



HAL
open science

The buffered chemostat with non-monotonic response functions

Alain Rapaport, Ihab Haidar, Jérôme Harmand

► **To cite this version:**

Alain Rapaport, Ihab Haidar, Jérôme Harmand. The buffered chemostat with non-monotonic response functions. 9th IFAC Symposium on Nonlinear Control Systems (NOLCOS 2013), Sep 2013, Toulouse, France. Paper WeB2.6. hal-00766243v1

HAL Id: hal-00766243

<https://hal.science/hal-00766243v1>

Submitted on 17 Dec 2012 (v1), last revised 25 May 2014 (v2)

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

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

The buffered chemostat with non-monotonic response functions

A. Rapaport^{1,*}, I. Haidar² and J. Harmand^{3,*}

¹ MISTEA, UMR INRA-SupAgro, Montpellier, France

² LSS-Supelec, Gif-sur-Yvette, France

³ LBE, INRA, Narbonne, France

* MODEMIC, INRIA Sophia-Antipolis Méditerranée, France

December 17, 2012

Abstract

We show how a particular spatial structure with a buffer globally stabilizes the chemostat dynamics with non-monotonic response function, while this is not possible with single, serial or parallel chemostats of the same total volume and input flow. We give a characterization of the set of such configurations that satisfy this property, as well as the configuration that ensures the best nutrient conversion. Furthermore, we characterize the minimal buffer volume to be added to a single chemostat for obtaining the global stability. These results are illustrated with the Haldane kinetic function.

Key-words. chemostat, interconnection, bi-stability, global asymptotic stability, optimization.

AMS subject classifications. 92D25, 34D23, 93A30, 90B05.

1 Introduction

The chemostat has been introduced in the fifties as an experimental device to study of microbial growth on a limiting resource [29, 32]. It is also often used as a mean to reproduce situations where (limiting) nutrients are fed to micro-organisms, typically in a liquid medium, such as in natural ecosystems [16, 5] or anthropized environments [23]. More generally, the chemostat is largely used as a scientific investigation tool in microbial ecology [21, 45].

The mathematical model of the chemostat has been extensively studied (see e.g. [40]) and used as a reference model in microbiology [33], microbial ecology [10] or biotechnological industries such as the wastewater treatment industry [7]. However, in many applications, the assumption of perfectly mixed chemostats has to be relaxed. In the eighties, the gradostat, as an experimental device composed of a set of chemostats of identical volume interconnected in series, has been proposed to represent spatial gradient [25]. For instance, it has been used to reproduce marine environment [18] or to model rhizosphere [11], and has motivated several mathematical studies [43, 20, 9, 37, 47, 39, 17, 41, 12]. Similarly, an interest for series of bioreactors appeared in biochemical industry, with tanks of possibly different volumes to be optimized [26, 19, 4, 15, 8]. Although island models have been proposed in ecology since the late sixties [27], relatively few studies have considered non-serial interconnections of chemostats [38]. In natural reservoirs such as in undergrounds or ground-waters, a spatial structure with interconnections between several volumes is often considered, each of them being approximated as perfectly mixed. Those interconnections can be parallel, series or built up in more complex networks. To our knowledge, the influence of the topology of a network of chemostats on the overall dynamics has been sparsely investigated in the literature. However, the simple consideration of two different habitats can lead to non-intuitive behaviors [42, 30, 35, 22] and influence significantly the overall performances [31, 13].

It is also well-known since the seventies that microbial growth can be inhibited by large concentrations of nutrient. Such inhibition can be modeled by non-monotonic response functions [1, 3] and lead to instability in the chemostat [2, 46, 24]. Several control strategies of the input flow have been proposed in the literature to globally stabilize such systems [6, 14, 34, 36] but the ability of a spatial structure to passively stabilize such dynamics has not been yet studied (in [38] a general structure of networks of chemostats is considered but with monotonic growth rates, while in [44] non-monotonic functions are considered but for the serial gradostat only).

The present work considers non-monotonic response functions with a particular interconnection of two chemostats of different volumes, one being a buffer tank. To our knowledge, this spatial structure, that is neither serial nor parallel, has not yet been considered in the literature. The idea is to decouple the residence time of microorganisms in two vessels such that the wash-out equilibrium is repulsive in both tanks. We prove that this is possible with such a configuration, while any serial, parallel or single tank structures with the same total volume exhibits bi-stability. This result brings new insights in microbial ecology for the understanding of the role of spatial patterns in the stability of bio-conversion processes in natural environments, where natural buffers can occur, such as in soil ecosystems. It has also potential impact on the design of robust industrial bio-processes.

The paper is organized as follows. Section 2 presents the hypotheses and the buffered configuration, comparing with serial and parallel interconnections. Section 3 studies the multiplicity of equilibria and provides a complete characterization of the set \mathcal{C} of configurations that have a unique positive equilibrium. It is then shown that this equilibrium is globally asymptotically stable. Section 4 analyzes the performance of the buffered chemostat, characterizing first the best configurations among \mathcal{C} in terms of nutrient conversion at steady state, and then the minimal volume of a buffer to be added to a single chemostat to make the interconnected system globally stable. Finally, in Section 5 numerical simulations illustrate the results on the Haldane function.

2 General considerations

We consider the chemostat model with a single strain growing on a single limiting nutrient. The system is fed with nutrient of concentration S_{in} with flow rate Q . The total volume V is assumed to be constant (i.e. input and output flow rates are supposed to be identical). When the concentrations of nutrient (or substrate) and biomass, denoted respectively S and X , are homogeneous, as it is the case in perfectly mixed tanks, the system can be modeled by the well-known equations:

$$\begin{aligned}\dot{S} &= -\frac{\mu(S)}{Y}X + \frac{Q}{V}(S_{in} - S), \\ \dot{X} &= \mu(S)X - \frac{Q}{V}X,\end{aligned}\tag{1}$$

where $\mu(\cdot)$ is the uptake function and Y the yield coefficient of the transformation of nutrient into biomass. Changing the units in which time t , growth $\mu(\cdot)$ and biomass X are measured, one can assume without any loss of generality that $D = Q/V = 1$ and $Y = 1$ (replacing t by t/D , $\mu(\cdot)$ by $D\mu(\cdot)$ and X by YX).

In this work, we consider specifically strain growths that present an inhibition, described by the following assumption.

Assumption A1. The function $\mu(\cdot)$ is $C^\infty([0, +\infty))$ and such that $\mu(0) = 0$, $\mu(S) > 0$ for any $S > 0$. Moreover there exists a number $\hat{S} > 0$ such that μ is increasing on $(0, \hat{S})$ and decreasing on $(\hat{S}, +\infty)$.

For instance, the Haldane function [1]

$$\mu(S) = \frac{\bar{\mu}S}{K + S + S^2/K_I}\tag{2}$$

fulfills Assumption A1 (see Figure 1). Classically, we consider the set

$$\Lambda = \{S > 0 \mid \mu(S) > 1\} \quad (3)$$

that plays an important role in the determination of the equilibriums of the system. Under Assumption A1, the set Λ is either empty of an open interval that we shall denote

$$\Lambda = (\lambda_-, \lambda_+) ,$$

where λ_+ can be equal to $+\infty$.

We recall from the theory of the chemostat model [40] the three kinds of solutions flow of the dynamics (1) under Assumption A1, depending on the parameter S_{in} .

Proposition 1. *Assume that Hypothesis A1 is fulfilled.*

- *Case 1: $\Lambda = \emptyset$ or $\lambda_+ > S_{in}$. The wash-out equilibrium $E_0 = (S_{in}, 0)$ is the unique non negative equilibrium of system (1). Furthermore it is globally attracting.*
- *Case 2: $S_{in} > \lambda_+$. The system (1) has three non-negative equilibriums $E_- = (\lambda_-, S_{in} - \lambda_-)$, $E_+ = (\lambda_+, S_{in} - \lambda_+)$ and $E_0 = (S_{in}, 0)$. Only E_- and E_0 are attracting, and the dynamics is bistable.*
- *Case 3: $S_{in} \in \Lambda$. The system (1) has two non negative equilibriums $E_- = (\lambda_-, S_{in} - \lambda_-)$ and $E_0 = (S_{in}, 0)$. E_- is globally attracting on the positive quadrant.*

In case 2, the issue of the growth can change radically depending on the initial condition. Throughout the paper, we shall consider uptake functions $\mu(\cdot)$ and values of S_{in} that fulfill the conditions of Case 2, so that the system exhibits bi-stability in the classical chemostat model (1).

Assumption A2. $\lambda_- < \lambda_+ < S_{in}$.

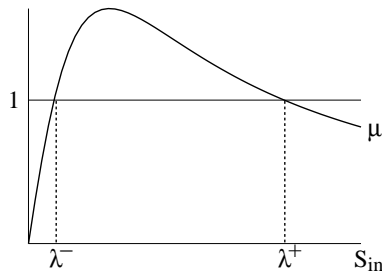


Figure 1: Graph of the Haldane function and illustration of Assumption A2.

The question we investigate in this paper is related to the assumption that the vessel is perfectly mixed, and to the role that a spatial structure could have on the stability of the dynamics. We shall consider spatial configurations with the same input flow and residence time than the perfectly mixed case, that is to say with the same total volume V and input flow Q . Under Assumption A2, one can check that having a volume V split in several smaller volumes V_i interconnected in series or in parallel, assuming that each of them is perfectly mixed, leads necessarily to a global dynamics having bi-stability or the wash-out as the only equilibrium in at least one of the tanks.

- In the series connection, the dynamics of the first tank of volume V_1 is given by equations (1) where V is replaced by $V_1 \leq V$. Its dilution rate is then equal to Q/V_1 , that is greater than $Q/V = 1$. According to Proposition 1 only Cases 1 or 2 can occur.

- In the parallel connection, the dynamics of each tank of volume V_i and flow rate Q_i is given by equations (1) where V and Q are replaced by V_i and Q_i . Denote $r_i = V_i/V$ and $\alpha_i = Q_i/Q$, and note that one has $\sum_i r_i = \sum_i \alpha_i = 1$. Then, the dilution rate D_i in the tank i is equal to α_i/r_i . According to Proposition 1, a necessary condition for having a single attracting equilibrium in each tank is to have $D_i < 1$ for any i , that contradicts $\sum_i r_i = \sum_i \alpha_i = 1$.

In the present work, we study a particular spatial configuration with a asymmetry created by two interconnected volumes, one of them serving as a buffer (see Figure 2), that we shall call the “buffered chemostat”, to be compared with the “single chemostat”. V_1 and V_2 are respectively the volumes of the main tank and

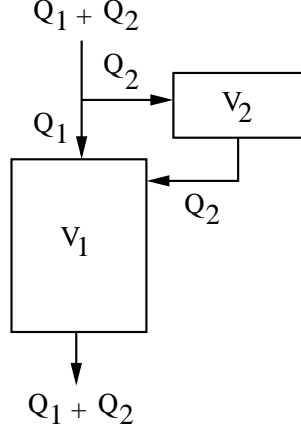


Figure 2: The buffered chemostat.

the buffer, and Q_1 and Q_2 denote the input flow rates of each tank. We assume that each vessel is perfectly mixed. Straightforwardly, the dynamical equations of the buffered chemostat are

$$\begin{aligned}
 \dot{S}_1 &= -\mu(S_1)X_1 + \frac{Q_1 S_{in} + Q_2 S_2 - Q S_1}{V_1}, \\
 \dot{X}_1 &= \mu(S_1)X_1 + \frac{Q_2 X_2 - Q X_1}{V_1}, \\
 \dot{S}_2 &= -\mu(S_2)X_2 + \frac{Q_2 S_{in} - Q_2 S_2}{V_2}, \\
 \dot{X}_2 &= \mu(S_2)X_2 - \frac{Q_2 X_2}{V_2}.
 \end{aligned} \tag{4}$$

Note that the limiting case $V_1 = 0$ consists in a by-pass of the volume V_2 with a flow Q_1 .

In the following sections, we shall consider configurations with the same total volume $V = V_1 + V_2$ and input flow $Q = Q_1 + Q_2$, to be compared with the single chemostat ($V_1 = V$ and $V_2 = 0$). In Section 4, we shall also consider configurations with a fixed volume $V_1 = V$ and study the benefit of adding a buffer of volume V_2 , under a constant total input flow $Q = Q_1 + Q_2$.

3 Study of multiplicity of equilibriums

Given $\mu(\cdot)$ and S_{in} such that Assumptions A1 and A2 are fulfilled, we describe the set of all possible configurations such that $Q = Q_1 + Q_2$ and $V = V_1 + V_2$ (with $Q/V = 1$) by two parameters $r \in (0, 1)$ and $\alpha > 0$ defined as follows

$$r = \frac{V_1}{V}, \quad \alpha = \frac{Q_2}{(1-r)Q}.$$

Dynamics (4) can then be written in the following way

$$\begin{aligned}
\dot{S}_1 &= -\mu(S_1)X_1 + \frac{\alpha(1-r)(S_2 - S_1) + (1 - \alpha(1-r))(S_{in} - S_1)}{r}, \\
\dot{X}_1 &= \mu(S_1)X_1 + \frac{\alpha(1-r)(X_2 - X_1) + (1 - \alpha(1-r))X_1}{r}, \\
\dot{S}_2 &= -\mu(S_2)X_2 + \alpha(S_{in} - S_2), \\
\dot{X}_2 &= \mu(S_2)X_2 - \alpha X_2.
\end{aligned} \tag{5}$$

One can easily see that an equilibrium $(S_1^*, X_1^*, S_2^*, X_2^*)$ of dynamics (5) is the solution of the following equations:

$$1 + \frac{1-r}{r} \left(1 - \alpha \frac{S_{in} - S_2^*}{S_{in} - S_1^*} \right) = \mu(S_1^*) \text{ or } S_1^* = S_{in}, \tag{6}$$

$$X_1^* = S_{in} - S_1^*, \tag{7}$$

$$\mu(S_2^*) = \alpha \text{ or } S_2^* = S_{in}, \tag{8}$$

$$X_2^* = S_{in} - S_2^*. \tag{9}$$

Due to the cascade structure of the model (4), the study of the dynamics of the second reactor can be done independently of the first one. Depending of the value of α , the three cases depicted in Proposition 1 for the single chemostat are possible.

Under Assumptions A1 and A2, one can straightforwardly check from equations (8) and (9) that there exists an unique positive equilibrium (S_2^*, X_2^*) in the second tank exactly when α belongs to the set $(0, \mu(S_{in}))$. For any number $\alpha \in (0, \mu(S_{in}))$, we then denote $S_2^*(\alpha) = S_2^* \in (0, S_{in})$ the unique solution of the equation

$$\mu(S_2^*(\alpha)) = \alpha. \tag{10}$$

We shall consider the family of hyperbola $H_{\alpha,r}$ that are the graphs of the functions

$$\phi_{\alpha,r}(s) = 1 + \frac{1-r}{r} \left(1 - \alpha \frac{S_{in} - S_2^*(\alpha)}{S_{in} - s} \right) \tag{11}$$

parametrized by $\alpha \in (0, \mu(S_{in}))$ and $r \in (0, 1)$. From equations (6) and (7), a positive equilibrium (S_1^*, X_1^*) of (5) has to fulfill precisely

$$\phi_{\alpha,r}(S_1^*) = \mu(S_1^*)$$

that is to claim that S_1^* is the abscissa of an intersection of the graph of $\mu(\cdot)$ with the hyperbola $H_{\alpha,r}$. To each solution S_1^* corresponds an unique $X_1^* = S_{in} - S_1^*$. We define the family of sets

$$\mathcal{R}(\alpha) = \{r \in (0, 1) \mid \exists! s \in (0, S_{in}) \text{ s.t. } \phi_{\alpha,r}(s) = \mu(s)\} \tag{12}$$

parametrized by $\alpha \in (0, \mu(S_{in}))$. The set \mathcal{C} of pairs (α, r) such that dynamics (5) admits an unique positive equilibrium is then defined by

$$\mathcal{C} = \{(\alpha, r) \mid \alpha \in (0, \mu(S_{in})), r \in \mathcal{R}(\alpha)\}. \tag{13}$$

For convenience, we shall consider the set of s at which the hyperbola $H_{\alpha,r}$ is tangent to the graph of the function $\mu(\cdot)$ and is locally on one side (that amounts to have 0 as a local extremum of the function $\phi_{\alpha,r}(\cdot) - \mu(\cdot)$ at s):

$$\mathcal{S}_{\alpha,r} = \left\{ s \in (\lambda_-, S_{in}) \text{ s.t. } \min \left\{ n \in \mathbb{N} \mid \frac{d^n \phi_{\alpha,r}}{ds^n}(s) \neq \frac{d^n \mu}{ds^n}(s) \right\} \text{ is even and larger than } 1 \right\} \tag{14}$$

and define the number

$$\underline{S}(\alpha) = \alpha S_2^*(\alpha) + (1 - \alpha) S_{in} . \quad (15)$$

We consider two subsets of values of r such that the hyperbola $H_{\alpha,r}$ is tangent to the graph of $\mu(\cdot)$.

$$R^-(\alpha) = \{r \in (0, 1) \mid \exists s \in \mathcal{S}_{\alpha,r} \text{ with } (s - \underline{S}(\alpha))(\lambda_+ - \underline{S}(\alpha)) < 0\} , \quad (16)$$

$$R^+(\alpha) = \{r \in (0, 1) \mid \exists s \in \mathcal{S}_{\alpha,r} \text{ with } (s - \lambda_+)(\lambda_+ - \underline{S}(\alpha)) \geq 0\} . \quad (17)$$

We state now our main result about the multiplicity of equilibriums of system (5) and give a characterization of the sets $\mathcal{R}(\alpha)$ defined in (12), depending on the sets $R^-(\alpha)$, $R^+(\alpha)$ and their interlacing. In Section 5, this result is applied to the Haldane function (2).

Proposition 2. *Assume that Hypotheses A1 and A2 are fulfilled. For any $\alpha \in (0, \mu(S_{in})]$ and $r \in (0, 1)$ the dynamics (5) admits a positive equilibrium with S_1^* such that*

$$(\underline{S}(\alpha) - S_1^*)(\lambda_+ - \underline{S}(\alpha)) \geq 0 . \quad (18)$$

The set $R^+(\alpha)$ is non empty, and the set $R^-(\alpha)$ is not reduced to a singleton when it is not empty. One has

$$\mathcal{R}(\alpha) = \begin{cases} (0, \min R^+(\alpha)) & \text{when } R^-(\alpha) = \emptyset , \\ (0, \min R^+(\alpha)) \cap (0, 1) \setminus [\min R^-(\alpha), \max R^-(\alpha)] & \text{when } R^-(\alpha) \neq \emptyset . \end{cases} \quad (19)$$

Furthermore,

- for any $r \in (\min R^+(\alpha), 1)$, there exists at least two equilibriums such that $(\underline{S}(\alpha) - S_1^*)(\lambda_+ - \underline{S}(\alpha)) \geq 0$, and at least four for r in a subset of $(\min R^+(\alpha), 1)$ when $R^+(\alpha)$ is not reduced to a singleton,
- when $R^-(\alpha)$ is non empty, for any $r \in (\min R^-(\alpha), \max R^-(\alpha))$, there exists at least three equilibriums such that $(\underline{S}(\alpha) - S_1^*)(\lambda_+ - \underline{S}(\alpha)) < 0$.

Proof. Fix $\alpha \in (0, \mu(S_{in})]$ and simply denote by S_2^* and \underline{S} the values of $S_2^*(\alpha)$ and $\underline{S}(\alpha)$. For each $r \in (0, 1)$, we define the function

$$f_r(s) = \phi_{\alpha,r}(s) - \mu(s) .$$

A positive equilibrium for the first tank has then to satisfy $f_r(S_1^*) = 0$.

One can easily check that $\phi_{\alpha,r}(\underline{S}) = 1$ whatever the value of $r \in (0, 1)$. $\phi_{\alpha,r}(\cdot)$ being decreasing, one has $\phi_{\alpha,r}(s) > 1$ for $s < \underline{S}$ and $\phi_{\alpha,r}(s) < 1$ for $s > \underline{S}$. For convenience, we shall also consider the function

$$\gamma(s) = \frac{\underline{S} - s}{\underline{S} - S_{in} + (S_{in} - s)\mu(s)} \quad (20)$$

that is defined on the set of $s \in (0, S_{in})$ such that $(S_{in} - s)\mu(s) \neq S_{in} - \underline{S}$. On this set, one can easily check that the following equivalence is fulfilled

$$f_r(s) = 0 \iff \gamma(s) = r .$$

From (20), one can also write

$$\gamma(s) = \frac{(\phi_{\alpha,r}(s) - 1)\frac{r}{1-r}}{(\phi_{\alpha,r}(s) - 1)\frac{r}{1-r} - 1 + \mu(s)}$$

and deduce the property

$$\gamma'(s) = 0 \iff \phi'_{\alpha,r}(s)(\mu(s) - 1) = (\phi_{\alpha,r}(s) - 1)\mu'(s) . \quad (21)$$

Recursively, one obtains for every integer n

$$\left\{ \frac{d^p \gamma}{ds^p}(s) = 0, p = 1 \cdots n \right\} \iff \left\{ \frac{d^p \phi_{\alpha,r}}{ds^p}(s)(\mu(s) - 1) = (\phi_{\alpha,r}(s) - 1) \frac{d^p \mu}{ds^p}(s), p = 1 \cdots n \right\} .$$

Consequently, the set $\mathcal{S}_{\alpha,r}$ defined in (14) can be characterized as

$$\mathcal{S}_{\alpha,r} = \left\{ s \in (\lambda_-, S_{in}) \text{ s.t. } \gamma(s) = r \text{ and } \min \left\{ n \in \mathbb{N}^* \mid \frac{d^n \gamma}{ds^n}(s) \neq 0 \right\} \text{ is even} \right\}$$

or equivalently

$$\mathcal{S}_{\alpha,r} = \{s \in (\lambda_-, S_{in}) \text{ s.t. } \gamma(s) = r \text{ is a local extremum} \} . \quad (22)$$

We distinguish now several case depending on the position of \underline{S} with respect to λ_+ .

Case 1: $\underline{S} < \lambda_+$.

If $\underline{S} \leq \lambda_-$, one has $f_r(\underline{S}) \geq 0$ and $f_r(S) < 0$ for any $S \in \Lambda$. $f_r(\cdot)$ being decreasing on $[0, \lambda_-]$, one deduces that there exists exactly one solution S_1^* of $f_r(S) = 0$ on the interval $[0, \lambda_+]$, whatever is r . Furthermore, this solution has to belong to $[\underline{S}, \lambda_-]$. The functions $\phi_r(\cdot)$ and $\mu(\cdot)$ being respectively decreasing and increasing on this interval, one has necessarily $\gamma'(S_1^*) \neq 0$ and then $R^-(\alpha) = \emptyset$.

If $\underline{S} > \lambda_-$, one has $f_r(S) > 0$ for any $S \in [0, \lambda_-]$, and $f_r(S) < 0$ for any $S \in [\underline{S}, \lambda_+]$. On the interval $I = (\lambda_-, \underline{S})$, the function $\gamma(\cdot)$ is well defined and $\gamma(I) = (0, 1)$ with $\gamma(\lambda_-) = 1$ and $\gamma(\underline{S}) = 0$. If $R^-(\alpha)$ is empty, then $\gamma(\cdot)$ is decreasing on I , and for any $r \in (0, 1)$ there exists a unique $S_1^* \in I$ such that $\gamma(S_1^*) = r$. If $R^-(\alpha)$ is not empty, property (22) implies that γ admits local extrema. Let r_m^-, r_M^- be respectively the smallest local minimum and the largest local maximum of $\gamma(\cdot)$ on the interval I . Note that $r_m^- > 0$ and $r_M^- < 1$ because $\gamma(I) = (0, 1)$, and that one has $r_m^- = \min R^-(\alpha) < r_M^- = \max R^-(\alpha)$. By the Mean Value Theorem, there exists exactly one solution S_1^* of $\gamma(s) = r$ on the interval $[0, \lambda_+]$ for any $r \notin [r_m^-, r_M^-]$, and there are at least three solutions for $r \in (r_m^-, r_M^-)$.

Consider now the interval $J = (\lambda_+, S_{in})$ where the function $\gamma(\cdot)$ is well defined and positive with $\gamma(\lambda_+) = 1$ and $\lim_{s \rightarrow S_{in}} \gamma(s) = 1$. We define

$$r^+ = \min\{\gamma(s) \mid s \in J\}$$

that belongs to $(0, 1)$. Then r^+ belongs to $R^+(\alpha)$, and for any $r < r^+$ there is no solution of $\gamma(s) = r$ on J . Thus r^+ is the minimal element of $R^+(\alpha)$. By the Mean Value Theorem there are at least two solutions of $\gamma(s) = r$ on J when $r > r^+$. When $R^+(\alpha)$ is not reduced to a singleton, the function γ has at least one local maximum r_M and one local minimum r_m , in addition to r^+ . By the Mean Value Theorem, there are at least four solutions of $\gamma(s) = r$ on J for $r \in (r_m, r_M)$.

Finally, we have shown that the set $R^+(\alpha)$ is non empty, and that the uniqueness of the solution of $\gamma(S_1^*) = r$ occurs exactly for values of r that do not belong to the set $[\min R^-(\alpha), \max R^-(\alpha)] \cup [\min R^+(\alpha), 1]$.

Case 2: $\underline{S} = \lambda_+$.

One has $f_r(\underline{S}) = 0$ for any r , so there exists a positive equilibrium with $S_1^* = \underline{S}$. $f_r(S) > 0$ for any $S \in [0, \lambda_-]$ and the function $\gamma(\cdot)$ is well defined on $I \cup J = (\lambda_-, \underline{S}) \cup (\underline{S}, S_{in})$ with $\gamma(I \cup J) = (0, 1)$, $\gamma(\lambda_-) = 1$ and $\lim_{s \rightarrow S_{in}} \gamma(s) = 1$. Using the L'Hôpital's rule, we show that the function $\gamma(\cdot)$ can be continuously extended at \underline{S} :

$$\lim_{s \rightarrow \underline{S}} \gamma(s) = \lim_{s \rightarrow \underline{S}} \frac{-1}{-\mu(s) + (S_{in} - s)\mu'(s)} = \frac{1}{1 - (S_{in} - \underline{S})\mu'(\underline{S})} .$$

Note that $\mu'(\underline{S}) < 0$ so that $\gamma(\underline{S})$ belongs to $(0, 1)$, and we pose

$$\bar{r} = \min\{\gamma(s) \mid s \in (\lambda_-, S_{in})\} .$$

Then, for $r < \bar{r}$, there is no solution of $\gamma(s) = r$ on (λ_-, S_{in}) , and \underline{S} is the only solution of $f_r(s) = 0$ on $(0, S_{in})$. On the contrary, for $r > \bar{r}$, there are at least two solutions of $\gamma(s) = r$ on (λ_-, S_{in}) and the dynamics has at least two positive equilibriums.

Similarly, the function $\gamma(\cdot)$ is C^1 on (λ_-, S_{in}) because it is differentiable at \underline{S} :

$$\gamma'(\underline{S}) = \frac{(S_{in} - \underline{S})\mu''(\underline{S}) - 2\mu'(\underline{S})}{[1 - (S_{in} - \underline{S})\mu'(\underline{S})]^2}$$

(and recursively as many time differentiable as the function $\mu(\cdot)$ is, minus one). Then \bar{r} is the minimal element of the set $R^+(\alpha)$, and the set $R^-(\alpha)$ is empty by definition. As previously, when $R^+(\alpha)$ is not reduced to a singleton, $\gamma(s) = r$ has at least four solutions for r in a subset of $(r^+, 1)$.

Case 3: $\underline{S} > \lambda_+$.

We proceed similarly as in case 1. Note first that there exists no solution of $f_r(s) = 0$ on the intervals $(0, \lambda_-)$ and $(\lambda_+, \underline{S})$ whatever is r .

On the set Λ , $\gamma(\cdot)$ is well defined with $\gamma(\Lambda) \subset (0, 1)$, $\gamma(\lambda_-) = 1$ and $\gamma(\lambda_+) = 1$ and we pose

$$r^+ = \min\{\gamma(s) \mid s \in \Lambda\}$$

that belongs to $(0, 1)$. One has necessarily $r^+ = \min R^+(\alpha)$, and there is no solution of $\gamma(S_1^*) = r$ exactly when $r < r^+$. For $r > r^+$, there exists at least two solutions by the Mean Value Theorem, and four for a subset of $(r^+, 1)$ when $R^+(\alpha)$ is not reduced to a singleton.

On the interval $J = (\underline{S}, S_{in})$, the function $\gamma(\cdot)$ is well defined with $\gamma(J) = (0, 1)$, $\gamma(\underline{S}) = 0$ and $\gamma(S_{in}) = 1$. There exists at least one solution of $f_r(s) = 0$ on this interval. If $R^-(\alpha) = \emptyset$, $\gamma(\cdot)$ is increasing and there exists a unique solution of $\gamma(S_1^*) = r$ on J whatever is r . Otherwise, $\min R^-(\alpha)$ and $\max R^-(\alpha)$ are the smallest local minimum and largest local maximum of the function γ on the interval J , respectively. Then, uniqueness of S_1^* on J is achieved exactly for r that does not belong to $[\min R^-(\alpha), \max R^-(\alpha)]$, and for $r \in (\min R^-(\alpha), \max R^-(\alpha))$, there are at least three solutions by the Mean Value Theorem. \square

For each $\alpha \in (0, \mu(S_{in})]$, we define the number

$$\bar{r}(\alpha) = \sup \mathcal{R}(\alpha) . \tag{23}$$

The following remark deals with the continuity of the map $\bar{r}(\cdot)$.

Remark 1. According to Proposition 2, for any α and r such that $r \in \mathcal{R}(\alpha)$, one can define uniquely a number $S_1^*(\alpha, r) \in (0, S_{in})$ such that

$$\phi_{\alpha, r}(S_1^*(\alpha, r)) = \mu(S_1^*(\alpha, r)) .$$

The map $(\alpha, r) \mapsto S_1^*(\alpha, r)$ is clearly continuous and one can then consider the limiting map:

$$\bar{S}_1^*(\alpha) = \lim_{r < \bar{r}(\alpha), r \rightarrow \bar{r}(\alpha)} S_1^*(\alpha, r) .$$

Furthermore, accordingly to Proposition 2, one has $\bar{S}_1^*(\alpha) \leq \lambda_+$ (resp. $\bar{S}_1^*(\alpha) \geq \lambda_+$) when $\underline{S}(\alpha) < \lambda_+$ (resp. $\underline{S}(\alpha) > \lambda_+$). Consider, if it exists, a value of α , denoted by $\underline{\alpha}$, that is such that $\underline{S}(\underline{\alpha}) = \lambda_+$. Although one has $\phi_{\underline{\alpha}, r}(\lambda_+) = \mu(\lambda_+)$ for any r , there is no reason to have

$$\lim_{\alpha < \underline{\alpha}, \alpha \rightarrow \underline{\alpha}} \bar{S}_1^*(\alpha) = \lambda_+ \quad \text{or} \quad \lim_{\alpha > \underline{\alpha}, \alpha \rightarrow \underline{\alpha}} \bar{S}_1^*(\alpha) = \lambda_+ .$$

Consequently, the map $\alpha \mapsto \bar{r}(\alpha)$ might be discontinuous at such point $\underline{\alpha}$.

We consider now pairs $(\alpha, r) \in \mathcal{C}$ and denote by $S_1^*(\alpha, r)$ the corresponding value of S_1 at the unique positive steady state, that we denote $E^*(\alpha, r)$.

Let us consider first the (S_2, X_2) sub-system, that is the the single chemostat model. Several results in the literature have shown the global stability of the positive equilibrium for this model, provided that the condition $\mu(S_{in}) > \alpha$ is satisfied (see for instance proofs for the non-monotonic case in [2, 46, 24]). We present here a little extension of this result that shows global stability in the limiting case $\alpha = \mu(S_{in})$, that is not covered in the statement of Proposition 1.

Lemma 1. *For any configuration $(\alpha, r) \in \mathcal{C}$ and non-negative initial condition with $X_2(0) > 0$, the solution $S_2(t)$ and $X_2(t)$ of (5) is non negative for any $t > 0$ and one has*

$$\lim_{t \rightarrow +\infty} (S_2(t), X_2(t)) = (S_2^*(\alpha), S_{in} - S_2^*(\alpha)) .$$

Proof. From equations (5) one can write the properties

$$\begin{aligned} S_2 = 0 &\implies \dot{S}_2 > 0 , \\ X_2 = 0 &\implies \dot{X}_2 = 0 , \end{aligned}$$

and deduces that the variables $S_2(t)$ and $X_2(t)$ remain non negative for any positive time. Considering the variable $Z_2 = S_2 + X_2 - S_{in}$ whose dynamics is $\dot{Z}_2 = -\alpha Z_2$, we conclude that $S_2(t)$ and $X_2(t)$ are bounded and satisfy

$$\lim_{t \rightarrow +\infty} S_2(t) + X_2(t) = S_{in} .$$

The dynamics of the variable S_2 can thus be written as an non autonomous scalar equation:

$$\dot{S}_2 = (\alpha - \mu(S_2))(S_{in} - S_2) - \mu(S_2)Z_2(t)$$

that is asymptotically autonomous. The study of his asymptotic dynamics is straightforward: any trajectory that converges forwardly to the domain $[0, S_{in}]$ has to converge to $S_2^*(\alpha)$ or to S_{in} . Then, the application of Theorem 6 (see Appendix) allows to conclude that forward trajectories of the (S_2, X_2) sub-system converge asymptotically either to the positive steady state $(S_2^*(\alpha), S_{in} - S_2^*(\alpha))$ or to the “wash-out” equilibrium $(S_{in}, 0)$. We show now that for any initial condition such that $X_2(0) > 0$, the forward trajectory cannot converge to the wash-out equilibrium. From equations (5) one can write

$$X_2(t) = X_2(0) e^{\int_0^t (\mu(S_2(\tau)) - \alpha) d\tau} .$$

If $X_2(\cdot)$ tends to 0, then one should have

$$\int_T^{+\infty} (\mu(S_2(\tau)) - \alpha) d\tau = -\infty \tag{24}$$

for any finite positive T . Using Taylor-Lagrange Theorem, there exists a continuous function $\theta(\cdot)$ in $(0, 1)$ such that

$$\mu(S_2(\tau)) = \mu(S_{in}) + \mu'(\tilde{S}_2(\tau))(S_2(\tau) - S_{in}) + \mu(S_{in}) - \alpha \text{ with } \tilde{S}_2(\tau) = S_{in} + \theta(\tau)(S_{in} - S_2(\tau)) .$$

One can then write

$$\begin{aligned} \int_T^{+\infty} (\mu(S_2(\tau)) - \alpha) d\tau &= \int_T^{+\infty} (\mu(S_{in}) - \alpha) d\tau - \int_T^{+\infty} \mu'(\tilde{S}_2(\tau)) X_2(\tau) d\tau + \int_T^{+\infty} \mu'(\tilde{S}_2(\tau)) Z_2(\tau) d\tau \\ &\geq - \int_T^{+\infty} \mu'(\tilde{S}_2(\tau)) X_2(\tau) d\tau - \frac{1}{\alpha} \int_T^{+\infty} \mu'(\tilde{S}_2(\tau)) \dot{Z}_2(\tau) d\tau . \end{aligned}$$

Note that $S_2(\cdot)$ tends to S_{in} when $X_2(\cdot)$ tends to 0. So there exists $T > 0$ such that $\tilde{S}_2(\tau) > \hat{S}$ for any $\tau > T$, and accordingly to Assumption A1, there exist positive numbers a, b such that $-\mu'(\tilde{S}_2(\tau)) \in [a, b]$ for any $\tau > T$. The following inequality is obtained

$$\int_T^{+\infty} (\mu(S_2(\tau)) - \alpha) d\tau \geq a \int_T^{+\infty} X_2(\tau) - \frac{b}{\alpha} |Z_2(T)|$$

leading to a contradiction with (24). \square

Then the global stability of the positive equilibrium of dynamics (5) can be proved.

Proposition 3. *For any configuration $(\alpha, r) \in \mathcal{C}$, any trajectory of the dynamics (5) with $X_2(0) > 0$ converges exponentially to the steady state $E^*(\alpha, r)$ in forward time.*

Proof. Let us consider the vector

$$Z = \begin{bmatrix} X_1 + S_1 - S_{in} \\ X_2 + S_2 - S_{in} \end{bmatrix}$$

whose dynamics is linear:

$$\dot{Z} = \underbrace{\begin{pmatrix} -\frac{1}{r} & \frac{\alpha(1-r)}{r} \\ 0 & -\alpha \end{pmatrix}}_A Z .$$

The matrix A is clearly Hurwitz and consequently Z converges exponentially towards 0 in forward time. Furthermore, variables S_2 and X_2 being non negative (see Lemma 1), one has also from (5) the following properties

$$\begin{aligned} S_1 = 0 &\implies \dot{S}_1 \geq 0 , \\ X_1 = 0 &\implies \dot{X}_1 \geq 0 , \end{aligned}$$

and deduces that variables S_1 and X_1 stay also non negative in forward time. The definition of Z allows us to conclude that variables S_1, X_1, S_2, X_2 are bounded.

From equations (5), the dynamics of the variable S_1 can be written as an non-autonomous scalar equation:

$$\dot{S}_1 = \left(-\mu(S_1) + \frac{1 - \alpha(1-r)}{r} \right) (S_{in} - S_1) + \frac{\alpha(1-r)}{r} (S_2(t) - S_1) - \mu(S_1)Z_1(t)$$

or equivalently:

$$\dot{S}_1 = (\phi_{\alpha,r}(S_1) - \mu(S_1))(S_{in} - S_1) + \alpha \frac{1-r}{r} (S_2(t) - S_2^*(\alpha)) - \mu(S_1)Z_1(t) . \quad (25)$$

By Lemma 1, we know that $S_2(t)$ converges towards $S_2^*(\alpha)$. So the dynamics (25) is asymptotically autonomous with the limiting equation

$$\dot{S}_1 = (\phi_{\alpha,r}(S_1) - \mu(S_1))(S_{in} - S_1) . \quad (26)$$

Proposition 2 guarantees that $S_1^*(\alpha, r)$ is the only solution of $\phi_{\alpha,r}(S_1) = \mu(S_1)$ on the domain $(0, S_{in})$. So any trajectory of (26) that converges forwardly to the domain $[0, S_{in}]$ has to converges to $S_1^*(\alpha, r)$ or to S_{in} . Along with Theorem 6 (see Appendix), we conclude that forward trajectories of the (S_1, X_1) sub-system converge asymptotically either to the positive steady state $(S_1^*(\alpha, r), S_{in} - S_1^*(\alpha, r))$ or to $(S_{in}, 0)$. We show that this last case is not possible. From equations (5), one has

$$X_1 = 0 \implies \dot{X}_1 = \frac{\alpha(1-r)}{r} X_2$$

but from Lemma 1, we know that $X_2(t)$ converges to a positive value and consequently X_1 cannot converges towards 0.

Finally, we write the Jacobian matrix J^* of dynamics (5) at steady state $E^*(\alpha, r)$ in (Z, S_1, S_2) coordinates:

$$J^* = \left(\begin{array}{cc|cc} & & & \\ & A & & 0 \\ \hline & & & \\ -\mu(S_1^*) & 0 & (\phi'_{\alpha,r}(S_1^*) - \mu'(S_1^*))(S_{in} - S_1^*) & \frac{\alpha(1-r)}{r} \\ 0 & -\mu(S_2^*) & 0 & -\mu'(S_2^*)(S_{in} - S_2^*) \end{array} \right).$$

Remind the following facts:

- i. A is Hurwitz,
 - ii. S_2^* is less than \hat{S} , so one has $\mu'(S_2^*) < 0$ (cf Assumption A1)
 - iii. S_1^* is the only zero of $\phi_{\alpha,r}(S_1) = \mu(S_1)$ on $(0, S_{in})$, the graph of $\phi_{\alpha,r}$ is not tangent to the graph of μ (Proposition 2) and $\phi_{\alpha,r}(0) > \mu(0) = 0$, so one has $\phi'_{\alpha,r}(S_1^*) - \mu'(S_1^*) < 0$
- One can then conclude that J^* is Hurwitz. \square

4 Study of performance of the buffered chemostat

We first aim at characterizing among all the configurations in the set \mathcal{C} the ones that provide the best conversion of the nutrient at steady state, that is the smallest value of $S_1^*(\alpha, r)$.

For convenience, we consider the function

$$\psi(s) = \mu(s)(S_{in} - s) \quad (27)$$

and define the number

$$\psi^* = \max_{s \in [0, \bar{s}]} \psi(s) \quad (28)$$

where \bar{s} is defined by

$$\bar{s} = \lim_{\alpha \rightarrow \mu(S_{in})} S_2^*(\alpha). \quad (29)$$

The number \bar{s} is such that $\mu(\bar{s}) = \mu(S_{in})$ with $\bar{s} < S_{in}$.

Assumptions A1 and A2 provide the uniqueness of s^* realizing the maximum in (28), and one can then define the number

$$\alpha^* = \mu(s^*). \quad (30)$$

Lemma 2. *Assume that Hypotheses A1 and A2 are fulfilled. For any $\alpha \in (0, \mu(S_{in})]$, one has the following property*

- if $\underline{S}(\alpha) < \lambda_+$,

$$\inf_{r \in \mathcal{R}(\alpha)} S_1^*(\alpha, r) = \lim_{r \rightarrow \bar{r}(\alpha)} S_1^*(\alpha, r),$$

- if $\underline{S}(\alpha) = \lambda_+$, $S_1^*(\alpha, r)$ is equal to λ_+ whatever is $r \in \mathcal{R}(\alpha)$,

- if $\underline{S}(\alpha) > \lambda_+$,

$$\inf_{r \in \mathcal{R}(\alpha)} S_1^*(\alpha, r) = \lim_{r \rightarrow 0} S_1^*(\alpha, r) = \underline{S}(\alpha).$$

where $\underline{S}(\cdot)$ is defined in (15).

Proof. Fix $\alpha \in (0, \mu(S_{in}))$. One can check from expression (11) that the map

$$r \longmapsto \phi_{\alpha,r}(s) \text{ is } \begin{cases} \text{decreasing for } s \in [0, \underline{S}(\alpha)] , \\ \text{increasing for } s \in (\underline{S}(\alpha), S_{in}] . \end{cases}$$

Along with property (18) of Proposition 2, one deduces the following properties.

- i. When $\underline{S}(\alpha) < \lambda_+$ and $r \in \mathcal{R}(\alpha)$, the unique positive $S_1^*(\alpha, r)$ solution of $\phi_{\alpha,r}(S_1) = \mu(S_1)$ belongs to $[0, \underline{S}(\alpha)]$ and $r \mapsto S_1^*(\alpha, r)$ is decreasing.
- ii. When $\underline{S}(\alpha) = \lambda_+$ and $r \in \mathcal{R}(\alpha)$, $S_1^*(\alpha, r) = \lambda_+$ is the only positive solution of $\phi_{\alpha,r}(S_1) = \mu(S_1)$.
- iii. When $\underline{S}(\alpha) > \lambda_+$ and $r \in \mathcal{R}(\alpha)$, the unique positive $S_1^*(\alpha, r)$ solution of $\phi_{\alpha,r}(S_1) = \mu(S_1)$ belongs to $[\underline{S}(\alpha), S_{in}]$ and $r \mapsto S_1^*(\alpha, r)$ is increasing.

The conclusion comes then straightforwardly. \square

Lemma 3. *Assume that Hypotheses A1 and A2 are fulfilled. The following property is then satisfied.*

$$\inf_{(\alpha,r) \in \mathcal{C}} S_1^*(\alpha, r) = \inf_{r \in \mathcal{R}(\alpha^*)} S_1^*(\alpha^*, r) .$$

Proof. Remark first from (10) and (15) that one has

$$\underline{S}(\alpha) = S_{in} - \psi(S_2^*(\alpha))$$

and

$$\alpha \in (0, \mu(S_{in})) \iff S_2^*(\alpha) \in (0, \bar{s}) .$$

We consider now three cases depending on ψ^* and λ_+ .

If $\psi^* < S_{in} - \lambda_+$, then for any $\alpha \in (0, \mu(S_{in}))$, one has $\underline{S}(\alpha) > \lambda_+$ and according to Lemma 2 one has

$$\inf_{r \in \mathcal{R}(\alpha)} S_1^*(\alpha, r) = \underline{S}(\alpha) .$$

Then one can write

$$\inf_{(\alpha,r) \in \mathcal{C}} S_1^*(\alpha, r) = \inf_{\alpha \in (0, \mu(S_{in}))} \underline{S}(\alpha) = \underline{S}(\alpha^*) = \inf_{r \in \mathcal{R}(\alpha^*)} S_1^*(\alpha^*, r) .$$

If $\psi^* = S_{in} - \lambda_+$ then $\underline{S}(\alpha) \geq \lambda_+$ for any $\alpha \in (0, \mu(S_{in}))$, and according to Lemma 2 one has $S_1^*(\alpha, r) \geq \lambda_+$ for any $r \in \mathcal{R}(\alpha)$. Furthermore, for $\alpha = \alpha^*$, one has $\underline{S}(\alpha^*) = \lambda_+$ for any $r \in \mathcal{R}(\alpha)$. This implies the equality

$$\inf_{(\alpha,r) \in \mathcal{C}} S_1^*(\alpha, r) = \lambda_+ = \underline{S}(\alpha^*) = \inf_{r \in \mathcal{R}(\alpha^*)} S_1^*(\alpha^*, r) .$$

If $\psi^* > S_{in} - \lambda_+$, then according to Proposition 2 there exist values of $\alpha \in (0, \mu(S_{in}))$ such that $S_1^*(\alpha, r) < \underline{S}(\alpha)$ for any $r \in \mathcal{R}(\alpha)$. Then one can write the following inequality

$$\begin{aligned} \phi_{\alpha,r}(S_1^*(\alpha, r)) &= 1 + \frac{1-r}{r} \left(\frac{\underline{S}(\alpha) - S_1^*(\alpha, r)}{S_{in} - S_1^*(\alpha, r)} \right) \\ &> 1 + \frac{1-r}{r} \left(\frac{\underline{S}(\alpha^*) - S_1^*(\alpha, r)}{S_{in} - S_1^*(\alpha, r)} \right) = \phi_{\alpha^*,r}(S_1^*(\alpha, r)) . \end{aligned}$$

Remind that one has $\phi_{\alpha,r}(S_1^*(\alpha, r)) = \mu(S_1^*(\alpha, r))$. This implies that the root $S_1^*(\alpha^*, r)$ of the function $s \mapsto \phi_{\alpha^*,r}(s) - \mu(s)$ is necessarily such that $S_1^*(\alpha^*, r) < S_1^*(\alpha, r)$, for any $r \in \mathcal{R}(\alpha)$. Finally, we obtain the equality

$$\inf_{(\alpha,r) \in \mathcal{C}} S_1^*(\alpha, r) = \inf_{r \in \mathcal{R}(\alpha^*)} S_1^*(\alpha^*, r) .$$

□

Lemmas 2 and 3 give the following characterization of the best configurations.

Proposition 4. *Assume that Hypotheses A1 and A2 are fulfilled. The best stable configuration consists in choosing $\alpha = \alpha^*$ (or α arbitrarily close to $\mu(S_{in})$ if $\alpha^* = \mu(S_{in})$) and*

- *having a by-pass of the volume V with a flow rate equal to $(1 - \alpha)Q$, when $\psi^* < S_{in} - \lambda_+$. The output concentration at steady state is then equal (or arbitrarily close) to $S_{in} - \psi^*$.*
- *choosing any value of $r \in \mathcal{R}(\alpha)$, when $\psi^* = S_{in} - \lambda_+$. The output concentration at steady state is then equal (or arbitrarily close) to λ_+ .*
- *taking r smaller and arbitrarily close to $\bar{r}(\alpha)$, when $\psi^* > S_{in} - \lambda_+$. The output at steady state is then arbitrary close to the infimum of S_1^* on \mathcal{S} (that is necessarily less than λ_+).*

Under Assumptions A1 and A2, we study now the benefit of adding to a single chemostat of volume V a buffer of volume V_2 under a constant total input flow $Q = Q_1 + Q_2$, and characterize the minimal value of V_2/V to obtain a global stability of the positive equilibrium. Similarly to Section 3, we describe the set of configurations by two non-negative parameters:

$$\alpha = \frac{Q_2}{V_2}, \quad \beta = \frac{V_2}{V},$$

but here one has $V_1 = V$ whatever is the volume V_2 . For any number $\alpha \in (0, \mu(S_{in})]$, there exists a unique $S_2^*(\alpha) \in (0, \bar{s})$ defined by (10) and (29), and consequently there exists a unique positive equilibrium in the second tank. The parameter α being fixed, one can straightforwardly check on equations (4) that a positive equilibrium in the first tank fulfills

$$\varphi(S_1^*) = \alpha\beta(S_{in} - S_2^*(\alpha)) \tag{31}$$

where the function φ is defined as

$$\varphi(s) = (S_{in} - s)(1 - \mu(s)). \tag{32}$$

Consequently, we are looking for the smallest value of β such that there exists a unique positive solution of (31) on the interval $(0, S_{in})$.

Proposition 5. *Under Assumptions A1 and A2, there exists a buffered configuration with an additional tank of volume V_2 that possesses a unique globally exponentially stable positive equilibrium from any initial condition with $S_2(0) > 0$, exactly when V_2 fulfills the condition*

$$\beta = \frac{V_2}{V} > \frac{\max_{s \in (\lambda_+, S_{in})} \varphi(s)}{\psi^*}, \tag{33}$$

where ψ^* is defined in (28), with any $\alpha \in (0, \mu(S_{in})]$ such that

$$\max_{s \in (\lambda_+, S_{in})} \varphi(s) < \alpha\beta(S_{in} - S_2^*(\alpha)) < S_{in}.$$

Proof. Let us examine first some properties of the function φ on the interval $(0, S_{in})$:

- . φ is negative exactly on the interval Λ ,
- . φ' is negative on $(0, \lambda_-)$ with $\varphi(0) = S_{in}$ and $\varphi(\lambda_-) = 0$,
- . $\varphi(\lambda_+) = \varphi(S_{in}) = 0$ and φ reaches its maximum m^+ on the sub-interval (λ_+, S_{in}) , that is strictly less than $S_{in} = \varphi(0)$,

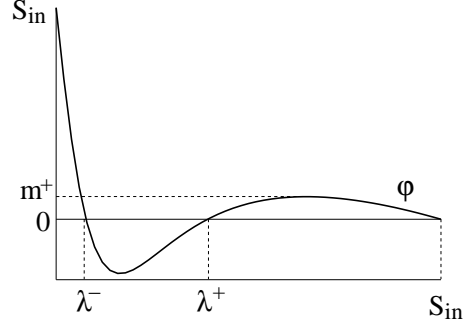


Figure 3: Illustration of the graph of the function φ

from which we deduce that there exists a unique solution of $\varphi(s) = c$ on the whole interval $(0, S_{in})$ exactly when $c \in (m^+, S_{in})$ (see Figure (3) as an illustration).

Consequently, the configurations (α, β) for which there exists a unique $S_1^*(\alpha, \beta) \in (0, S_{in})$ solution of the equation (31) are exactly those that fulfill the condition

$$\frac{m^+}{\alpha(S_{in} - S_2^*(\alpha))} < \beta < \frac{S_{in}}{\alpha(S_{in} - S_2^*(\alpha))}$$

with $\alpha \in (0, \mu(S_{in}))$. Then, Proposition 3 with $r = 1/(1 + \beta)$ guarantees that the unique positive equilibrium $(S_1^*(\alpha, \beta), S_{in} - S_1^*(\alpha, \beta), S_2^*(\alpha), S_{in} - S_2^*(\alpha))$ is globally exponentially stable on the domain $\mathbb{R}_+^2 \times \mathbb{R}_+^* \times \mathbb{R}_+$.

Among all such configurations, the infimum of β can be approached arbitrarily close when α is maximizing the function

$$\alpha \mapsto \alpha(S_{in} - S_2^*(\alpha))$$

on $(0, \mu(S_{in}))$, that exactly amounts to maximize the function ψ defined in (27) on the interval $(0, \bar{s}]$. \square

Remark 2. Section 3 has shown the benefit of the buffered chemostat in terms of global stability of the system, but with a price to pay in performances when one imposes to have the same residence time (i.e. the nutrient concentration at steady state is larger than λ_-). When adding a buffer, this is no longer true (i.e. the steady state necessarily exhibits a better performance than λ_-): there always exists a solution $S_1^* \in (0, \lambda_-)$ of (31), that is unique under conditions of Proposition 5, because $\varphi(0) = S_{in}$, $\varphi(\lambda_-) = 0$ and $\alpha\beta(S_{in} - S_2^*) \in (0, S_{in})$.

5 Illustration and discussion

We illustrate the results of the former sections on a non-monotonic uptake function given by the Haldane expression (2). One can easily check that for this function the set Λ defined in (3) is non empty exactly when the condition

$$\bar{\mu} > 1 + 2\sqrt{\frac{K}{K_I}}$$

is fulfilled. Then, λ_- , λ_+ are given by the following expressions:

$$\lambda_{\pm} = \frac{K_I(\bar{\mu} - 1) \pm \sqrt{K_I^2(\bar{\mu} - 1)^2 - 4KK_I}}{2}.$$

Remind that Assumption A2 is fulfilled for values of S_{in} larger than λ_+ .

Lemma 4. Assume that $\mu(\cdot)$ is an Haldane function and that Assumptions A1 and A2 are fulfilled. For any $\alpha \in (0, \mu(S_{in})]$, the following properties are satisfied.

- the set $R^+(\alpha)$ defined in (17) is a singleton,
- for any $r \in (0, 1)$, the set $\mathcal{S}_{r,\alpha}$ defined in (14) is either empty or a singleton,
- if the set $R^-(\alpha)$ defined in (16) is non empty, then one has $\max R^-(\alpha) < R^+(\alpha)$.

Proof. In the case of the Haldane function, the equality $\phi_{\alpha,r}(s) = \mu(s)$ can be rewritten as

$$(S_{in} - s - \alpha(1 - r))(S_{in} - S_2^*(\alpha))(K + S + S^2/K_I) = r\bar{\mu}s(S_{in} - s) .$$

So S_1^* is the root of a polynomial P of degree three, and there exists at most three solutions of $\phi_{\alpha,r}(s) = \mu(s)$. We then deduce from Proposition 2 that $R^+(\alpha)$ is a singleton.

Requiring to have $\phi_{\alpha,r}(s) = \mu(s)$ and $\phi'_{\alpha,r}(s) = \mu'(s)$ simultaneously implies that s is solution of $P = 0$ and $P'(s) = 0$ i.e. that s is a double root of P . P being of degree three, there is at most one such solution. So the set $\mathcal{S}_{r,\alpha}$ possesses at most one element, and this implies $R^-(\alpha) \cap R^+(\alpha) = \emptyset$.

When $R^-(\alpha)$ is non empty, we know from Proposition 2 that for $r \in (\min R^-(\alpha), \max R^-(\alpha))$, $\phi_{\alpha,r}(s) = \mu(s)$ has at least three solutions on an interval I , and for $r \in (\min R^+(\alpha), 1)$ at least two on another interval J , where I and J are disjoint. Consequently, one should have $\max R^-(\alpha) < \min R^+(\alpha)$, otherwise there would exist at least 5 solutions of $\phi_{\alpha,r}(s) = \mu(s)$ on $(0, S_{in})$. \square

Lemma 4 implies that for any $\alpha \in (0, \mu(S_{in})]$, the number $\bar{r}(\alpha)$ defined in (23) is the single element of $R^+(\alpha)$. It can then be determined numerically as the unique minimizer of the function

$$F_\alpha(r, s) = (\mu(s) - \phi_{\alpha,r}(s))^2 + (\mu'(s) - \phi'_{\alpha,r}(s))^2$$

on $(0, 1) \times \{s \in (\lambda^-, S_{in}) \text{ s.t. } (s - \lambda^+)(\lambda^+ - \underline{S}(\alpha)) \geq 0\}$ that is, for the Haldane function:

$$F_\alpha(r, s) = \left(\frac{\bar{\mu}s}{K + s + s^2/K_I} - \frac{1}{r} + \alpha \frac{1-r}{r} \frac{S_{in} - S_2^*(\alpha)}{S_{in} - s} \right)^2 + \left(\frac{\bar{\mu}(K - s^2/K_I)}{(K + s + s^2/K_I)^2} + \alpha \frac{1-r}{r} \frac{S_{in} - S_2^*(\alpha)}{(S_{in} - s)^2} \right)^2$$

where S_2^* defined in (10) is given by the formula

$$S_2^*(\alpha) = \frac{K_I(\bar{\mu} - \alpha) - \sqrt{K_I^2(\bar{\mu} - \alpha)^2 - 4\alpha^2 K K_I}}{2\alpha} ,$$

and $\underline{S}(\alpha)$ is defined in (15).

One can also easily check that for the Haldane growth, the function ψ defined in (27) is increasing up to ψ^* and decreasing. Its maximum on the interval $[0, S_{in}]$ is achieved for the value

$$\bar{s}^* = \frac{\sqrt{K^2 + K S_{in}(1 + S_{in}/K_I)} - K}{1 + S_{in}/K_I} .$$

Consequently, one has

$$s^* = \min(\bar{s}^*, \bar{s}) ,$$

that allows to determine the optimal value $\alpha^* = \mu(s^*)$.

The parameters given in Table 1 have been chosen for the numerical simulations.

Figure 4 illustrates the family of functions $\phi_{\alpha,r}(\cdot)$ and the tangent property with the graph of an Haldane function. On Figure 5, the domain \mathcal{C} defined in (13) is drawn for different values of S_{in} . According to Remark 1, one can see that the map $\alpha \mapsto \bar{r}(\alpha)$ is discontinuous at $\alpha = \underline{\alpha}$, where $\underline{\alpha}$ is such that $\underline{S}(\underline{\alpha}) = \lambda_+$

$\bar{\mu}$	K	K_I	λ_-	λ_+
12	1	0.8	$\simeq 0.103$	$\simeq 0.777$

Table 1: Parameters of the Haldane function and the corresponding values of λ_- , λ_+ .

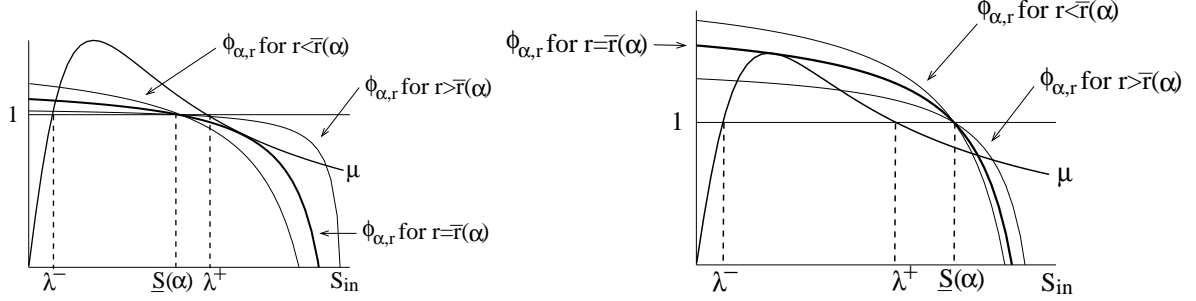


Figure 4: Family of functions $\phi_{\alpha,r}(\cdot)$ when $\underline{S}(\alpha) < \lambda_+$ (in the left) and $\underline{S}(\alpha) > \lambda_+$ (in the right).

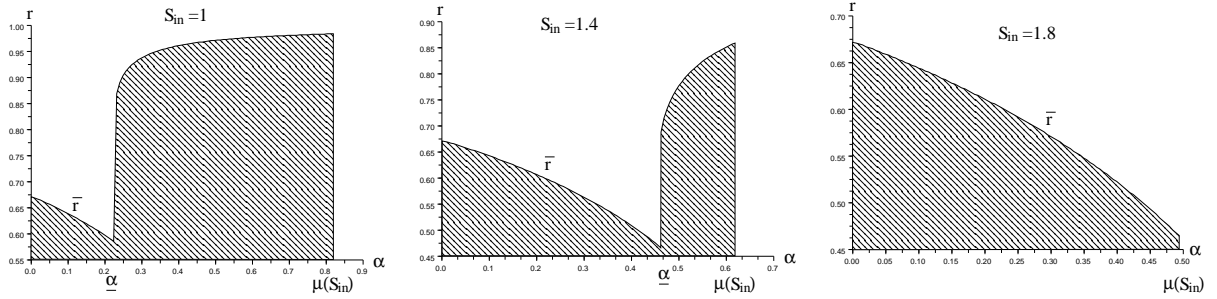


Figure 5: Domain \mathcal{C} of stable configurations for different values of S_{in} .

(when it exists). On Figure 6 one can see that the two limiting hyperbolas $H_{\alpha,\bar{r}(\alpha)}$ about $\underline{\alpha}$ are different for such a case.

Our study has revealed the role of the input concentration S_{in} on the shape of the domain \mathcal{C} . So we have computed numerically the best configurations (α^*, r^*) given by Proposition 4 as functions of S_{in} , as well as the corresponding output concentration S_1^* (see Figure 7). The map $S_{in} \mapsto \alpha^*$ given by (28) and (30) being continuous, the discontinuity of the map $\alpha \mapsto \bar{r}(\alpha)$ leads to a discontinuity of the map $S_{in} \mapsto S_1^*$. Consequently, there exists a threshold of S_{in} such that

- below the threshold, the optimal buffered chemostat provides global stability, with performance close to the single chemostat i.e. S_1^* is close to λ_- ;
- above the threshold, the optimal stable configuration consists in a by-pass of the single chemostat without any buffer. The performance is significantly modified as S_1^* is larger than λ_+ .

According to Propositions 2 and 4, this threshold corresponds to a value of S_{in} such that $\underline{S}^*(\alpha^*) = \lambda_+$, where \underline{S} is defined in (15). For values of S_{in} smaller than this threshold, the output concentration at steady state S_1^* of the best configuration is thus bounded by the one computed for the limiting case when S_{in} get arbitrary close to the threshold (see Figure 8). The values of S_{in} and S_1^* obtained at the threshold are given in Table 2. One can see on this example that the buffered chemostat allows a global stability for any value of S_{in} in the interval $[0.777, 1.641]$ with an output at steady state less than 0.167, to be compared with the

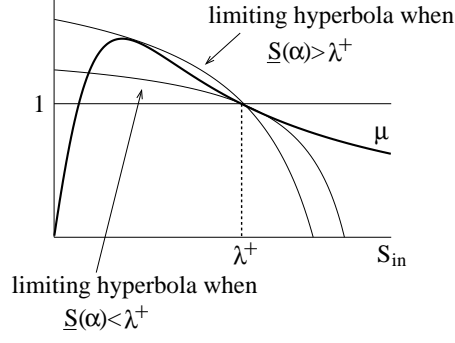


Figure 6: The limiting hyperbolas $H_{\alpha, \bar{r}(\alpha)}$ about $\alpha = \underline{\alpha}$ (for $S_{in} = 1.4$).

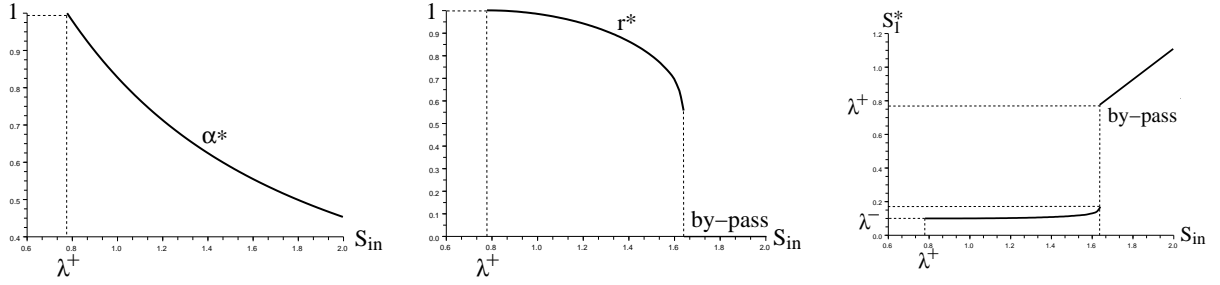


Figure 7: The best configuration (α^*, r^*) with S_1^* , as functions of S_{in} .

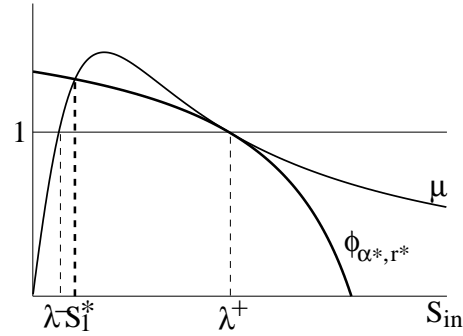


Figure 8: Determination of S_1^* for S_{in} at the threshold.

S_{in}	α^*	r^*	S_1^*
$\simeq 1.641$	$\simeq 0.543$	$\simeq 0.561$	$\simeq 0.167$

Table 2: Characteristics of the best configuration at the threshold value of S_{in} .

value 0.103 of the locally stable equilibrium of the single chemostat (see also Figure 7).

In industrial applications, the attraction of the wash-out equilibrium is undesired because it presents a risk that may ruin the culture in case of disturbance, temporarily pump breakdown or presence of toxic

material that could drive the state in the attracting basin of the wash-out equilibrium. It imposes also to ensure that initial condition belongs to the attracting basin of the desired equilibrium. A common technique to overcome these difficulties and allow an initial stage with a small concentration of biomass, is to control the input flow rate Q with a stabilizing feedback [6, 36] (it consists in finding a feedback law that reduces the flow rate when the state belongs to the attracting basin of the wash-out equilibrium). But this solution requires an upstream storage and an actuator. The design of a buffered chemostat is thus an alternative that does not require any upstream storage nor feedback control. In real world applications, it may happen that the growth function $\mu(\cdot)$ is not perfectly known or uncertain. Then choosing a buffered configuration not too close from the boundary of the domain \mathcal{C} provides a robustness margin for the global stability.

When the characteristics of the input flow cannot be changed, a simple solution consists in increasing the volume of the vessel, so that the dilution rate is small enough to ensure that condition of Case 3 of Proposition 1 is fulfilled. The relative increment $\Delta V/V$ has then to satisfy the condition

$$S_{in} \notin \left\{ S > 0 \mid \mu(S) > \frac{1}{1 + \frac{\Delta V}{V}} \right\} \quad (34)$$

that is equivalent to have

$$\frac{\Delta V}{V} > \frac{1}{\mu(S_{in})} - 1. \quad (35)$$

Note that under Assumptions A1 and A2, this last number is positive. This solution increases significantly the residence time in the tank and induces additional financial costs. Instead of choosing a larger volume V , we show that adding a buffer can be an interested alternative to improve the stability of a given bioprocess. For the parameters given in Table 1, we have compared numerically

- the smallest relative increment of the volume of the single chemostat to be globally stable, given in (35),
- the smallest relative size of the buffer to be added for the buffered chemostat to be globally stable, given by Proposition 5 (that imposes to choose $\alpha = \alpha^*$),

as functions of the input concentration S_{in} (for values larger than λ_+ for which the bi-stability occurs with a dilution rate equal to one, cf Proposition 2). One can clearly see on Figure 9 the advantage of the buffered

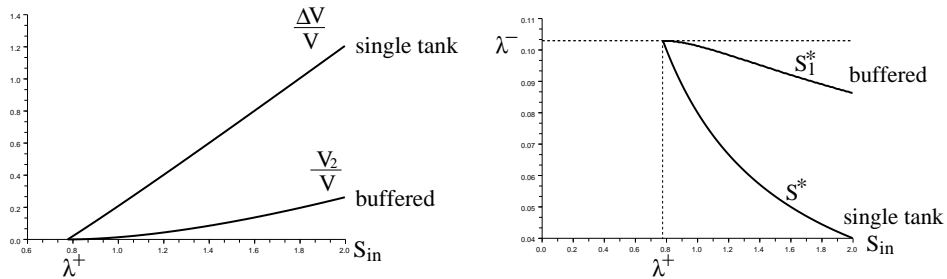


Figure 9: Comparison of minimal increase of volume, and output nutrient concentration, to obtain the global stability.

chemostat that requires less volume augmentation. The output concentrations are also drawn for both configurations with the minimal volume augmentation. According to Remark 2, these concentrations are always smaller than λ_- . This example demonstrates the flexibility of the buffered chemostat in the choice of possible configurations, with two parameters than can be tuned (see Figure 10), while the single chemostat is penalized with only one parameter, requiring larger increments of volume and providing (too) low output concentrations.

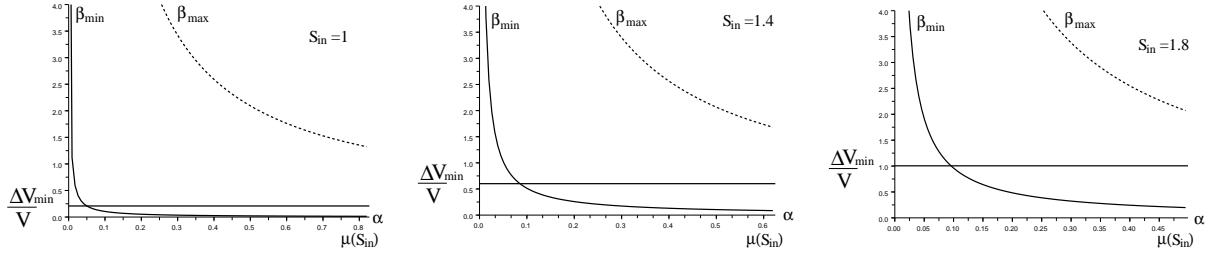


Figure 10: The sets of configurations (α, β) that ensure global stability, to be compared with the minimal relative increase of volume of the single chemostat (for different values of S_{in}).

Finally note that due to the robustness property that is obtained for the stability in the first tank when using a buffered chemostat, the presence of biomass at initial time is necessarily only in the buffer tank (see Proposition 3). This property possesses some advantages for the practitioners in industrial frameworks for the seeding phase.

6 Conclusion

The main message of the present study is that particular spatial structures can bring stability to unstable bioprocesses:

- We have shown that a buffered interconnection of two volumes can globally stabilize the chemostat model when it exhibits a bi-stability, while preserving the same total volume and input flow, which is not possible with parallel or serial interconnections.
- We have provided a characterization of the set \mathcal{C} of all buffered configurations that enjoy this property, and study their performances at steady-state in terms of nutrient conversion.
- Our study has emphasized the influence of the input concentration S_{in} on the shape of the set \mathcal{C} and the design of the best configurations, that could exhibit a threshold on the value of S_{in} above it a by-pass is more efficient. More precisely, we have shown that this threshold can be computed from the function

$$\psi(s) = (S_{in} - s)\mu(s)$$

(where $\mu(\cdot)$ is the nutrient uptake rate assumed to be non-monotonic).

- We have studied the minimal buffer volume to add to a single chemostat to obtain a global stability, and show how the flexibility of the buffered interconnection allows significantly less volume augmentation than increasing the size of the single chemostat.

Those results provide new insights on the role of spatial structures in natural ecosystems, and new strategies for piloting bioprocesses designing volume and input flow of a buffer. Our study has considered single strain. According to the Competitive Exclusion Principle, it is not (generically) possible to observe more than one species at steady state in the buffer tank, but this does not prevent to have coexistence with another dominant species in the main tank, which is not possible with a single chemostat. Consequently, it might be relevant to study the performances of the buffered chemostat choosing a different strain in the buffer, that could be the matter of a future work.

Appendix

We recall a result from [28, Theorem 1.8] about asymptotic behavior of trajectories of asymptotically autonomous dynamical system.

Theorem. Let Φ be an asymptotically autonomous semi-flow with limit semi-flow Θ , and let the orbit $\mathcal{O}_\Phi(\tau, \xi)$ have compact closure. Then the ω -limit set $\omega_\Phi(\tau, \xi)$ is non-empty, compact, connected, invariant and chain-recurrent by the semi-flow Θ and attracts $\Phi(t, \tau, \xi)$ when $t \rightarrow \infty$.

Acknowledgment

The authors are grateful to the INRA and INRIA supports within the French VITELBIO (VIRtual TELluric BIOreactors) research program. The authors thank also Prof. Denis Dochain, CESAME, Univ. Louvain-la-Neuve, for having fruitful discussions.

References

- [1] J.F. ANDREWS, *A mathematical model for the continuous culture of microorganisms utilizing inhibitory substrates*, Biotech. Bioengrg., 10 (1968), 707–723.
- [2] G. J. BUTLER AND G. S. K. WOLKOWICZ, *A mathematical model of the chemostat with a general class of functions describing nutrient uptake*, SIAM J. Appl. Math. 45 (1985), 138–151.
- [3] A. BUSH AND A. COOK *The effect of time delay and growth rate inhibition in the bacterial treatment of wastewater* J. Theor Biol. 63(2) (1976), 385–395.
- [4] C. DE GOOIJER, W. BAKKER, H. BEEFTINK AND J. TRAMPER, *Bioreactors in series: An overview of design procedures and practical applications*, Enzyme and Microbial Technology, 18 (1996), 202–219.
- [5] E. DI MATTIA, S. GREGO AND I. CACCIARI, *Eco-physiological characterization of soil bacterial populations in different states of growth* Microb. Ecol. 43(1) (2002), 34–43.
- [6] D. DOCHAIN AND G. BASTIN *Adaptive identification and control algorithms for non linear bacterial growth systems*. Automatica, 20 (5) (1984), 621–634.
- [7] D. DOCHAIN AND P. VANROLLEGHEM, *Dynamical Modelling and Estimation in Wastewater treatment Processes*, IWA Publishing, U.K. (2001).
- [8] A. DRAMÉ, J. HARMAND, A. RAPAPORT AND C. LOBRY, *Multiple steady state profiles in interconnected biological systems*, Mathematical and Computer Modelling of Dynamical Systems, 12 (2006), 379–393.
- [9] H. EL-OWAIDY AND O. EL-LEITHY, *Theoretical studies on extinction in the gradostat* Mathematical Biosciences, 101(1) (1990), 1–26.
- [10] A. FREDRICKSON AND G. STEPHANOPOULOS, *Microbial Competition* Science, 213 (1981), 972–979.
- [11] C. FRITZSCHE, K. HUCKFELDT AND E.-G. NIEMANN, *Ecophysiology of associative nitrogen fixation in a rhizosphere model in pure and mixed culture*, FEMS Microbiology Ecology, 8(4) (2011), 279–290.
- [12] A. GAKI, AL. THEODOROU, D. VAYENAS AND S. PAVLOU, *Complex dynamics of microbial competition in the gradostat*, Journal of Biotechnology, 139(1) (2009) pp 38–46.
- [13] , I. HAIDAR, A. RAPAPORT AND F. GÉRARD, *Effects of spatial structure and diffusion on the performances of the chemostat*, Mathematical Biosciences and Engineering, 8(4) (2011), 953–971.
- [14] J. HARMAND, A. RAPAPORT AND F. MAZENC *Output tracking of continuous bioreactors through recirculation and by-pass*, Automatica, 42(7) (2006) 1025–1032.
- [15] J. HARMAND, A. RAPAPORT AND A. TROFINO, *Optimal design of two interconnected bioreactors—some new results*, American Institute of Chemical Engineering Journal, 49 (1999), 1433–1450.

- [16] A. HASLER AND W. JOHNSON, *The in situ chemostat – a self-contained continuous culturing and water sampling system*. *Limnol. Oceanogr.* 79 (1954), 326–331.
- [17] J. HOFBAUER AND W. SO, *Competition in the gradostat: the global stability problem* *Original Research Nonlinear Analysis: Theory, Methods & Applications*, 22(8) (1994) 1017–1031.
- [18] Y. HIGASHI, N. YTOW, H. SAIDA AND H. SEKI *In situ gradostat for the study of natural phytoplankton community with an experimental nutrient gradient* *Environmental Pollution*, 99 (1998), 395–404.
- [19] G. HILL AND C. ROBINSON, *Minimum tank volumes for CFST bioreactors in series*, *The Canadian Journal of Chemical Engineering*, 67 (1989), 818–824.
- [20] W. JAEGER, J.-H. SO, B. TANG AND P. WALTMAN, *Competition in the gradostat*, *J. Math. Biol.* 25 (1987) 23–42.
- [21] H. JANNASH AND R. MATELES, *Experimental bacterial ecology studies in continuous culture*, *Advanced in Microbial Physiology* 11 (1974), 165–212.
- [22] P. LENAS, N. THOMOPOULOS, D. VAYENAS AND S. PAVLOU, *Oscillations of two competing microbial populations in configurations of two interconnected chemostats*, *Mathematical Biosciences*, 148(1) (1998), 43–63.
- [23] J. LA RIVIÈRE, *Microbial ecology of liquid waste*, *Advances in Microbial Ecology*, 1 (1977), 215–259.
- [24] B. LI, *Global asymptotic behavior of the chemostat: General response functions and differential removal rates*, *SIAM J. Appl. Math.* 59 (1998) 411–22.
- [25] R. LOVITT AND J. WIMPENNY, *The gradostat: A bidirectional compound chemostat and its applications in microbial research*, *Journal of General Microbiology*, 127 (1981), 261–268.
- [26] K. LUYBEN AND J. TRAMPER, *Optimal design for continuously stirred tank reactors in series using Michaelis-Menten kinetics*, *Biotechnology and Bioengineering*, 24 (1982), 1217–1220.
- [27] R. MACARTHUR AND E. WILSON, *The Theory of Island Biogeography*, Princeton University Press (1967).
- [28] M. MISCHAIKOW, H. SMITH AND H. THIEME, *Asymptotically autonomous semiflows: chain recurrence and Lyapunov functions*, *Transactions of the American Mathematical Society*, 347(5) (1995), 1669–1685.
- [29] J. MONOD, *La technique de la culture continue: Théorie et applications*, *Annales de l’Institut Pasteur*, 79 (1950), 390–410.
- [30] S. NAKAOKA AND Y. TAKEUCHI, *Competition in chemostat-type equations with two habitats*, *Mathematical Bioscience*, 201 (2006), 157–171.
- [31] M. NELSON AND H. SIDHU, *Evaluating the performance of a cascade of two bioreactors*, *Chemical Engineering Science*, 61 (2006), 3159–3166.
- [32] A. NOVICK AND L. SZILARD, *Description of the chemostat*, *Science*, 112 (1950), 715–716.
- [33] J. PIRT, *Principles of Microbe and Cell Cultivation*, Blackwell Scientific Publications (1975).
- [34] A. RAPAPORT AND J. HARMAND, *Biological control of the chemostat with non-monotonic response and different removal rates*, *Mathematical Biosciences and Engineering*, 5(3) (2008), 539–547.
- [35] A. RAPAPORT, J. HARMAND AND F. MAZENC, *Coexistence in the design of a series of two chemostats*, *Nonlinear Analysis, Real World Applications*, 9 (2008), 1052–1067.

- [36] A. SCHAUM, J. ALVAREZ AND T. LOPEZ-ARENAS, *Saturated PI control of continuous bioreactors with Haldane kinetics* Chem. Eng. Science, 68 (2012), 520–529.
- [37] H. SMITH AND B. TANG, *Competition in the gradostat: the role of the communication rate*, J. Math. Biol. 27(2) (1989) 139–165.
- [38] H. SMITH, B. TANG AND P. WALTMAN, *Competition in an n-vessel gradostat*, SIAM J. Appl. Math. 51 (1991) 1451–1471.
- [39] H. SMITH AND P. WALTMAN, H.L. Smith, P. Waltman, *The gradostat: a model of competition along a nutrient gradient*, J. Microb. Ecol. 22 (1991) 207–226.
- [40] H. SMITH AND P. WALTMAN, *The theory of chemostat, dynamics of microbial competition*, Cambridge Studies in Mathematical Biology, Cambridge University Press (1995).
- [41] H. SMITH AND P. WALTMAN, *Competition in the periodic gradostat*, Nonlinear Analysis: Real World Applications, 1(1) (2000) 177–188.
- [42] G. STEPHANOPOULOS AND A. FREDRICKSON, *Effect of inhomogeneities on the coexistence of competing microbial populations*, Biotechnology and Bioengineering, 21 (1979), 1491–1498.
- [43] B. TANG, *Mathematical investigations of growth of microorganisms in the gradostat*, J. Math. Biol. Vol 23 (1986) 319–339.
- [44] B. TANG, *Competition models in the gradostat with general nutrient uptake functions*, Rocky Mountain J. Math. 24(1) (1994) 335–349.
- [45] H. VELDCAMP, *Ecological studies with the chemostat*, Advances in Microbial Ecology, 1 (1977), 59–95.
- [46] G. WOLKOWICZ AND Z. LU, *Global dynamics of a mathematical model of competition in the chemostat: general response functions and differential death rates*, SIAM J. Appl. Math. 52 (1992) 222–233.
- [47] A. ZAGHROUT, *Asymptotic behavior of solutions of competition in gradostat with two limiting complementary substrates* Applied Mathematics and Computation, 49 (1) (1992) 19–37.