



**HAL**  
open science

# Stabilization of the chemostat system with mutations and application to microbial production

T Bayen, J Coville, F Mairet

► **To cite this version:**

T Bayen, J Coville, F Mairet. Stabilization of the chemostat system with mutations and application to microbial production. 2022. hal-03767646

**HAL Id: hal-03767646**

**<https://univ-avignon.hal.science/hal-03767646>**

Preprint submitted on 2 Sep 2022

**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.

# Stabilization of the chemostat system with mutations and application to microbial production\*

T. Bayen<sup>†</sup>, J. Coville<sup>‡</sup>, F. Mairet<sup>§</sup>

August 11, 2022

## Abstract

In this paper, we consider the chemostat system with  $n \geq 1$  species, one limiting substrate, and mutations between species. Our objective is to globally stabilize the corresponding dynamical system around a desired equilibrium point. Doing so, we introduce *auxostat feedback controls* which are controllers allowing the regulation of the substrate concentration. We prove that such feedback controls globally stabilize the resulting closed-loop system near the desired equilibrium point. This result is obtained by combining the theory of asymptotically autonomous systems and an explicit computation of solutions to the limit system. The performance of such controllers is illustrated on an optimal control problem of Lagrange type which consists in maximizing the production of species over a given time period w.r.t. the dilution rate chosen as control variable.

**Keywords** : Dynamical system, chemostat system, global stabilization, feedback control, optimal control, Pontryagin maximum principle.

## 1 Introduction

The chemostat system describes the evolution in time of bacteria interacting with a resource, and it was introduced simultaneously by Monod and Novick & Szilard in the fifties [31, 32, 33]. Such a dynamical system is widely used for ecological purpose for analyzing the growth of micro-organisms in natural environments (mountain lakes, lagoon, see, *e.g.*, [43]). It also serves in biotechnology to describe processes such as anaerobic digestion, waste water treatment, or engineering systems related to the production of molecules of industrial interest [2, 16, 17].

An important part of the literature related to the chemostat model deals with the stabilization of the corresponding dynamical system at a nominal point [17]. Such a questioning is of great interest, typically for maximizing microbial growth at steady state in anaerobic digestion models [4, 37], but also to cope with uncertainties (which is often encountered in bioprocesses) and delays on measurements [19, 27, 28, 29, 36]. The main objective of this paper is to study the stabilization of the chemostat system whenever mutations for microorganisms are incorporated [3, 5, 6]. This means that, in addition to the classical equations of the chemostat, each species is able to convert into neighbor ones. It is relevant whenever the number of species is high in order to take into account evolution (see [1, 3, 25]). Such a phenomenon leading to a coupling term between species may be related to transposable elements, gene transfer [12, 13], or mutations (among other possible factors). In the sequel, we employ the wording “mutations” to describe the coupling between species that is considered throughout this work. Note that the resulting system with mutations can be viewed as an approximation of population dynamics models involving a phenotypic trait (see, *e.g.*, [30]).

In order to stabilize the chemostat system with mutations near a desired equilibrium point, we introduce a linear feedback control of the state that maintains constant the substrate concentration at a desired value. Such

---

\*This research benefited from the support of Avignon Université (AAP Agro&Sciences) and from the support of the FMJH Program PGM0 and from the support to this program from EDF-THALES-ORANGE.

<sup>†</sup>Avignon Université, Laboratoire de Mathématiques d’Avignon (EA 2151), F-84018 Avignon, France. [terence.bayen@univ-avignon.fr](mailto:terence.bayen@univ-avignon.fr)

<sup>‡</sup>UR 546 Biostatistique et Processus Spatiaux, INRAE, Domaine St Paul Site Agroparc, F-84000 Avignon, France. [jerome.coville@inrae.fr](mailto:jerome.coville@inrae.fr)

<sup>§</sup>PHYSALG, PHYTOX, Ifremer, F-44000 Nantes, France. [francis.mairet@ifremer.fr](mailto:francis.mairet@ifremer.fr)

a regulating feedback is usually called an *auxostat* (see [5] and references herein). Our main result is Theorem 2.1 which asserts that the auxostat globally stabilizes (in the corresponding forward invariant domain) the resulting closed-loop system at the corresponding steady state. The proof of this result combines the theory of asymptotically autonomous systems (see [41]) and an explicit computation of solutions to the limit system (see [35]) after proving the convergence of the substrate concentration to the desired value. The same convergence result is also established replacing the preceding feedback by a non-linear variant of practical interest when one has access to measurements of the global biological activity (*e.g.*, biogas production or oxygen consumption, depending on which process is considered). It is worth mentioning that Theorem 2.1 is proved with Monod kinetics for the species because in that case, the Perron-Frobenius Theorem guarantees the uniqueness of a steady-state apart the washout. When considering Haldane’s kinetics, it is well-known that stability properties of the chemostat system are more involved since various stable equilibria exist [23, 40] (bistability for instance). When mutations are present, the system with a constant dilution rate also exhibits several equilibria. For each feedback control, we provide in this case a discussion about global stability of the resulting closed-loop system. To conclude the exposition about stability properties of the chemostat system with mutations, we would like to emphasize that global stability is an open question whenever the dilution rate is constant (except for small enough values of the dilution rate [3] or in the case where kinetics are close to a nominal one [12]). Thus, it is of great interest to consider feedback controls that globally stabilize the resulting closed-loop system.

The second issue addressed in this paper is concerned with analyzing the benefit of employing auxostat controllers in a real-world microbial production problem (see, *e.g.*, [20, 22] for related problems). Doing so, we consider an optimal control problem which consists in maximizing w.r.t. the dilution rate (chosen as an open loop control) the total production of species in a chemostat system with mutations over a time frame (see, *e.g.*, [6, 14, 15]). We start by giving properties of optimal controls that can be found using the Pontryagin Maximum Principle (PMP), see [34]. We also inspect the Legendre-Clebsch condition which allows us to obtain insights into the optimal synthesis and the turnpike structure of optimal controls [42]. Next, we turn to the definition of auxostat controllers via the resolution of the static optimization problem that is naturally associated to the optimal control problem under investigation. Its solution provides a nominal equilibrium point allowing the startup of the feedback controls. We then compare for various time frames the value of the cost function computed for the auxostat controls to the value function of the optimal control problem (computed numerically via a direct solver `bocop` [8]). We observe that whenever the time period increases, the gap between the value function and the cost function computed for the auxostat controls tends to be very small. In contrast, when the dilution rate is fixed as an optimization parameter (maximizing the cost function at steady-state), numerical simulations indicate that the approximation of the value function is less accurate.

The paper is structured as follows. In Section 2, we introduce the stabilization problem and we prove Theorem 2.1 which is our main result. We end-up this section by relating the desired steady-state (defined thanks to a given nominal value of the substrate) to the coexistence equilibrium when considering a constant dilution rate (see [3]). We also discuss global stability properties when Haldane’s kinetics are considered in place of Monod functions. In section 3, we introduce the optimal control problem related to the maximization of species and we first derive properties on optimal controls using the PMP. Next, we compare the performance of the auxostat controllers defined in Section 2 to the optimal solution computed numerically via a direct method. We end up the paper with conclusions and perspectives (Section 4).

## 2 Stabilization via auxostat controllers

### 2.1 Statement of the problem and main result

Throughout this paper, we consider a chemostat system with  $n \geq 1$  species, one limiting substrate, and mutations between species (represented by a linear coupling). The resulting dynamical system can be written:

$$\begin{cases} \dot{x}_i &= \mu_i(s)x_i - ux_i + \varepsilon(Mx)_i, & 1 \leq i \leq n, \\ \dot{s} &= - \sum_{j=1}^n \mu_j(s)x_j + u(1-s), \end{cases} \quad (2.1)$$

where  $x_i$ , resp.  $s$  denotes the concentration of species  $i$ , resp. the substrate concentration,  $u$  is the dilution rate (at this step,  $u$  is constant),  $\mu_i : \mathbb{R}_+ \rightarrow \mathbb{R}_+$  is the growth function associated with species  $i$ ,  $M$  is the

mutation matrix<sup>1</sup>

$$M := \begin{bmatrix} -1 & 1 & 0 & \cdots & 0 \\ 1 & -2 & 1 & \cdots & 0 \\ \vdots & \ddots & \ddots & \ddots & \vdots \\ 0 & \cdots & 1 & -2 & 1 \\ 0 & \cdots & 0 & 1 & -1 \end{bmatrix}, \quad (2.2)$$

and the input substrate concentration  $s_{in}$  has been normalized to 1 (with regards to classical notation in the literature, see, *e.g.*, [23], this amounts to take  $s_{in} = 1$  which is always possible from a mathematical point of view). Note also that we assumed yield coefficients to be all equal (based on experimental studies (see, *e.g.*, [33] and remarks about this point in [3]) and thus also normalized to 1. The positive number  $\varepsilon$  is called the mutation parameter.

**Remark 2.1.** *When  $\varepsilon = 0$ , we retrieve the classical chemostat system whose properties such as the competitive exclusion principle can be found in [23, 40]. The mutation matrix  $M$  is related to the discretization of the one-dimensional Laplace equation (Poisson problem) with Neumann boundary conditions [30]. A more general mutation matrix could be considered in place of  $M$  as long as the equality  $\sum_{j=1}^n m_{i,j} = 0$  remains valid for every  $1 \leq i \leq n$  in order to ensure mass conservation. Here, we can see that for every  $2 \leq i \leq n-1$ , species  $i$  converts into  $i-1$  and  $i+1$  whereas for  $i=1$  and  $i=n$ , it converts into species 2 and  $n-1$ . The particular expression of the mutation matrix in the first and last row is required for mass conservation. If yield coefficients are not equal, the study of (2.1) may be more involved since mass conservation will no longer hold true. So we chose not to address this issue here.*

In what follows (except in Sections 2.3 and 3.5), we suppose that for  $1 \leq i \leq n$ ,  $\mu_i$  is of Monod type, *i.e.*,

$$\mu_i(s) = \frac{m_i s}{k_i + s},$$

where parameters  $m_i, k_i$  are positive for all  $1 \leq i \leq n$ . Following [3], initial conditions for (2.1) are in the set

$$\mathcal{D} := \mathbb{R}_+^n \setminus \{0_{\mathbb{R}^n}\} \times (0, 1],$$

that is forward invariant by (2.1). The next property can be easily obtained from [3, Property 2.2].

**Property 2.1.** *Let  $v : \mathbb{R} \times \mathbb{R}^n \times \mathbb{R} \rightarrow \mathbb{R}_+$ ,  $(t, x, s) \mapsto v(t, x, s)$  be a real-valued measurable function with non-negative values,  $\varepsilon > 0$ , and  $(x^0, s^0) \in \mathcal{D}$ . If there exists a global<sup>2</sup> solution  $(x(\cdot), s(\cdot))$  to (2.1) with  $u = v$  such that  $(x(0), s(0)) = (x^0, s^0)$ , then,*

$$\forall t > 0, \forall 1 \leq i \leq n, x_i(t) > 0. \quad (2.3)$$

Our main aim in this work is to stabilize system (2.1) near a desired steady-state via an autonomous feedback control of the state. Doing so, we consider (2.1) in which  $u$  is replaced by a function  $\varphi : \mathbb{R}^n \times \mathbb{R} \rightarrow \mathbb{R}$ ,  $(x, s) \mapsto \varphi(x, s)$ , which amounts to write:

$$\begin{cases} \dot{x}_i &= \mu_i(s)x_i - \varphi(x, s)x_i + \varepsilon(Mx)_i, & 1 \leq i \leq n, \\ \dot{s} &= -\sum_{j=1}^n \mu_j(s)x_j + \varphi(x, s)(1-s). \end{cases} \quad (2.4)$$

Let us now introduce a feedback control  $\varphi_\sigma$  as

$$\varphi_\sigma(x) := \frac{1}{1-\sigma} \sum_{j=1}^n \mu_j(\sigma)x_j, \quad x \in \mathbb{R}^n, \quad (2.5)$$

where  $\sigma \in (0, 1)$  is fixed. Such a linear control w.r.t.  $x$  is called *auxostat* [5]. This terminology is justified by the following property : if we put  $\varphi_\sigma$  in place of  $\varphi$  in (2.4), then, for every  $t_0 \geq 0$ , one has

$$s(t_0) = \sigma \quad \Rightarrow \quad s(t) = \sigma, \quad \forall t \geq t_0,$$

<sup>1</sup>As usual, matrices are named using capital letters and coefficients are represented by lower case letters.

<sup>2</sup>*i.e.*, defined over  $\mathbb{R}_+$

by uniqueness of solutions in Cauchy-Lipschitz's Theorem. For practical interest in bioprocesses [26], it is also relevant to introduce the feedback control  $\psi_\sigma$  given by

$$\psi_\sigma(s, x) := \frac{1}{1-\sigma} \sum_{j=1}^n \mu_j(s) x_j, \quad (s, x) \in (0, 1] \times \mathbb{R}^n, \quad (2.6)$$

where  $\sigma \in (0, 1)$  is fixed. Its interest comes from the fact that  $\psi_\sigma$  is related to microbial growth in a bioreactor, and one can have easily access to measurements of this quantity. Our main result about global stabilization in this paper can be stated as follows.

**Theorem 2.1.** *For every  $(\varepsilon, \sigma) \in \mathbb{R}_+^* \times (0, 1)$ , the system (2.4) in which  $\varphi = \varphi_\sigma$  or  $\varphi = \psi_\sigma$  has a unique steady-state  $(x^{\sigma, \varepsilon}, \sigma) \in \mathcal{D}$  that is globally asymptotically stable in the set  $\mathcal{D}$ .*

As we will see in Section 2.2, the equilibrium point  $(x^{\sigma, \varepsilon}, \sigma) \in \mathcal{D}$  is defined as the unique solution to the system

$$D_s x - ux + \varepsilon M x = 0 \quad ; \quad \sum_{j=1}^n x_j + s = 1 \quad ; \quad s = \sigma, \quad (2.7)$$

where  $D_s \in \mathbb{R}^{n \times n}$  is the diagonal matrix  $D_s := \text{diag}(\mu_1(s), \dots, \mu_n(s))$  and  $u$  is the Perron root of the quasi-positive irreducible matrix<sup>3</sup>

$$A_{\varepsilon, s} := D_s + \varepsilon M \in \mathbb{R}^{n \times n},$$

(see, e.g., [3]). Note that the first equality in (2.7) defines the vector  $x$  as the Perron vector of  $A_{\varepsilon, \sigma}$  up to a positive multiplicative constant, i.e.,  $x = \kappa a_{\varepsilon, \sigma}$  for some  $\kappa > 0$  where  $a_{\varepsilon, \sigma}$  is the unitary Perron vector of  $A_{\varepsilon, \sigma}$ . The second equality in (2.7) allows to uniquely define the eigenvector vector  $x$  as  $x = \frac{1}{\kappa} a_{\varepsilon, \sigma}$  where  $\kappa := a_{\varepsilon, \sigma} \cdot \bar{1}$ ,  $\bar{1} := (1, \dots, 1) \in \mathbb{R}^n$ , and  $\cdot$  denotes the standard inner product of  $\mathbb{R}^n$ .

## 2.2 Proof of Theorem 2.1

To prove Theorem 2.1, we start by studying the asymptotic behavior of the total mass of the system

$$b := \sum_{j=1}^n x_j + s,$$

and of  $s(\cdot)$  in the case where  $\varphi = \varphi_\sigma$  in (2.4).

**Lemma 2.1.** *Let  $(\varepsilon, \sigma) \in \mathbb{R}_+^* \times (0, 1)$  and let  $(x^0, s^0) \in \mathcal{D}$ . Then, the unique solution of (2.4) associated with the feedback control given by (2.5) starting from  $(x^0, s^0)$  at time  $t = 0$  satisfies*

$$\lim_{t \rightarrow +\infty} b(t) = 1 \quad \text{and} \quad \lim_{t \rightarrow +\infty} s(t) = \sigma.$$

*Proof.* From (2.4),  $b$  satisfies  $\dot{b} = \varphi_\sigma(x)(1-b)$ , therefore  $b(t) = 1 + (b(0) - 1)e^{-\int_0^t \varphi_\sigma(x(\tau)) d\tau}$  for all  $t \geq 0$ . Let us then consider the three following cases.

*Case 1:*  $b(0) = 1$ . Then,  $b(t) = 1$  for every  $t \geq 0$  so that  $\lim_{t \rightarrow +\infty} b(t) = 1$ .

*Case 2:*  $b(0) > 1$ . It follows that  $b > 1$  and that  $b(\cdot)$  decreases over  $\mathbb{R}_+$ . Thus there exists  $\ell \geq 1$  such that  $\lim_{t \rightarrow +\infty} b(t) = \ell$ . We deduce that  $b$  and  $x$  are bounded over  $\mathbb{R}_+$  and so is  $\dot{x}$ . We suppose by contradiction that  $\ell > 1$ . By applying Barbalat's Lemma (see [24]), one must have  $\dot{b}(t) \rightarrow 0$  as  $t \rightarrow +\infty$  which implies that  $\varphi_\sigma(x(t)) \rightarrow 0$  as  $t \rightarrow +\infty$ . Hence, for every  $1 \leq k \leq n$ , one has  $x_k(t) \rightarrow 0$  as  $t \rightarrow +\infty$ . It follows that  $s(t) \rightarrow \ell > 1$  as  $t \rightarrow +\infty$  which contradicts the fact that  $[0, 1]$  is invariant by the equation of  $\dot{s}$  in (2.4) (see Property 2.1).

*Case 3:*  $b(0) < 1$ . Similarly as in case 2, one has  $b < 1$  and  $b(\cdot)$  also increases over  $\mathbb{R}_+$ , thus, there is  $\ell \in (0, 1]$  such that  $\lim_{t \rightarrow +\infty} b(t) = \ell$ . We again argue by contradiction supposing that  $\ell < 1$ . By Barbalat's Lemma, one must have  $\dot{b}(t) \rightarrow 0$  as  $t \rightarrow +\infty$ , and similarly as previously, we deduce that  $\varphi_\sigma(x(t)) \rightarrow 0$  as  $t \rightarrow +\infty$ ,

<sup>3</sup>Recall that given a quasi-positive irreducible matrix  $A \in \mathbb{R}^{n \times n}$ , the Perron-Frobenius Theorem ensures that the Perron root of  $A$  denoted by  $\lambda(A)$  (its greatest eigenvalue) is simple and that the Perron vector  $x$  (the associated eigenvector) is with positive coordinates. The fact that  $A_{\varepsilon, s}$  is quasi-positive irreducible is standard (see, e.g., [3]).

hence, for every  $1 \leq k \leq n$ , one must have  $x_k(t) \rightarrow 0$  as  $t \rightarrow +\infty$ . This implies that  $s(t) \rightarrow \ell$  as  $t \rightarrow +\infty$ . Observe now that one has

$$\sum_{j=1}^n \dot{x}_j(t) = \sum_{j=1}^n \mu_j(s(t))x_j(t) - \left( \sum_{j=1}^n \frac{\mu_j(\sigma)}{1-\sigma} x_j(t) \right) \left( \sum_{j=1}^n x_j(t) \right).$$

Now, there is  $\alpha > 0$  and  $t_0 \geq 0$  such that for every  $t \geq t_0$ , one has  $\mu_i(s(t)) \geq \alpha$  for every  $1 \leq i \leq n$  and every  $t \geq t_0$ . If we set  $\beta := \max_{1 \leq i \leq n} \frac{\mu_i(\sigma)}{1-\sigma}$ , we obtain for every  $t \geq t_0$ :

$$\sum_{j=1}^n \dot{x}_j(t) \geq \alpha \sum_{j=1}^n x_j(t) - \beta \left( \sum_{j=1}^n x_j(t) \right)^2 = \alpha \left( \sum_{j=1}^n x_j(t) \right) \left( 1 - \frac{\beta}{\alpha} \sum_{j=1}^n x_j(t) \right).$$

From the previous inequality, we deduce that  $\sum_{j=1}^n x_j$  is necessarily increasing over some time interval  $[t'_0, +\infty)$  where  $t'_0 \geq t_0$ . This contradicts the fact that it converges to zero as  $t \rightarrow +\infty$ . Hence, one must have  $\ell = 1$  as was to be proved.

We now address the convergence of  $s(\cdot)$ . From (2.4) with  $\varphi = \varphi_\sigma$  and using Property 2.1, one has for every time  $t \geq 0$ ,  $\dot{s}(t)|_{s=1} = -\sum_{j=1}^n \mu_j(1)x_j(t) < 0$ . We deduce that  $s(t) < 1$  for every  $t > 0$ . Now, the ODE<sup>4</sup> satisfied by  $s$  can be equivalently written as follows:

$$\dot{s}(t) = (1-s(t)) \sum_{j=1}^n x_j(t) \left[ \frac{\mu_j(\sigma)}{1-\sigma} - \frac{\mu_j(s(t))}{1-s(t)} \right] = (1-s(t))(\sigma-s(t)) \sum_{j=1}^n a_j(t)x_j(t), \quad (2.8)$$

where for  $1 \leq j \leq n$ ,

$$a_j(t) := \int_0^1 \rho'_j(s(t) + \xi(\sigma - s(t))) d\xi \quad \text{and} \quad \rho_j(\zeta) := \frac{\mu_j(\zeta)}{1-\zeta}, \quad \zeta \in (0, 1). \quad (2.9)$$

By a direct computation, we find that for  $\zeta \in [0, 1)$ , one has

$$\rho'_j(\zeta) = \frac{\mu'_j(\zeta)(1-\zeta) + \mu_j(\zeta)}{(1-\zeta)^2}.$$

Hence, there exists  $m > 0$  such that for every  $\zeta \in [0, 1)$ , one has  $\rho'_j(\zeta) \geq m$  for every  $\zeta \in [0, 1)$  implying that for every  $t \geq 0$ , one has  $a_j(t) \geq m$ .

*Case 1:*  $s(0) = \sigma$ . Then,  $s(t) = \sigma$  for every time  $t \geq 0$  implying that  $s(t) \rightarrow \sigma$  as  $t \rightarrow +\infty$ .

*Case 2:*  $s(0) > \sigma$ . In that case,  $s(\cdot)$  decreases over  $\mathbb{R}_+$ , thus it converges to some value  $\tilde{s} \in [\sigma, 1)$ . Without any loss of generality, we can assume that  $s(0) < 1$  (since  $s(\cdot)$  decreases). Now, let  $\eta > 0$ . Because  $s(\cdot)$  decreases and  $\sum_{j=1}^n x_j(t) \rightarrow 1 - \tilde{s}$  as  $t \rightarrow +\infty$ , we deduce that there exists  $t_0 \geq 0$  such that for every  $t \geq t_0$ , one has  $s(t) \leq 1 - \eta$  and  $\sum_{j=1}^n x_j(t) \geq \frac{1-\tilde{s}}{2}$ . We obtain the inequality

$$\dot{s}(t) \leq \frac{m\eta(1-\tilde{s})}{2}(\sigma - s(t)),$$

for every  $t \geq t_0$ , thus  $s(t) \rightarrow \sigma$  as  $t \rightarrow +\infty$  as was to be proved.

*Case 3:*  $s(0) < \sigma$ . In that case,  $s(\cdot)$  increases over  $\mathbb{R}_+$ , thus it converges to some value  $\tilde{s} \leq \sigma$  (and we can assume that  $\tilde{s} > 0$ ). Similarly as in case 2, there is  $t_0 \geq 0$  such that  $\sum_{j=1}^n x_j(t) \geq \frac{1-\tilde{s}}{2}$  for every  $t \geq t_0$ . We deduce that for  $t \geq t_0$ , one has

$$\dot{s}(t) \geq \frac{m(1-\tilde{s})(1-\sigma)}{2}(\sigma - s(t)).$$

We conclude in the same way. □

The same properties as in Lemma 2.1 hold true with  $\varphi = \psi_\sigma$  in (2.4). For sake of completeness, we give details of the proof in that case because the closed loop system (2.4) with  $\varphi = \psi_\sigma$  is not identical to (2.4) with  $\varphi = \varphi_\sigma$ , thus, the proof slightly differs.

---

<sup>4</sup>Ordinary Differential Equation.

**Lemma 2.2.** Let  $(\varepsilon, \sigma) \in \mathbb{R}_+^* \times (0, 1)$  and let  $(x^0, s^0) \in \mathcal{D}$ . Then, the unique solution of (2.4) associated with the feedback control given by (2.6) starting from  $(x^0, s^0)$  at time  $t = 0$  satisfies

$$\lim_{t \rightarrow +\infty} b(t) = 1 \quad \text{and} \quad \lim_{t \rightarrow +\infty} s(t) = \sigma.$$

*Proof.* First, we check that

$$\dot{b} = \psi_\sigma(x, s)(1 - b) \quad \text{and} \quad \dot{s} = -\psi_\sigma(x, s)(s - \sigma), \quad (2.10)$$

from which we deduce that  $b$  and  $s$  are monotone and bounded, so there exist  $\ell := \lim_{t \rightarrow +\infty} b(t)$  and  $\tilde{s} := \lim_{t \rightarrow +\infty} s(t)$ . In addition, we claim that  $\tilde{s} \in (0, 1)$ . Indeed, if  $s(0) = \sigma$ , the result is clear from Cauchy-Lipschitz's Theorem. If  $s(0) < \sigma$ , resp.  $s(0) > \sigma$ , then,  $s(\cdot)$  is increasing, resp. decreasing, whence the result.

*Case 1 :*  $b(0) = 1$ . It follows that  $b(t) = 1$  for  $t \geq 0$ . If  $\tilde{s} \neq \sigma$ , according to Barbalat's Lemma in (2.10), we obtain that  $\psi_\sigma(x(t), s(t)) \rightarrow 0$  as  $t \rightarrow +\infty$  and  $\sum_{j=1}^n x_j(t) \rightarrow 0$  as well. Using that  $b(t) = 1 = \sum_{j=1}^n x_j(t) + s(t)$  for  $t \geq 0$ , we obtain that  $s(t) \rightarrow 1$  as  $t \rightarrow +\infty$  which is a contradiction. Thus,  $\tilde{s} = \sigma$ .

*Case 2 :*  $b(0) > 1$ . It follows that  $\ell \geq 1$ . Suppose that  $\ell > 1$ . Using Barbalat's Lemma, we obtain that  $\psi_\sigma(x(t), s(t)) \rightarrow 0$  as  $t \rightarrow +\infty$  and  $\sum_{j=1}^n x_j(t) \rightarrow 0$  as  $t \rightarrow +\infty$ . We deduce that  $s(t) \rightarrow \ell \geq 1$  as  $t \rightarrow +\infty$  in contradiction with  $\tilde{s} \in (0, 1)$ . Hence,  $\ell = 1$ . Going back to  $b = \sum_{j=1}^n x_j + s$ , we obtain that  $\sum_{j=1}^n x_j(t) \rightarrow 1 - \tilde{s} > 0$  as  $t \rightarrow +\infty$ . Again by Barbalat's lemma, if  $\tilde{s} \neq \sigma$ , then, one should have  $\dot{s}(t) \rightarrow 0$  when  $t \rightarrow +\infty$  as well as  $\psi_\sigma(x(t), s(t)) \rightarrow 0$  when  $t \rightarrow +\infty$  from which we deduce that  $\sum_{j=1}^n x_j(t) \rightarrow 0$  as  $t \rightarrow +\infty$  which is a contradiction. Hence, we obtain  $\tilde{s} = \sigma$ .

*Case 3 :*  $b(0) < 1$ . In that case, suppose that  $\ell < 1$ . Then, again, we obtain that  $\psi_\sigma(x(t), s(t)) \rightarrow 0$  as  $t \rightarrow +\infty$ , thus  $x_i(t) \rightarrow 0$  as  $t \rightarrow +\infty$  for every  $1 \leq i \leq n$ . Hence, the following expression

$$\sum_{j=1}^n \dot{x}_j(t) = \sum_{j=1}^n \mu_j(s(t)) x_j(t) \left[ 1 - \frac{1}{1 - \sigma} \sum_{j=1}^n x_j(t) \right]$$

is necessarily positive for  $t$  large enough which contradicts the fact that  $\psi_\sigma(x(t), s(t)) \rightarrow 0$  as  $t \rightarrow +\infty$ . This concludes the proof.  $\square$

We now turn to the existence of steady-states for the closed loop system (2.4) with the feedback control  $\varphi_\sigma$  or  $\psi_\sigma$ .

**Proposition 2.1.** For every  $(\varepsilon, \sigma) \in \mathbb{R}_+^* \times (0, 1)$  system (2.4) with  $\varphi = \varphi_\sigma$  or  $\varphi = \psi_\sigma$  has a unique steady-state  $(x^{\sigma, \varepsilon}, \sigma) \in \mathcal{D}$  defined by (2.7).

*Proof.* Fix  $(\varepsilon, \sigma) \in \mathbb{R}_+^* \times (0, 1)$  and let  $(x, s) \in \mathcal{D}$  be a steady-state of (2.4) with  $\varphi = \varphi_\sigma$  and note that  $x \neq 0_{\mathbb{R}^n}$ . It follows that  $(x, s)$  is a solution to the system

$$\sum_{j=1}^n x_j + s = 1 \quad ; \quad \sum_{j=1}^n [\rho_j(s) - \rho_j(\sigma)] x_j = 0, \quad (2.11)$$

where  $\rho_j$  is defined in (2.9). Since the kinetics are of Monod type, the second above equality implies that  $s = \sigma$ . Now, in the case where  $\varphi = \psi_\sigma$ , a steady-state  $(x, s) \in \mathcal{D}$  of (2.4) with  $\varphi = \varphi_\sigma$  necessarily verifies

$$\sum_{j=1}^n x_j + s = 1 \quad ; \quad \sum_{j=1}^n \mu_j(s) x_j \left[ 1 - \frac{1 - s}{1 - \sigma} \right] = 0, \quad (2.12)$$

implying also that  $s = \sigma$  (since in the set  $\mathcal{D}$ ,  $s \neq 0$  and  $x \neq 0_{\mathbb{R}^n}$ ). It follows that a point  $(x, s) \in \mathcal{D}$  is a steady-state of (2.4) with  $\varphi = \varphi_\sigma$  or  $\varphi = \psi_\sigma$  if and only if

$$A_{\varepsilon, \sigma} x = u x \quad \text{and} \quad \sum_{j=1}^n x_j + \sigma = 1,$$

where  $u = \varphi_\sigma(x)$  or  $u = \psi_\sigma(x, \sigma)$ . By the Perron-Frobenius Theorem, the matrix  $A_{\varepsilon, \sigma}$  has a unique positive eigenvector  $x^{\sigma, \varepsilon}$  (the Perron eigenvector of  $A_{\varepsilon, \sigma}$ ) up to a positive multiplicative constant and thus  $u$  is necessarily the Perron root associated with  $A_{\varepsilon, \sigma}$ . Using that  $b = 1$ , we deduce that  $x^{\sigma, \varepsilon} \in \mathbb{R}^n \setminus \{0_{\mathbb{R}^n}\}$  is unique. We can conclude that for every  $\varepsilon > 0$  and every  $\sigma \in (0, 1)$ , system (2.4) with  $\varphi = \varphi_\sigma$  or  $\varphi = \psi_\sigma$  has a unique steady-state  $(x^{\sigma, \varepsilon}, \sigma)$  in  $\mathcal{D}$  and that  $\lambda(A_{\varepsilon, \sigma}) = \varphi_\sigma(x^{\sigma, \varepsilon}) = \psi_\sigma(x^{\sigma, \varepsilon}, \sigma)$ .  $\square$

**Remark 2.2.** If the invariant domain  $\mathcal{D}$  is replaced by the set  $\mathbb{R}^n \setminus \{0_{\mathbb{R}^n}\} \times [0, 1]$ , then, (2.4) with  $\varphi = \psi_\sigma$  has another equilibrium point  $(x, s)$  such that  $x_i = 1/n$  for all  $1 \leq i \leq n$  and  $s = 0$ . But we have seen in Lemma 2.2 that for every initial condition such that  $s(0) > 0$ , then,  $s(t) \rightarrow \sigma$  as  $t \rightarrow +\infty$ . So, convergence to this equilibrium point occurs only if  $s(0) = 0$  which is excluded by definition of  $\mathcal{D}$ . We shall also see in Section 3 that  $s = 0$  is not interesting from an application point of view because in that case, the cost function to be maximized is minimal at  $s = 0$  (see Proposition 3.2). That is why, we have excluded  $s = 0$  in the set  $\mathcal{D}$ .

Our next aim is to establish a global convergence result for the system

$$\dot{x} = D_\sigma x - \varphi_\sigma(x)x + \varepsilon Mx \quad (2.13)$$

in the invariant and attractive set

$$\mathcal{D}_\sigma := \left\{ x \in \mathbb{R}_+^n \setminus \{0_{\mathbb{R}^n}\}; \sum_{j=1}^n x_j = 1 - \sigma \right\}.$$

**Lemma 2.3.** For every  $(\varepsilon, \sigma) \in \mathbb{R}_+^* \times (0, 1)$  and for every  $x^0 \in \mathcal{D}_\sigma$ , the unique solution to (2.13) such that  $x(0) = x^0$  verifies

$$x(t) = (1 - \sigma) \frac{e^{tA_{\varepsilon, \sigma}} x^0}{\sum_{j=1}^n (e^{tA_{\varepsilon, \sigma}} x^0)_j}, \quad t \geq 0. \quad (2.14)$$

In addition, one has  $\lim_{t \rightarrow +\infty} x(t) = x^{\sigma, \varepsilon}$ .

*Proof.* The proof of the next Lemma is adapted from [35] taking into account the presence of mutations. For  $1 \leq i \leq n$ , the function  $q_i := \frac{x_i}{1 - \sigma}$  verifies the ODE:

$$\dot{q}_i = q_i \left( \mu_i(\sigma) - \sum_{j=1}^n \mu_j(\sigma) q_j \right) + \varepsilon (Mq)_i. \quad (2.15)$$

Let then  $r(\cdot)$  denotes the unique solution to the (linear) Cauchy problem

$$\begin{cases} \dot{r} &= A_{\varepsilon, \sigma} r, \\ r(0) &= q(0). \end{cases}$$

A direct computation shows that if we set  $z := \frac{r}{\sum_{i=1}^n r_i}$ , then  $z$  satisfies (2.15). In addition,  $\sum_{j=1}^n r_j(0) = \sum_{j=1}^n \frac{x_j(0)}{1 - \sigma} = 1$  because  $x^0 \in \mathcal{D}_\sigma$ . It follows that  $z(0) = r(0) = q(0)$ , hence, by uniqueness of a solution to a Cauchy problem, we deduce that  $q(t) = z(t)$  for every  $t \geq 0$ . Since  $x = (1 - \sigma)q$ , we obtain (2.14).

Next, we prove the convergence of  $x(\cdot)$  to  $x^{\sigma, \varepsilon}$ . Doing so, let us denote by  $\lambda_1 > \lambda_2 \cdots \geq \lambda_n$  the  $n$  eigenvalues of  $A_{\varepsilon, \sigma}$  (we omit hereafter the dependency w.r.t.  $\varepsilon$  and  $\sigma$  to simplify the exposition). Recall that  $\lambda_1$  is the Perron root of  $A_{\varepsilon, \sigma}$ , hence the first inequality between  $\lambda_1$  and  $\lambda_2$  is strict by the Perron-Frobenius Theorem. Let  $P \in \mathbb{R}^{n \times n}$  be the eigenvector matrix such that  $P^{-1} A_{\varepsilon, \sigma} P = H$  where  $H$  is the diagonal matrix  $H = \text{diag}(\lambda_1, \dots, \lambda_n)$ . The first column of  $P$  is a column vector that we denote by  $v$  and that is proportional to  $x^{\sigma, \varepsilon}$ , i.e.,  $v = \nu x^{\sigma, \varepsilon}$  for some  $\nu > 0$ . If we set  $y := P^{-1}x$ , we obtain from (2.14)

$$y(t) = (1 - \sigma) \frac{e^{tH} y(0)}{\sum_{i=1}^n (P e^{tH} y(0))_i}, \quad t \geq 0.$$

Hence, for every  $1 \leq k \leq n$ , one has

$$y_k(t) = (1 - \sigma) \frac{e^{\lambda_k t} y_k(0)}{\sum_{j=1}^n \tilde{p}_j e^{\lambda_j t} y_j(0)}, \quad t \geq 0,$$

where  $\tilde{p}_j := \sum_{i=1}^n p_{i,j}$  for  $1 \leq j \leq n$ . It follows that for  $2 \leq k \leq n$ , one has  $y_k(t) \rightarrow 0$  as  $t \rightarrow +\infty$  and for  $k = 1$ , we find that  $y_1(t) \rightarrow \frac{1 - \sigma}{\tilde{p}_1}$ . Going back to  $x(\cdot)$ , we obtain that  $x(t) = y_1(t)v + o(1)$  as  $t \rightarrow +\infty$  implying that

$$1 - \sigma = \sum_{j=1}^n x_j(t) = y_1(t) \sum_{j=1}^n v_j + o(1) = y_1(t) \tilde{p}_1 + o(1) = y_1(t) \nu (1 - \sigma) + o(1).$$

Consequently,  $y_1(t) \rightarrow \frac{1}{\nu}$  as  $t \rightarrow +\infty$ . Since  $x = Py$ , we deduce that  $x(t) \rightarrow \frac{v}{\nu} = x^{\sigma, \varepsilon}$  as wanted.  $\square$



*Proof of Theorem 2.1.* Take an initial condition in  $\mathcal{D}$ . System (2.4) with  $\varphi = \varphi_\sigma$  can be written

$$\dot{x} = D_{s(t)}x - \varphi_\sigma(x)x + \varepsilon Mx,$$

whereas with  $\varphi = \psi_\sigma$ , (2.4) becomes

$$\dot{x} = D_{s(t)}x - \psi_\sigma(x, s(t))x + \varepsilon Mx.$$

From Lemmas 2.1 and 2.2, one has  $s(t) \rightarrow \sigma$  as  $t \rightarrow +\infty$ , thus the two preceding systems are asymptotically autonomous systems and the limit equation is (2.13) (since  $s(t) \rightarrow \sigma$  as  $t \rightarrow +\infty$  and the convergence of the dynamics is also uniform in  $x$ ). From Lemmas 2.1 and 2.2, one also has  $b(t) \rightarrow 1$  as  $t \rightarrow +\infty$ . Now, every solution to (2.4) is bounded and is such that its  $\omega$ -limit set is a subset of  $\mathcal{D}_\sigma \times \{\sigma\}$ . We deduce from Lemma 2.3 and from the theory of asymptotically autonomous systems (see [41]) that  $x(t)$  converges to  $x^{\sigma, \varepsilon}$  as  $t \rightarrow +\infty$ , which ends the proof.  $\square$

### 2.3 Comments and remarks about Theorem 2.1

We start by giving a relationship between steady-states of the closed-loop system (2.4) with  $\varphi(s, x) = \varphi_\sigma(x)$  or  $\varphi(s, x) = \psi_\sigma(s, x)$  and steady-states of (2.1) associated with a constant control  $u$ . For  $\varepsilon > 0$  and  $s \in [0, 1]$ , set  $u_c(\varepsilon) := \lambda(D_1 + \varepsilon M)$  and  $\hat{\mu}(s) := \max(\mu_1(s), \dots, \mu_n(s))$ . Remind from [3] that for every  $(\varepsilon, u) \in \mathbb{R}_+^* \times \mathbb{R}_+^*$  such that  $u < u_c(\varepsilon)$ , system (2.1) has a unique locally asymptotically stable equilibrium point  $E_{\varepsilon, u} \in \mathbb{R}^{n+1}$  that is called coexistence steady-state (since every species is present asymptotically).

**Proposition 2.2.** (i) *Given  $(\varepsilon, \sigma) \in \mathbb{R}_+^* \times (0, 1)$  such that  $\hat{\mu}(\sigma) < \hat{\mu}(1) - 2\varepsilon$ , there is a unique  $u_{\sigma, \varepsilon} \in (0, u_c(\varepsilon))$  such that the steady-state  $(x^{\sigma, \varepsilon}, \sigma)$  defined by (2.7) verifies  $(x^{\sigma, \varepsilon}, \sigma) = E_{\varepsilon, u_{\sigma, \varepsilon}}$ .*

(ii) *Conversely, given  $u \in (0, u_c(\varepsilon))$ , there is a unique  $\sigma := s^{\varepsilon, u} \in (0, 1)$  such that  $E_{\varepsilon, u} = (x^{\sigma, \varepsilon}, \sigma)$ .*

*Proof.* Let us prove (i). Thanks to Theorem 2.1, the Perron root of  $A_{\varepsilon, \sigma}$  fulfills the equality

$$\lambda(A_{\varepsilon, \sigma}) = \varphi_\sigma(x^{\sigma, \varepsilon}) = \psi_\sigma(\sigma, x^{\sigma, \varepsilon}) = \frac{\sum_{j=1}^n \mu_j(\sigma)(x^{\sigma, \varepsilon})_j}{\sum_{j=1}^n (x^{\sigma, \varepsilon})_j}.$$

Thus, if we set  $u_{\sigma, \varepsilon} := \frac{\sum_{j=1}^n \mu_j(\sigma)(x^{\sigma, \varepsilon})_j}{\sum_{j=1}^n (x^{\sigma, \varepsilon})_j}$ , we have

$$u_{\sigma, \varepsilon} \leq \hat{\mu}(\sigma) < \hat{\mu}(1) - 2\varepsilon \leq \lambda(D_1 + \varepsilon M),$$

where the last inequality follows from [3, Proposition 3.2]. It follows that  $(x^{\sigma, \varepsilon}, \sigma)$  is a steady-state of (2.1) with  $u = u_{\sigma, \varepsilon}$ , hence  $(x^{\sigma, \varepsilon}, \sigma) = E_{\varepsilon, u_{\sigma, \varepsilon}}$ . In order to show the uniqueness property, suppose that there is  $u \in (0, u_c(\varepsilon))$  such that  $(x^{\sigma, \varepsilon}, \sigma) = E_{\varepsilon, u}$ . We then have  $u = \lambda(A_{\varepsilon, \sigma})$ , hence  $u = u_{\sigma, \varepsilon}$  as was to be proved.

Conversely, given  $u \in (0, u_c(\varepsilon))$ , let us set  $\sigma := s^{\varepsilon, u}$  where  $s^{\varepsilon, u}$  is the unique solution to the equation  $\lambda(D_s + \varepsilon M) = u$ . Since Monod kinetics are increasing, the mapping  $s \mapsto \lambda(D_s + \varepsilon M)$  is increasing over  $[0, 1]$ . Because  $0 < u < \lambda(D_1 + \varepsilon M)$ , we deduce that  $0 < s^{\varepsilon, u} < 1$ . Finally, observe that the steady-state  $E_{\varepsilon, u}$  is the unique solution to the system

$$(D_s + \varepsilon M)x = ux \quad ; \quad \sum_{j=1}^n x_j + s = 1 \quad u = \lambda(D_s + \varepsilon M). \quad (2.16)$$

By uniqueness of the solution to (2.7), we thus deduce the desired equality  $E_{\varepsilon, u} = (x^{\sigma, \varepsilon}, \sigma)$ .  $\square$

Thanks to the preceding proposition, instead of considering a nominal substrate concentration  $\sigma \in (0, 1)$  (giving raise to the unique equilibrium given in Theorem 2.1), we can also pick up a dilution rate  $u \in (0, u_c(\varepsilon))$  defining a unique coexistence steady-state  $E_{\varepsilon, u}$ , and, next, we can apply an auxostat controller that globally stabilizes the closed-loop system (2.4) around  $E_{\varepsilon, u}$  (remind that it is still an open question if  $E_{\varepsilon, u}$  is globally asymptotically stable for (2.1)). We end-up this section by two remarks in the case where  $\varepsilon = 0$  and whenever the kinetics is of Haldane type.

- For  $\varepsilon = 0$ , the techniques above still apply to globally stabilize (2.4) around a desired equilibrium point for which only the dominant species survives. More precisely, let us given  $\sigma \in (0, 1)$  such that  $\mu_1(\sigma) > \dots > \mu_n(\sigma)$ . From Lemmas 2.1 and 2.2, one has  $s(t) \rightarrow \sigma$  and  $b(t) \rightarrow 1$  as  $t \rightarrow +\infty$ . Now, in contrast with Proposition 2.1, system (2.4) with  $\varepsilon = 0$  has  $n$  equilibria apart the washout. But, Lemma 2.3 still applies with  $\varepsilon = 0$  showing that  $\lim_{t \rightarrow +\infty} x(t) = (1 - \sigma, 0, \dots, 0)$  (which is also the limit of (2.1) with  $u := \varphi_\sigma(x^{\sigma, \varepsilon})$  thanks to the competitive exclusion principle [23, 40]). The rest of the proof using the theory of asymptotically autonomous systems remains unchanged. More generally, note that as long as  $\mu_i(\sigma) \neq \mu_j(\sigma)$  for  $i \neq j$ , then,  $\lim_{t \rightarrow +\infty} x(t) = (0, \dots, 0, 1 - \sigma, 0, \dots, 0)$  where the only non-zero coordinate corresponds to the unique  $i_0 \in \{1, \dots, n\}$  such that  $\hat{\mu}(\sigma) = \mu_{i_0}(\sigma)$ .
- In this remark, we highlight the interest of considering auxostat controllers in the case where kinetics are of Haldane type (although hypotheses of Theorem 2.1 are no longer fulfilled in that case). Recall that Haldane's kinetics are given by

$$\mu_i(s) := \frac{m_i s}{k_i + s + \frac{s^2}{k_i}},$$

where all parameters are positive and  $1 \leq i \leq n$ . In contrast with Monod's kinetics,  $\mu_i$  is no longer monotone (see Fig. 2). This implies that given  $(\varepsilon, u) \in \mathbb{R}_+^* \times (0, u_c(\varepsilon))$ , the mapping  $s \mapsto \lambda(D_s + \varepsilon M)$  is no longer strictly monotone, so, the equation  $\lambda(D_s + \varepsilon M) = u$  may have several solutions over  $[0, 1]$ . It follows that the coexistence equilibrium  $E_{\varepsilon, u}$  may no longer be unique (apart the washout). But, one can still consider (2.4) in closed-loop form with  $\varphi = \psi_\sigma$ . Lemma 2.2 and Proposition 2.1 still hold true in that case, so that Lemma 2.3 can be applied to prove that  $\psi_\sigma$  renders (2.4) globally asymptotically stable. In contrast, when  $\varphi = \varphi_\sigma$ , the corresponding closed-loop system may have several equilibria apart the washout (the mapping  $\rho_j$  is no longer monotone in (2.11)). Because  $\mu_i$  is non-monotone, the convergence of  $s(t)$  to  $\sigma$  as  $t \rightarrow +\infty$  is no longer guaranteed (Lemma 2.1 no longer holds true). In conclusion, both feedbacks may still be implemented in practice, however, in that case, only  $\psi_\sigma$  globally stabilizes the resulting closed-loop system at the desired steady-state point.

### 3 Optimization of microbial production

In this section, we study the problem of maximizing biomass production (*i.e.*, the production of every species) w.r.t. the dilution rate  $t \mapsto u(t)$ . Remind that the dilution rate can be easily controlled from an application point of view, so, it is commonly used as a control variable in bioprocesses. Our aim is to show the efficiency of the feedback controls (2.5) and (2.6) in this context w.r.t. optimal strategies giving that way an alternative to classical approaches using the PMP or numerical methods. Let  $T > 0$  and let us consider the optimal control problem of Lagrange type:

$$\max_{u(\cdot) \in [0, u_{max}]} J_T(u) := \int_0^T u(t) \sum_{j=1}^n x_j(t) dt, \quad (3.1)$$

where  $(x(\cdot), s(\cdot))$  is the unique solution to (2.1) starting at some fixed initial condition in  $\mathcal{D}$  and associated with a measurable function  $t \mapsto u(t)$  with values within  $[0, u_{max}]$  over  $[0, T]$ . Here  $u_{max} > 0$  is fixed and represents the maximal feed rate of the reactor that can always be chosen large enough from a practical point of view (in particular, in such a way that singular arcs are always admissible, see, *e.g.*, [7] in which saturation in the chemostat occurs). Now, the existence of an optimal control of (3.1) is straightforward since the problem is linear w.r.t. the entry  $u$ . Our approach to study (3.1) is as follows.

- In Section 3.1, we apply the Pontryagin Maximum Principle on (3.1) which allows us to derive properties on optimal controls.
- In Section 3.2, we study the Legendre-Clebsch condition along any singular arc. This enables us to formulate a conjecture about the structure of optimal controls.
- In section 3.4, we describe an alternative approach for studying (3.1) based on Section 2 and the utilization of feedback control laws.
- In Section 3.5, we provide numerical results about the comparison between the value function associated with (3.1) (*i.e.*,  $\max_{u(\cdot)} J_T(u)$ ) and the value of  $J_T$  computed with the feedback controls  $\varphi_\sigma$  and  $\psi_\sigma$ .

### 3.1 Application of the Pontryagin Maximum Principle

In its present form, the most convenient way to derive necessary optimality conditions on optimal solutions of (3.1) is to apply the PMP (see [34]). Since there are no terminal constraints on the state, the Hamiltonian  $H = H(x, s, \lambda, u)$  associated with (3.1) can be written in normal form as follows:

$$H = \sum_{j=1}^n (\lambda_j - \lambda_{n+1}) \mu_j(s) x_j + \varepsilon \sum_{j=1}^n \lambda_j (Mx)_j + u \left[ \sum_{j=1}^n (1 - \lambda_j) x_j + \lambda_{n+1} (1 - s) \right], \quad (3.2)$$

where  $\lambda \in \mathbb{R}^{n+1}$  is the covector. Let  $(x, s, u)$  be an optimal triple of (3.1). According to the PMP, there is an absolutely continuous function  $\lambda : [0, T] \rightarrow \mathbb{R}^{n+1}$  (the adjoint vector) such that the quadruple  $(x, s, \lambda, u)$  fulfills the following conditions:

- The adjoint vector satisfies the terminal condition  $\lambda(T) = 0$  together with the adjoint equation

$$\dot{\lambda}(t) = -\nabla_{x,s} H(x(t), s(t), \lambda(t), u(t)) \quad \text{a.e. } t \in [0, T],$$

that can be equivalently written

$$\begin{cases} \dot{\lambda}_i &= (\lambda_{n+1} - \lambda_i) \mu_i(s) - \varepsilon (M\lambda)_i + u(\lambda_i - 1), \quad 1 \leq i \leq n, \\ \dot{\lambda}_{n+1} &= \sum_{j=1}^n (\lambda_{n+1} - \lambda_j) \mu'_j(s) x_j + u \lambda_{n+1}. \end{cases}$$

- The optimal control  $u$  satisfies the Hamiltonian maximization condition:

$$u(t) \in \operatorname{argmax}_{v \in [0, u_{max}]} H(x(t), s(t), \lambda(t), v) \quad \text{a.e. } t \in [0, T].$$

In order to make more explicit this condition, let us denote by  $\phi$  the switching function

$$\phi := \sum_{j=1}^n (1 - \lambda_j) x_j + (1 - s) \lambda_{n+1}.$$

The Hamiltonian condition then implies that every optimal control  $u$  satisfies the following property

$$\begin{aligned} \phi(t) > 0 &\Rightarrow u(t) = u_{max}, \\ \phi(t) < 0 &\Rightarrow u(t) = 0, \end{aligned} \quad (3.3)$$

for almost every  $t \in [0, T]$ , except along (possible) singular arcs<sup>5</sup>  $\mathcal{S}_{[t_1, t_2]}$  which are sub-arcs such that  $\phi \equiv 0$  on the corresponding time interval  $[t_1, t_2]$  (recall that (3.1) is affine w.r.t. the control). Next, we denote by  $\mathcal{B}_-$ , resp.  $\mathcal{B}_+$  a bang arc  $u = 0$ , resp.  $u = u_{max}$ . Remind that a *switching time*  $t_s$  is an instant such that the control is non-constant in every neighborhood of  $t = t_s$ , so that we necessarily have  $\phi(t_s) = 0$ . Before developing further the analysis of singular arcs, we give the following property about optimal controls in a neighborhood of the terminal time.

**Property 3.1.** *For every initial condition in  $\mathcal{D}$  and for every optimal control  $u$  of (3.1), there is  $\eta \in (0, T)$  such that*

$$\forall t \in [T - \eta, T], \quad u(t) = u_{max}. \quad (3.4)$$

*In addition, for every  $1 \leq i \leq n$ , the function  $\rho_i := \lambda_{n+1} - \lambda_i$  is negative over  $[T - \eta, T)$ .*

*Proof.* Since  $\lambda(T) = 0$ , we deduce that  $\phi(T) > 0$  which implies (3.4). Now, using the adjoint equation, we deduce that for  $1 \leq i \leq n$ ,  $\rho_i$  satisfies the ODE:

$$\dot{\rho}_i = \sum_{j=1}^n \mu'_j(s(t)) x_j(t) \rho_j + (u(t) - \mu_i(s(t))) \rho_i + u(t) + \varepsilon (M\lambda)_i. \quad (3.5)$$

Thanks to the terminal condition, we obtain that for  $1 \leq i \leq n$ , one has  $\rho_i(T) = 0$  and  $\dot{\rho}_i(t) = u_{max} + o(1)$  when  $t \rightarrow T$ . Hence, taking  $\eta$  small enough if necessary, we get that  $\rho_i < 0$  over  $[T - \eta, T)$  for every  $1 \leq i \leq n$  as wanted.  $\square$

Thanks to this property, every optimal trajectory ends up with a bang arc  $\mathcal{B}_+$ . Thus, a maximum of biomass is harvested at the end of the culture.

<sup>5</sup>We refer to [9, 10, 38] for more details about singular arcs.

### 3.2 Optimality of singular arcs

In this section, we investigate the structure of optimal controls through the analysis of singular arcs. We would like to know if an optimal control is rather of bang-bang type, or if it can contain a singular arc. To study qualitative properties of singular arcs, we need to differentiate twice the switching function w.r.t. the time  $t$  following geometric control theory for mono-input control-affine systems (see [9, 10, 38]). We find that  $\dot{\phi}$  and  $\ddot{\phi}|_u$  are given by the following expressions (next, we omit the variable  $t$  to simplify the notation):

$$\begin{aligned}\dot{\phi} &= \sum_{j=1}^n \mu_j(s)x_j + (1-s) \sum_{j=1}^n (\lambda_{n+1} - \lambda_j)\mu'_j(s)x_j, \\ \ddot{\phi}|_u &= 2(1-s) \sum_{j=1}^n \mu'_j(s)x_j + (1-s)^2 \sum_{j=1}^n (\lambda_{n+1} - \lambda_j)\mu''_j(s)x_j,\end{aligned}$$

over  $[0, T]$ . Recall from [9, 10, 38] that if a singular arc is optimal over a time interval  $[t_1, t_2]$ , then one must have

$$\phi(t) = \dot{\phi}(t) = 0 \quad \text{and} \quad \ddot{\phi}|_u(t) \geq 0,$$

for every  $t \in [t_1, t_2]$ . The non-negativity of  $\ddot{\phi}|_u$  is known as the *Legendre-Clebsch* necessary optimality condition. If one has  $\ddot{\phi}|_u > 0$  over  $[t_1, t_2]$ , we say that the strict Legendre-Clebsch condition is verified.

**Proposition 3.1.** *Let  $u$  be an optimal control of (3.1) with  $\varepsilon = 0$ . If  $u$  has a non-saturated<sup>6</sup> singular arc over a time interval  $[t_1, t_2]$  and if  $\rho_i < 0$  over  $[t_1, t_2]$ , the strict Legendre-Clebsch necessary optimality condition is verified over  $[t_1, t_2]$ .*

*Proof.* In view of the expressions of  $\mu_i$ , one has  $\mu''_i < 0$  for every  $1 \leq i \leq n$ . It follows that the second sum in the expression of  $\ddot{\phi}|_u$  is positive. Since the first sum in the expression of  $\ddot{\phi}|_u$  is also positive, the result follows.  $\square$

**Remark 3.1.** (i) *Showing that  $\rho_i < 0$  along a singular arc is an open question that could be addressed using the fact that (3.5) is cooperative (see [39]). This property is not straightforward : we only know that  $\rho_i < 0$  in some time interval  $[T - \eta, T]$  (Property 3.1). Establishing this property globally is more involved and out of the scope of the paper.*

(ii) *Under the hypotheses of Proposition 3.1, only singular arcs of first order may occur. This means that the corresponding singular control can be derived from the expression of  $\dot{\phi}$  since  $\dot{\phi}|_u > 0$  as a feedback control of the state and covector, see [9, 10, 38]). In particular, no chattering occurs.*

(iii) *From a numerical point of view, the Legendre-Clebsch condition can be used to confirm the optimality of a singular arc once a candidate has been computed numerically (via a direct or an indirect shooting method).*

Based on the preceding remarks and on numerical simulations (see Section 3.5), we posit the following conjecture related to the structure of an optimal control.

**Conjecture 3.1.** *Given  $T > 0$  and  $(x_0, s_0) \in \mathcal{D}$ , an optimal control  $u$  of (3.1) is of turnpike type, i.e., it consists of three arcs concatenated as follows:*

$$\mathcal{B}_\pm - \mathcal{S}_{[t_1, t_2]} - \mathcal{B}_+.$$

The structure of the control given above is in line with the *turnpike phenomenon* [42]. This means that an optimal control is everywhere almost stationary (i.e., the extremal  $(x, s, \lambda, u)$  is close to a stationary point of the associated static optimization problem, see Section 3.3 and Fig. 4 and 5) except in a neighborhood of  $t = 0$  and  $t = T$ . However, the optimal control problem under investigation is linear w.r.t. the entry, so, it is not clear a priori how to apply the results of [42] in our setting. At time  $t = 0$ , the bang arc  $\mathcal{B}_\pm$  depends on the initial condition and it is difficult to know in advance if one has  $u(t) = 0$  or  $u(t) = u_{max}$  in a neighborhood of  $t = 0$ . Nevertheless, the existence of a last bang arc  $\mathcal{B}_+$  is given by Property 3.1. In any case, providing an optimal synthesis (i.e., the construction of an optimal control in feedback form) in a high dimensional (non-linear) optimal control problem is in general non tractable. The theoretical properties obtained from the PMP serve to have insights into the structure of an optimal control. The study of optimal controls can be completed by a numerical study in two steps:

<sup>6</sup>i.e., the associated singular control is with admissible values within 0 and  $u_{max}$  over  $[t_1, t_2]$ .

- the optimal control problem is solved numerically by a direct method (like `bocop`, see [8]) which provides a control  $u_\varepsilon$  (see Section 3.5 for the numerical results) ;
- the control  $u_\varepsilon$  serves to initialize an indirect shooting method (taking as parameters  $\lambda(0)$ ,  $t_1$  and  $t_2$ ) to compute an optimal control  $u_\varepsilon^*$ .

Following this procedure, we would obtain that way a numerical approximation of an optimal control  $t \mapsto u(t)$  in open loop associated with some initial condition. Due to uncertainties in the process and measurements in the reactor (biogas or concentration), it is desirable from an application point of view to synthesize an efficient strategy in feedback form (also called sub-optimal strategy). This is what we do in what follows.

### 3.3 Static optimization problem

Our objective now is to implement the feedback controls  $\varphi_\sigma$  and  $\psi_\sigma$  in (3.1) in order to compare the corresponding value of the cost function to the value function associated with (3.1). Note that the controllers  $\varphi_\sigma$  and  $\psi_\sigma$  depend on a parameter  $\sigma$  that needs to be determined. This parameter will be defined as the solution to the following (static) optimization problem associated with (3.1). Remind that system (2.7) uniquely characterizes the coexistence steady-state. Hence, the static optimization problem associated with (3.1) can be gathered into

$$\max_{(u,x,s) \in \Omega} u \sum_{j=1}^n x_j \quad \text{s.t.} \quad \begin{cases} A_{\varepsilon,s} x & = ux, \\ \sum_{j=1}^n x_j + s & = 1, \\ \lambda(A_{\varepsilon,s}) & = u, \end{cases} \quad (3.6)$$

where  $\Omega := [0, u_{max}] \times \mathbb{R}_+^n \times [0, 1]$  (remind that  $\lambda(A)$  denotes the greatest eigenvalue of a quasi-positive irreducible matrix  $A \in \mathbb{R}^{n \times n}$ ).

**Proposition 3.2.** *The optimization problem (3.6) has a solution and it is equivalent to*

$$\max_{s \in (0,1)} \lambda(A_{\varepsilon,s})(1-s). \quad (3.7)$$

*Proof.* Clearly, (3.6) is equivalent to (3.7). Moreover, the existence is straightforward using the continuity of  $s \mapsto \lambda(A_{\varepsilon,s})(1-s)$  over  $[0, 1]$  (see, e.g., [3]) and the fact that  $s \mapsto \lambda(A_{\varepsilon,s})(1-s)$  is non-negative, vanishes at  $s = 0$  (because in that case  $\lambda(A_{\varepsilon,s}) = \lambda(M) = 0$ ) and at  $s = 1$ .  $\square$

**Remark 3.2.** *Based on numerical simulations, we believe that the solution to (3.6) is unique in the case of Monod kinetics for (2.4). Nevertheless, we have not detailed this issue here to avoid complicated computations involving the second derivative of the Perron root  $\lambda$ .*

On Fig. 1, we depict the mapping  $s \mapsto \lambda(A_{\varepsilon,s})(1-s)$  over  $[0, 1]$  verifying numerically the existence and uniqueness of a maximizer of this function over  $[0, 1]$  for various values of the parameters defining the kinetics.

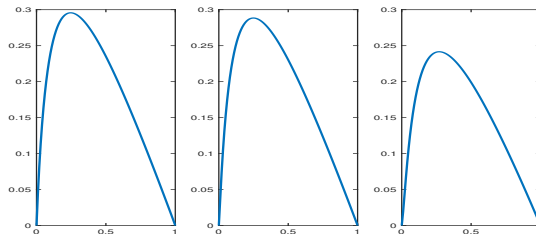


Figure 1: Plot of  $s \mapsto \lambda(A_{\varepsilon,s})(1-s)$  over  $[0, 1]$  for  $\varepsilon = 0$  (left),  $\varepsilon = 0.01$  (middle), and  $\varepsilon = 0.1$  (right). The number of species is  $n = 5$  and kinetics are arbitrary Monod functions.

### 3.4 Definition of sub-optimal strategies

We now furnish an alternative method to the PMP or numerical simulations via a direct or indirect method for studying (3.1). First, we suppose that  $u_{max}$  fulfills the inequality

$$u_{max} > \hat{\mu}(1), \quad (3.8)$$

in order to ensure the admissibility of the feedback controls (without saturation). As mentioned previously,  $u_{max}$  can always be taken large enough from a practical point of view. The next property is related to the admissibility of the feedback controls  $\varphi = \varphi_\sigma$  and  $\varphi = \psi_\sigma$

**Property 3.2.** *Given  $(\varepsilon, \sigma) \in \mathbb{R}_+^* \times (0, 1)$ , there is a time  $t_0 \geq 0$  such that for every solution to (2.4) with  $\varphi = \varphi_\sigma$  or  $\varphi = \psi_\sigma$ , one has:*

$$\forall t \geq t_0, \varphi_\sigma(x(t)) \leq u_{max} \quad \text{and} \quad \psi_\sigma(x(t), s(t)) \leq u_{max}.$$

*Proof.* Consider a solution to (2.1) starting in  $\mathcal{D}$  with  $u = \varphi_\sigma(x)$  or  $u = \psi_\sigma(x, s)$ . Theorem 2.1 implies that when  $t \rightarrow +\infty$ , one has  $\varphi_\sigma(x(t)) \rightarrow u_{\sigma, \varepsilon}$  and  $\psi_\sigma(x(t), s(t))$  as well. Now,  $u_{\sigma, \varepsilon} \leq \hat{\mu}(\sigma) < \hat{\mu}(1) < u_{max}$  and the result follows.  $\square$

So, if the feedbacks are non

Next, given  $\varepsilon \in \mathbb{R}_+^*$  and  $T \in \mathbb{R}_+^*$ , we propose the following sub-optimal strategy in feedback form:

- Step 1 : we compute  $\sigma_\varepsilon^* \in (0, 1)$  as a solution to (3.6) and we consider (2.1) with a single input  $u$  ;
- Step 2 : if the state  $x$  is an available output, we apply the feedback control (2.5) with  $\sigma = \sigma_\varepsilon^*$ . If microbial growth<sup>7</sup> is an available output, then we apply (2.6) with  $\sigma = \sigma_\varepsilon^*$ .

In the next section, we compare the optimal value of cost function to the cost function computed thanks to the above strategy.

### 3.5 Numerical simulations

We have carried out numerical simulations of (3.1) with four strategies:

- the functional  $J_T$  is computed with the constant dilution rate  $u(t) = u_\varepsilon^s$  corresponding to the optimal solution of the static problem ;
- the functional  $J_T$  is computed with the feedback control  $\varphi_{\sigma_\varepsilon^*}$  to regulate the substrate concentration at the optimal value of the static problem ;
- the functional  $J_T$  is computed with the feedback control  $\psi_{\sigma_\varepsilon^*}$ , with the same set-point ;
- the minimum of  $J_T$  is computed numerically with a direct solver `bocop` [8], using Lobatto IIIC discretization method with 200 time steps.

In the simulations, we consider 10 species and their specific growth rates are taken of Monod type, as follows:

$$\mu_i(s) = \frac{i \cdot s}{i^2 + s}, \quad i = 1, \dots, 10.$$

This represents a trade-off between substrate affinity and maximum growth rate (in line with [21]), so that each species is the best for certain substrate concentrations (see Fig. 2). The other parameters are taken as follows:  $\varepsilon = 0.01$ ,  $s_{in} = 100$  (in place of  $s_{in} = 1$  in (2.1)), and  $u_{max} = 5$ .

Trajectories are represented in Fig. 3. With the two feedbacks, the substrate concentration rapidly reaches its set-point, while this takes more time with the constant dilution rate. The optimal trajectory obtained with `bocop` is very similar to the feedback trajectories: the substrate concentration rapidly reaches the static optimal value  $\sigma_\varepsilon^*$ . The main difference is the Bang arc  $\mathcal{B}_+$  at the end, in order to harvest all the biomass.

We also compare the performances of the four above strategies as a function of the time horizon  $T$  (see Fig. 4), using the numerical optimal solution (obtained via `bocop`) as a reference. When the time horizon is

<sup>7</sup>Microbial growth and biogas activity in a continuous stirred tank reactor are quantities proportional to  $\sum_{j=1}^n \mu_j(s(t))x_j(t)$ .

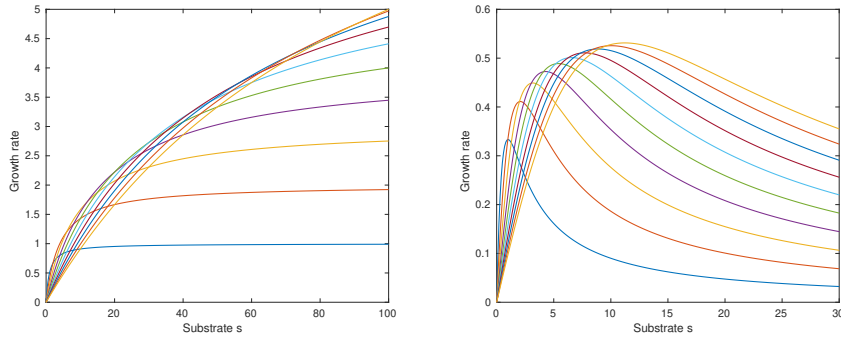


Figure 2: Specific growth rates  $\mu_i(\cdot)$  over  $[0, s_{in}]$  for the ten species used for our simulations. *Fig. left*: Monod functions. *Fig. right*: Haldane functions.

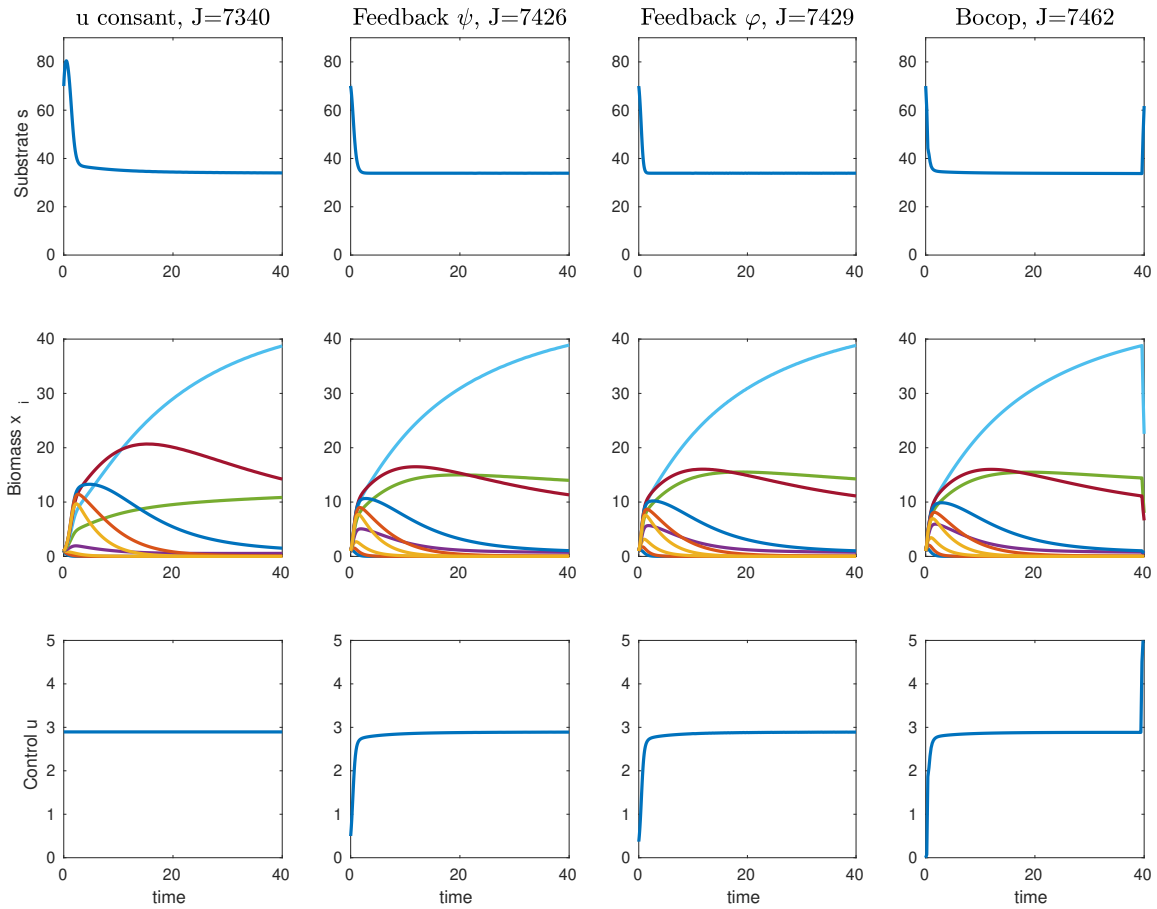


Figure 3: Trajectories with Monod growth functions using either a constant dilution rate  $u_\varepsilon^s$ , the feedbacks  $\varphi_{\sigma_\varepsilon^*}$  or  $\psi_{\sigma_\varepsilon^*}$ , or the optimal trajectory obtained numerically with `bocop`.

short, the constant dilution rate strategy performs the worst, while the two feedback strategies are similar, slightly under the optimal value of the cost function. When the time horizon becomes large enough, all the strategies give almost the same performance. All the trajectories stay the majority of the time on the optimal steady states, and the differences in the function appearing during the transients ( $t = 0$  and  $t = T$ ) become negligible.

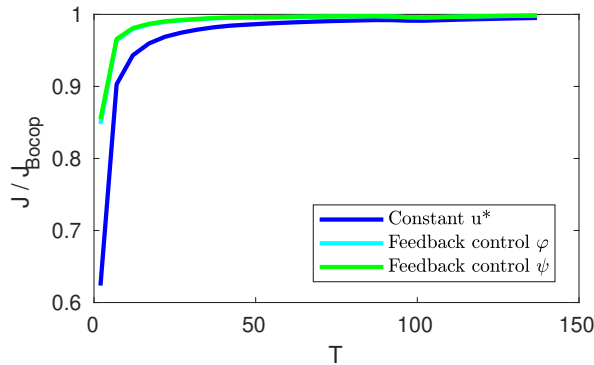


Figure 4: Comparison of the criteria between the different strategies, as a function of the time horizon. The curves for the two feedbacks are overlapped.

Finally, we have also considered specific growth rates with Haldane type (see Fig. 2):

$$\mu_i(s) = \frac{i \cdot s}{i^2 + s + i^{-0.1} \cdot s^2}, \quad i = 1, \dots, 10,$$

and for the other parameters, we have chosen  $\varepsilon = 0.01$ ,  $s_{in} = 30$  (in place of  $s_{in} = 1$  in (2.1)), and  $u_{max} = 1$ . The same four strategies are simulated and numerical results are depicted on fig. 5. When using a constant dilution rate, we observe that washout occurs. Actually, when considering inhibition, it is well known that the monospecies chemostat model presents bi-stability [18]. It appears that our system possesses a similar behavior : if we start with a high value of the dilution rate and a high input substrate concentration, the chemostat is washed out. On the other hand, the two feedback controls give accurate results with a production (*i.e.*, the value of  $J_T$ ) close to the optimal one computed numerically via `bocop`.

## 4 Conclusion and perspectives

In this paper, we have introduced feedback auxostat controllers in the chemostat system with mutations allowing the regulation of the substrate concentration at a desired value. The feedback control  $\varphi_\sigma$  requires measurements of the concentration of species  $x_i$  whereas the feedback  $\psi_\sigma$  can be used if one has access to measurements of the microbial growth. We have proved that the resulting closed-loop system is globally asymptotically stable around the desired steady-state of the system.

Next, we have considered an optimal control problem which consists in maximizing the production of all species in the chemostat system over a given time interval. Thanks to the PMP, we have given qualitative properties of optimal controls and singular arcs that confirm the turnpike structure of optimal controls obtained numerically. Since this approach furnishes an open loop control for each initial condition, it may suffer from lack of robustness in presence of uncertainties. So, we introduced sub-optimal strategies using the auxostat controllers regulated at the optimal solution to the static optimization problem associated with (3.1). We have seen that when the terminal time is large enough, these sub-optimal strategies provide an accurate approximation of the optimal cost value whereas with a constant control, the approximation of the optimal cost value is less accurate.

One main feature is that the proposed control strategies depend on the state only, so, once the static optimization problem has been solved, they can be used in practice to face uncertainties under the condition that one has access to one-line measurements of the state. Thanks to numerical simulations, we have also seen that the feedback controls are also of interest in the case where the kinetics are of Haldane type (in that case, global convergence results may no longer be guaranteed depending on the choice of the feedback control).

Future works related to the first part of the paper about the chemostat system with mutations could investigate the study of global convergence of (2.4) in the case where the control  $u$  is constant (this question remains difficult, see [3]) and in the more general case of Haldane's kinetics for the species. Another perspective could be to consider the case where yield coefficients are no longer equal to one (so that mass conservation is no longer guaranteed) and with a more general mutation matrix  $M$ .



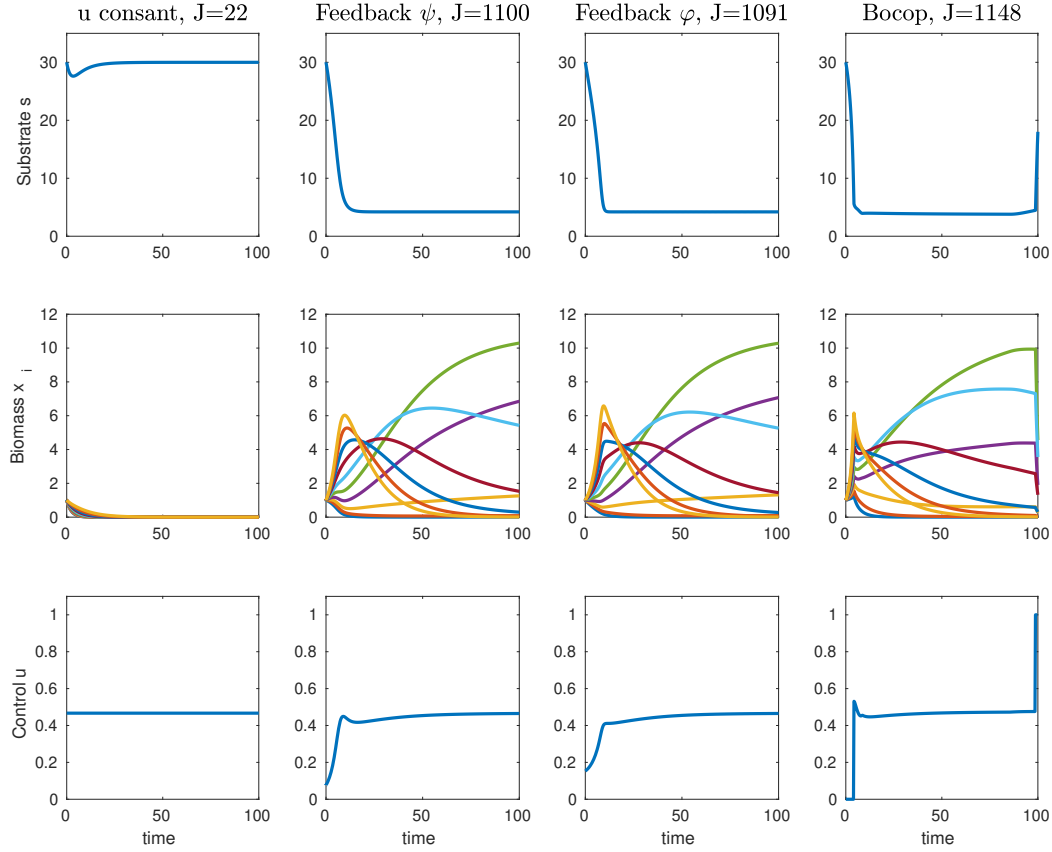


Figure 5: Trajectories with Haldane growth functions using either a constant dilution rate  $u^*$ , the feedbacks  $\varphi_{\sigma_\varepsilon^*}$  or  $\psi_{\sigma_\varepsilon^*}$ , or the optimal trajectory obtained numerically with `bocop`

Concerning the second part of the paper about the study of the maximization of the production of species, it could be interesting to refine our approach by incorporating two bang arcs (over  $[0, t_1]$  and  $[t_2, T]$  in line with Pontryagin's Principle) while keeping a stabilizing feedback control over  $[t_1, t_2]$  (or taking the constant static optimal value of the control), and to optimize the cost function w.r.t. the switching instants (see, *e.g.*, [11]).

## References

- [1] S.S. ARKIN, *Microbial evolution in the chemostat*, PhD Thesis, 2010, <http://hdl.handle.net/10044/1/11305>.
- [2] G. BASTIN, D. DOCHAIN, *On-line estimation and adaptive control of bioreactors*, Elsevier, New York, 1990.
- [3] T. BAYEN, H. CAZENAVE-LACROUTZ, J. COVILLE, *Stability of the chemostat system with a mutation factor*, to appear in *Discrete Contin. Dyn. Syst. Ser. B*, 2022, <https://arxiv.org/abs/2110.09582>.
- [4] T. BAYEN, P. GAJARDO, *A note on the steady state optimization of the biogas production in a two-stage anaerobic digestion model*, *J. Math. Biol.*, vol. 78, 4, pp. 1067–1087, 2019.
- [5] T. BAYEN, F. MAIRET, *Optimization of the separation of two species in a chemostat*, *Automatica J. IFAC*, vol. 50, 4, pp. 1243–1248, 2014.
- [6] T. BAYEN, F. MAIRET, *Optimization of strain selection in evolution experiments in chemostat*, *Internat. J. Control*, vol. 90, 12, pp. 2748–2759, 2017.

- [7] T. BAYEN, F. MAIRET, M. MAZADE, *Analysis of an optimal control problem connected to bioprocesses involving a saturated singular arc*, Discrete Contin. Dyn. Syst. Ser. B, vol. 20, 1, pp.39–58, 2015.
- [8] J.-F. BONNANS, V. GRELARD, P. MARTINON, Bocop, the optimal control solver, Open source toolbox for optimal control problems, <http://bocop.org>, 2011.
- [9] B. BONNARD, M. CHYBA, *Singular Trajectories and their role in Control Theory*, Springer, SMAI, vol. 40, 2002.
- [10] U. BOSCAIN, B. PICCOLI, *Optimal Syntheses for Control Systems on 2-D Manifolds*, Springer SMAI, vol. 43, 2004.
- [11] J.-B. CAILLAU, W. DJEMA, J.-L. GOUZÉ, S. MASLOVSKAYA, J.-B. POMET, *Turnpike Property in Optimal Microbial Metabolite Production*, J. Optim. Theory Appl., vol. 194, pp. 375–407, 2022.
- [12] P. DE LEENHEER, J. DOCKERY, T. GEDEON, S. PILYUGIN, *The chemostat with lateral gene transfer*, J. Biol. Dyn., vol. 4, 6, pp. 607–620, 2010.
- [13] P. DE LEENHEER, S.S. PILYUGIN, *Multistrain virus dynamics with mutations: A global analysis*, Math. Med. Biol., vol. 25, 4, pp. 285–322, 2008.
- [14] W. DJEMA, T. BAYEN, O. BERNARD, *Optimal Darwinian Selection of Microorganisms with Internal Storage*, Processes, vol. 10, 3, 461, 2022.
- [15] W. DJEMA, L. GIRALDI, S. MASLOVSKAYA, O. BERNARD, *Turnpike features in optimal selection of species represented by quota models*, Automatica J. IFAC, vol. 132, 109804, 2021.
- [16] D. DOCHAIN, P. VANROLLEGHEM, *Dynamical modelling and estimation in wastewater treatment processes*, IWA Publishing, vol. 4, London, 2001.
- [17] D. DOCHAIN, M. PERRIER, *Dynamical modelling, analysis, monitoring and control design for nonlinear bioprocesses*, Springer Berlin Heidelberg, Berlin, Heidelberg, 1997, pp. 147–197.
- [18] R. FEKIH-SALEM, N. ABDELLATIF, T. SARI, J. HARMAND, *Analyse mathématiques d’un modèle de digestion anaérobie ‘a trois étapes*, ARIMA, vol. 17, pp. 53–71, 2014.
- [19] P. GAJARDO, F. MAZENC, H. RAMIREZ, *Competitive exclusion principle in a model of chemostat with delays*, Discrete Contin. Dyn. Syst., vol. 16, pp. 253–272, 2009.
- [20] A. GHOULI, T. SARI, J. HARMAND, *Maximizing biogas production from the anaerobic digestion*, Journal of Process Control, vol. 36, pp. 79–86, 2015.
- [21] I. GUDELJ, R. BEARDMORE, S. ARKIN, C. MACLEAN, *Constraints on microbial metabolism drive evolutionary diversification in homogeneous environments*, Journal of Evolutionary Biology, vol. 20, pp. 1882–1889, 2007.
- [22] A. HADDON, H. RAMIREZ, A. RAPAPORT, *Optimal and sub-optimal feedback controls for biogas production*, J. of Optim. Theory and Appl., vol. 183, 2, pp. 642–670, 2020.
- [23] J. Harmand, C. Lobry, A. Rapaport, T. Sari, *The Chemostat: Mathematical Theory of Microorganism Cultures*, Wiley-ISTE, 2017.
- [24] H. KHALIL, *Nonlinear Systems*, 2nd ed. Prentice Hall, 1996.
- [25] C. LOBRY *La compétition dans le chémostat*, Travaux En Cours 81 : Des Nombres et des Mondes, pp. 119–187, édition Herman, Paris, 2013.
- [26] L. MAILLERET, O. BERNARD, J.-P. STEYER, *Nonlinear adaptive control for bioreactors with unknown kinetics*, Automatica J. IFAC, vol. 40, 8, pp. 1379–1385, 2004.
- [27] F. MAZENC, J. HARMAND, M. MALISOFF, *Stabilization in a chemostat with sampled and delayed measurements and uncertain growth functions*, Automatica J. IFAC, vol. 78, pp. 241–249, 2017.

- [28] F. MAZENC, M. MALISOFF, *Stability and stabilization for models of chemostats with multiple limiting substrates*, J. Biol. Dyn., 6, pp. 612–627, 2012.
- [29] F. MAZENC, S.I. NICULESCU, G; ROBLEDO, *Stability analysis of mathematical model of competition in a chain of chemostats in series with delay*, Appl. Math. Model., vol. 76, pp.311–329, 2019.
- [30] S. MIRRAHIMI, B. PERTHAME, J.Y. WAKANO, *Evolution of species trait through resource competition*, J. Math. Biol., vol. 64, 7, pp. 1189–1223, 2012.
- [31] J. MONOD, *Recherches sur la Croissance des Cultures Bactériennes*, Hermann, Paris 1942.
- [32] J. MONOD, *La technique de culture continue théorie et applications*, Ann. Inst. Pasteur, 79, pp. 390–410, 1950.
- [33] A. NOVICK, L. SZILARD, *Experiments with the chemostat on spontaneous mutations of bacteria*, PNAS 36: pp.708–719, 1950.
- [34] L.S. PONTRYAGIN, V.G. BOLTYANSKIY, R.V. GAMKRELIDZE, E.F. MISHCHENKO, *Mathematical theory of optimal processes*, The Macmillan Company, 1964.
- [35] A. RAPAPORT, D. DOCHAIN, J. HARMAND, *Practical coexistence in the chemostat with arbitrarily close growth functions*, ARIMA, vol. 9, 2007, Conference in honor of Claude Lobry, 2008.
- [36] G. ROBLEDO, *Feedback stabilization for a chemostat with delayed output*, Math. Biosci. Eng., vol. 6, 3, pp. 629–647, 2009.
- [37] T. SARI, *Best operating conditions for biogas production in some simple anaerobic digestion models*, Processes, vol. 10, 2, 258, 2022.
- [38] H. SCHATTLER, U. LEDZEWICZ, *Geometric Optimal Control*, Springer, 2012.
- [39] H. L. SMITH, *Monotone dynamical systems, an introduction to the theory of competitive and cooperative systems*, Mathematical Surveys and Monographs. American Mathematical Society, 1995.
- [40] H.L. SMITH, P. WALTMAN, *The theory of the chemostat, Dynamics of microbial competition*, Cambridge University Press, 1995.
- [41] H. THIEME, *Convergence results and a Poincaré Bendixson trichotomy for asymptotically autonomous differential equations*, J. Math. Biol, vol. 30, pp. 755–763, 1992.
- [42] E. TRELAT, E. ZUAZUA, *The turnpike property in finite-dimensional nonlinear optimal control*, J. Differential Equations, vol. 258, pp. 81–114, 2015.
- [43] H. VELDKAMP, *Ecological Studies with the Chemostat*, Springer US, Boston, MA, 1977, pp. 59–94.