



HAL
open science

Some non-intuitive properties of serial chemostats with and without mortality

Manel Dali-Youcef, Jérôme Harmand, Alain Rapaport, Tewfik Sari

► **To cite this version:**

Manel Dali-Youcef, Jérôme Harmand, Alain Rapaport, Tewfik Sari. Some non-intuitive properties of serial chemostats with and without mortality. 2021. hal-03697663v1

HAL Id: hal-03697663

<https://hal.inrae.fr/hal-03697663v1>

Preprint submitted on 26 Oct 2021 (v1), last revised 17 Jun 2022 (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.

Some non-intuitive properties of serial chemostats with and without mortality

M. Dali-Youcef^{*,**}, J. Harmand^{***}, A. Rapaport^{*} and T. Sari^{****}

Abstract

This paper discusses a model of two interconnected chemostats in series, characterized by biomass mortality. A comparison is established with a single chemostat of the same total volume in two different cases, that are with or without mortality rate. The outlet substrate concentration and the biogas flow rate are the main criteria for comparison. According to conditions depending on the operating parameters and the distribution of the total volume, our results show which structure, the series of chemostats or the single chemostat, performs better in terms of minimizing the outlet substrate concentration or maximizing the biogas flow rate, and this with or without account on mortality. Moreover, the differences and similarities in the results corresponding to the case with mortality and the one without mortality, are highlighted.

Keywords— chemostat, gradostat, biogas production, mortality.

1 Introduction

'Process design' means determining which type of ideal reactor (*e.g.* CSTR, PFR) - or interconnection of reactors - is best suited for a given 'task', the degrees of freedom being whether or not to multi-feed, the volumes of the tanks, the different interconnection points, etc... The 'task' in question here is the transformation of some matter - the 'substrate' - into a 'product' through a bioreaction auto-catalyzed by a microbial ecosystem, hereafter called the 'biomass'. This - very general - problem is of a crucial practical importance when dealing with large industries where the number of tanks, their volumes, the technology on which they rely on or the way they are connected to others directly impact not only the capital but also the operating costs. Being the first industry in terms of the volume of matter processed, the study of wastewater treatment plants (WWTP) have attracted a lot of attention for quite a long time, cf. for instance [11]. Because of their interest in producing energy from waste, the optimal design of anaerobic processes (AD) has also been extensively studied, cf. [10]. Such studies - among many others mainly conducted in the field of chemical and/or biochemical engineering - have in common to consider steady state characteristics of the bioprocess. Indeed, in these industries it is the rule rather than the exception to process continuously very large quantities of matter instead of stocking them, notably because the matter in question (liquid or solid waste in the examples here above) are produced continuously: the price to pay to stock them would be very high. They are however different in the objectives pursued: it is expected from a classical WWTP that the output concentration of pollutant (the output substrate concentration) be minimal while in the case of the AD, the objective is to maximize biogas production, the final product of the bioreaction. And looking at the results of the different available studies, it appears the best designs are not the same. In other words, given a bio-transformation process, different objectives may lead to different 'optimal' configurations. This is precisely summarized as a preamble by Gooijer and his coauthors in their 1996 survey on the design procedures of bioprocesses in series, citing what Herbert claimed in 1964 at a conference of bioengineering: 'If one fermenter gives good results, two fermenters will give better results and three fermenters better still. This is sometimes true, but often false.', cf. [5]. One difficulty when working with the design of bioprocesses comes from the fact that the quantities assumed to be known and the degrees of freedom are not the same depending on the specific problem to be solved. For instance, when dealing with water treatment, it is the rule rather than the exception to minimize the total volume of the system given input-output constraints specified by normative values. But, if the system considered is already existing, it could also be interested to find conditions such that the output pollution concentration be minimized, for instance in playing with operating conditions or with the interconnection of reactors. In addition, excluding a very few number of studies (cf. for instance [7] who worked with kinetics defined by qualitative properties), most available literature solve the problem in fixing kinetics once and for all, limiting the genericity of the results. Thus, it is particularly difficult to draw general conclusions and get a global idea about the advantages and drawbacks of a given configuration. In the present paper, we revisit the properties of a specific configuration - the two tanks in series - from a generic viewpoint. To do so, we adopt an approach in which we systematically compare the performances of a single CSTR with those obtained using two tanks in series in which both r , the fraction of the total volume used to calculate the volume of each tank as rV and $(1-r)V$,

respectively, and the input substrate concentration S^{in} are free. It should be noticed that it is easy to compare both configurations since the CSTR can be seen as a special case of the two tanks in series model when $r = 0$ or $r = 1$. The paper is organized as follows: first, we recall the model of the systems. It should be noticed that we consider here the presence of a mortality term which, as we will see, may play an important role in the design results. Then, considering the biomass growth rate follows a ‘Monod-like’ kinetics we establish the properties of the CSTR and the two tanks in series configuration for two different objectives pursued that are the search for the best design when we want to minimize the output substrate concentration and when we want to maximize biogas production. Finally, some conclusions and perspectives are drawn.

2 Presentation of the model and preliminaries

We consider two serial interconnected chemostats of total volume V . The first tank is of volume $V_1 := rV$ and the second tank is of volume $V_2 := (1-r)V$ where $r \in (0, 1)$. The substrate and the biomass concentrations in the tank i are respectively designated S_i and x_i , $i = 1, 2$. At the input, the first tank is fed by the substrate concentration denoted S^{in} . The dilution rate of the whole structure denoted D is defined by $D := Q/V$ where Q designating the flow rate is constant. The dilution rates of the tanks i are different and are defined by $D_i := Q/V_i$, $i = 1, 2$. The mortality of the biomass is denoted a such that $a \geq 0$. The mathematical model is given by the following equations:

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

Notice that for $r = 0$ or $r = 1$, the configuration is reduced to a single reactor, which corresponds to the single chemostat model given by the system

$$\begin{aligned}\dot{S} &= D(S^{in} - S) - f(S)x \\ \dot{x} &= -Dx + f(S)x - ax\end{aligned}\tag{2}$$

For sake of completeness, the analysis of system (2) is given in [4]. The considered growth function f is assumed to be of *Monod type* i.e. it verifies the following assumption.

Hypothesis 1 *The function f belongs to $C^1(\mathbb{R}_+, \mathbb{R}_+)$ and satisfies $f(0) = 0$, $f'(S) > 0$ for all $S > 0$.*

As f is increasing, then the *break-even concentration* is well defined by $\lambda(D) = f^{-1}(D)$ for $0 \leq D < m$ with $m := \sup_{S>0} f(S)$, (that may be $+\infty$).

System (1) can admits at most three steady states: the washout steady state $E_0 = (S^{in}, 0, S^{in}, 0)$, the steady state $E_1 = (S^{in}, 0, \bar{S}_2, \bar{x}_2)$ of washout in the first reactor but not in the second one and the steady state $E_2 = (S_1^*, x_1^*, S_2^*, x_2^*)$ of persistence of the biomass in both reactors. Expressions of equilibria E_1 and E_2 are given in Table 1 where E_2 requires the definition of the function h as

$$\begin{aligned}h(S_2) &= \frac{D + (1-r)a}{1-r} \frac{S_1^* - S_2}{\frac{DS^{in} + raS_1^*}{D+ra} - S_2} \\ &\text{with } S_1^* = \lambda(D/r + a).\end{aligned}\tag{3}$$

In order to simplify the notations, we posit $\delta := f(S^{in}) - a$.

Proposition 1 *Let $a \geq 0$, $D \geq 0$ and $r \in (0, 1)$ be fixed. For all $S^{in} > \lambda(D/r + a)$, the function $S^{in} \mapsto S_2^*(S^{in}, D, r)$ is decreasing.*

The proof of Proposition 1 is given in [3, 4]. This proposition asserts that increasing the input substrate concentration decreases the output substrate concentration at steady state of the series configuration.

3 Output substrate concentration

We consider the map $r \mapsto S_r^{out}(S^{in}, D)$ defined by (4), which represents the output substrate concentration at steady state of the serial configuration.

$$S_r^{out}(S^{in}, D) = \begin{cases} S^{in} & \text{if } D \geq r\delta \text{ and} \\ & D \geq (1-r)\delta \\ \lambda\left(\frac{D}{1-r} + a\right) & \text{if } D \geq r\delta \text{ and} \\ & D \leq (1-r)\delta \\ S_2^*(S^{in}, D, r) & \text{if } D < r\delta. \end{cases}\tag{4}$$

shown in Figure 1, there exists a unique value r_{min} of the parameter r which gives the smallest output substrate concentration of the series configuration, defined as

$$r_{min} := \operatorname{argmin}_{0 < r < 1} S_2^*(S^{in}, D, r). \quad (8)$$

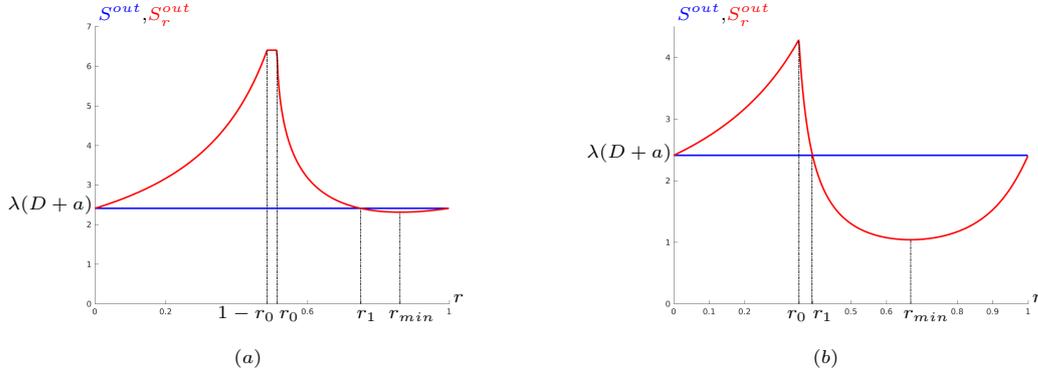


Figure 1: The output substrate concentrations of the serial configuration in red and the single chemostat in blue with $r_0 = D/\delta$, $f(S) = 4S/(5+S)$, $a = 0.3$, $D = 1$ and r_{min} is defined by (8). (a): $S^{in} = 6.4$, $r_0(6.4, 1) \approx 0.51$ and $r_1(18, 1) \approx 0.75$. (b): $S^{in} = 18$, $r_0(18, 1) \approx 0.35$ and $r_1(18, 1) \approx 0.39$.

According to Proposition 1, notice that for a fixed dilution rate D , the lowest output substrate concentration $S_{r_{min}}^{out}(S^{in}, D) = S_2^*(S^{in}, D, r_{min})$ gets smaller by increasing S^{in} . This was numerically highlighted in [12] for Monod and Contois growth functions.

4 Biogas flow rate

Recall that the biogas production rate of a single chemostat is proportional to the microbial activity $f(S)x$ and to its volume V (see [1, 9]). For the serial interconnection of two chemostats, the total biogas flow rates G_1 , G_2 corresponding respectively to the steady states E_1 , E_2 , are thus given by

$$\begin{aligned} G_1(S^{in}, D, r) &:= V_2 \bar{x}_2 f(\bar{S}_2), \\ G_2(S^{in}, D, r) &:= V_1 x_1^* f(S_1^*) + V_2 x_2^* f(S_2^*). \end{aligned} \quad (9)$$

According to Table 1 and (9), one deduces that for all $r\delta \leq D$ and $D < (1-r)\delta$, one has

$$G_1(S^{in}, D, r) := VD(S^{in} - \bar{S}_2) \quad (10)$$

and for all $D < r\delta$, one has

$$G_2(S^{in}, D, r) := VD(S^{in} - S_2^*). \quad (11)$$

Remark 1 According to the definitions (4), (10) and (11), one deduces that analyzing the output substrate concentration at steady state amounts to analyzing the biogas flow rate at steady state. In other words, minimizing the output substrate concentration at steady state allows to maximizing the biogas flow rate at steady state.

The biogas flow rate of the single chemostat at steady state being defined by

$$G_{chem}(S^{in}, D) := \begin{cases} 0 & \text{if } D \geq \delta \\ VD(S^{in} - \lambda(D+a)) & \text{if } D < \delta. \end{cases} \quad (12)$$

we have the following result as a direct consequence of Proposition 2, accordingly to Remark 1,

Proposition 3 Assume that Assumptions 1 and 2 are satisfied. For all $0 \leq D < \delta$ and $a \geq 0$, one has

- For every $r \in (0, 1)$, $G_1(S^{in}, D, r) < G_{chem}(S^{in}, D)$.
- If $S^{in} \leq g(D)$ then $G_2(S^{in}, D, r) < G_{chem}(S^{in}, D)$ for every $r \in (0, 1)$. If $S^{in} > g(D)$ then $G_2(S^{in}, D, r) < G_{chem}(S^{in}, D)$ if and only if $r_1(S^{in}, D) < r < 1$ where $r_1(S^{in}, D)$ is the unique solution of $S^{in} = g_r(D)$.

In addition, $G_1(S^{in}, D, r) = G_{chem}(S^{in}, D)$ for $r = 0$, and $G_2(S^{in}, D, r) = G_{chem}(S^{in}, D)$ for $r = r_1(S^{in}, D)$ and $r = 1$.

For a deeper analysis, we fix the input substrate concentration S^{in} and the parameter r , and consider the maps $D \mapsto G_{chem}(S^{in}, D)$, $D \mapsto G_1(S^{in}, D, r)$ and $D \mapsto G_2(S^{in}, D, r)$.

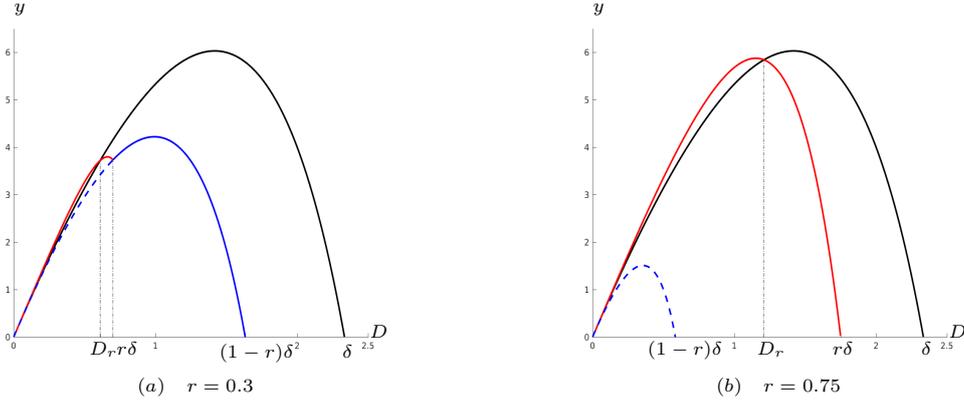


Figure 2: The curves of the functions $D \mapsto G_1(S^{in}, D, r)$ (in blue) and $D \mapsto G_2(S^{in}, D, r)$ (in red), for $S^{in} = 7$ and the corresponding value of $G_{chem}(S^{in}, D)$ (in black), with $D_r = D_r(S^{in})$, $f(S) = 4S/(5+S)$ and $a = 0$.

Proposition 4 For all $r \in (0, 1)$, $a \in [0, 1)$ and $0 \leq D < \delta$, one has

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

Proposition 4 is illustrated with Figures 2 and 3 which have been established for fixed S^{in} and parameter r (i.e. both volumes of the two reactors are fixed). The difference between the figures is due to the mortality rate which is null in Figure 2 and positive in Figure 3. On the one hand, observe that for any $D \in (0, \delta)$, the biogas flow rate of the serial configuration for the steady state E_1 with or without mortality (curves in blue) is always smaller than the biogas flow rate of the single chemostat (curves in black). On the other hand, notices that for any

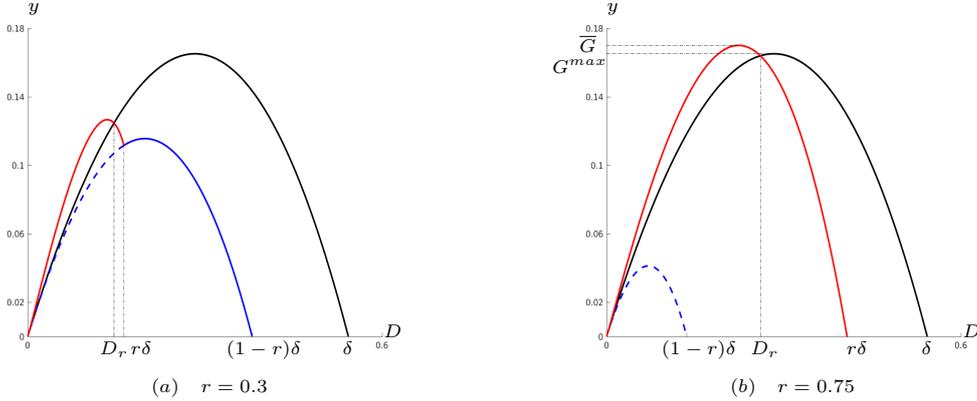


Figure 3: The curves of the functions $D \mapsto G_1(S^{in}, D, r)$ (in blue) and $D \mapsto G_2(S^{in}, D, r)$ (in red), for $S^{in} = 2$ and the corresponding value of $G_{chem}(S^{in}, D)$ (in black), with $G^{max} = G^{max}(S^{in})$, $\bar{G} = \bar{G}(r, S^{in})$, $f(S) = 4S/(5+S)$ and $a = 0.6$.

$D \in (0, D_r(S^{in}))$, the biogas flow rate of the serial configuration for the steady state E_2 , with or without mortality (curves in red) is greater than the biogas flow rate of the single chemostat (curves in black). Therefore, for any $D \in (0, D_r(S^{in}))$ the serial configuration is more efficient than the single chemostat. Notice that for fixed S^{in} , a and r , the value $D = D_r(S^{in})$ is solution of the equation $S^{in} = g_r(D)$ where g_r is defined by (6). This value exists and is unique under the following assumption which is satisfied by any concave growth function such as the Monod function, but also for the Hill function (for more details one can see Sections 5 of [3, 4]).

Hypothesis 3 For any $a \geq 0$ and $r \in (0, 1)$, the function $D \in [0, r(m-a)] \mapsto g_r(D) \in \mathbb{R}$ is increasing.

In addition, one can remark in Figure 2 that the biogas flow rate of the serial configuration without mortality never exceed the maximal biogas flow rate of the single chemostat. This observation is indeed always true and given by the Proposition 5 below.

For $S^{in} > 0$ and $a \geq 0$, let G^{max} be defined by

$$G^{max}(S^{in}) := \max_{D \in (0, \delta)} G_{chem}(S^{in}, D).$$

Proposition 5 Let S^{in} be fixed and $a = 0$. For any $D > 0$ and $r \in (0, 1)$, one has $G_1(S^{in}, D, r) < G^{max}(S^{in})$ and $G_2(S^{in}, D, r) < G^{max}(S^{in})$.

The proof is given in [3].

Thus, for a null mortality rate, when S^{in} and r are fixed, if the dilution rate D can be chosen then there is no interest to consider a serial configuration. Therefore, for optimizing the biogas flow rate production, the practitioner should consider a single chemostat (of same total volume). However, in Figure 3, one can notice that the maximal biogas flow rate of the serial configuration at the positive steady state E_2 with mortality (that is $\max_{D \geq 0} G_2(S^{in}, D, r)$, the maximum of the red curve), can exceed the maximal biogas flow rate of the single chemostat (which is $G^{max}(S^{in})$, the maximum of the black curve). This phenomenon can happen only when the mortality rate is non null. Proposition 6 asserts that without mortality, for a fixed r close enough to 1, the maximal biogas flow rate of the series configuration with mortality is certainly higher than the one of the single chemostat.

For $S^{in} > 0$ fixed, we assume that the maximum of the function $D \mapsto G_2(S^{in}, D, r)$ is unique, and we denote by $\bar{G}(r, S^{in})$ the maximal value of $G_2(S^{in}, D, r)$:

$$\bar{G}(r, S^{in}) = \max_{D \in [0, r\delta]} G_2(S^{in}, D, r). \quad (13)$$

Note that $G^{max}(S^{in}) = \bar{G}(1, S^{in})$. The following result follows from Proposition 8 of [4].

Proposition 6 Assume that f is \mathcal{C}^2 . If $a > 0$, then there exists $r^* \in (0, 1)$ such that for any $r \in (r^*, 1)$ we have $\bar{G}(r, S^{in}) > G^{max}(S^{in})$.

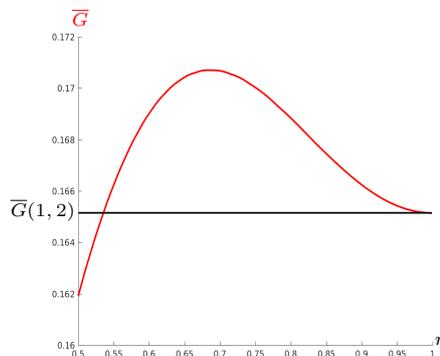


Figure 4: The map $r \mapsto \bar{G}(r, S^{in})$ with \bar{G} defined by (13) with $f(S) = 4S/(5 + S)$, $a = 0.6$ and $S^{in} = 2$.

Proposition 6 is illustrated with Figure 4, where one observes that the tangent of \bar{G} at $r = 1$ is horizontal i.e $\bar{G}'(1, 2) = 0$ and that $\bar{G}''(1, 2)$ is positive and remains positive in a neighborhood \mathcal{V}_1 of $r = 1$. In real ecosystems, a biomass mortality often occurs, but is sometimes neglected when it is very small. Indeed, this last result shows that the occurrence of a biomass mortality, even small, may be advantageous: considering two interconnected reactors in series where the volume of the first reactor rV is close to the total volume V (but not equal to, so that the second reactor has a small but non null volume $(1 - r)V$) gives the possibility to choosing a dilution rate sufficiently close or equal to $\bar{D}(r)$, defined in Proposition 6, which optimizes the production of the biogas flow rate and gives a better and more efficient functioning than a single tank configuration.

In Figure 5, graphs of the family of functions $r \mapsto G_2(S^{in}, D, r)$ for different values of D are plotted in black ($S^{in} > 0$ being fixed). The function $r \mapsto \bar{G}(r, S^{in})$ plotted in Figure 4 is thus the upper envelope of this family. Two particular curves are added on Figure 5. The green one corresponds to the value of D that maximizes the biogas production for the single chemostat, that is D which realizes the maximum of the function $D \mapsto G_{chem}(S^{in}, D)$. The blue one corresponds to the value of D of the maximizer of $(D, r) \mapsto G_2(S^{in}, D, r)$ (which has been obtained numerically), or equivalently D is the maximizer of $D \mapsto G_2(S^{in}, D, r)$ when r maximizes $r \mapsto \bar{G}(r, S^{in})$. One can see that the envelope of this family of functions is non monotonic and admits a maximum which is not reached for $r = 1$ (see the blue curve). Moreover, all the curves are locally decreasing about $r = 1$, excepted the one for D that realizes the maximum of $D \mapsto G_{chem}(S^{in}, D)$ (the green one), which is increasing with an horizontal slope at $r = 1$. Indeed, all the other curves reach 0 (wash-out) at smaller values of r and thus intersect the green one.

5 Conclusion

In this paper, we present different results following the study of a mathematical model of two interconnected chemostats in series. The particularity of our study is the consideration or not of mortality. On the one hand, we show that for fixed substrate concentration S^{in} and dilution rate D , whatever the mortality is; strictly positive

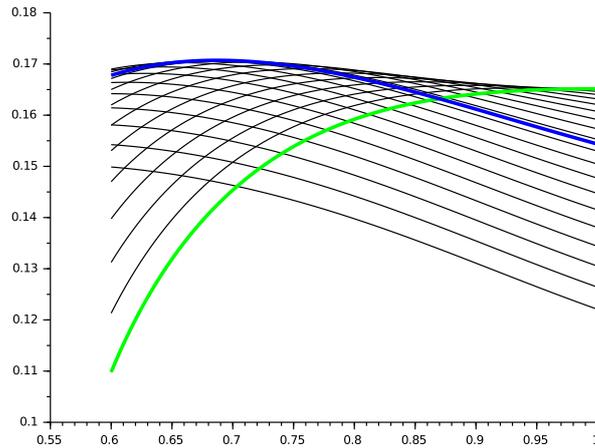


Figure 5: Curves $r \mapsto G_2(S^{in}, D, r)$ for different values of D , with $f(S) = 4S/(5 + S)$, $a = 0.6$ and $S^{in} = 2$. In blue, the one for $D \simeq 0.213$ such that $\max_r \bar{G}(r, S^{in}) = \max_r G_2(S^{in}, D, r)$. In green, the one for $D \simeq 0.283$ such that $G^{max}(S^{in}) = G_{chem}(S^{in}, D)$.

or null, for a volume distribution such that r is greater than the threshold $r_1(S^{in}, D)$, there exists a series configuration of two chemostats that gives a smaller substrate concentration than a single chemostat. In addition, with respect to the parameter r , we can even define the best performing series configuration which corresponds to $r = r_{min}$ defined by (8). This result was numerically noticed by [8] who built on the work done by [6] and [2]. Indeed, in the case with null mortality, using a growth function of type Monod, [8] found that two or three reactors in series optimally designed (e.g. the first reactor has the biggest volume), could provide substrate conversions similar to a PFR. Under the experimental study done by [6], where when using a Monod growth function, it is deduced that a PFR is always more efficient than a single chemostat, [8] deduced that under precise conditions, a configuration of two or three reactors in series more efficient than a single chemostat can exist. On the other hand, for a fixed inlet substrate concentration S^{in} and a previously defined volume distribution into the two tanks i.e. r , if the practitioner can choose the dilution rate D then, for null mortality, we prove that the single chemostat is always more efficient and allows to have a higher biogas flow rate than the serial device. Knowing that biogas flow rate is proportional to the microbial activity and as shown in [3], the biogas flow rate and the productivity of the biomass of the two series interconnected chemostats configuration are defined by the same equations at steady state, then all the results with no mortality, quoted so far, are valid for three different performances criteria: output substrate concentration, biogas flow rate and biomass productivity. Now, in the case where mortality is positive, the last result is not the same and changes significantly. Indeed, for a positive mortality, for a fixed S^{in} and r , if the practitioner can choose the dilution rate D , then the series configuration becomes the structure that should be considered. Indeed, for the right chosen dilution rate D , the series configuration gives a higher biogas flow rate than the single chemostat. This can only happen with presence of mortality. Therefore, considering mortality in a biological experiment corresponding to the hypotheses posed in our results, can represent a solution to improve the out-turn of the experiment.

Acknowledgements

These results are part of the JPI project Control4reuse (cf. <http://control4reuse.net>) financed by the French Research National Agency under the contract ANR-18-IC4W-0002. The authors thank also the support of the Euro-Mediterranean network TREASURE and the Algerian program "Bourses d'Excellence" for the funding of the PhD grant of Manel Dali Youcef.

References

- [1] G. Bastin and D. Dochain. *On-line estimation and adaptive control of bioreactors*. Elsevier, Amsterdam, 1990 (ISBN 0-444-88430-0). xiv+ 379 pp. Price US \$146.25/Dfl. 285.00, Elsevier, 1991.
- [2] Kenneth B Bischoff. Optimal continuous fermentation reactor design. *The Canadian Journal of Chemical Engineering*, 44(5):281–284, 1966.

- [3] M. Dali-Youcef, A. Rapaport, and T. Sari. Study of performance criteria of serial configuration of two chemostats. *Math. Biosci. Eng.*, 17(6):6278–6309, 2020.
- [4] M. Dali-Youcef, A. Rapaport, and T. Sari. Performances study of two serial interconnected chemostats with mortality. working paper or preprint, August 2021.
- [5] C. D. de Gooijer, W. A. M. Bakker, H. H. Beftink, and J. Tramper. Bioreactors in series : an overview of design procedures and practical applications. *Enzyme and Microbial Technology*, 18:202–219, 1996.
- [6] RB Grieves, WO Pipes, WF Milbury, and RK Wood. Piston-flow reactor model for continuous industrial fermentations. *Journal of Applied Chemistry*, 14(11):478–486, 1964.
- [7] J. Harmand, A. Rapaport, and A. Trofino. Optimal design of interconnected bioreactors : new results. *AIChE Journal*, 49(6):1433–1450, 2003.
- [8] Gordon A Hill and Campbell W Robinson. Minimum tank volumes for cfst bioreactors in series. *The Canadian Journal of Chemical Engineering*, 67(5):818–824, 1989.
- [9] M. Polihronakis, L. Petrou, and A. Deligiannis. Parameter adaptive control techniques for anaerobic digesters—real-life experiments. *Elsevier, Computers & chemical engineering*, 17(12):1167–1179, 1993.
- [10] A. Schievano, A. Tenca, S. Lonati, E. Manzini, and F. Adani. Can two-stage instead of one-stage anaerobic digestion really increase energy recovery from biomass? *Applied Energy*, 124:335–342, 2014.
- [11] S. E. Scuras, A. Jobbagy, and C. P. Leslie Grady. Optimization of activated sludge reactor configuration : kinetic considerations. *Water Research*, 35(18):4277–4284, 2001.
- [12] Jesús Zambrano and Bengt Carlsson. Optimizing zone volumes in bioreactors described by monod and contois growth kinetics. In *Proceeding of the IWA World Water Congress & Exhibition*, 2014.

*MISTEA, Univ Montpellier, INRAE, Institut Agro, Montpellier, France
Email addresses: manel.dali-youcef@inrae.fr/ alain.rapaport@inrae.fr

**LMA, Univ Avignon, Avignon, France
Email adress: manel.dali-youcef@univ-avignon.fr

***LBE, INRAE, Narbonne, France
Email adress: jerome.harmand@inrae.fr

****ITAP, Univ Montpellier, INRAE, Institut Agro, Montpellier, France
Email adress: tewfik.sari@inrae.fr