Performance Study of Two Serial Interconnected Chemostats with Mortality

Manel Dali-Youcef¹,³ · Alain Rapaport¹ · Tewfik Sari²

Received: 22 March 2022 / Accepted: 11 August 2022
© The Author(s), under exclusive licence to Society for Mathematical Biology 2022

Abstract
The present work considers the model of two chemostats in series when a biomass mortality is considered in each vessel. We study the performance of the serial configuration for two different criteria which are the output substrate concentration and the biogas flow rate production, at steady state. A comparison is made with a single chemostat with the same total volume. Our techniques apply for a large class of growth functions and allow us to retrieve known results obtained when the mortality is not included in the model and the results obtained for specific growth functions in both the mathematical literature and the biological literature. In particular, we provide a complete characterization of operating conditions under which the serial configuration is more efficient than the single chemostat, i.e., the output substrate concentration of the serial configuration is smaller than that of the single chemostat or, equivalently, the biogas flow rate of the serial configuration is larger than that of the single chemostat. The study shows that the maximum biogas flow rate, relative to the dilution rate, of the series device is higher than that of the single chemostat provided that the volume of the first tank is large enough. This non-intuitive property does not occur for the model without mortality.

Keywords Chemostat · Gradostat · Mortality · Bifurcations · Global stability · Operating diagram · Biogas production

Mathematics Subject Classification 34D20 · 34H20 · 65K10 · 92C75

Tewfik Sari
tewfik.sari@inrae.fr
Manel Dali-Youcef
daliyoucef.manel@gmail.com
Alain Rapaport
alain.rapaport@inrae.fr

¹ MISTEA, INRAE, Institut Agro, Univ Montpellier, Montpellier, France
² ITAP, INRAE, Institut Agro, Univ Montpellier, Montpellier, France
³ Laboratoire de Mathématiques, Université d’Avignon, Avignon, France

Published online: 28 August 2022


1 Introduction

The mathematical model of the chemostat has received a great attention in the literature for many years [see for instance Harmand et al. (2017) 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 (Fogler 2008; Levenspiel 1999).

Several extensions of the original model of the chemostat, considering spatial heterogeneity, have been proposed to better cope reality (see for instance Kung and Baltzis (1992)). Lovitt and Wimpenny have proposed the "gradostat" experimental device as a collection of chemostats of same volume interconnected in series (Lovitt and Wimpenny 1979, 1981), which has led to the so-called gradostat model representing in a more general framework a gradient of concentrations (Smith 1991; Tang 1986). The gradostat model has been further generalized as the "general gradostat model" representing more general interconnection graphs with tanks of different volumes (Smith et al. 1991; Smith and Waltman 1995).

Efficient use of a chemostat in practice relies on the analysis of its performance. The performance is considered for different criteria studied in the literature (Sari 2022), among which the most common are: the output substrate concentration, the residence time, the biogas flow rate and the biomass productivity. Particular interconnection structures have been investigated and compared for the properties in terms of input-output performances [see for instance Crespo and Rapaport (2020); Dali-Youcef et al. (2020); Haidar et al. (2011); Rapaport et al. (2015)]. 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 (de Gooijer et al. 1996; Harmand et al. 1999; Luyben and Tramper 1982; Nelson and Sidhu 2006; Zambrano et al. 2015).

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 (Li 1998; Rapaport and Harmand 2008; Sari and Mazenc 2011; Wolkowicz and Lu 1992). 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 chemostats 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 Pavlou (1999) for its importance for bioreactors. Indeed, such diagrams are more and more often constructed both in the biological literature (Pavlou 1999; Sbarciog et al. 2010; Wade et al. 2016; Xu et al. 2011) and the mathematical literature (Abdellatif et al. 2016; Bar and Sari 2020; Bornhöft et al. 2013; Daoud et al. 2018; Dellal et al. 2018; Fekih-Salem et al. 2021, 2017; Khedim et al. 2018; Sari 2022; Sari and Benyahia 2021; Sari and Harmand 2016; Sari and Wade 2017; Weedermann et al. 2013, 2015).

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.

Along the paper, we use the abbreviations LES for locally exponentially stable and GAS for globally asymptotically stable in the positive orthant.

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. Afterward, Sect. 3 focuses on the study of performances of the serial configuration with respect of the output substrate concentration. Then, Sect. 4 considers the performances of the serial configuration with respect of the biogas production. Next, Sect. 5 is devoted to illustrations and numerical simulations and a conclusion is given in Sect. 6. Moreover, we set up the single chemostat with mortality in Appendix A, while Appendix B is devoted to the existence and stability analysis of the steady states of the serial chemostat and Appendix C to its operating diagram. These results are extension of former results, in the case without mortality (Dali-Yousef et al. 2020), but that have required to revisit significantly the mathematical proofs. Finally, Appendix D contains technical proofs.

2 Presentation of the Model

We consider two serial interconnected chemostats where the total volume $V$ is divided into $V_1 = rV$ and $V_2 = (1 - r)V$, with $r \in (0, 1)$, as shown in Fig. 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 $S^{\text{in}}$, the flow rate is constant and is designated by $Q$. The output substrate concentration is the concentration of substrate in the second tank $S^{\text{out}} = S_2$.

The mathematical model is given by the following equations:

\begin{align}
\dot{S}_1 &= \frac{D}{r} (S^{\text{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{align}

(1)
Fig. 1 The serial configuration of two chemostats, respectively, of volumes \( rV \) and \((1-r)V \) (Color figure online)

where \( \dot{S}_i = \frac{dS_i}{dt} \), \( \dot{x}_i = \frac{dx_i}{dt} \), \( i = 1, 2 \), \( f \) is the growth function such that \( f(S_i) \) is the growth function of the substrate in the tank \( i = 1, 2 \), \( a \) is the mortality rate of the biomass and \( D = Q/V \) is the dilution rate of the whole structure. The dilution rate of the first tank is \( Q/V_1 = D/r \). The dilution rate of the second tank is \( Q/V_2 = D/(1-r) \).

Note that these equations are not valid for \( r = 0 \) and \( r = 1 \), which correspond to a single chemostat. For sake of completeness, the useful results on the single chemostat are given in Appendix A. 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

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

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

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

The particular case without mortality of the biomass (\( a = 0 \)) is studied in Dali-Youcef et al. (2020). The results on the existence and stability of steady states of system (1) are very similar to the case without mortality. The details are given in Appendix B. The system can have up to three steady states:

- The washout steady state \( E_0 = (S^{\text{in}}, 0, S^{\text{in}}, 0) \).
- The steady state \( E_1 = (S^{\text{in}}, 0, S_2^*, x_2^*) \) of washout in the first chemostat but not in the second one.
- The steady state \( E_2 = (S_1^*, x_1^*, S_2^*, x_2^*) \) of persistence of the species in both chemostats.

As in the case without mortality, see Table 3 in the Appendix, for any operating condition \((S^{\text{in}}, D)\), one and only one of the steady-states \( E_0, E_1 \) and \( E_2 \), is stable. It is then globally asymptotically stable (GAS).

The operating diagram of the system is described in Appendix C. The operating diagram has as coordinates the input substrate concentration \( S^{\text{in}} \) and the dilution rate.
Fig. 2 The operating diagram of system (1) and the curve $\Gamma_r$ defined by (14) under which the serial configuration is more efficient than the single chemostat (Color figure online)

$D$, 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. The operating diagram of system (1) is depicted in Fig. 2.

The aim of this work is to establish a comparison of the performance of the serial configuration with ones of the single chemostat. In the following, we compare both structures according to two different criteria; the output substrate concentration and the biogas flow rate.

### 3 Output Substrate Concentration

The output substrate concentration measures the biodegradation of the input substrate by the overall device. The reduction of the output substrate concentration is one of the main objectives of the biological wastewater treatment, and its minimization is often addressed in the literature, see, for example, Zambrano and Carlsson (2014). We assume that the serial configuration is functioning at a stable steady state. The output substrate concentration at steady state depends on the parameters $D$, $S^\text{in}$ and $r$, and will be denoted $S_r^\text{out}(S^\text{in}, D)$.

**Proposition 1** Assume that Assumption 1 is satisfied. The output substrate concentration at steady state of system (1) is given by

$$S_r^\text{out}(S^\text{in}, D) = \begin{cases} S^\text{in} & \text{if } S^\text{in} \leq \min \left( \lambda \left( \frac{D}{1-r} + a \right), \lambda \left( \frac{D}{r} + a \right) \right), \\ \overline{S}_2 & \text{if } \lambda \left( \frac{D}{1-r} + a \right) \leq S^\text{in} \leq \lambda \left( \frac{D}{r} + a \right), \\ S^*_2 & \text{if } S^\text{in} > \lambda \left( \frac{D}{r} + a \right), \end{cases}$$

(4)
where $S_2 = \lambda \left( D_1 - r + a \right)$ and $S_2^*$ is the unique solution of equation $h(S_2) = f(S_2)$. In this equation, the function $h$ is defined by:

\[ h(S_2) = \frac{D + (1 - r)a}{1 - r} \frac{S_1^* - S_2}{b - S_2}, \quad (5) \]

where $S_1^* = \lambda \left( D + a \right)$ and $b = \frac{D(S_1^* - S_2^*)}{D + ra} + S_1^*$.

**Proof** The output substrate concentration at steady state of system $(1)$ is equal to $S_{\text{in}}$, if $E_0$ is the GAS steady state. It is equal to $S_2$ if $E_1$ is the GAS steady state and to $S_2^*$ if $E_2$ is GAS. According to Theorem 3 in Appendix, $E_0$ is GAS if and only if

\[ D \geq \max\left( r, 1 - r \right) \left( f(S_{\text{in}}) - a \right), \]

which is equivalent to

\[ S_{\text{in}} \leq \min\left( \lambda \left( D + a \right), \lambda \left( D + a \right) \right). \]

On the other hand, using Theorem 3, $S_2$ depends on $D$ and $r$ and we have $S_2 = \lambda \left( D + a \right)$. $E_1$ is GAS if and only if

\[ r \left( f(S_{\text{in}}) - a \right) \leq D \leq (1 - r) \left( f(S_{\text{in}}) - a \right), \]

which is equivalent to

\[ \lambda \left( D + a \right) \leq S_{\text{in}} \leq \lambda \left( D + a \right). \]

Finally, using Theorem 3, we know that $S_2^*$ depends on parameters $S_{\text{in}}$, $D$, $r$. It is the unique solution of equation $h(S_2) = f(S_2)$, where $h$ is defined by $(5)$. On the other hand $E_2$ is GAS if and only if the condition $D < r \left( f(S_{\text{in}}) - a \right)$ is satisfied, which is equivalent to the condition $S_{\text{in}} > \lambda \left( D + a \right)$. \hfill \Box

Although $S_{\text{out}}(S_{\text{in}}, D)$ is defined only for $0 < r < 1$, we can extend it, by continuity, for $r = 0$ and $r = 1$ by

\[ S_{\text{out}}^0(S_{\text{in}}, D) = S_{\text{out}}^1(S_{\text{in}}, D) = S_{\text{out}}(S_{\text{in}}, D). \quad (6) \]

where $S_{\text{out}}(S_{\text{in}}, D)$, which is the output substrate concentration of the single chemostat, is given by

\[ S_{\text{out}}(S_{\text{in}}, D) = \left\{ \begin{array}{ll} S_{\text{in}} & \text{if } S_{\text{in}} \leq \lambda(D + a), \\ \lambda(D + a) & \text{if } S_{\text{in}} > \lambda(D + a). \end{array} \right. \quad (7) \]

For more information on $S_{\text{out}}(S_{\text{in}}, D)$, see Appendix A.
The proof of (6), comes from the following remarks. First, we have \( S_2^2(D, 0) = \lambda(D + a) \) and second, according to Lemma 9 in the Appendix, we can extend \( S_2^2(S^\text{in}, D, r) \), by continuity, to \( r = 1 \), by

\[
S_2^2(S^\text{in}, D, 1) = \lambda(D + a).
\]

Our aim in this section is to compare \( S^\text{out}_r \) defined by (4) and (6) and \( S^\text{out} \) defined by (7).

### 3.1 The Serial Configuration can be More Efficient Than the Single Chemostat

We fix \( r \) and we describe the set of operating conditions \((S^\text{in}, D)\) for which

\[
S^\text{out}_r(S^\text{in}, D) < S^\text{out}(S^\text{in}, D),
\]

that is to say, the serial configuration with volumes \( rV \) and \((1 - r)V\), is more efficient than the single chemostat of volume \( V \). For \( r \in (0, 1) \), let \( g_r : [0, r(m - a)) \mapsto \mathbb{R} \) defined by

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

**Lemma 1** For \( r \in (0, 1) \) we have \( g_r(D) > \lambda \left( \frac{D}{r} + a \right) \).

**Proof** As \( 0 < r < 1 \) and \( \lambda \) is an increasing function then, we have \( \lambda(D/r + a) > \lambda(D + a) \). Using (9), we have \( g_r(D) > \lambda(D/r + a) \).

**Theorem 1** Assume that Assumption 1 is satisfied. For any \( r \in (0, 1) \), we have

\[
S^\text{out}_r(S^\text{in}, D) = S^\text{out}(S^\text{in}, D) \iff S^\text{in} = g_r(D).
\]

Moreover,

\[
S^\text{out}_r(S^\text{in}, D) < S^\text{out}(S^\text{in}, D) \iff S^\text{in} > g_r(D).
\]

**Proof** Recall that \( S_2^2(S^\text{in}, D, r) \) is the unique solution of equation \( f(S_2) = h(S_2) \) with \( h \) defined by (5). Let us first prove that

\[
S_2^2(S^\text{in}, D, r) < \lambda(D + a) \iff S^\text{in} > g_r(D).
\]

Since \( f \) is increasing, see Assumption 1, and \( h \) is decreasing, see Lemma 8 in Appendix, then the condition \( S_2^2(S^\text{in}, D, r) < \lambda(D + a) \) is equivalent to the condition \( h(\lambda(D + a)) < f(\lambda(D + a)) = D + a \). Using (5), a straightforward computation shows that the condition \( h(\lambda(D + a)) < D + a \) is equivalent to \( S^\text{in} > g_r(D) \), where \( g_r \) is defined by (9). This proves (10).
Let us go now to the proof of the theorem. Assume that \( S_{\text{in}} > g_{r}(D) \). Using Lemma 1, we have

\[
S_{\text{in}} > \lambda(D/r + a) > \lambda(D + a).
\]

Using (4) and (7), we have

\[
\begin{align*}
S_{\text{out}}^r(S_{\text{in}}, D) &= S_{\text{in}}^r(S_{\text{in}}, D, r), \\
S_{\text{out}}(S_{\text{in}}, D) &= \lambda(D + a).
\end{align*}
\]

From (10), we have \( S_{\text{out}}^r(S_{\text{in}}, D) < S_{\text{out}}(S_{\text{in}}, D) \). Hence, we proved the following implication

\[
S_{\text{in}} > g_{r}(D) \implies S_{\text{out}}^r(S_{\text{in}}, D) < S_{\text{out}}(S_{\text{in}}, D).
\]

Assume now that \( S_{\text{in}} \leq g_{r}(D) \). When \( r < 1/2 \), three cases must be distinguished. First, if

\[
\lambda(D + a) < \lambda\left(\frac{D}{r} + a\right) < S_{\text{in}} \leq g_{r}(D),
\]

then, by (4) and (7), we obtain (11). Hence, using (10), we have \( S_{\text{out}}^r(S_{\text{in}}, D) \geq S_{\text{out}}(S_{\text{in}}, D) \). Secondly, if

\[
\lambda(D + a) < \lambda\left(\frac{D}{1-r} + a\right) \leq S_{\text{in}} \leq \lambda\left(\frac{D}{r} + a\right),
\]

then, by (4) and (7), we have

\[
\begin{align*}
S_{\text{out}}^r(S_{\text{in}}, D) &= \lambda\left(\frac{D}{1-r} + a\right), \\
S_{\text{out}}(S_{\text{in}}, D) &= \lambda(D + a).
\end{align*}
\]

Therefore, we have \( S_{\text{out}}^r(S_{\text{in}}, D) > S_{\text{out}}(S_{\text{in}}, D) \). Finally, if \( S_{\text{in}} \leq \lambda(D + a) \), then

\[
S_{\text{out}}^r(S_{\text{in}}, D) = S_{\text{out}}(S_{\text{in}}, D) = S_{\text{in}}.
\]

When \( r \geq 1/2 \), the proof is similar, excepted that we must distinguish only two cases, \( \lambda(D + a) < S_{\text{in}} \leq \lambda(D/r + a) \) and \( S_{\text{in}} \leq \lambda(D + a) \). Hence, we have proved the reciprocal implication of (12). This completes the proof of second equivalence in the theorem.

The same calculations show the equivalence if inequalities are replaced by equalities. \( \Box \)

Theorem 1 asserts that the serial configuration is more efficient than the single chemostat if and only if \( S_{\text{in}} > g_{r}(D) \). Let us illustrate this result in the operating diagram of system (1). Consider the curve of equation

\[
\Phi_{r} = \left\{(S_{\text{in}}, D) : S_{\text{in}} = \lambda(D/r + a)\right\}.
\]
According to the results given in Appendix C, the curves $\Phi_r$ and $\Phi_{1-r}$ defined by (13) separate the operating plane $(S^{\text{in}}, D)$ in four regions in which the system has different asymptotic behavior, see Table 3. To put it simply, in the $I_0(r)$ region, $E_0$ is GAS, in $I_1(r)$, $E_1$ is GAS, and in $I_2(r) \cup I_3(r)$, $E_3$ is GAS, see Fig. 2. This figure also shows the plot of the curve $\Gamma_r$, defined by

$$\Gamma_r := \left\{(S^{\text{in}}, D) : S^{\text{in}} = g_r(D)\right\}. \quad (14)$$

Using Lemma 1, we see that for all $r \in (0, 1)$, the curve $\Gamma_r$ is always at right of the curve $\Phi_r$. According to Theorem 1, the output substrate concentration of the serial configuration is smaller than the one of the single chemostat, if and only if $(S^{\text{in}}, D)$ is at right of the curve $\Gamma_r$ depicted in Fig. 2.

3.2 The Output Substrate Concentration as a Function of the Volume Fraction $r$

In this section we assume that $(S^{\text{in}}, D)$ is fixed and we look at the values of $r$ for which (8) holds. More precisely we are going to describe the function

$$r \mapsto S^{\text{out}}_r(S^{\text{in}}, D). \quad (15)$$

**Proposition 2** Assume that Assumption 1 is satisfied. Let $D > 0$, $S^{\text{in}} > \lambda(a)$. We denote $r_0 = D/(f(S^{\text{in}}) - a)$.

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

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

$$S^{\text{out}}_r(S^{\text{in}}, D) = \begin{cases} S^2 & \text{if } 0 \leq r \leq 1 - r_0, \\ S^{\text{in}} & \text{if } 1 - r_0 \leq r \leq r_0, \\ \bar{S}^*_2 & \text{if } r_0 \leq r \leq 1. \end{cases} \quad (16)$$

3. If $\lambda(2D+a) \leq S^{\text{in}}$, then $0 < r_0 \leq 1/2$ and one has

$$S^{\text{out}}_r(S^{\text{in}}, D) = \begin{cases} S^2 & \text{if } 0 \leq r \leq r_0, \\ \bar{S}^*_2 & \text{if } r_0 \leq r \leq 1. \end{cases} \quad (17)$$

Here $\bar{S}^2 = \lambda \left(\frac{D}{1-r} + a\right)$ and $S^*_2 = S^*_2(S^{\text{in}}, D, r)$ is the unique solution of equation $f(S^2) = h(S^2)$, where $h$ is defined by (5).

**Proof** If $S^{\text{in}} \leq \lambda(D+a)$, then, for all $r \in (0, 1)$, one has

$$S^{\text{in}} \leq \lambda(D+a) \leq \min \left\{\lambda \left(\frac{D}{1-r} + a\right), \lambda \left(\frac{D}{r} + a\right)\right\}.$$  

Then, according to (4), one has $S^{\text{out}}_r(S^{\text{in}}, D) = S^{\text{in}}$. This proves item 1 of the proposition.
Let \( r_0 = D/(f(S^{\text{in}}) - a) \), i.e., \( S^{\text{in}} = \lambda(D/r_0 + a) \).

If \( \lambda(D + a) < S^{\text{in}} < \lambda(2D + a) \), then \( r_0 \in (1/2, 1) \), so that \( 1 - r_0 < r_0 \). The interval \([0, 1]\) is subdivided into three sub-intervals. Firstly, if \( 0 \leq r \leq 1 - r_0 < r_0 \), then

\[
\lambda \left( \frac{D}{1-r} + a \right) \leq S^{\text{in}} = \lambda \left( \frac{D}{r_0} + a \right) < \lambda \left( \frac{D}{r} + a \right).
\]

Hence, according to (4), one has

\[
S'_r(S^{\text{in}}, D) = \lambda \left( \frac{D}{1-r} + a \right).
\]

Secondly, if \( 1 - r_0 \leq r \leq r_0 \), then \( r_0 \geq \max\{r, 1 - r\} \), so that

\[
S^{\text{in}} = \lambda \left( \frac{D}{r_0} + a \right) \leq \min \left\{ \lambda \left( \frac{D}{1-r} + a \right), \lambda \left( \frac{D}{r} + a \right) \right\}.
\]

Hence, according to (4), one has \( S'_r(S^{\text{in}}, D) = S^{\text{in}} \). Finally, if \( r_0 < r \leq 1 \), then one has

\[
S^{\text{in}} = \lambda \left( \frac{D}{r_0} + a \right) > \lambda \left( \frac{D}{r} + a \right).
\]

Hence, according to (4), one has

\[
S'_r(S^{\text{in}}, D) = S^*_r(S^{\text{in}}, D, r).
\]

This proves item 2 of the proposition.

If \( \lambda(2D + a) \leq S^{\text{in}} \), then \( r_0 \in (0, 1/2] \). Therefore, \( r_0 \leq 1 - r_0 \). The proof of item 3 of the proposition is the same as the proof of item 2 excepted that now, the interval \([0, 1]\) is subdivided now into two sub-intervals \([0, r_0]\) and \([r_0, 1]\), so that the interval for which \( S'_r(S^{\text{in}}, D) = S^{\text{in}} \) is empty. \( \square \)

We want to determine the values \( r \in (0, 1) \) for which the condition (8) is satisfied. We need the following Assumption that is satisfied by any concave growth function but also by non-concave growth functions, satisfying additional conditions, see Sect. 3.4.

**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 \( D < m - a \). Using \( g_r(D) > \lambda(D/r + a) \), we have

\[
\lim_{r \to D/(m-a)} g_r(D) > \lim_{r \to D/(m-a)} \lambda(D/r + a) = +\infty.
\]

On the other hand, using L’Hôpital’s rule, we have

\[
\lim_{r \to 1} g_r(D) = g(D).
\]
where \( g : [0, m - a) \to \mathbb{R}^+ \) is defined by

\[
g(D) = \lambda(D + a) + D\lambda'(D + a).
\] (19)

Therefore, from Assumption 2, the function \( r \mapsto g_r(D) \) is decreasing from \( (D/(m - a), 1) \) to \( (g(D), +\infty) \). Hence, it admits an inverse function

\[
S^\text{in} \in (g(D), +\infty) \mapsto r_1(S^\text{in}, D) \in (D/(m - a), 1).
\]

We use the notation \( r_1(\cdot, D) \) to recall the dependence of the inverse function in \( D \). For all \( D \in (0, m - a) \), \( r \in (D/(m - a), 1) \) and \( S^\text{in} > g(D) \), we have

\[
r = r_1(S^\text{in}, D) \iff S^\text{in} = g_r(D),
\] (20)

\[
r > r_1(S^\text{in}, D) \iff S^\text{in} > g_r(D).
\] (21)

**Theorem 2** Assume that Assumptions 1 and 2 are satisfied. Let \( g \) defined by (19).

- If \( S^\text{in} \leq g(D) \) then for any \( r \in (0, 1) \), we have \( S^\text{out}_r(S^\text{in}, D) > S^\text{out}(S^\text{in}, D) \). In addition, for \( r = 0 \) and \( r = 1 \) we have \( S^\text{out}_r(S^\text{in}, D) = S^\text{out}(S^\text{in}, D) \).
- If \( S^\text{in} > g(D) \) then \( S^\text{out}_r(S^\text{in}, D) < S^\text{out}(S^\text{in}, D) \) if and only if \( r_1(S^\text{in}, D) < r < 1 \), with \( r_1(S^\text{in}, D) \), defined by (20). In addition, for \( r = 0 \), \( r = r_1(S^\text{in}, D) \) and \( r = 1 \), we have \( S^\text{out}_r(S^\text{in}, D) = S^\text{out}(S^\text{in}, D) \).

**Proof** The function \( r \mapsto g_r(D) \) is decreasing and tends to \( g(D) \) as \( r \) tends to 1, as shown by (18). Thus, for all \( r \in (0, 1) \), we have \( g(D) < g_r(D) \). If \( S^\text{in} \leq g(D) \), then \( S^\text{in} < g_r(D) \). According to Theorem 1, for all \( r \in (0, 1) \), we have \( S^\text{out}_r(S^\text{in}, D) > S^\text{out}(S^\text{in}, D) \).

Let \( S^\text{in} > g(D) \). Let \( r_1 = r_1(S^\text{in}, D) \). According to (21), for all \( r > r_1 \), we have \( S^\text{in} > g_r(D) \). Thus, according to Theorem 1, we have \( S^\text{out}_r(S^\text{in}, D) < S^\text{out}(S^\text{in}, D) \).

The equality \( S^\text{out}_r(S^\text{in}, D) = S^\text{out}(S^\text{in}, D) \) is verified for the \( r = 0 \) and \( r = 1 \), see (6). In addition, we have \( S^\text{in} = g_r(D) \), see (20). Hence, according to Theorem 1, we have \( S^\text{out}_r(S^\text{in}, D) = S^\text{out}(S^\text{in}, D) \).

Let us now describe the subsets of the operational space \( (S^\text{in}, D) \) for which the behavior described in the three cases of Proposition 2 occurs. For a complete description we will also distinguish the sub-cases for which there exists \( r_1 = r_1(S^\text{in}, D) \) such that, for \( r_1 < r < 1 \), (8) is satisfied, as shown in Theorem 2. Consider the curves \( \Phi_1 \) and \( \Phi_{1/2} \), defined by (13), and the curve \( \Gamma \) defined by

\[
\Gamma := \{(S^\text{in}, D) : S^\text{in} = g(D)\}.
\] (22)

These three curves intersect at \( (\lambda(a), 0) \) and, using the inequality \( g(D) > \lambda(D + a) \), which is satisfied for all \( D > 0 \), one deduces that \( \Gamma \) is at the right of \( \Phi_1 \). Therefore, the curves \( \Phi_1, \Phi_{1/2} \) and \( \Gamma \) separate the set of operating parameters \( (S^\text{in}, D) \) into the...
following four subsets, see Fig. 3.

\[
\begin{align*}
J_0 &= \{(S^{\text{in}}, D) : S^{\text{in}} \leq \lambda(D + a)\}, \\
J_1 &= \{(S^{\text{in}}, D) : \lambda(D + a) < S^{\text{in}} \leq \min\{g(D), \lambda(2D + a)\}\}, \\
J_2 &= \{(S^{\text{in}}, D) : g(D) < S^{\text{in}} < \lambda(2D + a)\}, \\
J_3 &= \{(S^{\text{in}}, D) : \max\{g(D), \lambda(2D + a)\} \leq S^{\text{in}}\}, \\
J_4 &= \{(S^{\text{in}}, D) : \lambda(2D + a) < S^{\text{in}} < g(D)\}. \\
\end{align*}
\]  

(23)

Combining the results of Proposition 2 and Theorem 2, we find that the function \( r \mapsto S^{\text{out}}_r(S^{\text{in}}, D) \) is as in Fig. 4. In the following we will comment on this figure.

- If \((S^{\text{in}}, D) \in J_1\), then when \( S^{\text{in}} < \lambda(2D + a) \), \( S^{\text{out}}_r(S^{\text{in}}, D) \) is given by (16) and when \( S^{\text{in}} = \lambda(2D + a) \), \( S^{\text{out}}_r(S^{\text{in}}, D) \) is given by (17). In addition, for all \( r \in (0, 1) \), \( S^{\text{out}}_r(S^{\text{in}}, D) > S^{\text{out}}(S^{\text{in}}, D) \). The equality is fulfilled for \( r = 0 \) and \( r = 1 \), see Fig. 4a.
- If \((S^{\text{in}}, D) \in J_2\), then \( S^{\text{out}}_r(S^{\text{in}}, D) \) is given by (16) and \( S^{\text{out}}_r(S^{\text{in}}, D) < S^{\text{out}}(S^{\text{in}}, D) \) if and only if \( r \in (r_1(S^{\text{in}}, D), 1) \), where \( r_1(S^{\text{in}}, D) \) is defined by (20). The equality is fulfilled for \( r = 0, r = r_1(S^{\text{in}}, D) \) and \( r = 1 \), see Fig. 4b.
- If \((S^{\text{in}}, D) \in J_3\) then \( S^{\text{out}}_r(S^{\text{in}}, D) \) is given by (17) and \( S^{\text{out}}_r(S^{\text{in}}, D) < S^{\text{out}}(S^{\text{in}}, D) \) if and only if \( S^{\text{in}} > g(D) \) and \( r \in (r_1(S^{\text{in}}, D), 1) \) where \( r = r_1(S^{\text{in}}, D) \) is defined by (20). The equality is fulfilled for \( r = 0, r = r_1(S^{\text{in}}, D) \) and \( r = 1 \), see Fig. 4c.
- If \((S^{\text{in}}, D) \in J_4\) then \( S^{\text{out}}_r(S^{\text{in}}, D) \) is given by (17) and for all \( r \in (0, 1) \), \( S^{\text{out}}_r(S^{\text{in}}, D) > S^{\text{out}}(S^{\text{in}}, D) \). The equality is fulfilled for \( r = 0 \) and \( r = 1 \), see Fig. 4d.

Note that if \((S^{\text{in}}, D) \in J_0\), then case 1 of Proposition 2 occurs. One remarks that the lowest value of the red curve, corresponding to the lowest output substrate concentration of the serial configuration, is obtained for \((S^{\text{in}}, D) \in J_2 \cap J_3\) and \( r > r_1(S^{\text{in}}, D) \). This lowest concentration is obtained with the best possible serial configuration.

Figures 2, 3 and 4 are made without graduations on the axes because they represent general situations where the growth function is only assumed to verify our hypotheses.
It should be noticed that regions $J_0$, $J_1$ and $J_3$ always exist and are connected. However, regions the $J_2$ and $J_4$ do not always exist or are necessarily connected. This depends on the number of points of intersection between curves $\Phi_{1/2}$ and $\Gamma$. For a linear growth rate, $\Phi_{1/2} = \Gamma$ and hence, regions $J_2$ and $J_4$ do not exist, see Fig. 7a. For a Monod growth function, curves $\Phi_{1/2}$ and $\Gamma$ intersect only at point $(\lambda(a), 0)$ and hence, region $J_2$ always exist and is connected but region $J_4$ does not exist, see Fig. 8a. For a Hill growth function, curves $\Phi_{1/2}$ and $\Gamma$ always intersect at $(\lambda(a), 0)$ and also at a unique positive point, Lemma 6. Hence, regions $J_2$ and $J_4$ both exist and are connected, see Fig. 9a–c.

### 3.3 The Output Substrate Concentration as a Function of the Dilution Rate

In this section we assume that $S^{in}$ and $r$ are fixed and we look at the values of the dilution rate $D$ for which (8) holds, i.e., the serial configuration, is more efficient than the single chemostat. More precisely we are going to describe the function

$$D \mapsto S^{out}_r (S^{in}, D).$$

(24)
We want to determine the subset of values of $D$ for which the condition (8) is satisfied. We need the following Assumption that is satisfied by any concave growth function, but also by non-concave growth functions, satisfying additional conditions, see Sect. 3.4.

**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.

Using $g_r(D) > \lambda(D/r + a)$, we have

$$
\lim_{D \to r(m-a)} g_r(D) > \lim_{D \to r(m-a)} \lambda(D/r + a) = +\infty.
$$

From Assumption 3, the function $D \mapsto g_r(D)$ is increasing from $[0, r(m - a))$ to $[g_r(0) = \lambda(a), +\infty)$. Hence, its admits an inverse function

$$
S^{in} \in (\lambda(a), +\infty) \mapsto D_r(S^{in}) \in [0, r(m - a)).
$$

For all $r \in (0, 1)$, $S^{in} \geq \lambda(a)$ and $D \in [0, r(m - a))$, we have

$$
D = D_r(S^{in}) \iff S^{in} = g_r(D),
$$

$$
D < D_r(S^{in}) \iff S^{in} > g_r(D).
$$

**Proposition 3** Assume that Assumptions 1 and 3 are satisfied. We have

$$
S^{out}_r(S^{in}, D) < S^{out}(S^{in}, D) \iff 0 < D < D_r(S^{in}),
$$

where $D_r(S^{in})$ is defined by (25).

**Proof** Let $r \in (0, 1)$. According to (26), if $D < D_r(S^{in})$, then $S^{in} > g_r(D)$. Consequently, according to Theorem 1, we have $S^{out}_r(S^{in}, D) < S^{out}(S^{in}, D)$.

\[\square\]

### 3.4 How to Check Assumptions 2 and 3

In this section we give sufficient conditions for Assumption 2 and 3 to be satisfied. These conditions will be useful for the applications given in Sect. 5. For this purpose we consider the function $\gamma$ defined by

$$
\gamma(r, D) = g_r(D),
$$

defined on

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

which consists simply in considering $g_r(D)$, given by (9), as a function of both variables $r$ and $D$. If

$$
\frac{\partial \gamma}{\partial r}(r, D) < 0 \text{ for all } (r, D) \in \text{dom}(\gamma),
$$
then Assumption 2 is satisfied. Similarly, if
\[ \frac{\partial \gamma}{\partial D}(r, D) > 0 \text{ for all } (r, D) \in \text{dom}(\gamma), \]
then Assumption 3 is satisfied. The following Lemmas give sufficient conditions, for partial derivatives of \( \gamma \) to have their signs as indicated above.

**Lemma 2** For \( D \in (0, m - a) \), let \( l_D \) be defined on \( \text{dom}(l_D) = (D/(m - a), 1) \) by \( l_D(r) = \lambda(D/r + a) \).

1. Assume that for \( D \in (0, m - a) \) and \( r \in \text{dom}(l_D) \) we have
\[ l_D(1) > l_D(r) + (1 - r)l_D'(r) \quad (28) \]
then, for all \( (r, D) \in \text{dom}(\gamma) \), we have \( \frac{\partial \gamma}{\partial r}(r, D) < 0 \).
2. If, for \( D \in (0, m - a) \), \( l_D \) is strictly convex on \( \text{dom}(l_D) \), then the condition (28) is satisfied.
3. If \( f \) is twice derivable, then \( l_D \) is twice derivable and the following conditions are equivalent
   1. For \( D \in (0, m - a) \) and \( r \in \text{dom}(l_D) \), \( l_D''(r) > 0 \).
   2. For \( S > \lambda(a) \), \( (f(S) - a)f''(S) < 2(f'(S))^2 \).

**Proof** Notice first that \( \gamma(r, D) \) can be written as follows
\[ \gamma(r, D) = \lambda(D + a) + \left( \frac{1}{1 - r} - \frac{ra}{D + a} \right) \left( \lambda\left( \frac{D}{r} + a \right) - \lambda(D + a) \right). \quad (29) \]
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 \( \gamma \), is given then by
\[ \frac{\partial \gamma}{\partial r}(r, D) = \frac{a(1 - 2r)}{D + a} l_D'(r) + \left( \frac{1}{(1 - r)^2} - \frac{a}{D + a} \right) \left( l_D(r) - l_D(1) + (1 - r)l_D'(r) \right) \quad (30) \]
Notice that \( \frac{1}{(1 - r)^2} - \frac{a}{D + a} > 0 \) for all \( r \in (0, 1) \). From \( l_D'(r) = -\frac{D}{r^2}\lambda'(\frac{D}{r} + a) \), it is deduced that \( l_D'(r) < 0 \). Therefore, if the condition (28) is satisfied, and, in addition \( 0 < r \leq 1/2 \), then, from (30), 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 (29):
\[ \gamma(r, D) = l_D(1) + B(r) \frac{l_D(1) - l_D(r)}{1 - r}, \]
where \( B(r) = \frac{D + a - ar(1 - r)}{D + a} \). Straightforward computation show that
\[ \frac{\partial \gamma}{\partial r}(r, D) = \frac{D + ar(2 - r)}{(D + a)(1 - r)^2} \left( l_D(r) - l_D(1) + (1 - r)C(r)l_D'(r) \right), \quad (31) \]
where $C(r) = \frac{D+a-ar(1-r)}{D+ar(2-r)}$. 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 $C(1/2) = C(1) = 1$, we have $0 < C(r) < 1$ for all $r \in (1/2, 1)$. Now, if we assume that (28) is satisfied, for $1/2 < r < 1$ we have

$$l_D(1) > l_D(r) + (1 - r)l_D'(r) > l_D(r) + (1 - r)C(r)l_D'(r).$$

Hence, from (31), it is deduced that $\frac{\partial y}{\partial r} (r, D) < 0$. This proves part a of the lemma.

Moreover, if $l_D$ is strictly convex on $\text{dom}(l_D)$ 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 $r \in \text{dom}(l_D)$ one obtains the condition (28). This proves part b of the lemma. Assume now that $f$, and hence $l_D$, are twice derivable. Using

$$\lambda'(D) = \frac{1}{f'(\lambda(D))}, \quad \lambda''(D) = -\frac{f''(\lambda(D))}{(f'(\lambda(D)))^3},$$

we can write

$$l_D''(r) = \frac{2D}{r^3} \lambda' \left( \frac{D}{r} + a \right) + \frac{D^2}{r^2} \lambda'' \left( \frac{D}{r} + a \right)$$

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

Therefore, the condition 1 in item c in the lemma is equivalent to the following condition: For all $D \in (0, m - a)$ and $r \in (D/(m - a), 1]$, we have

$$D \frac{r}{f'' \left( \lambda \left( \frac{D}{r} + a \right) \right)} < 2 f' \left( \lambda \left( \frac{D}{r} + a \right) \right)^2.$$

Using the notation $S = \lambda \left( \frac{D}{r} + a \right)$, which is the same as $D/r = f(S) - a$, the condition (33) is equivalent to: For all $S > 0$, $(f(S) - a) f''(S) < 2 \left( f'(S) \right)^2$, which is the condition 2 in c in the lemma.

\begin{lemma}
Assume that

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

\end{lemma}
Then, \( \frac{\partial y}{\partial D}(r, D) > 0 \). Hence Assumption 3 is satisfied. If \( f' \) is decreasing, then the condition (34) is satisfied.

**Proof** From (29) we deduce that

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

Notice that \( \frac{1}{1-r} - \frac{ra}{D+a} > 0, \lambda'(D + a) > 0 \) and \( \lambda \left( \frac{D}{r} + a \right) > \lambda(D + a) \). Therefore the condition

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

is sufficient to have \( \frac{\partial y}{\partial D}(r, D) > 0 \). Using (32), this condition is equivalent to (34). Note that if \( f' \) is decreasing, then this condition is satisfied. Indeed, we have

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

which is the condition (34). \( \square \)

**Remark 1** Notice that:

i) The condition 2 in part c of Lemma 2 is equivalent to the condition

\[
\text{For all } S > \lambda(a), \frac{d^2}{dS^2} \left( \frac{1}{f(S)-a} \right) > 0.
\] (35)

Therefore, if \( f \) satisfies the condition (35), then it verifies Assumption 2.

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 2 and the condition (34) in Lemma 3 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 non-negative on \((0, \hat{S})\) and non-positive on \((\hat{S}, +\infty)\). If moreover the condition 2 in part c of Lemma 2 is verified for \( a = 0 \), then this condition is also verified for any \( a > 0 \) and \( S \in (\lambda(a), \hat{S}) \). Therefore, if \((1/f)''' > 0\) on \((0, \hat{S})\), then Assumption 2 is satisfied.

We will see in Sect. 5, how to use Remark 1 and Lemmas 2 and 3 to show that a linear growth function, a Monod function and a Hill function satisfy Assumptions 2 and 3.

## 4 Biogas Flow Rate

Microbial activity often produces by-products such as biogas, which can be a valuable source of energy in certain contexts. For instance, the anaerobic digestion of organic
matter by microbial species produces methane and carbon dioxide.Valorizing biogas production while treating wastewater has received recently great attention, as a way of producing valuable energy and limiting the carbon footprint of the process (Reh and Muller 2013).

We recall that the biogas flow rate is proportional to the microbial activity, as defined for instance in Bastin and Dochain (1991), Polihronakis et al. (1993). We consider here the biogas flow rate as a function of the input substrate concentration $S^{\text{in}}$, the dilution rate $D$ and the parameter $r$.

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

$$G_1(S^{\text{in}}, D, r) = V_2 \overline{x}_2 f(\overline{S}_2),$$  \hspace{1cm} (36)

with $V_2 = (1 - r)V$, $\overline{x}_2$ and $\overline{S}_2$ defined in (B15).

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

$$G_2(S^{\text{in}}, D, r) = V_1 x_1^* f(S_1^*) + V_2 x_2^* f(S_2^*),$$  \hspace{1cm} (37)

with $V_1 = rV$, $V_2 = (1 - r)V$, $x_1^*$ and $S_1^*$ defined in (B17), $x_2^*$ defined by (B18) and $S_2^*$ the unique solution of $h(S_2) = f(S_2)$.

**Proposition 4** 1. When $r(f(S^{\text{in}}) - a) \leq D$ and $D < (1 - r)(f(S^{\text{in}}) - a)$ then

$$G_1(S^{\text{in}}, D, r) = V D(S^{\text{in}} - \overline{S}_2).$$  \hspace{1cm} (38)

2. When $D < r(f(S^{\text{in}}) - a)$ then

$$G_2(S^{\text{in}}, D, r) = V D(S^{\text{in}} - S_2^*).$$  \hspace{1cm} (39)

**Proof** From system (B10), considering equation $\dot{S}_2 = 0$, one obtains $\overline{x}_2 f(\overline{S}_2) = D(S^{\text{in}} - \overline{S}_2)/(1 - r)$. Thus,

$$G_1(S^{\text{in}}, D, r) = V_1 \frac{D}{r}(S^{\text{in}} - \overline{S}_2) = V D(S^{\text{in}} - \overline{S}_2).$$

From system (B10), considering $\dot{S}_1 = 0$ and $\dot{S}_2 = 0$ gives, respectively, $x_1^* f(S_1^*) = D(S^{\text{in}} - S_1^*)/r$ and $x_2^* f(S_2^*) = D(S_1^* - S_2^*)/(1 - r)$. Thus, one has

$$G_2(S^{\text{in}}, D, r) = V_1 \frac{D}{r}(S^{\text{in}} - S_1^*) + V_2 \frac{D}{1-r}(S_1^* - S_2^*) = V D(S^{\text{in}} - S_2^*).$$

This ends the proof of the proposition. 

Although $G_1(S^{\text{in}}, D, r)$ and $G_2(S^{\text{in}}, D, r)$, given by (36) and (37), respectively, are not defined for $r = 0$ or $r = 1$, the formulas (38) and (39) allow them to be extended to $r = 0$ and $r = 1$, as was done for $S^{\text{out}}_r$ in (6). We can write

$$G_1(S^{\text{in}}, D, 0) = G_2(S^{\text{in}}, D, 1) = G_{\text{chem}}(S^{\text{in}}, D),$$
where
\[ G_{\text{chem}}(S^{\text{in}}, D) = V D (S^{\text{in}} - \lambda (D + a)), \tag{40} \]

represents the biogas flow rate of the single chemostat when \( 0 < D < f(S^{\text{in}}) - a \). For more information on \( G_{\text{chem}}(S^{\text{in}}, D) \), see (A7) in Appendix A.

### 4.1 The Serial Configuration can be More Efficient Than the Single Chemostat

In this section, we prove that the biogas flow rate \( G_1 \) corresponding to the steady state \( E_1 \) is always smaller than the biogas flow rate of the single chemostat. However, the biogas flow rate \( G_2 \) corresponding to the steady state \( E_2 \) can be larger than the biogas flow rate of the single chemostat. More precisely, we have the following result.

**Proposition 5** Assume that Assumption 1 is satisfied. Let \( r \in (0, 1) \), \( 0 \leq D < f(S^{\text{in}}) - a \) and \( G_{\text{chem}} \) defined by (40).

1. If \( r(f(S^{\text{in}}) - a) \leq D \) and \( D < (1 - r)(f(S^{\text{in}}) - a) \), then \( G_1(S^{\text{in}}, D, r) < G_{\text{chem}}(S^{\text{in}}, D) \), where \( G_1 \) is given by (38).
2. If \( D < r(f(S^{\text{in}}) - a) \), then
\[ G_2(S^{\text{in}}, D, r) > G_{\text{chem}}(S^{\text{in}}, D) \iff S^{\text{in}} > g_r(D), \]

where \( G_2 \) is given by (39) and \( g_r \) is defined by (9).

- If, in addition, Assumption 2 is satisfied, and \( S^{\text{in}} > g(D) \), then \( G_2(S^{\text{in}}, D, r) > G_{\text{chem}}(S^{\text{in}}, D) \), if and only if \( r > r_1(S^{\text{in}}, D) \), where \( r_1(S^{\text{in}}, D) \) is defined by (20).
- If, in addition, Assumption 3 is satisfied, then \( G_2(S^{\text{in}}, D, r) > G_{\text{chem}}(S^{\text{in}}, D) \), if and only if \( D < D_r(S^{\text{in}}) \), where \( D_r(S^{\text{in}}) \) is defined by (25).

**Proof**

1. Since \( D/(1 - r) > D \) and \( \lambda \) is increasing, we have \( \lambda(D/(1 - r) + a) > \lambda(D + a) \). Then, using the formula for \( G_1 \) given in Proposition 4, this induces the inequality \( G_1(S^{\text{in}}, D, r) < G_{\text{chem}}(S^{\text{in}}, D) \).
2. According to Theorem 1, for any \( r \in (0, 1) \) and \( D < r(f(S^{\text{in}}) - a) \) one has \( S^*_2(S^{\text{in}}, D, r) < \lambda(D + a) \) if and only if \( S^{\text{in}} > g_r(D) \). Consequently, using the formula for \( G_2 \) given in Proposition 4, one has \( G_2(S^{\text{in}}, D, r) > G_{\text{chem}}(S^{\text{in}}, D) \) if and only if \( S^{\text{in}} > g_r(D) \). If Assumption 2 is satisfied, then, using (21), we see that \( G_2(S^{\text{in}}, D, r) > G_{\text{chem}}(S^{\text{in}}, D) \) if and only if \( r > r_1(S^{\text{in}}, D) \). If Assumption 3 is satisfied, then, using (26), we see that \( G_2(S^{\text{in}}, D, r) > G_{\text{chem}}(S^{\text{in}}, D) \) if and only if \( D < D_r(S^{\text{in}}) \).

This ends the proof of the proposition. \( \square \)

Let \( S^{\text{in}} \) and \( D \) be fixed. The graphs of the biogas flow rates functions
\[ r \mapsto G_1(S^{\text{in}}, D, r), \text{ and } r \mapsto G_2(S^{\text{in}}, D, r), \]
For $r$ and $S_{in}$ fixed, the curves of the maps $D \mapsto G_1(S_{in}, D, r)$, in green, $D \mapsto G_2(S_{in}, D, r)$, in orange, and $D \mapsto G_{chem}(S_{in}, D)$, in black, where $G_1, G_2$ and $G_{chem}$ are given by (38), (39) and (40), respectively (Color figure online)

are easily obtained from the graph of the output substrate concentration, $r \mapsto S_{out}(S_{in}, D)$, see Fig. 4. Indeed, the formulas given in Proposition 4 show that whenever these functions are defined, we have

$$G_1(S_{in}, D, r) = VD (S_{in} - S_{out}(S_{in}, D)),$$
$$G_2(S_{in}, D, r) = VD (S_{in} - S_{out}(S_{in}, D)).$$

We will see, in Sect. 5, some illustrative plots of the biogas flow rates $G_1$ and $G_2$ as functions of the parameter $r \in [0, 1]$, for linear growth, see Fig. 7, Monod growth, see Fig. 8 and Hill growth, see Fig. 9.

Let us illustrate the result of Proposition 5 by plotting the graphs of the biogas flow rates

$$D \mapsto G_1(S_{in}, D, r) \text{ and } D \mapsto G_2(S_{in}, D, r),$$

when $r$ and $S_{in}$ are fixed, see Fig. 5. This figure is made without graduations on the axes because its represents a general situation where the growth function is only assumed to verify our hypotheses. Indeed the behaviors of the functions, depicted in this figure, follow from our results and are not simply numerical illustrations.

Notice that for any $r \in (0, 1)$, the graph of $G_1$ (plotted in green in the figure) is always below the graph $G_{chem}$ (plotted in black). This illustrates item 1 of Proposition 5. Assuming that Assumption 3 is satisfied, then for all $0 < D < D_r(S_{in})$, the graph of $G_2$ (plotted in orange) is above the graph of $G_{chem}$ (plotted in black). This illustrates item 2 of Proposition 5.
4.2 The Maximal Biogas of the Serial Configuration Can Exceed That of the Single Chemostat

In Fig. 5(c) the plot shows that the maximum of $G_2$ (the red curve) is larger than the maximum of $G_{\text{chem}}$, as we want to emphasize that the following inequality is possible

$$\max_D G_2(S_{\text{in}}, D, r) > \max_D G_{\text{chem}}(S_{\text{in}}, D).$$  \hspace{1cm} (41)

Indeed we will show that there is a value $r^* \in (0, 1)$ such that this inequality is true for all $r \in (r^*, 1)$. The threshold $r^*$ obviously depends on $S_{\text{in}}$ and the rate of mortality $a$. It will be noted $r^*(S_{\text{in}}, a)$ when we want to highlight this dependence. This phenomenon never occurs in the case of no mortality, since we have $r^*(S_{\text{in}}, 0) = 1$. Indeed, in the case without mortality, we proved, see Proposition 6 of Dali-Youcef et al. (2020), that for all $S_{\text{in}} > 0$, and all $r \in (0, 1)$ we have

$$\max_D G_2(S_{\text{in}}, D, r) < \max_D G_{\text{chem}}(S_{\text{in}}, D),$$

that is to say, the maximal biogas flow rate of the serial configuration never exceed the maximal biogas flow rate of the single chemostat.

Let us prove that when $a > 0$, the inequality (41) is always true for $r$ sufficiently close to 1. Observe that for any fixed $S_{\text{in}} > \lambda(a)$ and $r \in (0, 1]$, the continuous function $D \mapsto G_2(S_{\text{in}}, D, r)$ is defined on the closed interval $[0, r(f(S_{\text{in}}) - a)]$. It is null at the extremities of this interval and positive on the open interval $(0, r(f(S_{\text{in}}) - a))$. Therefore, it reaches its maximum. For a given $S_{\text{in}} > \lambda(a)$, we then consider the function

$$\overline{G}(r) := \max_{D \in [0, r(f(S_{\text{in}}) - a)]} G_2(S_{\text{in}}, D, r).$$  \hspace{1cm} (42)

We want to ensure that this maximum is reached at a single value, denoted $\overline{D}(r)$. Note that $\overline{D}(1)$ represents the value, which we will assume to be unique, at which the function $D \mapsto G_{\text{chem}}(S_{\text{in}}, D)$ reaches its maximum. We need the following assumption.
Assumption 4 The function $f$ is $C^2$ and increasing and, for $S_{\text{in}} > \lambda(a)$, there exists $\overline{D}(1) \in (0, f(S_{\text{in}}) - a)$ such that $D \mapsto G_{\text{chem}}(S_{\text{in}}, D)$ is

- Strictly concave at $\overline{D}(1)$,
- Increasing on $(0, \overline{D}(1))$,
- Decreasing on $(\overline{D}(1), f(S_{\text{in}}) - a)$.

These conditions are related to the single chemostat model. They are verified for linear, Monod, or Hill growth functions, see Remark 3 in Appendix A.

If Assumption 4 is satisfied, then the maximum of the function $D \mapsto G_{\text{chem}}(S_{\text{in}}, D)$ is unique. The following lemma shows that the function $D \mapsto G_2(S_{\text{in}}, D, r)$ satisfies the same property for $r$ sufficiently close to 1.

**Lemma 4** Assume that Assumption 4 is satisfied, then for any $S_{\text{in}} > \lambda(a)$, there exists a neighborhood $\mathcal{V}_1$ of 1, such that for any $r \in \mathcal{V}_1 \cap \{r \leq 1\}$, the maximum of the function $D \mapsto G_2(S_{\text{in}}, D, r)$ is unique. We denote it by $\overline{D}(r)$. Moreover, $\overline{D}$ is differentiable on $\mathcal{V}_1 \cap \{r < 1\}$ with bounded derivative.

**Proof** The proof is given in in Appendix D.2.

**Proposition 6** Under Assumption 4, the function $\overline{G}$ admits left limits of its first and second derivatives at $r = 1$, which are

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

(43)

**Proof** The proof is given in Appendix D.3.

**Proposition 7** Under Assumption 4, there exists $r^*$ in $(0, 1)$ such that (41) is true for any $r \in (r^*, 1)$ and

$$\max_D G_2(S_{\text{in}}, D, r^*) = \max_D G_{\text{chem}}(S_{\text{in}}, D).$$

**Proof** From Proposition 6, there exist $\varepsilon > 0$ such that for all $r \in (1 - \varepsilon, 1)$, we have $\overline{G}(r) > \overline{G}(1)$. Therefore, the subset $I$ of $(0, 1)$ defined by

$$I = \{\rho \in (0, 1) : \forall r \in (\rho, 1), \overline{G}(r) > \overline{G}(1)\},$$

is non-empty. Let $r^*$ be the lower bound of $I$. We have $\overline{G}(r^*) = \overline{G}(1)$ and $\overline{G}(r) > \overline{G}(1)$ for $r \in (r^*, 1)$. Using (42), we deduce that the equality in the proposition is true and (41) is true for any $r \in (r^*, 1)$.

The function $r \mapsto \overline{G}(r)$ reaches its maximum at some $r_{\text{max}} \in (r^*, 1)$. Let $D_{\text{max}} = \overline{D}(r_{\text{max}})$ be the maximum of the function $D \mapsto G_2(S_{\text{in}}, D, r_{\text{max}})$. Therefore the maximal biogas flow rate of the serial chemostat is given by $G_2(S_{\text{in}}, D_{\text{max}}, r_{\text{max}})$. It satisfies

$$G_2(S_{\text{in}}, D_{\text{max}}, r_{\text{max}}) > G_{\text{chem}}(\overline{S}_{\text{in}}, \overline{D}(1)).$$
We have plotted the function \( r \mapsto \overline{G}(r) \) for the linear, Monod, and Hill growth functions considered in Fig. 6. It is seen in this figure that the tangent at \( r = 1 \) is horizontal which corresponds to \( \overline{G}'(1) = 0 \). In addition, one remarks that \( \overline{G}(r) > \overline{G}(1) \) for \( r \) in some interval \((r^*, 1)\) and \( \overline{G}(r^*) = \overline{G}(1) \). Thus, with presence of mortality rate, if practitioners are able to choose the dilution rate \( D \), the good strategy consists in working with a serial configuration and choose \( r \) in the interval \((r^*, 1)\). The serial configuration should be operated at \( D = \overline{D}(r) \), where \( \overline{D}(r) \) is defined in Lemma 4.

**Remark 2**

- If one is interested in increasing the flow of biogas, the best choice is \( r = r^\text{max}, D = D^\text{max} \).
- If one is interested in reducing the dilution rate, the best choice is \( r = r^* \) and \( D = D^* \), where \( D^* = \overline{D}(r^*) \).

Indeed, for the choice \( r = r^* \) and \( D = D^* \), we have

\[
G_2(S^\text{in}, D^*, r^*) = G_{\text{chem}}(S^\text{in}, \overline{D}(1)),
\]

but \( D^* \) is expected to be significantly smaller than \( \overline{D}(1) \), the dilution rate that maximizes biogas for the single chemostat. In fact, reducing \( D \) means that the flow rate \( Q \) has been reduced, and therefore energy has been saved to obtain the same result as with a single chemostat.

This result has an important message for practitioners: the serial configuration is worth considering when mortality is not negligible. To the best of our knowledge, this result is new in the literature. On the other hand, it is not intuitive. For more information on this issue, see Sect. 5.4. For biological comments on the heuristic underlying this non-intuitive behavior, the reader is referred to Dali-Youcef et al. (2021).

### 5 Illustrations and Numerical Simulations

This section illustrates 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.

#### 5.1 Linear Growth Function

Let consider a linear function \( f(S) = \alpha S, \alpha > 0 \). As it is concave, according to item \( ii \) in Remark 1, 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 \( \Phi_{1/2}, \Gamma \), defined by (13), and \( \Omega \), defined by (22), are identical. Consequently, the operating plane \((S^\text{in}, D)\) is divided in three regions \( J_i, i = 0, 1, 3 \) defined in (23) that describe the behavior of the output substrate concentration and the biogas flow rate, see Fig. 7a.

Consider the operating points \( \eta_1 \) and \( \eta_3 \), fixed, respectively, in regions \( J_1 \) and \( J_3 \), as shown in Fig. 7a. The behavior of the biogas flow rate for these operating points is depicted in Fig. 7b, c. It should be noticed that for any other point \((S^\text{in}, D) \in J_1\), the
curve representing the biogas flow rate with respect to \( r \) should be similar to the curve shown in Fig. 7a, and corresponding to \((S^{\text{in}}, D) = \eta_1\). Similarly, for any other point \((S^{\text{in}}, D) \in J_3\), it should be similar to the curve shown in Fig. 7b, and corresponding to \((S^{\text{in}}, D) = \eta_3\).

In the linear case, the equation \( S^{\text{in}} = g_r(D) \) is a second degree algebraic equation in \( r \) that gives two solutions, one corresponds to \( r_1(S^{\text{in}}, D) \) defined by (20) 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 \( S^{\text{in}} > g(D) \), as stated in item 2 of Proposition 5, the serial configuration has a higher biogas flow rate production than a single chemostat if and only if \( r \in (r_1, 1) \), where \( r_1(0.5, 0.6) \approx 0.5 \), see Fig. 7b.

### 5.2 Monod Function

The Monod function is \( f(S) = mS/(K+S) \). As it is concave, according to item \( ii \) in Remark 1, the Monod function verifies Assumptions 2 and 3. Therefore, our results apply for Monod function.

**Lemma 5** For any \( D > 0 \), the curve \( \Gamma \), defined by (22), is at left of the curve \( \Phi_{1/2} \), defined by (13).

**Proof** The curves \( \Phi_{1/2} \) and \( \Gamma \) are, respectively, defined by equations \( \bar{S}^{\text{in}} = \lambda(2D+a) \) and \( S^{\text{in}} = g(D) \). Let the function \( H : [0, (m-a)/2) \mapsto \mathbb{R} \) be defined by

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

Note that \( H(0) = 0 \) and, for any \( D \in (0, (m-a)/2) \), one has \( H(D) > 0 \), i.e., \( \lambda(2D + a) > g(D) \).

Hence, the curve \( \Gamma \) is at left of the curve \( \Phi_{1/2} \). \( \square \)

As a consequence of Lemma 5, the operating plane \((S^{\text{in}}, D)\) is divided in four regions \( J_i \), \( i = 0, 1, 2, 3 \) defined in (23) that describe the behavior of the output substrate concentration and the biogas flow rate, see Fig. 8a.
Consider the operating points \( \eta_1, \eta_2 \) and \( \eta_3 \), fixed, respectively, in regions \( J_1, J_2 \) and \( J_3 \), as shown in Fig. 8a. The behavior of the biogas flow rate for these points is depicted in Fig. 8b–d. It should be noticed that for any other point \((S_{\text{in}}, D) \in J_1\) (resp. \((S_{\text{in}}, D) \in J_2 \) and \((S_{\text{in}}, D) \in J_3 \)), the curve representing the biogas flow rate with respect to \( r \) should be similar to the curve shown in Fig. 8b (resp. 8c, d), and corresponding to \((S_{\text{in}}, D) = \eta_1\) (resp. \((S_{\text{in}}, D) = \eta_2 \) and \((S_{\text{in}}, D) = \eta_3 \)).

In the Monod case, the equation \( S_{\text{in}} = g_r(D) \) is a second-degree algebraic equation in \( r \) that gives two solutions: one corresponds to \( r_1(S_{\text{in}}, D) \) defined by (20) and the other one is not considered as it does not belong to \((0, 1)\).

Since the point \( \eta_2 \) (resp. \( \eta_3 \)) satisfies the condition \( S_{\text{in}} > g(D) \), as stated in item 2 of Proposition 5, the serial configuration has a higher biogas flow rate production than a single chemostat if and only if \( r \in (r_1, 1) \), with \( r_1(3.45, 0.7) \approx 0.87 \) in Fig. 8c and \( r_1(5, 0.7) \approx 0.54 \) in Fig. 8d.

### 5.3 Hill Function

The Hill function is \( f(S) = mS^p/(K^p + S^p) \). Note that if \( p = 1 \), this function reduces to the Monod function. For \( p > 1 \) it is non-concave. We have

\[
\lambda(a) = \left( \frac{a}{m-a} \right)^{1/p} K.
\]

**Proposition 8**  The Hill function satisfies the conditions (34) and (35). Therefore, according to item iii in Remark 1, it verifies Assumption 2 and according to Lemma 3, it satisfies Assumption 3.

**Proof**  Let us first prove that the Hill function satisfies the condition (35). Straightforward computation gives

\[
\frac{d^2}{dS^2} \left( \frac{1}{f(S)-a} \right) = mpK^p \frac{(p+1)(m-a)S^{2p-2}+(p-1)aK^pS^{p-2}}{((m-a)S^p-aK^p)^3}.
\]
Therefore, \( \frac{d^2}{dS^2} \left( \frac{1}{f(S) - a} \right) > 0 \) for all \( S > \lambda(a) \), that is to say, (35) is satisfied. This result can also be obtained without laborious calculations by using item iii of Remark 1. Let \( \hat{S} \in (0, +\infty) \) be the inflexion point of the Hill function \( f \). It is sufficient to show that \((1/f)'' > 0\) for all \( S \in (0, \hat{S}) \). One easily see that

\[
\left( \frac{1}{f} \right)''(S) = \frac{p(p+1)K_p}{mS^{p+2}} > 0,
\]

for any \( S > 0 \). Consequently, for all \( p > 1 \), the Hill function verifies Assumption 2.

Let us now prove that the Hill function verifies the condition (34). Straightforward computations give

\[
f'(\lambda(D + a)) = \frac{p}{Km}(D + a) \frac{p-1}{p} (m - a - D) \frac{p+1}{p}.
\]

Therefore,

\[
f'(\frac{\lambda}{r} (D + a)) = \frac{p}{Km} (\frac{D}{r} + a) \frac{p-1}{p} (m - a - \frac{D}{r}) \frac{p+1}{p}.
\]

Since \( p > 1 \), \( D + ra < D + a \) and

\[0 < rm - ra - D < m - a - D,\]

one has

\[
(D + ra) \frac{p-1}{p} < (D + a) \frac{p-1}{p},
\]

\[
(rm - ra - D) \frac{1}{p} < (m - a - D) \frac{1}{p}.
\]

From (45) one has

\[
\left( \frac{D}{r} + a \right) \frac{p-1}{p} = \left( \frac{1}{r} \right) \frac{p-1}{p} (D + ra) \frac{p-1}{p} < \left( \frac{1}{r} \right) \frac{p-1}{p} (D + a) \frac{p-1}{p}.
\]

On the other hand, we have

\[
(m - a - D) \frac{p+1}{p} = \left( \frac{1}{r} \right)^{\frac{1}{p}} (m - a - \frac{D}{r}) A,
\]

where \( A = (rm - ra - D) \frac{1}{p} \). From (46), and using

\[0 < m - a - D/r < m - a - D,\]

we then deduce

\[
(m - a - D) \frac{p+1}{p} < \left( \frac{1}{r} \right)^{\frac{1}{p}} (m - a - D) \frac{p+1}{p}.
\]
Therefore, using (44), (47) and (48) one obtains

\[ f'(\lambda \left(\frac{D}{r} + a\right)) = \frac{p}{K_m} \left(\frac{D}{r} + a\right)^{p-1} \left(m - a - \frac{D}{r}\right)^{\frac{p+1}{p}} < \frac{p}{K_m} \frac{1}{r} (D + a)^{p-1} \left(m - a - D\right)^{\frac{p+1}{p}} = \frac{1}{r} f'(\lambda(D + a)). \]

This ends the proof of (34). Consequently, according to Lemma 3, any Hill function satisfies Assumption 3.

Let now consider the case \( p = 2 \) of the Hill function: \( f(S) = mS^2/(K^2 + S^2) \).

**Lemma 6** Let \( D_1 = (3m - 4a - \sqrt{m(5m - 4a)})/4 \). If \( 0 < D < D_1 \) then the curve \( \Phi_{1/2} \), defined by (13), at left of the curve \( \Gamma \), defined by (22). In contrast, if \( D_1 < D < (m - a)/2 \) then the curve \( \Phi_{1/2} \) is at right of the curve \( \Gamma \).

**Proof** Let the function \( H : [0, (m - a)/2) \mapsto \mathbb{R} \) be defined by \( H(D) := \lambda(2D + a) - g(D) \). We have

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

Straightforward computation shows that this function is positive if and only if the polynomial

\[ Q(D) := 4D^2 - 2(3m - 4a)D + 4a^2 - 5am + m^2 \]

is negative. The solution of equation \( Q(D) = 0 \) are

\[ D_1 = \frac{3m-4a-\sqrt{\Delta}}{4} \quad \text{and} \quad D_2 = \frac{3m-4a+\sqrt{\Delta}}{4}, \]

where \( \Delta = 4m(5m - 4a) > 0 \), as \( a < m \). Notice that we have \( 0 < D_1 < (m - a)/2 \) 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 \( \Phi_{1/2} \) at right of the curve \( \Gamma \).

As a consequence of Lemma 6, the operating plane is divided in five regions \( J_i \) \( i = 0, 1, 2, 3, 4 \) defined in (23), see Fig. 9a–c.

Consider the operating points \( \eta_1, \eta_2, \eta_3 \) and \( \eta_4 \) fixed, respectively, in regions \( J_1, J_2, J_3 \) and \( J_4 \), as shown in Fig. 9a–c. It should be noticed that for any other point \( (S_{\text{in}}, D) \in J_1 \) (resp. \( (S_{\text{in}}, D) \in J_2, (S_{\text{in}}, D) \in J_3 \) and \( (S_{\text{in}}, D) \in J_4 \)), the curve representing the biogas flow rate with respect to \( r \) should be similar to the curve shown in Fig. 9a (resp. (b), (c) and (d)), and corresponding to \( (S_{\text{in}}, D) = \eta_1 \) (resp. \( (S_{\text{in}}, D) = \eta_2, (S_{\text{in}}, D) = \eta_3 \) and \( (S_{\text{in}}, D) = \eta_4 \)).

Recall that \( r_1(S_{\text{in}}, D) \) is defined by (20). It is obtained by solving numerically the equation \( S_{\text{in}} = g_r(D) \). Since the point \( \eta_2 \) (resp. \( \eta_3 \)) satisfies the condition \( S_{\text{in}} > g(D) \), as stated in item 2 of Proposition 5, the serial configuration has a higher biogas flow rate production than a single chemostat if and only if \( r \in (r_1, 1) \), with \( r_1(9, 1.6) \approx 0.81 \) in Fig. 9e and \( r_1(12, 1.6) \approx 0.61 \), in Fig. 9f.
The curves $\Gamma$ and $\Phi_{1/2}$ intersect for $D_1 = 0.69$ (see Lemma 6). b, c Zooms of a showing the region $J_4$. The biogas flow rates corresponding to points $\eta_1 = (7, 1.6) \in J_1$, $\eta_2 = (9, 1.6) \in J_2$, $\eta_3 = (12, 1.6) \in J_3$ and $\eta_4 = (2.33, 0.3) \in J_4$ are depicted in d–g, respectively. In these panels curves are colored and numbered as in Fig. 7. d $r_0 \approx 0.63$. e $r_0 \approx 0.54$ and $r_1 \approx 0.81$. f $r_0 \approx 0.48$ and $r_1 \approx 0.61$. g $r_0 \approx 0.49$ (Color figure online)

5.4 The Serial Configuration is Worth Considering When Mortality is not Negligible

In this section we numerically illustrate Remark 2. We fix $S_{in}^\text{in}$, and we adopt the following notations.

$$D_{\text{chem}}^{\text{max}} = \overline{D}(1), \quad G_{\text{chem}}^{\text{max}} = \overline{G}(1) = G_{\text{chem}} \left( S_{in}^\text{in}, D_{\text{chem}}^{\text{max}} \right)$$
Fig. 10 a The map \( r \mapsto \overline{G}(r) \) defined by (42), with \( f(S) = 4S/(5 + S) \), \( a = 0.1 \) and \( S_{\text{in}} = 1.5 \), showing the values \( r^* \) and \( r_{\text{max}} \). b The corresponding maps \( D \mapsto G_{\text{chem}}(S_{\text{in}}, D) \), in black, \( D \mapsto G_2(S_{\text{in}}, D, r^*) \), in blue and \( D \mapsto G_2(S_{\text{in}}, D, r_{\text{max}}) \), in red, show the values \( D^* < D_{\text{max}} < D_{\text{max}}^{\text{chem}} \). c The biogas flow rates for \( a = 0, 0.1, 0.2, 0.3 \) showing the effects of mortality (Color figure online)

Table 1 Numerical values

| \( a \)   | \( D_{\text{max}}^{\text{chem}} \) | \( G^* = G_{\text{max}}^{\text{chem}} \) | \( r^* \) | \( D^* \) | \( D_{\text{max}} \) | \( G^* \) | \( G_{\text{max}} \) | \( G_{\text{max}}^{\text{chem}} \) | \( G_{\text{max}} - G_{\text{max}}^{\text{chem}} \) | \( D_{\text{max}}^{\text{chem}} - D^* \) | \( D_{\text{max}}^{\text{chem}} - D_{\text{max}} \) |
|----------|-------------------------------|-----------------------------|---------|--------|----------------|----------|----------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| 0        | 0.4918                        | 0.3930                      | 1.00    | 0.4918 | 0.3930         | 0.3930   | 0.3930         | 0.3930                       | 0.3930                       | 0.3930                       | 0.3930                       |
| 0.1      | 0.4359                        | 0.3167                      | 0.91    | 0.3758 | 0.3167         | 0.3167   | 0.3167         | 0.3167                       | 0.3167                       | 0.3167                       | 0.3167                       |
| 0.2      | 0.3806                        | 0.2478                      | 0.82    | 0.3925 | 0.2478         | 0.2478   | 0.2478         | 0.2478                       | 0.2478                       | 0.2478                       | 0.2478                       |
| 0.3      | 0.3259                        | 0.1866                      | 0.73    | 0.2969 | 0.1866         | 0.1866   | 0.1866         | 0.1866                       | 0.1866                       | 0.1866                       | 0.1866                       |

where \( \overline{G}(r) \) is defined by (42) and \( \overline{D}(r) \) is as in Lemma 4. Recall that \( r^* \in (0, 1) \) satisfies

\[
\overline{G}(r^*) = \overline{G}(1) = G_{\text{max}}^{\text{chem}}, \tag{49}
\]

and \( \overline{G}(r) > \overline{G}(1) \) for \( r \in (r^*, 1) \), so that \( \overline{G}(r) \) attains its maximum for \( r = r_{\text{max}} \in (r^*, 1) \), see Fig. 10a, obtained with a Monod function and \( S_{\text{in}} = 1.5 \). We adopt the following notations.

\[
D_{\text{max}} = \overline{D}(r_{\text{max}}), \quad G_{\text{max}}^{\text{chem}} = G_2(S_{\text{in}}, D_{\text{max}}, r_{\text{max}}),
\]

\[
D^* = \overline{D}(r^*), \quad G^* = G_2(S_{\text{in}}, D^*, r^*) = G_{\text{max}}^{\text{chem}}.
\]

These notations are illustrated in Fig. 10a,b. The zoom in Fig. 10b shows that \( G_{\text{max}}^{\text{chem}} \) exceeds \( G^* = G_{\text{max}}^{\text{chem}} \) only slightly, but \( D^* \) is significantly smaller than \( D_{\text{max}} \), which is itself smaller than \( D_{\text{max}}^{\text{chem}} \). We give in Table 1 the numerical values of \( r^*, r_{\text{max}}, D^* \),
$D_{\text{max}}^\text{max}$, $G_{\text{max}}^\text{max}$ and $G^* = G_{\text{chem}}^\text{max}$, for various values of the mortality rate $a$. The table also gives the relative gains

$$\frac{G_{\text{max}}^\text{max} - G_{\text{chem}}^\text{max}}{G_{\text{chem}}^\text{max}}$$,

$$\frac{D_{\text{max}}^\text{max} - D_{\text{chem}}^\text{max}}{D_{\text{chem}}^\text{max}}$$,

$$\frac{D_{\text{chem}}^\text{max} - D^*}{D_{\text{chem}}^\text{max}}$$,

when replacing the single chemostat with the serial device using the ratios $r^*$ and $r_{\text{max}}$. The gain in biogas production is almost negligible, but the gain in bioreactor flow rate is significant.

The biogas flow rates $G_{\text{chem}}(S_{\text{in}}, D)$, $G_2(S_{\text{in}}, D, r^*)$ and $G_2(S_{\text{in}}, D, r_{\text{max}})$, for the various considered values of the mortality rate $a$, are depicted in Fig. 10c, in black, blue and red, respectively. This figure shows that mortality is a real problem as it considerably reduces biogas production. Where mortality cannot be avoided or reduced, instead of using the single chemostat, by using a serial device, biogas production can be slightly improved while significantly reducing the bioreactor flow rate.

### 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 Dali-Youcef et al. (2020), 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 is 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 $S_{\text{in}}$, 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 Dali-Youcef et al. (2020) 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 at steady state are not given by the same formulas. Therefore, if biomass productivity is taken into account as a performance criterion, the comparison between the serial chemostat and the single chemostat does not lead to the same conclusions. For more details on this issue the reader can refer to Dali-Youcef and Sari (2021).

Acknowledgements  The authors thank Jérôme Harmand for valuable and fruitful comments. The authors thank the Euro-Mediterranean research network Treasure (http://www.inra.fr/treasure).

Author Contributions  All authors contributed to the study conception, methodology and mathematical analysis. The first draft of the manuscript was written by Manel Dali-Youcef. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Funding  The authors declare that no funds, grants, or other support was received during the preparation of this manuscript.

Data Availability  Data is sharing not applicable to this article as no datasets were generated or analyzed during the current study.

Declarations

Conflict of interest  The authors have no relevant financial or non-financial interests to disclose.

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 mathematical equations are given by

\[
\begin{align*}
\dot{S} &= D(S_{\text{in}} - S) - f(S)x, \\
\dot{x} &= -Dx + f(S)x - ax,
\end{align*}
\] (A1)

where \( S \) and \( x \) denote, respectively, the substrate and the biomass concentration, \( S_{\text{in}} \) the input substrate concentration, \( a \) the mortality rate and \( D = Q/V \) the dilution rate, with \( Q \) the input flow rate and \( V \) the volume of the tank. The specific growth rate \( f \) of the microorganisms satisfies Assumption 1. It is well known [see Harmand et al. (2017); Smith and Waltman (1995)] that, besides the washout \( F_0 = (S_{\text{in}}, 0) \), this system can have a positive steady state

\[
F_1 = (S^*(D), x^*(S_{\text{in}}, D)),
\]
The map $D \mapsto S^\text{out}(S^\text{in}, D)$ is increasing on $[0, \delta]$, where $\delta = f(S^\text{in}) - a$. The map $D \mapsto x^\text{out}(S^\text{in}, D)$ with $f(S) = 4S/(5+S)$, $S^\text{in} = 10$ and $a = 0.6$. The curve $\Gamma$ in the operating plane $(S^\text{in}, D)$ of the single chemostat (color figure online).

### Table 2: Stability of steady states in the various regions of the operating diagram

|       | $I_0$ | $I_1$ |
|-------|-------|-------|
| $F_0$ | GAS   | U     |
| $F_1$ | GAS   |       |

where

$$S^* = \lambda(D + a) \quad \text{and} \quad x^* = \frac{D}{D+a}(S^\text{in} - \lambda(D + a)).$$

See Fig. 11a for the plot of the function $D \mapsto S^*(D)$ and Fig. 11(b) for the plot of the function $D \mapsto x^*(S^\text{in}, D)$ for $0 \leq D \leq \delta$, where $\delta = f(S^\text{in}) - a$.

The washout steady state $F_0$ always exists. It is GAS if and only if $D \geq \delta$. It is LES if and only if $D > \delta$. The positive steady state $F_1$ exists if and only if $D < \delta$. It is GAS and LES whenever it exists. Therefore, the curve $\Phi$ defined by

$$\Phi := \{(S^\text{in}, D) : D = f(S^\text{in}) - a\} \quad (A2)$$

splits the set of operating parameters $(S^\text{in}, D)$ into two regions, denoted $I_0$ and $I_1$, as depicted in 11c. These regions are defined by

$$I_0 := \{(S^\text{in}, D) : D \geq f(S^\text{in}) - a\},$$

$$I_1 := \{(S^\text{in}, D) : D < f(S^\text{in}) - a\}. \quad (A3)$$

The behavior of the system in each region is given in Table 2. Figure 11c, together with 2 is called the operating diagram of the single chemostat.

The particularity of this operating diagram is that the curve limiting both regions $I_0$ and $I_1$ is translated from zero, unlike the case with mortality, as shown in Figure 2.5 of Harmand et al. (2017). Thus, with presence of mortality rate, the region where the washout is GAS, is larger.
The output substrate concentration of the single chemostat, at its stable steady state is given by

\[
S_{\text{out}}(S_{\text{in}}, D) = \begin{cases} 
S_{\text{in}} & \text{if } D \geq \delta, \\
\lambda(D + a) & \text{if } D < \delta.
\end{cases}
\]

(A4)

Its output biomass concentration at steady state is then given by

\[
x_{\text{out}}(S_{\text{in}}, D) = \frac{D}{D + a}(S_{\text{in}} - S_{\text{out}}(D, a))
\]

(A5)

For all \(S_{\text{in}} > \lambda(a)\), one has

\[
\frac{\partial S_{\text{out}}}{\partial D}(S_{\text{in}}, D) = \begin{cases} 
0 & \text{if } D > \delta, \\
\lambda'(D + a) & \text{if } D < \delta.
\end{cases}
\]

Thus, for any growth function satisfying Assumption 1 the function \(D \mapsto S_{\text{out}}(S_{\text{in}}, D)\) is increasing on \([0, \delta]\), as shown in Fig. 11a. The function \(D \mapsto x_{\text{out}}(S_{\text{in}}, D)\) is illustrated in Fig. 11b for a Monod function.

The biogas flow rate of the single chemostat is defined, up to a multiplicative yield coefficient, by

\[
G_{\text{chem}}(S_{\text{in}}, D) := Vx_{\text{out}}f(S_{\text{out}}).
\]

(A6)

Using the expressions (A4) and (A5), respectively, of \(S_{\text{out}}\) and \(x_{\text{out}}\), the biogas flow rate of the single chemostat is given by:

\[
G_{\text{chem}}(S_{\text{in}}, D) = \begin{cases} 
0 & \text{if } D \geq \delta, \\
VD(S_{\text{in}} - \lambda(D + a)) & \text{if } D < \delta.
\end{cases}
\]

(A7)

For a given \(S_{\text{in}} > \lambda(a)\), the function \(D \mapsto G_{\text{chem}}(S_{\text{in}}, D)\) is null for \(D = 0\) or \(D \geq \delta\), and is positive for \(D \in (0, \delta)\). Therefore it admits a maximum in \((0, \delta)\), which is assumed to be unique. A characterization of the growth functions for which this uniqueness is satisfied can be found in Sari (2022).

**Proposition 9** Assume that for any \(S_{\text{in}} > \lambda(a)\), the maximum of \(D \mapsto G_{\text{chem}}(S_{\text{in}}, D)\) is unique, and define \(\overline{D}(S_{\text{in}}) \in (0, \delta)\), such that

\[
G_{\text{chem}}\left(S_{\text{in}}, \overline{D}\left(S_{\text{in}}\right)\right) = \max_{D \geq 0} G_{\text{chem}}\left(S_{\text{in}}, D\right).
\]

Then, the dilution rate \(D = \overline{D}(S_{\text{in}})\) is the solution of the equation \(S_{\text{in}} = g(D)\), where the function \(g : [0, m - a) \mapsto \mathbb{R}\) is given by

\[
g(D) := \lambda(D + a) + D\lambda'(D + a)).
\]

(A8)
Proof For any \( S^{\text{in}} > \lambda(a) \) and \( D \in (0, \delta) \), we have

\[
\frac{\partial G_{\text{chem}}}{\partial D}(S^{\text{in}}, D) = V \left( S^{\text{in}} - \lambda(D + a) - D\lambda'(D + a) \right).
\] (A9)

Therefore, \( \frac{\partial G_{\text{chem}}}{\partial D}(S^{\text{in}}, D) = 0 \) if and only if \( S^{\text{in}} = g(D) \), where \( g \) is defined by (A8).

Notice that the function \( g \) defined by (A8) is the same as the function \( g_r \), defined by (19), which was obtained as the limit, when \( r \) tends to 1, to the function \( g_r \), defined by (9). Recall that \( \Gamma \) is the curve of equation \( S^{\text{in}} = g(D) \), see (22). This curve is depicted in Fig. 11c. It is the set of operating conditions given the higher biogas of the single chemostat. More precisely, for any \( S^{\text{in}} > \lambda(a) \), the maximum \( D = \overline{D}(S^{\text{in}}) \) of the biogas satisfies the condition \((S^{\text{in}}, D) \in \Gamma\).

Therefore, a sufficient condition for the uniqueness of \( \overline{D}(S^{\text{in}}) \) is that the mapping \( g \) is increasing. If, in addition, \( f \) is \( C^2 \), then, deriving (A9) with respect of \( D \), we have

\[
\frac{\partial^2 G_{\text{chem}}}{\partial D^2}(S^{\text{in}}, D) = -V g'(D).
\]

Hence, a sufficient condition for Assumption 4 to be satisfied is that \( g'(D) > 0 \) for \( D \in [0, m - a) \). This last condition if satisfied whenever \( f'' \leq 0 \) on \((\lambda(a), +\infty)\), or \( \left( \frac{1}{f-a} \right)'' > 0 \) on \((\lambda(a), +\infty)\), see Lemma 1 in Sari (2022). Therefore we can make the following remark.

Remark 3 Linear and Monod growth functions satisfy Assumption 4, since they satisfy \( f'' \leq 0 \) on \((0, +\infty)\). On the other hand the Hill function satisfies Assumption 4, since it satisfies \( \left( \frac{1}{f-a} \right)'' > 0 \) on \((\lambda(a), +\infty)\), as shown in Proposition 8.

Appendix B The Serial Configuration

We consider a slight extension of system (1) with different mortality rates in the two tanks. Indeed, we assume 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 \( a_1 \) and \( a_2 \) the mortality rates. The mathematical model is given by the following equations.

\[
\begin{align*}
\dot{S}_1 &= \frac{D}{r} (S^{\text{in}} - S_1) - f(S_1)x_1, \\
\dot{x}_1 &= -\frac{D}{r} x_1 + f(S_1)x_1 - a_1 x_1, \\
\dot{S}_2 &= \frac{D}{r} (S_1 - S_2) - f(S_2)x_2, \\
\dot{x}_2 &= \frac{D}{r} (x_1 - x_2) + f(S_2)x_2 - a_2 x_2.
\end{align*}
\] (B10)

The following result is classical in the mathematical theory of the chemostat.

Lemma 7 For any non-negative initial condition, the solution of system (B10) \((S_1(t), x_1(t), S_2(t), x_2(t))\) is non-negative for any \( t > 0 \) and positively bounded.
Proof Since the vector field defined by (B10) is $C^1$, the uniqueness of the solution to an initial value problem holds. From (B10) and using $f(0) = 0$, we have:

$$\text{for } i = 1, 2, \quad S_i = 0 \implies \dot{S}_i > 0,$$

$$x_1 = 0 \implies \dot{x}_1 = 0,$$

$$x_1 \geq 0 \text{ and } x_2 = 0 \implies \dot{x}_2 \geq 0.$$ Therefore, for $i = 1, 2$, $S_i(t) \geq 0$ and $x_i(t) \geq 0$, for all $t \geq 0$, for which they are defined, provided $S_i(0) \geq 0$ and $x_i(0) \geq 0$, for $i = 1, 2$, see Prop. B.7 in Smith and Waltman (1995). This proves that the solutions of non-negative initial conditions are always non-negative. Let $z_i = S_i + x_i$, $i = 1, 2$. From system (B10), we have

$$\dot{z}_1 = D r (S^\text{in} - z_1) - a_1 x_1,$$

$$\dot{z}_2 = D_1 r (z_1 - z_2) - a_2 x_2.$$ Consequently, we have the differential inequality

$$\dot{z}_1 \leq D r (S^\text{in} - z_1),$$

It follows by comparison of solutions of ordinary differential equations (see, for instance, Walter (1998)) that one has

$$z_1(t) \leq S^\text{in} + (z_1(0) - S^\text{in}) e^{-D r t}.$$ Therefore, $z_1(t) \leq Z_1$, where $Z_1 = \max(S^\text{in}, z_1(0))$. Then, we also have the differential inequality

$$\dot{z}_2 \leq D_1 r (Z_1 - z_2).$$

It follows again by comparison of solutions of ordinary differential equations that one has also

$$z_2(t) \leq Z_1 + (z_2(0) - Z_1) e^{-D_1 r t}.$$ Therefore, $z_2(t) \leq Z_2$, where $Z_2 = \max(Z_1, z_2(0))$. Hence, the solutions of (B10) are positively bounded. Therefore, they are defined for all $t \geq 0$. \qed

For the description of the steady states, we shall consider the following function $h$ that will play a key role

$$h(S_2) = \frac{D + (1-r)a_2}{1-r} \frac{S^*_1 - S_2}{b - S_2}, \quad S_2 \in (0, b)$$

where $S^*_1 = \lambda \left( \frac{D}{r} + a_1 \right)$, $b = \frac{D(S^\text{in} - S^*_1)}{D + ra_1} + S^*_1$. (B11)

This function satisfies the following property.

}\[\text{Springer}]}
Lemma 8 Assume that \( D/r + a_1 < f(S^{\text{in}}) \). The function \( h \) is decreasing from \( h(0) > 0 \) to \( h(S^*_1) = 0 \), where \( h(0) \) is given by

\[
h(0) = \frac{D + (1-r)a_2}{1-r} \frac{(D + ra_1)S^*_1}{DS^{\text{in}} + ra_1 S^*_1}.
\]  
(B12)

Proof From the condition \( D/r + a_1 < f(S^{\text{in}}) \) it is deduced that \( S^*_1 < S^{\text{in}} \). Note that

\[
b = \frac{DS^{\text{in}} + ra_1 S^*_1}{D + ra_1}.
\]

Hence, \( b \) is a convex combination of \( S^{\text{in}} \) and \( S^*_1 \), and we have \( S^*_1 < b < S^{\text{in}} \). Therefore, the vertical asymptote \( S^*_2 = b \) of \( h \) is at right of \( S^*_1 \). The derivative of \( h \) is

\[
h'(S^*_2) = \frac{D + (1-r)a_2}{1-r} \frac{S^*_1 - b}{(b - S^*_2)^2}.
\]

Hence, we have \( h'(S^*_2) < 0 \) for all \( S^*_2 < b \). Therefore, \( h \) is defined on the interval \((0, S^*_1)\) and is decreasing from \( h(0) \), given by (B12) to \( h(S^*_1) = 0 \).

Therefore, if \( D/r + a_1 < f(S^{\text{in}}) \), equation \( f(S^*_2) = h(S^*_2) \) admits a unique solution, denoted by \( S^*_2(S^{\text{in}}, D, r) \), as shown in Fig. 12a. This solution satisfies the following property.

Lemma 9 Considering \( a_1 = a_2 = a \), for all \( 0 < D < f(S^{\text{in}}) - a \), one has

\[
\lim_{r \to 1} S^*_2(S^{\text{in}}, D, r) = \lambda(D + a).
\]

Proof Let \( 0 < D < f(S^{\text{in}}) - a \). Using (5), the condition \( h(S^*_2) = f(S^*_2) \) is equivalent to

\[
(D + (1-r)a)(S^*_1 - S^*_2) = (1-r) \left( \frac{D}{D + ra_1}(S^{\text{in}} - S^*_1) + S^*_1 - S^*_2 \right) f(S^*_2).
\]  
(B13)

For \( r = 1 \), we have \( S^*_1 = \lambda(D + a) \). As \( \lim_{r \to 1} f(S^*_2) < +\infty \) then, (B13) gives

\[
D(\lambda(D + a) - \lim_{r \to 1} S^*_2(S^{\text{in}}, D, r)) = 0.
\]

Consequently, since \( D > 0 \), one has \( \lim_{r \to 1} S^*_2(S^{\text{in}}, D, r) = \lambda(D + a) \).

The existence and stability of steady states of (B10) are given by the following result.

Theorem 3 Assume that Assumption 1 is satisfied. The steady states of (B10) are:
The washout steady state $E_0 = (S^{\text{in}}, 0, S^{\text{in}}, 0)$ which always exists. It is GAS if and only if
\[
D \geq \max\{r(f(S^{\text{in}}) - a_1), (1 - r)(f(S^{\text{in}}) - a_2)\}. \tag{B14}
\]

It is LES if and only if
\[
D > \max\{r(f(S^{\text{in}}) - a_1), (1 - r)(f(S^{\text{in}}) - a_2)\}.
\]

The steady state $E_1 = (S^{\text{in}}, 0, \overline{S}_2, \overline{x}_2)$ of washout in the first chemostat but not in the second one with
\[
\overline{S}_2 = \lambda \left( \frac{D}{1 - r} + a_2 \right), \quad \overline{x}_2 = \frac{D}{D + (1 - r)a_2} (S^{\text{in}} - \overline{S}_2). \tag{B15}
\]

It exists if and only if $D < (1 - r)(f(S^{\text{in}}) - a_2)$. It is GAS if and only if
\[
r(f(S^{\text{in}}) - a_1) \leq D \quad \text{and} \quad D < (1 - r)(f(S^{\text{in}}) - a_2). \tag{B16}
\]

It is LES if and only if
\[
r(f(S^{\text{in}}) - a_1) < D < (1 - r)(f(S^{\text{in}}) - a_2).
\]

The steady state $E_2 = (S_1^*, x_1^*, S_2^*, x_2^*)$ of persistence of the species in both chemostats with
\[
S_1^* = \lambda \left( \frac{D}{1 - r} + a_1 \right), \quad x_1^* = \frac{D}{D + ra_1} (S^{\text{in}} - S_1^*), \tag{B17}
\]
\[
x_2^* = \frac{D}{D + (1 - r)a_2} \left( \frac{D}{D + ra_1} (S^{\text{in}} - S_1^*) + S_1^* - S_2^* \right) \tag{B18}
\]
and $S_2^* = S_2^*(S^{\text{in}}, D, r)$ is the unique solution of the equation $h(S_2) = f(S_2)$ with $h$ defined by (B11). This steady state exists and is positive if and only if $D < r(f(S^{\text{in}}) - a_1)$. It is GAS and LES whenever it exists and is positive.

\textbf{Proof} The 4-dimensional system of ODEs (B10) has a cascade structure of two planar systems of ODEs, whose mathematical analysis is easy and well known in the mathematical theory of the chemostat (Harmand et al. 2017; Smith and Waltman 1995). Using this cascade structure, the global behavior of the system is deduced from the global behavior of planar systems and Thieme’s theory of asymptotically autonomous systems, all the solutions being bounded.

For the convenience of the reader the details of the proof are given in Appendix D.1.

\hfill \Box

\textbf{Proposition 10} The function $S^{\text{in}} \mapsto S_2^*(S^{\text{in}}, D, r)$ is decreasing.

\textbf{Proof} $D$ and $r$ are fixed. Let $S^{\text{in},1} > S^{\text{in},2}$ and $h_i$ defined by (B11), with $S^{\text{in}} = S^{\text{in},i}$, $i = 1, 2$. Let $S_2^{i}$, the solution of equation $f(S_2) = h_i(S_2)$, $i = 1, 2$. Using Lemma 8,
the graph of $h_i$ is a decreasing hyperbola from $h_i(0)$ defined by (B12), with $S^\text{in} = S^\text{in,i}$, to $h_i(S^*_1) = 0$. Since $h_1(0) < h_2(0)$, we have $h_1(S_2) < h_2(S_2)$ for all $S_2 \in (0, S^*_1)$. Therefore, $S^*_2 < S^*_1$, see Fig. 12b.  

This result means that the effluent steady-state concentration of substrate decreases when the influent concentration of substrate increases. This behavior is quite different from the single chemostat, where the effluent steady-state substrate concentration is independent of the influent substrate concentration.

### Appendix C Operating Diagram

For the chemostat model, the operating diagram has as coordinates the input substrate concentration $S^\text{in}$ 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 $(S^\text{in}, D)$. The input substrate concentration $S^\text{in}$ 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 $S^\text{in}$ 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 $(S^\text{in}, D)$ the regions in which the solution of system (B10) globally converges toward one of the steady state $E_0$, $E_1$ or $E_2$. From the results given in Theorem 3, it is seen that these regions are delimited by the curves $\Phi^1_r$ and $\Phi^2_{1-r}$ defined by:

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

$$\Phi^2_{1-r} := \left\{(S^\text{in}, D) \in \mathbb{R}^2_+ : D = (1 - r)(f(S^\text{in}) - a_2)\right\}.$$
When $a_1 = a_2 = 0$, as we have shown in Dali-Youcef et al. (2020), these curves cross only at one point (the origin) and merge when $r = 1/2$. Therefore, in this case the curves $\Phi^1_r$ and $\Phi^2_{1-r}$ separate the operating plane ($S^{\text{in}}$, $D$), in only three regions, see (Dali-Youcef et al. 2020, Figure 5). This property continues 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 $\Phi^1_r$ and $\Phi^2_{1-r}$ separate the operating plane ($S^{\text{in}}$, $D$), in only three regions, see Fig. 13c, d. The novelty when $a_1$ and $a_2$ are different and non-null, is...
that the intersection of the curves $\Phi_r^1$ and $\Phi_{1-r}^2$ can lie outside the $S^\text{in}$ axis. Therefore there can be four regions in the operating plane, as depicted in Fig. 13a, f. For the description of the intersection of the curves $\Phi_r^1$ and $\Phi_{1-r}^2$, we need some definitions and notations. Let $\bar{r} \in (0, 1)$ be defined by

$$\bar{r} := \frac{m-a_2}{2m-a_1-a_2}.$$  \hfill (C21)

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 $P = (S_P^\text{in}, D_P)$ of the operating plane by:

$$S_P^\text{in} := \lambda \left( \frac{ra_1-(1-r)a_2}{2r-1} \right), \quad D_P := \frac{r(1-r)(a_2-a_1)}{1-2r}.$$  \hfill (C22)

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

**Proposition 11** 1. If $a_1 < a_2$, then for all $r \in (0, \bar{r})$, the curves $\Phi_r^1$ and $\Phi_{1-r}^2$ intersect at the point $P$ and $\Phi_r^1$ is strictly below [resp. above] $\Phi_{1-r}^2$ for $S^\text{in} > S_P^\text{in}$ [resp. $S^\text{in} < S_P^\text{in}$], see Fig. 13a. For all $r \in (\bar{r}, 1)$, $\Phi_r^1$ is strictly above $\Phi_{1-r}^2$, see Fig. 13b. 2. If $a_1 > a_2$ then for all $r \in (\bar{r}, 1)$, the curves $\Phi_r^1$ and $\Phi_{1-r}^2$ intersect at the point $P$ and $\Phi_r^1$ is strictly above [resp. below] $\Phi_{1-r}^2$ for $S^\text{in} > S_P^\text{in}$ [resp. $S^\text{in} < S_P^\text{in}$], see Fig. 13f. For all $r \in (0, \bar{r})$, $\Phi_r^1$ is below $\Phi_{1-r}^2$, see Fig. 13e. 3. If $a_1 = a_2$ then, for $r = 1/2$, $\Phi_r^1 = \Phi_{1-r}^2$. Moreover, if $r < 1/2$ then $\Phi_r^1$ is strictly below $\Phi_{1-r}^2$, see Fig. 13c and, if $r > 1/2$, then $\Phi_r^1$ is strictly above $\Phi_{1-r}^2$, see Fig. 13d.

**Proof** For $0 < r < 1$ and $S^\text{in} > \lambda(a_i)$ we define the function $\varphi_i$, $i = 1, 2$, by

$$\varphi_1(S^\text{in}, r) = r \left( f(S^\text{in}) - a_1 \right), \quad \varphi_2(S^\text{in}, r) = (1-r) \left( f(S^\text{in}) - a_2 \right).$$  \hfill (C23)

The curves $\Phi_r^1$ and $\Phi_{1-r}^2$, defined, respectively, by (C19) and (C20), intersect if and only if there exists $r \in (0, 1)$ and $S^\text{in} > \text{max} \left( \lambda(a_1), \lambda(a_2) \right)$ such that $\varphi_1(S^\text{in}, r) = \varphi_2(S^\text{in}, r)$, that is to say

$$f(S^\text{in}) = A(r), \quad \text{with} \quad A(r) := \frac{ra_1-(1-r)a_2}{2r-1}.$$  \hfill (C24)

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

$$\text{max} \left( a_1, a_2 \right) < A(r) < m,$$  \hfill (C25)

where $m = \sup(f)$, as in (2). When these conditions are satisfied, the solution of (C24) is given by $S^\text{in} = \lambda(A(r))$, where $\lambda$ is the inverse function of $f$, i.e., the break-even concentration defined by (3). Hence, $S^\text{in} = S_P^\text{in}$, given in (C22). The corresponding
intersection point of \( \Phi^1_r \) and \( \Phi^2_{1-r} \) is given by \( DP = r \left( f(S^\text{in}_P) - a_1 \right) \), which is the value given in (C22).

Let us determine now for which value of \( r \), the conditions (C25) 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

\[
A'(r) = \frac{a_2 - a_1}{(2r - 1)^2}. \tag{C26}
\]

Note that \( A(r) = m \) if and only if \( r = \bar{r} \), where \( \bar{r} \) is defined by (C21). Therefore if \( a_1 < a_2 \), then, according to (C26), \( A \) is increasing. Since \( A(0) = a_2 \), \( A(\bar{r}) = m \), and \( \bar{r} < 1/2 \), the condition (C25) is satisfied if and only if \( 0 < r < \bar{r} \). Similarly, if \( a_1 > a_2 \), then, according to (C26), \( A \) is decreasing. Since \( A(1) = a_1 \), \( A(\bar{r}) = m \) and \( \bar{r} > 1/2 \), the condition (C25) is satisfied if and only if \( \bar{r} < r < 1 \). Finally, if \( a_1 = a_2 \) then \( A(r) = a_1 \) and the condition (C25) cannot be satisfied.

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

- If \( r \in (0, \bar{r}) \), then \( f(S^\text{in}) < A(r) \) if and only if \( S^\text{in} < S^\text{in}_P \), where \( S^\text{in}_P \) is defined by (C22). Hence, the curves \( \Phi^1_r \) and \( \Phi^2_{1-r} \) intersect at \( P = (S^\text{in}_P, DP) \) and the curve \( \Phi^1_r \) is strictly below [resp. above] the curve \( \Phi^2_{1-r} \), for all \( S^\text{in} > S^\text{in}_P \) [resp. \( S^\text{in} < S^\text{in}_P \)].
- If \( r \in [\bar{r}, 1/2) \), then \( f(S^\text{in}) < A(r) \) for all \( S^\text{in} > 0 \), so that the curve \( \Phi^1_r \) is strictly above the curve \( \Phi^2_{1-r} \).
- If \( r \in [1/2, 1) \), then, using \( r \geq 1-r \) and \( a_1 < a_2 \), one has \( \varphi_1(S^\text{in}, r) > \varphi_2(S^\text{in}, r) \). Therefore, the curve \( \Phi^1_r \) is strictly above the curve \( \Phi^2_{1-r} \).

If \( a_1 > a_2 \), the proof is similar to the case \( a_1 < a_2 \).

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

For any \( r \in (0, 1) \), the curves \( \Phi^1_r \) and \( \Phi^2_{1-r} \), defined by (C19) and (C20), respectively, split the plane \( (S^\text{in}, D) \) in the regions denoted \( I_0(r) \), \( I_1(r) \), \( I_2(r) \) and \( I_3(r) \) defined in Table 3. These regions are depicted in Fig. 13 in the cases \( a_1 < a_2 \), \( a_1 = a_2 \) and \( a_1 > a_2 \).

The behavior of the system in each region, when it is not empty, is given in Table 3. Notice that \( E_1 \) exists in both regions \( I_1(r) \) and \( I_2(r) \), but is stable only when \( (S^\text{in}, D) \) is in \( I_1(r) \).

When \( a_1 = a_2 = 0 \), \( \lambda(a_1) = \lambda(a_2) = 0 \) and the curves \( \Phi^1_r \) and \( \Phi^2_{1-r} \) of the operating diagram start from the origin of the plane \( (S^\text{in}, D) \) and merge for \( r = 1/2 \). Therefore, the diagrams shown in panels (a), (b), (c), (d), (e) and (f) of Fig. 13 are reduced to only two different cases characterized by \( 0 < r < 1/2 \) and \( 1/2 < r < 1 \),
as shown in Figure 5 of Dali-Youcef et al. (2020). There is no change 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, panels (a) and (f) of Fig. 13 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 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 \( r \), panels (a) and
(f) of Fig. 13 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 dysfuntioning
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.

When \( a_1 = a_2 = a \), which is the case corresponding to the system (1) considered
in Section 2, only panels (c,d) of Fig. 13 are encountered, as shown in Fig. 2. We
describe hereafter the bifurcations that occur in this particular case. The general case,
i.e., when \( a_1 \neq a_2 \) is similar.

**Remark 4** Transcritical bifurcations occur in the limit cases \( D = r(S^{in} - a) \) and
\( D = (1 - r)(S^{in} - a) \), for system (1). If \( 0 < r < 1/2 \) then, we have a transcritical
bifurcation of \( E_0 \) and \( E_1 \) when \( D = (1 - r)(S^{in} - a) \) and a transcritical bifurcation
of \( E_1 \) and \( E_2 \) when \( D = r(S^{in} - a) \). If \( 1/2 < r < 1 \) then, we have a transcritical
bifurcation of \( E_0 \) and \( E_1 \) when \( D = (1 - r)(S^{in} - a) \) and a transcritical bifurcation
of \( E_0 \) and \( E_2 \) when \( D = r(S^{in} - a) \). If \( r = 1/2 \) and \( D = (f(S^{in}) - a)/2 \) then,
we have transcritical bifurcations of \( E_0 \) and \( E_1 \), and \( E_0 \) and \( E_2 \), simultaneously.

| Regions | \( I_0(r) \) | \( I_1(r) \) | \( I_2(r) \) | \( I_3(r) \) |
|---------|-------------|-------------|-------------|-------------|
| \( E_0 \) | GAS         | U           | U           | U           |
| \( E_1 \) | GAS         | U           |             |             |
| \( E_2 \) |             |             | GAS         | GAS         |
Appendix D Proofs

D.1 Proof of Theorem 3

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 $\dot{S}_1 = 0$, 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 $S_1 = S_{\text{in}}$ 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 $\dot{S}_2 = 0$ it is deduced that $S_2 = S_{\text{in}}$. Hence we obtain the steady state $E_0 = (S_{\text{in}}, 0, S_{\text{in}}, 0)$, which always exist.

On the other hand, if $f(S_2) = D/(1-r) + a_2$, then $S_2 = \overline{S}_2$, defined in (B15). From equation $\dot{S}_2 = 0$, it is deduced that $x_2 = \overline{x}_2$, defined in (B15). Hence we obtain the steady state $E_1 = (S_{\text{in}}, 0, \overline{S}_2, \overline{x}_2)$. This steady state exists if and only if $S_{\text{in}} > \overline{S}_2$, that is $D < (1-r)(f(S_{\text{in}}) - a_2)$.

Suppose now that $f(S_1) = D/r + a_1$. Then $S_1 = S_{1*}$, defined in (B17). From equation $\dot{S}_1 = 0$, it is deduced that $x_1 = x_{1*}$, defined in (B17). From equation $\dot{S}_2 + \dot{x}_2 = 0$, it is deduced that

$$x_2 = \frac{D}{D + (1-r)a_2}(S_{1*} + x_{1*} - S_2).$$

(D27)

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 (B11). Hence $S_2 = S_{2*}$, which is the unique solution of the equation $f(S_2) = h(S_2)$, as shown in Fig. 12a. Replacing $S_2$ by $S_{2*}^*$ in (D27) gives $x_2 = x_{2*}^*$, defined by (B18). 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 $S_{\text{in}} > S_{1*}^*$, which is equivalent to $D < r(f(S_{\text{in}}) - a_1)$.

Let us now study the local stability. Since the system has a cascade structure, the stability analysis reduces to the study of square $2 \times 2$ matrices. Indeed, the Jacobian matrix associated to system (B10) is the lower triangular matrix by blocs, $J = \begin{pmatrix} A & 0 \\ B & C \end{pmatrix}$ where $B$ is the diagonal matrix whose diagonal elements are $D/(1-r)$, and $A$ and $C$ are given by:

$$A = \begin{pmatrix} -D/r - f'(S_1) & -f(S_1) \\ f'(S_1)x_1 & -D/r + f(S_1) - a_1 \end{pmatrix},$$

$$C = \begin{pmatrix} -D/r - f'(S_2)x_2 & -f(S_2) \\ f'(S_2)x_2 & -D/r + f(S_2) - a_2 \end{pmatrix}.$$  

Hence, the eigenvalues of $J$ are the ones of $A$ and $C$.

For $E_0$, the eigenvalues are $-D/r, -D/r + f(S_{\text{in}}) - a_1, -D/(1-r)$ and $-D/(1-r) + f(S_{\text{in}}) - a_2$. They are negative if and only if $D > r(f(S_{\text{in}}) - a_1)$ and $D > (1-r)(f(S_{\text{in}}) - a_2)$. Therefore, $E_0$ is LES if and only if the condition in the theorem is satisfied.

For $E_1$, the eigenvalues of $A$ are $-D/r + f(S_{\text{in}}) - a_1$ and $-D/r$. The first eigenvalue is negative if and only if $D > r(f(S_{\text{in}}) - a_1)$. On the other hand, since the determinant
of $C$ is positive, and its trace is negative, the eigenvalues of $C$ have negative real parts. Therefore, $E_1$ is LES if and only if the condition in the theorem is satisfied.

For $E_2$, the determinant of $A$ is positive and its trace is negative. On the other hand, using the notation $C_{E_2}$ for the matrix $C$ evaluated at $E_2$, we have

$$
\text{det}(C_{E_2}) = \left(-\frac{D}{1-r} - f'(S^*_2) x_2^* \right) \left(-\frac{D}{1-r} - a_2 + f(S^*_2) \right) + f(S^*_2) f'(S^*_2) x_2^*,
$$

$$
\text{tr}(C_{E_2}) = -2 \frac{D}{1-r} - a_2 - f'(S^*_2) x_2^* + f(S^*_2).
$$

Note that $h(S_2) < D/(1-r) + a_2$ for all $S_2 \in (0, S^*_2)$. Therefore, from (B11), we have $f(S^*_2) = h(S^*_2) < D/(1-r) + a_2$. Consequently, $\text{det}(C_{E_2})$ and $\text{tr}(C_{E_2})$ are, respectively, positive and negative. Therefore, $E_2$ is LES whenever it exists, that is $D < r(f(S^\text{in}) - a_1)$.

For the study of the global stability we use the cascade structure of the system (B10) and Thieme’s theorem (see Theorem A1.9 of Harmand et al. (2017)). In the rest of the proof, we denote by $(S_1(t), x_1(t), S_2(t), x_2(t))$ the solution of (B10) with the initial condition $(S^0_1, x^0_1, S^0_2, x^0_2)$.

Then, $(S_1(t), x_1(t))$ is the solution of system

$$
\dot{S}_1 = \frac{D}{r} (S^\text{in} - S_1) - f(S_1) x_1,
$$

$$
\dot{x}_1 = -\frac{D}{r} x_1 + f(S_1) x_1 - a_1 x_1, \quad (D28)
$$

with initial condition $(S^0_1, x^0_1)$ and $(S_2(t), x_2(t))$ is the solution of the non-autonomous system of differential equations

$$
\dot{S}_2 = \frac{D}{1-r} (S_1(t) - S_2) - f(S_2) x_2,
$$

$$
\dot{x}_2 = \frac{D}{1-r} (x_1(t) - x_2) + f(S_2) x_2 - a_2 x_2, \quad (D29)
$$

with the initial condition $(S^0_2, x^0_2)$. The system (D28) is the classical model of a single chemostat. Its asymptotic behavior is well known (see, for instance, Proposition 2.2 of Harmand et al. (2017)). This system admits the steady states:

$$
e^0_1 = (S^\text{in}, 0) \quad \text{and} \quad e^0_1 = (S^*_1, x^*_1), \quad (D30)
$$

where $S^*_1$ and $x^*_1$ are defined by (B17). Two cases must be distinguished.

Firstly, if $\lambda(D/r + a_1) \geq S^\text{in}$, that is $D \geq r(f(S^\text{in}) - a_1)$ then, $e^0_1$, defined in (D30), is GAS for (D28) in the non-negative quadrant. Hence, for any non-negative initial condition $(S^0_1, x^0_1)$,

$$
\lim_{t \to +\infty} (S_1(t), x_1(t)) = (S^\text{in}, 0). \quad (D31)
$$

Therefore, the system (D29) is asymptotically autonomous with the limiting system

$$
\dot{S}_2 = \frac{D}{1-r} (S^\text{in} - S_2) - f(S_2) x_2, 
$$

$$
\dot{x}_2 = -\frac{D}{1-r} x_2 + f(S_2) x_2 - a_2 x_2. \quad (D32)
$$
Recall that the solutions of (D29) are positively bounded. Therefore, we shall use Thieme’s results which apply for bounded solutions.

The system (D32) 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 (B15). Two subcases must be distinguished.

- If $\lambda (D/(1 - r) + a_2) \geq S_{in}^2$, that is $D \geq (1 - r)(f(S_{in}) - a_2)$, then $e_0^2$ is GAS in the non-negative quadrant. Using Thieme’s theorem, we deduce that for any non-negative $(S_0^2, x_0^2)$, the solution $(S_2(t), x_2(t))$ of (D29) converges toward $e_0^2 = (S_{in}, 0)$. Using (D31) 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 (B10) converges toward $E_0 = (S_{in}, 0, S_{in}, 0)$, which proves (B14).

- In contrast, if $\lambda (D/(1 - r) + a_2) < S_{in}^2$, that is $D < (1 - r)(f(S_{in}) - a_2)$ then, both steady states $e_0^2$ and $e_1^2$ exist and $e_2^2$ is GAS in the positive quadrant. Although system (D29) has the saddle point $e_0^2$, no polycycle can exist. Using Thieme’s theorem, for any positive $(S_0^2, x_0^2)$, the solution $(S_2(t), x_2(t))$ of (D29) converges toward $e_1^2 = (\overline{S}_2, \overline{x}_2)$. Using (D31) 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 (B10) converges toward $E_1 = (S_{in}, \overline{S}_2, \overline{x}_2)$, which proves (B16).

Secondly, if $\lambda (D/r + a_1) < S_{in}^2$, that is $D < r(f(S_{in}) - a_1)$, then $e_1^1$, defined in (D30), is GAS for (D28) in the positive quadrant. Hence, for any positive initial condition $(S_1^0, x_1^0)$

$$\lim_{t \to +\infty} (S_1(t), x_1(t)) = (S_1^*, x_1^*). \quad (D33)$$

Therefore, the system (D29) is asymptotically autonomous with the limiting system

$$\begin{align*}
\dot{S}_2 &= \frac{D}{1-r}(S_1^* - S_2) - f(S_2)x_2, \\
\dot{x}_2 &= \frac{D}{1-r}(x_1^* - x_2) + f(S_2)x_2 - a_2x_2. 
\end{align*} \quad (D34)$$

The system (D34) represents the classical model of a single chemostat with an input biomass. In this case, there is no washout and the system (D34) always admits one LES steady state $e_2 = (S_2^*, x_2^*)$ with positive biomass defined by (B18) and $S_2^*$ the unique solution of $h(S_2) = f(S_2)$.

Let us show that this steady state is GAS for (D34). Assume that $x_2 > 0$. Consider the change of variable $\xi = \ln(x_2)$. The system (D34) becomes as

$$\begin{align*}
\dot{S}_2 &= \frac{D}{1-r}(S_1^* - S_2) - f(S_2)e^\xi, \\
\dot{\xi} &= \frac{D}{1-r}(x_1^*e^{-\xi} - 1) + f(S_2) - a_2. 
\end{align*} \quad (D35)$$

The divergence of the vector field

$$\psi(S_2, \xi) = \left[\frac{D}{1-r}(S_1^* - S_2) - f(S_2)e^\xi, \frac{D}{1-r}(x_1^*e^{-\xi} - 1) + f(S_2) - a_2\right]$$
associated to (D35) is $\text{div}(S_2, \xi) = -\frac{D}{1-r}(1 + x_1^*e^\xi) - f'(S_2)e^\xi$. It is negative. Thus, using Bendixson–Dulac criterion, system (D35) cannot have a periodic solution. Hence, system (D34) has no cycle in the positive quadrant. For any non-negative initial condition $(S^0_2, x^0_2)$, the solution of (D34) is bounded. Hence, the $\omega$-limit set of $(S^0_2, x^0_2)$, denoted $\omega(S^0_2, x^0_2)$, is non-empty and included in the positive quadrant. If $e_2 \notin \omega(S^0_2, x^0_2)$ then, using Poincaré–Bendixson theorem, $\omega(S^0_2, x^0_2)$ is a limit cycle, but the system does not present any, due to the divergence property. One then deduces $e_2 \in \omega(S^0_2, x^0_2)$ and, as $e_2$ is LES, then $\omega(S^0_2, x^0_2) = \{e_2\}$. Consequently, $e_2$ is GAS for (D34) in the positive quadrant.

Using again Thieme’s theorem, for any positive $(S^0_2, x^0_2)$, the solution $(S_2(t), x_2(t))$ of (D29) converges toward $e_2 = (S^*_2, x^*_2)$. Using (D33) we deduce that, if $D < r(f(S^\text{in}) - a_1)$, then the solution $(S_1(t), x_1(t), S_2(t), x_2(t))$ of (B10) converges toward $E_2 = (S^*_1, x^*_1, S^*_2, x^*_2)$. This ends the proof of the theorem.

**D.2 Proof of Lemma 4**

Let $d$ be fixed such that $\delta := f(S^\text{in}) - a > 0$. The proof consists in showing that the function $(D, r) \mapsto G_2(S^\text{in}, D, r)$ can be formally extended as a $C^2$ function for values of $r$ larger than 1 (although such values have no physical meaning). Recall first that for any $D \in (0, \delta)$, one has $G_2(S^\text{in}, D, 1) = G_{\text{chem}}(S^\text{in}, D)$. As $G_2(S^\text{in}, D(1), 1) > 0$ and $G_2(S^\text{in}, 0, 1) = 0$, there exists by continuity of the function $G_2$, numbers $D \in (0, \bar{D}(1))$, $r \in (0, 1)$ such that

$$G_2(S^\text{in}, D, r) < \max_{d \in (0, r\delta)} G_2(S^\text{in}, d, r), \ (D, r) \in [0, D] \times [r, 1]. \quad (D36)$$

Let $\varepsilon > 0$ be such that

$$D_\varepsilon := \varepsilon \left(a + \max_{s \in [0, S^\text{in}]} f'(s)(S^\text{in} - s)\right) < D \quad (D37)$$

and consider the domain

$$\mathcal{D}_\varepsilon := \left\{(D, r); \ D \in (D_\varepsilon, \delta), \ r \in \left(\max \left(\frac{r}{\delta}, \frac{D}{\delta}\right), 1 + \varepsilon\right)\right\}.$$ 

Note that for any $(D, r) \in \mathcal{D}_\varepsilon$, the number $\lambda(D/r + a) = f^{-1}(D/r + a)$ is well defined. Posit the function

$$\varphi(S_2, D, r) = (D + (1 - r)a) (\lambda(D/r + a) - S_2) - (1 - r)f(S_2) \left(Dr\frac{S^\text{in}r\lambda(D/r + a)}{D + ra} - S_2\right),$$

where $(S_2, D, r) \in (0, S^\text{in}) \times \mathcal{D}_\varepsilon$. As $f$ is $C^2$, $\varphi$ is $C^2$ on $(0, S^\text{in}) \times \mathcal{D}_\varepsilon$.

For $r < 1$ and $(D, r) \in \mathcal{D}_\varepsilon$, one has

$$\varphi(S_2, D, r) = (1 - r) \left(Dr\frac{S^\text{in}r\lambda(D/r + a)}{D + ra} - S_2\right) (h(S_2) - f(S_2))$$
where $h$ is the function defined in (5). According to Lemma 8, $h$ is positive decreasing on $(0, \lambda(D/r+a))$, and $h-f$ admits an unique zero $S_2^* = S_2^*(S^{\text{in}}, D, r)$ on $(0, \lambda(D/r+a))$. Then, one can write

$$
\frac{\partial S_2 \phi}{S_2=S_2^*} = (1-r) \left( \frac{DS^{\text{in}} + ra\lambda(D/r+a)}{D + ra} - S_2 \right) \left( \frac{\partial S_2 h - f'}{S_2=S_2^*} < 0. \right.
$$

For $r \in [1, 1+\varepsilon)$ and $(D, r) \in \mathcal{D}_{\varepsilon}$, one has

$$
\frac{\partial S_2 \phi = - (D + (1-r)a) - (1-r)f'(S_2) \left( \frac{DS^{\text{in}} + ra\lambda(D/r+a)}{D + ra} - S_2 \right)
$$

which is negative for any $S_2 \in (0, S^{\text{in}})$ thanks to condition (D37). As $\phi(0, D, r) > 0$ and $\phi(S^{\text{in}}, D, r) < 0$, we deduce the existence of a unique $S_2^* = S_2^*(S^{\text{in}}, D, r)$ in $(0, S^{\text{in}})$ such that $\phi(S_2^*, D, r) = 0$, which also verifies $\partial S_2 \phi < 0$ at $S_2 = S_2^*$.

Then, by the implicit function theorem, the function $(D, r) \mapsto S_2^*(S^{\text{in}}, D, r)$ is $C^2$ on $\mathcal{D}_{\varepsilon}$. Recall that for $r < 1$ and $D < r\delta$, one has the expression $G_2(S^{\text{in}}, D, r) = VD(S^{\text{in}} - S_2^*(S^{\text{in}}, D, r))$ (see Proposition 4). We extend now the function $(D, r) \mapsto G_2(S^{\text{in}}, D, r)$ with this last $C^2$ expression on $\mathcal{D}_{\varepsilon}$. As $G_2(S^{\text{in}}, D, 1) = G_{\text{chem}}(S^{\text{in}}, D)$ for any $D \in (0, \delta)$, one deduces, by continuity of the partial derivatives of $G_2$ with respect to $D$ and property (D36), the existence of $\mathcal{V}_D$, $\mathcal{V}_r$ as neighborhoods, respectively, of $\overline{D}(1)$ and $1$ with $\mathcal{V}_D \times \mathcal{V}_r \subset \mathcal{D}_{\varepsilon}$ such that for any $r \in \mathcal{V}_r$, the function $D \mapsto G_2(S^{\text{in}}, D, r)$ possesses the following properties

1. it is strictly concave on $\mathcal{V}_D$,
2. it is increasing on $(D_{\varepsilon}, \overline{D}(1)) \setminus \mathcal{V}_D$ and decreasing on $(\overline{D}(1), r\delta) \setminus \mathcal{V}_D$,
3. its maximum over $(0, r\delta)$ is not reached for $D \leq D_{\varepsilon}$.

We thus deduce that $D \mapsto G_2(S^{\text{in}}, D, r)$ admits a unique maximum $\overline{D}(r)$ on $(0, r\delta)$, for any $r \in \mathcal{V}_r$.

Finally, for any $r \in \mathcal{V}_r$, $\overline{D}(r)$ is characterized as the zero of the map $D \mapsto F(D, r)$ where $F$ is the $C^1$ function

$$
F(D, r) := \partial_D G_2(S^{\text{in}}, D, r)
$$

From property 1. above, one obtains

$$
\partial_D F(\overline{D}(r), r) = \partial_D^2 G_2(S^{\text{in}}, \overline{D}(r), r) < 0, \quad r \in \mathcal{V}_r
$$

and by the implicit function theorem, there exists a neighborhood $\mathcal{V}_1 \subset \mathcal{V}_r$ of $1$ such that $\overline{D}$ is $C^1$ on $\mathcal{V}_1$, which ends the proof of the lemma.
D.3 Proof of Proposition 6

$S^\text{in}$ being fixed, we shall drop the $S^\text{in}$ dependency in the expressions of $S^*_i, x^*_i$ ($i = 1, 2$) and $G_2$. Thus, let us define

$$
G(D, r) := G_2(S^\text{in}, D, r),
F_i(D, r) := f(S^*_i(D, r)) x^*_i(D, r), \quad i = 1, 2,
$$
as functions of $D \geq 0$ and $r \in V_1 \cap \{r < 1\}$. 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 9, for all $D \geq 0$, one has

$$
S^*_2(D, 1) = S^*_1(D, 1) = \lambda(D + a)
$$
and

$$
x^*_2(D, 1) = x^*_1(D, 1) = \frac{D}{D+a} (S^\text{in} - \lambda(D + a)).
$$

(D38)

Thus, for all $D \geq 0$, one has

$$
F_1(D, 1) = F_2(D, 1),
$$
and $F_2$ is also well defined for $r = 1$. Thus, according to (37), for all $D \geq 0$ and $r \in V_1 \cap \{r \leq 1\}$, one has

$$
G(D, r) = r F_1(D, r) + (1 - r) F_2(D, r),
$$
and from Lemma 4, for $r \in V_1 \cap \{r < 1\}$, one has

$$
\overline{G}(r) = G(\overline{D}(r), r),
$$
with $\overline{G}$ defined by (42). For convenience, for a function $E$ of $(D, r)$ that is differentiable, we shall define the three following functions: $\overline{E}(r) := E(\overline{D}(r), r)$ and

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

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

$$
\overline{G}(r) = r \overline{F}_1(r) + (1 - r) \overline{F}_2(r), \quad \text{for } r \in V_1 \cap \{r < 1\}.
$$

(D41)

As the functions $F_i, i = 1, 2$, are differentiable and as $\overline{D}(r)$ is a maximizer of $D \mapsto r F_1(D, r) + (1 - r) F_2(D, r)$ on the interior of the interval $[0, f(S^\text{in}) - a]$, one has

$$
r \partial_D F_1(r) + (1 - r) \partial_D F_2(r) = 0, \quad \text{for } r \in V_1 \cap \{r < 1\},
$$
and $\partial_D F_1(1) = 0$. 

\[\square\] Springer
As $f$ is $C^2$ and $\overline{D}$ is assumed to be differentiable on $\mathcal{V}_1 \cap \{ r < 1 \}$, $G$ is differentiable and from (D41), for all $r \in \mathcal{V}_1 \cap \{ r < 1 \}$, one has

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

and with (D42), for all $r \in \mathcal{V}_1 \cap \{ r < 1 \}$, one has simply

$$G'(r) = F_1(r) - F_2(r) + r \partial_r F_1(r) + (1 - r) \partial_r F_2(r). \quad (D43)$$

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 (D39), one has in particular

$$F_1(1) = F_2(1). \quad (D44)$$

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 (B17)). 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

$$\partial_D F_1(r) = -r \frac{\partial}{\partial (D/r)} \tilde{F}_1(D/r). \quad (D45)$$

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

$$\partial_r F_1(1) = 0. \quad (D46)$$

Finally, from $\dot{S}_2 = 0$, for all $r \in \mathcal{V}_1 \cap \{ r < 1 \}$, one gets

$$F_2(D, r) = \frac{D}{1 - r} (S_1^*(D, r) - S_2^*(D, r)). \quad (D47)$$

Differentiating (D47) with respect to $r$ gives

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

which can be written equivalently as

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

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

$$(1 - r) \partial_r F_2(r) = \overline{D}(r) (\partial_r S_1^*(r) - \partial_r S_2^*(r)) + F_2(r).$$
Notice that for \( D = \bar{D}(r) \), (D47) gives
\[
F_2(r) = \frac{\bar{D}(r)}{1 - r} (\bar{S}_1^*(r) - \bar{S}_2^*(r)), \quad \text{for all } r \in V_1 \cup \{ r < 1 \}.
\] (D48)

Using L'Hôpital’s rule in (D48) when \( r \) tends to 1, one gets
\[
F_2(1) = \lim_{r \to 1^-} \frac{\bar{D}(r)(\bar{S}_1^*(r) - \bar{S}_2^*(r)) + \bar{D}(r)(\partial_r S_1^*(r) - \partial_r S_2^*(r))}{1 - r}.
\]
and using (D38) and (D44), one obtains
\[
F_1(1) = \lim_{r \to 1^-} -\bar{D}(r)(\partial_r S_1^*(r) - \partial_r S_2^*(r)).
\]

Consequently, one has
\[
\lim_{r \to 1^-} (1 - r)\partial_r F_2(r) = 0. \tag{D49}
\]

With (D44), (D46) and (D49), expression (D43) gives the existence of the limit of \( \bar{G}' \) when \( r \) tends to 1 with \( r < 1 \), which is
\[
\bar{G}'(1^-) = 0. \tag{D50}
\]

Note that \( \bar{G}''(1^-) \) exists if and only if \( \lim_{r \to 1^-} \frac{\bar{G}'(r) - \bar{G}'(1^-)}{r - 1} \) exists. Using (D50) and (D43), one has
\[
\frac{\bar{G}'(r) - \bar{G}'(1^-)}{r - 1} = -\frac{\bar{G}'(r)}{1 - r} = -\frac{F_1(r) - F_2(r) + \partial_r F_1(r) + (1 - r)\partial_r F_2(r)}{1 - r}. \tag{D51}
\]

On the one hand, using L’Hôpital’s rule, one has
\[
\lim_{r \to 1^-} \frac{\bar{F}_1(r) - \bar{F}_2(r)}{1 - r} = \lim_{r \to 1^-} \frac{\bar{F}_1'(r) - \bar{F}_2'(r)}{1 - r}.
\]

Recall that \( \partial_r F_1(1) = 0 \) and thus one has \( \bar{F}_1'(1) = 0 \). Consequently, one has
\[
\lim_{r \to 1^-} \frac{\bar{F}_1(r) - \bar{F}_2(r)}{1 - r} = \lim_{r \to 1^-} \bar{F}_2'(r) = \lim_{r \to 1^-} \partial_r F_2(r) + \partial_D F_2(r) \bar{D}'(r). \tag{D52}
\]

On the other hand, using (D42) and (D45), one has
\[
\frac{r}{1 - r} \partial_r F_1(r) = \frac{\bar{D}(r)}{r} \partial_D F_2(r). \tag{D53}
\]
Thus, according to (D51), (D52) and (D53), one gets

$$\lim_{r \to 1^-} \frac{\overline{G}'(r) - \overline{G}'(1^-)}{r - 1} = \lim_{r \to 1^-} -2\partial_r F_2(r) - \partial_D F_2(r) \left( \overline{D}'(r) + \frac{\overline{D}(r)}{r} \right)$$  \hspace{1cm} (D54)

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

$$\overline{F} = f' \left( S^*_2 \right) \frac{\partial S^*_2}{\partial r} x^*_2 + f \left( S^*_2 \right) \frac{\partial x^*_2}{\partial r}. $$

Let us use the expression \(G(D, r) = D(S^{\text{in}} - S^*_2(D, r))\) given by Proposition 4. As \(\overline{D}(r)\) is a maximizer, then one has

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

Using (D38), one then deduces

$$\partial_D S^*_2(1^-) = \frac{S^{\text{in}} - \lambda \left( \overline{D}(1) + a \right)}{\overline{D}(1)}. $$

In addition, using expressions (B18) and (D38), one gets

$$\partial_D x^*_2(1^-) = -\frac{\overline{D}(1)}{(\overline{D}(1) + a)^2} \left( S^{\text{in}} - \lambda \left( \overline{D}(1) + a \right) \right), $$

and hence the limit of \(\partial_D F_2\) when \(r\) tends to 1 exists:

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

where \(A = S^{\text{in}} - \lambda \left( \overline{D}(1) + a \right) - \frac{\overline{D}(1)}{f' \left( \lambda \left( \overline{D}(r) + a \right) \right)} \). Thus, one has

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

with \(g\) defined by (A8). According to Proposition 9, one has \(S^{\text{in}} - g \left( \overline{D}(1) \right) = 0\). 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

$$\frac{\partial F_2}{\partial r} = f' \left( S^*_2 \right) \frac{\partial S^*_2}{\partial r} x^*_2 + f \left( S^*_2 \right) \frac{\partial x^*_2}{\partial r}. $$

Let us use again the expression \(G(D, r) = D(S^{\text{in}} - S^*_2(D, r))\). According to (D41), one has

$$\overline{G}'(r) = \partial_r G(r) + \partial_D G(r) \overline{D}'(r)$$
where $\partial_D G(r) = 0$. According to (D50), we have $\partial_r G(1^-) = 0$, and thus $\partial_r S^*_2(1^-) = 0$. Using expression (B18), one gets

$$\partial_r x^*_2(1^-) = -a\bar{D}(1) \frac{S^\text{in} - \lambda(\bar{D}(1) + a)}{(\bar{D}(1) + a)^2},$$

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

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

As $\bar{D}'$ is assumed to be bounded on $V_1 \cup \{r < 1\}$, we thus obtain from (D54) the existence of $\bar{G}''(1^-)$ with

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

which is given by expression (43).

References

Abdellatif N, Fekih-Salem R, Sari T (2016) Competition for a single resource and coexistence of several species in the chemostat. Math Biosci Eng 13:631–652
Bar B, Sari T (2020) The operating diagram for a model of competition in a chemostat with an external lethal inhibitor. Discrete Contin Dyn Syst B 25:2093–2120
Bastin G, Dochain D (1991) On-line estimation and adaptive control of bioreactors. Elsevier, Amsterdam
Bornhöft A, Hanke-Rauschenbach R, Sundmacher K (2013) Steady state analysis of the anaerobic digestion model no. 1 (ADM1). Nonlinear Dyn 73:535–549
Crespo M, Rapaport A (2020) About the chemostat model with a lateral diffusive compartment. J Optim Theory Appl 185:597–621
Dali-Youcef M, Sari T (2021) The productivity of two serial chemostats. https://hal.inrae.fr/hal-03445797
Dali-Youcef M, Rapaport A, Sari T (2020) Study of performance criteria of serial configuration of two chemostats. Math Biosci Eng 17(6):6278–6309
Dali-Youcef M, Harmand J, Rapaport A, Sari T (2022) Some non-intuitive properties of serial chemostats with and without mortality. In: Mathmod 2022, 10th Vienna International Conference on Mathematical Modelling, Jul 2022, Vienna, Austria. https://hal.inrae.fr/hal-03404740
Daoud Y, Abdellatif N, Sari T, Harmand J (2018) Steady-state analysis of a syntrophic model: the effect of a new input substrate concentration. Math Model Nat Phenom 13:31
de Gooijer C, Bakker W, Beefink H, Tramper J (1996) Bioreactors in series: an overview of design procedures and practical applications. Enzyme Microb Technol 18:202–219
Dellal M, Lakrib M, Sari T (2018) The operating diagram of a model of two competitors in a chemostat with an external inhibitor. Math Biosci 302:27–45
Fekih-Salem R, Lobry C, Sari T (2017) A density-dependent model of competition for one resource in the chemostat. Math Biosci 286:104–122
Fekih-Salem R, Daoud Y, Abdellatif N, Sari T (2021) A mathematical model of anaerobic digestion with syntrophic relationship, substrate inhibition and distinct removal rates. SIAM J Appl Dyn Syst 20:621–1654
Fogler S (2008) Elements of chemical reaction engineering, 4th edn. Prentice Hall, New-York
Haidar I, Rapaport A, Gérard F (2011) Effects of spatial structure and diffusion on the performances of the chemostat. Math Biosci 286:104–122
Harmand J, Rapaport A, Trofino A (1999) Optimal design of two interconnected bioreactors-some new results. AIChE J 49(6):1433–1450

© Springer
Harmand J, Lobry C, Rapaport A, Sari T (2017) The chemostat: mathematical theory of microorganism cultures. Chemical engineering series. Wiley, New York
Khedim Z, Benyahia B, Cherki B, Sari T, Harmand J (2018) Effect of control parameters on biogas production during the anaerobic digestion of protein-rich substrates. Appl Math Model 61:351–376
Kung CM, Baltzis BC (1992) The growth of pure and simple microbial competitors in a moving and distributed medium. Math Biosci 111:295–313
Levenspiel O (1999) Chemical reaction engineering, 3rd edn. Wiley, New York
Li B (1998) Global asymptotic behavior of the chemostat: general response functions and differential removal rates. SIAM J Appl Math 59:411–4
Lovitt RW, Wimpenny JWT (1979) The gradostat: a tool for investigating microbial growth and interactions in solute gradients. Soc Gen Microbial Quart 6:80
Lovitt RW, Wimpenny JWT (1981) The gradostat: a bidirectional compound chemostat and its applications in microbiological research. J Gen Microbiol 127:261–268
Luyben K, Tramper J (1982) Optimal design for continuously stirred tank reactors in series using Michaelis–Menten kinetics. Biotechnol Bioeng 24:1217–1220
Nelson M, Sidhu H (2006) Evaluating the performance of a cascade of two bioreactors. Chem Eng Sci 61:3159–3166
Pavlou S (1999) Computing operating diagrams of bioreactors. J Biotechnol 71:7–16
Polihronakis M, Petrou L, Deligiannis A (1993) Parameter adaptive control techniques for anaerobic digesters-real-life experiments. Comput Chem Eng 17(12):1167–1179
Rapaport A, Harmand J (2008) Biological control of the chemostat with nonmonotonic response and different removal rates. Math Biosci Eng 5(3):539–547
Rapaport A, Haidar I, Harmand J (2015) Global dynamics of the buffered chemostat for a general class of growth functions. J Math Biol 71(1):69–98
Reh T, Muller J (2013) CO2 abatement costs of greenhouse gas (GHG) mitigation by different biogas conversion pathways. J Environ Manag 114(15):13–25
Sari T (2022) Best operating conditions for biogas production in some simple anaerobic digestion models. Processes 10:258
Sari T, Benyahia B (2021) The operating diagram for a two-step anaerobic digestion model. Nonlinear Dyn 105:2711–2737
Sari T, Harmand J (2016) A model of a syntrophic relationship between two microbial species in a chemostat including maintenance. Math Biosci 275:1–9
Sari T, Mazenc F (2011) Global dynamics of the chemostat with different removal rates and variable yields. Math Biosci Eng 8(3):827–40
Sari T, Wade MJ (2017) Generalised approach to modelling a three-tiered microbial food-web. Math Biosci 291:21–37
Sbarciog M, Loccuferi M, Noldus E (2010) Determination of appropriate operating strategies for anaerobic digestion systems. Biochem Eng J 51:180–188
Smith H (1991) The gradostat: a model of competition along a nutrient gradient. Microb Ecol 22(1):207–26
Smith H, Waltman P (1995) The theory of the chemostat, dynamics of microbial competition. Cambridge University Press, Cambridge
Smith H, Tang B, Waltman P (1991) Competition in a n-vessel gradostat. SIAM J Appl Math 91(5):1451–1471
Tang B (1986) Mathematical investigations of growth of microorganisms in the gradostat. J Math Biol 23:319–339
Wade MJ, Pattinson RW, Parker NG, Dolfing J (2016) Emergent behaviour in a chlorophenol-mineralising three-tiered microbial ‘food web’. J Theor Biol 389:171–186
Walter W (1998) Ordinary Differential Equations. Springer Graduate Texts in Mathematics, 182
Weedermann M, Seo G, Wolkwics GSK (2013) Mathematical model of anaerobic digestion in a chemostat: effects of syntrophy and inhibition. J Biol Dyn 7:59–85
Weedermann M, Wolkwicz GSK, Sasara J (2015) Optimal biogas production in a model for anaerobic digestion. Nonlinear Dyn 81:1097–1112
Wolkwicz GSK, Lu Z (1992) Global dynamics of a mathematical model of competition in the chemostat: general response functions and differential death rates. SIAM J Appl Math 52:222–23
Xu A, Dolfing J, Curtis TP, Montague G, Martin E (2011) Maintenance affects the stability of a two-tiered microbial ‘food chain’. J Theor Biol 276:35–41
Zambrano J, Carlsson B (2014) Optimizing zone volumes in bioreactors described by Monod and Contois growth kinetics. In: Proceeding of the IWA World Water Congress & Exhibition, Lisbon, Portugal
Zambrano J, Carlsson B, Diehl S (2015) Optimal steady-state design of zone volumes of bioreactors with Monod growth kinetics. Biochem Eng J 100:59–66

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Springer Nature or its licensor holds exclusive rights to this article under a publishing agreement with the author(s) or other rightsholder(s); author self-archiving of the accepted manuscript version of this article is solely governed by the terms of such publishing agreement and applicable law.