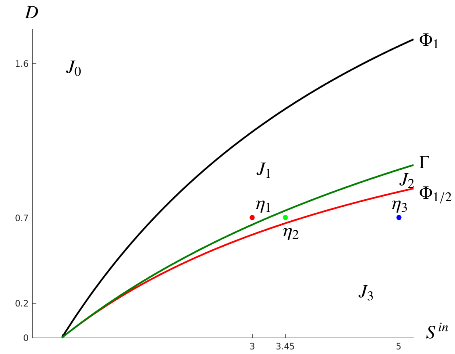
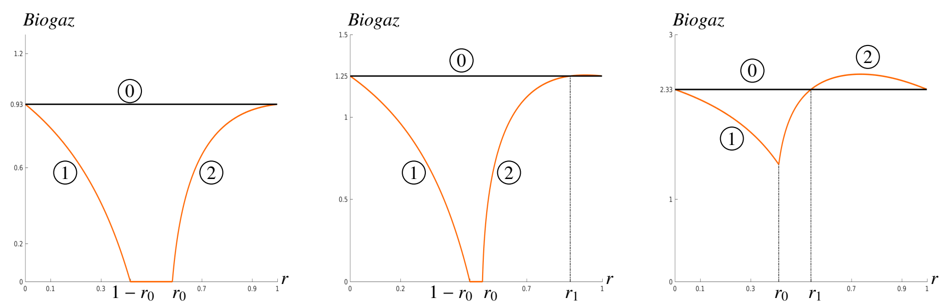


Figure 10. The four regions in the operating plane with $f(S) = 4S/(5+S)$ and $a = 0.3$. The biogas flow rate corresponding to points $\eta_1 = (3, 0.7)$, $\eta_2 = (3.45, 0.7)$ and $\eta_3 = (5, 0.7)$ is depicted in Figure 11.



(a) $(S^{in}, D) = \eta_1$ (b) $(S^{in}, D) = \eta_2$ (c) $(S^{in}, D) = \eta_3$

Figure 11. The biogas flow rate of the serial configuration with respect to $r \in [0, 1]$, corresponding to points η_1 ,η_2 and η_3 depicted in Figure 10. The numbered curves ⓪ (in black), and ①, ② (in orange) are respectively defined by $y = G_{chem}(S^{in}, D)$, $y = G_1(S^{in}, D, r)$ and $y = G_2(S^{in}, D, r)$. Recall that $r_0 = D/(f(S^{in}) - a)$ and $r = r_1$ is the solution of $S^{in} = g_r(D)$. In (a): $(S^{in}, D) = \eta_1$ and $r_0 \approx 0.58$. In (b): $(S^{in}, D) = \eta_2$, $r_0 \approx 0.53$ and $r_1(3.45, 0.7) \approx 0.87$. In (c): $(S^{in}, D) = \eta_3$, $r_0 \approx 0.41$ and $r_1(5, 0, 7) \approx 0.54$.

In the Monod case, $S^{in} = g_r(D)$ is a second degree algebraic equation in r that gives two solutions, one corresponds to $r_1(S^{in}, D)$ cited in Lemma 5 and the other one is not considered as it does not belong to $(0, 1)$.

Since the point η_2 (resp. η_3) satisfies the condition $S^{in} > g(D)$, as stated in Proposition 9, there exists a threshold $r = r_1(3.45, 0.7) \approx 0.87$ (resp. $r = r_1(5, 0.7) \approx 0.54$) solution of $3.45 = g_r(0.7)$ (resp. $5 = g_r(0.7)$) such that, the serial configuration has a higher biogas flow rate production than a single chemostat if and only if $r \in (r_1, 1)$, see Figure 11 (b) (resp. (c)).

5.3. **Hill function.** For all $p > 1$, the non-concave Hill function is given by $f(S) = mS^p/(K^p + S^p)$.

Proposition 10. *The Hill function verifies Assumptions 2 and 3.*

Proof. Let $\hat{S} \in (0, +\infty)$ be the inflexion point of the Hill function f. According to **iii/** in Remark 3, it is sufficient to show that $\frac{d^2}{dS^2}\left(\frac{1}{f(S)}\right) > 0$ for all $S \in (0, \hat{S})$. One has, $\frac{d^2}{dS^2}\left(\frac{1}{f(S)}\right) = \frac{p(p+1)K^p}{mS^{p+2}}$ which is positive for all $S > 0$. Then, $\frac{d^2}{dS^2}\left(\frac{1}{f(S)-a}\right)$ is positive for any $a > 0$ and $S \in (\lambda(a), \hat{S})$. Consequently, for all $p > 1$, the Hill function verifies Assumption 2. As an example, one notices in Figure 12 that for the Hill function, the map $S \mapsto 1/(f(S) - a)$ remains convex for different nonnegative values of a.

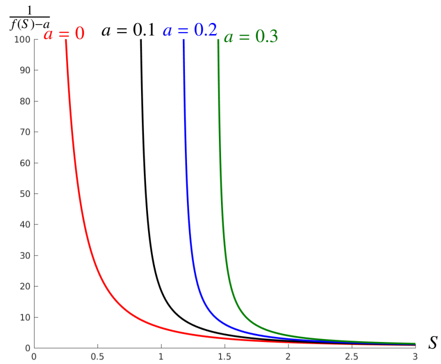


FIGURE 12. The maps $S \mapsto 1/(f(S) - a)$ with $f(S) = 4S^2/(25 + S^2)$ and different values of a.

Let us prove that the Hill function verifies the condition (4.21) of Lemma 7, which implies that it satisfies Assumption 3. Straightforward computations give

(5.1)

$$\lambda(D + a) = K\left(\frac{D+a}{m-a-D}\right)^{\frac{1}{p}}, \qquad f'(\lambda(D + a)) = \frac{p}{Km}(D + a)^{\frac{p-1}{p}}(m - a - D)^{\frac{p+1}{p}},$$

$$\lambda(D/r + a) = K\left(\frac{D/r+a}{m-a-D/r}\right)^{\frac{1}{p}}, \qquad f'(\lambda(D/r + a)) = \frac{p}{Km}(D/r + a)^{\frac{p-1}{p}}(m - a - D/r)^{\frac{p+1}{p}}.$$

Since $p > 1$, $D + ra < D + a$ and $0 < rm - ra - D < m - a - D$, one has

$$(D + ra)^{\frac{p-1}{p}} < (D + a)^{\frac{p-1}{p}} \quad \text{and} \quad (rm - ra - D)^{\frac{1}{p}} < (m - a - D)^{\frac{1}{p}}. \tag{5.2}$$

From the first inequality in (5.2) one has

$$(D/r + a)^{\frac{p-1}{p}} = (1/r)^{\frac{p-1}{p}}(D + ra)^{\frac{p-1}{p}} < (1/r)^{\frac{p-1}{p}}(D + a)^{\frac{p-1}{p}}. \tag{5.3}$$

From the second inequality in (5.2), and using $0 < m - a - D/r < m - a - D$, one has

$$(m - a - D/r)^{\frac{p+1}{p}} = (1/r)^{\frac{1}{p}}(rm - ra - D)^{\frac{1}{p}}(m - a - D/r) < (1/r)^{\frac{1}{p}}(m - a - D)^{\frac{p+1}{p}}. \tag{5.4}$$

Therefore, using (5.3) and (5.4) one obtains

$$(D/r+a)^{\frac{p-1}{p}}(m-a-D/r)^{\frac{p+1}{p}} < (1/r)^{\frac{p-1}{p}+\frac{1}{p}}(D+a)^{\frac{p-1}{p}}(m-a-D)^{\frac{p+1}{p}} = (D+a)^{\frac{p-1}{p}}(m-a-D)^{\frac{p+1}{p}}/r$$

Consequently, using (5.1), one has $f'(\lambda(D/r + a)) < f'(\lambda(D + a))/r$. This completes the proof of the proposition. □

Proposition 10 insures that our results apply for an Hill function. Let now consider the Hill function $f(S) = mS^2/(K^2 + S^2)$.

Lemma 9. *Let us denote* $D_1 := (3m - 4a - \sqrt{m(5m-4a)})/4$.

If $0 < D < D_1$ *then the curve* $\Phi_{1/2}$ *defined in* (4.13) *is strictly above the curve* Γ *defined by* (A.10)*. In contrast, if* $D_1 < D < (m-a)/2$ *then the curve* $\Phi_{1/2}$ *is strictly below the curve* Γ.

Proof. Let the function $H : [0, (m-a)/2) \mapsto \mathbb{R}$ be defined by $H(D) := \lambda(2D + a) - g(D)$ which is equivalent to

$$H(D) = K\left(\sqrt{\frac{2D+a}{m-a-D}} - \frac{(2D+a)(m-a-D) + (D+a)(m-a)}{2(m-a-D)^{3/2}\sqrt{D+a}}\right).$$

This function is positive if the polynomial $Q(D) := 4D^2 - 2(3m-4a)D + 4a^2 - 5am + m^2$ is negative. The discriminant Δ_Q of equation $Q(D) = 0$ is $\Delta_Q = 4m(5m-4a)$ and is positive as $a < m$. Therefore, $Q(D) = 0$ admits the two roots

$$D_1 = \frac{3m - 4a - \sqrt{m(5m-4a)}}{4} \quad \text{and} \quad D_2 = \frac{3m - 4a + \sqrt{m(5m-4a)}}{4},$$

such that $0 < D_1 < (m-a)/2$ and $(m-a)/2 < D_2$. Thus, for any $D \in (D_1, (m-a)/2)$, we have $H(D) > 0$ and then the curve $\Phi_{1/2}$ is strictly below the curve Γ. □

As a consequence of Lemma 9, the operating plane consists of five regions J_i $i = 0, 1, 2, 3, 4$ defined in (4.14), see Figure 13.

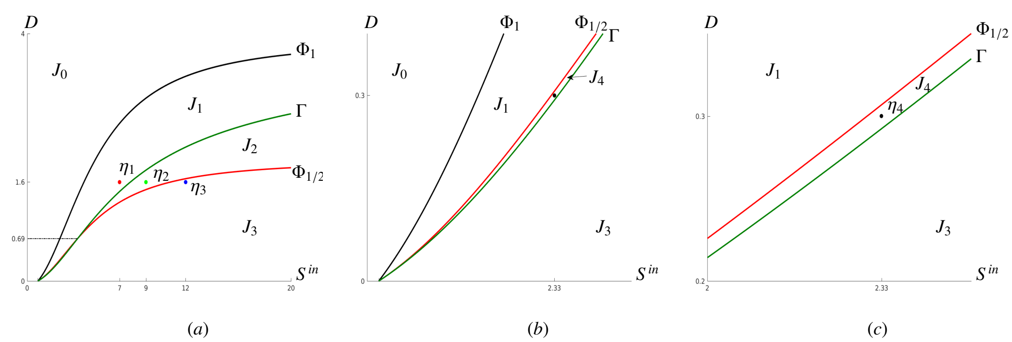
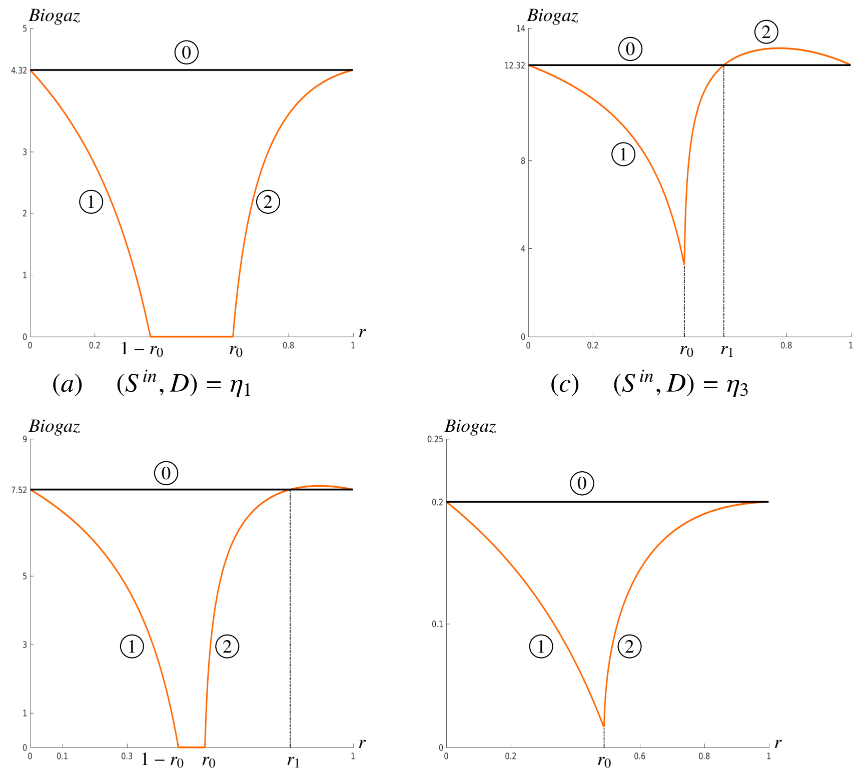


FIGURE 13. The five regions in the operating plane with $f(S) = 4S^2/(25 + S^2)$, $a = 0.1$ and $D_1 = 0.69$ (see Lemma 9). The biogas flow rate corresponding to points $\eta_1 = (7, 1.6)$, $\eta_2 = (9, 1.6)$, $\eta_3 = (12, 1.6)$ and $\eta_4 = (2.33, 0.3)$ is depicted in Figure 14.

Let η_1, η_2, η_3 and η_4 be four points fixed respectively in regions J_1, J_2, J_3 and J_4, as shown in Figure 13. It should be noticed that for any other point $(S^{in}, D) \in J_1$ (resp. $(S^{in}, D) \in J_2$, $(S^{in}, D) \in J_3$ and $(S^{in}, D) \in J_4$), the curve representing the biogas flow rate with respect to r should be similar to the curve shown in Figure 14 (a) (resp. (b), (c) and (d)), and corresponding to $(S^{in}, D) = \eta_1$ (resp. $(S^{in}, D) = \eta_2$, $(S^{in}, D) = \eta_3$ and $(S^{in}, D) = \eta_4$).

Recall that $r = r_1(S^{in}, D)$ is the solution of equation $S^{in} = g_r(D)$. The value of $r_1(S^{in}, D)$ is obtained numerically. Since the point η_2 (resp. η_3) satisfies the condition $S^{in} > g(D)$, as stated in Proposition 9, there exists a threshold $r = r_1(9, 1.6) \approx 0.81$ (resp. $r = r_1(12, 1.6) \approx$



(a) $(S^{in}, D) = \eta_1$ (c) $(S^{in}, D) = \eta_3$

(b) $(S^{in}, D) = \eta_2$ (d) $(S^{in}, D) = \eta_4$

Figure 14. The biogas flow rate of the serial configuration with respect to r, corresponding to points η_1, η_2, η_3 and η_4 depicted in Figure 13. The numbered curves ⓪ (in black), and ①, ② (in orange) are respectively defined by $y = G_{chem}(S^{in}, D)$, $y = G_1(S^{in}, D, r)$ and $y = G_2(S^{in}, D, r)$. Recall that $r_0 = D/(f(S^{in}) - a)$ and $r = r_1$ is the solution of $S^{in} = g_r(D)$. In (a): $(S^{in}, D) = \eta_1$ and $r_0 \approx 0.63$. In (b): $(S^{in}, D) = \eta_2$, $r_0 \approx 0.54$ and $r_1(9, 1.6) \approx 0.81$. In (c): $(S^{in}, D) = \eta_3$, $r_0 \approx 0.48$ and $r_1(12, 1.6) \approx 0.61$. In (d): $(S^{in}, D) = \eta_4$ and $r_0 \approx 0.49$.

0.61) solution of equation $9 = g_r(1.6)$ (resp. $12 = g_r(1.6)$) such that the serial configuration has a higher biogas flow rate production than a single chemostat if and only if $r \in (r_1, 1)$, see Figure 14 (b) (resp. (c)).

5.4. **Illustrations of Proposition 8.** Proposition 8 is illustrated in Figure 15. Indeed, it is shown that the tangent at $r = 1$ is horizontal which corresponds to the first stated result in the proposition: $\overline{G}'(1) = 0$. In addition, one remarks that $\overline{G}''(1) > 0$ and it remains positive in a neighborhood of $r = 1$. Thus, with presence of mortality rate, for a fixed input substrate concentration, if practitioners are able to choose the dilution rate D, to optimize the efficiency, they should consider a serial configuration where the volume rV of the first tank is quite close to the total volume V (i.e. r is quite close to 1) and a dilution rate defined by $D = \text{argmax}_{D \in (0, f(S^{in}) - a)} G_2(S^{in}, D, r)$. This result has an important message for practitioners which is new to the best of our knowledge of the literature: the serial configuration does worth to be considered when mortality is not negligible.

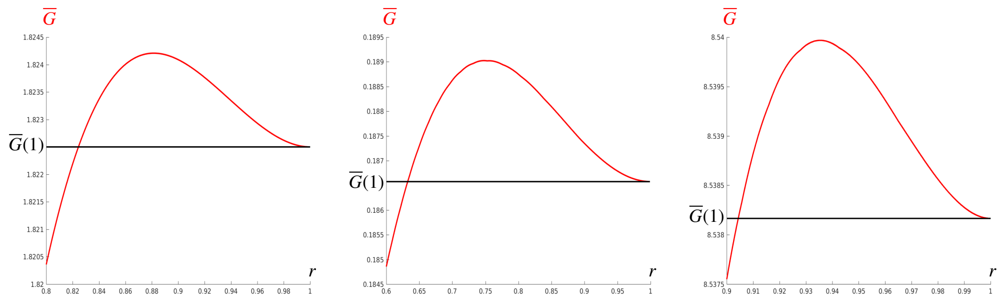


FIGURE 15. The map $r \mapsto \overline{G}(r)$ with $\overline{G}$ defined by (4.24). In (a): $f(S) = 4S$, $a = 0.6$ and $S^{in} = 1.5$. In (b): $f(S) = 4S/(5+S)$, $a = 0.3$ and $S^{in} = 1.5$. In (c): $f(S) = 4S^2/(25+S^2)$, $a = 0.3$ and $S^{in} = 10$.

6. CONCLUSION

In this work, an in-depth study is carried out on the mathematical model of two interconnected chemostats in serial with mortality. Equations contain a term representing the mortality rate of the species. Due to this added term characterizing the mathematical model, this paper is considered as an extension of the work done in [6], where the model does not consider the mortality rate. However, the mathematical analysis revealed that the proofs have had to be significantly revisited and reveal several new non intuitive differences compared to the case without mortality. Let us recall that without mortality, the dynamics admits a forward attractive invariant hyperplane related to the total mass conservation, which is no longer verified under mortality consideration. This at the core of the differences in the mathematical analysis. The study of the model is based on the analysis of the asymptotic behavior of its solutions, and is supported by an operating diagram which describes the number and stability of steady states. In a first step, we considered different mortality rates a_1, a_2 in each tank. Then, in view of comparing with the single configuration, we considered identical mortality rate $a = a_1 = a_2$. We analyzed the performances of the model at steady state for two different criteria: the output substrate concentration and the biogas flow rate (and compared them for the single chemostat with the same mortality rate a). Explicit expressions of criteria, depending on the dilution rate D and the input substrate concentration S^{in}, are provided. These new results provide conditions that insure the existence of a serial configuration more efficient than a single chemostat, in the sense of minimizing the output substrate concentration or maximizing the biogas flow rate.

Along the paper, the similarities, specificities and differences of our model compared to the model without mortality (i.e. for $a = 0$) studied in [6] are highlighted. Among the differences that attract attention, on the one hand, we have the operating diagram with different mortality which presents many more cases than the diagram without mortality where it is reduced to only two cases. Thus, the presence of the four regions of stability on the same diagram is now possible. On the other hand, we have the biogas production of the serial device in its maximum state which can be significantly larger than the largest biogas production of the single chemostat. This never happens in the case without mortality. Finally, unlike the case without mortality, the biomass productivity and the biogas flow rate are no more represented by the same stead-state equations with presence of mortality. Therefore, to broaden the present work, an in-depth analysis according to the mortality rate a and the parameter r can be considered for the performance's criterion: the productivity of the biomass and its analysis will be the subject of a further work.

Appendix

Appendix A. The single chemostat

In this section, we give a brief presentation of the mathematical model of the single chemostat with mortality rate. The goal is to write explicitly some definitions and properties that we use in the comparison with the series device of the two interconnected chemostats.

A.1. **Mathematical model.** The mathematical model representing the single chemostat with mortality rate is defined by

$$\begin{array}{rcl} \dot{S} & = & D(S^{in} - S) - f(S)x \\ \dot{x} & = & -Dx + f(S)x - ax \end{array} \tag{A.1}$$

where S and x denote respectively the substrate and the biomass concentrations, S^{in} the input substrate concentration, Q the input flow rate, V the total volume, a the mortality rate, D defined by $D := Q/V$ is the dilution rate. The specific growth rate $f(\cdot)$ of the microorganisms satisfies Assumption 1. Under this Assumption one can check that we have the existence and the uniqueness of the solution of system (A.1). The existence and stability of steady states of (A.1) are given by the following result.

Theorem 4. *The steady states of* (A.1) *are:*

1. *The washout steady state $E_0 = (S^{in}, 0)$ which always exists. It is GAS if and only if $D \geq f(S^{in}) - a$. It is LES if and only if $D > f(S^{in}) - a$.*
2. *The steady state $E_1 = (S_1^*, x_1^*)$ of persistence of the species with*

$$S_1^* := \lambda(D + a) \quad \textit{and} \quad x_1^* := \frac{D}{D + a}(S^{in} - \lambda(D + a)). \tag{A.2}$$

This steady state exists and is positive if and only if $D < f(S^{in}) - a$. It is GAS and LES whenever it exists and is positive.

The proof of the theorem is a classical result in the literature that can be found in [13].

We depict the operating diagram corresponding to system (A.1). Thus, the regions in which the solution of system (A.1) globally converges towards one of the steady states E_0 or E_1 are depicted in the plane (S^{in}, D). Let the curve Φ be defined by

$$\Phi := \{(S^{in}, D) : D = f(S^{in}) - a\}. \tag{A.3}$$

These curve split the plane (S^{in}, D) into two regions denoted I_0 and I_1, as depicted in Figure 17. These regions are defined by

$$I_0 := \{(S^{in}, D) : D \geq f(S^{in}) - a\}, \quad I_1 := \{(S^{in}, D) : D < f(S^{in}) - a\}. \tag{A.4}$$

The behavior of the system in each region is given in Table 2. The particularity of the operating diagram is that the curve limiting both regions I_0 and I_1 is translated from zero,

	I_0	I_1
E_0	GAS	U
E_1		GAS

TABLE 2. Stability of steady states in the various regions of the operating diagram. The letter U means that the steady state is unstable. The letters GAS mean that the steady state is globally asymptotically stable in the positive orthant. No letter means that the steady state does not exist.

unlike the case with mortality, as shown in Figure 2.5 of [13]. Thus, with presence of mortality rate, the region where the washout is GAS, is larger.

A.2. **Output substrate and biomass concentrations.** According to Theorem 4, at steady state, for all $S^{in} > \lambda(a)$, the output substrate concentration of the single chemostat is defined by

$$S^{out}(S^{in}, D) := \begin{cases} S^{in} & \text{if} \quad D \geq f(S^{in}) - a \\ \lambda(D + a) & \text{if} \quad D < f(S^{in}) - a, \end{cases} \tag{A.5}$$

and its output biomass is defined by

$$x^{out}(S^{in}, D) := \begin{cases} 0 & \text{if} \quad D \geq f(S^{in}) - a \\ \frac{D}{D+a}(S^{in} - \lambda(D + a)) & \text{if} \quad D < f(S^{in}) - a. \end{cases} \tag{A.6}$$

Thus, for all $S^{in} > \lambda(a)$, one has

$$\frac{\partial S^{out}}{\partial D}(S^{in}, D) = \begin{cases} 0 & \text{if} \quad D \geq f(S^{in}) - a \\ \lambda'(D + a) & \text{if} \quad D < f(S^{in}) - a, \end{cases}$$

and

$$\frac{\partial x^{out}}{\partial D}(S^{in}, D) = \begin{cases} 0 & \text{if} \quad D \geq f(S^{in}) - a \\ \frac{1}{D+a}\left(\frac{a}{D+a}(S^{in} - \lambda(D + a)) - \lambda'(D + a)\right) & \text{if} \quad D < f(S^{in}) - a. \end{cases}$$

Thus, for all $D < f(S^{in}) - a$, $D \mapsto S^{out}(S^{in}, D)$ is increasing, as shown in Figure 16 (a) but the output biomass depends on the chosen growth function. As an example, $D \mapsto x^{out}(S^{in}, D)$ is illustrated in Figure 16 (b) for Monod function.

A.3. **Biogas flow rate.** The biogas flow rate of the single chemostat is defined, up to a multiplicative yield coefficient, by

$$G_{chem}(S^{in}, D) := V x^{out} f(S^{out}). \tag{A.7}$$

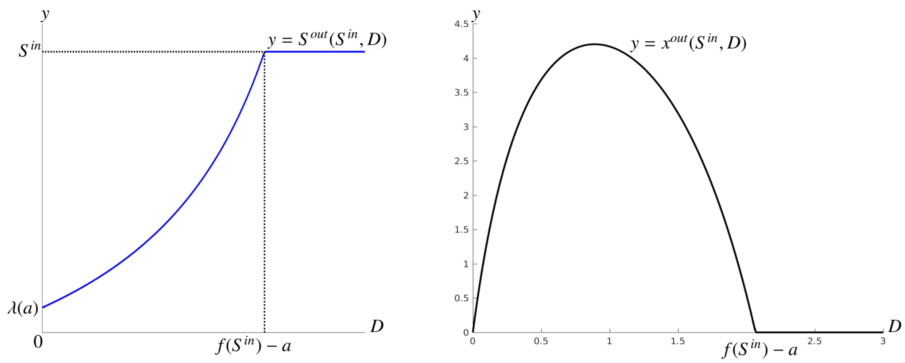
Using definitions (A.5) and (A.6) respectively of S^{out} and x^{out}, for all $S^{in} > \lambda(a)$, the biogas flow rate of the single chemostat is given by:

$$G_{chem}(S^{in}, D) = \begin{cases} 0 & \text{if} \quad D \geq f(S^{in}) - a \\ VD(S^{in} - \lambda(D + a)) & \text{if} \quad D < f(S^{in}) - a. \end{cases} \tag{A.8}$$

For a given $S^{in} > 0$, the function $D \mapsto G_{chem}(S^{in}, D)$ is null for $D = 0$ or $D \geq f(S^{in}) - a$, and is positive for $D \in (0, f(S^{in}) - a)$. Therefore it admits a maximum on $(0, f(S^{in}) - a)$.

Proposition 11. *Assume that for a given $S^{in} > 0$, the maximum of $D \mapsto G_{chem}(S^{in}, D)$ is unique, and define $D^*(S^{in})$ such that*

$$G_{chem}(S^{in}, D^*(S^{in})) = \max_{D \geq 0} G_{chem}(S^{in}, D).$$



(a) (b)

FIGURE 16. (a): The map $D \mapsto S^{out}(S^{in}, D)$. (b): The map $D \mapsto x^{out}(S^{in}, D)$ with $f(S) = 4S/(5+S)$, $S^{in} = 10$ and $a = 0.6$.

Then, the dilution rate $D = D^*(S^{in})$ is the solution of the equation $S^{in} = g(D)$, where the function $g : [0, m-a) \mapsto \mathbb{R}$ is given by the expression

$$g(D) := \lambda(D+a) + \frac{D}{f'(\lambda(D+a))}. \tag{A.9}$$

Proof. For a given $S^{in} > 0$, we have

$$\frac{\partial G_{chem}}{\partial D}(S^{in}, D) = V\left(S^{in} - \lambda(D+a) - \frac{D}{f'(\lambda(D+a))}\right) = V(S^{in} - g(D)),$$

for all $D < f(S^{in}) - a$ where g is defined by (A.9). Therefore, $\frac{\partial G_{chem}}{\partial D}(S^{in}, D) = 0$ is verified if and only if $S^{in} = g(D)$. Thus, $D = D^*(S^{in})$ is the unique solution of $S^{in} = g(D)$. □

Let the curve Γ be defined by

$$\Gamma := \{(S^{in}, D) : S^{in} = g(D)\}. \tag{A.10}$$

For any $S^{in} > \lambda(a)$, the higher biogas flow rate production is given for $(S^{in}, D) \in \Gamma$ where Γ is depicted in Figure 17.

APPENDIX B. PROOF OF THEOREM 1

We begin by the existence of steady states. The steady states are the solutions of the set of equations $\dot{S}_1 = 0$, $\dot{x}_1 = 0$, $\dot{S}_2 = 0$, $\dot{x}_2 = 0$. From equation $\dot{x}_1 = 0$, it is deduced that $x_1 = 0$ or $f(S_1) = D/r + a_1$. Suppose first that $x_1 = 0$. Then, from equation $\dot{S}_1 = 0$ it is deduced that $S_1 = S^{in}$ and from equation $\dot{x}_2 = 0$ it is deduced that $x_2 = 0$ or $f(S_2) = D/(1-r) + a_2$. If $x_2 = 0$, then from equation $S_2 = 0$ it is deduced that $S_2 = S^{in}$. Hence we obtain the equilibrium point $E_0 = (S^{in}, 0, S^{in}, 0)$, which always exist. On the other hand, if $f(S_2) = D/(1-r) + a_2$, then $S_2 = \lambda(D/(1-r) + a_2) = \overline{S}_2$, defined in (2.6). From equation $\dot{S}_2 = 0$, it is deduced that $x_2 = D(S^{in} - \overline{S}_2)/(D + (1-r)a_2) = \overline{x}_2$,defined in (2.6). Hence we obtain the equilibrium point $E_1 = (S^{in}, 0, \overline{S}_2, \overline{x}_2)$. This steady state exists if and only if $S^{in} > \overline{S}_2$, that is $D < (1-r)(f(S^{in}) - a_2)$.

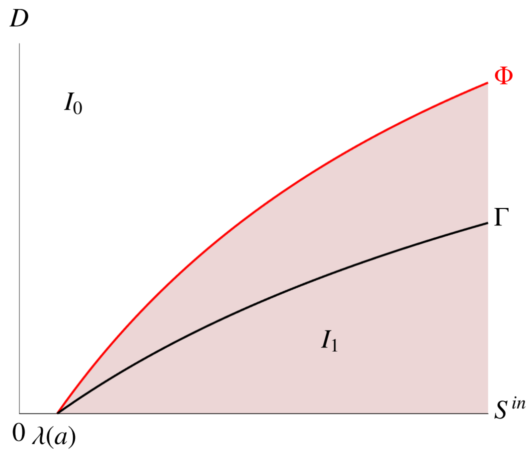


FIGURE 17. The map $D \mapsto g(D)$ in the operating plane (S^{in}, D) of the single chemostat.

Suppose now that $f(S_1) = D/r + a_1$. Then $S_1 = \lambda(D/r + a_1) = S_1^*$, defined in (2.8). From equation $\dot{S}_1 = 0$, it is deduced that $x_1 = (D/(D + ra_1))(S^{in} - S_1^*) = x_1^*$, defined in (2.8). From equation $\dot{S}_2 + \dot{x}_2 = 0$, it is deduced that

$$x_2 = \frac{D}{D + (1 - r)a_2}(S_1^* + x_1^* - S_2). \tag{B.1}$$

Replacing x_2 by this expression in the equation $\dot{S}_2 = 0$, it is deduced that $f(S_2) = h(S_2)$, where h is defined by (2.4). Hence $S_2 = S_2^*$, which is the unique solution of the equation $f(S_2) = h(S_2)$, as shown in Figure 2 (a). Replacing S_2 by S_2^* in (B.1) gives $x_2 = x_2^*$, defined by (2.9). Consequently, we obtain the steady state $E_2 = (S_1^*, x_1^*, S_2^*, x_2^*)$. This steady state is positive if and only if $S^{in} > S_1^*$, which is equivalent to $D < r(f(S^{in}) - a_1)$.

Let us now study the local stability. The Jacobian matrix associated to system (2.1) is given by:

$$J = \begin{pmatrix} A & 0 \\ B & C \end{pmatrix} \quad \text{with} \quad A = \begin{pmatrix} -\frac{D}{r} - f'(S_1)x_1 & -f(S_1) \\ f'(S_1)x_1 & -\frac{D}{r} + f(S_1) - a_1 \end{pmatrix},$$

$$B = \begin{pmatrix} \frac{D}{1-r} & 0 \\ 0 & \frac{D}{1-r} \end{pmatrix} \quad \text{and} \quad C = \begin{pmatrix} -\frac{D}{1-r} - f'(S_2)x_2 & -f(S_2) \\ f'(S_2)x_2 & -\frac{D}{1-r} + f(S_2) - a_2 \end{pmatrix}.$$

J is a lower triangular matrix by blocs and its eigenvalues are the ones of matrices A and C. For E_0, the eigenvalues are $-D/r$, $-D/r + f(S^{in}) - a_1$, $-D/(1-r)$ and $-D/(1-r) + f(S^{in}) - a_2$. They are negative if and only if $D > r(f(S^{in}) - a_1)$ and $D > (1-r)(f(S^{in}) - a_2)$. Therefore, E_0 is LES if and only if $D > \max\{r(f(S^{in}) - a_1), (1-r)(f(S^{in}) - a_2)\}$. For E_1, the eigenvalues of A are $-D/r$ and $-D/r + f(S^{in}) - a_1$. The second eigenvalue is negative if and only if $D > r(f(S^{in}) - a_1)$.

In the following we shall use the notations A_{E_i} and C_{E_i} to indicate the matrices A and C corresponding to the steady state E_i ($i = 0, 1, 2$), and det and tr to indicate respectively the determinant and the trace of the matrices. We have $\det(C_{E_1}) = (D/(1 - r) + a_2) f'(\overline{S}_2)\overline{x}_2$ and $\operatorname{tr}(C_{E_1}) = -D/(1-r) - f'(\overline{S}_2)\overline{x}_2$ which are respectively positive and negative. Therefore, E_1 is LES if and only if $r(f(S^{in}) - a_1) < D < (1 - r)(f(S^{in}) - a_2)$. For E_2, we have

$\det(A_{E_2}) = (D/r + a_1)f'(S_1^*)x_1^*$ and $\mathrm{tr}(A_{E_2}) = -D/r - f'(S_1^*)x_1^*$ which are respectively positive and negative. In addition, we have $\det(C_{E_2}) = (-D/(1-r) - f'(S_2^*)x_2^*)(-D/(1-r) - a_2 + f(S_2^*)) + f(S_2^*)f'(S_2^*)x_2^*$ and $\mathrm{tr}(C_{E_2}) = -2D/(1-r) - a_2 - f'(S_2^*)x_2^* + f(S_2^*)$. Note that $h(S_2) < D/(1-r) + a_2$ for all $S_2 \in (0, S_1^*)$. Therefore, from (2.4), we have $f(S_2^*) = h(S_2^*) < D/(1-r) + a_2$. Consequently, $\det(C_{E_2})$ and $\mathrm{tr}(C_{E_2})$ are respectively positive and negative. Therefore, E_2 is LES whenever it exists, that is $D < r(f(S^{in}) - a_1)$.

For the study of the global stability we use the cascade structure of the system (2.1) and Thieme's Theorem (see Theorem A1.9 of [13]). In the rest of the proof, we denote by $(S_1(t), x_1(t), S_2(t), x_2(t))$ the solution of (2.1) with the initial condition $(S_1^0, x_1^0, S_2^0, x_2^0)$. Then, $(S_1(t), x_1(t))$ is the solution of system

$$\begin{array}{lll} \dot{S}_1 & = & \frac{D}{r}(S^{in} - S_1) - f(S_1)x_1 \\ \dot{x}_1 & = & -\frac{D}{r}x_1 + f(S_1)x_1 - a_1x_1 \end{array} \tag{B.2}$$

with the initial condition (S_1^0, x_1^0) and $(S_2(t), x_2(t))$ is the solution of the non-autonomous system of differential equations

$$\begin{array}{lll} \dot{S}_2 & = & \frac{D}{1-r}(S_1(t) - S_2) - f(S_2)x_2 \\ \dot{x}_2 & = & \frac{D}{1-r}(x_1(t) - x_2) + f(S_2)x_2 - a_2x_2 \end{array} \tag{B.3}$$

with the initial condition (S_2^0, x_2^0). The system (B.2) is the classical model of a single chemostat. Its asymptotic behaviour is well known (see, for instance, Proposition 2.2 of [13]). This system admits the steady states:

$$e_0^1 = \left(S^{in}, 0\right) \quad \text{and} \quad e_1^1 = (S_1^*, x_1^*) \tag{B.4}$$

where S_1^* and x_1^* are defined by (2.8). Two cases must be distinguished.

Firstly, if $\lambda(D/r + a_1) \geq S^{in}$, that is $D \geq r(f(S^{in}) - a_1)$ then, e_0^1, defined in (B.4), is GAS for (B.2) in the nonnegative quadrant. Hence, for any non-negative initial condition (S_1^0, x_1^0),

$$\lim_{t\to+\infty}(S_1(t), x_1(t)) = (S^{in}, 0). \tag{B.5}$$

Therefore, the system (B.3) is asymptotically autonomous with the limiting system

$$\begin{array}{lll} \dot{S}_2 & = & \frac{D}{1-r}(S^{in} - S_2) - f(S_2)x_2 \\ \dot{x}_2 & = & -\frac{D}{1-r}x_2 + f(S_2)x_2 - a_2x_2. \end{array} \tag{B.6}$$

Recall that the solutions of (B.3) are positively bounded. Therefore, we shall use Thieme's results which apply for bounded solutions.

The system (B.6) represents the classical model of a single chemostat. It admits the two steady states $e_0^2 = (S^{in}, 0)$ and $e_1^2 = (\overline{S}_2, \overline{x}_2)$, with $(\overline{S}_2, \overline{x}_2)$ defined by (2.6). Two subcases must be distinguished

- If $\lambda(D/(1-r) + a_2) \geq S^{in}$, that is $D \geq (1-r)(f(S^{in}) - a_2)$ then, e_0^2 is GAS in the nonnegative quadrant. Using Thieme's Theorem, we deduce that for any nonnegative (S_2^0, x_2^0), the solution $(S_2(t), x_2(t))$ of (B.3) converges towards $e_0^2 = (S^{in}, 0)$. Using (B.5) we deduce that, when $D \geq \max(r(f(S^{in})-a_1), (1-r)(f(S^{in})-a_2))$, the solution $(S_1(t), x_1(t), S_2(t), x_2(t))$ of (2.1) converges towards $E_0 = (S^{in}, 0, S^{in}, 0)$, which proves (2.5).
- In contrast, if $\lambda(D/(1-r) + a_2) < S^{in}$, that is $D < (1-r)(f(S^{in}) - a_2)$ then, both steady states e_0^2 and e_1^2 exist and e_1^2 is GAS in the positive quadrant. Although system (B.3) has the saddle point e_0^2, no polycyle can exist. Using Thieme's Theorem, for any positive (S_2^0, x_2^0), the solution $(S_2(t), x_2(t))$ of (B.3) converges towards $e_1^2 = (\overline{S}_2, \overline{x}_2)$. Using (B.5) we deduce that, if $r(f(S^{in}) - a_1) \leq D$ and

$D < (1-r)(f(S^{in}) - a_2)$, then the solution $(S_1(t), x_1(t), S_2(t), x_2(t))$ of (2.1) converges towards $E_1 = \left(S^{in}, 0, \overline{S}_2, \overline{x}_2\right)$, which proves (2.7).

Secondly, if $\lambda(D/r + a_1) < S^{in}$, that is $D < r(f(S^{in}) - a_1)$ then, e_1^1, defined in (B.4), is GAS for (B.2) in the positive quadrant. Hence, for any positive initial condition (S_1^0, x_1^0)

$$\lim_{t\to+\infty}(S_1(t), x_1(t)) = (S_1^*, x_1^*)\,. \tag{B.7}$$

Therefore, the system (B.3) is asymptotically autonomous with the limiting system

$$\begin{array}{rcl} \dot{S}_2 &=& \frac{D}{1-r}(S_1^* - S_2) - f(S_2)\,x_2 \\ \dot{x}_2 &=& \frac{D}{1-r}(x_1^* - x_2) + f(S_2)\,x_2 - a_2 x_2. \end{array} \tag{B.8}$$

The system (B.8) represents the classical model of a single chemostat with an input biomass. In this case, there is no washout and the system (B.8) always admits one LES steady state $e_2 = (S_2^*, x_2^*)$ with positive biomass defined by (2.9) and S_2^* the unique solution of $h(S_2) = f(S_2)$.

Let us show that this steady state is GAS for (B.8). Assume that $x_2 > 0$. Consider the change of variable $\xi = \ln(x_2)$. The system (B.8) becomes as

$$\begin{array}{rcl} \dot{S}_2 &=& \frac{D}{1-r}(S_1^* - S_2) - f(S_2)\,e^{\xi} \\ \dot{\xi} &=& \frac{D}{1-r}(x_1^* e^{-\xi} - 1) + f(S_2) - a_2. \end{array} \tag{B.9}$$

The divergence of the vector field

$$\psi(S_2, \xi) = \left[\begin{array}{l} \frac{D}{1-r}(S_1^* - S_2) - f(S_2)\,e^{\xi} \\ \frac{D}{1-r}(x_1^* e^{-\xi} - 1) + f(S_2) - a_2 \end{array}\right]$$

associated to (B.9) is $\operatorname{div}\psi(S_2, \xi) = -\frac{D}{1-r}(1 + x_1^* e^{\xi}) - f'(S_2)e^{\xi}$. It is negative. Thus, using Bendixon-Dulac criterion, system (B.9) cannot have a periodic solution. Hence, system (B.8) has no cycle in the positive quadrant. For any non negative initial condition (S_2^0, x_2^0), the solution of (B.8) is bounded. Hence, the ω-limit set of (S_2^0, x_2^0), denoted $\omega(S_2^0, x_2^0)$, is non-empty and included in the positive quadrant. If $e_2 \notin \omega(S_2^0, x_2^0)$ then, using Poincaré-Bendixon Theorem, $\omega(S_2^0, x_2^0)$ is a limit cycle, but the system does not present any, due to the divergence property. One then deduces $e_2 \in \omega(S_2^0, x_2^0)$ and, as e_2 is LES, then $\omega(S_2^0, x_2^0) = \{e_2\}$. Consequently, e_2 is GAS for (B.8) in the positive quadrant.

Using again Thieme's Theorem, for any positive (S_2^0, x_2^0), the solution $(S_2(t), x_2(t))$ of (B.3) converges towards $e_2 = (S_2^*, x_2^*)$. Using (B.7) we deduce that, if $D < r(f(S^{in}) - a_1)$, then the solution $(S_1(t), x_1(t), S_2(t), x_2(t))$ of (2.1) converges towards $E_2 = (S_1^*, x_1^*, S_2^*, x_2^*)$. This ends the proof of the theorem.

Appendix C. Proof of Proposition 8

S^{in} being fixed, we shall drop the S^{in} dependency in the expressions of S_i^*, x_i^* $(i = 1, 2)$ and G_2. Thus, let us define $G(D, r) := G_2(S^{in}, D, r)$ and $F_i(D, r) := f(S_i^*(D, r))x_i^*(D, r)$ $(i = 1, 2)$ as functions of $D \geq 0$ and $r \in \mathcal{V}_1 \cap \{r < 1\}$. Remark from the expression of F_1, that it is well defined as well as its partial derivatives at $r = 1$. In addition, for the limiting case $r = 1$, using Lemma 3, for all $D \geq 0$, one has

$$S_2^*(D,1) = S_1^*(D,1) = \lambda(D+a) \text{ and } x_2^*(D,1) = x_1^*(D,1) = \frac{D}{D+a}(S^{in} - \lambda(D+a)). \tag{C.1}$$

Thus, one has

$$F_1(D,1) = F_2(D,1), \qquad \text{for all } D \geq 0 \tag{C.2}$$

and F_2 is also well defined for $r = 1$. Thus, according to (4.23), one has

$$G(D,r) = rF_1(D,r) + (1-r)F_2(D,r), \quad \text{for all } D \geq 0 \text{ and } r \in \mathcal{V}_1 \cap \{r \leq 1\}$$

and from Assumption 4, one has

$$\overline{G}(r) = G(\overline{D}(r), r), \quad r \in \mathcal{V}_1 \cap \{r < 1\} \tag{C.3}$$

with $\overline{G}$ defined (4.24). For convenience, for a function E of (D,r) that is differentiable, we shall define the three following functions.

$$\partial_r E(r) := \frac{\partial E}{\partial r}(\overline{D}(r), r), \quad \partial_D E(r) := \frac{\partial E}{\partial D}(\overline{D}(r), r), \quad \overline{E}(r) := E(\overline{D}(r), r).$$

Therefore, the function $\overline{G}$ writes

$$\overline{G}(r) = r\overline{F}_1(r) + (1-r)\overline{F}_2(r), \quad \text{for all } r \in \mathcal{V}_1 \cap \{r < 1\}. \tag{C.4}$$

As the functions F_i are differentiable and as $\overline{D}(r)$ is a maximizer of $D \mapsto rF_1(D,r) + (1-r)F_2(D,r)$ on the interior of the interval $[0, f(S^{in}) - a]$, one has

$$r\partial_D F_1(r) + (1-r)\partial_D F_2(r) = 0, \quad \text{for all } r \in \mathcal{V}_1 \cap \{r < 1\}, \tag{C.5}$$

and $\partial_D F_1(1) = 0$. As f is C^2 and $\overline{D}$ is assumed to be differentiable on $\mathcal{V}_1 \cap \{r < 1\}$, $\overline{G}$ is differentiable and from (C.4), one has

$$\overline{G}'(r) = \overline{F}_1(r) - \overline{F}_2(r) + r\partial_r F_1(r) + (1-r)\partial_r F_2(r) + (r\partial_D F_1(r) + (1-r)\partial_D F_2(r))\overline{D}'(r)$$

for all $r \in \mathcal{V}_1 \cap \{r < 1\}$, and with (C.5) one has simply

$$\overline{G}'(r) = \overline{F}_1(r) - \overline{F}_2(r) + r\partial_r F_1(r) + (1-r)\partial_r F_2(r), \quad \text{for all } r \in \mathcal{V}_1 \cap \{r < 1\}. \tag{C.6}$$

Let us now determine the limits of the terms of the right side of this last equality when r tends to 1. Firstly, according to (C.2), one has in particular

$$\overline{F}_1(1) = \overline{F}_2(1). \tag{C.7}$$

Secondly, remark that the dynamics of the first tank is parameterized by the single dilution rate $D_1 = D/r$, the other parameters being fixed (see the expression (2.8)). The function F_1 takes then the form $F_1(D,r) = \tilde{F}_1(D/r)$ where $\tilde{F}_1$ is a smooth function. Therefore, one has

$$\partial_D F_1(r) = -\frac{r}{\overline{D}(r)}\partial_r F_1(r). \tag{C.8}$$

As $\partial_D F_1(1) = 0$ then one deduces

$$\partial_r F_1(1) = 0. \tag{C.9}$$

Finally, from $\dot{S}_2 = 0$, one gets

$$F_2(D,r) = \frac{D}{1-r}(S_1^*(D,r) - S_2^*(D,r)), \quad \text{for all } r \in \mathcal{V}_1 \cap \{r < 1\}. \tag{C.10}$$

Differentiating (C.10) with respect to r gives

$$\frac{\partial F_2}{\partial r}(D,r) = \frac{D}{1-r}\left(\frac{\partial S_1^*}{\partial r}(D,r) - \frac{\partial S_2^*}{\partial r}(D,r)\right) + \frac{D}{(1-r)^2}(S_1^*(D,r) - S_2^*(D,r))$$

which can be written equivalently as

$$(1-r)\frac{\partial F_2}{\partial r}(D,r) = D\left(\frac{\partial S_1^*}{\partial r}(D,r) - \frac{\partial S_2^*}{\partial r}(D,r)\right) + F_2(D,r).$$

Thus, for $D = \overline{D}(r)$, one has

$$(1-r)\partial_r F_2(r) = \overline{D}(r)(\partial_r S_1^*(r) - \partial_r S_2^*(r)) + \overline{F}_2(r).$$

Notice that for $D = \overline{D}(r)$, (C.10) gives

$$\overline{F}_2(r) = \frac{\overline{D}(r)}{1-r}(\overline{S}_1^*(r) - \overline{S}_2^*(r)), \quad \text{for all } r \in \mathcal{V}_1 \cup \{r < 1\}. \tag{C.11}$$

Using L'Hôspital's rule in (C.11) when r tends to 1, one gets

$$\overline{F}_2(1) = \lim_{r\to 1^-} \frac{\overline{D}'(r)(\overline{S}_1^*(r) - \overline{S}_2^*(r)) + \overline{D}(r)(\partial_r S_1^*(r) - \partial_r S_2^*(r))}{-1}$$

and using (C.1) and (C.7), one obtains

$$\overline{F}_1(1) = \lim_{r\to 1^-} -\overline{D}(r)(\partial_r S_1^*(r) - \partial_r S_2^*(r)).$$

Consequently, one has

$$\lim_{r\to 1^-} (1-r)\partial_r F_2(r) = 0. \tag{C.12}$$

With (C.7), (C.9) and (C.12), expression (C.6) gives the existence of the limit of $\overline{G}'$ when r tends to 1 with $r < 1$, which is

$$\overline{G}'(1^-) = 0. \tag{C.13}$$

Note that $\overline{G}''(1^-)$ exists if and only if $\lim_{r\to 1^-} \frac{\overline{G}'(r)-\overline{G}'(1)}{r-1}$ exists. Using (C.13) and (C.6), one has

$$\frac{\overline{G}'(r) - \overline{G}'(1^-)}{r-1} = -\frac{\overline{G}'(r)}{1-r} = -\frac{\overline{F}_1(r) - \overline{F}_2(r) + r\partial_r F_1(r) + (1-r)\partial_r F_2(r)}{1-r}. \tag{C.14}$$

On the one hand, using L'Hôspital's rule, one has

$$\lim_{r\to 1^-} \frac{\overline{F}_1(r) - \overline{F}_2(r)}{1-r} = \lim_{r\to 1^-} \frac{\overline{F}_1'(r) - \overline{F}_2'(r)}{-1}.$$

Recall that $\partial_r F_1(1) = 0$ and thus one has $\overline{F}_1'(1) = 0$. Consequently, one has

$$\lim_{r\to 1^-} \frac{\overline{F}_1(r) - \overline{F}_2(r)}{1-r} = \lim_{r\to 1^-} \overline{F}_2'(r) = \lim_{r\to 1^-} \partial_r F_2(r) + \partial_D F_2(r)\overline{D}'(r). \tag{C.15}$$

On the other hand, using (C.5) and (C.8), one has

$$\frac{r}{1-r}\partial_r F_1(r) = \frac{\overline{D}(r)}{r}\partial_D F_2(r). \tag{C.16}$$

Thus, according to (C.14), (C.15) and (C.16), one gets

$$\lim_{r\to 1^-} \frac{\overline{G}'(r) - \overline{G}'(1^-)}{r-1} = \lim_{r\to 1^-} -2\partial_r F_2(r) - \left(\frac{\overline{D}(r)}{r} + \overline{D}'(r)\right)\partial_D F_2(r). \tag{C.17}$$

Let us show now that the limit of $\partial_D F_2(r)$ tends to 0 when r tends to 1. One has

$$\frac{\partial F_2}{\partial D} = f'(S_2^*)\frac{\partial S_2^*}{\partial D}x_2^* + f(S_2^*)\frac{\partial x_2^*}{\partial D}.$$

Let use the expression $G(D,r) = D(S^{in} - S_2^*(D,r))$ given by Proposition 6. As $\overline{D}(r)$ is a maximizer then one has

$$\partial_D G(r) = S^{in} - \overline{S}_2^*(r) - \overline{D}(r)\partial_D S_2^*(r) = 0.$$

Using (C.1), one then deduces

$$\partial_D S_2^*(1^-) = \frac{S^{in} - \lambda\left(\overline{D}(1) + a\right)}{\overline{D}(1)}.$$

In addition, using expressions (2.9) and (C.1), one gets

$$\partial_D x_2^*(1^-) = -\frac{\overline{D}(1)}{\left(\overline{D}(1) + a\right)^2}\left(S^{in} - \lambda\left(\overline{D}(1) + a\right)\right),$$

and hence the existence of the limit of $\partial_D F_2$ when r tends to 1:

$$\partial_D F_2(1^-) = \frac{S^{in} - \lambda\left(\overline{D}(1) + a\right)}{\overline{D}(1) + a} f'\left(\lambda\left(\overline{D}(1) + a\right)\right)\left(S^{in} - \lambda\left(\overline{D}(1) + a\right) - \frac{\overline{D}(1)}{f'\left(\lambda\left(\overline{D}(1) + a\right)\right)}\right).$$

This is equivalent to

$$\partial_D F_2(1^-) = \frac{S^{in} - \lambda\left(\overline{D}(1) + a\right)}{\overline{D}(1) + a} f'\left(\lambda\left(\overline{D}(r) + a\right)\right)\left(S^{in} - g\left(\overline{D}(1)\right)\right),$$

with g defined by (A.9). According to Proposition 11, one has $S^{in} - g\left(\overline{D}(1)\right) = 0$. Consequently, one has $\partial_D F_2(1^-) = 0$.

Finally, it remains to calculate the limit of $\partial_r F_2(r)$ when r tends to 1. One has

$$\frac{\partial F_2}{\partial r} = f'(S_2^*)\frac{\partial S_2^*}{\partial r}x_2^* + f(S_2^*)\frac{\partial x_2^*}{\partial r}.$$

Let use again the expression $G(D, r) = D(S^{in} - S_2^*(D, r))$. According to (C.4), one has

$$\overline{G}'(r) = \partial_r G(r) + \partial_D G(r)\overline{D}'(r)$$

where $\partial_D G(r) = 0$. According to (C.13), we deduce $\partial_r G(1^-) = 0$, and thus $\partial_r S_2^*(1^-) = 0$. Using expression (2.9), one gets

$$\partial_r x_2^*(1^-) = -a\overline{D}(1)\frac{S^{in} - \lambda\left(\overline{D}(1) + a\right)}{\left(\overline{D}(1) + a\right)^2},$$

and then the existence of the limit of $\partial_r F_2$ when r tends to 1:

$$\partial_r F_2(1^-) = -a\overline{D}(1)\frac{S^{in} - \lambda\left(\overline{D}(1) + a\right)}{\overline{D}(1) + a}.$$

As $\overline{D}'$ is assumed to be bounded on $\mathcal{V}_1 \cup \{r < 1\}$, we thus obtain from (C.17) the existence of $\overline{G}''(1^-)$ with

$$\overline{G}''(1^-) = -2\partial_r F_2(1^-)$$

which is given by expression (4.25).

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